DISSECTING THE ETIOLOGY OF ATRIAL FIBRILLATION

A POPULATION PERSPECTIVE ON RISK FACTORS AND SEX DIFFERENCES

SVEN GEURTS

Dissecting the Etiology of Atrial Fibrillation

A population perspective on risk factors and sex differences

Sven Geurts

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Dissecting the Etiology of Atrial Fibrillation

A population perspective on risk factors and sex differences

De ontleding van de etiologie van atriumfibrilleren

Een populatie perspectief op risicofactoren en sekse verschillen

Proefschrift

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Copromotor:	dr. M. Kavousi

To my loving parents, brother, and Anouk

Aut cum scuto aut in scuto

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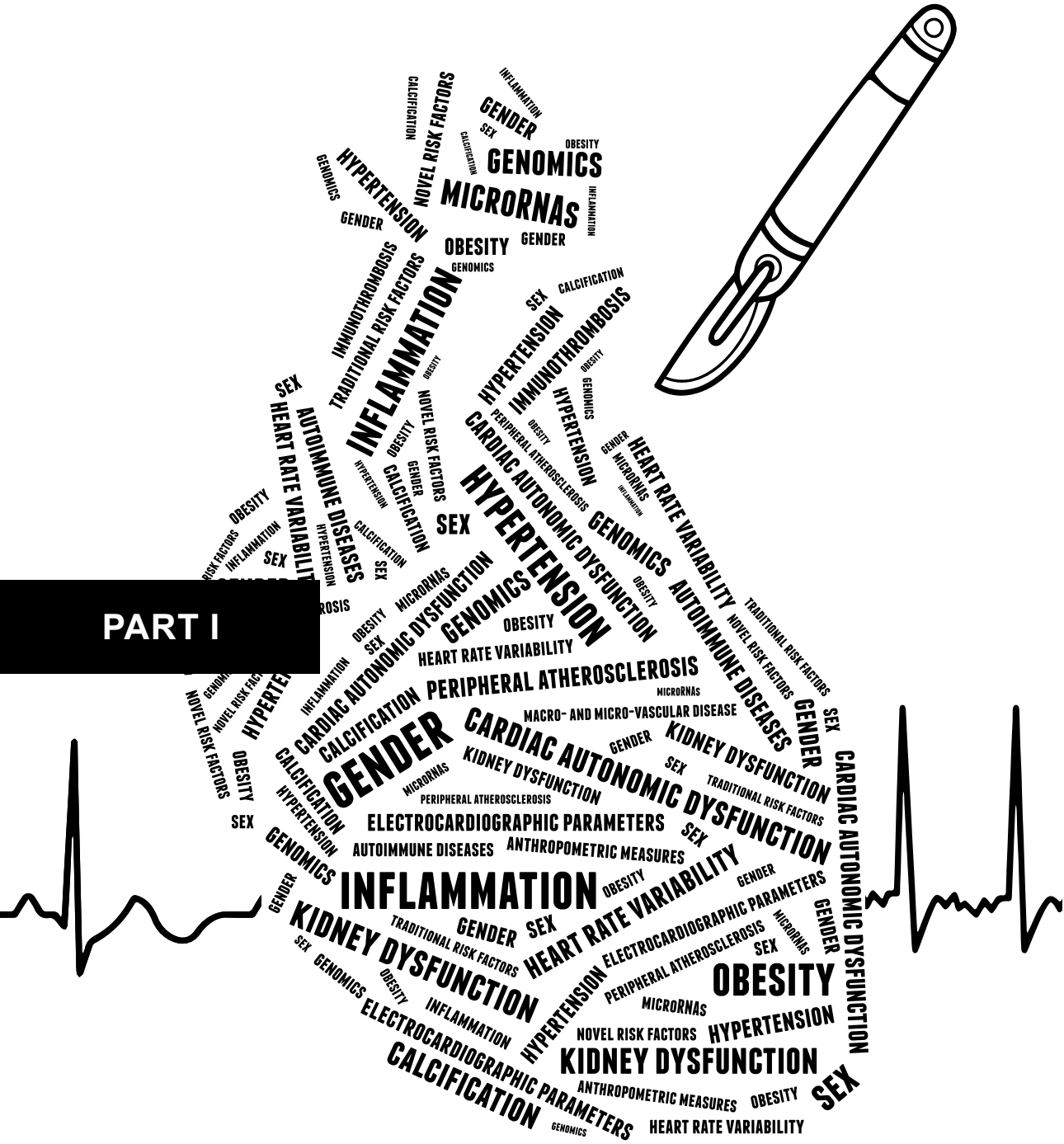
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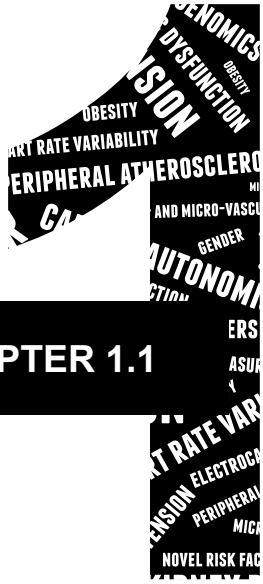
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC MEASURES
MACRO- AND MICRO-VASCULAR DISEASE
MICRORNAS

General introduction and thesis objective



CHAPTER 1.1

General introduction

General introduction

Atrial fibrillation

Atrial fibrillation is defined as a supraventricular tachyarrhythmia with chaotic atrial excitation, unproductive atrial contraction, and subsequently irregularly irregular ventricular excitation and ventricular contraction.(1, 2) Symptoms of atrial fibrillation include palpitations, chest discomfort, dyspnea, and fatigue, although some individuals are asymptomatic and do not experience any complaints at the time of atrial fibrillation occurrence.(1, 3) Next to the aforementioned symptoms, atrial fibrillation substantially increases the risk of hospitalization, morbidity such as myocardial infarction, heart failure, stroke, and dementia, and mortality.(1, 4-6)

Globally, atrial fibrillation is the most common cardiac arrhythmia with an estimated prevalence of 2-4% and its prevalence is expected to even further increase due to the extended longevity of the population.(1, 4-7) This increasing prevalence of atrial fibrillation highlights the growing disease burden of atrial fibrillation for the decades to come. During the last two decades, major advancement in knowledge regarding the epidemiology, prediction, pathophysiology, and treatment of atrial fibrillation has been generated in an attempt to gain a better understanding of atrial fibrillation and to reduce its burden. Aging is the most prominent atrial fibrillation risk factor, but the increasing burden of other atrial fibrillation risk factors such as obesity, hypertension, diabetes mellitus, coronary heart disease, and heart failure also contributes to atrial fibrillation initiation, maintenance, and progression.(1) The lifetime risk of atrial fibrillation is estimated to be 1 in 3 individuals at an index age of 55 years when individuals have at least one risk factor for atrial fibrillation (hypertension, smoking, alcohol intake, obesity, history of diabetes mellitus, history of myocardial infarction, or history of heart failure).(1, 8) In individuals with an optimal risk factor profile (in other words, no atrial fibrillation risk factors) this lifetime risk is estimated to be 1 in 5 individuals at the same index age.(8) These findings stress the essential role of better understanding and decreasing the atrial fibrillation risk factor burden in an attempt to reduce the lifetime risk of atrial fibrillation.

Besides age and modifiable risk factors, genetic predisposition to atrial fibrillation also seems to play a profound role in atrial fibrillation risk.(9) Thus far, more than 160 genes have been associated with atrial fibrillation in the past years by linkage studies, functional studies, and genome-wide association studies (GWAS).(9) More specifically, genetic variance in genes encoding for ion-channels, transcription factors, and myocardial structural integrity may lead to increased cardiac automaticity and re-entry which are known to predispose to atrial fibrillation.(9)

While atrial fibrillation may exist in the absence of electrical, or structural abnormalities of the atria, previous evidence has indicated that electrical, and

structural atrial remodeling are at the root of the substrate that initiates, and perpetuates atrial fibrillation.(1, 2) Further, it has been highlighted that risk factors of atrial fibrillation, which frequently co-exist, can aggravate this electrical, and structural remodeling and thereby increase an individual's risk to develop, maintain, and exacerbate atrial fibrillation.(1, 2, 4-7) Despite the global effort to advance the knowledge within the scientific atrial fibrillation field, the etiology of atrial fibrillation remains incompletely understood which, to some extent, compromises the development of effective treatment modalities. This also further complicates the prediction, prevention, and management of atrial fibrillation. Generating insight is therefore of utmost importance to dissect the etiology of atrial fibrillation which is a complex polygenetic arrhythmia.

Part II Macro- and micro-vascular disease and the risk of atrial fibrillation

Coronary heart disease, peripheral vascular disease, and cerebrovascular disease are three common diseases among the macro-vascular disease spectrum. Furthermore, micro-vascular disease includes retinopathy, neuropathy, and nephropathy. Macro- and micro-vascular diseases are common conditions in the general population. The increasing prevalence of atrial fibrillation is partly attributed to the increase of atrial fibrillation risk factors such as coronary heart disease. The role of coronary heart disease such as myocardial infarction in atrial fibrillation pathogenesis is well established. This role is further highlighted by the greatly increased risk of 60-77% in myocardial infarction patients to develop new-onset atrial fibrillation.(1, 10)

Two underlying mechanisms are suggested to underlie myocardial infarction and atrial fibrillation. First, the main determinant of atrial fibrillation development during myocardial infarction is atrial ischemia/infarction.(11) This ischemia/infarction induces slowed, and heterogeneous conduction, and subsequently increases the propensity to develop atrial fibrillation. Second, myocardial ischemia/infarction, independent of atrial ischemia/infarction, causes hemodynamic changes, atrial stretch, and neurohumoral activation that may also contribute to atrial fibrillation occurrence.(11)

Yet, the relationship between other forms of macro- and micro-vascular disease in atrial fibrillation development is not yet fully understood. It has been hypothesized that other forms of (sub)clinical) macro-vascular disease such as arteriosclerotic calcification, and peripheral vascular disease also increase atrial fibrillation susceptibility by causing hypoperfusion and ischemia of the atria and subsequent atrial fibrosis.(11, 12) Moreover, they may increase the systolic cardiac afterload through arterial stiffness which in turn gives rise to ventricular and atrial remodeling of the heart.(13-17) Inflammation, endothelial dysfunction, and platelet-mediated thrombosis also have been suggested as part of the underlying mechanisms that

relate (peripheral) atherosclerosis with atrial fibrillation.(17-20)

In the micro-vascular domain. Nephropathy or reduced kidney function may initiate atrial fibrillation through increased activity of the renin-angiotensin-aldosterone system,(21-28) hypertension,(25, 26) left ventricular hypertrophy,(26) inflammation,(26, 29-31) and by promoting cardiovascular diseases such as coronary heart disease, and heart failure.(26, 32, 33)

Although, these hypotheses seem plausible, previous studies that assessed the link between macro- and micro-vascular disease with atrial fibrillation using various methodological approaches remain inconclusive. This reiterates the complexity of atrial fibrillation pathophysiology, as many etiological questions regarding the role of macro- and micro-vascular disease in atrial fibrillation pathogenesis still remain to be answered.

Part III Cardiac autonomic dysfunction and the risk of atrial fibrillation

The heart is extensively innervated by autonomic nerves, composed of extrinsic and intrinsic ganglion cells which are vital for physiological cardiac functioning.(34, 35) Extrinsic cardiac nerves originate from the paravertebral ganglia while the intrinsic cardiac nerves are found predominantly within the atria. Both play a role in arrhythmia, although the latter are in particular closely entangled with atrial arrhythmogenesis.(34, 35) Increasing evidence suggests that dysfunction of the autonomic nervous system, including the sympathetic, parasympathetic, extrinsic, and intrinsic cardiac neural network, is involved in the pathophysiology of atrial fibrillation.(36-38)

The electrocardiogram is non-invasive, readily and rapidly available, and an inexpensive tool that is viable to assess cardiac autonomic dysfunction. It enables measurement of multiple metrics of cardiac autonomic dysfunction such as heart rate variability, and various electrocardiographic parameters that represent cardiac conduction. Moreover, heart rate variability, the reflection between the autonomic nervous system and the heart,(39-41) has been suggested to modify atrial fibrillation risk.(38, 42-47) Specifically, lower and higher heart rate variability may lead to a decline in cardiac function, and subsequently give rise to atrial fibrillation.(38, 42-47) Previous evidence also indicated that a complex relationship between various electrocardiographic parameters mirrored by atrioventricular conduction (PR interval), ventricular depolarization (QRS), and ventricular repolarization (QT, QT corrected for heart rate (QTc), and JT interval), and variation in successive cardiac contractions (RR interval, and heart rate) with atrial fibrillation exists.(48-53)

Various mechanisms may explain the complex interplay between cardiac autonomic dysfunction and atrial fibrillation. Left atrial enlargement has been associated with heart rate variability. This could suggest a role for heart rate variability in atrial

fibrillation pathogenesis that is mediated by the left atrium.(47) Autonomic imbalance represented by heart rate variability could also trigger an inflammatory response that may cause atrial fibrillation.(41)

The PR interval reflects the atrioventricular conduction and its interferences. PR interval prolongation (PR >200 ms or first-degree atrioventricular block) may surface from conduction interferences within the atria, the atrioventricular node, His bundle, and/or at multiple sites which may be produced by atrial fibrosis.(54, 55) Atrial fibrosis can be primary (idiopathic) or secondary to conditions such as aging, coronary heart disease, calcification, and inflammation.(55, 56) Alternately, prolonged ventricular repolarization may result in atrioventricular dyssynchrony which may give rise to left ventricular diastolic dysfunction and this could result in increased atrial pressure.(57) This increased atrial pressure and atrial wall tension may then provoke remodeling of the left atrium and thereby produce a substrate for atrial fibrillation.(57) Myocardial excitation, and contraction, reflected by the RR interval, and heart rate, and its relationship with atrial fibrillation is also well established.(58) Heart rate control is a complex dance between sympathetic activation and vagal withdrawal during physical activity.(58, 59) On one hand, a low heart rate is commonly associated with a healthy body mass index, increased exercise tolerance, reduced morbidity, and mortality.(58) Nonetheless, a low heart rate during physical exertion might represent an altered response to physical activity due to prolonged vagal activation.(58, 60) This prolonged vagal activation supplements acetylcholine-dependent potassium currents which decrease the action potential duration which may facilitate conduction abnormalities and thereby development of atrial fibrillation.(58, 61) On the other hand, a high heart rate is associated with comorbidities such as hypertension, diabetes mellitus, coronary heart disease, and heart failure which are all known to be involved in atrial fibrillation pathogenesis.(58) Additionally, a high heart rate might be a marker of increased sympathetic activation which may decrease the atrial refractory period and thereby trigger atrial fibrillation.(58, 62)

Lastly, a role for the sinoatrial node, the pacemaker of the heart, is suggested in atrial fibrillation pathophysiology. More specifically, it has been suggested that sinus node disease is intertwined with cardiac autonomic dysfunction and may cause atrial fibrillation by promoting atrial extrasystoles.(63, 64) Atrial extrasystoles, in the presence of sinus node disease, may arise during the slow atrial cycle and are followed by a compensatory pause. This compensatory pause may be prolonged which allows other atrial ectopic activity to occur which possibly set off atrial fibrillation.(63) Early premature beats that originate from areas other than the sinus node, the pacemaker of the heart may give rise to conduction block and re-entry, in turn imposing atrial fibrillation.(63) Noteworthy, sinus nodal artery stenosis is also common in patients with atrial fibrillation which implies that ischemic damage to the sinus node alone without atrial fibrosis, stretch or muscle loss may initiate atrial fibrillation.(63)

Taken together, the combination of atrial enlargement, inflammation, atrial extrasystoles, conduction abnormalities, and sinus node ischemia may explain the mechanisms through which heart rate variability and electrocardiographic parameters may promote atrial fibrillation. In addition, it has been shown that modulating cardiac autonomic nerve function by ganglionated plexus ablation may aid in atrial fibrillation control, nonetheless the treatment of atrial fibrillation using these therapies remains difficult.(35) This implies that the exact underlying mechanisms are still unclear and that there is still room to improve our knowledge regarding the link between cardiac autonomic dysfunction with atrial fibrillation.

Part IV Inflammation and the risk of atrial fibrillation

Inflammation is hypothesized as one of the underlying key conditions involved in atrial fibrillation pathogenesis.(31, 65) Recent evidence further indicates the role of two proxies of inflammation; namely immunothrombosis, and autoimmune diseases, in atrial fibrillation etiology.

Immunothrombosis is the complex interplay of the innate immune system and the coagulation system.(66-68) Another important role is played by neutrophil extracellular traps that start inflammatory processes while stimulating the activation of platelets and the coagulation cascade.(66, 69) This synergy between inflammation and coagulation, called immunothrombosis, may cause atrial remodeling, and contribute to the development of atrial fibrillation.(70-73) Nonetheless, the exact role of immunothrombosis on atrial fibrillation risk remains unclear.

Autoimmune diseases such as rheumatoid arthritis are suggested to be associated with atrial fibrillation through atrial electrical and structural remodeling.(74-77) Nonetheless, conclusive evidence is lacking as there are only a few studies examining the relationship between autoimmune diseases and atrial fibrillation probably due to the low prevalence of most autoimmune diseases which limits strong causal inferences.

Therefore, the presence of immunothrombosis and autoimmune diseases may indeed suggest to increase atrial fibrillation vulnerability through infiltration of immune cells, proteins, and other inflammatory processes that may negatively impact the atria.(31) Nonetheless their exact potential role in atrial fibrillation development is not yet examined in detail.

Part V Traditional and novel risk factors for atrial fibrillation

Recent evidence suggest the whole spectrum of anthropometric measures,(78-81) the shape and slope of trajectories of obesity-related measures(82, 83) and blood pressure,(1, 82, 84-88) and microRNAs(89-96) as emerging traditional and potential novel risk factors for atrial fibrillation.

Obesity or increasing body mass index is an established risk factor for atrial fibrillation.(1) Ventricular remodeling, impaired left ventricular relaxation, and increased left ventricular diastolic filling pressure are closely related to obesity.(97) It has also been shown that obesity leads to hypoxia of the expanding adipose tissue, results in adipose fibrosis, produces adipocytokines, contributes to increased epicardial fat accumulation, and myocardium damage.(98, 99) In particular, epicardial fat and myocardial damage are known to be implicated in atrial fibrillation occurrence.(1, 100) Yet, the relationship between the whole spectrum of anthropometric measures, in addition to body mass index, such as weight, height, waist circumference, hip circumference, and waist-to-hip ratio with atrial fibrillation remains unclear. Moreover, the impact of longitudinal trajectories of obesity-related measures on new-onset atrial fibrillation remains unknown.

Hypertension or blood pressure is another well-recognized risk factor for atrial fibrillation. The underlying mechanisms of the link between hypertension and atrial fibrillation are not fully understood, but it has been suggested that various factors could contribute to this relationship. Hypertension accelerates atrial remodeling by inflammatory changes, fibrosis, and atrial hypertrophy.(101) Furthermore, long-term hypertension with increased cardiac afterload in the left ventricle contributes to left ventricular hypertrophy.(101) Moreover, hypertension may dysregulate the autonomic nervous system and thereby trigger atrial fibrillation.(102) Yet, the impact of longitudinal trajectories blood pressure on new-onset atrial fibrillation remains undetermined.

Given the aforementioned mechanisms that may link hypertension with atrial fibrillation.(1, 85-88) It seems biologically plausible that antihypertensive drugs are of therapeutic value to prevent atrial fibrillation. Yet, conclusive clinical evidence in the form of large randomized clinical trials (RCTs) and comprehensive meta-analyses investigating the role of blood pressure reduction through various antihypertensive drugs for atrial fibrillation prevention is lacking.(87, 88) Not surprisingly, as RCTs examining (new) drugs are challenging, costly, often restricted to high-risk patients (older, and/or with underlying heart disease), have a relatively short duration of follow-up, and often lead to inconclusive results.

MicroRNAs are a class of small non-coding RNAs that post-transcriptionally regulate gene expression by complementary binding to target transcripts. During the past years, the role of microRNAs in cardiovascular disease has received a major interest.(103) MicroRNAs have a suggested role in atrial fibrillation pathophysiology as microRNAs are key regulators of electrical remodeling,(104) structural remodeling,(105) autonomic nerve remodeling,(106) calcium handling abnormalities,(107) and inflammation (108) of the heart. Nonetheless, their exact role in atrial fibrillation remains to be further elucidated.

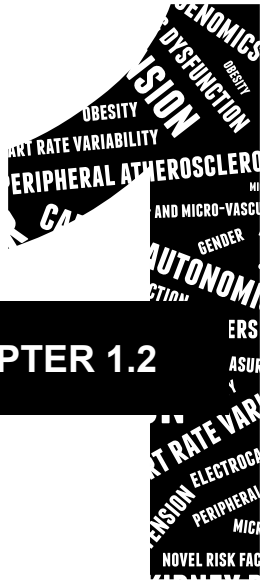
These underlying mechanisms seem promising to indeed flag the whole spectrum of anthropometric measures and trajectories of obesity-related measures and blood pressure as emerging traditional risk factors as well as microRNAs as novel risk factors for atrial fibrillation, although definitive answers remain there to be found.

Part VI Sex and gender implications and the risk of atrial fibrillation

The atrial fibrillation lifetime risk of 1 in 3 individuals at index age of 55 years is dependent on an individual's genetic profile, age, sex, and coexistence of other atrial fibrillation risk factors.(1) Particularly, the age-adjusted prevalence, incidence, and lifetime risk of atrial fibrillation are higher in men than in women. Nonetheless, a larger proportion of women will end up living with atrial fibrillation due to their increased lifespan in comparison to men.(72, 109) Moreover, the lifetime risk of atrial fibrillation in each cardiovascular risk factor profile (optimal, borderline, and elevated) seems to be higher among men than women. Additionally, the increase in lifetime risk from optimal to borderline risk profiles seems to be larger among men than women.(8)

Recent evidence indicates that differences in atrial fibrillation pathophysiology between men and women exist.(72, 109) Sex hormones are considered to play a vital role in cardiovascular health and disease.(110) The suggested beneficial effect of estrogen on endothelial function and cholesterol metabolism dissipates as women age.(111) This natural decline in estrogen levels due to aging, in particular after menopause has been profoundly associated with an increased risk of cardiovascular disease.(112) Reproductive lifespan factors, as a reflection of the cumulative exposure to endogenous sex hormones, have been suggested as emerging risk factors for cardiovascular disease development.(113-115) Despite the lack of direct evidence, it has been hypothesized that estrogen may confer a beneficial effect on atrial fibrillation by extending atrial conduction time, action potential duration, and the atrial refractory period.(116)

These findings suggest the sex- and gender-specific nature of atrial fibrillation development and the differential contribution of various risk factors to atrial fibrillation pathogenesis in men and women. However, findings on this matter warrant further research as evident sex- and gender-specific atrial fibrillation risk factors remain elusive.


OBESITY
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CHAPTER 1.2


PERIPHERAL ATHEROSCLEROSIS


NOVEL RISK FACTORS
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AUTOIMMUNE DISEASES
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Thesis objective

Thesis objective

The objective of this thesis is to dissect the etiology of atrial fibrillation using a population perspective on risk factors and sex differences. This thesis focuses on vascular- (**Part II**), cardiac autonomic- (**Part III**), inflammatory- (**Part IV**), traditional-, novel- (**Part V**), and sex- and gender-specific (**Part VI**) risk factors in the general population and their role in atrial fibrillation pathophysiology. This division is made as these factors have been implicated in atrial fibrillation pathophysiology.(1) Therefore, these factors were used to conceptualize the studies and parts described throughout this thesis.

The research included in this thesis was conducted using data from the Rotterdam Study, the UK Biobank, and the publicly available genome-wide association studies (GWAS). Before mentioning the main findings of my studies, I would like to dedicate a few words in this section to properly introduce the used studies.

First, the Rotterdam Study, locally also called Erasmus Rotterdam Gezondheid Onderzoek (ERGO), was founded in 1989 as a prospective population-based cohort study to investigate the occurrence, and progression of determinants that underlie common diseases in middle-aged and elderly individuals.(71, 117) The study area is geographically bound to the Ommoord district in the city of Rotterdam, The Netherlands. At present the Rotterdam Study incorporates four cohorts that were established in 1989 (RS-I), 2000 (RS-II), 2006 (RS-III), and 2015 (RS-IV), respectively.(71) Since 1989, over 18,000 inhabitants, aged ≥40 years were included and participants attended follow-up examinations every 3-5 years.(71) In addition, the participants' morbidity and mortality was continuously collected through linkage with digital files from general practitioners in Ommoord.(71) The fundamental strength of the Rotterdam Study is the commitment and dedication of its participants who have allowed for in-depth evaluation of common diseases for ≥3 decades. It goes without question that such a large sample size, long follow-up time, and meticulous and continuously-updated assessment of determinants, as well as outcomes, are of unspoken value to chart the burden of the most common cardiac arrhythmia (i.e. atrial fibrillation) and to dissect its pathogenesis. Noteworthy, the Rotterdam Study complies with the Declaration of Helsinki, has been approved by the Medical Ethics Committee of the Erasmus MC, and by the Dutch Ministry of Health, Welfare and Sport. All its participants provided written informed consent to participate, prior to inclusion, in the study, and to have their information obtained from treating physicians.

Second, the UK Biobank, a very large prospective population-based cohort study. It includes over 500,000 inhabitants aged between 37 and 73 years across England, Scotland, and Wales that were recruited between 2007 and 2010.(118) These

participants provided medical history, behavioral habits, physical examinations, and biological samples at the time of inclusion.(118) Of note, the UK Biobank complies with the Declaration of Helsinki, and has received ethics approval from the North West Multi-Centre Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland. All participants provided written informed consent, prior to inclusion, in the study.

Lastly, publicly available summary statistics from multiple GWAS were extracted and used to conduct the various MR studies throughout this thesis.(119-130) In short, a GWAS is a study that involves scanning the genome with a lot of markers to identify genetic variants that are associated with a disease of interest. These identified genetic variants could then be taken forward to better understand the underlying biology of a disease using various methodologies.

Part II embarks by unravelling the link between macro- and micro-vascular disease and atrial fibrillation. In **Chapter 2.1**, we studied baseline assessments of coronary- and extra-coronary arteriosclerotic calcification (macro-vascular disease) in relation to atrial fibrillation. In **Chapter 2.2**, baseline and longitudinal measurements of peripheral atherosclerosis (macro-vascular disease) and their associated atrial fibrillation risk are examined. In **Chapter 2.3**, kidney function (micro-vascular disease) and atrial fibrillation within the general population are bidirectionally investigated to determine their influence on each other. In **Chapter 2.4**, we additionally sought to assess the bidirectional association between genetically predicted kidney function (micro-vascular disease) and genetically predicted atrial fibrillation.

Part III aims to disentangle the relationship between cardiac autonomic dysfunction and atrial fibrillation. In **Chapter 3.1**, we determined the link between longitudinal measurements of heart rate variability and genetically predicted heart rate variability and atrial fibrillation risk. In **Chapter 3.2**, baseline and longitudinal measurements of electrocardiographic parameters in relation to atrial fibrillation are studied to evaluate their influence on atrial fibrillation.

Part IV evaluates the role of inflammation in atrial fibrillation pathogenesis. In **Chapter 4.1**, immunothrombosis and atrial fibrillation risk are studied within the general population. In **Chapter 4.2**, we extensively evaluated the available literature and meta-analyzed the contribution of immunothrombosis in atrial fibrillation pathophysiology. In **Chapter 4.3**, we examined the role of various autoimmune diseases in atrial fibrillation development.

Part V continues with application of novel methods for investigating (shape of) trajectories of traditional risk factors in association with atrial fibrillation and

identifying emerging potential novel risk factors for atrial fibrillation. In **Chapter 5.1**, we evaluated longitudinal measurements of the whole spectrum of anthropometric measures and its impact on atrial fibrillation to extent previous findings that solely focused on body mass index while assessing atrial fibrillation risk. In **Chapter 5.2**, trajectories of obesity-related measures and blood pressure are investigated to quantify their risk on new-onset atrial fibrillation. In **Chapter 5.3**, we investigated the effects of antihypertensive drugs for the prevention of atrial fibrillation using a drug target Mendelian randomization (MR) study approach. In **Chapter 5.4**, we assessed how well-expressed circulatory microRNAs in plasma relate to atrial fibrillation and provided a literature review on the link between microRNAs and atrial fibrillation.

Part VI highlights the implications of sex- and gender in atrial fibrillation. In **Chapter 6.1**, we assessed women-specific risk factors and their impact on atrial fibrillation occurrence. In **Chapter 6.2,** we determined the current status and future directions of sex- and gender-specific atrial fibrillation prediction by leveraging big data.

Part VII discusses and summarizes the research in this thesis. In **Chapter 7.1**, the main results of the studies included in this thesis are discussed, and placed in a broader perspective. Further, we address methodological considerations, review potential implications, and elaborate on future directions for atrial fibrillation research. Lastly, the main findings of this thesis are summarized in English and in Dutch in **Chapter 7.2**, and **Chapter 7.3**, respectively.

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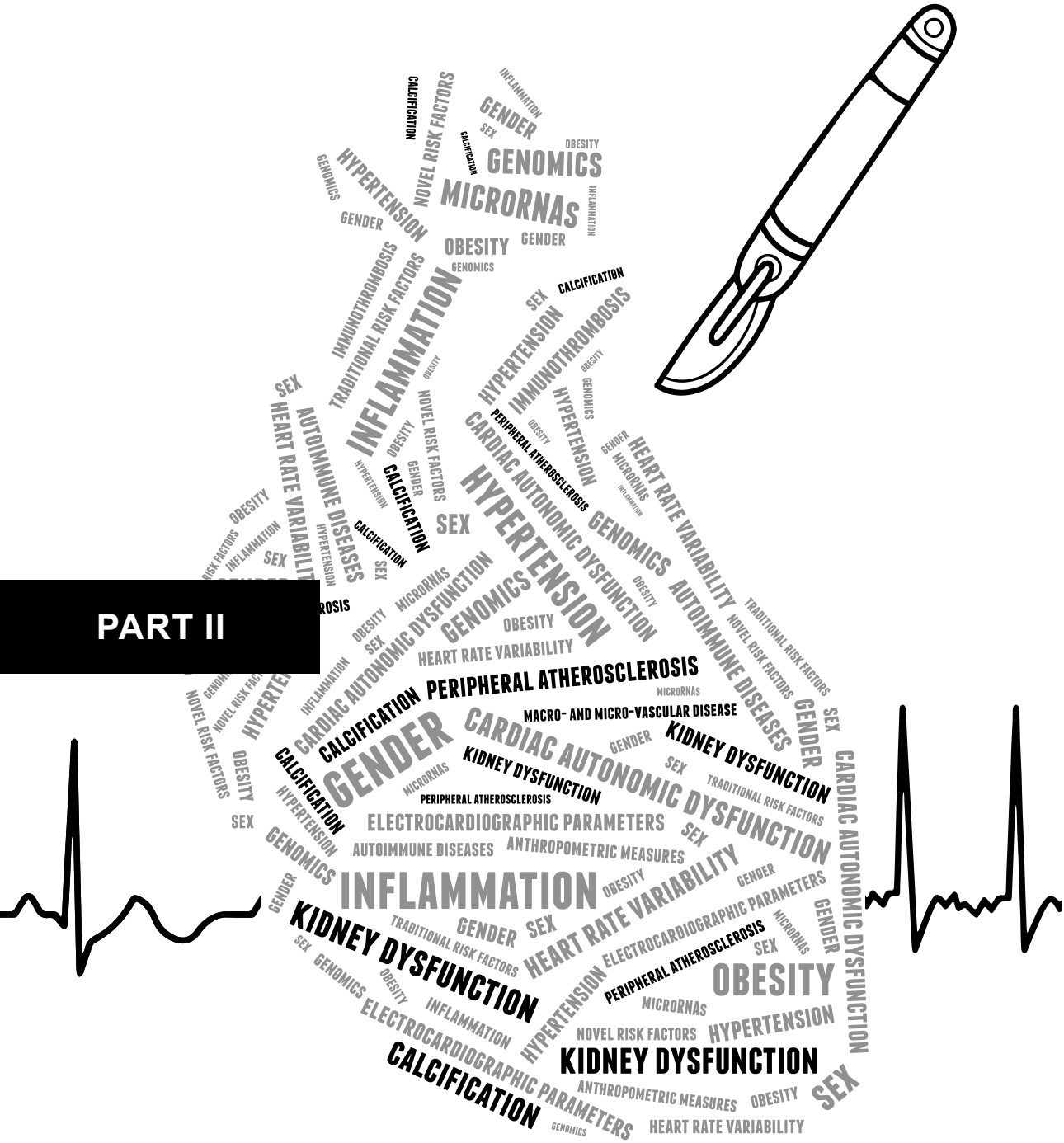
PART II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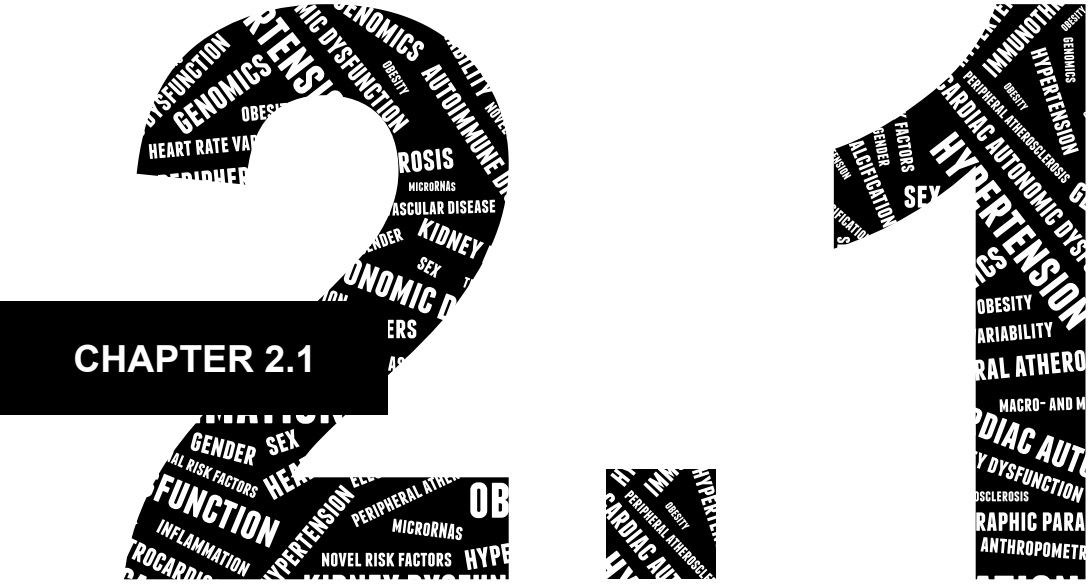
CALCIFICATION
NOVEL RISK FACTORS
IMMUNOTHROMBOSIS
TRADITIONAL RISK FACTORS
HEART RATE VARIABILITY
AUTOIMMUNE DISEASES
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
MACRO- AND MICRO-VASCULAR DISEASE
KIDNEY DYSFUNCTION
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC MEASURES

Macro- and micro-vascular disease and the risk of atrial fibrillation


CHAPTER 2.1
GENOMICS
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CARDIAC AUTONOMIC DYSFUNCTION
OBESITY
VARIABILITY
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ANTHROPOMETR

Coronary and extra-coronary arteriosclerotic calcification and the risk of atrial fibrillation

Arteriosclerotic calcification and atrial fibrillation in the general population: the Rotterdam Study.

Geurts S, Bos MM, van der Toorn JE, Stricker BHC, Ghanbari M, Kors JA, Deckers JW, Ikram MA, Bos D, Kavousi M.

Submitted.

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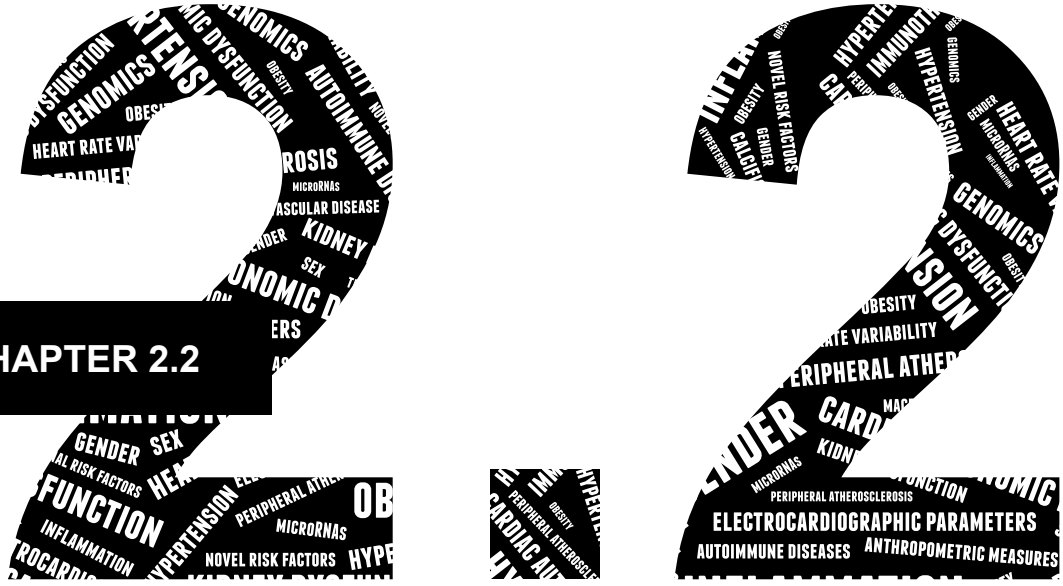
CHAPTER 2.2
GENOMICS
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DYSFUNCTION
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Peripheral atherosclerosis and the risk of atrial fibrillation

Subclinical measures of peripheral atherosclerosis and the risk of new-onset atrial fibrillation in the general population: the Rotterdam Study.

Geurts S, Brunborg C, Papageorgiou G, Ikram MA, Kavousi M.

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ABSTRACT

Background
Limited population-based data on the (sex-specific) link between subclinical measures of peripheral atherosclerosis and new-onset atrial fibrillation (AF) exist.

Methods
Subclinical measures of peripheral atherosclerosis including carotid intima-media thickness (cIMT), carotid plaque, and ankle-brachial index (ABI) were assessed at baseline and follow-up examinations. A total of 12,840 participants free of AF at baseline from the population-based Rotterdam Study were included. Cox proportional hazards models and joint models, adjusted for cardiovascular risk factors, were used to determine the associations between baseline and longitudinal measures of cIMT, carotid plaque, and ABI with new-onset AF.

Results
During a median follow-up of 9.2 years, 1,360 incident AF cases occurred among 12,840 participants (mean age 65.2 years, 58.3% women). Higher baseline cIMT (hazard ratio (HR), 95% confidence interval (CI), 1.81, 1.21-2.71, p=0.0042), presence of carotid plaque (HR, 95% CI, 1.19, 1.04-1.35, p=0.0089), lower ABI (HR, 95% CI, 1.57, 1.14-2.18, p=0.0061) and longitudinal measures of higher cIMT (HR, 95% CI, 2.14, 1.38-3.29, p=0.0021), presence of carotid plaque (HR, 95% CI, 1.61, 1.12-2.43, p=0.0112), and lower ABI (HR, 95% CI, 4.43, 1.83-10.49, p=0.0007) showed significant associations with new-onset AF in the general population. Sex-stratified analyses showed that the associations for cIMT, carotid plaque, and ABI were mostly prominent among women.

Conclusions
Baseline and longitudinal subclinical measures of peripheral atherosclerosis (carotid atherosclerosis, and lower extremity peripheral atherosclerosis) were significantly associated with an increased risk of new-onset AF, especially among women.

INTRODUCTION

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia.(1, 2) Parallel to the aging of the population, the prevalence of AF is expected to increase steeply in the coming years.(1-3) Despite improvements in the management of patients with AF, it still confers a large morbidity and mortality risk.(1, 2, 4) Notably, recent evidence points towards sex differences in the pathophysiology and prognosis of AF.(5, 6) Women with AF are older at diagnosis, have a higher prevalence of hypertension and valvular heart disease, and have an increased risk of stroke, myocardial infarction, and mortality in comparison with men.(5)

Atherosclerosis of the peripheral vasculature is a largely prevalent condition in the general population that is associated with increased risk of morbidity and mortality.(7) Peripheral atherosclerosis and AF share common major risk factors.(8) Previous reports have suggested a relationship between peripheral atherosclerosis and AF mainly based on subgroup or post hoc analyses of various patient studies.(8) Few population-based studies have shown associations between subclinical measures of peripheral atherosclerosis; carotid intima-media thickness (cIMT)(9-12), carotid plaque(9, 10), and ankle-brachial index (ABI)(7, 13, 14) with increased risk of new-onset AF. To date, limited data on the link between longitudinal measures of peripheral atherosclerosis with new-onset AF in the general population exist. Moreover, comprehensive assessment of the sex-specific association between the 2 conditions is sparse.

We thus aimed to investigate the associations between baseline and longitudinal measures of subclinical peripheral atherosclerosis including cIMT, carotid plaque, and ABI with the risk of new-onset AF among participants from the large population-based Rotterdam Study. Additionally, we sought to evaluate sex differences with regard to the association between subclinical peripheral atherosclerosis and new-onset AF.

METHODS

Study design

We used data from the Rotterdam Study.(15, 16) The Rotterdam Study is a prospective population-based cohort study that aims to assess the occurrence and determinants of age-related diseases in the general population. During 1990-1993, all inhabitants of the Ommoord district in the city of Rotterdam in The Netherlands aged ≥55 years were invited for the study. A total of 7,983 (78% of all invitees) agreed to participate (RS-I). In 2000, the cohort was extended with 3,011 participants who had become ≥55 years or had migrated into the research area (RS-II). In 2006, the cohort was again extended with 3,932 participants that were ≥45 years (RS-III). The overall response rate at baseline was 72%. Participants attended follow-up examinations every 3-6 years. Outcome data on morbidity and mortality were continuously collected through linkage with digital files from general practitioners in the study area.(15, 16)

The Rotterdam Study complies with the declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Study population

For the current study, we included participants at study entry of the three recruitment waves. Participants with prevalent AF at baseline (n=559), no informed consent for follow-up data collection (n=305), no follow-up time (n=6) or no subclinical measures of peripheral atherosclerosis (n=1,216), mainly due to logistic reasons, were excluded. Among the 12,840 participants free of AF at baseline that were included, 11,971 had at least 1 available measurement for cIMT, 11,947 had at least one available measurement for carotid plaque, and 8,532 had at least 1 available measurement for ABI; 6,832 participants had 2 measurements for cIMT, 6,311 had 2 measurements for carotid plaque, and 3,123 had 2 measurements for ABI; 1,075 participants had 3 measurements for cIMT, and 961 had 3 measurements for carotid plaque.

Subclinical measures of peripheral atherosclerosis

Participants were assessed for cIMT, carotid plaque, and ABI at baseline and follow-up examinations. Measurement of cIMT was performed with ultrasonography of both the left and right carotid arteries using a 7.5 MHz linear array transducer with a Duplex scanner (ATL UltraMark IV, Advanced Technology Laboratories, Bethel, Washington). The cIMT was calculated as the mean from the near and far walls measurements of both the left and right carotid arteries.(17) Carotid plaque was assessed by examining the ultrasonographic images at common, internal, and bifurcation sites of the carotid artery for presence of atherosclerotic lesions. Presence of carotid plaque was defined as a focal widening relative to adjacent segments with protrusion into the lumen composed of only calcified deposits or combination of calcified and non-calcified material.(17)

ABI was defined as the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm and was calculated for each leg. Ankle systolic blood pressure was measured in both right and left posterior tibial arteries using a Doppler ultrasound transducer with random-zero sphygmomanometer with the patients in supine position. The lowest ABI in either leg was used in the analyses. Peripheral artery disease (PAD) was defined as ABI ≤0.9. Values of ABI >1.4 were excluded, because high ABI may represent a different underlying pathology related to calcified, non-compressible arterial vessels.(18, 19)

Assessment of atrial fibrillation

AF was defined in accordance with the European Society of Cardiology (ESC) guidelines.(4) Methods on event adjudication for prevalent and incident AF have been described previously.(15) In short, to assess AF at baseline and follow-up examinations a 10-second 12-lead electrocardiogram (ECG) was used with an ACTA Gnosis IV ECG recorder (Esaote Biomedica, Florence, Italy). The ECG records were stored digitally, and analyzed with the Modular ECG Analysis System (MEANS).(20) Subsequently, 2 research physicians, blinded to the MEANS diagnosis, validated the diagnosis of AF. In case of disagreement a cardiologist was consulted.(3, 9) Additional follow-up data was obtained from medical files of participating general practitioners, hospitals, outpatient clinics, national registration of all hospitals discharge diagnoses, and follow-up examinations at the research center. The date of incident AF was defined as the date of the first occurrence of symptoms suggestive of AF with subsequent ECG verification obtained from the medical records. Participants were followed from the date of enrolment in the Rotterdam Study until the date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of cardiovascular risk factors

The cardiovascular risk factors included in the study were body mass index (BMI), total cholesterol, high-density lipoprotein (HDL) cholesterol, hypertension, smoking status, history of diabetes mellitus (DM), history of coronary heart disease (CHD), history of heart failure (HF), left ventricular hypertrophy (LVH) on the ECG, use of cardiac medication, and use of lipid lowering medication. Methods for measurements of cardiovascular risk factors are explained in details in the supplementary material (**Methods S1**).

Statistical analyses

Baseline characteristics

Participant characteristics at study entry are presented as mean with standard deviation (SD) or number (n) with percentages as appropriate. Differences between men and women were examined by Student's T-test for continuous variables and Chi-Square test for categorical variables. The distribution of cIMT, and ABI were normal. Therefore, no transformation was needed.

Cox proportional hazards models

Competing risk analyses were performed using Cox proportional hazards models to investigate the relationship between subclinical measures of peripheral atherosclerosis at baseline (cIMT, carotid plaque, and ABI) with incident AF. Cause-specific hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to quantify the associations. For continuous exposure variables an examination of the shape of relation with incident AF was performed using natural cubic splines. No deviation from linearity was found. The proportional hazard assumptions were assessed using Schoenfeld residuals and were found to be satisfied.

Joint models

Further, to investigate the associations between repeated measurements of peripheral atherosclerosis over time with the risk of incident AF, joint models for longitudinal and time to event data were used. First, an appropriate, for each outcome, mixed effects model was used to analyze the longitudinal measures of peripheral atherosclerosis over time and to account for the correlation of repeated measurements. More specifically, for cIMT a linear mixed effects model was used with random intercepts and random slopes, including a linear effect of time. Moreover, for carotid plaque a logistic mixed effects model was used with random intercepts and random slopes, including a linear effect of time. Finally, for ABI a linear mixed effects model was used with random intercepts and random slopes, including a linear effect of time. Time was measured in years after baseline and cardiovascular risk factors/covariates were treated as fixed effects in all models. Likelihood-ratio tests were used to assess whether random slopes could be dropped from the model. Due to the low number of repeated measurements per individual (range, 1-3), non-linear functions of time using splines were not used. Next, the estimated subject-

specific trajectories from the mixed effects models were included in the Cox models as time-varying covariates under the joint modelling framework.(21)
Analyses were performed in the total study population and for men and women separately. In addition, for the Cox proportional hazards models we tested the interaction of sex using the likelihood-ratio test with the individual subclinical measures of peripheral atherosclerosis in the total study population in model 1 and model 2. Similarly, we also reported the p values of sex interaction from the joint model in both models. All models (mixed- and survival models) were adjusted for age, sex (if applicable), and cohort (model 1) and additionally for cardiovascular risk factors including BMI, total cholesterol, HDL cholesterol, hypertension, smoking status, history of DM, history of CHD, history of HF, LVH on the ECG, use of cardiac medication, and use of lipid lowering medication (model 2). Missing baseline covariate values were imputed under the assumption of missing at random and were imputed using predictive mean matching ("pmm"), binary logistic regression ("logreg"), and a proportional odds model ("polyr") for continuous, binary, and ordered categorical covariates, respectively, from the "mice" package in R.(22) For imputation all available data were used to generate one imputed dataset. Missing values: BMI (1.9%), total cholesterol (2.5%), HDL cholesterol (2.5%), systolic blood pressure (0.6%), diastolic blood pressure (0.6%), smoking status (1.4%), history of CHD (3.5%), history of HF (0.2%), LVH on the ECG (18.7%), use of cardiac medication (0.7%), use of antihypertensive medication (0.7%), and use of lipid lowering medication (0.7%).

Sensitivity analyses
Multiple sensitivity analyses were performed. We compared the analyses with imputed data and complete-case analyses. Moreover, we reran our analyses after excluding participants with prevalent and incident CHD (prior to incident AF) to evaluate if this would attenuate the observed associations. Finally, we also calculated the cause-specific HRs for mortality to evaluate the competing risk of mortality with incident AF.

Statistical significance was considered at two-tailed $p<0.05$ or for the Bayesian joint models, a tail probability of <0.05. The data management was done using IBM SPSS Statistics version 25.0 for Windows (IBM Corp, Armonk, New York). The statistical analyses were performed using the R package "JMbayes2"(23) in R software (R 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).(24)

RESULTS

Baseline characteristics

A total of 12,840 participants free of AF at baseline, 5,359 men (41.7%) and 7,481 women (58.3%), were eligible for the analyses. The baseline characteristics for the total study population and for the study population stratified by sex are depicted in **Table 1**. The mean age of the total study population was 65.2 ± 9.8 years and 58.3% were women.

Atrial fibrillation incidence

During a median follow-up time of 9.2 years (interquartile range (IQR), 6.1-14.3), 1,360 incident AF cases (10.6%) (640 in men and 720 in women) and 4,348 mortality cases (33.9%) (1,879 in men and 2,469 in women) occurred. The incidence rate of AF was 9.8 per 1,000 person-years in the total study population (11.8 per 1,000 person-years in men, 8.6 per 1,000 person-years in women) and the incidence rate of mortality was 31.5 per 1,000 person-years in the total study population (34.6 per 1,000 person-years in men, 29.5 per 1,000 person-years in women).

Cox proportional hazards models

The Cox proportional hazards analyses showed significant associations between higher baseline cIMT (HR, per one unit increase, 95% CI, 2.98, 2.01-4.42, $p=5.22x10^{-08}$), presence of carotid plaque (HR, per one unit increase in the probability, 95% CI, 1.30, 1.15-1.48, $p=4.06x10^{-05}$), and lower ABI (HR, per one unit decrease, 95% CI, 2.11, 1.55-2.87, $p=2.32x10^{-06}$) with an increased risk of new-onset AF in the total study population in model 1. For the Cox proportional hazards models, the results of the sex interaction testing for cIMT, carotid plaque, and ABI in the total study population were p=0.0047, p=0.5534, p=0.7621, respectively. After adjusting for additional cardiovascular risk factors in model 2, the effect estimates attenuated, but higher cIMT (HR, per one unit increase, 95% CI, 1.81, 1.21-2.71, p=0.0042), presence of carotid plaque (HR, per one unit increase in the probability, 95% CI, 1.19, 1.04-1.35, p=0.0089), and lower ABI (HR, per one unit decrease, 95% CI, 1.57, 1.14-2.18, p=0.0061) remained significantly associated with the risk of new-onset AF in the total study population (**Tables 2** and **3**). In model 2, the results of the sex interaction testing for cIMT, carotid plaque, and ABI in the total study population were p=0.0011, p=0.2535, p=0.9317, respectively. Additionally, analyses of quartiles of cIMT (HR, 95% CI, 1.31, 1.10-1.57, p=0.0030, for the highest vs. lowest quartile) and categories of ABI (HR, 95% CI, 1.21, 1.02-1.42, p=0.0249 for the lowest (≤0.90) vs. highest (1.00-1.40) category) showed significant graded associations between increased quartiles of cIMT and lower categories of ABI with incident AF in model 2 (**Tables 2** and **3**).

The sex-stratified analyses from model 2 showed that the associations for higher cIMT (HR, per one unit increase, 95% CI, 1.00, 0.56-1.80, p=0.9989), and presence of carotid plaque (HR, per one unit increase in the probability, 95% CI, 1.10, 0.91-1.33, p=0.3399) were not significant in men, while significant associations for higher cIMT (HR, per one unit increase, 95% CI, 3.32 , 1.90-5.80, $p=2.49x10^{-05}$), and presence of carotid plaque (HR, per one unit increase in the probability, 95% CI, 1.27, 1.07-1.51, p=0.0065) were found in women. Analyses of lower ABI (HR, per one unit decrease, 95% CI, 1.62, 1.01-2.59, p=0.0447) showed a significant association in men and a borderline significant association was found in women (HR, per one unit decrease, 95% CI, 1.53, 0.97-2.39, p=0.0654). Again, we observed graded associations between increased quartiles of cIMT and lower categories of ABI with incident AF in the sex-stratified analyses (**Tables 2** and **3**).

Joint models

The joint model analyses also showed significant associations between longitudinal measures of higher cIMT (HR, per one unit increase, 95% CI, 3.38, 2.20-5.23, p<0.0001), presence of carotid plaque (HR, per one unit increase in the probability, 95% CI, 2.05, 1.42-3.03, p=0.0028), and lower ABI (HR, per one unit decrease, 95% CI, 7.53, 3.65-16.10, p<0.0001) with an increased risk of new-onset AF in the total study population in model 1. The p values of the sex interaction in model 1 in the joint model for cIMT, carotid plaque, and ABI in the total study population were p<0.0001, p=0.0027, p=0.0053, respectively. Adjusting for additional cardiovascular risk factors in model 2 did also attenuate the effect estimates, but higher cIMT (HR, per one unit increase, 95% CI, 2.14, 1.38-3.29, p=0.0021), presence of carotid plaque (HR, per one unit increase in the probability, 95% CI, 1.61, 1.12-2.43, p=0.0112), and lower ABI (HR, per one unit decrease, 95% CI, 4.43, 1.83-10.49, p=0.0007) remained significantly associated with the risk of new-onset AF in the total study population (**Tables 2** and **3**). In model 2, the p values of the sex interaction in the joint model for cIMT, carotid plaque, and ABI in the total study population were p<0.0001, p=0.0200, p=0.0302, respectively. The corresponding HRs from the sex-stratified joint model analyses were somewhat higher, but more or less comparable to the HRs obtained in the Cox proportional hazards analyses (**Tables 2** and **3**).

Sensitivity analyses

In our sensitivity analyses, the results after imputation did not differ substantially from the complete-case analyses (**Tables S1** and **S2**). In addition, excluding participants with prevalent and incident CHD (prior to incident AF) from our analyses did not substantially change our original results (**Tables S3** and **S4**). Lastly, we evaluated the competing risk of mortality with incident AF and larger cIMT, presence of carotid plaque, and lower ABI were all significantly associated with mortality in both model 1 and 2 (**Tables S5** and **S6**).

Table 1. Baseline characteristics of the total study population and stratified by sex

Baseline characteristics *	Total study population n=12,840	Men n=5,359	Women n=7,481	p ¶
Age, years	65.2 ± 9.8	64.4 ± 9.1	65.8 ± 10.3	<0.001
Women, n (%)	7,481 (58.3)	NA	7,481 (100)	NA
Body mass index, kg/m^2	26.9 ± 4.1	26.6 ± 3.6	27.2 ± 4.5	<0.001
Total cholesterol, mmol/L †	6.1 ± 1.2	5.8 ± 1.2	6.3 ± 1.2	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.4 ± 0.4	1.2 ± 0.3	1.5 ± 0.4	<0.001
Systolic blood pressure, mmHg	139.0 ± 21.7	139.8 ± 20.9	138.5 ± 22.3	0.001
Diastolic blood pressure, mmHg	77.6 ± 12.0	78.8 ± 12.0	76.7 ± 11.9	<0.001
Hypertension, n (%)	7,628 (59.4)	3,218 (60.0)	4,410 (58.9)	0.211
Smoking status				<0.001
Never, n (%)	4,114 (32.5)	751 (14.1)	3,363 (45.7)	
Former, n (%)	5,514 (43.5)	3,010 (56.7)	2,504 (34.0)	
Current, n (%)	3,038 (24.0)	1,550 (29.2)	1,488 (20.2)	
History of diabetes mellitus, n (%)	1,334 (10.4)	632 (11.8)	702 (9.4)	<0.001
History of coronary heart disease, n (%)	804 (6.5)	572 (11.0)	232 (3.2)	<0.001
History of heart failure, n (%)	220 (1.7)	84 (1.6)	136 (1.8)	0.278
Left ventricular hypertrophy, n (%)	683 (6.5)	394 (9.0)	289 (4.8)	<0.001
Cardiac medication, n (%)	810 (6.4)	366 (6.9)	444 (6.0)	0.039
Antihypertensive medication, n (%)	779 (6.1)	329 (6.2)	450 (6.1)	0.762
Lipid lowering medication, n (%)	1,376 (10.8)	658 (12.4)	718 (9.7)	<0.001
Carotid intima-media thickness, mm ‡	0.82 ± 0.15	0.85 ± 0.15	0.80 ± 0.14	<0.001
Carotid plaque, n (%) §	7,918 (66.3)	3,660 (72.7)	4,258 (61.6)	<0.001
Ankle-brachial index ‖	1.05 ± 0.19	1.08 ± 0.19	1.04 ± 0.19	<0.001
Peripheral artery disease, n (%) ‖	1,366 (16.0)	507 (14.4)	859 (17.2)	0.001

Values are shown before imputation and therefore not always add up to 100%.

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.
† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.
‡ The baseline population for carotid intima-media thickness measurements included 5,048 men and 6,923 women.
§ The baseline population for carotid plaque measurements included 5,033 men and 6,914 women.
‖ The baseline population for ankle-brachial index measurements included 3,525 men and 5,007 women. Peripheral artery disease was defined as ankle-brachial index ≤0.9.
¶ Statistical significance for continuous variables was tested using the Student's T-test and for categorical variables was tested using the Chi-Square test.

Table 2. Association between baseline and longitudinal measures of carotid intima-media thickness and carotid plaque with the risk of new-onset atrial fibrillation in the total study population and stratified by sex

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
cIMT ‖	**2.98 (2.01-4.42), p=5.22x10^{-08}**	**1.81 (1.21-2.71), p=0.0042**	1.70 (0.97-2.99), p=0.0642	1.00 (0.56-1.80), p=0.9989	**5.26 (3.05-9.09), p=2.66x10^{-09}**	**3.32 (1.90-5.80), p=2.49x10^{-05}**
Carotid plaque ‖	**1.30 (1.15-1.48), p=4.06x10^{-05}**	**1.19 (1.04-1.35), p=0.0089**	**1.25 (1.03-1.50), p=0.0218**	1.10 (0.91-1.33), p=0.3399	**1.35 (1.14-1.59), p=0.0006**	**1.27 (1.07-1.51), p=0.0065**
cIMT, quartiles §						
Q1 ¶	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Q2 ¶	1.16 (0.97-1.38), p=0.1021	1.07 (0.90-1.27), p=0.4755	1.15 (0.90-1.46), p=0.2571	1.06 (0.83-1.34), p=0.6633	1.21 (0.95-1.55), p=0.1210	1.11 (0.87-1.42), p=0.4131
Q3 ¶	**1.32 (1.11-1.57), p=0.0019**	1.18 (0.99-1.40), p=0.0672	1.21 (0.95-1.54), p=0.1179	1.05 (0.82-1.34), p=0.6832	**1.33 (1.04-1.71), p=0.0218**	1.21 (0.95-1.55), p=0.1259
Q4 ¶	**1.58 (1.33-1.89), p=3.43x10^{-07}**	**1.31 (1.10-1.57), p=0.0030**	**1.31 (1.03-1.68), p=0.0301**	1.07 (0.83-1.38), p=0.6104	**1.77 (1.38-2.27), p=6.96x10^{-06}**	**1.50 (1.16-1.92), p=0.0016**
Joint models §						
cIMT ‖	**3.38 (2.20-5.23), p<0.0001**	**2.14 (1.38-3.29), p=0.0021**	**1.87 (1.01-3.47), p=0.0449**	1.12 (0.58-2.22), p=0.7460	**6.59 (3.58-12.18), p<0.0001**	**4.31 (2.23-8.12), p<0.0001**
Carotid plaque ‖	**2.05 (1.42-3.03), p=0.0028**	**1.61 (1.12-2.43), p=0.0112**	**1.86 (1.12-3.11), p=0.0049**	1.23 (0.78-1.96), p=0.3796	**2.11 (1.38-3.33), p<0.0001**	**1.82 (1.17-2.81), p=0.0084**

Abbreviations: CI, confidence interval; cIMT, carotid intima-media thickness; HR, hazard ratio; Q, quartiles.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Association between ‡ baseline carotid intima-media thickness and § longitudinal measures of carotid intima-media thickness, and carotid plaque for up to 3 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit increase in carotid intima-media thickness, and 1 unit increase in the probability of carotid plaque with the risk of new-onset atrial

fibrillation.
¶Quartiles in the total study population were Q1: ≤0.72mm, Q2: 0.73-0.80mm, Q3: 0.81-0.90mm, Q4: ≥0.91mm.
Quartiles in men were Q1: ≤0.74mm, Q2: 0.75-0.83mm, Q3: 0.84-0.94mm, Q4: ≥0.95mm.
Quartiles in women were Q1: ≤0.70mm, Q2: 0.71-0.78mm, Q3: 0.79-0.88mm, Q4: ≥0.89mm.
The associations with a $p<0.05$ are highlighted in **bold**.

Table 3. Association between baseline and longitudinal measures of ankle-brachial index with the risk of new-onset atrial fibrillation in the total study population and stratified by sex

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
ABI ‖	**2.11 (1.55-2.87), p=2.32x10^{-06}**	**1.57 (1.14-2.18), p=0.0061**	**2.29 (1.47-3.57), p=0.0003**	**1.62 (1.01-2.59), p=0.0447**	**1.95 (1.27-3.00), p=0.0023**	1.53 (0.97-2.39), p=0.0654
ABI, categories ‖						
≤0.90	**1.34 (1.14-1.57), p=0.0004**	**1.21 (1.02-1.42), p=0.0249**	**1.45 (1.14-1.85), p=0.0029**	1.27 (0.99-1.63), p=0.0625	**1.25 (1.01-1.56), p=0.0397**	1.17 (0.94-1.45), p=0.1707
0.91-0.99	**1.29 (1.09-1.53), p=0.0037**	1.17 (0.99-1.40), p=0.0696	**1.40 (1.08-1.83), p=0.0113**	1.25 (0.96-1.63), p=0.1011	1.21 (0.97-1.52), p=0.0961	1.11 (0.88-1.39), p=0.3757
1.00-1.40	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Joint models §						
ABI ‖	**7.53 (3.65-16.10), p<0.0001**	**4.43 (1.83-10.49), p=0.0007**	**6.53 (2.47-19.01), p<0.0001**	**3.72 (1.20-11.95), p=0.0225**	**7.84 (2.61-22.07), p<0.0001**	**5.03 (1.61-16.80), p=0.0042**

Abbreviations: ABI, ankle-brachial index; CI, confidence interval; HR, hazard ratio.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Association between ‡ baseline ankle-brachial index and § longitudinal measures of ankle-brachial index for up to 2 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit decrease in ankle-brachial index with the risk of new-onset atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

DISCUSSION

In this large prospective population-based cohort study, baseline and longitudinal measures of subclinical peripheral atherosclerosis were significantly associated with an increased risk of new-onset AF in the general population. Sex-stratified analyses indicated that the associations were mostly prominent among women. Our findings imply that treatment to reduce subclinical peripheral atherosclerosis might carry a potential for prevention of AF in the general population, especially among women.

The relationship between peripheral atherosclerosis and AF is not yet fully understood. It has been suggested that the association between peripheral atherosclerosis and AF can be in part attributable to the several shared cardiovascular risk factors.(8, 14, 25) Common risk factors including age, sex, obesity, hypertension, and diabetes mellitus, that contribute to (peripheral) atherosclerosis do also contribute to AF development.(26) Inflammation, endothelial dysfunction, and platelet-mediated thrombosis have been suggested as part of the underlying mechanisms that relate peripheral atherosclerosis with AF.(8, 14, 25, 27) Indeed, in our study, the associations between cIMT, carotid plaque, and ABI with incident AF attenuated after adjustment for traditional cardiovascular risk factors. However, the associations remained significant after taking into account cardiovascular risk factors. Nonetheless, it seems plausible that the combination of these aforementioned mechanisms reflects the association between peripheral atherosclerosis and AF, but further research to elucidate underlying mechanisms is warranted.

CIMT, carotid plaque, and ABI are perceived as subclinical measures of peripheral atherosclerosis and have also been linked to coronary artery disease (CAD).(8, 28-30) All 3 measures provide information regarding the extent of atherosclerosis even during the early phases of atheroma formation.(31) However, atherosclerosis of the carotid arteries may carry a stronger association with coronary atherosclerosis, and therefore CAD, than lower extremity atherosclerosis.(31, 32) Atherosclerosis, specifically CAD, induces an increase in left ventricular filling pressure, as reflected by an enlarged left atrium. Myocardial ischemia as well induces electrical and structural remodeling of the AF substrate. These aforementioned phenomena are among the mechanisms linking atherosclerosis and CAD with AF occurrence and maintenance.(33) Notably, besides its stronger association with CAD, cIMT has shown to be associated with pan-vascular atherosclerosis.(34) Notably, excluding participants with prevalent and incident CHD (prior to incident AF) did not change our original results.

Our study assessed the baseline and longitudinal measures of subclinical peripheral atherosclerosis during a long follow-up time in relation to new-onset AF. Considering repeated measurements of peripheral atherosclerosis in relation to new-onset AF by using joint models may provide more insight and give more prognostic information over a single baseline measurement. Longitudinal measures of carotid atherosclerosis, and lower extremity peripheral atherosclerosis, during follow-up were associated with an increased risk of incident AF, especially among women. These findings extend previous evidence by additionally reporting on repeated measurements and sex differences while assessing the relationship between peripheral atherosclerosis and AF.(7-14) We are not able to fully explain these sex differences, but one possible explanation could be due to differences in sex hormones. Women might benefit from the anti-atherosclerotic characteristics of higher estrogen levels during their life span. However, this protection is rapidly lost after menopause which gives rise to various forms of cardiovascular disorders. It has been demonstrated that estrogen affects the coronary arteries, aorta, and cerebral arteries differently.(35) We therefore hypothesize that the higher estrogen levels before menopause among women may have a larger protective effect on coronary and carotid atherosclerosis than lower extremity atherosclerosis.(35) This might explain why cIMT and carotid plaque is only associated with incident AF in women and not in men and that ABI is associated with AF in both men and women. We further hypothesize that the association in women may be caused through a distinct pathway, other than the pathways observed in men. In particular, the effect estimates observed in men in our study attenuated the most after adjusting for traditional cardiovascular risk factors. This might imply that the pathways involved in AF pathophysiology in women might not be solely via the traditional cardiovascular risk factors. Further, previous evidence has suggested competing risk of death as a plausible explanation for these sex differences.(9, 11) Since AF is also strongly associated with age, there may be a possibility that men die of other (cardiovascular) diseases prior to AF development and this hypothesis was supported by our competing risk analyses which showed that cIMT, carotid plaque, and ABI were significantly associated with mortality. Nevertheless, we observed a higher incidence of AF in men than women in this study.

The major strengths of this study are its population-based nature, large sample size with detailed information on cardiovascular risk factors, meticulous adjudication of AF events, and long follow-up time. Availability of both carotid atherosclerosis and lower extremity peripheral atherosclerosis measures allowed for direct comparison of various vascular beds in the same population. Availability of repeated measurements for subclinical peripheral atherosclerosis during follow-up enabled us to study longitudinal measures of peripheral atherosclerosis in association with new-onset AF. Additionally, we performed multiple sensitivity analyses including complete-case analyses, excluding prevalent and incident CHD prior to AF events, and the use of competing risk analyses to calculate cause-specific hazards.

There are also some limitations. We could not distinguish between paroxysmal, persistent, long-standing persistent, and permanent AF as Holter monitoring has not been performed in this large population-based cohort. Although, we adjusted for several cardiovascular risk factors, we cannot entirely rule out the possibility of residual confounding by other unmeasured risk factors. Finally, since our study population includes mainly elderly subjects that are mainly from European descent, our results may not be generalizable to younger populations or other ethnicities.

In this large population-based cohort study we assessed baseline and longitudinal measures of subclinical peripheral atherosclerosis during follow-up in relationship to new-onset AF. We found that baseline and longitudinal measures of subclinical peripheral atherosclerosis were significantly associated with an increased risk of new-onset AF. Our findings imply that treatment to reduce subclinical peripheral atherosclerosis might carry a potential for prevention of AF in the general population, especially among women, but future experimental studies are warranted to confirm our findings.

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SUPPLEMENTARY MATERIAL

Chapter 2.2 Peripheral atherosclerosis and the risk of atrial fibrillation

Methods S1. Assessment of cardiovascular risk factors

All participants responded to comprehensive computerized questionnaires at baseline about their current health status, medical history, medication, and life style. They were interviewed at home by trained interviewers, and underwent more extensive clinical examination and laboratory assessments at the research center.

Standardized measurements of height (in m) and weight (in kg) were performed and body mass index (BMI) was calculated as weight divided by height squared. Serum total and high-density lipoprotein (HDL) cholesterol were measured with an automated enzymatic method. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressures were calculated as the mean of the 2 consecutive measurements. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or use of antihypertensive drugs prescribed for hypertension.(3,9) Smoking information derived from baseline questionnaires was categorized into never, former, and current smokers. Diabetes mellitus (DM) was defined as fasting serum glucose levels ≥7.0 mmol/L (126 mg/dL) (or non-fasting serum glucose levels ≥11.1 mmol/L (200 mg/dL) if fasting samples were unavailable) or the use of antidiabetic therapy. The assessment and definition of prevalent coronary heart disease (CHD) and heart failure (HF) has been described in detail previously.(15) Left ventricular hypertrophy (LVH) on the electrocardiogram (ECG) was diagnosed using the MEANS program with an algorithm that takes into accounts QRS voltages, with an age-dependent correction and repolarization. Medication use was derived from baseline questionnaires, pharmacy data, and was categorized and defined according to the World Health Organization Anatomical Therapeutic Chemical (WHO ATC) classifications. Specifically, cardiac medication, antihypertensive medication, and lipid lowering medication were defined according to the WHO ATC categories c01, c02, and c10, respectively.

Table S1. Association between baseline and longitudinal measures of carotid intima-media thickness and carotid plaque with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with non-imputed data

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
cIMT ‖	**3.13 (2.01-4.88), p=4.61x10^{-07}**	**2.02 (1.28-3.19), p=0.0026**	**1.99 (1.08-3.66), p=0.0273**	1.26 (0.66-2.38), p=0.4841	**5.51 (2.88-10.56), p=2.65x10^{-07}**	**3.77 (1.93-7.35), p=9.70x10^{-05}**
Carotid plaque ‖	**1.33 (1.15-1.54), p=0.0002**	**1.22 (1.05-1.42), p=0.0084**	**1.33 (1.07-1.64), p=0.0106**	1.18 (0.95-1.48), p=0.1317	**1.33 (1.09-1.62), p=0.0057**	**1.27 (1.03-1.56), p=0.0238**
cIMT, quartiles ‖						
Q1 ¶	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Q2 ¶	1.14 (0.93-1.39), p=0.2166	1.06 (0.86-1.30), p=0.5904	1.23 (0.94-1.60), p=0.1395	1.15 (0.87-1.50), p=0.3284	1.26 (0.94-1.68), p=0.1257	1.15 (0.86-1.55), p=0.3390
Q3 ¶	**1.36 (1.11-1.65), p=0.0028**	1.22 (1.00-1.50), p=0.0515	1.30 (0.99-1.71), p=0.0569	1.15 (0.87-1.52), p=0.3239	**1.34 (1.00-1.79), p=0.0494**	1.24 (0.93-1.67), p=0.1437
Q4 ¶	**1.61 (1.31-1.96), p=4.08x10^{-06}**	**1.37 (1.11-1.68), p=0.0029**	**1.43 (1.08-1.89), p=0.0112**	1.20 (0.91-1.60), p=0.2012	**1.81 (1.35-2.42), p=7.3x10^{-05}**	**1.56 (1.16-2.09), p=0.0032**
Joint models §						
cIMT ‖	**3.43 (1.98-5.63), p<0.0001**	**2.26 (1.39-3.75), p=0.0028**	**2.13 (1.06-4.10), p=0.0330**	1.34 (0.65-2.68), p=0.4154	**6.42 (3.11-12.95), p<0.0001**	**4.44 (2.02-9.31), p<0.0001**
Carotid plaque ‖	**2.19 (1.49-3.46), p<0.0001**	**1.65 (1.13-2.47), p=0.0077**	**2.01 (1.18-3.73), p=0.0126**	1.47 (0.89-2.68), p=0.1488	**1.86 (1.18-3.10), p=0.0042**	**1.72 (1.07-2.93), p=0.0239**

Abbreviations: CI, confidence interval; cIMT, carotid intima-media thickness; HR, hazard ratio; Q, quartiles.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Association between ‡ baseline carotid intima-media thickness and § longitudinal measures of carotid intima-media thickness, and carotid plaque for up to 3 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit increase in carotid intima-media thickness, and 1 unit increase in the probability of carotid plaque with the risk of new-onset atrial

fibrillation.
¶ Quartiles in the total study population were Q1: ≤0.72mm, Q2: 0.73-0.80mm, Q3: 0.81-0.90mm, Q4: ≥0.91mm.
Quartiles in men were Q1: ≤0.74mm, Q2: 0.75-0.83mm, Q3: 0.84-0.94mm, Q4: ≥0.95mm.
Quartiles in women were Q1: ≤0.70mm, Q2: 0.71-0.78mm, Q3: 0.79-0.88mm, Q4: ≥0.89mm.
The associations with a $p<0.05$ are highlighted in **bold**.

Table S2. Association between baseline and longitudinal measures of ankle-brachial index with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with non-imputed data

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
ABI ‖	**2.18 (1.53-3.10), p=1.62x10^{-05}**	**1.66 (1.15-2.41), p=0.0071**	**2.71 (1.66-4.44), p=7.06x10^{-05}**	**1.97 (1.17-3.31), p=0.0107**	**1.73 (1.04-2.88), p=0.0337**	1.41 (0.83-2.39), p=0.2084
ABI, categories ‖						
≤0.90	**1.39 (1.16-1.66), p=0.0004**	**1.25 (1.04-1.51), p=0.0181**	**1.59 (1.22-2.06), p=0.0006**	**1.40 (1.07-1.83), p=0.0139**	1.22 (0.95-1.57), p=0.1210	1.13 (0.87-1.46), p=0.3643
0.91-0.99	**1.34 (1.10-1.63), p=0.0037**	1.22 (1.00-1.48), p=0.0507	**1.60 (1.20-2.13), p=0.0013**	**1.43 (1.07-1.91), p=0.0154**	1.15 (0.88-1.51), p=0.2990	1.07 (0.81-1.40), p=0.6422
1.00-1.40	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Joint models §						
ABI ‖	**8.91 (3.31-23.10), p<0.0001**	**5.52 (1.93-15.69), p=0.0014**	**9.48 (2.91-34.97), p<0.0001**	**5.45 (1.67-18.88), p=0.0098**	**7.03 (1.96-28.57), p=0.0028**	5.11 (0.97-22.93), p=0.0533

Abbreviations: ABI, ankle-brachial index; CI, confidence interval; HR, hazard ratio.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Association between ‡ baseline ankle-brachial index and § longitudinal measures of ankle-brachial index for up to 2 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit decrease in ankle-brachial index with the risk of new-onset atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

Table S3. Association between baseline and longitudinal measures of carotid intima-media thickness and carotid plaque with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with exclusion of prevalent and incident coronary heart disease cases prior to incident atrial fibrillation

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
cIMT ‖	**3.14 (1.99-4.95), p=8.26x10^{-07}**	**1.99 (1.24-3.18), p=0.0043**	1.47 (0.73-2.95), p=0.2810	0.88 (0.42-1.83), p=0.7235	**5.76 (3.17-10.46), p=9.14x10^{-09}**	**3.66 (1.99-6.76), p=3.26x10^{-05}**
Carotid plaque ‖	**1.25 (1.09-1.43), p=0.0013**	**1.17 (1.02-1.34), p=0.0259**	1.16 (0.94-1.43), p=0.1680	1.08 (0.87-1.33), p=0.4995	**1.31 (1.10-1.57), p=0.0026**	**1.23 (1.03-1.48), p=0.0224**
cIMT, quartiles ‖						
Q1 ¶	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Q2 ¶	1.20 (0.99-1.45), p=0.0641	1.12 (0.93-1.35), p=0.2466	1.23 (0.93-1.61), p=0.1417	1.13 (0.86-1.49), p=0.3962	1.19 (0.91-1.54), p=0.2000	1.10 (0.85-1.43), p=0.4575
Q3 ¶	**1.37 (1.14-1.66), p=0.0010**	**1.24 (1.03-1.51), p=0.0254**	1.30 (0.99-1.71), p=0.0643	1.13 (0.85-1.50), p=0.4039	**1.38 (1.07-1.79), p=0.0143**	1.27 (0.98-1.64), p=0.0724
Q4 ¶	**1.64 (1.35-1.99), p=8.97x10^{-07}**	**1.37 (1.12-1.67), p=0.0021**	1.30 (0.97-1.75), p=0.0775	1.07 (0.79-1.46), p=0.6545	**1.81 (1.39-2.36), p=1.08x10^{-05}**	**1.53 (1.17-1.99), p=0.0018**
Joint models §						
cIMT ‖	**3.61 (2.22-5.83), p<0.0001**	**2.44 (1.52-3.99), p<0.0001**	1.45 (0.67-3.20), p=0.3488	0.94 (0.41-2.08), p=0.8646	**7.63 (4.03-14.89), p<0.0001**	**5.11 (2.52-10.05), p<0.0001**
Carotid plaque ‖	**1.83 (1.22-2.74), p=0.0028**	**1.62 (1.12-2.40), p=0.0084**	1.51 (0.93-2.53), p=0.0933	1.26 (0.75-2.16), p=0.4084	**1.99 (1.22-3.28), p=0.0035**	**1.78 (1.15-2.78), p=0.0105**

Abbreviations: CI, confidence interval; cIMT, carotid intima-media thickness; HR, hazard ratio; Q, quartiles .

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication. Association between ‡ baseline carotid intima-media thickness and § longitudinal measures of carotid intima-media thickness, and carotid plaque for up to 3 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit increase in carotid intima-media thickness, and 1 unit increase in the probability of carotid plaque with the risk of new-onset atrial fibrillation.
¶ Quartiles in the total study population were Q1: ≤0.72mm, Q2: 0.73-0.80mm, Q3: 0.81-0.90mm, Q4: ≥0.91mm.
Quartiles in men were Q1: ≤0.74mm, Q2: 0.75-0.83mm, Q3: 0.84-0.94mm, Q4: ≥0.95mm.
Quartiles in women were Q1: ≤0.70mm, Q2: 0.71-0.78mm, Q3: 0.79-0.88mm, Q4: ≥0.89mm.
The associations with a $p<0.05$ are highlighted in **bold**.

Table S4. Association between baseline and longitudinal measures of ankle brachial-index with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with exclusion of prevalent and incident coronary heart disease cases prior to incident atrial fibrillation

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
ABI ‖	**2.38 (1.68-3.36), p=1.15x10^{-06}**	**1.83 (1.27-2.64), p=0.0012**	**2.82 (1.67-4.77), p=0.0001**	**2.24 (1.28-3.92), p=0.0049**	**2.08 (1.31-3.31), p=0.0020**	1.62 (0.99-2.64), p=0.0531
ABI, categories ‖						
≤0.90	**1.34 (1.12-1.61), p=0.0015**	**1.24 (1.03-1.49), p=0.0241**	**1.56 (1.17-2.07), p=0.0026**	**1.42 (1.06-1.92), p=0.0191**	1.21 (0.96-1.53), p=0.1014	1.14 (0.90-1.44), p=0.2837
0.91-0.99	**1.32 (1.09-1.60), p=0.0044**	1.19 (0.98-1.44), p=0.0787	**1.47 (1.07-2.02), p=0.0191**	1.30 (0.94-1.80), p=0.1107	1.24 (0.98-1.57), p=0.0732	1.12 (0.88-1.42), p=0.3465
1.00-1.40	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Joint models §						
ABI ‖	**11.52 (4.11-31.96), p<0.0001**	**7.70 (2.38-25.71), p<0.0001**	**12.10 (3.43-40.05), p<0.0001**	**9.58 (2.59-38.28), p<0.0001**	**9.21 (2.79-29.16), p<0.0001**	**5.72 (1.58-22.81), p=0.0049**

Abbreviations: ABI, ankle-brachial index; CI, confidence interval; HR, hazard ratio.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication. Association between ‡ baseline ankle-brachial index and § longitudinal measures of ankle-brachial index for up to 2 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit decrease in ankle-brachial index with the risk of new-onset atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

Table S5. Association between baseline and longitudinal measures of carotid intima-media thickness and carotid plaque with the risk of mortality in the total study population and stratified by sex

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
cIMT ‖	**2.99 (2.39-3.75), p<2.00x10^{-16}**	**2.46 (1.96-3.09), p=8.21x10^{-15}**	**3.46 (2.52-4.76), p=1.94x10^{-14}**	**3.02 (2.17-4.20), p=4.47x10^{-11}**	**2.56 (1.86-3.51), p=6.22x10^{-09}**	**2.14 (1.56-2.93), p=2.20x10^{-06}**
Carotid plaque ‖	**1.31 (1.22-1.41), p=6.10x10^{-13}**	**1.21 (1.13-1.31), p=4.13x10^{-07}**	**1.33 (1.18-1.49), p=1.22x10^{-06}**	**1.24 (1.10-1.39), p=0.0003**	**1.29 (1.17-1.42), p=1.89x10^{-07}**	**1.19 (1.08-1.31), p=0.0005**
cIMT, quartiles ‖						
Q1 ¶	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Q2 ¶	1.00 (0.90-1.11), p=0.9914	0.98 (0.88-1.09), p=0.6890	1.04 (0.89-1.22), p=0.6088	1.01 (0.87-1.19), p=0.8578	0.98 (0.85-1.13), p=0.7694	0.97 (0.84-1.12), p=0.6771
Q3 ¶	**1.22 (1.10-1.35), p=0.0002**	**1.19 (1.07-1.32), p=0.0009**	**1.33 (1.14-1.54), p=0.0002**	**1.28 (1.10-1.49), p=0.0014**	1.13 (0.99-1.30), p=0.0804	1.14 (0.99-1.31), p=0.0747
Q4 ¶	**1.36 (1.22-1.50), p=1.08x10^{-08}**	**1.28 (1.15-1.42), p=5.82x10^{-06}**	**1.45 (1.25-1.69), p=1.16x10^{-06}**	**1.40 (1.19-1.63), p=2.73x10^{-05}**	**1.25 (1.08-1.44), p=0.0023**	**1.21 (1.05-1.39), p=0.0107**
Joint models §						
cIMT ‖	**2.78 (2.14-3.61), p<0.0001**	**2.25 (1.74-2.93), p<0.0001**	**3.15 (2.18-4.55), p<0.0001**	**2.66 (1.85-3.83), p<0.0001**	**2.52 (1.74-3.61), p<0.0001**	**2.13 (1.46-3.13), p<0.0001**
Carotid plaque ‖	**2.94 (2.19-4.10), p<0.0001**	**1.99 (1.50-2.71), p<0.0001**	**2.97 (1.93-4.92), p<0.0001**	**2.07 (1.36-3.34), p=0.0007**	**2.50 (1.72-3.80), p<0.0001**	**1.75 (1.23-2.58), p=0.0014**

Abbreviations: CI, confidence interval; cIMT, carotid intima-media thickness; HR, hazard ratio; Q, quartiles.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Association between ‡ baseline carotid intima-media thickness and § longitudinal measures of carotid intima-media thickness, and carotid plaque for up to 3 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit increase in carotid intima-media thickness, and 1 unit increase in the probability of carotid plaque with the risk of mortality.
¶ Quartiles in the total study population were Q1: ≤0.72mm, Q2: 0.73-0.80mm, Q3: 0.81-0.90mm, Q4: ≥0.91mm.
Quartiles in men were Q1: ≤0.74mm, Q2: 0.75-0.83mm, Q3: 0.84-0.94mm, Q4: ≥0.95mm.
Quartiles in women were Q1: ≤0.70mm, Q2: 0.71-0.78mm, Q3: 0.79-0.88mm, Q4: ≥0.89mm.
The associations with a $p<0.05$ are highlighted in **bold**.

Table S6. Association between baseline and longitudinal measures of ankle-brachial index with the risk of mortality in the total study population and stratified by sex

	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Cox proportional hazards models ‡						
ABI ‖	**3.25 (2.78-3.79), p<2.00x10^{-16}**	**2.63 (2.24-3.09), p<2.00x10^{-16}**	**3.66 (2.90-4.62), p<2.00x10^{-16}**	**2.76 (2.16-3.52), p=3.64x10^{-16}**	**2.90 (2.35-3.57), p<2.00x10^{-16}**	**2.51 (2.02-3.11), p<2.00x10^{-16}**
ABI, categories ‖						
≤0.90	**1.64 (1.52-1.78), p<2.00x10^{-16}**	**1.49 (1.37-1.62), p<2.00x10^{-16}**	**1.73 (1.53-1.97), p<2.00x10^{-16}**	**1.51 (1.33-1.72), p=4.94x10^{-10}**	**1.57 (1.42-1.75), p<2.00x10^{-16}**	**1.47 (1.32-1.64), p=2.13x10^{-12}**
0.91-0.99	**1.28 (1.16-1.41), p=7.73x10^{-07}**	**1.22 (1.11-1.34), p=7.35x10^{-05}**	**1.28 (1.09-1.49), p=0.00195**	**1.19 (1.02-1.39), p=0.0287**	**1.27 (1.12-1.44), p=0.0002**	**1.25 (1.10-1.41), p=0.0005**
1.00-1.40	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Joint models §						
ABI ‖	22.79 (13.99-39.40), p<0.0001	15.67 (9.18-28.03), p<0.0001	17.26 (9.25-33.46), p<0.0001	10.46 (5.30-22.70), p<0.0001	22.89 (11.55-49.67), p<0.0001	17.95 (7.83-42.54), p<0.0001

Abbreviations: ABI, ankle-brachial index; CI, confidence interval; HR, hazard ratio.

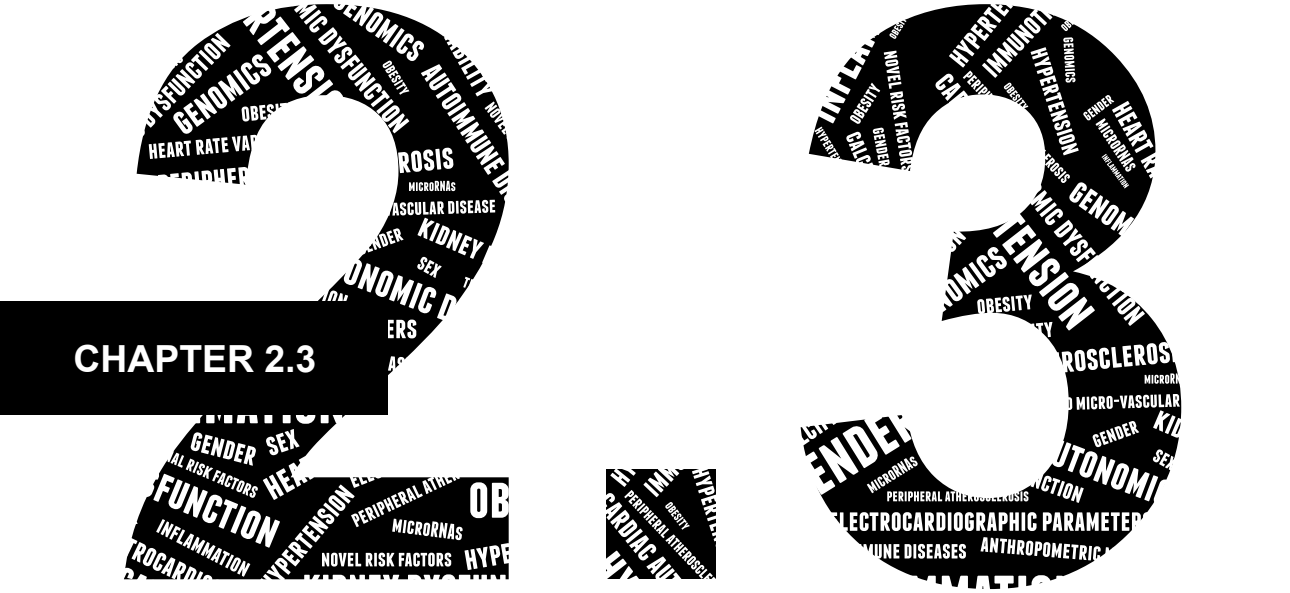
* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Association between ‡ baseline ankle-brachial index and § longitudinal measures of ankle-brachial index for up to 2 repeated measurements during follow-up with incident atrial fibrillation, assessed by ‡ Cox proportional hazards models and § joint models.

‖ Hazard ratios represent 1 unit decrease in ankle-brachial index with the risk of mortality.

The associations with a p<0.05 are highlighted in **bold**.


CHAPTER 2.3
GENOMICS
AUTOIMMUNE
HEART RATE
MICRORNAS
KIDNEY
SEX
GENDER
INFLAMMATION
NOVEL RISK FACTORS
OBESITY
IMMUNOTHERAPY
HYPERTENSION
HEART
MICRO-VASCULAR
PERIPHERAL ATHEROSCLEROSIS
ANTHROPOMETRIC

Kidney function and the risk of atrial fibrillation

Bidirectional association between kidney function and atrial fibrillation: a population-based cohort study.

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ABSTRACT

Background
Consensus lacks concerning a bidirectional association between kidney function and atrial fibrillation (AF), but this is crucial information for prevention/treatment efforts for both chronic kidney disease and AF. Therefore, we investigated the bidirectional association between kidney function and AF.

Methods
This study was a prospective cohort study including 9,228 participants (mean age 64.9 years, 57.2% women) with information on kidney function (estimated glomerular filtration rate (eGFR) based on serum creatinine (eGFRcreat), cystatin C (eGFRcys), or both (eGFRcreat-cys), and urine albumin-to-creatinine ratio) and AF. Reduced kidney function was defined as eGFRcreat <60 ml/min per 1.73 m^2. Cox proportional hazards, logistic regression, linear mixed, and joint models were used to investigate the association of kidney function with AF and vice versa.

Results
During a median follow-up 8.0 years, 780 events of incident AF occurred. Lower eGFRcys and eGFRcreat-cys were associated with increased AF risk (hazard ratio (HR), 1.08 (95% CI, 1.03-1.14) and HR, 1.07 (95% CI, 1.01-1.14), respectively, per 10 ml/min per 1.73 m^2 eGFR decrease). For eGFRcys and eGFRcreat-cys, 10-year cumulative incidence of AF was 16% (eGFR <60) and 6% (eGFR ≥60). Prevalent AF (vs. no prevalent AF) was associated with 2.85 ml/min per 1.73 m^2 lower eGFRcreat and with a faster decline of eGFRcreat with age. Prevalent AF was associated with a 1.3-fold increased risk of incident reduced kidney function.

Conclusions
Kidney function, especially eGFRcys, and AF are bidirectionally associated. There are currently no targeted prevention efforts for AF in patients with mild chronic kidney disease and vice versa. Our results could provide the first step to improve prediction/prevention of both conditions.

INTRODUCTION

Atrial fibrillation (AF) and chronic kidney disease (CKD) are highly prevalent diseases.(1, 2) More specifically, an estimated number of 5 million new AF cases occur annually worldwide(1) and CKD is affecting 11-13% of the global population.(2) Furthermore, both diseases are associated with substantial morbidity and mortality from cardiovascular and cerebrovascular disease.(3-5) Moreover, AF and CKD share several important and potential modifiable risk factors, such as hypertension and diabetes mellititus (DM),(6-9) and management of these risk factors is a cornerstone in the prevention of both diseases. However, despite efforts to prevent AF and CKD mainly by managing traditional risk factors, the prevalence of both is expected to increase in the upcoming years.(10, 11) This highlights the need for identifying additional risk factors to improve the prediction and prevention of AF as well as of CKD.

Interestingly, a bidirectional association between the 2 diseases may exist, revealing the potential of kidney function to be a modifiable risk factor for AF and vice versa. However, the presence of a bidirectional association between kidney function and AF in the general population is incompletely understood. Only a small number of studies have investigated a possible bidirectional association between kidney function and AF in the general population, and conflicting results were reported.(12, 13) Moreover, previous population-based studies investigating the association between kidney function and AF have several limitations, including calculating estimated glomerular filtration rate (eGFR) based on serum creatinine only (eGFRcreat). Currently, serum creatinine is widely used in clinical practice as a marker of kidney function, although it is suggested that eGFR based on cystatin C (eGFRcys) might be a stronger predictor of cardiovascular events.(14) Therefore, it is worth investigating both markers of kidney function (i.e. eGFRcreat and eGFRcys) to investigate the potential of both in determining AF risk. Furthermore, previous studies relied on a single assessment of kidney function by which potential variation and transient declines in kidney function over time are not taken into account, which could lead to misclassification bias.

Therefore, in this study, we aimed to investigate the bidirectional association between different assessments of kidney function and AF within the general population. Moreover, we included both single and multiple assessments of kidney function to reduce the potential bias that can occur when including single assessments of kidney function only.

METHODS

Study design

The Rotterdam Study is a prospective, population-based cohort study designed to investigate the occurrence and determinants of age-related diseases in the general population. Details regarding the design and rationale of the Rotterdam Study have been described in detail previously.(15) In summary, the Rotterdam Study is ongoing since 1990 and includes 14,926 participants aged 45 years and older, living in Ommoord, a district in Rotterdam, The Netherlands. The study consists of 3 independent cohorts: RS-I, RS-II, and RS-III. The original cohort, RS-I, comprised 7,983 participants aged 55 years and older. In 2000, this cohort was extended with RS-II, including 3,011 participants who had become 55 years old or moved into Ommoord since the start of the study. In 2006, the cohort was further enlarged with RS-III, including 3,392 participants aged 45 years and older who had not been invited to participate previously. Follow-up examinations are planned every 3-6 years and the participants are continuously monitored for relevant outcomes, including cardiovascular diseases.

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Study population

Participants were eligible for inclusion if they had measurements of serum creatinine and serum cystatin C available at baseline, which was defined as the third visit of RS-I (1997-1999), the first visit of RS-II (2000-2001), and the first visit of RS-III (2006-2008). In addition, information on prevalent and incident AF had to be available at baseline and during follow-up. All participants were followed up from the day of baseline laboratory measurement to the date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of kidney function

eGFR was calculated for serum creatinine (eGFRcreat), serum cystatin C (eGFRcys), or both (eGFRcreat-cys), according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.(16, 17) Serum creatinine was measured using an enzymatic assay method and expressed in micromoles per liter (µmol/L).(18) Serum cystatin C was measured using a particle-enhanced immunonephelometric assay and expressed in milligrams per liter (mg/L). We categorized eGFRcreat using a cut-off of 60 ml/min per 1.73 m^2, because eGFRcreat <60 ml/min per 1.73 m^2 is a well-accepted definition for reduced kidney function in population-based research settings.(19) The same cut-off was used for the categorization of eGFRcys and eGFRcreat-cys. Assessments of eGFRcreat from the Rotterdam Study were supplemented with those of the Star-MDC database, which is a database from a center for medical diagnostics for outpatients in the city of Rotterdam, providing multiple assessments of eGFRcreat over time.(17) In this database, serum creatinine was determined by using an enzymatic assay method as well. Incident reduced kidney function was defined as the first time eGFRcreat dropped below 60 ml/min per 1.73 m^2. Baseline urine albumin and creatinine were determined in timed overnight urine by a turbidimetric method and measured by a Hitachi Modular P analyzer (Roche/Hitachi Diagnostics, Mannheim, Germany).(20, 21) The urine albumin-to-creatinine ratio (UACR) was calculated by dividing urine albumin by urine creatinine (mg/g).

Assessment of atrial fibrillation

Ascertainment of prevalent and incident AF within the Rotterdam Study has been reported elsewhere in detail.(22) In short, AF ascertainment is in accordance with the European Society of Cardiology (ESC) guidelines(23) and cases were determined using 3 methods. First, ECGs that were obtained at baseline and during follow-up examinations at the research center were stored digitally and processed by the Modular ECG Analysis System (MEANS).(24, 25) As verification of the AF diagnosis, all ECGs with a MEANS diagnosis of AF, atrial flutter, or any other rhythm disorder were independently reviewed by 2 research physicians who were blinded to the MEANS diagnosis. In case of a persisting disagreement between the coding physicians, the judgement of a cardiologist was sought and taken as decisive. Second, additional information on AF was obtained from general practitioners' records, including their own results and the results from other physicians practicing in hospitals and outpatient clinics. Finally, information was obtained from a national registry of all hospital discharge diagnoses as well. The occurrence of AF during a serious disease resulting in death, during myocardial infarction, or during cardiac operative procedures of patients who recovered during the hospital admission was not considered as a case. These participants were censored on the date of the detection of AF. We did not distinguish between AF and atrial flutter when identifying cases, because both conditions are similar with respect to risk factors and

consequences.(26, 27)

Assessment of cardiovascular risk factors

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Information on educational level, alcohol intake, smoking status, and medication use was collected during home interviews. Educational level was categorized into 4 categories: primary education, lower or intermediate general and lower vocational education, higher general and intermediate vocational education, and higher vocational education or university. The highest achieved educational level was taken as a proxy for socio-economic status. Alcohol intake was measured in grams per day and smoking status was categorized into never, former, and current smokers. The World Health Organization's Anatomical Therapeutic Chemical code C01 was used to define cardiac medication use. Physical activity levels were assessed with a validated adapted version of the Zutphen Physical Activity Questionnaire and the Longitudinal Aging Study Amsterdam (LASA) Physical Activity Questionnaire, and expressed in total metabolic equivalent hours per week. Systolic and diastolic blood pressure were measured twice on the right arm using a random-zero sphygmomanometer and the mean of these measurements was taken as the final measurement. Hypertension was defined as a systolic blood pressure of at least 140 mmHg, a diastolic blood pressure of at least 90 mmHg, or the use of antihypertensive drugs prescribed for hypertension. Serum cholesterol levels were measured by the department of Clinical Chemistry of the Erasmus MC using standard laboratory techniques. DM cases were defined by a previous diagnosis of the disease, a fasting serum glucose level ≥7.0 mmol/L (126 mg/dL), a non-fasting serum glucose level ≥11.1 mmol/L (200 mg/dL when fasting samples were absent), or the use of blood glucose lowering medication. History of coronary heart disease (CHD) was defined as a history of myocardial infarction or a history of a coronary revascularization procedure.(28) Heart failure (HF) was defined in accordance with the ESC guidelines as a combination of the presence of typical symptoms and signs of HF (such as shortness of breath at rest or during exertion, ankle edema, or pulmonary crepitation), confirmed by objective evidence of cardiac dysfunction or a positive response to the initiated treatment.(28, 29)

Statistical analyses

To assess the potential bidirectional association between kidney function and AF, we investigated the association between kidney function and incident AF, as well as the association between prevalent AF and kidney function. To account for missing values in the covariates (missingness for all covariates <2%, except for physical activity and alcohol use, which was <20%), multiple imputation using the “mice” package in R(30) was performed. Data were imputed using Bayesian linear regression (“norm”) or predictive mean matching (“pmm”) for continuous covariates, binary logistic regression (“logreg”) for binary categorical covariates, polytomous

logistic regression ("polyreg") for unordered categorical covariates with >2 levels, and a proportional odds model ("polyr") for ordered categorical covariates with >2 levels. We generated 5 imputed data sets and results for each data set were pooled to obtain single estimates. Statistical significance was considered at a two-sided p<0.05. Statistical analyses were performed using R software (R 3.6.3; R Foundation for Statistical Computing, Vienna, Austria.

Kidney function and incident atrial fibrillation

Cox proportional hazards models were used to obtain hazard ratios (HRs) with their 95% confidence intervals (CIs) for the associations of continuous and categorized baseline eGFRcys, eGFRcreat, and eGFRcreat-cys with incident AF. The same approach was used to study the association between UACR and incident AF. UACR was not normally distributed and therefore, a natural logarithmic transformation was used. We added 1 mg/g to the non-transformed values to account for zero values of UACR. All HRs were reported per 10 ml/min per 1.73 m^2 decrease in eGFR and per 1 unit increase in log UACR (mg/g). The proportional hazards assumptions were checked using Schoenfeld residual testing and by assessing the Schoenfeld plots. We repeated the analyses using non-transformed UACR and HRs were reported per 1 mg/g increase in UACR. Participants with prevalent AF were excluded in the analyses regarding incident AF. Primary models were adjusted for age, sex, and cohort. We additionally adjusted for the potential confounders, educational level, BMI, smoking status, alcohol, total cholesterol, history of DM, physical activity, and cardiac medication use in a second model, and for the potential confounders that could also act as mediators, hypertension, history of CHD, and history of HF in a third model. Age- and sex-adjusted cumulative incidences for categorized (cut-off 60 ml/min per 1.73 m^2) eGFRcys and eGFRcreat-cys were extracted from standardized Cox proportional hazards models. Joint models were used to study the association between repeated assessments of eGFRcreat only and the risk of incident AF, because no repeated measurements of serum cystatin C were available. The longitudinal submodel was defined as a linear mixed effects model. The fixed effects in the longitudinal submodel included age, sex, and cohort, and the random effects included a random intercept and linear random slopes (i.e. of time). The survival submodel was defined as a Cox proportional hazards model and analyses were performed using the same models to adjust for confounders as described above. The joint model was fit under a maximum likelihood approach. Pre-defined stratification by age and sex was performed and interaction terms of these variables with the 3 GFR estimates were used to assess effect modification. A p for interaction <0.10 was considered to be statistically significant.

In sensitivity analyses, we added age, BMI, and smoking status as time-varying covariates to the abovementioned models instead of only the baseline assessments of these covariates. In addition, we restricted the analyses to participants with eGFRcreat <120 ml/min per 1.73 m^2 and excluded participants with prevalent CHD and HF. We also excluded participants with both prevalent and incident CHD and HF, and as a separate sensitivity analysis, we added CHD and HF as time-varying covariates to the second model. Furthermore, we excluded the first 2 and 4 years of follow-up to assess the possibility of reverse causality.

Prevalent atrial fibrillation and kidney function

Linear regression models were used to study the associations between prevalent AF and baseline levels of eGFRcys, eGFRcreat, and eGFRcreat-cys, and linear mixed models were used to investigate the trajectories of eGFRcreat in participants with and without prevalent AF. The same 3 models to adjust for covariates as described for the analyses regarding kidney function and incident AF were used. Age was used as the time variable in the linear mixed models. First, all linear mixed models included prevalent AF as determinant and no interaction term between prevalent AF and age. Second, the analyses were repeated and an interaction term between prevalent AF and age was added to the models. The association between prevalent AF and eGFRcreat on average with age is presented by the effect estimate of prevalent AF, whereas the association between prevalent AF and change in eGFRcreat with age is represented by the effect estimate of the interaction term. Cox proportional hazards models were used to study the association between prevalent AF and reduced kidney function, and cases with prevalent reduced kidney function were excluded for these analyses. In a sensitivity analysis, we excluded participants with incident AF during follow-up.

RESULTS

Kidney function and incident atrial fibrillation

For the incident AF analyses, we included 9,288 participants with a mean age of 64.9 years, of whom 57.2% were women (**Table 1**). A total of 780 cases of incident AF occurred during a median follow-up time of 8.0 years (interquartile range (IQR), 6.1-13.3 years), with an incidence rate of 8.9 per 1,000 person-years. The total number of repeated assessments of eGFR of all participants included in the analyses regarding incident AF was 55,917, with a median of 4 assessments per participant. Lower levels of baseline eGFRcys and eGFRcreat-cys were associated with an increased risk of incident AF, with an adjusted HR of 1.08 (95% CI, 1.03-1.14) and an adjusted HR of 1.07 (95% CI, 1.01-1.14), respectively, per 10 ml/min per 1.73 m^2 decrease in eGFR (**Table 2**). Additional adjustment for hypertension, CHD, and HF did not substantially change the results (**Table S1**). There was no association between baseline eGFRcreat and incident AF, and similar results were reported when using repeated assessments of eGFRcreat over time (**Table 2**). Adding age, BMI, and smoking status as time-varying covariates to the model, restricting the analyses to participants with eGFRcreat <120 ml/min per 1.73 m^2, excluding participants with prevalent CHD and HF, excluding participants with prevalent and incident CHD and HF, adding CHD and HF as time-varying covariates to the second model, and excluding the first 2 and 4 years of follow-up did not change the risk estimates substantially (**Table S2**). Stratification analyses for age and sex did not show differential risks (p for interaction for all analyses >0.35, **Table S3**). Assessments of UACR were available in a subset of 3,065 participants. No association between UACR and incident AF was shown (**Table S4**).

Table 1. Baseline characteristics of the total study population

Baseline characteristics *	**Participants without prevalent AF (n=9,288)**	**Participants with prevalent AF (n=409)**
Age, years	64.9 ± 9.7	73.0 ± 10.0
Women, n (%)	5,317 (57.2)	185 (45.2)
Educational level		
Primary education, n (%)	1,145 (12.4)	69 (17.0)
Lower/ intermediate general and lower vocational education, n (%)	3,718 (40.4)	174 (42.9)
Higher general and intermediate vocational education, n (%)	2,688 (29.2)	105 (25.9)
Higher vocational education and university, n (%)	1,654 (18.0)	58 (14.3)
Body mass index, kg/m^2	27.2 ± 4.2	27.4 ± 4.2
Systolic blood pressure, mmHg	140 ± 21	142 ± 23
Diastolic blood pressure, mmHg	79 ± 11	77 ± 13
Hypertension, n (%)	5,871 (62.0)	349 (86.6)
History of diabetes mellitus, n (%)	1,101 (11.9)	77 (18.8)
History of coronary heart disease, n (%)	514 (5.6)	56 (13.8)
History of heart failure, n (%)	184 (2.0)	87 (21.3)
Smoking status		
Current, n (%)	1,796 (19.5)	52 (12.9)
Former, n (%)	4,377 (47.6)	223 (55.5)
Never, n (%)	3,017 (32.8)	127 (31.6)
Alcohol use, g/day	5.7 (0.5-14.6)	5.1 (0.3-14.3)
eGFRcreat, ml/min per 1.73 m^2	81.1 ± 14.7	70.6 ± 18.8
eGFRcys, ml/min per 1.73 m^2	77.3 ± 18.8	61.9 ± 19.5
eGFRcreat-cys, ml/min per 1.73 m^2	79.0 ± 16.2	66.2 ± 18.1
Total cholesterol, mmol/L	5.7 ± 1.0	5.4 ± 1.0
Cardiac medication use, n (%)	508 (5.6)	166 (42.9)

Abbreviations: AF, atrial fibrillation; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C.
Values are shown before imputation and therefore not always add up to 100%.
* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table 2. Association between continuous and categorized eGFRcys, eGFRcreat, and eGFRcreat-cys and the risk of incident atrial fibrillation

eGFR	AF events/ Total n	HR (95% CI), Model 1 *	HR (95% CI), Model 2 †
Continuous, baseline assessment ‡			
eGFRcys, ml/min per 1.73 m^2 ‖	780/9,288	**1.11 (1.06-1.17)**	**1.08 (1.03-1.14)**
eGFRcreat, ml/min per 1.73 m^2 ‖	780/9,288	1.05 (0.99-1.11)	1.04 (0.98-1.10)
eGFRcreat-cys, ml/min per 1.73 m^2 ‖	780/9,288	**1.10 (1.04-1.16)**	**1.07 (1.01-1.14)**
Continuous, repeated assessments §			
eGFRcreat, ml/min per 1.73 m^2 ‖	780/9,288	1.03 (0.97-1.09)	1.02 (0.97-1.09)
Categorical, baseline assessment ‡			
Categories of eGFRcys			
eGFRcys ≥60 ml/min per 1.73 m^2	545/7,599	Reference	Reference
eGFRcys <60 ml/min per 1.73 m^2	235/1,689	**1.45 (1.21-1.73)**	**1.37 (1.14-1.64)**
Categories of eGFRcreat			
eGFRcreat ≥60 ml/min per 1.73 m^2	657/8,422	Reference	Reference
eGFRcreat <60 ml/min per 1.73 m^2	123/866	**1.27 (1.03-1.56)**	1.22 (0.99-1.50)
Categories of eGFRcreat-cys			
eGFRcreat-cys ≥60 ml/min per 1.73 m^2	624/8,186	Reference	Reference
eGFRcreat-cys <60 ml/min per 1.73 m^2	156/1,102	**1.35 (1.11-1.64)**	**1.27 (1.04-1.55)**

Abbreviations: AF, atrial fibrillation; CI, confidence interval; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C; HR, hazard ratio; n, number.

* Adjusted for age, sex, and cohort. † Adjusted for age, sex, cohort, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication. ‡ Cox proportional hazards models were used to investigate the associations between continuous/categorical eGFR at baseline and incident AF and § joint models were used to investigate the association between repeated assessments of eGFRcreat and incident AF. ‖ Hazard ratios given per 10 ml/min per 1.73 m^2 decrease in eGFR with the risk of incident atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

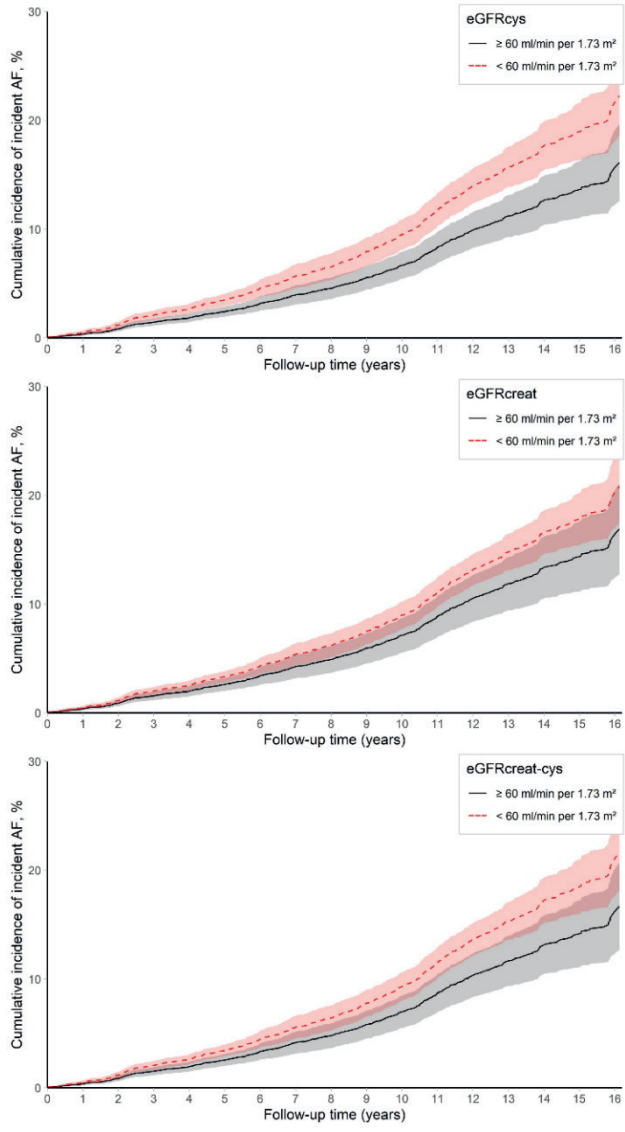
Categories of eGFR and incident atrial fibrillation

Categorization of eGFRcys using a cutoff of 60 ml/min per 1.73 m^2 was associated with an increased risk of incident AF in participants with eGFRcys <60 ml/min per 1.73 m^2 (HR, 1.37 (95% CI, 1.14-1.64)), compared with participants with eGFRcys ≥60 ml/min per 1.73 m^2 (**Table 2**). Similar results were reported for eGFRcreat-cys (HR, 1.27 (95% CI, 1.04-1.55)). In addition, cumulative incidences were higher for participants with eGFRcys <60 ml/min per 1.73 m^2 compared with participants with eGFRcys ≥60 ml/min per 1.73 m^2 (**Figure 1**). For eGFRcys, 10-year cumulative incidence of AF was 9.5% (95% CI, 8.1-10.9) for participants with eGFR <60 ml/min per 1.73 m^2 and 6.8% (95% CI, 5.4-7.9) for participants with eGFR ≥60 ml/min per 1.73 m^2, with a cumulative incidence ratio of 1.40 (95% CI, 1.38-1.49). The absolute risk difference at 15 years was 5.4% (95% CI, 5.3-5.4). Categorization of eGFRcreat using a cutoff of 60 ml/min per 1.73 m^2 was not significantly associated with an increased risk of incident AF in participants with eGFRcreat <60 ml/min per 1.73 m^2 (HR, 1.22 (95% CI, 0.99-1.50)). When additionally adjusting for hypertension, CHD, and HF, only the association between categorized eGFRcys and incident AF remained statistically significant (data not shown).

Prevalent atrial fibrillation and kidney function

For analyses regarding the association between prevalent AF and kidney function, we included 9,697 participants, of whom 409 had prevalent AF at baseline (mean age, 65.3 years, 56.7% women). In a cross-sectional analysis, eGFRcys was 4.24 ml/min per 1.73 m^2 (95% CI, -5.68 to -2.81 ml/min per 1.73 m^2) lower in participants with prevalent AF compared with participants without prevalent AF (**Table 3**). In addition, eGFRcreat and eGFRcreat-cys were 1.93 ml/min per 1.73 m^2 (95% CI, -3.23 to -0.63 ml/min per 1.73 m^2) and 3.36 ml/min per 1.73 m^2 (95% CI, -4.64 to -2.07 ml/min per 1.73 m^2) lower in participants with prevalent AF compared with participants without prevalent AF, respectively (**Table 3**). The total number of repeated assessments of eGFRcreat of all participants included in the analyses with prevalent AF was 70,687, with a median of 5 assessments per participant. When studying the trajectories of eGFRcreat with age in participants with and without prevalent AF, eGFRcreat was 2.85 ml/min per 1.73 m^2 (95% CI, -4.10 to -1.60 ml/min per 1.73 m^2) lower in participants with prevalent AF compared with participants without prevalent AF (**Table 3**). Inclusion of an interaction term between AF and age also revealed a faster decline of eGFRcreat with aging in participants with prevalent AF compared with participants without prevalent AF (**Figure 2**). Furthermore, prevalent AF was associated with an increased risk of incident reduced kidney function, with an adjusted HR of 1.33 (95% CI, 1.1-1.58) (**Table 3**). Excluding participants with incident AF during follow-up did not change the risk estimates substantially (**Table S5**).

Figure 1. Cumulative incidence of incident AF by eGFRcys, eGFRcreat, and eGFRcreat-cys



Abbreviations: AF, atrial fibrillation; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C. Cumulative incidence by eGFRcys, eGFRcreat, and eGFRcreat-cys at baseline, adjusted for age and sex.

Table 3. Association between prevalent atrial fibrillation and 1) eGFRcys, eGFRcreat, and eGFRcreat-cys at baseline, 2) eGFRcreat with age, and 3) incident reduced kidney function

	Total n	Beta (95% CI), Model 1 *	Beta (95% CI), Model 2 †
Outcome: eGFR at baseline (cross-sectional) ‡			
eGFRcys			
No prevalent AF	9,288	Reference	Reference
Prevalent AF	409	**-5.46 (-6.89 to -4.03)**	**-4.24 (-5.68 to -2.81)**
eGFRcreat			
No prevalent AF	9,288	Reference	Reference
Prevalent AF	409	**-2.80 (-4.07 to -1.53)**	**-1.93 (-3.23 to -0.63)**
eGFRcreat-cys			
No prevalent AF	9,288	Reference	Reference
Prevalent AF	409	**-4.46 (-5.72 to -3.19)**	**-3.36 (-4.64 to -2.07)**
Outcome: eGFRcreat with age (longitudinal) § ¶			
	Total n	**Beta (95% CI), Model 1** *	**Beta (95% CI), Model 2** †
No prevalent AF	9,288	Reference	Reference
Prevalent AF	409	**-4.08 (-5.29 to -2.86)**	**-2.85 (-4.10 to -1.60)**
Outcome: incident reduced kidney function (longitudinal) ‖ #			
	Events / total n	**HR (95% CI), Model 1** *	**HR (95% CI), Model 2** †
No prevalent AF	2,535 / 8,422	Reference	Reference
Prevalent AF	157 / 306	**1.50 (1.27-1.77)**	**1.33 (1.12-1.58)**

Abbreviations: AF, atrial fibrillation; CI, confidence interval; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C; HR, hazard ratio; n, number.

* Adjusted for age, sex, and cohort.

† Adjusted for age, sex, cohort, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication.

‡ Linear regression models were used to investigate the associations between prevalent atrial fibrillation and eGFR at baseline and § linear mixed models were used to investigate the association between prevalent atrial fibrillation and eGFRcreat with age.

‖ Cox proportional hazards models were used to investigate the associations between prevalent atrial fibrillation and incident reduced kidney function.

¶ Total of 70,687 repeated assessments of eGFR (median of 5 repeated assessments).

Participants with prevalent reduced kidney function were excluded from the analysis (n=969). The associations with a p<0.05 are highlighted in **bold**.

Figure 2. Longitudinal changes in eGFRcreat according to prevalent AF

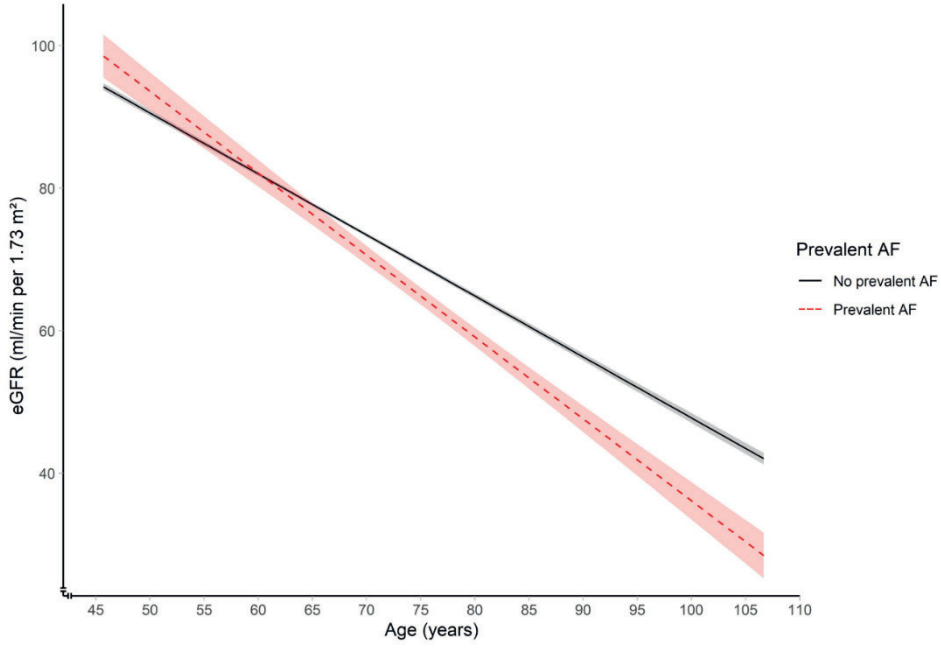


Figure is based on an unadjusted model (p for interaction <0.001). Linear mixed models were used to investigate the association between prevalent AF and eGFRcreat with age. AF indicates atrial fibrillation; eGFR, estimated glomerular filtration rate; and eGFRcreat, eGFR based on serum creatinine.

DISCUSSION

In the current study, lower levels of eGFRcys and eGFRcreat-cys were associated with an increased risk of incident AF. The direction of the association with eGFRcreat suggests an increased risk of incident AF with lower levels of eGFRcreat as well, although the association was less strong and not statistically significant. In cross-sectional and longitudinal analyses, prevalent AF was associated with lower levels of all 3 GFR estimates and a faster decline of eGFRcreat with age was revealed in participants with prevalent AF.

Our findings strongly suggest the presence of a bidirectional relationship between kidney function and AF in middle-aged and elderly individuals from the general population. Previous studies that have investigated this bidirectional association within the general population have shown conflicting results and did not include different and multiple assessments of kidney function.(12, 13) One study was conducted in participants taking part in a voluntary health checkup program in Japan and reported a potential bidirectional association between kidney function assessed by eGFRcreat and AF, that is, kidney dysfunction increased the risk of incident AF and vice versa.(12) Conversely, the second study, which included participants from a population-based cohort of ambulatory elderly, reported no association between kidney dysfunction and both prevalent and incident AF when kidney dysfunction was defined as eGFRcreat <60 ml/min per 1.73 m^2.(13) They did report an association of serum cystatin C with prevalent AF, but not with incident AF. In the current study, we report that prevalent AF is associated with reduced kidney function. The availability of a high number of eGFRcreat assessments over time allowed us to investigate the trajectories of eGFRcreat with age in participants with and without prevalent AF. Our eGFRcreat trajectories revealed that eGFRcreat was lower and declined faster in participants with prevalent AF compared with participants without prevalent AF. These findings reveal that prevalent AF could be a modifiable risk factor for kidney function decline. Potentially, further studies might investigate whether early treatment of prevalent AF with appropriate drugs could prevent further deterioration of kidney function over time.

As previously mentioned, there are only a few studies investigating the bidirectional association of kidney function and AF in the general population. Several other studies investigating the association of kidney function with AF in the general population(31-36) were unidirectional and did not include multiple assessments of kidney function, which could have resulted in misclassification bias. Furthermore, some were limited in generalizability, because they included only women or predominantly younger participants,(31, 32, 35) although AF and CKD are typically diseases of older age. In addition, previous studies adjusted for different sets of confounders, which complicates an accurate comparison of their findings. In the

current study, we included different and multiple assessments of kidney function and report that reduced kidney function increases the risk of incident AF in middle-aged and elderly men and women. Moreover, the direction of the association of all 3 GFR estimates with the risk of incident AF was the same, with the strongest association reported for eGFRcys and no significant association reported for eGFRcreat. A possible explanation for our results can be found in the differences between serum cystatin C and serum creatinine, because serum cystatin C has been suggested to be a better marker of kidney function(37) and a stronger predictor of cardiovascular events and mortality risk(14) when compared with serum creatinine. The mechanism behind this phenomenon is not completely understood, but cystatin C appears to be less affected by age, sex, and muscle mass than serum creatinine.(14, 38-40) In addition, serum cystatin C may be more sensitive for detecting small changes in eGFR.(41)

One of the mechanisms explaining the bidirectional relationship between kidney function and AF could be the presence of shared cardiovascular risk factors. However, adjusting the analyses for various cardiovascular risk factors, including in a time-varying fashion, did not alter our risk estimates, suggesting that other and potentially more causal mechanisms could underlie the reported associations. Potential other mechanisms might be captured by the cardiorenal syndrome, an umbrella term that is used to describe the pathological interplay between the cardiovascular system and the kidneys.(42) The pathological mechanisms involved in the cardiorenal syndrome are comprehensive and include 2 important mechanisms that could also explain the reported bidirectional association: inflammation and renin-angiotensin aldosterone system activation. First, reduced kidney function induces a pro-inflammatory state with a central role for inflammatory cytokines.(43-45) Moreover, an increase in inflammatory markers and thus an inflammatory state was also shown to be related to a decrease in eGFR in the general population.(46) Increased levels of pro-inflammatory cytokines have also been associated with an increased risk of AF(47-50) and AF itself can also promote inflammation.(51) Second, increased renin-angiotensin aldosterone system activity is often reported in CKD,(52) which could promote structural and electrical atrial remodeling.(52-54) In addition, the expression of the angiotensin-converting enzyme(55) and the levels of plasma aldosterone(56-58) are increased in patients with AF, suggesting an increased activity of the renin-angiotensin aldosterone system in patients with AF as well. In turn, this increased activity could have pathological consequences for the kidneys, because especially excessive levels of aldosterone and angiotensin II could have pro-inflammatory and pro-fibrotic effects on the kidneys.(59)

Our study has several strengths. First, the population-based design of the Rotterdam Study including a high number of participants with a high participation rate provides

sufficient statistical power to study our research questions and makes the results generalizable to the general population of middle-aged and elderly individuals. Second, a high number of meticulously adjudicated AF events were included in the study, due to the long follow-up time and the extensive evaluation of AF cases. Third, the high number of eGFRcreat assessments over time allowed us to study the association between kidney function and AF in more detail and reduced the potential bias that can occur when including only a single assessment of kidney function. Although not every participant had the same number of repeated eGFRcreat assessments available, we were still able to provide valid results by using statistical methods that can handle such unbalanced data. Several limitations should be mentioned as well. First, residual confounding cannot be excluded, even though we adjusted for a wide variety of confounders. Second, eGFRcys and eGFRcreat-cys were determined only at baseline and therefore changes in these assessments over time could not be analyzed. Third, data on UACR were only available in a subset of the population and only a low number of incident AF events occurred in this population. Therefore, the power to detect an association between UACR and incident AF could have been limited. Fourth, part of the included participants had missing values in one or more confounders, although the missingness was expected to be at random and was <2% for most confounders. Furthermore, we performed multiple imputation to account for missing values in the covariates. Finally, the Rotterdam Study includes mainly White middle-aged and elderly subjects, which limits the generalizability of our results to other ethnicities, and younger populations.

In conclusion, we report an increased risk of incident AF with lower levels of eGFRcys and eGFRcreat-cys. This reveals that kidney function, especially when assessed by eGFRcys, could be a modifiable risk factor for incident AF. In addition, we report that prevalent AF is associated with reduced kidney function, both at baseline and over time, which reveals that prevalent AF could be a modifiable risk factor for decreased kidney function. Because the prevalence of both AF and CKD is expected to increase in the upcoming years despite efforts to prevent both diseases by managing traditional risk factors, our findings may be highly clinically relevant, because they could improve the prediction/prevention of both AF and CKD. In addition, it could also change the preferred treatment strategy when both conditions are present simultaneously. Although our findings suggest a bidirectional association between kidney function and AF, future studies are needed to investigate the causality of this association, for example with Mendelian randomization analyses. In addition, future studies are needed to investigate potential underlying mechanisms, first focusing on whether cardiovascular risk factors mediate the association between kidney function and AF and vice versa. Prediction studies are needed to explore whether adding eGFR, and especially eGFRcys, to screening models for incident AF could improve these models and vice versa.

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SUPPLEMENTARY MATERIAL

Chapter 2.3 Kidney function and the risk of atrial fibrillation

Table S1. Association between eGFRcys, eGFRcreat, and eGFRcreat-cys at baseline and the risk of incident atrial fibrillation

eGFR ‖	**AF events / total n**	**HR (95% CI), Model 1** *	**HR (95% CI), Model 2** †	**HR (95% CI), Model 3** ‡
eGFRcys, ml/min per 1.73 m^2 §	780/9,288	**1.11 (1.06-1.17)**	**1.08 (1.03-1.14)**	**1.07 (1.02-1.13)**
eGFRcreat, ml/min per 1.73 m^2 §	780/9,288	1.05 (0.99-1.11)	1.04 (0.98-1.10)	1.02 (0.96-1.08)
eGFRcreat-cys, ml/min per 1.73 m^2 §	780/9,288	**1.10 (1.04-1.16)**	**1.07 (1.01-1.14)**	**1.07 (1.02-1.13)**

Abbreviations: AF, atrial fibrillation; CI, confidence interval; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C; HR, hazard ratio; n, number.

* Adjusted for age, sex, and cohort.

† Adjusted for age, sex, cohort, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication.

‡ Adjusted for age, sex, cohort, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication, hypertension, history of coronary heart disease, and history of heart failure.

§ Hazard ratios given per 10 ml/min per 1.73 m^2 decrease in eGFR with the risk of incident atrial fibrillation.

‖ Cox proportional hazards models were used to investigate the associations between eGFR at baseline and the risk of incident AF.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S2. Sensitivity analyses for the association between eGFRcys, eGFRcreat, and eGFRcreat-cys at baseline and the risk of incident atrial fibrillation

Sensitivity analyses ‡	AF events / total n	HR (95% CI), Model 1 *	HR (95% CI), Model 2 †
Correcting for time-varying effects of age, BMI, and smoking status			
eGFRcys, ml/min per 1.73 m^2 §	780/9,288	**1.11 (1.06-1.17)**	**1.09 (1.03-1.15)**
eGFRcreat, ml/min per 1.73 m^2 §	780/9,288	1.05 (0.99-1.12)	1.04 (0.99-1.11)
eGFRcreat-cys ml/min per 1.73 m^2	780/9,288	**1.10 (1.04-1.17)**	**1.08 (1.02-1.14)**
Restricting to participants with eGFRcreat below 120 ml/min per 1.73 m^2			
eGFRcys, ml/min per 1.73 m^2	780/9,281	**1.11 (1.06-1.17)**	**1.08 (1.03-1.14)**
eGFRcreat, ml/min per 1.73 m^2	780/9,281	1.05 (0.99-1.11)	1.04 (0.98-1.10)
eGFRcreat-cys ml/min per 1.73 m^2	780/9,281	**1.10 (1.04-1.16)**	**1.07 (1.01-1.14)**
Excluding participants with prevalent CHD and HF**			
eGFRcys, ml/min per 1.73 m^2	664/8,596	**1.09 (1.03-1.16)**	**1.07 (1.01-1.14)**
eGFRcreat, ml/min per 1.73 m^2	664/8,596	1.04 (0.98-1.11)	1.04 (0.97-1.10)
eGFRcreat-cys ml/min per 1.73 m^2	664/8,596	**1.08 (1.02-1.16)**	1.07 (1.00-1.14)
Excluding participants with prevalent and incident CHD and HF §			
eGFRcys, ml/min per 1.73 m^2	546/7,618	**1.08 (1.01-1.15)**	1.06 (0.99-1.13)
eGFRcreat, ml/min per 1.73 m^2	546/7,618	1.02 (0.95-1.09)	1.01 (0.94-1.01)
eGFRcreat-cys ml/min per 1.73 m^2	546/7,618	1.06 (0.99-1.96)	1.04 (0.96-1.12)
Correcting for time-varying effects of CHD and HF ¶			
eGFRcys, ml/min per 1.73 m^2	780/9,288	NA	**1.05 (1.00-1.10)**
eGFRcreat, ml/min per 1.73 m^2	780/9,288	NA	1.02 (0.97-1.07)
eGFRcreat-cys ml/min per 1.73 m^2	780/9,288	NA	1.04 (0.99-1.10)
Excluding first 2 years of follow-up			
eGFRcys, ml/min per 1.73 m^2	697/8,965	**1.13 (1.07-1.20)**	**1.10 (1.04-1.17)**
eGFRcreat, ml/min per 1.73 m^2	697/8,965	**1.07 (1.00-1.13)**	1.05 (0.99-1.12)
eGFRcreat-cys ml/min per 1.73 m^2	697/8,965	**1.12 (1.06-1.19)**	**1.09 (1.03-1.16)**
Excluding first 4 years of follow-up			
eGFRcys, ml/min per 1.73 m^2	597/8,554	**1.12 (1.05-1.19)**	**1.09 (1.02-1.16)**
eGFRcreat, ml/min per 1.73 m^2	597/8,554	**1.07 (1.01-1.15)**	**1.06 (1.00-1.14)**
eGFRcreat-cys, ml/min per 1.73 m^2	597/8,554	**1.12 (1.05-1.19)**	**1.09 (1.02-1.17)**

Abbreviations: AF, atrial fibrillation; CHD, coronary heart disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C; HF, heart failure; HR, hazard ratio; n, number; NA, not applicable.

* Adjusted for age, sex, and cohort.

† Adjusted for age, sex, cohort, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication.

‡ Cox proportional hazards models were used to investigate the associations between eGFR at baseline and the risk of incident AF. § The non-imputed data is used to exclude the participants with coronary heart disease and heart failure at baseline.

‖ Hazard ratios given per 10 ml/min per 1.73 m^2 decrease in eGFR with the risk of incident atrial fibrillation.

¶ The models are additionally adjusted for the time-varying effect of coronary heart disease and heart failure. The associations with a $p<0.05$ are highlighted in **bold**.

Table S3. Stratified analyses by age and sex for the association between eGFRcys, eGFRcreat, and eGFRcreat-cys at baseline and the risk of incident atrial fibrillation

Stratification variable	eGFR ‡	AF events / total n	HR (95% CI), Model 1 *	HR (95% CI), Model 2 †
Age	eGFRcys, ml/min per 1.73 m^2 ‖			
<65		225/5,226	**1.12 (1.02-1.23)**	1.08 (0.98-1.19)
≥65		555/4,062	**1.11 (1.04-1.18)**	**1.09 (1.02-1.16)**
	p for interaction		0.97	0.93
	eGFRcreat, ml/min per 1.73 m^2 ‖			
<65		225/5,226	1.06 (0.95-1.18)	1.06 (0.95-1.19)
≥65		555/4,062	1.04 (0.98-1.12)	1.03 (0.96-1.10)
	p for interaction		0.92	0.92
	eGFRcreat-cys, ml/min per 1.73 m^2 ‖			
<65		225/5,226	1.11 (1.00-1.24)	1.08 (0.97-1.21)
≥65		555/4,062	**1.10 (1.02-1.17)**	**1.07 (1.00-1.15)**
	p for interaction		0.82	0.69
Sex	eGFRcys, ml/min per 1.73 m^2 ‖			
Men		399/3,971	**1.12 (1.04-1.20)**	**1.10 (1.03-1.19)**
Women		381/5,317	**1.10 (1.02-1.19)**	1.06 (0.98-1.15)
	p for interaction		0.42	0.65
	eGFRcreat, ml/min per 1.73 m^2 ‖			
Men		399/3,971	1.05 (0.97-1.14)	1.05 (0.97-1.14)
Women		381/5,317	1.05 (0.97-1.14)	1.03 (0.95-1.12)
	p for interaction		0.46	0.58
	eGFRcreat-cys, ml/min per 1.73 m^2 ‖			
Men		399/3,971	**1.11 (1.03-1.20)**	**1.10 (1.01-1.19)**
Women		381/5,317	**1.09 (1.00-1.18)**	1.05 (0.97-1.14)
	p for interaction		0.38	0.36

Abbreviations: AF, atrial fibrillation; CI, confidence interval; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C; HR, hazard ratio; n, number.

* Adjusted for age, sex and cohort.

† Adjusted for age, sex, cohort, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication.

‡ Cox proportional hazards models were used to investigate the associations between eGFR at baseline and the risk of incident AF.

‖ Hazard ratios given per 10 ml/min per 1.73 m^2 decrease in eGFR with the risk of incident atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

Table S4. Association between the UACR at baseline and the risk of incident atrial fibrillation

UACR [§]	AF events / total n	HR (95% CI), Model 1 [*]	HR (95% CI), Model 2 [†]	HR (95% CI), Model 3 [‡]
Ln(UACR) (mg/g) [‖]	71/3,065	1.10 (0.88-1.38)	1.08 (0.86-1.37)	1.06 (0.83-1.34)
UACR, mg/g [‖]	71/3,065	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)

Abbreviations: AF, atrial fibrillation; BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; DM, diabetes mellitus; HF, heart failure; HR, hazard ratio; n, number; UACR, albumin-to-creatinine ratio.

[*] Adjusted for age, and sex.

[†] Adjusted for age, sex, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication.

[‡] Adjusted for age, sex, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, use of cardiac medication, hypertension, history of coronary heart disease, and history of heart failure.

[§] Cox proportional hazards models were used to investigate the association between UACR at baseline and the risk of incident atrial fibrillation.

[‖] Hazard ratios given per 1 unit increase in ln(UACR) with the risk of incident atrial fibrillation. The associations with a $p<0.05$ are highlighted in **bold**.

Table S5. Association between prevalent atrial fibrillation and eGFRcys, eGFRcreat, and eGFRcreat-cys at baseline, eGFRcreat with age, and incident reduced kidney function, excluding participants with incident atrial fibrillation during follow-up

	Total n	**Beta (95% CI), Model 1** [*]	**Beta (95% CI), Model 2** [†]
Outcome: eGFR at baseline (cross-sectional) [‡]			
eGFRcys			
No prevalent AF	8,508	Reference	Reference
Prevalent AF	409	**-5.53 (-6.96 to -4.09)**	**-4.09 (-5.54 to -2.65)**
eGFRcreat			
No prevalent AF	8,508	Reference	Reference
Prevalent AF	409	**-2.83 (-4.11 to -1.56)**	**-1.79 (-3.09 to -0.48)**
eGFRcreat-cys			
No prevalent AF	8,508	Reference	Reference
Prevalent AF	409	**-4.46 (-5.72 to -3.19)**	**-3.20 (-4.49 to -1.90)**
Outcome: eGFRcreat with age (longitudinal) [§]			
	Total n	**Beta (95% CI), Model 1** [*]	**Beta (95% CI), Model 2** [†]
No prevalent AF	8,508	Reference	Reference
Prevalent AF	409	**-2.51 (-3.89 to -1.15)**	**-1.53 (-2.95 to -0.11)**
Outcome: incident reduced kidney function (longitudinal) [‖] [¶]			
	Events / total n	**HR (95% CI), Model 1** [*]	**HR (95% CI), Model 2** [†]
No prevalent AF	2,169/7,765	Reference	Reference
Prevalent AF	157/306	**1.53 (1.30-1.81)**	**1.36 (1.14-1.62)**

Abbreviations: AF, atrial fibrillation; BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; eGFRcreat, eGFR based on serum creatinine; eGFRcreat-cys, eGFR based on serum creatinine and serum cystatin C; eGFRcys, eGFR based on serum cystatin C; HR, hazard ratio; n, number.

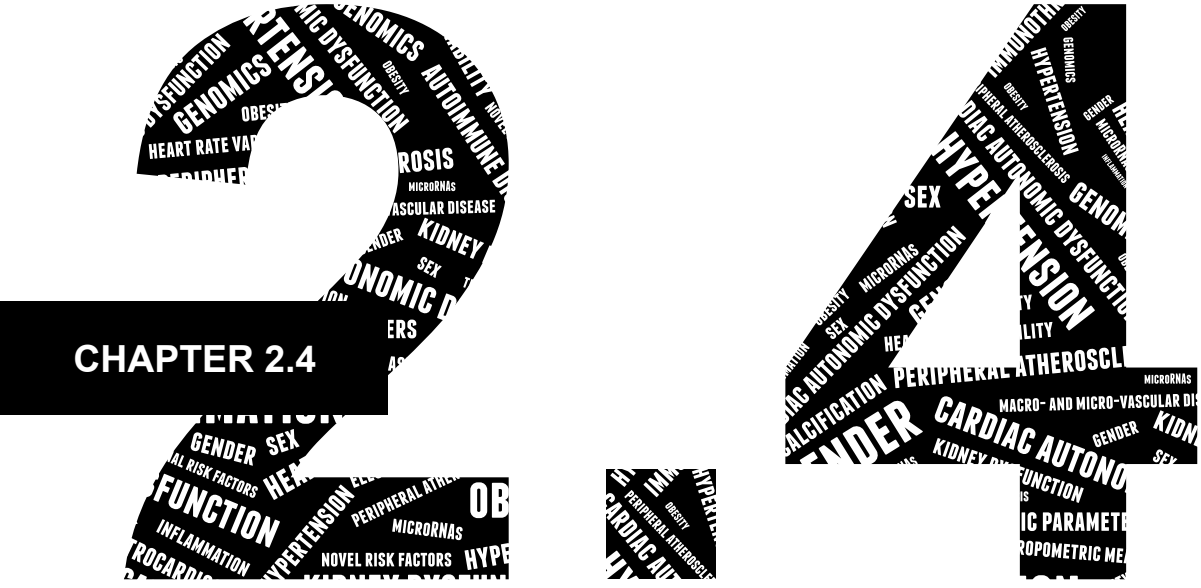
[*] Adjusted for age, sex, and cohort.

[†] Adjusted for age, sex, and cohort, educational level, body mass index, smoking status, alcohol, total cholesterol, history of diabetes mellitus, physical activity, and use of cardiac medication.

[‡] Linear regression models were used to investigate the associations between prevalent atrial fibrillation and eGFR at baseline and [§] linear mixed models were used to investigate the association between prevalent atrial fibrillation and eGFRcreat with age.

[‖] Cox proportional hazards models were used to investigate the associations between prevalent atrial fibrillation and incident reduced kidney function.

[¶] Participants with prevalent reduced kidney function were excluded from the analysis (n=969). The associations with a p<0.05 are highlighted in **bold**.


CHAPTER 2.4

Genetically predicted kidney function and the risk of atrial fibrillation

Disentangling the association between kidney function and atrial fibrillation: a bidirectional Mendelian randomization study.

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ABSTRACT

Background
The potential bidirectional causal association between kidney function and atrial fibrillation (AF) remains unclear.

Methods
We conducted a bidirectional two-sample Mendelian randomization (MR) analysis. From multiple genome-wide association studies (GWAS), we retrieved genetic variants associated with kidney function (estimated glomerular filtration rate based on creatinine (eGFRcreat), blood urea nitrogen (BUN), chronic kidney disease (CKD stage ≥G3): n=1,045,620, eGFR based on cystatin C: n=24,063-32,861, urine albumin-to-creatinine ratio (UACR), and microalbuminuria: n=564,257), and AF (n=1,030,836). The inverse variance weighted method was used as our main analysis.

Results
MR analyses supported a causal effect of CKD (n=9 SNPs, odds ratio (OR): 1.10, 95% confidence interval (CI): 1.04-1.17, $p=1.97x10^{-03}$), and microalbuminuria (n=5 SNPs, OR: 1.26, 95% CI: 1.10-1.46, $p=1.38x10^{-03}$) on AF risk. We also observed a causal effect of AF on eGFRcreat (n=97 SNPs, OR: 1.00, 95% CI: 1.00-1.00, $p=6.78x10^{-03}$), CKD (n=107 SNPs, OR: 1.06, 95% CI: 1.03-1.09, $p=2.97x10^{-04}$), microalbuminuria (n=83 SNPs, OR: 1.07, 95% CI: 1.04-1.09, $p=2.49 \times 10^{-08}$), and a suggestive causal effect on eGFRcys (n=103 SNPs, OR: 0.99, 95% CI: 0.99-1.00, $p=4.61x10^{-02}$). Sensitivity analyses, including weighted median estimator, MR-Egger, the MR pleiotropy residual sum and outlier test, and excluding genetic variants associated with possible confounders and/or horizontal mediators (myocardial infarction/coronary artery disease, heart failure) indicated that these findings were robust.

Conclusions
Our results supported a bidirectional causal association between kidney function and AF. The shared genetic architecture between kidney dysfunction and AF might represent potential important therapeutic targets to prevent both conditions in the general population.

INTRODUCTION

Chronic kidney disease (CKD) and atrial fibrillation (AF) are both common conditions which carry independent risks for cardiovascular morbidity and mortality.(1-7) The public health burden of both diseases is expected to rise as the incidence of CKD and AF increases due to aging of the population.(1-7) Additionally, the increasing incidence of obesity, diabetes mellitus, and hypertension may also contribute to the rise of CKD and AF incidence.(7, 8)

On the one hand, reduced kidney function may lead to AF through increased activity of the renin-angiotensin-aldosterone system (RAAS),(9-16) hypertension,(9, 16) left ventricular hypertrophy,(9) inflammation(9, 17-19) and by promoting cardiovascular diseases such as coronary heart disease, and heart failure.(5, 9, 20) On the other hand, AF may give rise to kidney dysfunction through activation of RAAS,(9) hypoperfusion,(9) thromboembolism,(9) inflammation,(9) and by inducing other cardiovascular diseases.(5, 9, 20) This complex interplay between the kidneys and the heart may result in a vicious cycle in which each condition promotes initiation and progression of the other condition.(5, 9, 20) Indeed, unidirectional and bidirectional associations between kidney function and AF have been described in several observational studies.(8, 21-28) However, observational studies are prone to residual confounding and reverse causality and therefore cannot support a causal association between the 2 conditions.(29)

Mendelian randomization (MR) has emerged as a reliable genetic research method to leverage genetic variation to overcome some of the limitations of observational studies and to estimate causal associations.(29, 30) The only previous MR study(28) that assessed the bidirectional causal association between kidney function and AF, described an unidirectional association, identifying AF as a causal risk factor for kidney function, but not vice versa. However, this study used an older trans-ethnic AF genome-wide association study (GWAS) with a smaller sample size and was based on fewer genetic instruments for AF for the MR analyses.(31) Moreover, this study was not comprehensive, as it only investigated the causal association between estimated glomerular filtration rate (eGFR) based on serum creatinine (eGFRcreat), CKD and AF.(28)

In this study, we performed a comprehensive two-sample MR analysis using summary level data from the largest to date GWAS on kidney function,(32-35) CKD,(32) and AF(36) to investigate the potential bidirectional causal role of kidney function on AF and vice versa. Assessments of kidney function and CKD included eGFR based on serum creatinine (eGFRcreat),(32) blood urea nitrogen (BUN),(32) CKD stage ≥G3,(32) eGFR based on serum cystatin C (eGFRcys),(34, 35) urine albumin-to-creatinine ratio (UACR),(33) and microalbuminuria.(33)

METHODS

Study design

This study complies with the declaration of Helsinki and has been conducted using publicly available summary statistics from multiple GWAS.(32-36) The summary statistics from 4 GWAS meta-analyses on kidney function(32-35) are available at URL: https://ckdgen.imbi.uni-freiburg.de/. The summary statistics from the GWAS meta-analysis on AF(36) are available at URL: http://csg.sph.umich.edu/willer/public/afib2018/. No original data were collected for this bidirectional MR study. Ethical approval and informed consent from each participant for each of the studies included in the current investigation can be found in the original publications.(32-36) The analysis of anonymous publicly available summary statistics did not require additional ethical approval, therefore the requirement for informed consent was waived.

Genome-wide association study meta-analysis for kidney function

The 4 GWAS meta-analyses that were used for this study were part of the CKDGen Consortium and have investigated different assessments of kidney function (eGFRcreat, BUN, eGFRcys, UACR, and microalbuminuria) and CKD (CKD stage ≥G3; in accordance with the Kidney Disease: Improving Global Outcomes (KDIGO) classification).(37) The study characteristics of the 4 GWAS meta-analyses are extensively discussed in the **Methods S1-3**.(32-35)

The kidney function genetic variants that were reported in the various GWAS involved genes that are expressed in renal tissues, such as the kidneys and urinary tract, and may thereby affect eGFR, kidney physiology or kidney morphology.(32-35) We implemented these genetic variants as instrumental variables for eGFRcreat, BUN, CKD, eGFRcys, UACR, and microalbuminuria as an exposure. Additionally, we also used the summary statistics of eGFRcreat, BUN, CKD, eGFRcys, UACR, and microalbuminuria as an outcome in our bidirectional MR analyses.

Genome-wide association study meta-analysis for atrial fibrillation

The study characteristics of the GWAS meta-analysis of AF are shown in **Methods S4**. The AF genetic variants implicated genes that are expressed within the heart and have been suggested to affect cardiac development, cardiac ion channels, cardiac calcium signaling, structural integrity of the heart, and skeletal muscles.(36) We also utilized these genetic variants as instrumental variables for AF as an exposure. In addition, the summary statistics of AF were also used as an outcome in our bidirectional MR analyses.

Statistical analyses

Mendelian randomization

Multiple bidirectional two-sample MR analyses were conducted to examine the causality between kidney function and AF. MR analyses has 3 assumptions that should be fulfilled to provide valid causal estimates. The first assumption is that the genetic variant is strongly associated with the exposure. The second assumption is that the genetic variant only affects the outcome through its effect on the exposure. Finally, the third assumption is that the genetic variant is not associated with any confounders of the exposure-outcome relationship. We selected genetic variants that were genome-wide significantly associated with the trait of interest. We next clumped the genetic variants to ensure that the instrumental variables for the exposure were independent ($p < 5.0 \times 10^{-08}$ for genome-wide significance and $r2 < 0.1$) to avoid the use of correlated genetic variants that are in linkage disequilibrium.(29, 30) In addition, palindromic genetic variants were removed during the harmonization of the genetic variants. Moreover, European ancestry genetic variants and summary statistics were selected in the subsequent MR analyses, if available, to avoid possible bias due to population stratification.(29, 30)

We calculated the F-statistic of each genetic instrument, as a strength measure for the genetic instruments, to limit weak instrument bias. We included genetic variants with sufficient strength and considered $F > 10$ as sufficient strength.(38) We used the "TwoSampleMR" package(38, 39) to combine the effects of the individual genetic variants on the exposure and outcome using the inverse variance weighted (IVW) method.(40) The IVW method was our main MR method and it includes a meta-analysis of all the Wald ratios from the individual genetic variants on the exposure and outcome. In other words, the IVW method represents a weighted mean estimate of the effect of genetically determined kidney function on AF risk and vice versa. In addition, we used the random effect IVW method to account for possible heterogeneity between genetic variants and to relax the assumption of no horizontal pleiotropy.

MR estimates are presented as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Statistical significance was considered at a two-sided $p<0.05$. All MR analyses and data management were done using R statistical software (R 4.0.2: R Foundation for Statistical Computing, Vienna, Austria).

Sensitivity analyses

The rationale, assumptions, and sensitivity analyses of the MR analyses are depicted in detail in **Methods S5** and **S6**.(41-46)

RESULTS

Mendelian randomization

Within the European study sample, a total of 256 genome-wide significant index genetic variants were associated with eGFRcreat of which 19 were also genome-wide significantly associated with CKD stage ≥G3. A total of 111 and 5 genome-wide significant genetic variants were associated with BUN and eGFRcys, respectively. A total of 59 genome-wide significant index genetic variants were associated with UACR of which 17 were genome-wide significantly associated with microalbuminuria. The genetic variants associated with BUN, UACR, and microalbuminuria were retrieved from a trans-ethnic study sample, because no summary statistics were available from an European study sample. The AF GWAS identified 111 genome-wide significant genetic variants that were associated with AF.

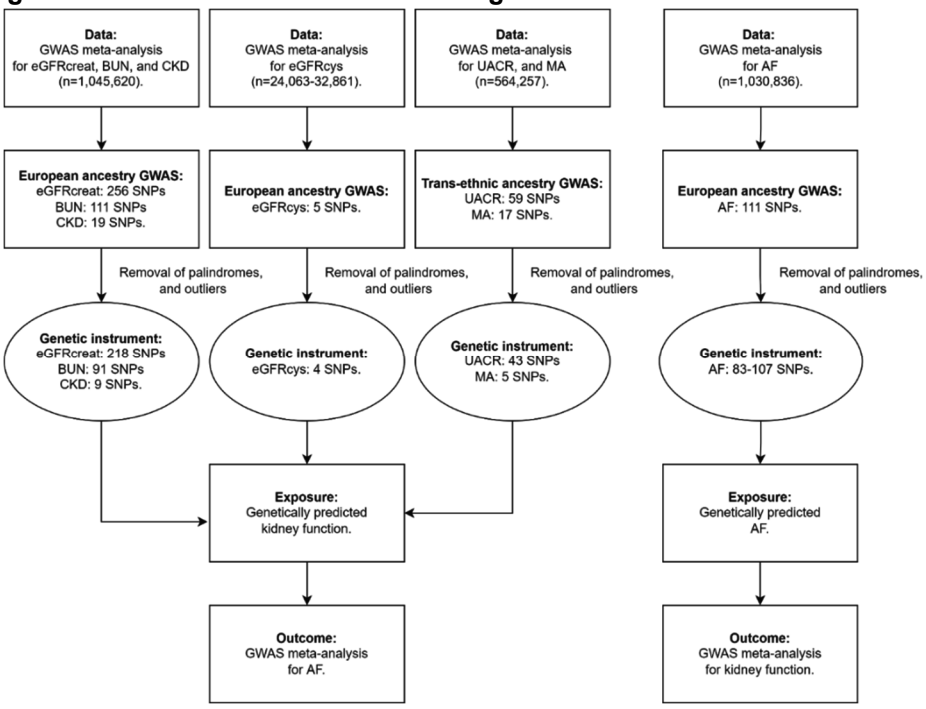
As aforementioned, we clumped all genetic variants and removed palindromes. In addition, we removed potential outliers by using the MR pleiotropy residual sum and outlier (MR-PRESSO) test and examined sensitivity plots to select our genetic variants. This provided a total of 218 genetic variants for eGFRcreat, 91 for BUN, 9 for CKD, 4 for eGFRcys, 43 for UACR, and 5 for microalbuminuria, which were available in the AF GWAS and were subsequently used as instrumental variables in the MR analyses. In addition, from the 111 genetic variants for AF, a total of 97 genetic variants for eGFRcreat, 99 for BUN, 107 for CKD, 103 for eGFRcys, 100 for UACR, and 83 for microalbuminuria were available in the kidney function GWAS and were subsequently used in the MR analyses (**Figure 1**).

All genetic instruments/instrumental variables had a F-statistic >10 and were, therefore considered of sufficient strength to be used in the MR analyses (range 23-2648).(38) Our MR analyses based on the IVW method supported a causal effect of CKD and microalbuminuria on AF risk (CKD: n=9 SNPs, OR: 1.10, per 1 unit increase in the odds for CKD, 95% CI: 1.04-1.17, $p=1.97x10^{-03}$; microalbuminuria: n=5 SNPs, OR: 1.26, per 1 unit increase in the odds for microalbuminuria, 95% CI: 1.10-1.46, $p=1.38x10^{-03}$) (**Table 1**). Moreover, we observed causal effects of AF on eGFRcreat (n=97 SNPs, OR: 1.00, per 1 unit increase in the odds for AF, 95% CI: 1.00-1.00, $p=6.78x10^{-03}$), CKD risk (n=107 SNPs, OR: 1.06, per 1 unit increase in the odds for AF, 95% CI: 1.03-1.09, $p=2.97x10^{-04}$), and microalbuminuria risk (n=83 SNPs, OR: 1.07, per 1 unit increase in the odds for AF, 95% CI: 1.04-1.09, $p=2.49x10^{-08}$) (**Table 1**, **Figures 2** and **3**). We found a suggestive causal effect of AF on eGFRcys (n=103 SNPs, OR: 0.99, per 1 unit increase in the odds for AF, 95% CI: 0.99-1.00, $p=4.61x10^{-02}$). MR analyses did not support a significant causal effect of the other kidney function assessments (eGFRcreat, BUN, eGFRcys, and UACR) on AF risk (**Table 1**, **Figures 2** and **3**). The effect estimates of the genetic variants associated with eGFRcreat, BUN, CKD, eGFRcys, UACR, microalbuminuria, and AF that were used in our bidirectional MR analyses are extensively presented in **Tables S1-S12**.

Sensitivity analyses

Our MR sensitivity analyses based on the WME and MR-Egger slope method were in general concordant with the results of the IVW method (**Table 1**, **Figures 2** and **3**). More specifically, the WME sensitivity analyses also supported a causal effect of CKD, and microalbuminuria on AF risk (CKD: n=9 SNPs, OR: 1.08, per 1 unit increase in the odds for CKD, 95% CI: 1.00-1.16, $p=4.43x10^{-02}$; microalbuminuria: 5 SNPs, OR: 1.23, per 1 unit increase in the odds for microalbuminuria, 95% CI: 1.03-1.47, $p=2.57x10^{-03}$). The point estimates of the MR-Egger slope method were also in general in line with the point estimates of the IVW method. This is reassuring, because valid MR estimates rely on sensitivity analyses that are concordant with its main analysis (i.e. the IVW method). In addition, the MR-Egger intercept and MR-PRESSO did not provide evidence for the presence of directional pleiotropy after clumping, removal of palindromes, and removal of potential outliers (**Table 1**, **Figures 2** and **3**). Similar results were observed when we excluded genetic variants that were also associated with potential confounders and/or horizontal mediators such as myocardial infarction/coronary artery disease(44) and heart failure(45) (data not shown). The exact extent of sample overlap could not be determined due to unavailability of individual level data. The potential overlap could be estimated based on the description of the individual studies included within the different GWAS. There was potential overlap between eGFRcreat and AF for 291,146 individuals and between CKD and AF for 296,258 individuals. Potential overlap was present between eGFRcys from Gorski et al.(35) and AF for 15,470 individuals, between eGFRcys from Li et al.(34) and AF for 16,335 individuals. Potential overlap was present between UACR and AF for 439,298 individuals, and between microalbuminuria and AF for 290,249 individuals (**Methods S1-4**).

Figure 1. Flow chart for the selection of genetic variants



Abbreviations: AF, atrial fibrillation; BUN, blood urea nitrogen; CKD, chronic kidney disease; creat, creatinine; cys, cystatin; eGFR, estimated glomerular filtration rate; GWAS, genome-wide association study; MA, microalbuminuria; n, number; SNP, single nucleotide polymorphism; UACR, urine albumin-to-creatinine ratio.

Table 1. Mendelian randomization analyses between kidney function and atrial fibrillation

			IVW			WME		MR-Egger slope		MR-Egger intercept
Exposure	**Outcome**	**n of SNPs**	**OR (95% CI) ***	**p**	**p †**	**OR (95% CI) ***	**p**	**OR (95% CI) ***	**p**	**p**
eGFRcreat	AF	218	0.88 (0.58-1.34)	$5.54x10^{-01}$	$4.17x10^{-12}$	0.70 (0.42-1.17)	$1.71x10^{-01}$	0.80 (0.28-2.34)	$6.86x10^{-01}$	$8.49x10^{-01}$
BUN	AF	91	1.22 (0.92-1.62)	$1.71x10^{-01}$	$4.16x10^{-05}$	1.29 (0.89-1.86)	$1.78x10^{-01}$	1.25 (0.62-2.53)	$5.30x10^{-01}$	$9.35x10^{-01}$
CKD	AF	9	**1.10 (1.04-1.17)**	**$1.97x10^{-03}$**	$9.62x10^{-01}$	**1.08 (1.00-1.16)**	**$4.43x10^{-02}$**	1.07 (0.84-1.37)	$6.04x10^{-01}$	$8.18x10^{-01}$
eGFRcys	AF	4	0.86 (0.69-1.08)	$1.99x10^{-01}$	$6.52x10^{-01}$	0.85 (0.67-1.08)	$1.88x10^{-01}$	0.88 (0.63-1.21)	$5.10x10^{-01}$	$9.15x10^{-01}$
UACR	AF	43	1.16 (0.97-1.40)	$1.07x10^{-01}$	$7.76x10^{-03}$	1.03 (0.83-1.27)	$8.02x10^{-01}$	1.06 (0.51-2.20)	$8.82x10^{-01}$	$7.94x10^{-01}$
MA	AF	5	**1.26 (1.10-1.46)**	**$1.38x10^{-03}$**	$4.72x10^{-01}$	**1.23 (1.03- 1.47)**	**$2.57x10^{-02}$**	0.94 (0.45-1.97)	$8.84x10^{-01}$	$4.85x10^{-01}$
AF	eGFRcreat	97	**1.00 (1.00-1.00)**	**$6.78x10^{-03}$**	$3.28x10^{-06}$	**1.00 (1.00-1.00)**	**$9.26x10^{-03}$**	1.00 (1.00-1.00)	$3.50x10^{-01}$	$5.98x10^{-01}$
AF	BUN	99	1.00 (1.00-1.00)	$7.06x10^{-01}$	$1.15x10^{-02}$	1.00 (1.00-1.01)	$4.97x10^{-01}$	1.00 (1.00-1.01)	$6.12x10^{-01}$	$4.05x10^{-01}$
AF	CKD	107	**1.06 (1.03-1.09)**	**$2.97x10^{-04}$**	$1.50x10^{-01}$	**1.07 (1.02-1.13)**	**$5.03x10^{-03}$**	**1.08 (1.02-1.15)**	**$1.14x10^{-02}$**	$4.13x10^{-01}$
AF	eGFRcys	103	**0.99 (0.99-1.00)**	**$4.61x10^{-02}$**	$6.94x10^{-01}$	0.99 (0.98-1.01)	$2.92x10^{-01}$	0.99 (0.98-1.01)	$2.99x10^{-01}$	$9.96x10^{-01}$
AF	UACR	100	1.00 (0.99-1.01)	$9.28x10^{-01}$	$6.36x10^{-08}$	1.00 (0.99-1.01)	$7.98x10^{-01}$	1.00 (0.98-1.01)	$5.47x10^{-01}$	$4.49x10^{-01}$
AF	MA	83	**1.07 (1.04-1.09)**	**$2.49x10^{-08}$**	$5.67x10^{-01}$	**1.04 (1.00-1.08)**	**$4.34x10^{-02}$**	1.04 (1.00-1.09)	$7.50x10^{-02}$	$1.92x10^{-01}$

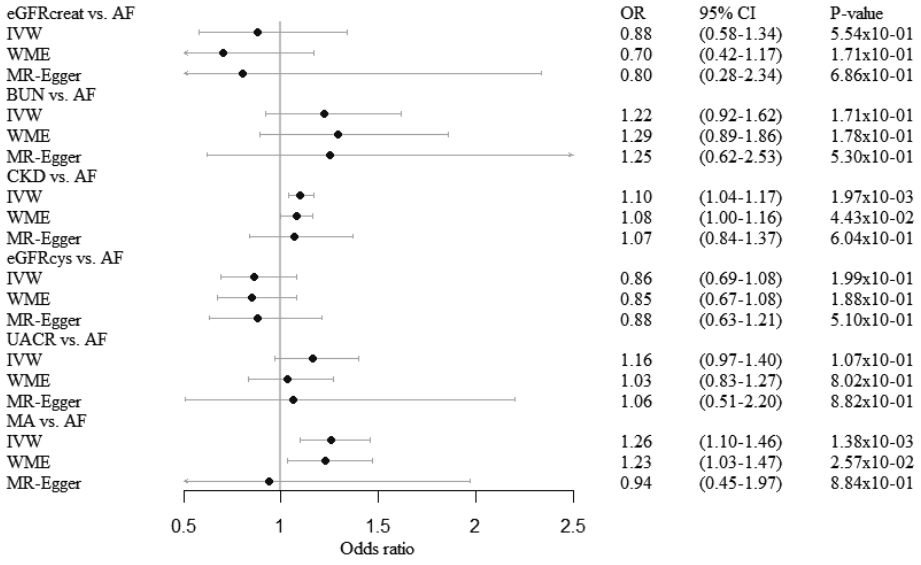
Abbreviations: AF, atrial fibrillation; BUN, blood urea nitrogen; CI, confidence interval; CKD, chronic kidney disease; creat, creatinine; cys, cystatin; eGFR, estimated glomerular filtration rate; IVW, inverse variance weighted; MA, microalbuminuria; n, number; OR, odds ratio; SNP, single nucleotide polymorphism; UACR, urine albumin-to-creatinine ratio; WME, weighted median estimator.

* Odds ratios represent a genetically determined 1 unit increase of ln(eGFRcreat), 1 unit increase of BUN, 1 unit increase in the odds of CKD, 1 unit increase of ln(eGFRcys), 1 unit increase of ln(UACR), and 1 unit increase in the odds of MA, respectively (kidney function as exposure) with the odds of atrial fibrillation (atrial fibrillation as outcome). Alternatively, the odds ratios represent a genetically determined 1 unit increase in the odds of atrial fibrillation (atrial fibrillation as exposure) with a lower ln(eGFRcreat), higher BUN, higher odds of CKD, lower ln(eGFRcys), higher ln(UACR), higher odds of MA, respectively (kidney function as outcome).

† p for heterogeneity.

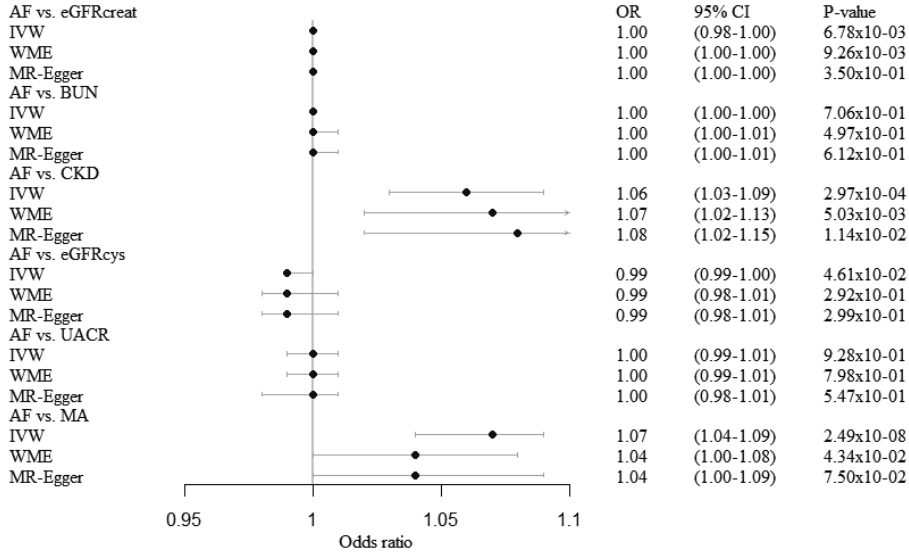
The associations with a $p<0.05$ are highlighted in **bold**.

Figure 2. Forest plot which visualizes the Mendelian randomization analyses between kidney function and atrial fibrillation



Abbreviations: AF, atrial fibrillation; BUN, blood urea nitrogen; CI, confidence interval; CKD, chronic kidney disease; creat, creatinine; cys, cystatin; eGFR, estimated glomerular filtration rate; IVW, inverse variance weighted; MA, microalbuminuria; n, number; OR, odds ratio; SNP, single nucleotide polymorphism; UACR, urine albumin-to-creatinine ratio; WME, weighted median estimator.

Figure 3. Forest plot which visualizes the Mendelian randomization analyses between atrial fibrillation and kidney function



Abbreviations: AF, atrial fibrillation; BUN, blood urea nitrogen; CI, confidence interval; CKD, chronic kidney disease; creat, creatinine; cys, cystatin; eGFR, estimated glomerular filtration rate; IVW, inverse variance weighted; MA, microalbuminuria; n, number; OR, odds ratio; SNP, single nucleotide polymorphism; UACR, urine albumin-to-creatinine ratio; WME, weighted median estimator.

DISCUSSION

Our study sheds light on the complex bidirectional interplay between kidney function and AF by leveraging genetic variants to infer causality. In this comprehensive bidirectional MR analysis, we found evidence to support the bidirectional causal relationship between kidney function and AF. Specifically, our MR analysis supports a causal effect of CKD stage ≥G3 and microalbuminuria on AF risk. Moreover, we found causal effects of AF on various kidney function assessments and CKD including eGFRcreat, CKD stage ≥G3 and microalbuminuria, and a suggestive causal effect of AF on eGFRcys. Our study therefore extends our knowledge about the unidirectional and bidirectional association that has been suggested by previous observational studies.(8, 21-27) Our results confirm that CKD, in particular its more severe forms such as CKD stage ≥G3 and increased urinary albumin excretion defined as microalbuminuria, are independent risk factors for AF and vice versa. The shared genetic architecture between kidney dysfunction and AF could be used to identify therapeutic targets to prevent both diseases, as well as their complications, in the general population.

Several mechanisms potentially underlie the bidirectional causal relationship between kidney function and AF. One mechanism could be the presence of shared cardiovascular risk factors such as obesity, hypertension, myocardial infarction, heart failure, and diabetes mellitus which all have been suggested as risk factors for CKD and AF.(8) Another possible link between kidney function and AF is sodium retention.(5, 20) Kidney dysfunction increases the risk of sodium retention which may lead to extracellular volume expansion, hypertension, left ventricular hypertrophy, and cardiac dilation.(5, 20) This cardiac remodeling that occurs due to left ventricular hypertrophy and cardiac dilation may then increase the myocardial oxygen demand and potentially create myocardial ischemia.(5, 20) In addition, cardiac dilation may also cause mitral insufficiency which may further impair left and right ventricular function.(5, 8) These aforementioned mechanisms thereby increase the risk of AF.(5, 20) AF may cause thromboembolism which could lead to renal infarction and a decline in cardiac function. Both thromboembolism and a decline in cardiac function may then have deleterious effects on the kidneys.(8) Further, it has been suggested that AF induces angiotensin II type 1 receptor-mediated oxidative stress and impairs microvascular blood flow of the ventricles.(47) This mechanism was also extended to the renal microvasculature as AF also affects renal microvascular blood flow, down-regulates renal neutral endopeptidase expression, induces renal profibrotic structural changes, and ultimately may impact renal function over time.(48) In addition, the application of aldosterone, atrial natriuretic peptide, asymmetric dimethylarginine, and angiotensin peptides during AF did not prevent the down-regulation of renal neutral endopeptidase expression. This could imply that the irregularly irregular rhythm caused by AF is the direct effect that induces

structural renal changes rather than indirect humoral changes that may be induced during this process.(48, 49) Other possible links that could link kidney dysfunction to AF is inflammation.(16-19, 50) Kidney dysfunction and AF are both associated with a pro-inflammatory state through increased levels of pro-inflammatory cytokines, which further cause a decline in kidney and cardiac function, respectively and thereby may increase the risk of one another.(16-19, 50) The increased activity of the RAAS is also among the suggested mechanisms. On the one hand, activation of RAAS caused by CKD could lead to atrial remodeling through hypertension, increased atrial pressure, atrial enlargement, cardiac fibrosis, and by modulation of cardiac ion channels. On the other hand, activation of the RAAS caused by AF could also have a detrimental effect on the kidneys.(9-15)

We did not find evidence for a causal role of eGFRcreat and eGFRcys on AF, although the effect estimates of both assessments were in line with each other. However, we did find a causal effect of CKD stage ≥G3 and microalbuminuria on AF risk. This could be due to the fact that the presence of CKD stage ≥G3 and microalbuminuria represent a greater level of impaired kidney function and therefore may be more strongly associated with AF risk. A possible explanation for these discrepancies could be that indeed only more pathological levels of eGFR (CKD stage ≥G3, defined as eGFRcreat <60 ml/min per 1.73m2) and UACR (microalbuminuria is defined as UACR >30 mg/g) may lead to AF and vice versa. This hypothesis is supported by previous studies that reported a J-shaped or graded relationship between impaired kidney function and incident AF.(9, 21, 26) Participants with CKD stage ≥G3 were shown to have a significant graded increasing risk of incident AF while such a significant increased risk for AF was not observed in participants with CKD stage G2 (eGFRcreat levels of 60-89 ml/min per 1.73m2) or with CKD stage G1 (>90 ml/min per 1.73m2).(9, 21, 26) Similarly, the presence of microalbuminuria (30-299 mg/g) or macroalbuminuria (≥300 mg/g) also showed a significant graded association with increased incident AF risk with increasing levels of albuminuria.(9, 21, 26, 27) These findings indeed suggest that there is a pathological kidney function threshold that has to be surpassed. Subsequently, surpassing this threshold would then trigger the pathological cascades that are set in motion by reduced kidney function such as activation of the RAAS, hypertension, ischemia, heart failure, and inflammation which may then lead to incident AF. Moreover, we found that both CKD and microalbuminuria were significantly associated with AF and vice versa, which further supports the idea that both markers represent independent risk factors for AF and vice versa.(27) Reason for this might be that albuminuria is a reflection of microvascular damage, endothelial dysfunction, or cardiometabolic syndrome whereas CKD may be a better representation of intrinsically impaired kidney function.(27)

The causal effect estimates that we obtained from the MR analysis were different than the effect estimates that were obtained from previous observational epidemiological studies.(8, 21-27) This could be due to the differences in the time window between MR studies and traditional observational studies. MR studies calculate a risk estimate from a lifetime exposure to a certain risk factor where traditional observational studies estimate a risk estimate of an exposure with a certain follow-up time, for example a 10-years risk. Another possible explanation could be the unmeasured confounding and reverse causation that could still be present in traditional observational studies. MR analysis avoids these biases by using genetic proxies of risk factors that are not prone to these biases, because of the random distribution of genetic variants at conception. In addition, MR analysis is a helpful and insightful research method to assess causality of associations which are not possible or feasible to be investigated with randomized clinical trials due to limitations such as being unethical, unpractical and/or too expensive. Furthermore, our results also differ from the results of a previous bidirectional MR.(28) Park et al.(28) found that AF is a causal risk factor for kidney function impairment, however a causal effect of kidney function on AF was not observed. Specifically, there are several noteworthy differences between the study of Park et al.(28) and our study. First, Park et al.(28) used fewer assessments of kidney function (eGFRcreat and CKD) while we used a more comprehensive kidney function panel (eGFRcreat, BUN, CKD, eGFRcys, UACR, and microalbuminuria) to assess the bidirectional association. Second, in contrast to Park et al.,(28) we used the most recent available GWAS for AF. The GWAS for AF that was used by Park et al.(28) was trans-ethnic, had a smaller sample size (n=588,190), and had fewer genetic variants (n=94 SNPs) that could be used for the MR analyses. Park et al.(28) focused primarily on trans-ethnic ancestry while we focused on European ancestry, when available.

Unravelling the bidirectional casual association between kidney function and AF could have some clinical implications. As kidney dysfunction and AF are causal risk factors for one another, appropriate management of kidney dysfunction may lead to a reduced risk of AF and the other way around. On the one hand, appropriate management of kidney dysfunction includes managing CKD-related risk factors such as obesity, dyslipidemia, hypertension, and lifestyle advice.(37) On the other hand, management of AF is based on the ABC pathway as suggested by the ESC guidelines which consists of: (A) avoid stroke (anticoagulation), (B) better symptom management with patient-centered, symptom directed decisions on rate or rhythm control, and (C) cardiovascular and comorbidity risk optimization.(4) Future randomized clinical trials could support our findings by evaluating kidney dysfunction outcomes when performing AF-targeted interventions and evaluating AF outcomes when performing interventions that target kidney dysfunction. Moreover, early screening for kidney dysfunction and monitoring of kidney function in AF patients or early screening for AF and monitoring of AF in patients with kidney dysfunction is warranted.

Major strengths of this study include the use of summary statistics from the largest to date GWAS meta-analyses. With these large study samples we were able to extract a substantial amount of genetic instruments that we could use for the subsequent MR analyses. By using a bidirectional MR approach, we were also able to disentangle the complex interplay between the kidneys and the heart. In addition, by using MR, we were more likely to avoid certain biases that are more common in traditional observational epidemiological studies such as residual confounding and reverse causation. However, our study also has some limitations. First, we cannot rule out unobserved horizontal pleiotropy, although we tried to address horizontal pleiotropy through current best practices for MR sensitivity analyses. We used the WME, MR-Egger, MR-PRESSO, and sensitivity plots to identify and correct for horizontal pleiotropy. Additionally, we excluded genetic variants that were associated with potential confounders and/or horizontal mediators such as myocardial infarction/coronary artery disease, and heart failure. Second, there was a potential partial overlap in the samples that were used to obtain the genetic instruments which may cause bias towards the observational findings.(43) However, this bias is difficult to avoid with the ongoing collaborations between large scale genetic consortia which combine their study samples in an attempt to increase their sample sizes. Additionally, to what extent this might have led to weak instrument bias is uncertain, although considerable weak instrument bias may be of less concern given the aforementioned range of the F-statistic of the included genetic instruments used in our analyses.(43) Third, we were unable to perform MR analyses for BUN, UACR, and microalbuminuria with European summary statistics due to unavailability of these statistics, which may have caused some stratification bias in those analyses, nonetheless the GWAS of Wuttke et al.(32) (BUN) and Teumer et al.(33) (UACR, and microalbuminuria) both included mainly European participants (74% and 97%, respectively). Fourth, mostly single assessments of eGFRcreat, BUN, CKD, eGFRcys, UACR, microalbuminuria and AF were used in the various GWAS from which we derived the genetic variants. This may have caused misclassification bias to some extent, although this would have probably led to an underestimation of the true association. Fifth, our results may not be generalizable to younger populations and other ethnicities, because our analysis included older participants mostly from European descent. Lastly, it is also worth noting that the limited amount of genetic variants that we were able to use for some of the analyses to evaluate the associations could have led to insufficient power to detect some significant associations. Future GWAS with even larger sample sizes could aid in identification of additional genetic variants to further increase the power of future MR studies.

In summary, our study confirms a bidirectional causal relationship between kidney function and AF that has been suggested by previous observational studies. The shared genetic architecture between kidney dysfunction and AF might represent important therapeutic targets to prevent both diseases, as well as their complications, in the general population.

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SUPPLEMENTARY MATERIAL

Chapter 2.4 Genetically predicted kidney function and the risk of atrial fibrillation

Table S6. Effect estimates for the associations of the genetic variants with microalbuminuria and atrial fibrillation

Table S7. Effect estimates for the associations of the genetic variants with atrial fibrillation and estimated glomerular filtration rate based on creatinine

Table S8. Effect estimates for the associations of the genetic variants with atrial fibrillation and blood urea nitrogen

Table S9. Effect estimates for the associations of the genetic variants with atrial fibrillation and chronic kidney disease

Table S10. Effect estimates for the associations of the genetic variants with atrial fibrillation and estimated glomerular filtration rate based on cystatin C

Table S11. Effect estimates for the associations of the genetic variants with atrial fibrillation and urine albumin-to-creatinine ratio

Table S12. Effect estimates for the associations of the genetic variants with atrial fibrillation and microalbuminuria

Methods S1. Study population of the genome-wide association study from which the genetic instruments for estimated glomerular filtration rate based on creatinine, blood urea nitrogen, and chronic kidney disease were obtained

The first GWAS meta-analysis that we used, investigated single continuous assessments of eGFRcreat (log transformed eGFRcreat in ml/min per 1.73 m^2), BUN (in mg/dL), and a single binary assessment of kidney disease CKD stage ≥G3 (based on a single assessment of eGFRcreat <60 ml/min per 1.73 m^2). This trans-ethnic GWAS by Wuttke et al.(33) included 121 studies and encompassed a total of n=765,348 participants of European (n=567,460, 74.1%), East Asian (n=165,726, 21.7%), African-American (n=13,842, 1.8%), South Asian (n=13,359, 1.8%), and Hispanic (n=4,961, 0.6%) descent. The median age of the population was 54.0 years and 50.0% were women. In addition, the median of the study-specific mean of eGFRcreat was 88.9 ml/min per 1.73 m^2, the median of the study-specific mean of BUN was 14.6 mg/dL, and the prevalence of CKD stage ≥G3 was 8.1%. The GWAS identified 256 index genetic variants that were genome-wide significantly associated with eGFRcreat within the European ancestry study sample. From the 256 index genetic variants 19 were also genome-wide significantly associated with CKD stage ≥G3. A total of 111 genome-wide significant genetic variants were associated with BUN and were derived from the trans-ethnic study sample, since no summary statistics were available from an European study sample.

N of studies	N of participants	Ethnicity	Phenotype definition
121	eGFRcreat: discovery analysis in 765,348 individuals. BUN: discovery analysis in 416,178 individuals. CKD: discovery analysis in 625,219 individuals.	13,842 African-American, 567,460 European, 165,726 East Asian, 13,359 South Asian, and 4,961 Hispanic ancestry.	eGFRcreat was winsorized at 15 and 200 ml/min per 1.73m^2 and eGFRcreat was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)(50) equation based on serum creatinine values. eGFRcreat was log transformed. BUN was derived as blood urea multiplied by 2.8, with units expressed as mg dL/L. CKD was defined as eGFR <60 mL/min per 1.73m^2.
Abbreviations of the included studies *			
AA-DHS, ADVANCE-5. ADVANCE-6, ADVANCE-UKB, AFTER EU, **AGES**, Airwave, Amish, **ARIC AA, ARIC EA**, ASPS, ASPS-Fam, BES 610, BES omniexpress, **Biobank Japan**, **BioMe-Omni AA, BioMe Omni-EA**, **BioMe-Omni HA**, **BioVU/Vanderbilt 660**, **BioVU/Vanderbilt AA1M**, **BioVU/Vanderbilt Omni1**, **BioVU/Vanderbilt Omni5**, CHNS, CHRIS, **CHS AA**, **CHS EA**, Cilento, CoLaus, CROATIA-Korcula, CROATIA-Split, CROATIA-Vis, Czech post-MONICA, DC 1M, DC 610, **deCODE**, DIACORE, EGCUT 370, EGCUT Exome, EGCUT OMNI, ERF, ESTHER, FamHS, **FHS**, **FINCAVAS normal**, **FINCAVAS HCE**, Finrisk, GCKD, Generation R, **GS:SFHS**, GSK cases, GSK controls, HANDLS, HYPERGENES cases, HYPERGENES controls, INGI-CARL, INGI-FVG, INGI-VBI, INTERVAL, JHS, JUPITER, **KORA F3**, **KORA F4**, LIFE-Adult, LIFE-Child, LIFE-Heart, Lifelines, Living-Biobank-Chinese, Living-Biobank-Malay, LLFS, LOLIPOP EW610, LOLIPOP EWA, LOLIPOP EWP, LOLIPOP IA317, LOLIPOP IA610, LOLIPOP IAP, LOLIPOP OmniEE, **LURIC**, **MDC-CC/MDCS**, **MESA AFR**, **MESA EAS**, **MESA EUR**, **MESA HIS**, METSIM, MICROS, MyCode (Geisinger Research), NEO, NESDA, OGP, ORCADES, **PIVUS**, POPGEN, **PREVEND**, QIMR adolescent, QIMR adult, **RS I**, **RS II**, **RS III**, SCES 610, SCES omniexpress, SCHS - CHD cases, SCHS - CHD controls, **SHIP 0**, **SHIP 1**, SHIP-Trend, SiMES, SINDI, SKIPOGH, SOLID-TIMI 52 EA, SOLID-TIMI 52 EAS, SOLID-TIMI 52 SA, Sorbs, SP2 1M, SP2 550, SP2 610, STABILITY EA, STABILITY EAS, STABILITY SA, TRAILS, **TwinGene**, **ULSAM**, VIKING, **WGHS**, YFS		

The data with the potential overlapping samples with GWAS for atrial fibrillation are marked in **bold**. The exact extent of sample overlap could not be determined due to unavailability of individual level data, but based on the individual studies included within the different GWAS. There was potential overlap between eGFRcreat and AF for 291,146 individuals, between CKD and AF for 296,258 individuals. The current list includes some possible trans-ethnic studies, so these numbers might be a slight overestimation, since the studies that were used for the European summary statistics were not separately listed for the GWAS of Wuttke et al. The number of individuals included from the individual studies for BUN was not reported in the GWAS, so the potential overlap could therefore not be determined.

* For further details, please see Supplementary Table 1 of the GWAS from Wuttke et al.(33)

Methods S2. Study population of the genome-wide association study from which the genetic instruments for estimated glomerular filtration rate based on cystatin C were obtained

The second and third GWAS meta-analyses examined the continuous assessment of eGFRcys (log transformed eGFRcys in ml/min per 1.73 m^2). The 2 European GWAS by Gorski et al.(36) and Li et al.(35) included 11 and 10 studies, and included a total of n=24,063, and n=32,861 participants, respectively. The baseline characteristics in the study from Gorski et al.(36) were as follows: the median of the study-specific mean age was 56.2 years, 52.0% were women, and the median of the study-specific mean eGFRcys was 86.3 ml/min per 1.73 m^2. For Li et al.(35) the baseline characteristics were as follows: the median of the study-specific mean age was 54.1 years, 53.7% were women, and the median of the study-specific mean eGFRcys was 94.9 ml/min per 1.73 m^2. Both GWAS identified 5 genetic variants that were genome-wide significantly associated with eGFRcys.

N of studies	N of participants	Ethnicity	Phenotype definition
Gorski: 11 Li: 10	Gorski: eGFRcys: discovery analysis in 24,063 individuals. Li: eGFRcys: discovery analysis in 32,861 individuals.	Gorski: European. Li: European.	Gorski: eGFRcys was winsorized at 15 and 200 ml/min per 1.73m^2 and eGFRcys was calculated as 76.7 multiplied by (serum cystatin C). eGFRcys was log transformed. Li: eGFRcys was winsorized at 15 and 200 ml/min per 1.73m^2 and eGFRcys was calculated as 76.7 multiplied by (serum cystatin C). eGFRcys was log transformed.
Abbreviations of the included studies * †			
Gorski: ARIC EA, EGCUT1, FamHS, FHS, GENDIAN, HABC, KORA-F3, KORA-F4, MESA, SHIP, SHIP-Trend. Li: Amish, ARIC EA, CHS EA, EGCUT, FamHS, FHS, HRS, KORA-F4, SHIP, SHIP-Trend.			

The data with the potential overlapping samples with GWAS for atrial fibrillation are underlined. The exact extent of sample overlap could not be determined due to unavailability of individual level data, but based on the individual studies included within the different GWAS. There was potential overlap between eGFRcys from Gorski and AF for a total of 15,470 individuals, and between eGFRcys from Li and AF for 16,335 individuals.

* For further details, please see Supplementary Table 1 of the GWAS from Gorski et al.(36)

† For further details, please see Supplementary Table 1 of the GWAS from and Li et al.(35)

Methods S3. Study population of the genome-wide association study from which the genetic instruments for urine albumin-to-creatinine ratio and microalbuminuria were obtained

The fourth GWAS meta-analysis evaluated the continuous assessment of UACR (log transformed UACR in mg/g) and a binary assessment of microalbuminuria (UACR >30mg/g). This trans-ethnic GWAS by Teumer et al.(34) included 54 studies and a total of n=564,257 participants of European (n=547,361, 97%), African-American (n=6,795, 1.2%), East Asian (n=6,324, 1.1%), South Asian (n=2,335, 0.4%), and Hispanic (n=1,442, 0.3%) ancestry. The median of the study-specific mean age was 58.3 years, 51.9% were women. The median of UACR was 7.5mg/g, and the prevalence of microalbuminuria was 15.0%.(34) A total of 59 index genetic variants were genome-wide significantly associated with UACR, of which 17 were also genome-wide significantly associated with microalbuminuria. Furthermore, the genetic variants from UACR and microalbuminuria were retrieved from a trans-ethnic study sample, since no summary statistics were available from an European study sample.

N of studies	N of participants	Ethnicity	Phenotype definition
54	UACR: discovery analysis in 564,257 individuals. Microalbuminuria: discovery analysis in 347,283 individuals.	6,795 African-American, 547,361 European, 6,324 East Asian, 2,335 South Asian, and 1,442 Hispanic ancestry.	Urinary albumin values below the detection limit of the used assays were set to the lower limit of detection, and the UACR was assessed in mg/g and calculated as urinary albumin (mg/l)/urinary creatinine (mg/dl) multiplied by 100. UACR was log transformed. Microalbuminuria cases were defined as UACR >30, and controls as UACR <10 mg/g.
Abbreviations of the included studies *			
AA-DHS, ADVANCE-5, ADVANCE-6, ADVANCE-UKB, *AGES*, Amish, *ARIC AA*, *ARIC EA*, *BioVU/Vanderbilt 660*, *BioVU/Vanderbilt AA1M, BioVU/Vanderbilt Omni1, BioVU/Vanderbilt Omni5,* CHRIS, *CHS AA*, *CHS EA*, *deCODE*, DIACORE, ESTHER, *FHS*, *FINCAVAS*, GCKD, Generation R, JHS 24-hour urine measurement, JHS Spot urine measurement, *KORA-F3, KORA-F4,* LIFE-Adult, LIFE-Child, Lifelines, Living-Biobank-CHS, *MESA AA*, *MESA EA*, *MESA EAS*, *MESA HIS*, MICROS, MyCode (Geisinger Research), NEO, *PIVUS*, POPGEN, *PREVEND*, *RS III*, SCES 610, SCES omniexpress, *SHIP*, SHIP-Trend, SiMES, SINDI, SKIPOGH, Sorbs, SP2-1M, SP2-550, SP2-610, *UK Biobank*, *ULSAM*.			

The data with the potential overlapping samples with GWAS for atrial fibrillation are in *italics*. The exact extent of sample overlap could not be determined due to unavailability of individual level data, but based on the individual studies included within the different GWAS. There was potential overlap between UACR and AF for a total of 439,298 individuals, and between MA and AF for 290,249 individuals.

* For further details, please see Supplementary Table 1 of the GWAS from Teumer et al.(34)

Methods S4. Study population of the genome-wide association study from which the genetic instruments for atrial fibrillation were obtained

The GWAS meta-analysis of AF investigated the binary assessment of AF, and AF cases were defined as individuals with ICD-9: 427.31 or ICD-10: I48. This GWAS by Nielsen et al.[37] included 40 studies and encompassed n=1,030,836 participants from mainly European descent (n=1,029,399, 99.9%). The median age was not provided, 53% were women, and the prevalence of AF was 6%.(37) A total of 111 genetic variants were genome-wide significantly associated with AF. The AF genetic variants implicated genes that are expressed within the heart and have been suggested to affect cardiac development, cardiac ion channels, cardiac calcium signaling, structural integrity of the heart, and skeletal muscles.(37) We also utilized these genetic variants as instrumental variables for AF as an exposure. In addition, the summary statistics of AF were also used as an outcome in our bidirectional MR.

N of studies	N of participants	Ethnicity	Phenotype definition
40	Discovery analysis in 1,030,836 individuals. AF: 60,620 cases, and 970,216 controls.	Mainly European.	AF cases were defined as patients with ICD-9: 427.31 or ICD-10: I48.
Abbreviations of the included studies * †			
AGES, ANGES, ***ARIC AA***, ***ARIC EA***, Beat-AF, **Biobank Japan**, **BioMe-Omni AA**, **BioMe Omni-EA**, **BioMe-Omni HA**, ***BioVU***, CCAF, ***CHS AA***, ***CHS EA***, COROGENE, ***deCODE***, DiscovEHR, ***FHS***, ***FINCAVAS***, **GS:SFHS**, HUNT, ***KORA***, **LURIC**, **MDC-CC/MDCS**, ***MESA***, MGH AF study, MGH CAMP, MGI, ***PIVUS***, ***PREVEND***, PROSPER, **RS I**, **RS II**, ***RS III***, ***SHIP***, SPHFC, **TwinGene**, *UK Biobank*, ***ULSAM***, **WGHS**, WTCCC2 Munich.			

The data with the potential overlapping samples with GWAS for kidney function eGFRcreat, BUN, and CKD are marked in **bold**. The data with the potential overlapping samples with GWAS for kidney function eGFRcys are underlined. The data with the potential overlapping samples with GWAS for kidney function UACR, and MA are in *italics*. The exact extent of sample overlap could not be determined due to unavailability of individual level data, but based on the individual studies included within the different GWAS. There was potential overlap between eGFRcreat and AF for 291,146 individuals, between CKD and AF for 296,258 individuals, between eGFRcys from Gorski and AF for 15,470 individuals and between eGFRcys from Li and AF for 16,335 individuals, between UACR and AF for 439,298 individuals, and between MA and AF for 290,249 individuals. The current list includes some possible trans-ethnic studies, so these numbers might be a slight overestimation, since the studies that were used for the European summary statistics were not separately listed for the GWAS of Wuttke et al. The number of individuals included from the individual studies for BUN was not reported in the GWAS, so the potential overlap could therefore not be determined.

* For further details, please see Supplementary Table 1 of the original GWAS from Nielsen et al.(37)

† For further details, please see Supplementary Table 1 of the original GWAS from Christophersen et al.(38)

Methods S5. Mendelian randomization sensitivity analyses

We performed multiple sensitivity analyses. First, the MR estimates could be biased if genetic variants have horizontal pleiotropic effects that affect the outcome via other pathways than through the exposure. Therefore, in an attempt to satisfy the second and third MR assumption, additional analyses were performed including weighted median estimator (WME), MR-Egger and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) to account and formally test for potential pleiotropy.(43, 44) More specifically, the weighted median estimator (WME) analysis gives a weighted median effect of genetically determined kidney function on AF risk and vice versa,(45) and assumes that only half of the genetic variants needs to be valid genetic instruments (in other words, no violation of the 3 MR assumptions for half of the genetic variants). Furthermore, if pleiotropy is absent, the MR-Egger intercept will not significantly deviate from zero and the MR-Egger slope will be in line with the IVW and WME. In short, similar effect estimates from the IVW, WME, MR-Egger slope indicate that the MR results are robust.(45) Moreover, we used MR-PRESSO and examined the sensitivity plots such as the scatter plots to identify and remove horizontal pleiotropic outliers to provide an outlier-corrected estimate.(44) Cochran's Q test was used to test for heterogeneity between genetic variants. Third, we excluded genetic variants that were also associated with potential confounders and/or horizontal mediators of the exposure-outcome association (myocardial infarction/coronary artery disease,(46) and heart failure(47)), since this may bias our estimates. Finally, we investigated the potential overlap between the study samples that were used to identify the genetic variants, because sample overlap in two-sample MR analyses might potentially cause bias towards the observational findings.(48)

Table S1. Effect estimates for the associations of the genetic variants with estimated glomerular filtration rate based on creatinine and atrial fibrillation

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs6667182	1	15914545	eGFRcreat	T	C	0.3200	-0.0043	0.0004	AF	0.3181	-0.0091	0.0072
rs11260709	1	16557691	eGFRcreat	T	C	0.6800	0.0024	0.0004	AF	0.6745	-0.0042	0.0071
rs11261022	1	18807953	eGFRcreat	A	C	0.3600	-0.0027	0.0004	AF	0.3580	-0.0019	0.0069
rs9887775	1	23702531	eGFRcreat	A	G	0.8100	-0.0035	0.0004	AF	0.8116	0.0153	0.0084
rs78614739	1	27174180	eGFRcreat	T	C	0.1700	0.0026	0.0005	AF	0.1699	-0.0038	0.0091
rs499600	1	46039077	eGFRcreat	T	G	0.1500	-0.0037	0.0005	AF	0.1764	-0.0031	0.0091
rs688540	1	48002447	eGFRcreat	A	G	0.8700	-0.0031	0.0006	AF	0.8542	0.0103	0.0105
rs17413465	1	55718708	eGFRcreat	A	C	0.1800	0.0025	0.0004	AF	0.1893	0.0011	0.0086
rs2792796	1	56715908	eGFRcreat	T	C	0.6100	-0.0021	0.0004	AF	0.6057	-0.0120	0.0068
rs1887252	1	82957871	eGFRcreat	C	G	0.6400	-0.0029	0.0004	AF	0.6205	-0.0142	0.0069
rs7543734	1	94050911	eGFRcreat	C	G	0.2000	0.0031	0.0005	AF	0.2108	-0.0045	0.0082
rs11166440	1	100808363	eGFRcreat	A	G	0.6300	0.0021	0.0004	AF	0.6272	-0.0080	0.0069
rs407102	1	109846278	eGFRcreat	T	C	0.7000	0.0031	0.0004	AF	0.6920	-0.0009	0.0073
rs12736457	1	113258293	eGFRcreat	C	G	0.8700	0.0056	0.0005	AF	0.8719	-0.0007	0.0103
rs509345	1	150276022	eGFRcreat	A	G	0.5200	0.0024	0.0003	AF	0.5063	0.0106	0.0066
rs267738	1	150940625	eGFRcreat	T	G	0.7900	-0.0050	0.0004	AF	0.7819	-0.0070	0.0081
rs3845534	1	163738950	eGFRcreat	A	G	0.4900	-0.0019	0.0003	AF	0.4873	0.0023	0.0067
rs3795503	1	180905694	eGFRcreat	T	C	0.3300	0.0022	0.0004	AF	0.3322	0.0112	0.0072
rs78444298	1	184672098	eGFRcreat	A	G	0.0200	-0.0107	0.0014	AF	0.0175	0.0813	0.0283
rs1119066	1	186658212	eGFRcreat	A	C	0.1500	0.0027	0.0005	AF	0.1522	-0.0154	0.0092
rs3850625	1	201016296	eGFRcreat	A	G	0.1200	0.0048	0.0006	AF	0.1134	0.0011	0.0105
rs12024377	1	205537858	eGFRcreat	A	G	0.3700	0.0020	0.0004	AF	0.3690	0.0038	0.0070
rs78986840	1	208051123	eGFRcreat	T	C	0.9400	-0.0045	0.0007	AF	0.9377	-0.0050	0.0144
rs7535253	1	214744893	eGFRcreat	T	C	0.2100	0.0023	0.0004	AF	0.2269	0.0203	0.0081
rs7514450	1	220991171	eGFRcreat	T	C	0.4300	0.0022	0.0003	AF	0.4241	-0.0072	0.0067

rs417237	1	228532195	eGFRcreat	T	G	0.6100	0.0020	0.0004	AF	0.6194	0.0029	0.0068
rs2490391	1	243469669	eGFRcreat	A	C	0.4600	-0.0025	0.0003	AF	0.4637	0.0135	0.0067
rs3791221	2	226933	eGFRcreat	A	G	0.6500	0.0021	0.0004	AF	0.6440	-0.0122	0.0069
rs1595810	2	12115479	eGFRcreat	A	G	0.2000	-0.0024	0.0004	AF	0.2125	0.0027	0.0084
rs807624	2	15782471	eGFRcreat	T	G	0.3400	0.0034	0.0004	AF	0.3554	0.0043	0.0069
rs4441471	2	16715408	eGFRcreat	A	G	0.7200	0.0022	0.0004	AF	0.7253	0.0176	0.0074
rs4567937	2	18676265	eGFRcreat	A	G	0.3200	-0.0032	0.0004	AF	0.3140	0.0036	0.0073
rs780094	2	27741237	eGFRcreat	T	C	0.3800	0.0046	0.0004	AF	0.3782	-0.0010	0.0068
rs6722113	2	28417504	eGFRcreat	A	G	0.3400	-0.0022	0.0004	AF	0.3260	-0.0048	0.0077
rs2301343	2	40680149	eGFRcreat	T	G	0.7400	-0.0023	0.0004	AF	0.7511	0.0069	0.0077
rs168505	2	54920968	eGFRcreat	T	C	0.4000	-0.0027	0.0004	AF	0.3998	-0.0001	0.0068
rs72841902	2	73372212	eGFRcreat	A	T	0.2900	0.0022	0.0004	AF	0.2849	0.0109	0.0073
rs6546869	2	73895765	eGFRcreat	A	G	0.2200	0.0061	0.0004	AF	0.2320	0.0164	0.0080
rs72995641	2	103166325	eGFRcreat	A	G	0.2000	-0.0026	0.0004	AF	0.2048	-0.0043	0.0083
rs140179699	2	120936492	eGFRcreat	A	G	0.9500	0.0073	0.0011	AF	0.9654	-0.0186	0.0199
rs11694902	2	121988884	eGFRcreat	A	G	0.1400	0.0041	0.0005	AF	0.1391	-0.0091	0.0097
rs12989250	2	148776438	eGFRcreat	A	G	0.3100	-0.0026	0.0004	AF	0.3035	0.0263	0.0072
rs10432479	2	152365775	eGFRcreat	T	C	0.3700	-0.0021	0.0004	AF	0.3746	0.0025	0.0069
rs7565830	2	159810691	eGFRcreat	A	G	0.7200	-0.0022	0.0004	AF	0.7276	0.0056	0.0075
rs35472707	2	169995581	eGFRcreat	T	C	0.0500	-0.0075	0.0008	AF	0.0531	0.0210	0.0157
rs187355703	2	176993583	eGFRcreat	C	G	0.9700	0.0101	0.0011	AF	0.9767	-0.0350	0.0228
rs34468415	2	178125142	eGFRcreat	A	G	0.6400	-0.0028	0.0004	AF	0.6428	0.0051	0.0070
rs75267082	2	188129669	eGFRcreat	A	T	0.8900	0.0034	0.0006	AF	0.8884	0.0017	0.0107
rs1047891	2	211540507	eGFRcreat	A	C	0.3100	-0.0065	0.0004	AF	0.3032	-0.0107	0.0074
rs1548945	2	217665788	eGFRcreat	T	C	0.4100	0.0037	0.0004	AF	0.4200	-0.0147	0.0068
rs17462630	2	219286541	eGFRcreat	C	G	0.3400	0.0024	0.0004	AF	0.3434	0.0045	0.0071
rs1050816	2	220358198	eGFRcreat	T	C	0.3300	0.0029	0.0004	AF	0.3354	-0.0123	0.0070
rs13029395	2	227344207	eGFRcreat	T	C	0.1800	0.0034	0.0006	AF	0.2063	0.0366	0.0134
rs7592697	2	230665303	eGFRcreat	T	C	0.6500	-0.0020	0.0004	AF	0.6531	-0.0097	0.0070

rs6780429	3	30750404	eGFRcreat	A	C	0.5300	-0.0020	0.0003	AF	0.5353	-0.0129	0.0067
rs9838792	3	38546726	eGFRcreat	A	G	0.3900	0.0031	0.0004	AF	0.3827	-0.0072	0.0069
rs7651407	3	48479039	eGFRcreat	T	C	0.4500	0.0027	0.0004	AF	0.4625	-0.0066	0.0067
rs4625	3	49572140	eGFRcreat	A	G	0.6800	-0.0023	0.0004	AF	0.6919	0.0007	0.0071
rs62257555	3	51593113	eGFRcreat	A	G	0.9400	0.0048	0.0009	AF	0.9428	-0.0029	0.0163
rs35004449	3	52852897	eGFRcreat	T	G	0.2700	0.0027	0.0004	AF	0.2637	0.0163	0.0075
rs66473811	3	64000464	eGFRcreat	T	C	0.8400	0.0031	0.0005	AF	0.8318	0.0066	0.0090
rs9868185	3	121657593	eGFRcreat	A	G	0.5400	0.0027	0.0003	AF	0.5284	0.0031	0.0067
rs3905668	3	135931586	eGFRcreat	A	G	0.7200	-0.0025	0.0004	AF	0.7154	0.0144	0.0074
rs1397764	3	141750810	eGFRcreat	A	G	0.2800	0.0047	0.0004	AF	0.2962	-0.0019	0.0074
rs6779368	3	185298868	eGFRcreat	A	G	0.6600	0.0033	0.0004	AF	0.6609	0.0041	0.0071
rs112545201	3	185803532	eGFRcreat	T	C	0.1300	-0.0042	0.0005	AF	0.1370	0.0015	0.0097
rs11919484	3	186432839	eGFRcreat	T	G	0.3100	-0.0023	0.0004	AF	0.3101	-0.0073	0.0071
rs363092	4	3196029	eGFRcreat	A	C	0.4200	-0.0022	0.0004	AF	0.4222	0.0055	0.0067
rs6833292	4	10272429	eGFRcreat	T	C	0.4400	0.0020	0.0003	AF	0.4332	-0.0184	0.0067
rs7667050	4	23813109	eGFRcreat	T	C	0.4700	0.0020	0.0003	AF	0.4701	0.0080	0.0066
rs1910738	4	52687939	eGFRcreat	T	G	0.7100	0.0023	0.0004	AF	0.7064	-0.0057	0.0074
rs7687209	4	109693926	eGFRcreat	T	C	0.4200	0.0022	0.0004	AF	0.4289	-0.0012	0.0069
rs71606723	4	115498457	eGFRcreat	A	T	0.7600	0.0029	0.0004	AF	0.7777	-0.0051	0.0079
rs6555317	5	498235	eGFRcreat	A	G	0.6900	0.0024	0.0004	AF	0.6894	0.0001	0.0075
rs13157326	5	34504277	eGFRcreat	A	G	0.4800	-0.0027	0.0004	AF	0.4663	0.0100	0.0067
rs11951093	5	39421736	eGFRcreat	A	G	0.4200	-0.0056	0.0004	AF	0.4082	-0.0080	0.0068
rs495237	5	39968812	eGFRcreat	T	G	0.2500	0.0029	0.0004	AF	0.2108	-0.0009	0.0081
rs12520984	5	52787358	eGFRcreat	C	G	0.3300	0.0022	0.0004	AF	0.3152	0.0058	0.0071
rs79760705	5	53298716	eGFRcreat	T	G	0.1100	0.0056	0.0006	AF	0.1084	0.0212	0.0107
rs55938024	5	67742038	eGFRcreat	A	G	0.1200	-0.0065	0.0006	AF	0.1181	-0.0019	0.0104
rs76215063	5	68265211	eGFRcreat	T	C	0.9200	-0.0041	0.0007	AF	0.9328	-0.0065	0.0136
rs3797537	5	78322650	eGFRcreat	A	G	0.7100	0.0021	0.0004	AF	0.7188	-0.0093	0.0073
rs419291	5	131633355	eGFRcreat	T	C	0.3900	0.0021	0.0004	AF	0.3889	0.0002	0.0069

rs12163971	5	132226669	eGFRcreat	A	C	0.1600	-0.0032	0.0005	AF	0.1702	0.0074	0.0089
rs3812036	5	176813404	eGFRcreat	T	C	0.2600	-0.0069	0.0004	AF	0.2647	0.0051	0.0077
rs6921580	6	7203714	eGFRcreat	C	G	0.4100	0.0027	0.0004	AF	0.4193	-0.0013	0.0068
rs3134605	6	32159956	eGFRcreat	T	C	0.8000	0.0033	0.0004	AF	0.7967	-0.0066	0.0091
rs13200335	6	41690823	eGFRcreat	A	C	0.4200	0.0024	0.0003	AF	0.4191	-0.0111	0.0068
rs77915916	6	43287722	eGFRcreat	A	T	0.9200	0.0047	0.0006	AF	0.9135	0.0252	0.0122
rs881858	6	43806609	eGFRcreat	A	G	0.7000	-0.0056	0.0004	AF	0.6837	-0.0052	0.0073
rs6458868	6	52632213	eGFRcreat	T	C	0.6500	-0.0021	0.0004	AF	0.6409	-0.0048	0.0071
rs1268176	6	109018046	eGFRcreat	A	G	0.3400	0.0027	0.0004	AF	0.3347	0.0137	0.0071
rs9375694	6	130356608	eGFRcreat	A	G	0.7000	0.0026	0.0004	AF	0.6899	-0.0215	0.0072
rs9375818	6	131882078	eGFRcreat	A	G	0.2300	-0.0026	0.0004	AF	0.2250	0.0061	0.0080
rs3822939	6	133849789	eGFRcreat	A	G	0.4600	-0.0028	0.0003	AF	0.4599	-0.0036	0.0067
rs62432759	6	154858365	eGFRcreat	A	G	0.7800	-0.0025	0.0004	AF	0.7775	-0.0074	0.0082
rs12207180	6	160633107	eGFRcreat	A	T	0.1200	-0.0085	0.0005	AF	0.1230	-0.0033	0.0102
rs13230509	7	1286192	eGFRcreat	C	G	0.6900	-0.0055	0.0004	AF	0.6776	0.0103	0.0076
rs4410790	7	17284577	eGFRcreat	T	C	0.3700	-0.0023	0.0004	AF	0.3743	-0.0095	0.0069
rs6948759	7	33095688	eGFRcreat	T	C	0.2100	-0.0026	0.0004	AF	0.2225	-0.0103	0.0079
rs700753	7	46753684	eGFRcreat	C	G	0.3400	0.0033	0.0004	AF	0.3477	0.0055	0.0070
rs73116829	7	50739738	eGFRcreat	A	G	0.1100	-0.0043	0.0006	AF	0.0963	-0.0038	0.0114
rs55759218	7	77453357	eGFRcreat	A	G	0.2700	-0.0039	0.0004	AF	0.2745	0.0163	0.0074
rs325442	7	127457228	eGFRcreat	A	G	0.4000	0.0021	0.0003	AF	0.4070	0.0182	0.0068
rs3757387	7	128576086	eGFRcreat	T	C	0.5500	0.0029	0.0004	AF	0.5424	-0.0044	0.0067
rs62491533	7	129564134	eGFRcreat	T	C	0.8300	-0.0027	0.0005	AF	0.8193	0.0074	0.0088
rs10224002	7	151415041	eGFRcreat	A	G	0.7200	0.0068	0.0004	AF	0.7144	-0.0049	0.0074
rs6971211	7	155664686	eGFRcreat	T	C	0.4100	-0.0029	0.0004	AF	0.4088	-0.0029	0.0069
rs2365286	7	156258179	eGFRcreat	A	G	0.7400	-0.0033	0.0004	AF	0.7310	-0.0210	0.0076
rs2442604	8	6388533	eGFRcreat	T	C	0.5500	-0.0020	0.0003	AF	0.5473	-0.0033	0.0066
rs11784052	8	8671962	eGFRcreat	T	C	0.4600	0.0027	0.0004	AF	0.4579	-0.0169	0.0067
rs7838146	8	22492143	eGFRcreat	T	C	0.3600	-0.0021	0.0004	AF	0.3603	0.0067	0.0069

rs1913641	8	76483239	eGFRcreat	T	G	0.4800	-0.0020	0.0003	AF	0.4947	-0.0022	0.0066
rs4566	8	86361082	eGFRcreat	T	G	0.6100	0.0020	0.0004	AF	0.5936	-0.0146	0.0068
rs10086569	8	87247209	eGFRcreat	T	C	0.2400	0.0028	0.0004	AF	0.2413	0.0066	0.0078
rs78936994	8	120894208	eGFRcreat	T	G	0.2200	0.0024	0.0004	AF	0.2224	0.0169	0.0080
rs2954017	8	126476873	eGFRcreat	T	C	0.4600	0.0026	0.0004	AF	0.4710	0.0011	0.0067
rs10964603	9	20559727	eGFRcreat	T	C	0.7800	-0.0025	0.0004	AF	0.7833	-0.0248	0.0082
rs544169	9	33956791	eGFRcreat	A	G	0.7400	0.0024	0.0004	AF	0.7428	-0.0147	0.0075
rs2039424	9	71432174	eGFRcreat	A	G	0.6200	0.0048	0.0004	AF	0.6136	0.0055	0.0068
rs4836732	9	119266695	eGFRcreat	T	C	0.5300	0.0025	0.0003	AF	0.5286	-0.0035	0.0066
rs11794652	9	133496402	eGFRcreat	A	G	0.1600	-0.0028	0.0005	AF	0.1587	0.0063	0.0090
rs28404308	9	140103272	eGFRcreat	A	T	0.6200	0.0027	0.0005	AF	0.6207	0.0023	0.0076
rs80282103	10	899071	eGFRcreat	A	T	0.9200	0.0081	0.0006	AF	0.9218	-0.0093	0.0125
rs6481598	10	29781798	eGFRcreat	C	G	0.7800	0.0023	0.0004	AF	0.7754	-0.0031	0.0080
rs3793805	10	51049027	eGFRcreat	A	G	0.5700	-0.0020	0.0004	AF	0.5693	-0.0012	0.0067
rs10994860	10	52645424	eGFRcreat	T	C	0.1900	0.0039	0.0004	AF	0.1878	0.0043	0.0086
rs7084764	10	69960430	eGFRcreat	A	G	0.5000	0.0026	0.0003	AF	0.4991	-0.0020	0.0067
rs816828	10	79291868	eGFRcreat	T	C	0.5100	-0.0020	0.0004	AF	0.5098	0.0029	0.0066
rs2068888	10	94839642	eGFRcreat	A	G	0.4500	-0.0026	0.0003	AF	0.4476	-0.0135	0.0066
rs284859	10	104573017	eGFRcreat	T	G	0.1900	0.0027	0.0004	AF	0.1868	0.0038	0.0085
rs10430743	10	126456997	eGFRcreat	T	G	0.4300	0.0025	0.0003	AF	0.4437	0.0167	0.0067
rs11564722	11	2178330	eGFRcreat	T	C	0.2400	0.0038	0.0004	AF	0.2307	-0.0118	0.0082
rs233438	11	2794392	eGFRcreat	A	G	0.8100	0.0043	0.0004	AF	0.8004	-0.0086	0.0086
rs396341	11	5571897	eGFRcreat	T	C	0.2600	0.0030	0.0004	AF	0.2711	0.0172	0.0075
rs12361687	11	9890052	eGFRcreat	A	G	0.3600	0.0021	0.0004	AF	0.3690	-0.0002	0.0070
rs3925584	11	30760335	eGFRcreat	T	C	0.5500	-0.0055	0.0003	AF	0.5568	0.0057	0.0067
rs6484504	11	31424823	eGFRcreat	T	C	0.2800	-0.0032	0.0004	AF	0.2821	0.0157	0.0075
rs10838702	11	47410888	eGFRcreat	T	G	0.3800	-0.0023	0.0004	AF	0.3828	0.0059	0.0068
rs1783827	11	57409538	eGFRcreat	A	G	0.5700	-0.0021	0.0004	AF	0.5576	-0.0010	0.0068
rs11227260	11	65461158	eGFRcreat	T	G	0.3500	-0.0032	0.0004	AF	0.3355	0.0067	0.0071

rs3018667	11	68912221	eGFRcreat	A	G	0.3200	-0.0024	0.0004	AF	0.3353	-0.0094	0.0071
rs2509851	11	118966780	eGFRcreat	A	C	0.6300	0.0021	0.0004	AF	0.6272	0.0132	0.0069
rs11062167	12	364739	eGFRcreat	A	G	0.5300	-0.0042	0.0003	AF	0.5407	-0.0028	0.0068
rs632887	12	3392351	eGFRcreat	A	G	0.5900	0.0033	0.0004	AF	0.6005	0.0068	0.0068
rs11063193	12	4591100	eGFRcreat	T	C	0.8800	0.0029	0.0005	AF	0.8697	0.0090	0.0100
rs117113238	12	12209203	eGFRcreat	A	G	0.0900	0.0039	0.0006	AF	0.0923	0.0031	0.0118
rs10846157	12	15325031	eGFRcreat	A	C	0.8100	-0.0036	0.0004	AF	0.8037	-0.0092	0.0084
rs2634675	12	48740855	eGFRcreat	A	G	0.4600	0.0028	0.0004	AF	0.4504	-0.0201	0.0067
rs7966357	12	51209838	eGFRcreat	C	G	0.6600	0.0024	0.0004	AF	0.6505	-0.0156	0.0071
rs7974833	12	57791833	eGFRcreat	T	C	0.7600	-0.0032	0.0004	AF	0.7381	0.0081	0.0077
rs17696736	12	112486818	eGFRcreat	A	G	0.5700	0.0020	0.0004	AF	0.5796	-0.0080	0.0068
rs303937	13	72372524	eGFRcreat	A	T	0.4100	0.0027	0.0004	AF	0.4101	0.0047	0.0068
rs7326821	13	96068204	eGFRcreat	A	G	0.8300	0.0026	0.0005	AF	0.8275	-0.0014	0.0089
rs72683923	14	50735947	eGFRcreat	T	C	0.9800	-0.0076	0.0014	AF	0.9818	-0.0257	0.0276
rs2071047	14	54418411	eGFRcreat	A	G	0.4100	0.0020	0.0003	AF	0.4055	0.0095	0.0067
rs1569011	14	81853291	eGFRcreat	A	G	0.4400	0.0020	0.0003	AF	0.4352	-0.0021	0.0067
rs1028455	14	88829975	eGFRcreat	A	T	0.3300	0.0021	0.0004	AF	0.3353	0.0002	0.0071
rs35629566	14	93072317	eGFRcreat	C	G	0.8300	0.0030	0.0005	AF	0.8265	0.0194	0.0090
rs61993680	14	100752644	eGFRcreat	A	C	0.6500	-0.0022	0.0004	AF	0.6506	-0.0138	0.0071
rs12913015	15	39305443	eGFRcreat	T	C	0.4400	0.0028	0.0004	AF	0.4486	0.0023	0.0068
rs6492982	15	41399951	eGFRcreat	T	C	0.5500	-0.0032	0.0004	AF	0.5424	-0.0104	0.0068
rs1153855	15	45660758	eGFRcreat	C	G	0.6200	0.0086	0.0004	AF	0.6244	-0.0060	0.0068
rs10851543	15	53962748	eGFRcreat	A	G	0.5600	0.0030	0.0003	AF	0.5613	0.0043	0.0067
rs1994887	15	57793765	eGFRcreat	A	C	0.2800	-0.0024	0.0004	AF	0.2887	0.0020	0.0074
rs956006	15	62808539	eGFRcreat	T	C	0.3400	0.0022	0.0004	AF	0.3337	0.0012	0.0072
rs11071738	15	63580155	eGFRcreat	T	C	0.5300	-0.0025	0.0003	AF	0.5370	0.0056	0.0067
rs11071939	15	67463391	eGFRcreat	T	C	0.9200	-0.0038	0.0007	AF	0.9232	0.0145	0.0127
rs4886425	15	74124543	eGFRcreat	A	G	0.1700	-0.0027	0.0005	AF	0.1839	-0.0012	0.0088
rs2472297	15	75027880	eGFRcreat	T	C	0.2600	0.0039	0.0004	AF	0.2640	-0.0062	0.0077

rs4886699	15	75692303	eGFRcreat	A	C	0.7500	0.0031	0.0004	AF	0.7625	-0.0023	0.0077
rs10851885	15	76304503	eGFRcreat	A	G	0.7600	0.0050	0.0004	AF	0.7503	-0.0197	0.0078
rs506000	15	76817788	eGFRcreat	T	C	0.9100	-0.0038	0.0006	AF	0.9114	-0.0021	0.0116
rs113956264	16	1997004	eGFRcreat	T	C	0.0400	0.0081	0.0012	AF	0.0343	0.0192	0.0214
rs1635404	16	3747042	eGFRcreat	T	G	0.7000	-0.0024	0.0004	AF	0.6853	0.0180	0.0073
rs77924615	16	20392332	eGFRcreat	A	G	0.2000	0.0096	0.0005	AF	0.1965	-0.0093	0.0087
rs7188071	16	28917644	eGFRcreat	T	C	0.3600	0.0024	0.0004	AF	0.3703	0.0152	0.0070
rs12920176	16	51761084	eGFRcreat	A	C	0.5900	-0.0026	0.0004	AF	0.5783	-0.0174	0.0068
rs7203398	16	53189672	eGFRcreat	A	C	0.7300	0.0027	0.0004	AF	0.7256	-0.0034	0.0075
rs7185391	16	68323115	eGFRcreat	T	G	0.2900	-0.0026	0.0004	AF	0.2925	0.0055	0.0075
rs56140069	16	69795323	eGFRcreat	A	T	0.8200	0.0025	0.0005	AF	0.8114	-0.0055	0.0087
rs28581385	16	79942679	eGFRcreat	A	T	0.8500	-0.0033	0.0005	AF	0.8508	-0.0197	0.0093
rs72817412	16	89141490	eGFRcreat	T	C	0.0500	0.0049	0.0009	AF	0.0494	0.0036	0.0162
rs9894634	17	1967501	eGFRcreat	T	C	0.6000	-0.0021	0.0003	AF	0.5885	0.0051	0.0067
rs1242484	17	17351643	eGFRcreat	T	C	0.6900	-0.0025	0.0004	AF	0.6756	0.0081	0.0072
rs2252281	17	19437187	eGFRcreat	T	C	0.6100	0.0041	0.0004	AF	0.5980	0.0070	0.0069
rs72834794	17	38211383	eGFRcreat	A	C	0.9100	-0.0041	0.0006	AF	0.9093	-0.0208	0.0120
rs35662455	17	56755223	eGFRcreat	C	G	0.8800	0.0030	0.0005	AF	0.8878	-0.0017	0.0107
rs9907229	17	58917399	eGFRcreat	T	C	0.8500	-0.0049	0.0005	AF	0.8464	-0.0167	0.0091
rs11657044	17	59450105	eGFRcreat	T	C	0.1700	-0.0075	0.0005	AF	0.1837	-0.0151	0.0090
rs6501468	17	66427696	eGFRcreat	T	C	0.2300	0.0024	0.0004	AF	0.2440	-0.0197	0.0080
rs1719934	18	5585158	eGFRcreat	A	G	0.5400	0.0028	0.0003	AF	0.5389	-0.0140	0.0067
rs9807656	18	42346956	eGFRcreat	T	C	0.9000	-0.0034	0.0006	AF	0.8993	0.0029	0.0110
rs1377164	18	59328934	eGFRcreat	T	C	0.2100	0.0034	0.0004	AF	0.2205	0.0015	0.0080
rs3111316	19	13038415	eGFRcreat	A	G	0.5900	-0.0019	0.0004	AF	0.5721	-0.0105	0.0069
rs4808154	19	18843752	eGFRcreat	T	C	0.7100	0.0026	0.0004	AF	0.7164	0.0066	0.0075
rs8101667	19	33402419	eGFRcreat	T	C	0.3300	0.0050	0.0004	AF	0.3471	-0.0085	0.0071
rs113445505	19	38157969	eGFRcreat	T	C	0.3700	0.0038	0.0004	AF	0.3817	-0.0003	0.0069
rs281380	19	49214470	eGFRcreat	T	C	0.6300	-0.0022	0.0004	AF	0.6408	-0.0040	0.0070

rs62187541	20	1340244	eGFRcreat	A	G	0.9300	-0.0037	0.0007	AF	0.9296	0.0382	0.0134
rs1509117	20	8303120	eGFRcreat	A	T	0.3000	0.0025	0.0004	AF	0.3065	-0.0056	0.0077
rs6135224	20	14677650	eGFRcreat	A	G	0.6900	-0.0020	0.0004	AF	0.6948	0.0037	0.0072
rs6088528	20	33156742	eGFRcreat	A	G	0.5000	-0.0033	0.0003	AF	0.4903	-0.0096	0.0067
rs6029640	20	39970385	eGFRcreat	A	G	0.5800	-0.0021	0.0004	AF	0.5833	0.0143	0.0069
rs736820	20	43034016	eGFRcreat	A	G	0.3700	-0.0021	0.0004	AF	0.3690	0.0081	0.0070
rs6127099	20	52731402	eGFRcreat	A	T	0.7200	-0.0051	0.0004	AF	0.7255	0.0050	0.0077
rs2235826	20	56143169	eGFRcreat	A	T	0.8100	-0.0033	0.0005	AF	0.7951	0.0070	0.0087
rs2236521	20	60892116	eGFRcreat	A	G	0.5500	-0.0022	0.0004	AF	0.5461	0.0100	0.0069
rs2261092	20	62353933	eGFRcreat	A	G	0.0700	-0.0045	0.0007	AF	0.1023	0.0039	0.0128
rs1570521	20	62911019	eGFRcreat	T	G	0.4100	0.0020	0.0004	AF	0.4287	-0.0068	0.0069
rs2823139	21	16576783	eGFRcreat	A	G	0.3400	-0.0027	0.0004	AF	0.3386	0.0163	0.0070
rs2834317	21	35356706	eGFRcreat	A	G	0.1500	-0.0031	0.0005	AF	0.1533	0.0107	0.0094
rs2244237	21	37818141	eGFRcreat	T	G	0.2200	0.0027	0.0004	AF	0.2246	0.0003	0.0080
rs2074204	22	30403996	eGFRcreat	T	C	0.2600	-0.0025	0.0004	AF	0.2631	0.0136	0.0076
rs80576	22	36539804	eGFRcreat	A	G	0.1600	-0.0027	0.0005	AF	0.1871	0.0126	0.0091
rs2267372	22	38598234	eGFRcreat	A	G	0.4000	0.0024	0.0004	AF	0.4031	0.0106	0.0069
rs112880707	22	40884662	eGFRcreat	T	C	0.1100	0.0056	0.0006	AF	0.1014	-0.0165	0.0111
rs1883991	22	43112818	eGFRcreat	A	C	0.6900	-0.0032	0.0004	AF	0.6777	-0.0108	0.0074

Abbreviations: AF, atrial fibrillation; Chr, chromosome; creat, creatinine; EA, effect allele; EAF, effect allele frequency; eGFR, estimated glomerular filtration rate; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S2. Effect estimates for the associations of the genetic variants with blood urea nitrogen and atrial fibrillation

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs72658302	1	33883746	BUN	T	C	0.8096	0.0060	0.0009	AF	0.8680	0.0113	0.0099
rs80020578	1	54848827	BUN	A	G	0.2154	0.0070	0.0009	AF	0.1030	0.0040	0.0110
rs2755256	1	67471692	BUN	A	T	0.2278	-0.0071	0.0009	AF	0.2842	-0.0025	0.0075
rs10874312	1	82944571	BUN	A	G	0.6250	0.0055	0.0007	AF	0.6435	-0.0129	0.0070
rs10922532	1	89442733	BUN	T	C	0.3876	0.0048	0.0009	AF	0.3416	-0.0032	0.0097
rs2383531	1	186729401	BUN	A	G	0.9351	-0.0093	0.0017	AF	0.9084	-0.0192	0.0138
rs34277475	1	205416664	BUN	A	C	0.1978	-0.0081	0.0014	AF	0.1906	-0.0164	0.0094
rs17528077	1	227192280	BUN	T	C	0.7635	-0.0054	0.0010	AF	0.7569	-0.0172	0.0078
rs3791760	2	10118424	BUN	T	C	0.7192	0.0050	0.0008	AF	0.7506	-0.0106	0.0076
rs11127126	2	28023284	BUN	T	C	0.3777	-0.0057	0.0008	AF	0.2157	-0.0089	0.0080
rs11123170	2	113978940	BUN	C	G	0.6449	-0.0077	0.0007	AF	0.6449	0.0109	0.0069
rs1078442	2	121988924	BUN	A	C	0.4554	0.0050	0.0007	AF	0.5271	0.0069	0.0066
rs72929920	2	177097022	BUN	T	C	0.0308	0.0137	0.0025	AF	0.0324	0.0174	0.0199
rs1047891	2	211540507	BUN	A	C	0.2779	-0.0068	0.0008	AF	0.3032	-0.0107	0.0074
rs832805	2	219473737	BUN	T	C	0.3501	-0.0046	0.0008	AF	0.3984	0.0083	0.0069
rs1609783	3	25095911	BUN	A	G	0.5364	0.0052	0.0007	AF	0.5331	0.0129	0.0067
rs998394	3	64801187	BUN	A	G	0.4015	-0.0095	0.0008	AF	0.4631	0.0130	0.0067
rs11720938	3	66806828	BUN	T	C	0.8185	-0.0118	0.0009	AF	0.8125	-0.0097	0.0086
rs2332036	3	121714391	BUN	T	C	0.5368	0.0053	0.0007	AF	0.4708	-0.0017	0.0067
rs35320690	3	135932494	BUN	T	C	0.7247	0.0087	0.0009	AF	0.7158	0.0134	0.0074
rs16853637	3	169110019	BUN	A	G	0.1552	0.0106	0.0010	AF	0.1288	0.0059	0.0099
rs9290867	3	187721537	BUN	A	T	0.3140	-0.0161	0.0007	AF	0.3176	0.0015	0.0071
rs4498196	4	3747842	BUN	A	C	0.5335	-0.0045	0.0007	AF	0.5992	0.0045	0.0069
rs11940694	4	39414993	BUN	A	G	0.4654	0.0048	0.0007	AF	0.3983	-0.0097	0.0068
rs1229984	4	100239319	BUN	T	C	0.5710	0.0079	0.0014	AF	0.0674	-0.0389	0.0227
rs115403343	5	39591602	BUN	T	C	0.9783	0.0182	0.0030	AF	0.9801	0.0111	0.0251

rs79575541	5	40672422	BUN	C	G	0.9225	0.0304	0.0016	AF	0.9264	0.0041	0.0127
rs1168404	5	68706825	BUN	T	C	0.3809	0.0045	0.0008	AF	0.3446	0.0145	0.0077
rs17663555	5	72432036	BUN	C	G	0.6814	-0.0077	0.0007	AF	0.6920	0.0210	0.0073
rs62374016	5	90211273	BUN	T	C	0.6668	0.0043	0.0007	AF	0.7257	0.0003	0.0074
rs4976646	5	176788570	BUN	T	C	0.6477	-0.0066	0.0007	AF	0.6465	-0.0030	0.0070
rs283558	6	51044467	BUN	T	C	0.4370	0.0079	0.0007	AF	0.5092	0.0194	0.0066
rs4454139	6	55043236	BUN	A	C	0.2120	0.0048	0.0009	AF	0.2025	0.0049	0.0085
rs7766720	6	107172979	BUN	T	C	0.8573	-0.0064	0.0012	AF	0.9244	-0.0335	0.0126
rs162185	6	134226147	BUN	T	C	0.5534	-0.0041	0.0007	AF	0.5940	-0.0160	0.0069
rs300143	6	166421127	BUN	A	G	0.6225	0.0096	0.0008	AF	0.5703	-0.0125	0.0068
rs13230625	7	1286244	BUN	A	G	0.5269	0.0139	0.0009	AF	0.6845	0.0108	0.0078
rs6974343	7	29590944	BUN	A	G	0.2381	-0.0045	0.0008	AF	0.2401	0.0031	0.0077
rs700753	7	46753684	BUN	C	G	0.3066	-0.0049	0.0008	AF	0.3477	0.0055	0.0070
rs55914958	7	101237753	BUN	T	C	0.2932	-0.0064	0.0008	AF	0.3401	-0.0100	0.0071
rs2395811	7	106627029	BUN	C	G	0.2740	0.0050	0.0008	AF	0.2567	-0.0163	0.0078
rs73728279	7	151411494	BUN	T	G	0.2742	0.0148	0.0010	AF	0.2811	0.0048	0.0075
rs7834797	8	23759535	BUN	A	G	0.4854	0.0065	0.0007	AF	0.5739	-0.0091	0.0067
rs56411466	8	30280065	BUN	A	G	0.5445	-0.0050	0.0008	AF	0.4825	-0.0039	0.0067
rs6473252	8	81800193	BUN	T	C	0.4385	0.0043	0.0007	AF	0.4240	-0.0057	0.0067
rs11989898	8	127489030	BUN	A	G	0.7844	-0.0080	0.0009	AF	0.8513	-0.0102	0.0095
rs2978981	8	143759137	BUN	T	C	0.4781	-0.0041	0.0007	AF	0.4479	-0.0090	0.0067
rs703037	10	29196655	BUN	A	C	0.1845	-0.0065	0.0010	AF	0.1873	-0.0103	0.0085
rs7084402	10	60265404	BUN	A	G	0.5159	0.0051	0.0007	AF	0.5454	0.0086	0.0067
rs10821944	10	63785089	BUN	T	G	0.6893	-0.0042	0.0007	AF	0.7135	0.0047	0.0074
rs35451331	10	94842046	BUN	A	G	0.1574	-0.0067	0.0011	AF	0.1565	0.0104	0.0092
rs7096822	10	126664166	BUN	T	C	0.7537	0.0045	0.0008	AF	0.7568	-0.0063	0.0077
rs3925584	11	30760335	BUN	T	C	0.5968	0.0104	0.0007	AF	0.5568	0.0057	0.0067
rs11039216	11	47406592	BUN	T	C	0.4448	0.0044	0.0008	AF	0.5250	-0.0056	0.0067
rs7123489	11	65524252	BUN	A	C	0.3085	-0.0051	0.0008	AF	0.3419	0.0067	0.0070

rs4567493	11	86634423	BUN	A	G	0.3789	-0.0050	0.0007	AF	0.3678	-0.0073	0.0070
rs7931938	11	111207105	BUN	A	G	0.3915	-0.0042	0.0007	AF	0.4604	0.0089	0.0066
rs7936300	11	122601034	BUN	A	G	0.6340	-0.0044	0.0008	AF	0.5839	-0.0122	0.0068
rs1551210	12	42865874	BUN	T	C	0.4020	0.0059	0.0007	AF	0.4043	-0.0030	0.0068
rs836968	12	50267335	BUN	T	C	0.3856	0.0046	0.0008	AF	0.2705	-0.0052	0.0075
rs73114872	12	56861034	BUN	T	C	0.8386	-0.0076	0.0009	AF	0.8286	0.0015	0.0089
rs2122982	12	57781893	BUN	A	G	0.2077	-0.0079	0.0009	AF	0.2645	-0.0094	0.0077
rs1275609	12	76271183	BUN	A	G	0.4278	-0.0046	0.0008	AF	0.3308	-0.0072	0.0071
rs2264750	12	121450165	BUN	T	C	0.3861	0.0066	0.0007	AF	0.2967	0.0114	0.0072
rs963740	13	51096095	BUN	A	T	0.6024	-0.0052	0.0007	AF	0.7178	0.0080	0.0073
rs584480	13	72345505	BUN	T	C	0.5001	-0.0060	0.0007	AF	0.4080	0.0069	0.0068
rs7327286	13	73713447	BUN	A	G	0.7192	-0.0052	0.0008	AF	0.7918	-0.0008	0.0085
rs9517448	13	99438618	BUN	A	C	0.7605	-0.0070	0.0009	AF	0.8590	0.0098	0.0096
rs12856221	13	111094489	BUN	A	G	0.2624	0.0052	0.0010	AF	0.2787	-0.0083	0.0075
rs17730281	15	53907948	BUN	A	G	0.2930	-0.0078	0.0008	AF	0.2307	-0.0011	0.0078
rs17237465	15	61190011	BUN	T	C	0.6666	-0.0055	0.0007	AF	0.7027	-0.0029	0.0072
rs2470893	15	75019449	BUN	T	C	0.3070	-0.0064	0.0010	AF	0.3229	-0.0063	0.0072
rs4886755	15	76298132	BUN	A	G	0.4953	-0.0080	0.0007	AF	0.4912	-0.0051	0.0066
rs77924615	16	20392332	BUN	A	G	0.2056	-0.0123	0.0009	AF	0.1965	-0.0093	0.0087
rs7359387	16	69733665	BUN	T	G	0.7662	-0.0046	0.0008	AF	0.8488	-0.0301	0.0092
rs7213526	17	45011157	BUN	T	C	0.4345	0.0077	0.0007	AF	0.4073	0.0117	0.0067
rs6504021	17	59240473	BUN	T	C	0.7700	0.0082	0.0008	AF	0.7840	-0.0116	0.0083
rs28680305	17	65579728	BUN	A	G	0.4151	-0.0075	0.0008	AF	0.3559	0.0103	0.0072
rs148703751	17	80166293	BUN	T	C	0.2022	0.0067	0.0011	AF	0.2261	-0.0015	0.0083
rs16942713	18	24386535	BUN	T	G	0.2054	0.0067	0.0010	AF	0.0835	0.0049	0.0120
rs1484873	18	43206985	BUN	A	G	0.1351	-0.0169	0.0011	AF	0.0858	0.0138	0.0140
rs7243437	18	45658047	BUN	A	G	0.6646	0.0055	0.0007	AF	0.6504	0.0109	0.0070
rs7259714	19	817394	BUN	T	C	0.4350	-0.0047	0.0008	AF	0.3954	0.0120	0.0071
rs8106700	19	7200558	BUN	A	G	0.2894	0.0044	0.0007	AF	0.2822	-0.0064	0.0075

rs2238691	19	46179043	BUN	A	G	0.2120	-0.0089	0.0008	AF	0.2095	-0.0020	0.0082
rs838144	19	49250239	BUN	T	C	0.5299	0.0087	0.0009	AF	0.5006	-0.0033	0.0068
rs4811741	20	55287399	BUN	A	G	0.3877	-0.0047	0.0007	AF	0.3216	-0.0022	0.0073
rs6026580	20	57468150	BUN	T	C	0.6572	-0.0052	0.0009	AF	0.6534	-0.0093	0.0100
rs2823139	21	16576783	BUN	A	G	0.3165	0.0050	0.0007	AF	0.3386	0.0163	0.0070
rs2834310	21	35343934	BUN	A	G	0.5470	-0.0041	0.0007	AF	0.5159	0.0067	0.0067
rs219778	21	37834641	BUN	A	G	0.7406	0.0067	0.0009	AF	0.7456	0.0027	0.0077

Abbreviations: AF, atrial fibrillation; BUN, blood urea nitrogen; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S3. Effect estimates for the associations of the genetic variants with chronic kidney disease and atrial fibrillation

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs2490391	1	243469669	CKD	A	C	0.4700	0.0754	0.0092	AF	0.4637	0.0135	0.0067
rs187355703	2	176993583	CKD	C	G	0.9800	-0.1987	0.0312	AF	0.9767	-0.0350	0.0228
rs3812036	5	176813404	CKD	T	C	0.2700	0.0806	0.0109	AF	0.2647	0.0051	0.0077
rs13230509	7	1286192	CKD	C	G	0.6900	0.0645	0.0117	AF	0.6776	0.0103	0.0076
rs10224002	7	151415041	CKD	A	G	0.7200	-0.1083	0.0102	AF	0.7144	-0.0049	0.0074
rs80282103	10	899071	CKD	A	T	0.9200	-0.1136	0.0169	AF	0.9218	-0.0093	0.0125
rs3925584	11	30760335	CKD	T	C	0.5600	0.0800	0.0092	AF	0.5568	0.0057	0.0067
rs11227260	11	65461158	CKD	T	G	0.3400	0.0574	0.0096	AF	0.3355	0.0067	0.0071
rs1153855	15	45660758	CKD	C	G	0.6200	-0.0771	0.0094	AF	0.6244	-0.0060	0.0068

Abbreviations: AF, atrial fibrillation; Chr, chromosome; CKD, chronic kidney disease; EA, effect allele; EAF, effect allele frequency; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S4. Effect estimates for the associations of the genetic variants with estimated glomerular filtration rate based on cystatin C and atrial fibrillation

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs3925584	11	30760335	eGFRcys	T	C	0.6520	-0.0080	0.0022	AF	0.5568	0.0057	0.0067
rs653178	12	112007756	eGFRcys	T	C	0.7811	0.0129	0.0023	AF	0.5327	-0.0033	0.0068
rs4293393	16	20364588	eGFRcys	A	G	0.8315	-0.0177	0.0029	AF	0.8148	-0.0064	0.0085
rs1158167	20	23578189	eGFRcys	A	G	0.7445	-0.0661	0.0026	AF	0.7571	0.0106	0.0080

Abbreviations: AF, atrial fibrillation; Chr, chromosome; cys, cystatin C; EA, effect allele; EAF, effect allele frequency; eGFR, estimated glomerular filtration rate; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S5. Effect estimates for the associations of the genetic variants with urine albumin-to-creatinine ratio and atrial fibrillation

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs1337526	1	47965130	UACR	A	G	0.2102	-0.0271	0.0024	AF	0.2160	0.0106	0.0083
rs2070803	1	155174106	UACR	A	G	0.5720	-0.0167	0.0020	AF	0.5717	0.0076	0.0067
rs16864515	1	171435542	UACR	A	C	0.0968	-0.0185	0.0033	AF	0.1032	0.0201	0.0111
rs819636	1	200271408	UACR	T	C	0.6612	-0.0120	0.0021	AF	0.6546	0.0025	0.0071
rs3850625	1	201016296	UACR	A	G	0.1185	0.0177	0.0031	AF	0.1134	0.0011	0.0105
rs4665972	2	27598097	UACR	T	C	0.3941	0.0182	0.0021	AF	0.3862	0.0005	0.0070
rs12714144	2	85754578	UACR	A	T	0.8687	0.0219	0.0029	AF	0.8760	0.0005	0.0100
rs2880119	2	111809330	UACR	A	C	0.8578	-0.0169	0.0028	AF	0.8347	-0.0084	0.0095
rs10207567	2	203714973	UACR	C	G	0.8138	0.0191	0.0025	AF	0.8059	0.0198	0.0087
rs1047891	2	211540507	UACR	A	C	0.3140	-0.0187	0.0021	AF	0.3032	-0.0107	0.0074
rs57858280	2	227941981	UACR	T	C	0.1298	0.0191	0.0030	AF	0.1206	-0.0029	0.0103
rs73065147	3	46894939	UACR	T	C	0.9262	-0.0261	0.0038	AF	0.9302	-0.0389	0.0129
rs1010553	3	52540773	UACR	T	C	0.5131	0.0115	0.0020	AF	0.5241	0.0067	0.0067
rs112607182	3	170027407	UACR	T	C	0.0749	0.0306	0.0041	AF	0.0767	0.0190	0.0142
rs6535594	4	149132756	UACR	A	G	0.5016	0.0148	0.0020	AF	0.4939	0.0074	0.0067
rs40480	5	53325481	UACR	C	G	0.6333	-0.0132	0.0021	AF	0.6281	-0.0110	0.0069
rs162890	5	131623658	UACR	T	C	0.3352	0.0132	0.0021	AF	0.3510	0.0016	0.0072
rs2240060	6	31114900	UACR	A	G	0.2883	0.0137	0.0022	AF	0.3164	-0.0050	0.0074
rs2760995	6	32574358	UACR	A	G	0.2003	-0.0140	0.0025	AF	0.1973	-0.0078	0.0093
rs1544935	6	39124448	UACR	T	G	0.7853	-0.0167	0.0024	AF	0.7824	0.0073	0.0080
rs3734692	6	43817791	UACR	A	T	0.6891	-0.0173	0.0022	AF	0.6907	0.0008	0.0073
rs4410790	7	17284577	UACR	T	C	0.3752	-0.0209	0.0020	AF	0.3743	-0.0095	0.0069
rs17158386	7	29805361	UACR	A	G	0.2623	0.0200	0.0023	AF	0.2499	0.0013	0.0079
rs13230845	7	69869513	UACR	C	G	0.1892	-0.0156	0.0025	AF	0.1941	-0.0135	0.0085
rs10110261	8	23739375	UACR	A	G	0.4971	-0.0116	0.0020	AF	0.5069	0.0005	0.0067

rs28412751	8	61639875	UACR	T	C	0.4503	-0.0116	0.0020	AF	0.4482	0.0036	0.0067
rs6998967	8	81364205	UACR	A	G	0.1692	-0.0148	0.0027	AF	0.1524	-0.0204	0.0093
rs2793351	10	22151578	UACR	A	G	0.6849	0.0117	0.0021	AF	0.6669	-0.0124	0.0078
rs67339103	10	77893686	UACR	A	G	0.2296	0.0170	0.0024	AF	0.2130	0.0050	0.0081
rs2068888	10	94839642	UACR	A	G	0.4537	-0.0124	0.0020	AF	0.4476	-0.0135	0.0066
rs113139575	11	10296221	UACR	C	G	0.9364	-0.0250	0.0040	AF	0.9339	0.0001	0.0136
rs11030024	11	27508681	UACR	T	C	0.2194	-0.0134	0.0024	AF	0.2019	-0.0074	0.0082
rs7115200	11	71752160	UACR	T	G	0.5608	-0.0123	0.0020	AF	0.5544	0.0053	0.0069
rs508205	11	120057343	UACR	A	G	0.5572	-0.0131	0.0020	AF	0.5498	-0.0031	0.0067
rs10491967	12	3368093	UACR	A	G	0.1174	-0.0184	0.0033	AF	0.0974	0.0086	0.0110
rs3784283	15	41867782	UACR	A	T	0.5918	0.0148	0.0020	AF	0.5953	0.0201	0.0069
rs1145078	15	45682277	UACR	T	C	0.2718	-0.0179	0.0022	AF	0.2639	0.0058	0.0075
rs2470893	15	75019449	UACR	T	C	0.3247	0.0231	0.0021	AF	0.3229	-0.0063	0.0072
rs2460448	16	89700881	UACR	A	G	0.4416	-0.0112	0.0020	AF	0.4643	-0.0157	0.0069
rs11078597	17	1618363	UACR	T	C	0.8118	-0.0159	0.0026	AF	0.8061	0.0135	0.0086
rs11659764	18	53335512	UACR	A	T	0.0525	0.0297	0.0045	AF	0.0490	0.0183	0.0160
rs15052	19	41813375	UACR	T	C	0.8254	0.0173	0.0027	AF	0.8191	-0.0085	0.0093
rs6142630	20	30761183	UACR	A	G	0.4018	0.0112	0.0020	AF	0.4167	-0.0063	0.0068

Abbreviations: AF, atrial fibrillation; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism; UACR, urine albumin-to-creatinine-ratio.

Table S6. Effect estimates for the associations of the genetic variants with microalbuminuria and atrial fibrillation

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs112607182	3	170027407	MA	T	C	0.0749	0.0758	0.0139	AF	0.0767	0.0190	0.0142
rs6535594	4	149132756	MA	A	G	0.5016	0.0431	0.0067	AF	0.4939	0.0074	0.0067
rs4410790	7	17284577	MA	T	C	0.3752	-0.0417	0.0069	AF	0.3743	-0.0095	0.0069
rs67339103	10	77893686	MA	A	G	0.2296	0.0554	0.0083	AF	0.2130	0.0050	0.0081
rs3784283	15	41867782	MA	A	T	0.5918	0.0402	0.0068	AF	0.5953	0.0201	0.0069

Abbreviations: AF, atrial fibrillation; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; MA, microalbuminuria; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S7. Effect estimates for the associations of the genetic variants with atrial fibrillation and estimated glomerular filtration rate based on creatinine

			Exposure effect estimates							Outcome effect estimates		
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs284277	1	10790797	AF	C	A	0.3830	0.039221	0.007324	eGFRcreat	0.3700	-0.0005	0.0004
rs7529220	1	22282619	AF	C	T	0.8470	0.058269	0.009628	eGFRcreat	0.8500	-0.0002	0.0005
rs2885697	1	41544279	AF	G	T	0.3520	0.039221	0.007324	eGFRcreat	0.3400	0.0008	0.0004
rs11590635	1	49309764	AF	A	G	0.0240	0.148420	0.024314	eGFRcreat	0.0240	0.0013	0.0014
rs1545300	1	112464004	AF	C	T	0.6910	0.058269	0.007255	eGFRcreat	0.6900	0.0000	0.0004
rs4073778	1	116297758	AF	A	C	0.5640	0.048790	0.004859	eGFRcreat	0.5700	-0.0007	0.0003
rs79187193	1	147255831	AF	G	A	0.9430	0.113329	0.015878	eGFRcreat	0.9400	0.0011	0.0008
rs11264280	1	154862952	AF	T	C	0.3330	0.131028	0.006684	eGFRcreat	0.3300	0.0001	0.0004
rs72700114	1	170193825	AF	C	G	0.0760	0.198851	0.014581	eGFRcreat	0.0760	0.0009	0.0007
rs10753933	1	203026214	AF	T	G	0.4480	0.058269	0.007186	eGFRcreat	0.4400	0.0001	0.0003
rs4951258	1	205691316	AF	A	G	0.4160	0.039221	0.007395	eGFRcreat	0.4000	-0.0005	0.0004
rs7578393	2	26165528	AF	T	C	0.7960	0.058269	0.007186	eGFRcreat	0.7900	0.0004	0.0005
rs11125871	2	61470126	AF	C	T	0.6050	0.039221	0.004906	eGFRcreat	0.6000	-0.0008	0.0004
rs2540949	2	65284231	AF	A	T	0.6150	0.067659	0.007186	eGFRcreat	0.6100	-0.0003	0.0004
rs6747542	2	70106832	AF	T	C	0.5360	0.058269	0.007255	eGFRcreat	0.5400	0.0002	0.0004
rs72926475	2	86594487	AF	G	A	0.8770	0.067659	0.009538	eGFRcreat	0.8700	-0.0005	0.0005
rs28387148	2	127433465	AF	T	C	0.1050	0.076961	0.011867	eGFRcreat	0.1000	-0.0002	0.0006
rs67969609	2	145760353	AF	G	C	0.0710	0.067659	0.011867	eGFRcreat	0.0800	-0.0009	0.0007
rs56181519	2	175555714	AF	C	T	0.7320	0.067659	0.007186	eGFRcreat	0.7300	-0.0007	0.0004
rs2288327	2	179411665	AF	G	A	0.1560	0.095310	0.009277	eGFRcreat	0.1600	0.0007	0.0005
rs3820888	2	201180023	AF	C	T	0.3920	0.067659	0.007120	eGFRcreat	0.3900	0.0000	0.0004
rs35544454	2	213266003	AF	A	T	0.8080	0.058269	0.009628	eGFRcreat	0.8100	-0.0005	0.0004
rs7650482	3	12841804	AF	G	A	0.6400	0.067659	0.007120	eGFRcreat	0.6400	-0.0012	0.0004
rs73041705	3	24463235	AF	T	C	0.7020	0.048790	0.007324	eGFRcreat	0.7000	-0.0004	0.0004
rs6790396	3	38771925	AF	G	C	0.5960	0.058269	0.007186	eGFRcreat	0.5900	-0.0006	0.0004

rs34080181	3	66454191	AF	G	A	0.6210	0.048790	0.007324	eGFRcreat	0.6200	-0.0002	0.0004
rs17005647	3	69406181	AF	T	C	0.3640	0.039221	0.007324	eGFRcreat	0.3600	-0.0003	0.0004
rs6771054	3	89489529	AF	T	C	0.5960	0.048790	0.007324	eGFRcreat	0.5900	0.0001	0.0004
rs10804493	3	111554426	AF	A	G	0.6510	0.058269	0.007255	eGFRcreat	0.6500	0.0001	0.0004
rs1278493	3	135814009	AF	G	A	0.4360	0.039221	0.004906	eGFRcreat	0.4500	-0.0005	0.0003
rs7612445	3	179172979	AF	T	G	0.1880	0.048790	0.009719	eGFRcreat	0.1900	-0.0002	0.0004
rs60902112	3	194800853	AF	T	C	0.2260	0.048790	0.007324	eGFRcreat	0.2200	0.0003	0.0004
rs67249485	4	111699685	AF	T	A	0.1990	0.364643	0.007087	eGFRcreat	0.2100	-0.0010	0.0004
rs6829664	4	114448656	AF	G	A	0.2620	0.058269	0.007255	eGFRcreat	0.2600	0.0002	0.0004
rs10213171	4	148937537	AF	G	C	0.0610	0.095310	0.011651	eGFRcreat	0.0700	0.0008	0.0007
rs12648245	4	174641184	AF	T	C	0.9240	0.095310	0.011651	eGFRcreat	0.9100	-0.0008	0.0008
rs6596717	5	106427609	AF	C	A	0.3950	0.039221	0.007324	eGFRcreat	0.3900	0.0003	0.0004
rs337705	5	113737062	AF	G	T	0.3750	0.058269	0.007255	eGFRcreat	0.3800	0.0002	0.0004
rs2012809	5	128190363	AF	G	A	0.7900	0.058269	0.009628	eGFRcreat	0.8100	0.0000	0.0005
rs2040862	5	137419989	AF	T	C	0.1780	0.104360	0.006864	eGFRcreat	0.1800	-0.0004	0.0005
rs6580277	5	142818123	AF	G	A	0.2370	0.067659	0.009538	eGFRcreat	0.2300	0.0004	0.0004
rs12188351	5	168386089	AF	A	G	0.0560	0.086178	0.014046	eGFRcreat	0.0550	-0.0008	0.0008
rs6891790	5	172670745	AF	G	T	0.7170	0.076961	0.007120	eGFRcreat	0.7000	-0.0003	0.0004
rs73366713	6	16415751	AF	G	A	0.8600	0.104360	0.009194	eGFRcreat	0.8600	0.0004	0.0005
rs34969716	6	18210109	AF	A	G	0.3050	0.067659	0.007120	eGFRcreat	0.3100	0.0002	0.0004
rs3176326	6	36647289	AF	G	A	0.8020	0.058269	0.007186	eGFRcreat	0.8000	-0.0008	0.0004
rs2031522	6	87821501	AF	A	G	0.6240	0.039221	0.007324	eGFRcreat	0.6200	0.0000	0.0004
rs13195459	6	122403559	AF	G	A	0.6380	0.058269	0.007186	eGFRcreat	0.6500	-0.0003	0.0004
rs117984853	6	149399100	AF	T	G	0.1010	0.122218	0.013548	eGFRcreat	0.1000	-0.0007	0.0006
rs5734480	7	14372009	AF	A	G	0.2490	0.058269	0.007255	eGFRcreat	0.2500	-0.0002	0.0004
rs6462079	7	28415827	AF	A	G	0.7210	0.048790	0.007324	eGFRcreat	0.7400	-0.0005	0.0004
rs35005436	7	74134911	AF	C	T	0.1550	0.058269	0.009628	eGFRcreat	0.1500	0.0013	0.0005
rs56201652	7	92278116	AF	G	A	0.7330	0.048790	0.007255	eGFRcreat	0.7300	0.0010	0.0004
rs11773845	7	116191301	AF	A	C	0.5860	0.104360	0.006864	eGFRcreat	0.5900	-0.0003	0.0003

rs55985730	7	128417044	AF	G	T	0.0600	0.086178	0.014046	eGFRcreat	0.0600	-0.0003	0.0008
rs7789146	7	150661409	AF	G	A	0.8210	0.058269	0.009628	eGFRcreat	0.8100	-0.0003	0.0005
rs35620480	8	11499908	AF	C	A	0.1570	0.058269	0.007255	eGFRcreat	0.1600	-0.0013	0.0005
rs7508	8	17913970	AF	A	G	0.7110	0.067659	0.007120	eGFRcreat	0.7200	-0.0006	0.0004
rs7834729	8	21821778	AF	G	T	0.8850	0.067659	0.009538	eGFRcreat	0.8800	-0.0016	0.0005
rs62521286	8	124551975	AF	G	A	0.0660	0.122218	0.013548	eGFRcreat	0.0600	-0.0004	0.0007
rs6994744	8	141740868	AF	C	A	0.4950	0.039221	0.004906	eGFRcreat	0.5000	0.0003	0.0003
rs10821415	9	97713459	AF	A	C	0.4130	0.086178	0.007054	eGFRcreat	0.4200	-0.0003	0.0003
rs7096385	10	69664881	AF	T	C	0.0920	0.067659	0.011867	eGFRcreat	0.0780	-0.0016	0.0007
rs10458660	10	77936576	AF	G	A	0.1730	0.058269	0.007255	eGFRcreat	0.1700	0.0013	0.0005
rs11598047	10	105342672	AF	G	A	0.1620	0.157004	0.008722	eGFRcreat	0.1600	0.0005	0.0005
rs10749053	10	112576695	AF	T	C	0.1580	0.058269	0.009628	eGFRcreat	0.1400	0.0003	0.0005
rs10741807	11	20011445	AF	T	C	0.2450	0.076961	0.007120	eGFRcreat	0.2300	-0.0003	0.0004
rs76097649	11	128764570	AF	A	G	0.0930	0.113329	0.011340	eGFRcreat	0.0900	-0.0007	0.0007
rs4963776	12	24779491	AF	G	T	0.8180	0.095310	0.006990	eGFRcreat	0.8200	0.0011	0.0005
rs17380837	12	26345526	AF	C	T	0.6930	0.048790	0.007255	eGFRcreat	0.6900	-0.0006	0.0004
rs12809354	12	32978437	AF	C	T	0.1440	0.067659	0.009538	eGFRcreat	0.1500	-0.0002	0.0005
rs2860482	12	57105938	AF	A	C	0.2740	0.058269	0.007255	eGFRcreat	0.2700	-0.0011	0.0004
rs71454237	12	70013415	AF	G	A	0.7910	0.058269	0.007186	eGFRcreat	0.7900	-0.0001	0.0004
rs12426679	12	76237987	AF	C	T	0.4720	0.039221	0.004906	eGFRcreat	0.4800	0.0001	0.0003
rs883079	12	114793240	AF	T	C	0.7070	0.095310	0.006926	eGFRcreat	0.7100	-0.0008	0.0004
rs10773657	12	123327900	AF	C	A	0.1380	0.058269	0.009628	eGFRcreat	0.1200	-0.0005	0.0005
rs6560886	12	133150210	AF	C	T	0.7880	0.048790	0.009719	eGFRcreat	0.8000	0.0005	0.0005
rs9506925	13	23368943	AF	T	C	0.2670	0.048790	0.007324	eGFRcreat	0.2700	-0.0003	0.0004
rs35569628	13	113872712	AF	T	C	0.7770	0.048790	0.007324	eGFRcreat	0.7600	0.0003	0.0004
rs422068	14	23864804	AF	C	T	0.3490	0.039221	0.007324	eGFRcreat	0.3500	0.0001	0.0004
rs11156751	14	32990437	AF	C	T	0.2850	0.067659	0.007120	eGFRcreat	0.3000	-0.0002	0.0004
rs73241997	14	35173775	AF	T	C	0.1420	0.076961	0.009449	eGFRcreat	0.1500	0.0009	0.0005
rs2738413	14	64679960	AF	A	G	0.4950	0.076961	0.007054	eGFRcreat	0.4800	-0.0002	0.0003

rs74884082	14	73249419	AF	C	T	0.7500	0.048790	0.009719	eGFRcreat	0.7500	0.0011	0.0004
rs10873298	14	77426525	AF	C	T	0.3660	0.039221	0.007324	eGFRcreat	0.3700	0.0004	0.0004
rs7170477	15	64103777	AF	A	G	0.3040	0.039221	0.004906	eGFRcreat	0.3100	0.0000	0.0004
rs74022964	15	73677264	AF	T	C	0.1570	0.113329	0.009112	eGFRcreat	0.1600	-0.0002	0.0005
rs12908004	15	80676925	AF	G	A	0.1640	0.076961	0.009449	eGFRcreat	0.1700	-0.0005	0.0005
rs2359171	16	73053022	AF	A	T	0.1760	0.173953	0.008576	eGFRcreat	0.1800	-0.0008	0.0005
rs7225165	17	1309850	AF	G	A	0.8870	0.067659	0.011979	eGFRcreat	0.8900	0.0003	0.0006
rs9899183	17	7452977	AF	T	C	0.7140	0.048790	0.007324	eGFRcreat	0.7300	-0.0011	0.0004
rs72811294	17	12618680	AF	G	C	0.8870	0.067659	0.011867	eGFRcreat	0.8900	0.0010	0.0005
rs1563304	17	44874453	AF	T	C	0.1780	0.067659	0.009538	eGFRcreat	0.1700	-0.0002	0.0005
rs12604076	17	76773638	AF	T	C	0.4780	0.039221	0.007395	eGFRcreat	0.4700	-0.0009	0.0004
rs8088085	18	48708548	AF	A	C	0.5350	0.039221	0.007395	eGFRcreat	0.5400	0.0000	0.0003
rs2834618	21	36119111	AF	T	G	0.8940	0.095310	0.009277	eGFRcreat	0.8900	0.0002	0.0006
rs464901	22	18597502	AF	T	C	0.6650	0.048790	0.007255	eGFRcreat	0.6600	-0.0004	0.0004

Abbreviations: AF, atrial fibrillation; Chr, chromosome; creat, creatinine; EA, effect allele; EAF, effect allele frequency; eGFR, estimated glomerular filtration rate, N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S8. Effect estimates for the associations of the genetic variants with atrial fibrillation and blood urea nitrogen

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs284277	1	10790797	AF	C	A	0.3830	0.039221	0.007324	BUN	0.4800	0.000700	0.000800
rs7529220	1	22282619	AF	C	T	0.8470	0.058269	0.009628	BUN	0.7700	-0.000100	0.000900
rs2885697	1	41544279	AF	G	T	0.3520	0.039221	0.007324	BUN	0.3200	-0.000200	0.000700
rs1545300	1	112464004	AF	C	T	0.6910	0.058269	0.007255	BUN	0.7000	-0.000400	0.000900
rs4073778	1	116297758	AF	A	C	0.5640	0.048790	0.004859	BUN	0.4800	0.000900	0.000700
rs79187193	1	147255831	AF	G	A	0.9430	0.113329	0.015878	BUN	0.9420	0.004400	0.001800
rs72700114	1	170193825	AF	C	G	0.0760	0.198851	0.014581	BUN	0.0720	0.000000	0.001600
rs10753933	1	203026214	AF	T	G	0.4480	0.058269	0.007186	BUN	0.5300	-0.001100	0.000700
rs4951258	1	205691316	AF	A	G	0.4160	0.039221	0.007395	BUN	0.3800	-0.000500	0.000700
rs7578393	2	26165528	AF	T	C	0.7960	0.058269	0.007186	BUN	0.6200	-0.001600	0.001000
rs11125871	2	61470126	AF	C	T	0.6050	0.039221	0.004906	BUN	0.6200	-0.000500	0.000700
rs2540949	2	65284231	AF	A	T	0.6150	0.067659	0.007186	BUN	0.6300	-0.000900	0.000700
rs6747542	2	70106832	AF	T	C	0.5360	0.058269	0.007255	BUN	0.4700	-0.000900	0.000700
rs72926475	2	86594487	AF	G	A	0.8770	0.067659	0.009538	BUN	0.8600	0.001500	0.001300
rs28387148	2	127433465	AF	T	C	0.1050	0.076961	0.011867	BUN	0.1000	0.002600	0.001500
rs56181519	2	175555714	AF	C	T	0.7320	0.067659	0.007186	BUN	0.7400	-0.001100	0.001000
rs2288327	2	179411665	AF	G	A	0.1560	0.095310	0.009277	BUN	0.3700	-0.000400	0.000800
rs3820888	2	201180023	AF	C	T	0.3920	0.067659	0.007120	BUN	0.4800	-0.000400	0.000700
rs35544454	2	213266003	AF	A	T	0.8080	0.058269	0.009628	BUN	0.8100	0.001000	0.001100
rs7650482	3	12841804	AF	G	A	0.6400	0.067659	0.007120	BUN	0.5300	-0.001700	0.000700
rs73041705	3	24463235	AF	T	C	0.7020	0.048790	0.007324	BUN	0.7000	0.000600	0.000800
rs6790396	3	38771925	AF	G	C	0.5960	0.058269	0.007186	BUN	0.6500	0.000000	0.000800
rs34080181	3	66454191	AF	G	A	0.6210	0.048790	0.007324	BUN	0.6600	0.000800	0.000800
rs17005647	3	69406181	AF	T	C	0.3640	0.039221	0.007324	BUN	0.4700	0.000400	0.000800
rs6771054	3	89489529	AF	T	C	0.5960	0.048790	0.007324	BUN	0.6600	-0.000200	0.000900
rs10804493	3	111554426	AF	A	G	0.6510	0.058269	0.007255	BUN	0.6000	-0.001000	0.000700

rs7612445	3	179172979	AF	T	G	0.1880	0.048790	0.009719	BUN	0.2000	0.000100	0.000900
rs60902112	3	194800853	AF	T	C	0.2260	0.048790	0.007324	BUN	0.3800	-0.000900	0.000800
rs67249485	4	111699685	AF	T	A	0.1990	0.364643	0.007087	BUN	0.3900	0.000500	0.000800
rs6829664	4	114448656	AF	G	A	0.2620	0.058269	0.007255	BUN	0.2300	0.000300	0.000900
rs10213171	4	148937537	AF	G	C	0.0610	0.095310	0.011651	BUN	0.0900	0.000900	0.001300
rs12648245	4	174641184	AF	T	C	0.9240	0.095310	0.011651	BUN	0.9000	0.002600	0.001300
rs6596717	5	106427609	AF	C	A	0.3950	0.039221	0.007324	BUN	0.3400	0.002300	0.000800
rs337705	5	113737062	AF	G	T	0.3750	0.058269	0.007255	BUN	0.3500	0.000400	0.000700
rs2012809	5	128190363	AF	G	A	0.7900	0.058269	0.009628	BUN	0.8300	-0.000600	0.001100
rs2040862	5	137419989	AF	T	C	0.1780	0.104360	0.006864	BUN	0.1700	0.000700	0.001100
rs6580277	5	142818123	AF	G	A	0.2370	0.067659	0.009538	BUN	0.2200	0.000000	0.001000
rs12188351	5	168386089	AF	A	G	0.0560	0.086178	0.014046	BUN	0.0650	0.000000	0.001400
rs6891790	5	172670745	AF	G	T	0.7170	0.076961	0.007120	BUN	0.5900	-0.001400	0.000800
rs73366713	6	16415751	AF	G	A	0.8600	0.104360	0.009194	BUN	0.8600	-0.000100	0.001400
rs34969716	6	18210109	AF	A	G	0.3050	0.067659	0.007120	BUN	0.3000	-0.001400	0.001100
rs3176326	6	36647289	AF	G	A	0.8020	0.058269	0.007186	BUN	0.8200	0.000700	0.001000
rs2031522	6	87821501	AF	A	G	0.6240	0.039221	0.007324	BUN	0.5900	0.000600	0.000700
rs13195459	6	122403559	AF	G	A	0.6380	0.058269	0.007186	BUN	0.6700	0.000100	0.000800
rs117984853	6	149399100	AF	T	G	0.1010	0.122218	0.013548	BUN	0.0890	-0.001400	0.001700
rs55734480	7	14372009	AF	A	G	0.2490	0.058269	0.007255	BUN	0.2900	0.000400	0.000800
rs6462079	7	28415827	AF	A	G	0.7210	0.048790	0.007324	BUN	0.7400	-0.000100	0.000900
rs35005436	7	74134911	AF	C	T	0.1550	0.058269	0.009628	BUN	0.1500	-0.000800	0.001100
rs56201652	7	92278116	AF	G	A	0.7330	0.048790	0.007255	BUN	0.7400	-0.000100	0.000900
rs11773845	7	116191301	AF	A	C	0.5860	0.104360	0.006864	BUN	0.6100	0.000400	0.000700
rs55985730	7	128417044	AF	G	T	0.0600	0.086178	0.014046	BUN	0.0600	-0.001900	0.002200
rs7789146	7	150661409	AF	G	A	0.8210	0.058269	0.009628	BUN	0.7600	-0.001000	0.000900
rs35620480	8	11499908	AF	C	A	0.1570	0.058269	0.007255	BUN	0.1600	-0.002700	0.001200
rs7508	8	17913970	AF	A	G	0.7110	0.067659	0.007120	BUN	0.6600	-0.000500	0.000700
rs7834729	8	21821778	AF	G	T	0.8850	0.067659	0.009538	BUN	0.8600	0.000100	0.001100

rs62521286	8	124551975	AF	G	A	0.0660	0.122218	0.013548	BUN	0.0600	-0.003300	0.001800
rs6994744	8	141740868	AF	C	A	0.4950	0.039221	0.004906	BUN	0.5700	-0.000300	0.000700
rs10821415	9	97713459	AF	A	C	0.4130	0.086178	0.007054	BUN	0.3700	0.001200	0.000700
rs2274115	9	139094773	AF	G	A	0.7000	0.048790	0.009719	BUN	0.6300	-0.000100	0.000800
rs12245149	10	65321147	AF	C	A	0.5260	0.048790	0.007324	BUN	0.5700	0.001600	0.000700
rs7096385	10	69664881	AF	T	C	0.0920	0.067659	0.011867	BUN	0.2300	0.000100	0.001000
rs60212594	10	75414344	AF	G	C	0.8560	0.113329	0.011340	BUN	0.8400	-0.000400	0.000900
rs10458660	10	77936576	AF	G	A	0.1730	0.058269	0.007255	BUN	0.3500	-0.002000	0.000800
rs11598047	10	105342672	AF	G	A	0.1620	0.157004	0.008722	BUN	0.1500	-0.000600	0.001000
rs10749053	10	112576695	AF	T	C	0.1580	0.058269	0.009628	BUN	0.1400	0.000600	0.001200
rs10741807	11	20011445	AF	T	C	0.2450	0.076961	0.007120	BUN	0.3700	0.000100	0.000800
rs76097649	11	128764570	AF	A	G	0.0930	0.113329	0.011340	BUN	0.0870	-0.001100	0.001800
rs4963776	12	24779491	AF	G	T	0.8180	0.095310	0.006990	BUN	0.8300	-0.000200	0.000900
rs17380837	12	26345526	AF	C	T	0.6930	0.048790	0.007255	BUN	0.7000	0.000400	0.000800
rs12809354	12	32978437	AF	C	T	0.1440	0.067659	0.009538	BUN	0.1400	-0.000300	0.001000
rs2860482	12	57105938	AF	A	C	0.2740	0.058269	0.007255	BUN	0.3100	0.000800	0.000800
rs71454237	12	70013415	AF	G	A	0.7910	0.058269	0.007186	BUN	0.7900	-0.000500	0.000900
rs12426679	12	76237987	AF	C	T	0.4720	0.039221	0.004906	BUN	0.5600	-0.001400	0.000700
rs883079	12	114793240	AF	T	C	0.7070	0.095310	0.006926	BUN	0.6000	0.000300	0.000700
rs10773657	12	123327900	AF	C	A	0.1380	0.058269	0.009628	BUN	0.1800	-0.000900	0.000900
rs6560886	12	133150210	AF	C	T	0.7880	0.048790	0.009719	BUN	0.8100	-0.002600	0.001300
rs9506925	13	23368943	AF	T	C	0.2670	0.048790	0.007324	BUN	0.2700	0.000800	0.000900
rs35569628	13	113872712	AF	T	C	0.7770	0.048790	0.007324	BUN	0.6600	-0.001100	0.000800
rs422068	14	23864804	AF	C	T	0.3490	0.039221	0.007324	BUN	0.3200	-0.000800	0.000800
rs11156751	14	32990437	AF	C	T	0.2850	0.067659	0.007120	BUN	0.3100	0.001100	0.000900
rs73241997	14	35173775	AF	T	C	0.1420	0.076961	0.009449	BUN	0.1800	-0.001800	0.000900
rs2738413	14	64679960	AF	A	G	0.4950	0.076961	0.007054	BUN	0.4400	0.001100	0.000700
rs74884082	14	73249419	AF	C	T	0.7500	0.048790	0.009719	BUN	0.7600	0.000300	0.000900
rs10873298	14	77426525	AF	C	T	0.3660	0.039221	0.007324	BUN	0.3700	-0.001400	0.000700

rs7170477	15	64103777	AF	A	G	0.3040	0.039221	0.004906	BUN	0.3000	-0.001000	0.001000
rs74022964	15	73677264	AF	T	C	0.1570	0.113329	0.009112	BUN	0.1500	-0.000500	0.001100
rs12908004	15	80676925	AF	G	A	0.1640	0.076961	0.009449	BUN	0.1500	0.002100	0.001100
rs2359171	16	73053022	AF	A	T	0.1760	0.173953	0.008576	BUN	0.2400	-0.000300	0.000900
rs7225165	17	1309850	AF	G	A	0.8870	0.067659	0.011979	BUN	0.8600	0.000400	0.001100
rs9899183	17	7452977	AF	T	C	0.7140	0.048790	0.007324	BUN	0.7400	-0.000300	0.000900
rs72811294	17	12618680	AF	G	C	0.8870	0.067659	0.011867	BUN	0.8900	-0.000200	0.001100
rs11658278	17	38031164	AF	T	C	0.4790	0.048790	0.007324	BUN	0.5700	0.000700	0.000700
rs1563304	17	44874453	AF	T	C	0.1780	0.067659	0.009538	BUN	0.1700	0.002400	0.001300
rs12604076	17	76773638	AF	T	C	0.4780	0.039221	0.007395	BUN	0.5300	0.001300	0.000700
rs9953366	18	46474192	AF	C	T	0.6630	0.048790	0.007255	BUN	0.5800	-0.000700	0.000800
rs8088085	18	48708548	AF	A	C	0.5350	0.039221	0.007395	BUN	0.6100	-0.001400	0.000700
rs2834618	21	36119111	AF	T	G	0.8940	0.095310	0.009277	BUN	0.9000	0.000400	0.001200
rs464901	22	18597502	AF	T	C	0.6650	0.048790	0.007255	BUN	0.6700	0.000800	0.000700
rs133902	22	26164079	AF	T	C	0.4270	0.039221	0.007324	BUN	0.5300	0.001000	0.000700

Abbreviations: AF, atrial fibrillation; BUN, blood urea nitrogen; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S9. Effect estimates for the associations of the genetic variants with atrial fibrillation and chronic kidney disease

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs284277	1	10790797	AF	C	A	0.3830	0.039221	0.007324	CKD	0.3800	0.017800	0.009700
rs7529220	1	22282619	AF	C	T	0.8470	0.058269	0.009628	CKD	0.8600	0.005600	0.013600
rs2885697	1	41544279	AF	G	T	0.3520	0.039221	0.007324	CKD	0.3400	-0.014700	0.009600
rs11590635	1	49309764	AF	A	G	0.0240	0.148420	0.024314	CKD	0.0250	0.056400	0.037000
rs146518726	1	51535039	AF	A	G	0.0330	0.157004	0.019549	CKD	0.0310	0.059400	0.027600
rs1545300	1	112464004	AF	C	T	0.6910	0.058269	0.007255	CKD	0.6900	0.000900	0.009900
rs4073778	1	116297758	AF	A	C	0.5640	0.048790	0.004859	CKD	0.5700	0.008000	0.009200
rs79187193	1	147255831	AF	G	A	0.9430	0.113329	0.015878	CKD	0.9370	0.009600	0.019200
rs11264280	1	154862952	AF	T	C	0.3330	0.131028	0.006684	CKD	0.3300	0.007000	0.010300
rs72700114	1	170193825	AF	C	G	0.0760	0.198851	0.014581	CKD	0.0760	0.007500	0.018500
rs10753933	1	203026214	AF	T	G	0.4480	0.058269	0.007186	CKD	0.4400	-0.001700	0.009300
rs4951258	1	205691316	AF	A	G	0.4160	0.039221	0.007395	CKD	0.4200	0.009400	0.009300
rs7578393	2	26165528	AF	T	C	0.7960	0.058269	0.007186	CKD	0.8000	0.009200	0.012500
rs11125871	2	61470126	AF	C	T	0.6050	0.039221	0.004906	CKD	0.6100	0.010000	0.009300
rs2540949	2	65284231	AF	A	T	0.6150	0.067659	0.007186	CKD	0.6000	0.010700	0.009400
rs6747542	2	70106832	AF	T	C	0.5360	0.058269	0.007255	CKD	0.5400	0.003500	0.009700
rs72926475	2	86594487	AF	G	A	0.8770	0.067659	0.009538	CKD	0.8700	0.004700	0.014500
rs28387148	2	127433465	AF	T	C	0.1050	0.076961	0.011867	CKD	0.1100	0.016400	0.015500
rs67969609	2	145760353	AF	G	C	0.0710	0.067659	0.011867	CKD	0.0800	-0.003900	0.018200
rs56181519	2	175555714	AF	C	T	0.7320	0.067659	0.007186	CKD	0.7200	-0.001200	0.010800
rs2288327	2	179411665	AF	G	A	0.1560	0.095310	0.009277	CKD	0.1600	-0.000300	0.012500
rs3820888	2	201180023	AF	C	T	0.3920	0.067659	0.007120	CKD	0.3900	0.004900	0.009300
rs35544454	2	213266003	AF	A	T	0.8080	0.058269	0.009628	CKD	0.8000	0.001500	0.012200
rs7650482	3	12841804	AF	G	A	0.6400	0.067659	0.007120	CKD	0.6500	0.021200	0.009600
rs73041705	3	24463235	AF	T	C	0.7020	0.048790	0.007324	CKD	0.7000	0.001500	0.010500
rs6790396	3	38771925	AF	G	C	0.5960	0.058269	0.007186	CKD	0.6000	0.017000	0.009800

rs34080181	3	66454191	AF	G	A	0.6210	0.048790	0.007324	CKD	0.6300	0.003600	0.010000
rs17005647	3	69406181	AF	T	C	0.3640	0.039221	0.007324	CKD	0.3600	0.005300	0.010000
rs6771054	3	89489529	AF	T	C	0.5960	0.048790	0.007324	CKD	0.6000	-0.003500	0.011100
rs10804493	3	111554426	AF	A	G	0.6510	0.058269	0.007255	CKD	0.6600	-0.000600	0.009600
rs1278493	3	135814009	AF	G	A	0.4360	0.039221	0.004906	CKD	0.4400	0.009800	0.009300
rs7612445	3	179172979	AF	T	G	0.1880	0.048790	0.009719	CKD	0.1900	-0.007000	0.011700
rs60902112	3	194800853	AF	T	C	0.2260	0.048790	0.007324	CKD	0.2300	-0.004600	0.011400
rs10006327	4	103890980	AF	C	T	0.4900	0.039221	0.007395	CKD	0.4800	-0.021300	0.009700
rs67249485	4	111699685	AF	T	A	0.1990	0.364643	0.007087	CKD	0.2100	0.032400	0.011200
rs6829664	4	114448656	AF	G	A	0.2620	0.058269	0.007255	CKD	0.2600	0.012200	0.010400
rs10213171	4	148937537	AF	G	C	0.0610	0.095310	0.011651	CKD	0.0700	-0.035900	0.018700
rs12648245	4	174641184	AF	T	C	0.9240	0.095310	0.011651	CKD	0.9200	-0.000300	0.020200
rs6596717	5	106427609	AF	C	A	0.3950	0.039221	0.007324	CKD	0.4000	0.004400	0.009800
rs337705	5	113737062	AF	G	T	0.3750	0.058269	0.007255	CKD	0.3700	0.001300	0.009500
rs2012809	5	128190363	AF	G	A	0.7900	0.058269	0.009628	CKD	0.8100	-0.000600	0.015800
rs2040862	5	137419989	AF	T	C	0.1780	0.104360	0.006864	CKD	0.1800	0.007000	0.011900
rs6580277	5	142818123	AF	G	A	0.2370	0.067659	0.009538	CKD	0.2200	-0.008400	0.011500
rs12188351	5	168386089	AF	A	G	0.0560	0.086178	0.014046	CKD	0.0580	-0.000900	0.019500
rs6891790	5	172670745	AF	G	T	0.7170	0.076961	0.007120	CKD	0.7100	0.016200	0.011100
rs73366713	6	16415751	AF	G	A	0.8600	0.104360	0.009194	CKD	0.8600	-0.014500	0.014100
rs34969716	6	18210109	AF	A	G	0.3050	0.067659	0.007120	CKD	0.2900	-0.008000	0.011600
rs3176326	6	36647289	AF	G	A	0.8020	0.058269	0.007186	CKD	0.8100	0.015400	0.012300
rs2031522	6	87821501	AF	A	G	0.6240	0.039221	0.007324	CKD	0.6300	0.001200	0.009400
rs13195459	6	122403559	AF	G	A	0.6380	0.058269	0.007186	CKD	0.6400	0.001500	0.010000
rs117984853	6	149399100	AF	T	G	0.1010	0.122218	0.013548	CKD	0.1000	-0.003800	0.016900
rs5734480	7	14372009	AF	A	G	0.2490	0.058269	0.007255	CKD	0.2400	0.003600	0.011400
rs6462079	7	28415827	AF	A	G	0.7210	0.048790	0.007324	CKD	0.7400	0.007700	0.010500
rs35005436	7	74134911	AF	C	T	0.1550	0.058269	0.009628	CKD	0.1600	-0.004200	0.013300
rs56201652	7	92278116	AF	G	A	0.7330	0.048790	0.007255	CKD	0.7300	-0.009000	0.010300

rs11773845	7	116191301	AF	A	C	0.5860	0.104360	0.006864	CKD	0.5900	0.014100	0.009300
rs55985730	7	128417044	AF	G	T	0.0600	0.086178	0.014046	CKD	0.0600	-0.001600	0.021000
rs7789146	7	150661409	AF	G	A	0.8210	0.058269	0.009628	CKD	0.8200	0.021000	0.012600
rs35620480	8	11499908	AF	C	A	0.1570	0.058269	0.007255	CKD	0.1600	0.012000	0.012600
rs7508	8	17913970	AF	A	G	0.7110	0.067659	0.007120	CKD	0.7200	0.004000	0.010100
rs7834729	8	21821778	AF	G	T	0.8850	0.067659	0.009538	CKD	0.8800	-0.019800	0.013900
rs62521286	8	124551975	AF	G	A	0.0660	0.122218	0.013548	CKD	0.0600	-0.021900	0.020000
rs6994744	8	141740868	AF	C	A	0.4950	0.039221	0.004906	CKD	0.5000	0.000900	0.009200
rs10821415	9	97713459	AF	A	C	0.4130	0.086178	0.007054	CKD	0.4100	0.017000	0.009300
rs2274115	9	139094773	AF	G	A	0.7000	0.048790	0.009719	CKD	0.7000	-0.020900	0.011300
rs12245149	10	65321147	AF	C	A	0.5260	0.048790	0.007324	CKD	0.5300	-0.006100	0.009100
rs7096385	10	69664881	AF	T	C	0.0920	0.067659	0.011867	CKD	0.0670	0.030200	0.018400
rs10458660	10	77936576	AF	G	A	0.1730	0.058269	0.007255	CKD	0.1700	-0.015300	0.012200
rs11598047	10	105342672	AF	G	A	0.1620	0.157004	0.008722	CKD	0.1700	-0.008400	0.012200
rs10749053	10	112576695	AF	T	C	0.1580	0.058269	0.009628	CKD	0.1500	0.007300	0.013500
rs10741807	11	20011445	AF	T	C	0.2450	0.076961	0.007120	CKD	0.2300	0.002400	0.010900
rs76097649	11	128764570	AF	A	G	0.0930	0.113329	0.011340	CKD	0.0910	0.038900	0.017700
rs4963776	12	24779491	AF	G	T	0.8180	0.095310	0.006990	CKD	0.8200	0.002800	0.011800
rs17380837	12	26345526	AF	C	T	0.6930	0.048790	0.007255	CKD	0.6900	0.014000	0.010400
rs12809354	12	32978437	AF	C	T	0.1440	0.067659	0.009538	CKD	0.1500	0.012000	0.013400
rs2860482	12	57105938	AF	A	C	0.2740	0.058269	0.007255	CKD	0.2600	-0.007300	0.010500
rs71454237	12	70013415	AF	G	A	0.7910	0.058269	0.007186	CKD	0.7900	-0.000800	0.012000
rs12426679	12	76237987	AF	C	T	0.4720	0.039221	0.004906	CKD	0.4700	-0.010300	0.009200
rs883079	12	114793240	AF	T	C	0.7070	0.095310	0.006926	CKD	0.7100	0.025600	0.010100
rs10773657	12	123327900	AF	C	A	0.1380	0.058269	0.009628	CKD	0.1200	0.019700	0.014200
rs6560886	12	133150210	AF	C	T	0.7880	0.048790	0.009719	CKD	0.8000	-0.015500	0.013400
rs9506925	13	23368943	AF	T	C	0.2670	0.048790	0.007324	CKD	0.2700	0.006500	0.010300
rs35569628	13	113872712	AF	T	C	0.7770	0.048790	0.007324	CKD	0.7700	0.002500	0.011500
rs422068	14	23864804	AF	C	T	0.3490	0.039221	0.007324	CKD	0.3500	0.008700	0.009600

rs11156751	14	32990437	AF	C	T	0.2850	0.067659	0.007120	CKD	0.3000	-0.006100	0.010700
rs73241997	14	35173775	AF	T	C	0.1420	0.076961	0.009449	CKD	0.1500	-0.010600	0.013000
rs2738413	14	64679960	AF	A	G	0.4950	0.076961	0.007054	CKD	0.4900	0.008700	0.009100
rs74884082	14	73249419	AF	C	T	0.7500	0.048790	0.009719	CKD	0.7400	-0.006600	0.011100
rs10873298	14	77426525	AF	C	T	0.3660	0.039221	0.007324	CKD	0.3700	0.011600	0.009600
rs147301839	15	57924714	AF	C	A	0.0070	0.329304	0.052843	CKD	0.0100	0.046000	0.067300
rs7170477	15	64103777	AF	A	G	0.3040	0.039221	0.004906	CKD	0.3100	0.000100	0.010100
rs74022964	15	73677264	AF	T	C	0.1570	0.113329	0.009112	CKD	0.1600	-0.002000	0.013300
rs12908004	15	80676925	AF	G	A	0.1640	0.076961	0.009449	CKD	0.1600	0.003500	0.013100
rs4965430	15	99268850	AF	C	G	0.3860	0.048790	0.007324	CKD	0.3800	0.008800	0.009900
rs140185678	16	2003016	AF	A	G	0.0350	0.165514	0.021632	CKD	0.0380	-0.004300	0.033500
rs2359171	16	73053022	AF	A	T	0.1760	0.173953	0.008576	CKD	0.1800	0.005800	0.012600
rs7225165	17	1309850	AF	G	A	0.8870	0.067659	0.011979	CKD	0.8900	-0.018800	0.016000
rs9899183	17	7452977	AF	T	C	0.7140	0.048790	0.007324	CKD	0.7300	-0.000200	0.010500
rs72811294	17	12618680	AF	G	C	0.8870	0.067659	0.011867	CKD	0.8900	-0.035500	0.014300
rs11658278	17	38031164	AF	T	C	0.4790	0.048790	0.007324	CKD	0.4900	-0.016500	0.009100
rs1563304	17	44874453	AF	T	C	0.1780	0.067659	0.009538	CKD	0.1700	0.020900	0.013300
rs12604076	17	76773638	AF	T	C	0.4780	0.039221	0.007395	CKD	0.4800	0.009800	0.009600
rs9953366	18	46474192	AF	C	T	0.6630	0.048790	0.007255	CKD	0.6700	-0.027500	0.010400
rs8088085	18	48708548	AF	A	C	0.5350	0.039221	0.007395	CKD	0.5400	0.003900	0.009100
rs2834618	21	36119111	AF	T	G	0.8940	0.095310	0.009277	CKD	0.8900	0.004700	0.014800
rs464901	22	18597502	AF	T	C	0.6650	0.048790	0.007255	CKD	0.6600	0.000900	0.009700
rs133902	22	26164079	AF	T	C	0.4270	0.039221	0.007324	CKD	0.4300	0.030600	0.009300

Abbreviations: AF, atrial fibrillation; Chr, chromosome; CKD, chronic kidney disease; EA, effect allele; EAF, effect allele frequency; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S10. Effect estimates for the associations of the genetic variants with atrial fibrillation and estimated glomerular filtration rate based on cystatin C

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs284277	1	10790797	AF	C	A	0.3830	0.039221	0.007324	eGFRcys	0.5838	-0.006900	0.002400
rs7529220	1	22282619	AF	C	T	0.8470	0.058269	0.009628	eGFRcys	0.7500	-0.004300	0.003200
rs2885697	1	41544279	AF	G	T	0.3520	0.039221	0.007324	eGFRcys	0.2830	0.002000	0.002400
rs11590635	1	49309764	AF	A	G	0.0240	0.148420	0.024314	eGFRcys	0.0119	-0.011200	0.009000
rs146518726	1	51535039	AF	A	G	0.0330	0.157004	0.019549	eGFRcys	0.0220	-0.002400	0.007100
rs1545300	1	112464004	AF	C	T	0.6910	0.058269	0.007255	eGFRcys	0.8201	0.000900	0.002400
rs4073778	1	116297758	AF	A	C	0.5640	0.048790	0.004859	eGFRcys	0.3581	0.000600	0.002200
rs79187193	1	147255831	AF	G	A	0.9430	0.113329	0.015878	eGFRcys	0.9643	0.001400	0.004700
rs11264280	1	154862952	AF	T	C	0.3330	0.131028	0.006684	eGFRcys	0.1941	-0.000400	0.002500
rs72700114	1	170193825	AF	C	G	0.0760	0.198851	0.014581	eGFRcys	0.0517	-0.003100	0.004300
rs10753933	1	203026214	AF	T	G	0.4480	0.058269	0.007186	eGFRcys	0.5820	0.000600	0.002200
rs4951258	1	205691316	AF	A	G	0.4160	0.039221	0.007395	eGFRcys	0.3530	0.001000	0.002300
rs7578393	2	26165528	AF	T	C	0.7960	0.058269	0.007186	eGFRcys	0.4936	-0.000100	0.003100
rs11125871	2	61470126	AF	C	T	0.6050	0.039221	0.004906	eGFRcys	0.6648	0.004000	0.002300
rs2540949	2	65284231	AF	A	T	0.6150	0.067659	0.007186	eGFRcys	0.6227	0.001800	0.002300
rs6747542	2	70106832	AF	T	C	0.5360	0.058269	0.007255	eGFRcys	0.4327	-0.000500	0.002300
rs72926475	2	86594487	AF	G	A	0.8770	0.067659	0.009538	eGFRcys	0.9089	-0.004200	0.003200
rs67969609	2	145760353	AF	G	C	0.0710	0.067659	0.011867	eGFRcys	0.2793	-0.000600	0.004100
rs56181519	2	175555714	AF	C	T	0.7320	0.067659	0.007186	eGFRcys	0.7962	0.003400	0.002700
rs2288327	2	179411665	AF	G	A	0.1560	0.095310	0.009277	eGFRcys	0.3027	-0.003300	0.002900
rs3820888	2	201180023	AF	C	T	0.3920	0.067659	0.007120	eGFRcys	0.4354	-0.004200	0.002300
rs35544454	2	213266003	AF	A	T	0.8080	0.058269	0.009628	eGFRcys	0.8974	-0.000900	0.002900
rs7650482	3	12841804	AF	G	A	0.6400	0.067659	0.007120	eGFRcys	0.5929	0.003800	0.002300
rs73041705	3	24463235	AF	T	C	0.7020	0.048790	0.007324	eGFRcys	0.7276	0.001000	0.002400
rs6790396	3	38771925	AF	G	C	0.5960	0.058269	0.007186	eGFRcys	0.7005	0.000000	0.002200
rs34080181	3	66454191	AF	G	A	0.6210	0.048790	0.007324	eGFRcys	0.7582	-0.001400	0.002300
rs17005647	3	69406181	AF	T	C	0.3640	0.039221	0.007324	eGFRcys	0.3544	0.001700	0.002400
rs6771054	3	89489529	AF	T	C	0.5960	0.048790	0.007324	eGFRcys	0.5856	-0.002300	0.002300

rs10804493	3	111554426	AF	A	G	0.6510	0.058269	0.007255	eGFRcys	0.5403	-0.000100	0.002400
rs1278493	3	135814009	AF	G	A	0.4360	0.039221	0.004906	eGFRcys	0.4959	-0.002100	0.002200
rs7612445	3	179172979	AF	T	G	0.1880	0.048790	0.009719	eGFRcys	0.2431	-0.002100	0.002900
rs60902112	3	194800853	AF	T	C	0.2260	0.048790	0.007324	eGFRcys	0.3590	0.003800	0.002700
rs1458038	4	81164723	AF	T	C	0.3090	0.039221	0.007324	eGFRcys	0.2514	-0.002800	0.002500
rs67249485	4	111699685	AF	T	A	0.1990	0.364643	0.007087	eGFRcys	0.4002	-0.002300	0.002700
rs6829664	4	114448656	AF	G	A	0.2620	0.058269	0.007255	eGFRcys	0.1635	-0.002400	0.002600
rs10213171	4	148937537	AF	G	C	0.0610	0.095310	0.011651	eGFRcys	0.1136	-0.007000	0.004300
rs6596717	5	106427609	AF	C	A	0.3950	0.039221	0.007324	eGFRcys	0.3091	-0.000800	0.002300
rs337705	5	113737062	AF	G	T	0.3750	0.058269	0.007255	eGFRcys	0.4199	-0.001300	0.002300
rs2012809	5	128190363	AF	G	A	0.7900	0.058269	0.009628	eGFRcys	0.8791	-0.003400	0.002800
rs2040862	5	137419989	AF	T	C	0.1780	0.104360	0.006864	eGFRcys	0.0810	-0.003100	0.002900
rs6580277	5	142818123	AF	G	A	0.2370	0.067659	0.009538	eGFRcys	0.1676	0.001400	0.002700
rs12188351	5	168386089	AF	A	G	0.0560	0.086178	0.014046	eGFRcys	0.0398	0.001200	0.004700
rs6891790	5	172670745	AF	G	T	0.7170	0.076961	0.007120	eGFRcys	0.5174	-0.002700	0.002900
rs73366713	6	16415751	AF	G	A	0.8600	0.104360	0.009194	eGFRcys	0.8878	-0.003100	0.003300
rs34969716	6	18210109	AF	A	G	0.3050	0.067659	0.007120	eGFRcys	0.2303	0.002700	0.002900
rs3176326	6	36647289	AF	G	A	0.8020	0.058269	0.007186	eGFRcys	0.8288	-0.001900	0.002800
rs2031522	6	87821501	AF	A	G	0.6240	0.039221	0.007324	eGFRcys	0.5870	-0.004500	0.002300
rs13195459	6	122403559	AF	G	A	0.6380	0.058269	0.007186	eGFRcys	0.7262	-0.004500	0.002300
rs117984853	6	149399100	AF	T	G	0.1010	0.122218	0.013548	eGFRcys	0.0403	-0.002300	0.004500
rs55734480	7	14372009	AF	A	G	0.2490	0.058269	0.007255	eGFRcys	0.2143	0.002200	0.002900
rs6462079	7	28415827	AF	A	G	0.7210	0.048790	0.007324	eGFRcys	0.8168	0.004200	0.002600
rs35005436	7	74134911	AF	C	T	0.1550	0.058269	0.009628	eGFRcys	0.0838	-0.004600	0.003800
rs56201652	7	92278116	AF	G	A	0.7330	0.048790	0.007255	eGFRcys	0.7679	0.000600	0.002400
rs11773845	7	116191301	AF	A	C	0.5860	0.104360	0.006864	eGFRcys	0.5545	-0.001200	0.002300
rs55985730	7	128417044	AF	G	T	0.0600	0.086178	0.014046	eGFRcys	0.0215	0.000400	0.005000
rs7789146	7	150661409	AF	G	A	0.8210	0.058269	0.009628	eGFRcys	0.7418	-0.005500	0.003100
rs35620480	8	11499908	AF	C	A	0.1570	0.058269	0.007255	eGFRcys	0.0962	-0.000600	0.003200
rs7508	8	17913970	AF	A	G	0.7110	0.067659	0.007120	eGFRcys	0.7024	0.003500	0.002500
rs7834729	8	21821778	AF	G	T	0.8850	0.067659	0.009538	eGFRcys	0.7967	0.003300	0.003300
rs62521286	8	124551975	AF	G	A	0.0660	0.122218	0.013548	eGFRcys	0.0504	-0.001900	0.004700
rs6994744	8	141740868	AF	C	A	0.4950	0.039221	0.004906	eGFRcys	0.6328	0.002300	0.002200

rs10821415	9	97713459	AF	A	C	0.4130	0.086178	0.007054	eGFRcys	0.3082	0.000400	0.002200
rs2274115	9	139094773	AF	G	A	0.7000	0.048790	0.009719	eGFRcys	0.6534	-0.001000	0.002800
rs12245149	10	65321147	AF	C	A	0.5260	0.048790	0.007324	eGFRcys	0.5540	-0.003300	0.002200
rs7096385	10	69664881	AF	T	C	0.0920	0.067659	0.011867	eGFRcys	0.1703	-0.005200	0.004400
rs60212594	10	75414344	AF	G	C	0.8560	0.113329	0.011340	eGFRcys	0.8443	-0.001500	0.003100
rs10458660	10	77936576	AF	G	A	0.1730	0.058269	0.007255	eGFRcys	0.2843	0.000700	0.002900
rs11598047	10	105342672	AF	G	A	0.1620	0.157004	0.008722	eGFRcys	0.1387	0.004400	0.003000
rs10749053	10	112576695	AF	T	C	0.1580	0.058269	0.009628	eGFRcys	0.1543	0.000000	0.003500
rs10741807	11	20011445	AF	T	C	0.2450	0.076961	0.007120	eGFRcys	0.3681	-0.002300	0.002700
rs4935786	11	121661507	AF	T	A	0.2670	0.048790	0.007324	eGFRcys	0.3993	-0.001600	0.002700
rs76097649	11	128764570	AF	A	G	0.0930	0.113329	0.011340	eGFRcys	0.0696	0.003900	0.004900
rs4963776	12	24779491	AF	G	T	0.8180	0.095310	0.006990	eGFRcys	0.8672	-0.001100	0.002900
rs17380837	12	26345526	AF	C	T	0.6930	0.048790	0.007255	eGFRcys	0.7527	-0.001900	0.002500
rs12809354	12	32978437	AF	C	T	0.1440	0.067659	0.009538	eGFRcys	0.1131	0.003100	0.003100
rs2860482	12	57105938	AF	A	C	0.2740	0.058269	0.007255	eGFRcys	0.2738	-0.002700	0.002600
rs71454237	12	70013415	AF	G	A	0.7910	0.058269	0.007186	eGFRcys	0.7990	-0.000600	0.002800
rs12426679	12	76237987	AF	C	T	0.4720	0.039221	0.004906	eGFRcys	0.6772	-0.000400	0.002200
rs883079	12	114793240	AF	T	C	0.7070	0.095310	0.006926	eGFRcys	0.6053	-0.002300	0.002500
rs10773657	12	123327900	AF	C	A	0.1380	0.058269	0.009628	eGFRcys	0.2111	-0.001200	0.003400
rs6560886	12	133150210	AF	C	T	0.7880	0.048790	0.009719	eGFRcys	0.8837	0.004500	0.004000
rs9506925	13	23368943	AF	T	C	0.2670	0.048790	0.007324	eGFRcys	0.1319	-0.002400	0.002500
rs35569628	13	113872712	AF	T	C	0.7770	0.048790	0.007324	eGFRcys	0.6845	0.001800	0.002600
rs422068	14	23864804	AF	C	T	0.3490	0.039221	0.007324	eGFRcys	0.3507	-0.001900	0.002400
rs73241997	14	35173775	AF	T	C	0.1420	0.076961	0.009449	eGFRcys	0.2358	0.002200	0.003100
rs2738413	14	64679960	AF	A	G	0.4950	0.076961	0.007054	eGFRcys	0.3283	-0.000400	0.002200
rs74884082	14	73249419	AF	C	T	0.7500	0.048790	0.009719	eGFRcys	0.7473	-0.000300	0.002600
rs10873298	14	77426525	AF	C	T	0.3660	0.039221	0.007324	eGFRcys	0.5114	-0.002000	0.002300
rs7170477	15	64103777	AF	A	G	0.3040	0.039221	0.004906	eGFRcys	0.2001	-0.000800	0.002400
rs74022964	15	73677264	AF	T	C	0.1570	0.113329	0.009112	eGFRcys	0.1406	0.002600	0.003000
rs12908004	15	80676925	AF	G	A	0.1640	0.076961	0.009449	eGFRcys	0.1543	-0.004700	0.003100
rs140185678	16	2003016	AF	A	G	0.0350	0.165514	0.021632	eGFRcys	0.0114	0.005300	0.009000
rs2359171	16	73053022	AF	A	T	0.1760	0.173953	0.008576	eGFRcys	0.2276	-0.005300	0.003000
rs7225165	17	1309850	AF	G	A	0.8870	0.067659	0.011979	eGFRcys	0.8828	-0.001300	0.004100

rs9899183	17	7452977	AF	T	C	0.7140	0.048790	0.007324	eGFRcys	0.8123	0.000000	0.002600
rs72811294	17	12618680	AF	G	C	0.8870	0.067659	0.011867	eGFRcys	0.8645	0.005500	0.003500
rs11658278	17	38031164	AF	T	C	0.4790	0.048790	0.007324	eGFRcys	0.5980	0.000800	0.002300
rs12604076	17	76773638	AF	T	C	0.4780	0.039221	0.007395	eGFRcys	0.5778	0.003900	0.002200
rs9953366	18	46474192	AF	C	T	0.6630	0.048790	0.007255	eGFRcys	0.6516	0.004700	0.002700
rs8088085	18	48708548	AF	A	C	0.5350	0.039221	0.007395	eGFRcys	0.6900	-0.000200	0.002200
rs2834618	21	36119111	AF	T	G	0.8940	0.095310	0.009277	eGFRcys	0.9057	0.002200	0.003700
rs464901	22	18597502	AF	T	C	0.6650	0.048790	0.007255	eGFRcys	0.6094	-0.001100	0.002500
rs133902	22	26164079	AF	T	C	0.4270	0.039221	0.007324	eGFRcys	0.6813	-0.000700	0.002300

Abbreviations: AF, atrial fibrillation; Chr, chromosome; cys, cystatin C; EA, effect allele; EAF, effect allele frequency; eGFR, estimated glomerular filtration rate; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

Table S11. Effect estimates for the associations of the genetic variants with atrial fibrillation and urine albumin-to-creatinine ratio

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs7529220	1	22282619	AF	C	T	0.8470	0.058269	0.009628	UACR	0.8547	0.000200	0.002800
rs146518726	1	51535039	AF	A	G	0.0330	0.157004	0.019549	UACR	0.0234	-0.013300	0.006800
rs1545300	1	112464004	AF	C	T	0.6910	0.058269	0.007255	UACR	0.6876	0.003000	0.002100
rs4073778	1	116297758	AF	A	C	0.5640	0.048790	0.004859	UACR	0.5643	0.000300	0.002000
rs79187193	1	147255831	AF	G	A	0.9430	0.113329	0.015878	UACR	0.9483	-0.001600	0.004600
rs11264280	1	154862952	AF	T	C	0.3330	0.131028	0.006684	UACR	0.3211	-0.004500	0.002100
rs72700114	1	170193825	AF	C	G	0.0760	0.198851	0.014581	UACR	0.0747	0.008500	0.003900
rs4951258	1	205691316	AF	A	G	0.4160	0.039221	0.007395	UACR	0.3892	-0.006000	0.002000
rs7578393	2	26165528	AF	T	C	0.7960	0.058269	0.007186	UACR	0.8022	0.005100	0.002700
rs11125871	2	61470126	AF	C	T	0.6050	0.039221	0.004906	UACR	0.5952	-0.006000	0.002000
rs2540949	2	65284231	AF	A	T	0.6150	0.067659	0.007186	UACR	0.6223	0.004400	0.002000
rs6747542	2	70106832	AF	T	C	0.5360	0.058269	0.007255	UACR	0.5379	0.002100	0.002000
rs72926475	2	86594487	AF	G	A	0.8770	0.067659	0.009538	UACR	0.8726	-0.000200	0.003000
rs28387148	2	127433465	AF	T	C	0.1050	0.076961	0.011867	UACR	0.0997	0.004300	0.003400
rs67969609	2	145760353	AF	G	C	0.0710	0.067659	0.011867	UACR	0.0982	0.001700	0.003800
rs56181519	2	175555714	AF	C	T	0.7320	0.067659	0.007186	UACR	0.7402	0.001400	0.002300
rs2288327	2	179411665	AF	G	A	0.1560	0.095310	0.009277	UACR	0.1727	-0.001200	0.002600
rs3820888	2	201180023	AF	C	T	0.3920	0.067659	0.007120	UACR	0.3911	0.001800	0.002000
rs35544454	2	213266003	AF	A	T	0.8080	0.058269	0.009628	UACR	0.8161	0.002800	0.002600
rs7650482	3	12841804	AF	G	A	0.6400	0.067659	0.007120	UACR	0.6441	-0.005400	0.002100
rs73041705	3	24463235	AF	T	C	0.7020	0.048790	0.007324	UACR	0.6998	-0.003100	0.002200
rs6790396	3	38771925	AF	G	C	0.5960	0.058269	0.007186	UACR	0.5913	-0.002800	0.002000
rs34080181	3	66454191	AF	G	A	0.6210	0.048790	0.007324	UACR	0.6141	-0.004600	0.002100
rs17005647	3	69406181	AF	T	C	0.3640	0.039221	0.007324	UACR	0.3563	0.000100	0.002100
rs6771054	3	89489529	AF	T	C	0.5960	0.048790	0.007324	UACR	0.5904	0.002700	0.005400

rs10804493	3	111554426	AF	A	G	0.6510	0.058269	0.007255	UACR	0.6583	0.006500	0.002100
rs1278493	3	135814009	AF	G	A	0.4360	0.039221	0.004906	UACR	0.4364	0.001000	0.002000
rs7612445	3	179172979	AF	T	G	0.1880	0.048790	0.009719	UACR	0.1932	-0.007400	0.002500
rs60902112	3	194800853	AF	T	C	0.2260	0.048790	0.007324	UACR	0.2313	0.001200	0.002400
rs1458038	4	81164723	AF	T	C	0.3090	0.039221	0.007324	UACR	0.2938	0.002900	0.002200
rs10006327	4	103890980	AF	C	T	0.4900	0.039221	0.007395	UACR	0.4916	0.000700	0.002000
rs67249485	4	111699685	AF	T	A	0.1990	0.364643	0.007087	UACR	0.2105	-0.000600	0.002400
rs6829664	4	114448656	AF	G	A	0.2620	0.058269	0.007255	UACR	0.2551	-0.001100	0.002300
rs10213171	4	148937537	AF	G	C	0.0610	0.095310	0.011651	UACR	0.0669	-0.001500	0.004200
rs12648245	4	174641184	AF	T	C	0.9240	0.095310	0.011651	UACR	0.9246	-0.006900	0.003900
rs6596717	5	106427609	AF	C	A	0.3950	0.039221	0.007324	UACR	0.3831	0.000300	0.002000
rs337705	5	113737062	AF	G	T	0.3750	0.058269	0.007255	UACR	0.3906	0.002200	0.002000
rs2012809	5	128190363	AF	G	A	0.7900	0.058269	0.009628	UACR	0.8112	-0.001200	0.002600
rs2040862	5	137419989	AF	T	C	0.1780	0.104360	0.006864	UACR	0.1769	0.001600	0.002600
rs6580277	5	142818123	AF	G	A	0.2370	0.067659	0.009538	UACR	0.2400	0.001200	0.002300
rs12188351	5	168386089	AF	A	G	0.0560	0.086178	0.014046	UACR	0.0524	-0.001700	0.004400
rs6891790	5	172670745	AF	G	T	0.7170	0.076961	0.007120	UACR	0.7054	-0.004300	0.002200
rs73366713	6	16415751	AF	G	A	0.8600	0.104360	0.009194	UACR	0.8585	-0.001200	0.002900
rs34969716	6	18210109	AF	A	G	0.3050	0.067659	0.007120	UACR	0.3144	0.003700	0.002300
rs3176326	6	36647289	AF	G	A	0.8020	0.058269	0.007186	UACR	0.8006	0.001700	0.002500
rs2031522	6	87821501	AF	A	G	0.6240	0.039221	0.007324	UACR	0.6123	0.005700	0.002000
rs13195459	6	122403559	AF	G	A	0.6380	0.058269	0.007186	UACR	0.6500	-0.002600	0.002100
rs117984853	6	149399100	AF	T	G	0.1010	0.122218	0.013548	UACR	0.0896	-0.000400	0.003600
rs55734480	7	14372009	AF	A	G	0.2490	0.058269	0.007255	UACR	0.2653	0.002200	0.002300
rs6462079	7	28415827	AF	A	G	0.7210	0.048790	0.007324	UACR	0.7385	0.000400	0.002200
rs11773845	7	116191301	AF	A	C	0.5860	0.104360	0.006864	UACR	0.5892	-0.003500	0.002000
rs55985730	7	128417044	AF	G	T	0.0600	0.086178	0.014046	UACR	0.0559	0.003100	0.004500
rs7789146	7	150661409	AF	G	A	0.8210	0.058269	0.009628	UACR	0.8211	0.004800	0.002600
rs35620480	8	11499908	AF	C	A	0.1570	0.058269	0.007255	UACR	0.1589	-0.002100	0.002700

rs7508	8	17913970	AF	A	G	0.7110	0.067659	0.007120	UACR	0.7210	0.000900	0.002200
rs7834729	8	21821778	AF	G	T	0.8850	0.067659	0.009538	UACR	0.8733	-0.000800	0.003000
rs62521286	8	124551975	AF	G	A	0.0660	0.122218	0.013548	UACR	0.0662	-0.006000	0.004000
rs6994744	8	141740868	AF	C	A	0.4950	0.039221	0.004906	UACR	0.4935	0.006500	0.002000
rs10821415	9	97713459	AF	A	C	0.4130	0.086178	0.007054	UACR	0.4175	0.005200	0.002000
rs2274115	9	139094773	AF	G	A	0.7000	0.048790	0.009719	UACR	0.7059	-0.000600	0.002300
rs12245149	10	65321147	AF	C	A	0.5260	0.048790	0.007324	UACR	0.5179	-0.001100	0.002000
rs7096385	10	69664881	AF	T	C	0.0920	0.067659	0.011867	UACR	0.0763	-0.001200	0.003900
rs60212594	10	75414344	AF	G	C	0.8560	0.113329	0.011340	UACR	0.8554	0.003700	0.002800
rs10458660	10	77936576	AF	G	A	0.1730	0.058269	0.007255	UACR	0.1786	-0.000800	0.002600
rs11598047	10	105342672	AF	G	A	0.1620	0.157004	0.008722	UACR	0.1556	0.002400	0.002700
rs10749053	10	112576695	AF	T	C	0.1580	0.058269	0.009628	UACR	0.1440	-0.003400	0.002900
rs10741807	11	20011445	AF	T	C	0.2450	0.076961	0.007120	UACR	0.2350	-0.002300	0.002300
rs4935786	11	121661507	AF	T	A	0.2670	0.048790	0.007324	UACR	0.2728	0.003000	0.002300
rs76097649	11	128764570	AF	A	G	0.0930	0.113329	0.011340	UACR	0.0890	-0.000700	0.003600
rs4963776	12	24779491	AF	G	T	0.8180	0.095310	0.006990	UACR	0.8216	0.000000	0.002600
rs17380837	12	26345526	AF	C	T	0.6930	0.048790	0.007255	UACR	0.7024	0.000000	0.002200
rs12809354	12	32978437	AF	C	T	0.1440	0.067659	0.009538	UACR	0.1461	-0.000800	0.002800
rs2860482	12	57105938	AF	A	C	0.2740	0.058269	0.007255	UACR	0.2771	0.000600	0.002200
rs71454237	12	70013415	AF	G	A	0.7910	0.058269	0.007186	UACR	0.7892	-0.007700	0.002500
rs12426679	12	76237987	AF	C	T	0.4720	0.039221	0.004906	UACR	0.4935	0.003100	0.002000
rs883079	12	114793240	AF	T	C	0.7070	0.095310	0.006926	UACR	0.7218	-0.001300	0.002200
rs10773657	12	123327900	AF	C	A	0.1380	0.058269	0.009628	UACR	0.1211	-0.000300	0.003100
rs6560886	12	133150210	AF	C	T	0.7880	0.048790	0.009719	UACR	0.8064	-0.007100	0.002700
rs9506925	13	23368943	AF	T	C	0.2670	0.048790	0.007324	UACR	0.2779	0.000900	0.002200
rs35569628	13	113872712	AF	T	C	0.7770	0.048790	0.007324	UACR	0.7645	-0.000800	0.002400
rs422068	14	23864804	AF	C	T	0.3490	0.039221	0.007324	UACR	0.3599	0.000100	0.002100
rs11156751	14	32990437	AF	C	T	0.2850	0.067659	0.007120	UACR	0.2779	0.002100	0.002300
rs73241997	14	35173775	AF	T	C	0.1420	0.076961	0.009449	UACR	0.1553	0.000700	0.002700

rs2738413	14	64679960	AF	A	G	0.4950	0.076961	0.007054	UACR	0.4842	0.001700	0.002000
rs74884082	14	73249419	AF	C	T	0.7500	0.048790	0.009719	UACR	0.7504	-0.002300	0.002300
rs10873298	14	77426525	AF	C	T	0.3660	0.039221	0.007324	UACR	0.3772	0.002300	0.002100
rs74022964	15	73677264	AF	T	C	0.1570	0.113329	0.009112	UACR	0.1579	-0.002400	0.002700
rs12908004	15	80676925	AF	G	A	0.1640	0.076961	0.009449	UACR	0.1620	0.004500	0.002700
rs4965430	15	99268850	AF	C	G	0.3860	0.048790	0.007324	UACR	0.3807	-0.002500	0.002100
rs2359171	16	73053022	AF	A	T	0.1760	0.173953	0.008576	UACR	0.1741	-0.003100	0.002600
rs7225165	17	1309850	AF	G	A	0.8870	0.067659	0.011979	UACR	0.8862	0.002000	0.003200
rs9899183	17	7452977	AF	T	C	0.7140	0.048790	0.007324	UACR	0.7311	-0.003700	0.002200
rs72811294	17	12618680	AF	G	C	0.8870	0.067659	0.011867	UACR	0.8849	0.001000	0.003100
rs1563304	17	44874453	AF	T	C	0.1780	0.067659	0.009538	UACR	0.1767	0.001300	0.002700
rs12604076	17	76773638	AF	T	C	0.4780	0.039221	0.007395	UACR	0.4782	0.002700	0.002000
rs9953366	18	46474192	AF	C	T	0.6630	0.048790	0.007255	UACR	0.6734	0.007700	0.002200
rs8088085	18	48708548	AF	A	C	0.5350	0.039221	0.007395	UACR	0.5487	0.000800	0.002000
rs2834618	21	36119111	AF	T	G	0.8940	0.095310	0.009277	UACR	0.8978	0.004500	0.003300
rs464901	22	18597502	AF	T	C	0.6650	0.048790	0.007255	UACR	0.6532	-0.001700	0.002100
rs133902	22	26164079	AF	T	C	0.4270	0.039221	0.007324	UACR	0.4392	-0.001200	0.002000

Abbreviations: AF, atrial fibrillation; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism; UACR, urine albumin-to-creatinine-ratio.

Table S12. Effect estimates for the associations of the genetic variants with atrial fibrillation and microalbuminuria

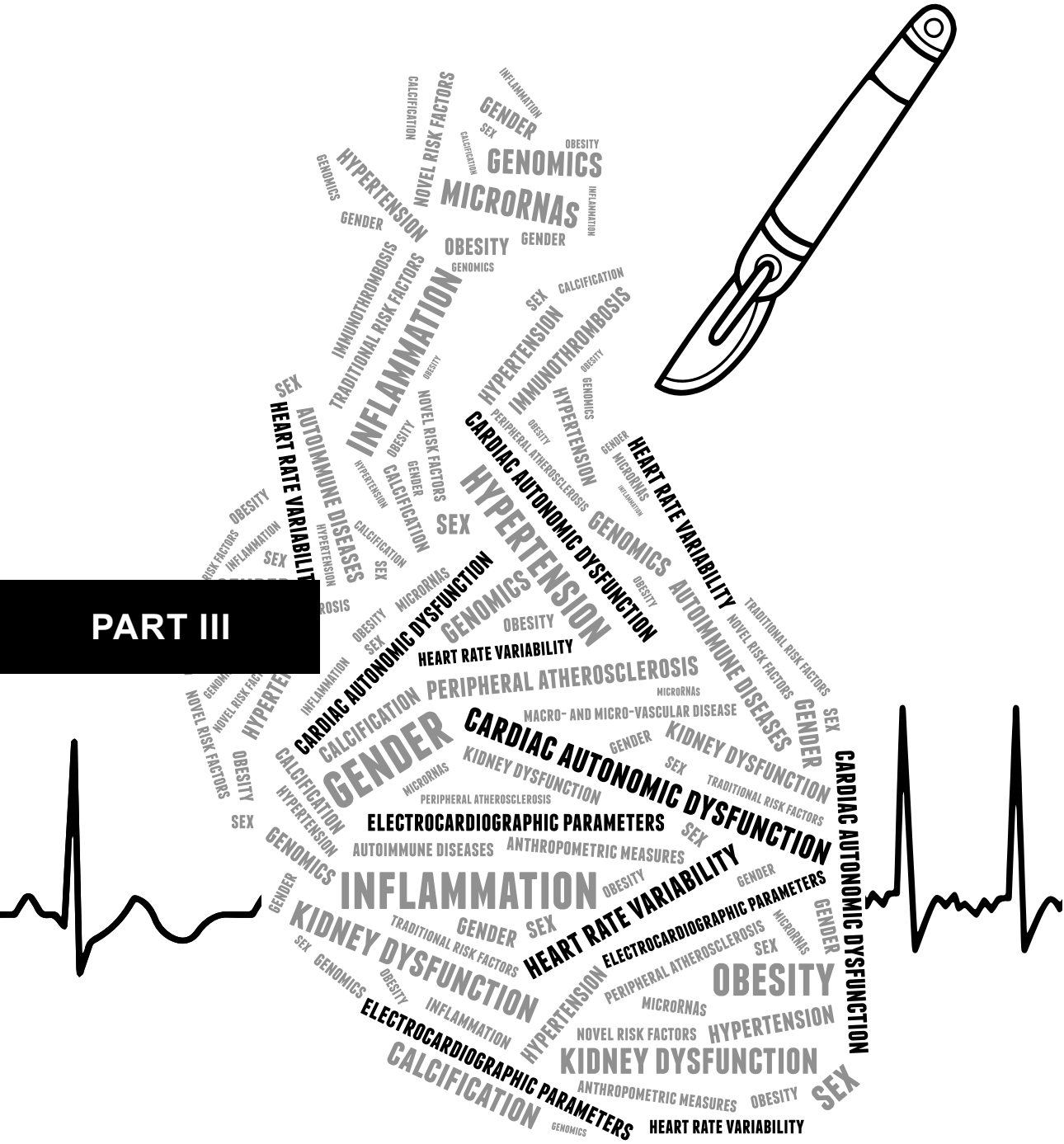
			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs7529220	1	22282619	AF	C	T	0.8470	0.058269	0.009628	MA	0.8555	0.005400	0.009700
rs1545300	1	112464004	AF	C	T	0.6910	0.058269	0.007255	MA	0.6875	0.014000	0.007300
rs4073778	1	116297758	AF	A	C	0.5640	0.048790	0.004859	MA	0.5648	-0.002100	0.006700
rs72700114	1	170193825	AF	C	G	0.0760	0.198851	0.014581	MA	0.0743	0.023800	0.013300
rs7578393	2	26165528	AF	T	C	0.7960	0.058269	0.007186	MA	0.8020	0.005200	0.009000
rs11125871	2	61470126	AF	C	T	0.6050	0.039221	0.004906	MA	0.5964	-0.006800	0.006800
rs2540949	2	65284231	AF	A	T	0.6150	0.067659	0.007186	MA	0.6225	0.015600	0.006900
rs6747542	2	70106832	AF	T	C	0.5360	0.058269	0.007255	MA	0.5372	0.004900	0.006900
rs72926475	2	86594487	AF	G	A	0.8770	0.067659	0.009538	MA	0.8739	0.009500	0.010400
rs28387148	2	127433465	AF	T	C	0.1050	0.076961	0.011867	MA	0.0999	0.024500	0.011400
rs67969609	2	145760353	AF	G	C	0.0710	0.067659	0.011867	MA	0.0979	0.012000	0.013000
rs56181519	2	175555714	AF	C	T	0.7320	0.067659	0.007186	MA	0.7400	0.006900	0.007900
rs2288327	2	179411665	AF	G	A	0.1560	0.095310	0.009277	MA	0.1719	0.004500	0.009000
rs3820888	2	201180023	AF	C	T	0.3920	0.067659	0.007120	MA	0.3909	0.008100	0.006800
rs35544454	2	213266003	AF	A	T	0.8080	0.058269	0.009628	MA	0.8165	0.000600	0.008900
rs73041705	3	24463235	AF	T	C	0.7020	0.048790	0.007324	MA	0.7005	-0.004400	0.007500
rs6790396	3	38771925	AF	G	C	0.5960	0.058269	0.007186	MA	0.5921	-0.002600	0.007000
rs17005647	3	69406181	AF	T	C	0.3640	0.039221	0.007324	MA	0.3565	-0.003500	0.007200
rs10804493	3	111554426	AF	A	G	0.6510	0.058269	0.007255	MA	0.6581	0.016800	0.007100
rs1278493	3	135814009	AF	G	A	0.4360	0.039221	0.004906	MA	0.4358	0.011900	0.006800
rs60902112	3	194800853	AF	T	C	0.2260	0.048790	0.007324	MA	0.2313	0.012500	0.008000
rs1458038	4	81164723	AF	T	C	0.3090	0.039221	0.007324	MA	0.2936	0.013000	0.007400
rs10006327	4	103890980	AF	C	T	0.4900	0.039221	0.007395	MA	0.4908	0.006200	0.006800
rs67249485	4	111699685	AF	T	A	0.1990	0.364643	0.007087	MA	0.2097	0.012900	0.008300
rs6829664	4	114448656	AF	G	A	0.2620	0.058269	0.007255	MA	0.2556	0.001900	0.007700
rs10213171	4	148937537	AF	G	C	0.0610	0.095310	0.011651	MA	0.0669	0.006300	0.014100

rs12648245	4	174641184	AF	T	C	0.9240	0.095310	0.011651	MA	0.9253	-0.001500	0.013500
rs6596717	5	106427609	AF	C	A	0.3950	0.039221	0.007324	MA	0.3828	0.008300	0.007100
rs337705	5	113737062	AF	G	T	0.3750	0.058269	0.007255	MA	0.3907	0.001900	0.006800
rs2012809	5	128190363	AF	G	A	0.7900	0.058269	0.009628	MA	0.8121	-0.006500	0.009000
rs2040862	5	137419989	AF	T	C	0.1780	0.104360	0.006864	MA	0.1770	0.013400	0.008800
rs6580277	5	142818123	AF	G	A	0.2370	0.067659	0.009538	MA	0.2390	-0.006500	0.008100
rs12188351	5	168386089	AF	A	G	0.0560	0.086178	0.014046	MA	0.0526	0.009200	0.015000
rs6891790	5	172670745	AF	G	T	0.7170	0.076961	0.007120	MA	0.7065	0.002600	0.007700
rs73366713	6	16415751	AF	G	A	0.8600	0.104360	0.009194	MA	0.8585	0.004200	0.009900
rs34969716	6	18210109	AF	A	G	0.3050	0.067659	0.007120	MA	0.3141	0.013600	0.007800
rs3176326	6	36647289	AF	G	A	0.8020	0.058269	0.007186	MA	0.8001	0.000500	0.008600
rs13195459	6	122403559	AF	G	A	0.6380	0.058269	0.007186	MA	0.6501	-0.007100	0.007200
rs117984853	6	149399100	AF	T	G	0.1010	0.122218	0.013548	MA	0.0908	0.015500	0.012200
rs55734480	7	14372009	AF	A	G	0.2490	0.058269	0.007255	MA	0.2653	0.012200	0.007800
rs6462079	7	28415827	AF	A	G	0.7210	0.048790	0.007324	MA	0.7395	-0.000600	0.007600
rs56201652	7	92278116	AF	G	A	0.7330	0.048790	0.007255	MA	0.7286	0.017600	0.007500
rs55985730	7	128417044	AF	G	T	0.0600	0.086178	0.014046	MA	0.0559	0.022800	0.015400
rs7789146	7	150661409	AF	G	A	0.8210	0.058269	0.009628	MA	0.8204	0.016700	0.009000
rs35620480	8	11499908	AF	C	A	0.1570	0.058269	0.007255	MA	0.1595	0.003300	0.009200
rs7508	8	17913970	AF	A	G	0.7110	0.067659	0.007120	MA	0.7208	0.003000	0.007500
rs7834729	8	21821778	AF	G	T	0.8850	0.067659	0.009538	MA	0.8723	-0.004200	0.010300
rs6994744	8	141740868	AF	C	A	0.4950	0.039221	0.004906	MA	0.4930	0.007200	0.006700
rs10821415	9	97713459	AF	A	C	0.4130	0.086178	0.007054	MA	0.4168	0.013500	0.006800
rs2274115	9	139094773	AF	G	A	0.7000	0.048790	0.009719	MA	0.7070	-0.009400	0.007800
rs7096385	10	69664881	AF	T	C	0.0920	0.067659	0.011867	MA	0.0750	0.003200	0.013100
rs60212594	10	75414344	AF	G	C	0.8560	0.113329	0.011340	MA	0.8550	0.009800	0.009500
rs10458660	10	77936576	AF	G	A	0.1730	0.058269	0.007255	MA	0.1773	-0.003700	0.008800
rs11598047	10	105342672	AF	G	A	0.1620	0.157004	0.008722	MA	0.1562	0.007600	0.009200
rs10749053	10	112576695	AF	T	C	0.1580	0.058269	0.009628	MA	0.1451	0.006500	0.009700

rs10741807	11	20011445	AF	T	C	0.2450	0.076961	0.007120	MA	0.2346	-0.006200	0.007900
rs4935786	11	121661507	AF	T	A	0.2670	0.048790	0.007324	MA	0.2718	0.009800	0.007900
rs76097649	11	128764570	AF	A	G	0.0930	0.113329	0.011340	MA	0.0895	0.010900	0.012300
rs17380837	12	26345526	AF	C	T	0.6930	0.048790	0.007255	MA	0.7023	0.002000	0.007500
rs12809354	12	32978437	AF	C	T	0.1440	0.067659	0.009538	MA	0.1458	0.005100	0.009700
rs2860482	12	57105938	AF	A	C	0.2740	0.058269	0.007255	MA	0.2756	0.000300	0.007500
rs12426679	12	76237987	AF	C	T	0.4720	0.039221	0.004906	MA	0.4927	-0.003300	0.006700
rs883079	12	114793240	AF	T	C	0.7070	0.095310	0.006926	MA	0.7215	-0.004800	0.007500
rs10773657	12	123327900	AF	C	A	0.1380	0.058269	0.009628	MA	0.1208	-0.001500	0.010500
rs9506925	13	23368943	AF	T	C	0.2670	0.048790	0.007324	MA	0.2780	0.008600	0.007500
rs35569628	13	113872712	AF	T	C	0.7770	0.048790	0.007324	MA	0.7651	0.000400	0.008100
rs422068	14	23864804	AF	C	T	0.3490	0.039221	0.007324	MA	0.3595	0.005500	0.007000
rs11156751	14	32990437	AF	C	T	0.2850	0.067659	0.007120	MA	0.2777	0.010600	0.007900
rs73241997	14	35173775	AF	T	C	0.1420	0.076961	0.009449	MA	0.1553	-0.000600	0.009300
rs2738413	14	64679960	AF	A	G	0.4950	0.076961	0.007054	MA	0.4845	0.007200	0.006700
rs74884082	14	73249419	AF	C	T	0.7500	0.048790	0.009719	MA	0.7501	-0.006600	0.008000
rs10873298	14	77426525	AF	C	T	0.3660	0.039221	0.007324	MA	0.3770	0.004400	0.007000
rs7170477	15	64103777	AF	A	G	0.3040	0.039221	0.004906	MA	0.2955	0.017500	0.007500
rs12908004	15	80676925	AF	G	A	0.1640	0.076961	0.009449	MA	0.1620	0.011100	0.009300
rs7225165	17	1309850	AF	G	A	0.8870	0.067659	0.011979	MA	0.8859	0.017000	0.010900
rs9899183	17	7452977	AF	T	C	0.7140	0.048790	0.007324	MA	0.7308	-0.008100	0.007600
rs1563304	17	44874453	AF	T	C	0.1780	0.067659	0.009538	MA	0.1777	0.021500	0.009100
rs12604076	17	76773638	AF	T	C	0.4780	0.039221	0.007395	MA	0.4780	0.005300	0.006800
rs9953366	18	46474192	AF	C	T	0.6630	0.048790	0.007255	MA	0.6733	0.017400	0.007500
rs8088085	18	48708548	AF	A	C	0.5350	0.039221	0.007395	MA	0.5495	0.001200	0.006700
rs2834618	21	36119111	AF	T	G	0.8940	0.095310	0.009277	MA	0.8975	-0.009500	0.011200
rs464901	22	18597502	AF	T	C	0.6650	0.048790	0.007255	MA	0.6534	-0.009700	0.007000
rs133902	22	26164079	AF	T	C	0.4270	0.039221	0.007324	MA	0.4390	0.000400	0.006800

Abbreviations: AF, atrial fibrillation; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; MA, microalbuminuria; N, sample size; OA, other allele; Pos, genomic position; Pval: p-value, SE: standard error, SNP: single nucleotide polymorphism.

PART III


INFLAMMATION
GENOMICS
MICRORNAS
HYPERTENSION
OBESITY
GENDER
SEX
CALCIFICATION
NOVEL RISK FACTORS
IMMUNOTHROMBOSIS
TRADITIONAL RISK FACTORS
HEART RATE VARIABILITY
AUTOIMMUNE DISEASES
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
MACRO- AND MICRO-VASCULAR DISEASE
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC MEASURES
KIDNEY DYSFUNCTION

Cardiac autonomic dysfunction and the risk of atrial fibrillation


CHAPTER 3.1

Heart rate variability and the risk of atrial fibrillation

Heart rate variability and atrial fibrillation in the general population: a longitudinal and Mendelian randomization study.

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ABSTRACT

Background
Sex differences and causality of the association between heart rate variability (HRV) and atrial fibrillation (AF) in the general population remain unclear.

Methods
12,334 participants free of AF from the population-based Rotterdam Study were included. Measures of HRV including the standard deviation of normal RR intervals (SDNN), SDNN corrected for heart rate (SDNNc), RR interval differences (RMSSD), RMSSD corrected for heart rate (RMSSDc), and heart rate were assessed at baseline and follow-up examinations. Joint models, adjusted for cardiovascular risk factors, were used to determine the association between longitudinal measures of HRV with new-onset AF. Genetic variants for HRV were used as instrumental variables in a Mendelian randomization (MR) analysis using genome-wide association studies (GWAS) summary-level data.

Results
During a median follow-up of 9.4 years, 1,302 incident AF cases occurred among 12,334 participants (mean age 64.8 years, 58.3% women). In joint models, higher SDNN (hazard ratio (HR), 95% confidence interval (CI), 1.24, 1.04-1.47, p=0.0213), and higher RMSSD (HR, 95% CI, 1.33, 1.13-1.54, p=0.0010) were significantly associated with new-onset AF. Sex-stratified analyses showed that the associations were mostly prominent among women. In MR analyses, a genetically determined increase in SDNN (odds ratio (OR), 95% CI, 1.60, 1.27-2.02, $p=8.36x10^{-05}$), and RMSSD (OR, 95% CI, 1.56, 1.31-1.86, $p= 6.32x10^{-07}$) were significantly associated with an increased odds of AF.

Conclusions
Longitudinal measures of uncorrected HRV were significantly associated with new-onset AF, especially among women. MR analyses supported the causal relationship between uncorrected measures of HRV with AF. Our findings indicate that measures to modulate HRV might prevent AF in the general population, in particular in women.

INTRODUCTION

Atrial fibrillation (AF), the most common cardiac arrhythmia, is associated with substantial morbidity and mortality and represents a significant burden on healthcare.(1-4) The exact AF pathogenesis remains to be identified. It has recently been suggested that cardiac autonomic imbalance could play a role in AF pathophysiology by promoting a decline in cardiac function.(5-10)

Heart rate variability (HRV) is considered a non-invasive, accessible measure that may reflect the complex interaction between the autonomic nervous system and the heart.(11, 12) A complex relationship between HRV and AF has been suggested.(5-10, 13) Specifically, lower and higher levels of HRV may lead to decline in cardiac function and subsequently give rise to AF.(5-10, 13) Recent evidence shows that sex differences with regard to AF burden, pathophysiology, and prognosis exist.(14) However, the previous observational studies have been limited to either a cross-sectional design or a single measurement of HRV and did not evaluate sex differences. In addition, observational studies are prone to residual confounding and reverse causality.(15)

Genome-wide association studies (GWAS) have identified genetic variants/single nucleotide polymorphisms (SNPs) for multiple assessments of HRV(16) and AF.(17, 18) Pathway and tissue enrichment analyses suggest that HRV SNPs are preferentially expressed within the sinoatrial node.(16) Moreover, AF SNPs have been suggested to affect the cardiac ion channels, cardiac calcium signaling, and the heart and skeletal muscles.(17, 18) This suggests that there may be a genetic foundation underlying the association between HRV and AF.

We aimed to investigate the association between longitudinal measures of HRV and heart rate with the risk of new-onset AF in the general population. Additionally, we used a comprehensive Mendelian randomization (MR) analysis using summary-level data from GWAS on measures of HRV and AF to investigate the potential causal relationship between HRV and AF.

METHODS

Study design

The current study was embedded within the Rotterdam Study.(19, 20) The Rotterdam Study is a prospective population-based cohort study that aims to assess the occurrence and determinants of age-related diseases in the general population. During 1990-1993, all inhabitants of the Ommoord district in the city of Rotterdam in The Netherlands aged ≥55 years were invited for the study. A total of 7,983 (78% of all invitees) agreed to participate (RS-I). In 2000, the cohort was extended with 3,011 participants who had become ≥55 years or had migrated into the research area (RS-II). In 2006, the cohort was again extended with 3,932 participants who were ≥45 years (RS-III). The overall response rate at baseline was 72%. Participants attended follow-up examinations every 3-6 years. Outcome data on morbidity and mortality were continuously collected through linkage with digital files from general practitioners in the study area.(19, 20)

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Study population

For the present study, we included participants at study entry of the 3 recruitment waves. Participants with prevalent AF at baseline (n=559), no informed consent for follow-up data collection (n=305), no follow-up time (n=6) or no measures of HRV (n=1,722) were excluded. Among the 12,334 free of AF included participants, 12,334 had at least 1 measurement for standard deviation of normal RR intervals (SDNN), SDNN corrected for heart rate (SDNNc), RR interval differences (RMSSD), RMSSD corrected for heart rate (RMSSDc), and heart rate. 8,832 participants had 2 measurements, 3,837 had 3 measurements, 1,817 had 4 measurements, and 787 participants had 5 measurements that were available during follow-up (before date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of heart rate variability

Participants underwent a 10-second 12-lead resting electrocardiogram (ECG) using an ACTA Gnosis IV ECG recorder (Esaote Biomedica, Florence, Italy) and the ECG records were digitally stored. Subsequently, Modular ECG Analysis System (MEANS) was used to interpret the ECGs.(21) ECGs of individuals with a pacemaker, ECGs with <5 RR intervals between normal beats, ECGs with >5 premature supra- and/or ventricular complexes were excluded for the assessment of HRV.(22) In addition, the remaining ECGs marked as non-sinus arrhythmia and sinus arrhythmia by MEANS were manually assessed by 2 medical doctors to rule out and exclude atrial fibrillation/flutter, other arrhythmias, and ECGs with poor signal quality. Sinus rhythm (including sinus arrhythmia) is based on the detection by MEANS of regular P waves that have a fixed coupling interval with the following QRS complexes.(21) Furthermore, a random sample of 200 ECGs marked as sinus rhythm by MEANS were also manually checked by 2 medical doctors and 199 ECGs were found to be in sinus rhythm during the manual assessment indicating a very high positive predictive value of MEANS which was also demonstrated in earlier work.(21) RR intervals between 2 adjacent normal beats were used to compute the mean heart rate and time-domain indices of HRV; SDNN and RMSSD. Moreover, as HRV is potentially inversely and exponentially associated with heart rate, we additionally used heart rate corrected values of RMSSD (RMSSDc), and SDNN (SDNNc) using an exponential model.(22-26) The reproducibility of the HRV data was evaluated in a later cohort of the Rotterdam Study, in which ECG recordings of 3-5 min were made. From a sample of 310 3-5 min ECGs, we extracted from each recording 2 10-second ECGs, one after the first minute of recording, the second after 2 minutes. The sample of 310 pairs of 10-second ECGs was also manually assessed by 2 medical doctors to rule out and exclude arrhythmias, and ECGs with poor signal quality. After exclusion, 211 ECG pairs remained and were used to calculate the HRV measures. Differences were examined using the paired T-test. The HRV measures did not statistically significantly differ from each other (p=0.087 for RR, p=0.415 for SDNN, and p=0.427 for RMSSD).

Assessment of atrial fibrillation
AF was defined in accordance with the European Society of Cardiology (ESC) guidelines.(4) The methods on event adjudication for prevalent and incident AF within the Rotterdam Study have been described in detail earlier.(20) In short, AF was assessed at baseline and follow-up examinations using a 10-second 12-lead ECG with an ACTA Gnosis IV ECG recorder (Esaote Biomedica, Florence, Italy). The ECG records were then stored digitally and analyzed with Modular ECG Analysis System (MEANS).(21) Thereafter, 2 medical doctors validated the diagnosis of AF and in case of disagreement a cardiologist was consulted.(3) Additional follow-up data was obtained from medical files of participating general practitioners, hospitals, outpatient clinics, national registration of all hospitals discharge diagnoses, and follow-up examinations at the research center. The date of incident AF was defined as the date of the first occurrence of symptoms suggestive of AF with subsequent ECG verification obtained from the medical records. Participants were followed from the date of enrolment in the Rotterdam Study until the date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of cardiovascular risk factors
The cardiovascular risk factors included in this study were body mass index (BMI), total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the ECG, use of cardiac medication, use of antihypertensive medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication. Methods for measurements of cardiovascular risk factors are explained in detail in the **Methods S1**.(3, 19, 20)

Selection of genetic variants on heart rate variability and atrial fibrillation
Genetic variants associated with HRV were used as instrumental variables for the MR analyses. The genetic variants were retrieved from publically available summary statistics from 2 GWAS.(16-18) Details regarding the study populations are depicted in **Tables S4** and **S5**. For HRV, we retrieved independent genetic variants from a GWAS on HRV that assessed SDNN and RMSSD as log transformed continuous measures. This GWAS meta-analysis on HRV included 53,174 participants from European descent.(16) In addition, we retrieved independent genetic variants that were associated with AF from a GWAS that included 1,030,836 European participants (60,620 AF cases and 970,216 controls).(17) Only independent genetic variants in the subsequent MR analyses were included ($p<5.0x10^{-08}$ genome-wide significant and $r^2<0.1$).

Statistical analyses

Baseline characteristics

The baseline characteristics of the study population are presented as mean with standard deviation (SD) or number (n) with percentages as appropriate. Differences between men and women were examined by Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) for continuous variables, and Chi-Square test for categorical variables. The distributions of the different HRV measures and heart rate were skewed. Therefore, a natural logarithmic transformation was used to obtain a normal distribution.

Joint models

Competing risk analyses were employed using joint models for longitudinal and time to event data. To investigate the association between longitudinal measures of HRV with the risk of new-onset AF with mortality as a competing event, cause-specific hazard ratios (HRs) with their 95% confidence intervals (CIs) were calculated to quantify the associations (**Figure 1**). See the **Methods S2**. for details on the rationale, imputation and sensitivity analyses of the joint model analyses.(27, 28)

The analyses were done in the total study population and for men and women separately. Additionally, we reported the p values of sex interaction from the joint model. All models (mixed- and survival models) were adjusted for age, sex (if applicable), and cohort (model 1), and additionally for cardiovascular risk factors including body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the ECG, cardiac medication, beta blockers, calcium blockers, and use of lipid lowering medication (model 2). Time was measured in years after baseline and the variables from model 1 and 2 were treated as covariates in the subsequent models.

Mendelian randomization

We conducted two-sample MR analyses to examine the potential causal association between HRV and AF. The inverse variance weighted (IVW) method is the main method used in our analyses.(29) MR estimates were presented as odds ratios (ORs) with corresponding 95% CIs (**Figure 1**). See the **Methods S3**. for more details on the rationale, assumptions and sensitivity analyses of the MR analyses.(15, 29-35)

A two-tailed $p<0.05$ was considered statistically significant. The data management was done using IBM SPSS Statistics version 25.0 for Windows (IBM Corp, Armonk, New York). The statistical analyses were done using the R packages "JMbayes2",(36) and "TwoSampleMR" (30, 34, 35) in R software (R 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).(37)

RESULTS

Baseline characteristics

A total of 12,334 participants, 5,140 men (41.7%) and 7,194 women (58.3%), were eligible for the analyses. Baseline characteristics for the total study population and stratified by sex are presented in **Table 1**. The mean age of the total study population was 64.8 ± 9.5 years and 58.3% were women. Median values for SDNN, SDNNc, RMSSD, RMSSDc, and heart rate were 16.2 ms, 27.0 ms, 16.3 ms, 33.7 ms, and 69.0 beats/min, respectively. See **Table 1** for more details.

Atrial fibrillation incidence

During a median follow-up of 9.4 years (interquartile range (IQR), 6.2-15.1), 1,302 incident AF cases (10.6%) (613 in men and 691 in women) and 4,004 mortality cases (32.5%) (1,740 in men and 2,264 in women) occurred. The incidence rate of AF was 9.6 per 1,000 person-years in the total study population (11.5 per 1,000 person-years in men, 8.4 per 1,000 person-years in women) and the incidence rate of mortality was 29.5 per 1,000 person-years in the total study population (32.6 per 1,000 person-years in men, 27.5 per 1,000 person-years in women).

Joint models

Joint models showed significant associations in model 2 with the risk of new-onset AF in the total study population for a higher SDNN (HR, 95% CI, 1.24, 1.04-1.47, p=0.0213), and a higher RMSSD (HR, 95% CI, 1.33, 1.13-1.54, p=0.0010). However, a higher SDNNc (HR, 95% CI, 1.06, 0.89-1.23, p=0.4784), higher RMSSDc (HR, 95% CI, 1.09, 0.96-1.22, p=0.1774), and a lower heart rate (HR, 95% CI, 1.21, 0.74-1.99, p=0.4781) were not significantly associated with the risk of new-onset AF in the total study population. The effect estimates slightly attenuated in model 2 in comparison to model 1, but SDNN, and RMSSD remained significant. See **Table 2** for more details.

The sex-stratified analyses from model 2 showed that in men only the association for a higher RMSSD (HR, 95% CI, 1.23, 1.01-1.48, p=0.0414) with the risk of new-onset AF was significant. The analyses in women showed significant associations for a higher SDNN (HR, 95% CI, 1.36, 1.03-1.79, p=0.0278), higher RMSSD (HR, 95% CI, 1.47, 1.16-1.89, p=0.0018), and lower heart rate (HR, 95% CI, 1.88, 1.02-3.67, p=0.0408) with the risk of new-onset AF. See **Table 2** for more information. In model 2, the p values of the sex interaction in the joint model for SDNN, SDNNc, RMSSD, RMSSDc, and heart rate were p=0.1077, p=0.7638, p=0.0065, p=0.8465, and p=0.1298, respectively.

All results of the joint model sensitivity analyses are depicted in **Results S1**.

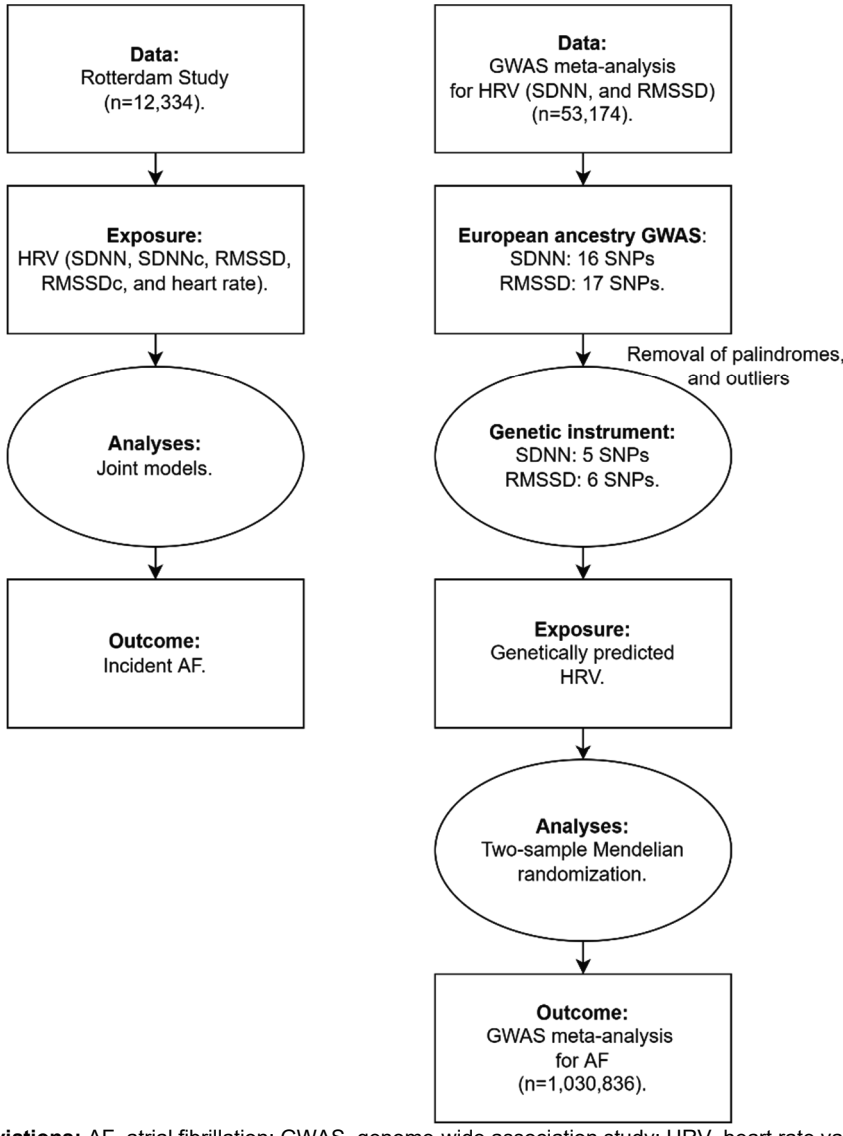
Mendelian randomization

A total of 33 genome-wide significant independent genetic variants were associated with HRV represented by SDNN (n=16) and RMSSD (n=17), respectively. A total of 5 SNPs for SDNN and 6 SNPs for RMSSD were available in the AF GWAS and were used for the MR analyses after removal of potential outliers. All individual genetic instruments for SDNN and RMSSD had a F-statistic >10 (median for SDNN, 53.2 (IQR, 51.8-70.8) and median for RMSSD, 54.7 (IQR, 43.1-69.3)) and were, therefore, considered to be of sufficient strength to be used in the MR analyses. The effect estimates of the genetic variants associated with SDNN, RMSSD and AF that were used in the MR analyses are presented in **Table S4**.

The MR estimates from the association between HRV and AF based on the IVW, weighted median estimator (WME), and MR-Egger methods are presented in **Table 3**. Specifically, MR analyses supported the causal effects of genetically determined SDNN and RMSSD on AF risk (for SDNN: n=5 SNPs, OR, 95% CI, 1.60, 1.27-2.02, $p=8.36x10^{-05}$ and for RMSSD: n=6 SNPs, OR, 95% CI, 1.56, 1.31-1.86, $p=6.32x10^{-07}$). A graphical presentation of the results can be found in **Figure 2**.

The results of the MR sensitivity analyses are depicted in **Results S2**.(33)

Figure 1. Flow chart for the conducted analyses



Abbreviations: AF, atrial fibrillation; GWAS, genome-wide association study; HRV, heart rate variability; SDNN, standard deviation of normal to normal RR intervals; SDNNc, standard deviation of normal to normal RR intervals corrected for heart rate; RMSSD, root mean square of successive RR interval differences; RMSSDc, root mean square of successive RR interval differences corrected for heart rate; SNPs, single nucleotide polymorphisms.

Table 1. Baseline characteristics of the total study population and stratified by sex

Baseline characteristics *	Total study population n=12,334	Men n=5,140	Women n=7,194	p §
Age, years	64.8 ± 9.5	64.0 ± 8.9	65.3 ± 10.0	<0.001
Women, n (%)	7,194 (58.3)	NA	7,194 (100)	NA
Body mass index, kg/m^2	27.0 ± 4.1	26.7 ± 3.6	27.2 ± 4.5	<0.001
Total cholesterol, mmol/L †	6.1 ± 1.2	5.9 ± 1.2	6.4 ± 1.2	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.4 ± 0.4	1.2 ± 0.3	1.5 ± 0.4	<0.001
Systolic blood pressure, mmHg	138.4 ± 21.4	139.0 ± 20.5	138.0 ± 22.1	0.011
Diastolic blood pressure, mmHg	77.7 ± 11.8	78.9 ± 11.8	76.9 ± 11.8	<0.001
Hypertension, n (%)	7225 (58.6)	3010 (58.6)	4215 (58.7)	0.928
Smoking status				<0.001
Never, n (%)	3,915 (32.3)	717 (14.1)	3,198 (45.3)	
Former, n (%)	5,293 (43.6)	2,884 (56.7)	2,409 (34.2)	
Current, n (%)	2,931 (24.1)	1,484 (29.2)	1,447 (20.5)	
History of diabetes mellitus, n (%)	1,268 (10.3)	595 (11.6)	673 (9.4)	<0.001
History of coronary heart disease, n (%)	756 (6.3)	547 (10.9)	209 (3.0)	<0.001
History of heart failure, n (%)	210 (1.7)	84 (1.6)	126 (1.8)	0.617
Left ventricular hypertrophy, n (%)	696 (6.2)	402 (8.7)	294 (4.5)	<0.001
Cardiac medication, n (%)	646 (5.5)	299 (6.2)	347 (5.1)	0.012
Antihypertensive medication, n (%)	3,344 (28.6)	1,345 (27.7)	1,999 (29.3)	0.068
Beta blockers, n (%)	1591 (13.6)	698 (14.4)	893 (13.1)	0.042
Calcium blockers, n (%)	1,006 (8.6)	453 (9.3)	553 (8.1)	0.019
Lipid lowering medication, n (%)	1,274 (10.9)	616 (12.7)	658 (9.6)	<0.001
SDNN, ms ‡	16.2 (10.3-26.8)	16.2 (10.1-27.5)	16.2 (10.4-26.2)	0.705
SDNNc, ms ‡	27.0 (16.8-45.9)	25.1 (14.9-43.7)	28.3 (18.2-47.6)	<0.001
RMSSD, ms ‡	16.3 (10.4-26.5)	16.0 (9.9-26.3)	16.4 (10.7-27.0)	0.002

RMSSDc, ms ‡	33.7 (20.5-59.4)	28.7 (17.2-53.3)	36.9 (23.3-63.3)	<0.001
Heart rate, beats/min ‡	69.0 (62.0-76.7)	67.0 (60.1-75.3)	70.3 (63.6-77.6)	<0.001

Values are shown before imputation and therefore not always add up to 100%.

Abbreviations: RMSSD, root mean square of successive RR interval differences; RMSSDc, root mean square of successive RR interval differences corrected for heart rate; SDNN, standard deviation of normal to normal RR intervals; SDNNc, standard deviation of normal to normal RR intervals corrected for heart rate.

* Values are mean (standard deviation) or number (percentages).

† SI conversion factors: To convert cholesterol to mg/dL, divide values by 0.0259.

‡ Non-transformed median with interquartile range.

§ Statistical significance for continuous variables was tested using the Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) and for categorical variables was tested using the Chi Square test.

Table 2. Association between longitudinal measures of heart rate variability with the risk of new-onset atrial fibrillation in the total study population and stratified by sex

Heart rate variability measures	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
SDNN ‡	**1.22 (1.02-1.48), p=0.0287**	**1.24 (1.04-1.47), p=0.0213**	1.09 (0.87-1.38), p=0.4659	1.16 (0.92-1.45), p=0.2103	**1.46 (1.13-2.03), p=0.0031**	**1.36 (1.03-1.79), p=0.0278**
SDNNc ‡	1.00 (0.86-1.17), p=0.9741	1.06 (0.89-1.23), p=0.4784	1.04 (0.86-1.26), p=0.7112	1.10 (0.90-1.32), p=0.3122	0.98 (0.77-1.27), p=0.8162	1.01 (0.81-1.27), p=0.9274
RMSSD ‡	**1.38 (1.20-1.60), p<0.0001**	**1.33 (1.13-1.54), p=0.0010**	**1.21 (1.01-1.46), p=0.0392**	**1.23 (1.01-1.48), p=0.0414**	**1.60 (1.29-2.00), p<0.0001**	**1.47 (1.16-1.89), p=0.0018**
RMSSDc ‡	1.06 (0.94-1.20), p=0.3302	1.09 (0.96-1.22), p=0.1774	1.10 (0.94-1.28), p=0.2320	1.13 (0.96-1.30), p=0.1411	1.02 (0.85-1.21), p=0.8413	1.04 (0.87-1.23), p=0.6994
Heart rate ‡	1.60 (0.90-2.80), p=0.1083	1.21 (0.74-1.99), p=0.4781	0.77 (0.42-1.40), p=0.3979	0.66 (0.36-1.19), p=0.1643	**2.21 (1.13-4.10), p=0.0261**	**1.88 (1.02-3.67), p=0.0408**

Abbreviations: CI, confidence interval; HR, hazard ratio; RMSSD, root mean square of successive RR interval differences; RMSSDc, root mean square of successive RR interval differences corrected for heart rate; SDNN, standard deviation of normal to normal RR intervals; SDNNc, standard deviation of normal to normal RR intervals corrected for heart rate.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, cardiac medication, beta blockers, calcium blockers, and use of lipid lowering medication.

‡ Hazard ratios represent 1 unit increase of ln(SDNN), ln(SDNNc), ln(RMSSD), ln(RMSSDc), and 1 unit decrease of ln(heart rate) with the risk of new-onset atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

Table 3. Mendelian randomization analyses between heart rate variability and atrial fibrillation

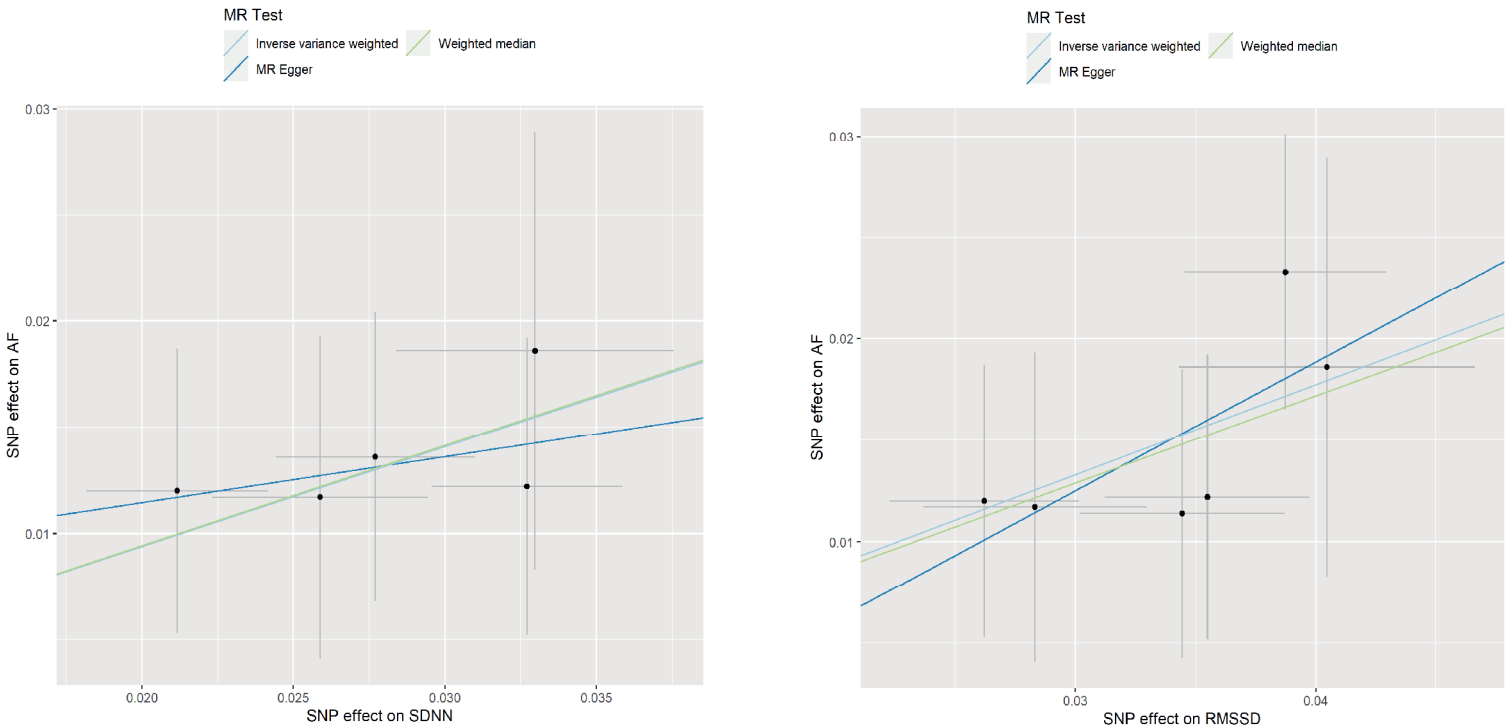
Method	SDNN (n=5 SNPs)		RMSSD (n=6 SNPs)	
	OR (95% CI) *	**p**	**OR (95% CI) ***	**p**
IVW	**1.60 (1.27-2.02)**	**$8.36x10^{-05}$**	**1.56 (1.31-1.86)**	**$6.32x10^{-07}$**
WME	**1.60 (1.22-2.11)**	**$8.28x10^{-04}$**	**1.54 (1.23-1.92)**	**$1.49x10^{-04}$**
MR-Egger slope	1.24 (0.29-5.40)	$7.92x10^{-01}$	1.89 (0.58-6.08)	$3.48x10^{-01}$
MR-Egger intercept	1.01 (0.97-1.05)	$7.55x10^{-01}$	0.99 (0.96-1.03)	$7.63x10^{-01}$
Heterogeneity	NA	$9.82x10^{-01}$	NA	$9.26x10^{-01}$
Global test MR-PRESSO	NA	$9.92x10^{-01}$	NA	$9.08x10^{-01}$

Abbreviations: CI, confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; NA, not applicable; OR, odds ratio; RMSSD, the root mean square of successive RR interval differences; SDNN, standard deviation of normal to normal RR intervals; SNPs, single nucleotide polymorphisms; WME, weighted median estimator.

* Odds ratios represent a genetically determined 1 unit increase of ln(SDNN), and ln(RMSSD) with the odds of atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

Figure 2. Scatter plot which visualizes the causal effect estimate for each individual genetic variant on SDNN, and RMSSD and its effect on atrial fibrillation



Abbreviations: IVW, inverse variance weighted; RMSSD, the root mean square of successive RR interval differences; SDNN, standard deviation of normal to normal RR intervals; SNPs, single nucleotide polymorphisms; WME, weighted median estimator.
The dots represent the corresponding effect estimates of each individual genetic variant. The crosses around the dots represent the 95% confidence intervals of the corresponding effect estimates of each individual genetic variant.

DISCUSSION

Our study shed light on the complex interaction between HRV and AF. Our joint model analyses showed that longitudinal measures of SDNN, and RMSSD were significantly associated with new-onset AF in the general population while SDNNc, RMSSDc, and heart rate were not significantly associated. Sex-stratified analyses showed that RMSSD among men, and SDNN, RMSSD, and heart rate among women were significantly associated with new-onset AF. MR analyses supported the causal association between SDNN, and RMSSD with AF. Our findings indicate that treatment to modulate HRV might prevent AF in the general population, in particular in women.

The exact mechanism that underlies the relationship between HRV and AF remains incompletely understood. Shared underlying risk factors such as obesity, diabetes mellitus, and coronary heart disease could influence HRV and are also implicated in AF pathophysiology.(1, 5, 12, 38, 39) In our study, however, the associations of HRV with incident AF slightly attenuated, but remained significant after extensive adjustment for shared cardiovascular risk factors. The increase in left atrial size that has been associated with HRV could suggest a role for HRV in AF pathogenesis that is mediated by the left atrium.(13) Moreover, autonomic imbalance could trigger an inflammatory response that can subsequently lead to AF.(12) Finally, the effect of the GWAS-identified HRV SNPs on the genes (especially, *GNG11*, *RGS6*) that are preferentially expressed within the sinoatrial node underlines the genetic basis that potentially underlies the association between HRV and AF. In short, these genes may affect acetylcholine release of the vagal nerves within the sinoatrial node and thereby influence HRV.(16) More specifically, *GNG11* codes for the γ11 subunit of the heterotrimeric G-protein complex Gαβγ and may cause a decreased expression of this subunit.(16) This lower availability of this subunit may then reduce Gβγ induced GIRK activation. This potentially blunts heart rate changes caused by oscillatory changes in cardiac vagal activity, ultimately decreasing HRV.(16) Furthermore, *RGS6* regulates the heterotrimeric G-protein complex signaling type 6 and may increase its availability. This leads to a decreased GIRK activation and potentially blunts the effects in cardiac vagal activation, and may thereby decrease HRV.(16) Subsequently, it has been suggested that sinus node disease (SND) may cause AF by promoting atrial extrasystoles, and re-entry.(40, 41) Atrial extrasystoles may occur during the slow atrial cycle in the presence of SND. Atrial extrasystoles are mostly followed by a compensatory pause. The pause may then be prolonged which allows other atrial ectopic activity to arise which possibly triggers AF.(40) Early premature beats that originate from areas other than the sinus node may result in conduction block and initiate re-entry, which may be a mechanism underlying AF.(40) Furthermore, stenosis in the sinus nodal artery is also common in patients with AF which implies that ischemic damage to the sinus node alone without atrial

fibrosis, stretch or muscle loss may result in AF.(40) Overall, a combination of atrial extrasystoles, re-entry, and ischemia to the sinus node are mechanisms by which SND may cause and promote AF.

We investigated the longitudinal measures of HRV during a long follow-up time in relation to new-onset AF. Taking into account repeated measurements of HRV in relation to new-onset AF may provide more insight and prognostic information over a single baseline measurement that has been done by most of the previous studies.(5-10, 13) Longitudinal measures of HRV during follow-up were associated with an increased risk of incident AF, especially among women. These findings extend previous evidence by simultaneously evaluating the repeated measurements of uncorrected and corrected HRV, heart rate, and sex differences while investigating the link between HRV and AF.(5-10, 13) To some extent our findings support the association between heart rate and AF that has been previously reported in observational,(6, 7, 42) and Mendelian randomization studies.(43) However, we only found a significant association for heart rate in association with AF among women. One potential explanation could be differences in sex hormones. It has been demonstrated that an acute ovarian hormone withdrawal induced by oophorectomy leads to decline in different measures of HRV (SDNN, RMSSD), and an increase in heart rate in women.(44) The same study also showed that estrogen replacement therapy for 3 months within the oophorectomized women restored the HRV and heart rate to a pre-surgery level.(44) This might explain why uncorrected HRV and heart rate were only associated with incident AF in women, and not in men, in our study. We further hypothesize that competing risk of death is a possible explanation for the observed sex differences. AF is strongly associated with age,(1-3) so it is likely that men die of other (cardiovascular) diseases before development of AF. This hypothesis was supported by our competing risk analyses which showed that SDNN, RMSSD, RMSSDc, and heart rate were significantly associated with mortality, especially among men. Nevertheless, we found a higher incidence of AF in men, than in women in our study.

Our MR approach sheds light on the causality of the association between HRV and AF. Our effect estimates were more or less in line with previous observational studies. However, we were unable to assess the association between SDNNc, RMSSDc and AF, since not enough instrumental variables for SDNNc and RMSSDc were available to be used for the MR analyses. Future GWAS with a larger sample size could identify new additional genetic variants that could be used to assess the association between heart rate corrected HRV and AF. This could be of importance, because of the strong inverse association that exists between HRV and heart rate.(24, 25) This relation is further underlined by Nolte et al. who showed attenuation in the HRV SNP associations when they corrected for heart rate.(16) This might imply that uncorrected measures of HRV may be, in part, confounded by heart rate. Although, we showed that heart rate itself was not significantly associated with new-

onset AF (except in women), we cannot rule out the possibility that heart rate is the overall determining factor instead of HRV after all. Since our uncorrected measures of HRV were indeed not significantly associated with new-onset AF. Further, as heart rate is also associated with AF and cardiovascular mortality proper adjustment for heart rate is of importance.(26, 43, 45, 46) However, excluding a genetic variant that was also associated with heart rate, a potential confounder or horizontal mediator, did not substantially change our MR results. Future studies on HRV measures corrected for heart rate could further aid in elucidating the exact mechanisms underlying HRV and AF.

The major strengths of this study are its population-based nature, large sample size with detailed information on cardiovascular risk factors, meticulous adjudication of incident AF and long follow-up time, multiple sensitivity analyses including complete-case analyses, excluding prevalent and incident CHD prior to AF diagnosis, use of competing risk analyses to compute cause-specific hazards, and use of large-scale GWAS summary-statistics. The availability of repeated measurements for different HRV measures during follow-up also enabled us to investigate longitudinal measures of HRV in association with new-onset AF in a joint modeling approach which may provide more insight and give more prognostic information over a single baseline measurement. Moreover, by using a MR approach we were able to gain more insight in the complex interaction between HRV and AF and to avoid certain biases that are more common in traditional observational epidemiological studies such as residual confounding and reverse causation.(15) However, our study also has some limitations that should be taken into consideration. Our HRV measures were based on 10-second ECGs, although HRV guidelines recommend that HRV measures are based on preferably 5-minute or 24-hour ECG recordings.(22) Nevertheless, 10-second ECGs are more commonly performed in healthcare, are cheaper, are faster, and thereby more patient friendly than longer ECG recordings. Additionally, HRV measures from 10-second ECGs have already been associated with left ventricular function,(47) heart failure,(47, 48) cardiac- (49) and all-cause mortality.(50) Additionally, other studies that investigated the reliability of 10-second ECGs in comparison to 5-minute ECGs to assess HRV showed that 10-second ECGs are also a reliable tool for HRV risk assessment, in particular within population-based studies.(51, 52) We could not distinguish between paroxysmal, persistent, long-term persistent, and permanent AF as Holter monitoring has not been done in this large population-based cohort. In our MR analyses, we cannot rule out unobserved horizontal pleiotropy, although we tried to address horizontal pleiotropy by using multiple MR sensitivity analyses such as MR-Egger, WME, MR-PRESSO, and sensitivity plots to identify and correct for horizontal pleiotropy. Additionally, not enough sex-stratified SNPs were available in the publically available genetic dataset to perform the MR for men and women separately. Furthermore, there was partial overlap in the samples that were used to obtain the genetic instruments which may cause bias towards observational findings.(33) However, the

potential bias was probably negligible given that the maximum potential overlap was 2.1%. Finally, our findings may not be generalizable to younger populations and other ethnicities, as our analysis included mainly older participants from European descent.

In conclusion, longitudinal measures of SDNN, RMSSD, but not SDNNc, RMSSDc, and heart rate, were significantly associated with new-onset AF. In sex-stratified analyses, RMSSD among men and SDNN, RMSSD, and heart rate among women were significantly associated with new-onset AF. MR analysis confirmed the complex association between HRV and AF that has been indicated by our and previous observational studies. These findings indicate that measures to modulate HRV might prevent AF in the general population, especially among women, but future MR studies that investigate the causality between heart rate corrected measures of HRV and AF are warranted.

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SUPPLEMENTARY MATERIAL

Chapter 3.1 Heart rate variability and the risk for atrial fibrillation

Methods S1. Assessment of cardiovascular risk factors

Methods S2. Joint model analyses

Methods S3. Mendelian randomization analyses

Methods S4. Study population of the genome-wide association study from which the genetic instruments for heart rate variability were obtained

Methods S5. Study population of the genome-wide association study from which the genetic instruments for atrial fibrillation were obtained

Results S1. Joint model sensitivity analyses

Results S2. Mendelian randomization sensitivity analyses

Table S1. Association between longitudinal measures of heart rate variability with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with non-imputed data

Table S2. Association between longitudinal measures of heart rate variability with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with exclusion of prevalent and incident coronary heart disease cases before atrial fibrillation

Table S3. Association between longitudinal measures of heart rate variability with the risk of mortality in the total study population and stratified by sex

Table S4. Effect estimates for the associations of the genetic variants with SDNN, RMSSD and atrial fibrillation

Methods S1. Assessment of cardiovascular risk factors

All participants responded to comprehensive questionnaires at baseline to evaluate their current health status, medical history, medication use, and lifestyle. In addition, the participants were interviewed at home by trained interviewers, underwent more extensive clinical examination, and laboratory assessments at the research center.(19, 20)

Standardized measurements of height (in m) and weight (in kg) were performed and body mass index (BMI) was calculated as weight divided by height squared. Serum total and high-density lipoprotein (HDL) cholesterol were measured with an automated enzymatic method. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressures were calculated as the mean of the 2 consecutive measurements. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or the use of antihypertensive medication prescribed for hypertension.(3) Smoking status was derived from baseline questionnaires and was categorized into never, former, and current smokers. Diabetes mellitus (DM) was defined as fasting serum glucose levels ≥7.0 mmol/L (126 mg/dL) (or non-fasting serum glucose levels ≥11.1 mmol/L (200 mg/dL) if fasting samples were unavailable) or the use of antidiabetic therapy. The assessment and definition of coronary heart disease (CHD), and heart failure (HF) has been described in detail elsewhere.(20) Left ventricular hypertrophy (LVH) was diagnosed using MEANS with an algorithm that takes into account QRS voltages, with an age-dependent correction and repolarization. Medication use was derived from baseline questionnaires, pharmacy data and was categorized and defined according to the World Health Organization Anatomical Therapeutic Chemical (WHO ATC) classifications. Specifically, cardiac medication, antihypertensive medication, use of beta blockers, use of calcium blockers, and lipid lowering medication were defined according to the WHO ATC categories c01, c02, c07, c08, and c10 respectively.

Methods S2. Joint model analyses

The baseline characteristics of the study population are presented as mean with standard deviation (SD) or number (n) with percentages as appropriate. Differences between men and women were examined by Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) for continuous variables and Chi-Square test for categorical variables. The distributions of the different HRV measures and heart rate were skewed. Therefore, a natural logarithmic transformation was used to obtain a normal distribution.

Competing risk analyses were performed using joint models for longitudinal and time to event data. To investigate the association between longitudinal measures of HRV with the risk of new-onset AF with mortality as a competing event. Cause-specific hazard ratios (HRs) with their 95% confidence intervals (CIs) were calculated to quantify the associations. First, linear mixed effects models were used to model the longitudinal measures of HRV and to account for the correlation of repeated measures. The outcome of interest in each mixed effects model was either SDNN, SDNNc, RMSSD, RMSSDc, and heart rate with up to 5 repeated measurements during follow-up. Time was measured in years after baseline and cardiovascular risk factors/covariates were treated as fixed effects in all models. The models investigating the longitudinal measures of SDNN, SDNNc, RMSSD, RMSSDc, and heart rate included a random intercept and slope, and an unstructured covariance matrix. Next, the results from the mixed effects models and Cox models were combined. This was done using the repeated measures for either SDNN, SDNNc, RMSSD, RMSSDc, and heart rate from the linear mixed effects models as a time-dependent covariate in the Cox models.(27)

The analyses were performed in the total study population and for men and women separately. Additionally, we reported the p of sex interaction from the joint model. All models (mixed- and survival models) were adjusted for age, sex (if applicable), and cohort (model 1), and additionally for cardiovascular risk factors including BMI, total cholesterol, HDL cholesterol, hypertension, smoking status, history of DM, history of CHD, history of HF, LVH on the ECG, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication (model 2). Time was measured in years after baseline and the variables from model 1 and 2 were treated as covariates in the subsequent models. Missing values of any covariates were imputed under the assumption of missing at random using the “mice” package in R.[28] For imputation, all available data were used to generate 1 imputed dataset. Missing values for various covariates were as follows: BMI (2.0%), total cholesterol (6.9%), HDL cholesterol (6.8%), systolic blood pressure (1.2%), diastolic blood pressure (1.2%), smoking status (1.6%), history of CHD (2.4%), history of HF (0.1%), LVH on the ECG (9.5%), use of cardiac medication (5.3%), use of antihypertensive medication (5.4%), use of beta blockers (5.3%), use of calcium blockers (5.3%), and use of lipid lowering medication (5.3%).

As sensitivity analyses, we assessed the associations using complete-case analyses based on non-imputed data. Moreover, we assessed the associations after exclusion of participants with prevalent CHD and incident CHD before the onset of AF to assess if this would attenuate our original findings. Finally, we calculated the cause-specific HRs for mortality to evaluate the competing risk of mortality with incident AF.

Methods S3. Mendelian randomization analyses

MR enables assessment of causality by using information on genetic variants. As genetic variants are randomly distributed from parents to offspring at conception, this random distribution of alleles is thereby not influenced by confounding or disease status (reverse causation).(15) We conducted two-sample MR analyses to examine the potential causal association between HRV and AF. MR analyses requires 3 assumptions to provide valid causal estimates.(15) The first assumption is that the genetic variant is strongly associated with the corresponding exposure (HRV). The second assumption is that the genetic variant only affects the outcome (AF) through its effect on the exposure (HRV) and the third assumption is that the genetic variant is not associated with any confounders of the exposure-outcome (HRV-AF) association.

We used the TwoSampleMR package(30, 34, 35) to estimate the effects of the individual genetic instruments using inverse-variance weighted (IVW) analyses.[29] The IVW method is a combined estimate of the Wald ratios from all the individual genetic variants. This means that the IVW method is a weighted mean estimate of the effect of a genetically determined HRV on AF risk. Moreover, we used the random effect IVW method to account for possible heterogeneity between the genetic variants and to relax the no horizontal pleiotropy assumption. MR estimates are presented as odds ratios (ORs) with corresponding 95% confidence intervals (CIs).

Furthermore, the F-statistic, a strength measure for the genetic instruments, was calculated for each genetic instrument and a value of F>10 was considered as sufficient strength.(30) We only included genetic variants with F>10 to limit weak instrument bias and to meet the first MR assumption. Furthermore, if the genetic variants show horizontal pleiotropic effects that influence the outcome through pathways other than through the exposure this may lead to biased MR estimates. Therefore, in an attempt to meet the second and third MR assumption, we performed additional analyses including weighted median estimator (WME), MR-Egger and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) to account and test for horizontal pleiotropy.(31, 32) We used the weighted median estimator (WME) method, which gives a weighted median effect of genetically determined HRV on AF risk.(15) The WME method assumes that only half the genetic variants need to be valid genetic instruments (so, no violation of the 3 MR assumptions for half of the genetic variants). The MR-Egger intercept will not significantly deviate from zero and the MR-Egger slope will be in line with the IVW and WME in the absence of horizontal pleiotropy. In short, if we obtain similar effect estimates from the IVW, WME, MR-Egger slope this would indicate that the MR results are robust.(32) In addition, we used MR-PRESSO to identify horizontal pleiotropic outliers and to provide an outlier-corrected estimate.[31] Moreover,

sensitivity plots such as scatter plots were also used to identify potential outliers, if needed. Heterogeneity between the genetic variants could also be an indication of horizontal pleiotropy and we therefore tested heterogeneity using Cochran's Q test. Furthermore, we excluded genetic variants that were also associated with heart rate, a potential confounder, horizontal mediator of the exposure-outcome association, since this may bias our estimates. Finally, we determined the potential overlap between the study samples that were used to identify the genetic variants, because sample overlap in two-sample MR analyses might potentially cause bias towards the observational results (**Methods S4** and **S5**).(33)

Methods S4. Study population of the genome-wide association study from which the genetic instruments for heart rate variability were obtained

N of studies	N of participants	Ethnicity	Phenotype definition
29	Discovery analysis in 28,700 individuals.	European (100%).	HRV traits were extracted from the IBI time series preferably based on 2-10 min periods of ECG in a standardized setting, at rest and in a sitting/supine position.
Abbreviations of the included studies *			
ARIC EA, CARLA, **CHS**, **FHS**, **FINCAVAS**, FINGESTURE, FLEMNGHO-EPOGH, GenR, GTR, **KORA S4**, Lifelines, **MESA**, MRC NSHD, MRS, NESDA, NFBC 1966, NTR, **PIVUS**, **PREVEND**, **RS I**, **RS II**, TRAILS-CC, TRAILS-Pop, UCSD TWINS, **ULSAM**, WHI CT-Garnet, WHI CT-MOPMAP, WHII, YFS.			

The data with the potential overlapping samples with GWAS for atrial fibrillation are marked in **bold**. The exact extent of overlap could not be determined due to unavailability of individual level data, however considering the names of the included studies in the GWAS. The potential overlap was possible between HRV and AF for 21,617 individuals.

* For further details, please see Table S2 of the GWAS from Nolte et al.(16)

Methods S5. Study population of the genome-wide association study from which the genetic instruments for atrial fibrillation were obtained

N of studies	N of participants	Ethnicity	Phenotype definition
40	Discovery analysis in 1,030,836 individuals. AF: 60,620 cases, and 970,216 controls.	Mainly European (>98%).	AF cases were defined as patients with ICD-9: 427.31 or ICD-10: I48.
Abbreviations of the included studies * †			
AGES, ANGES, ARIC AA, **ARIC EA**, Beat-AF, Biobank Japan, BioMe-Omni AA, BioMe Omni-EA, BioMe-Omni HA, BioVU, CCAF, **CHS AA**, **CHS EA**, COROGENE, deCODE, DiscovEHR, **FHS**, **FINCAVAS**, GS:SFHS, HUNT, **KORA**, LURIC, MDC-CC/MDCS, **MESA**, MGH AF study, MGH CAMP, MGI, **PIVUS**, **PREVEND**, PROSPER, **RS I**, **RS II**, RS III, SHIP, SPHFC, TwinGene, UK Biobank, **ULSAM**, WGHS, WTCCC2 Munich.			

The data with the potential overlapping samples with GWAS for HRV are marked in **bold**. The exact extent of overlap could not be determined due to unavailability of individual level data, however considering the names of the included studies in the GWAS. The potential overlap was possible between HRV and AF for 21,617 individuals.

* For further details, please see Table S1 of the original GWAS from Nielsen et al.(17)

† For further details, please see Table S1 of the original GWAS from Christophersen et al.(18)

Results S1. Joint model sensitivity analyses

Our sensitivity analyses showed that the results after imputation did not differ substantially from the complete-case analyses (**Table S1**). Moreover, excluding participants with prevalent and incident CHD (prior to incident AF) from the analyses did not change the original results noteworthy (**Table S2**). Lastly, in our competing risk analyses, SDNN, RMSSD, RMSSDc, and heart rate were all significantly associated with mortality in both model 1 and 2 which confirms that mortality is a potential competing risk for incident AF, especially among men (**Table S3**).

Results S2. Mendelian randomization sensitivity analyses

The estimates of the WME and MR-Egger slope method were in line with the IVW method after correcting for outliers using MR-PRESSO and additionally examining the sensitivity plots such as the scatter plots during the analyses. In addition, we found no evidence for the presence of directional horizontal pleiotropy after removal of outliers using the MR-Egger intercept (p for SDNN: $7.55x10^{-01}$, and p for RMSSD: $7.63x10^{-01}$) and MR-PRESSO (p for SDNN: $9.92x10^{-01}$, and p for RMSSD: $9.08x10^{-01}$). Moreover, we found no evidence for heterogeneity between the genetic variants using Cochran's Q test (p of the IVW method for SDNN: $9.82x10^{-01}$, and p of the IVW method for RMSSD: $9.26x10^{-01}$). See **Table 3** for more detailed information. Similar results were observed when we excluded a genetic variant in our analyses that was also associated with heart rate which could be a potential confounder and horizontal mediator (data not shown). Determination of the exact extent of sample overlap between the 2 study samples was not possible due to unavailability of individual level data. However, considering the study names included in both GWAS, there was potential overlap between HRV and AF for 21,617 individuals and to what extent this might have biased our results is uncertain. However, given the sample size of the largest GWAS which included 1,030,836 participants,(17) the potential overlap was probably negligible (21,617/1,030,836=2.10%) See **Methods** S**4** and **S5** for detailed information on both GWAS.

Table S1. Association between longitudinal measures of heart rate variability with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with non-imputed data

	Total study population		Men		Women	
Heart rate variability measures	**Cause-specific HR (95% CI)**					
	Model 1 *	**Model 2** †	**Model 1** *	**Model 2** †	**Model 1** *	**Model 2** †
SDNN ‡	1.19 (0.98-1.45), p=0.0808	1.22 (0.97-1.50), p=0.0798	1.00 (0.79-1.28), p=0.9539	1.02 (0.78-1.32), p=0.8994	**1.59 (1.12-2.23), p=0.0101**	**1.50 (1.10-2.02), p=0.0090**
SDNNc ‡	0.99 (0.84-1.17), p=0.9260	1.06 (0.87-1.26), p=0.4903	0.95 (0.77-1.18), p=0.6448	0.98 (0.79-1.19), p=0.8509	1.05 (0.80-1.37), p=0.6977	1.13 (0.88-1.46), p=0.3448
RMSSD ‡	**1.35 (1.16-1.59), p=0.0001**	**1.32 (1.11-1.58), p=0.0026**	1.11 (0.90-1.38), p=0.3262	1.10 (0.88-1.37), p=0.3813	**1.76 (1.34-2.28), p=<0.0001**	**1.60 (1.26-2.03), p=0.0001**
RMSSDc ‡	1.05 (0.92-1.21), p=0.4557	1.09 (0.94-1.25), p=0.2119	1.02 (0.87-1.20), p=0.8034	1.04 (0.87-1.22), p=0.6484	1.11 (0.88-1.38), p=0.3193	1.15 (0.95-1.40), p=0.1578
Heart rate ‡	1.41 (0.80-2.43), p=0.2511	1.09 (0.55-2.03), p=0.7599	0.68 (0.36-1.29), p=0.2459	0.65 (0.34-1.25), p=0.1986	1.91 (0.93-3.68), p=0.0806	1.35 (0.69-2.60), p=0.3777

Abbreviations: CI, confidence interval; HR, hazard ratio; RMSSD, root mean square of successive RR interval differences; RMSSDc, root mean square of successive RR interval differences corrected for heart rate; SDNN, standard deviation of normal to normal RR intervals; SDNNc, standard deviation of normal to normal RR intervals corrected for heart rate.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication use.

‡ Hazard ratios represent 1 unit increase of ln(SDNN), ln(SDNNc), ln(RMSSD), ln(RMSSDc), and 1 unit decrease of ln(heart rate) with the risk of new-onset atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

Table S2. Association between longitudinal measures of heart rate variability with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with exclusion of prevalent and incident coronary heart disease cases before atrial fibrillation

	Total study population		Men		Women	
Heart rate variability measures	**Cause-specific HR (95% CI)**					
	Model 1 *	**Model 2** †	**Model 1** *	**Model 2** †	**Model 1** *	**Model 2** †
SDNN ‡	**1.34 (1.11-1.62), p=0.0012**	**1.34 (1.11-1.60), p=0.0010**	1.26 (0.97-1.62), p=0.0790	1.26 (0.97-1.65), p=0.0766	**1.47 (1.11-1.99), p=0.0065**	1.32 (0.99-1.74), p=0.0612
SDNNc ‡	1.02 (0.86-1.19), p=0.8064	1.05 (0.91-1.22), p=0.4983	1.12 (0.89-1.37), p=0.3128	1.11 (0.88-1.39), p=0.3562	0.94 (0.72-1.23), p=0.6053	0.96 (0.73-1.22), p=0.7967
RMSSD ‡	**1.48 (1.27-1.73), p<0.0001**	**1.43 (1.23-1.66), p<0.0001**	**1.37 (1.09-1.70), p=0.0070**	**1.35 (1.10-1.67), p=0.0045**	**1.65 (1.30-2.05), p<0.0001**	**1.47 (1.15-1.87), p=0.0008**
RMSSDc ‡	1.09 (0.96-1.23), p=0.1935	1.09 (0.97-1.22), p=0.1506	1.16 (0.97-1.37), p=0.0943	1.15 (0.97-1.38), p=0.1122	1.00 (0.84-1.19), p=0.9921	1.00 (0.83-1.20), p=0.9713
Heart rate ‡	**1.73 (1.10-2.67), p=0.0223**	1.56 (0.91-2.55), p=0.0997	0.68 (0.36-1.32), p=0.2610	0.77 (0.37-1.52), p=0.4577	2.80 (1.24-5.68), p=0.0165	1.75 (0.93-3.24), p=0.0827

Abbreviations: CI, confidence interval; HR, hazard ratio; RMSSD, root mean square of successive RR interval differences; RMSSDc, root mean square of successive RR interval differences corrected for heart rate; SDNN, standard deviation of normal to normal RR intervals; SDNNc, standard deviation of normal to normal RR intervals corrected for heart rate.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication use.

‡ Hazard ratios represent 1 unit increase of ln(SDNN), ln(SDNNc), ln(RMSSD), ln(RMSSDc), and 1 unit decrease of ln(heart rate) with the risk of new-onset atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S3. Association between longitudinal measures of heart rate variability with the risk of mortality in the total study population and stratified by sex

	Total study population		Men		Women	
Heart rate variability measures	**Cause-specific HR (95% CI)**					
	Model 1 *	**Model 2** †	**Model 1** *	**Model 2** †	**Model 1** *	**Model 2** †
SDNN ‡	**0.82 (0.73-0.91), p<0.0001**	**0.82 (0.74-0.91), p<0.0001**	**0.74 (0.64-0.85), p<0.0001**	**0.76 (0.66-0.87), p=0.0004**	0.89 (0.76-1.03), p=0.1262	0.87 (0.74-1.02), p=0.0848
SDNNc ‡	1.00 (0.92-1.09), p=0.9970	1.01 (0.92-1.10), p=0.9046	1.02 (0.92-1.14), p=0.6745	1.01 (0.90-1.13), p=0.8441	0.97 (0.85-1.10), p=0.6114	0.98 (0.86-1.11), p=0.7008
RMSSD ‡	**0.91 (0.83-0.99), p=0.0218**	**0.89 (0.82-0.98), p=0.0119**	**0.82 (0.73-0.93), p=0.0011**	**0.83 (0.74-0.93), p=0.0019**	1.00 (0.89-1.13), p=0.9638	0.97 (0.85-1.10), p=0.6155
RMSSDc ‡	**1.09 (1.02-1.16), p=0.0079**	**1.08 (1.01-1.15), p=0.0234**	**1.13 (1.03-1.23), p=0.0074**	**1.10 (1.01-1.20), p=0.0287**	1.05 (0.96-1.15), p=0.3093	1.05 (0.95-1.16), p=0.3291
Heart rate ‡	**0.47 (0.34-0.65), p<0.0001**	**0.45 (0.33-0.62), p<0.0001**	**0.28 (0.19-0.43), p<0.0001**	**0.31 (0.21-0.48), p<0.0001**	0.90 (0.59-1.38), p=0.6154	0.71 (0.45-1.10), p=0.1204

Abbreviations: CI, confidence interval; HR, hazard ratio; RMSSD, root mean square of successive RR interval differences; RMSSDc, root mean square of successive RR interval differences corrected for heart rate; SDNN, standard deviation of normal to normal RR intervals; SDNNc, standard deviation of normal to normal RR intervals corrected for heart rate.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication use.

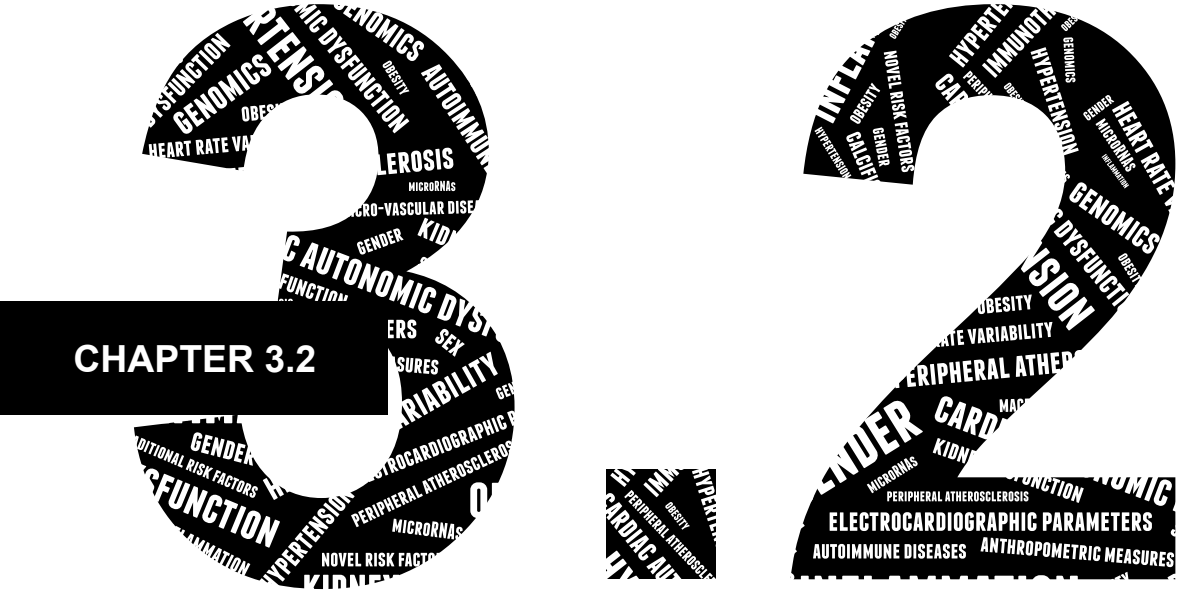
‡ Hazard ratios represent 1 unit increase of ln(SDNN), ln(SDNNc), ln(RMSSD), ln(RMSSDc), and 1 unit decrease of ln(heart rate) with the risk of new-onset atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

Table S4. Effect estimates for the associations of the genetic variants with SDNN, RMSSD and atrial fibrillation

			Exposure effect estimates						Outcome effect estimates			
SNP	**Chr**	**Pos**	**Trait**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**Trait**	**EAF**	**Beta**	**SE**
rs236349	6	36928543	SDNN	G	A	0.650985	-0.032710	0.003155	AF	0.650900	-0.012200	0.007000
rs4262	7	93389364	SDNN	C	T	0.389763	-0.027710	0.003294	AF	0.422700	-0.013600	0.006800
rs36423	14	71422955	SDNN	T	G	0.129193	-0.032977	0.004580	AF	0.141600	-0.018600	0.010300
rs4899412	14	71534015	SDNN	T	C	0.252324	-0.025882	0.003548	AF	0.273200	-0.011700	0.007600
rs2529471	14	71883022	SDNN	C	A	0.428286	-0.021160	0.003002	AF	0.429000	-0.012000	0.006700
rs236349	6	36928543	RMSSD	G	A	0.654958	-0.035488	0.004264	AF	0.650900	-0.012200	0.007000
rs180238	7	93388383	RMSSD	C	T	0.333966	-0.034439	0.004273	AF	0.350500	-0.011400	0.007100
rs7980799	12	33468257	RMSSD	A	C	0.391851	-0.038742	0.004202	AF	0.414900	-0.023300	0.006800
rs36423	14	71422955	RMSSD	T	G	0.127977	-0.040464	0.006163	AF	0.141600	-0.018600	0.010300
rs4899412	14	71534015	RMSSD	T	C	0.253558	-0.028307	0.004651	AF	0.273200	-0.011700	0.007600
rs2529471	14	71883022	RMSSD	C	A	0.424941	-0.026200	0.003934	AF	0.429000	-0.012000	0.006700

Abbreviations: SNP: single nucleotide polymorphism, Chr: chromosome, Pos: genomic position, EA: effect allele, OA: other allele, EAF: effect allele frequency, SE: standard error, Pval: p-value, N: sample size, SDNN: standard deviation of normal to normal RR intervals, RMSSD: the root mean square of successive RR interval differences.


CHAPTER 3.2
GENOMICS
AUTOIMMUNE
DYSFUNCTION
OBESITY
GENOMICS
HEART RATE
LEROSIS
MICRORNAS
GENDER
AUTONOMIC
SEX
RIABILITY
GENDER
PERIPHERAL ATHEROSCLEROSIS
MICRORNAS
NOVEL RISK FACTORS
HYPERTENSION
IMMUNOTHERAPY
GENOMICS
OBESITY
NOVEL RISK FACTORS
GENDER
HEART RATE
MICRORNAS
GENOMICS
DYSFUNCTION
OBESITY
VARIABILITY
PERIPHERAL
CARDIAC
KIDNEY
PERIPHERAL ATHEROSCLEROSIS
ELECTROCARDIOGRAPHIC PARAMETERS
AUTOIMMUNE DISEASES
ANTHROPOMETRIC MEASURES

Electrocardiographic parameters and the risk of atrial fibrillation

Electrocardiographic parameters and the risk of new-onset atrial in the general population: the Rotterdam Study.

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ABSTRACT

Background
The (shape of the) association and sex differences in the link between electrocardiographic parameters and new-onset atrial fibrillation (AF) remain incompletely understood.

Methods
A total of 12,212 participants free of AF at baseline from the population-based Rotterdam Study were included. Up to 5 repeated measurements of electrocardiographic parameters including PR, QRS, QT, QT corrected for heart rate (QTc), JT, RR interval, and heart rate were assessed at baseline and follow-up examinations. Cox proportional hazards models and joint models, adjusted for cardiovascular risk factors, were used to determine the (shape of the) association between baseline and longitudinal electrocardiographic parameters with new-onset AF. Additionally, we evaluated potential sex differences.

Results
During a median follow-up of 9.3 years, 1,282 incident AF cases occurred among 12,212 participants (mean age 64.9 years, 58.2% women). Penalized cubic splines revealed that associations between baseline electrocardiographic measures and risk of new-onset AF were generally U- and N-shaped. Sex differences in terms of the shape of the various associations were most apparent for baseline PR, QT, QTc, RR, and heart rate in relation to new-onset AF. Longitudinal measures of higher PR interval (hazard ratio (HR), 95% confidence interval (CI), 1.43, 1.02-2.04, p=0.0393), and higher QTc interval (HR, 95% CI, 5.23, 2.18-12.45, p=0.0002) were significantly associated with new-onset AF. Sex-stratified analyses indicated that the associations were more prominent among men.

Conclusions
Associations of baseline electrocardiographic measures and risk of new-onset AF were mostly U- and N-shaped. Longitudinal electrocardiographic measures of PR, and QTc interval were significantly associated with new-onset AF, in particular among men.

INTRODUCTION

Atrial fibrillation (AF), the most frequently encountered cardiac arrhythmia, is associated with increased hospitalization, morbidity and mortality risk.(1) Although, the exact etiology of AF remains to be elucidated, it has been suggested that both structural and electrical remodeling are crucial in AF pathophysiology.(1) In particular, electrical abnormalities and/or structural endophenotypes, represented by electrocardiographic parameters, could play a role in the development of AF.(2-5)

The electrocardiogram (ECG) is a non-invasive, readily available, and inexpensive measure that provides detailed information about cardiac conduction. A complex relationship between electrocardiographic parameters that reflect atrioventricular conduction (PR interval), ventricular depolarization (QRS), and ventricular repolarization (QT, QT corrected for heart rate (QTc), and JT interval), and cardiac contractions (RR interval, and heart rate) and AF has been suggested.(2-5) Nonetheless, the associations with new-onset AF and the shape of these associations remain incompletely understood. Furthermore, previous studies on the association of electrocardiographic parameters with AF have relied on a single measurement of electrocardiographic parameters, by which biological variation, and cardiac decline over time are not taken into account, which could have led to misclassification bias of these parameters. While sex differences with regard to AF burden, pathophysiology, and prognosis have been indicated,(6) sex differences in the association of electrocardiographic parameters with new-onset AF have not been investigated.

We therefore aimed to investigate (the shape of) the association between baseline and longitudinal measures of electrocardiographic parameters including PR, QRS, QT, QTc, JT, RR interval, and heart rate with the risk of new-onset AF among men and women from the large population-based Rotterdam Study.

METHODS

Study design

Our study was embedded within the framework of the Rotterdam Study.(7, 8) See **Methods S1** for more details.

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Study population

For the present study, we included participants at study entry of the 3 recruitment waves. Participants with prevalent AF at baseline (n=559), no informed consent for follow-up data collection (n=305), no follow-up time (n=6) or no electrocardiographic measures (n=1,843), mainly due to logistic reasons, were excluded. A total of 24,407 ECGs were available among the 12,212 participants free of AF at baseline who were included. 12,212 participants had at least 1 measurement for PR, QRS, QT, QTc, JT, RR interval, and heart rate, respectively; 6,354 participants had 2 measurements; 3,462 had 3 measurements; 1,637 had 4 measurements, and 742 participants had 5 measurements that were available during follow-up.

Assessment of electrocardiographic parameters

Participants underwent a 10-second 12-lead resting ECG using an ACTA Gnosis IV ECG recorder (Esaote Biomedica, Florence, Italy), which were digitally stored. Subsequently, Modular ECG Analysis System (MEANS) was used to analyze and interpret the ECGs. MEANS determines the PR interval from the start of the P wave until the start of QRS complex, the QRS duration from the start of the QRS complex until the end, and the QT interval from the start of the QRS complex until the end of the T wave.(9) To correct the QT interval for heart rate (QTc), Hodges' formula, QTc=QT+0.00175 ([60/RR]-60), was used to calculate QTc interval.(10) JT interval was calculated as QT interval-QRS duration.(9) The RR interval was computed as the averaged time between two subsequent QRS complexes, from which the heart rate (in beats per minute) was derived.

Assessment of atrial fibrillation

The definition of AF was in accordance with the European Society of Cardiology (ESC) guidelines.(1) The methodology on event adjudication for prevalent and incident AF within the Rotterdam Study have been described in detail previously.(8, 11) In short, AF was assessed at baseline and follow-up examinations using a 10-second 12-lead ECG with an ACTA Gnosis IV ECG recorder (Esaote; Biomedica, Florence, Italy). The ECG records were then stored digitally and analyzed with the MEANS. Thereafter, two medical doctors validated the diagnosis of AF and in case of disagreement a cardiologist was consulted.(8, 11) Additional follow-up data was obtained from medical files of participating general practitioners, hospitals, outpatient clinics, national registration of all hospitals discharge diagnoses, and follow-up examinations at the research center. The date of incident AF was defined as the date of the first occurrence of symptoms suggestive of AF with subsequent ECG verification obtained from the medical records. Participants were followed from the date of enrolment in the Rotterdam Study until the date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of cardiovascular risk factors

The cardiovascular risk factors included in this study were body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the ECG, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication. Methods for measurements of cardiovascular risk factors are explained in detail in the **Methods S2**.(7, 8, 11)

Statistical analyses

Baseline characteristics

The baseline characteristics of the study population are presented as mean with standard deviation (SD) or number (n) with percentages as appropriate. The differences between men and women were evaluated by Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) for continuous variables and Chi-Square test for categorical variables. As the distribution of the different electrocardiographic parameters were skewed, a natural logarithmic transformation was used to obtain a normal distribution.

Cox proportional hazards and joint models

Cox proportional hazard models with and without penalized cubic splines were used to investigate the shape of the association (for example linear, J-shaped or U-shaped) between baseline measures of electrocardiographic parameters and the risk of new-onset AF. Further, we conducted competing risk analyses using joint models to investigate the association between longitudinal measures of

electrocardiographic parameters and the risk of new-onset AF with mortality as a competing event. Cause-specific hazard ratios (HRs) with their 95% confidence intervals (CIs) were calculated to quantify the associations.

The analyses were conducted in the total study population and for men and women separately. Additionally, we presented the p-values of the sex interaction in the total study population from the joint model. Mixed models were adjusted for age, and sex (if applicable) while survival models were adjusted for age, sex (if applicable), and cohort (model 1), and additionally for cardiovascular risk factors including body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the ECG, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication (model 2). Time was measured in years after baseline and the variables from model 1 and 2 were treated as covariates in the subsequent models. See the **Methods S3** for more details on the rationale, imputation and sensitivity analyses of the Cox proportional hazards models and joint models.

Sensitivity analyses

See the **Methods S3** for more details on the rationale, imputation and sensitivity analyses of the Cox proportional hazards models and joint models.

RESULTS

Baseline characteristics

A total of 12,212 participants were eligible for the analyses. The baseline characteristics for the total study population and stratified by sex are presented in **Table 1**. The mean age of the total study population was 64.9 ± 9.6 years and 58.2% were women. The median values of the electrocardiographic parameters were: PR 164.0 ms, QRS 98.0 ms, QT 400.0 ms, QTc 417.4 ms, JT 302.0 ms, RR interval 870.0 ms, and heart rate 69.0 beats/min.

Atrial fibrillation incidence

During a median follow-up of 9.3 years (interquartile range (IQR), 6.2-14.7), 1,282 incident AF cases (10.5%) (609 in men and 673 in women) and 3,912 mortality cases (1,714 men and 2,198 women) occurred. The incidence rate of AF was 9.7 per 1,000 person-years in the total study population (11.6 per 1,000 person-years in men, 8.4 per 1,000 person-years in women) and the incidence rate of mortality was 29.5 per 1,000 person-years in the total study population (32.7 per 1,000 person-years in men, 27.4 per 1,000 person-years in women).

Cox proportional hazards models

The non-linear associations in the total study population and stratified by sex are depicted in **Figures 1-4**. Cox proportional hazards models using penalized cubic splines in model 2 revealed that associations between baseline electrocardiographic measures and risk of new-onset AF were mostly U- and N-shaped. More specifically, an U-shape was observed for ln(PR) interval. A ln(PR) interval below 5.0 and above 5.2 was associated with a higher risk of new-onset AF. For ln(QRS) interval an inverted U-shape was found. It was found that below 4.5 and above 5.1, there was lower risk of new-onset AF. A N-shape was identified for ln(QT) interval. A value below approximately 5.8 conferred a lower risk for new-onset AF while between 5.8 and 5.9 the risk was neutral, values between 5.9 and 6.05 conferred a lower risk and above 6.05 again a higher risk for new-onset AF. We also observed a U-shape for ln(QTc) interval. Having a value below 5.9 and above 6.05 led to a lower or higher risk of new-onset AF, respectively. For ln(JT) interval a N-shape was found where values below 5.4, between 5.5 and 5.6, between 5.6 and 5.8, and above 5.8 translated to a lower, higher, lower or higher risks for new-onset AF, respectively. For ln(RR) interval a N-shape was also found where values below 6.3, between 6.3 and 6.6, between 6.6 and 6.9, and above 6.9 translated to lower, higher, lower or higher new-onset AF risk, respectively. Lastly, for ln(heart rate) a N-shape was identified. Values below approximately 4.1 seemed to be associated with a larger risk of new-onset AF, values between 4.1 and 4.4 with a lower risk, between 4.4 and 4.7 with a higher risk, and above 4.7 again with a lower risk of new-onset AF.

The sex differences in terms of the shape of the various associations were mostly apparent for baseline PR, QT, QTc, RR interval, and heart rate in relationship to new-onset AF.

Joint models

Joint models showed significant associations between longitudinal measures of higher PR interval (HR, per 1 unit increase, 95% CI, 1.91, 1.34-2.91, p=0.0002), higher QTc interval (HR, per 1 unit increase, 95% CI, 11.88, 5.24-27.39, p<0.0001), and lower heart rate (HR, per 1 unit increase, 95% CI, 1.68, 1.05-2.75, p=0.0279) with an increased risk of new-onset AF in the total study population in model 1. The p-values of the sex interaction in model 1 in the joint model for PR, QRS, QT, QTc, JT, RR interval, and heart rate in the total study population were p=0.0215, p=0.0425, p=0.0502, p=0.3150, p=0.1426, p=0.0240, p=0.0032, respectively. Adjusting for additional cardiovascular risk factors in model 2 did attenuate the effect estimates, but higher PR interval (HR, per 1 unit increase, 95% CI, 1.43, 1.02-2.04, p=0.0393), and higher QTc interval (HR, per 1 unit increase, 95% CI, 5.23, 2.18-12.45, p=0.0002) remained significantly associated with the risk of new-onset AF in the total study population (**Table 2**). In model 2, the p-values of the sex interaction in the joint model for PR, QRS, QT, QTc, JT, RR interval, and heart rate in the total study population were p=0.1441, p=0.3670, p=0.1381, p=0.4046, p=0.0794, p=0.0296, p=0.0065, respectively.

The sex stratified analyses from model 2 showed significant associations for a higher QTc interval (HR, per 1 unit increase, 95% CI, 11.35, 3.76-34.78, p<0.0001), and higher RR interval (HR, per 1 unit increase, 95% CI, 0.55, 0.32-0.94, p=0.0286) in men. The analyses in women showed borderline significant associations for a higher QTc interval (HR, per 1 unit increase, 95% CI, 2.81, 0.94-8.52, p=0.0647), and lower heart rate (HR, 95% CI, per 1 unit decrease, 1.80, 0.99-3.27, p=0.0538) (**Table 2**).

Sensitivity analyses

The results of our Cox proportional hazards and joint model sensitivity analyses are depicted in **Results S1**, and **Tables S1-S3**.

Table 1. Baseline characteristics of the total study population and stratified by sex

Baseline characteristics *	Total study population n=12,212	Men n=5,107	Women n=7,105	p §
Age, years	64.9 ± 9.6	64.1 ± 8.9	65.4 ± 10.1	<0.001
Women, n (%)	7,105 (58.2)	NA	7,105 (100)	NA
Body mass index, kg/m^2	26.9 ± 4.1	26.6 ± 3.5	27.2 ± 4.5	<0.001
Total cholesterol, mmol/L †	6.1 ± 1.2	5.8 ± 1.2	6.3 ± 1.2	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.4 ± 0.4	1.2 ± 0.3	1.5 ± 0.4	<0.001
Systolic blood pressure, mmHg	139.0 ± 21.6	139.6 ± 20.7	138.5 ± 22.2	0.004
Diastolic blood pressure, mmHg	78.1 ± 11.8	79.2 ± 11.8	77.3 ± 11.7	<0.001
Hypertension, n (%)	7,218 (59.2)	3,009 (59.0)	4,209 (59.3)	0.722
Smoking status, n (%)				<0.001
Never	3,868 (32.2)	719 (14.2)	3,149 (45.3)	
Former	5,236 (43.6)	2,855 (56.5)	2,381 (34.2)	
Current	2,903 (24.2)	1,479 (29.3)	1,424 (20.5)	
History of diabetes mellitus, n (%)	1,244 (10.2)	594 (11.6)	650 (9.2)	<0.001
History of coronary heart disease, n (%)	745 (6.2)	544 (10.9)	201 (2.9)	<0.001
History of heart failure, n (%)	208 (1.7)	90 (1.8)	118 (1.7)	0.673
Left ventricular hypertrophy, n (%)	724 (6.2)	424 (8.7)	300 (4.4)	<0.001
Cardiac medication, n (%)	579 (5.3)	268 (5.8)	311 (4.9)	0.032
Antihypertensive medication, n (%)	3,528 (29.4)	1,432 (28.5)	2,096 (30.0)	0.083
Beta blockers, n (%)	1,476 (13.4)	647 (14.0)	829 (13.0)	0.125
Calcium blockers, n (%)	916 (8.3)	422 (9.1)	494 (7.7)	0.009
Lipid lowering medication, n (%)	1,235 (11.2)	600 (13.0)	635 (9.9)	<0.001
PR, ms ‡	164.0 (150.0-180.0)	168.0 (152.0-184.0)	162.0 (148.0-176.0)	<0.001
QRS, ms ‡	98.0 (88.0-106.0)	102.0 (94.0-110.0)	94.0 (86.0-102.0)	<0.001
QT, ms ‡	400.0 (382.0-420.0)	400.0 (382.0-420.0)	400.0 (384.0-420.0)	0.335

QTc, ms ‡	417.4 (406.1-430.0)	414.1 (402.9-427.1)	419.7 (409.0-431.9)	<0.001
JT, ms ‡	302.0 (284.0-320.0)	296.0 (278.0-314.0)	306.0 (288.0-324.0)	<0.001
RR, ms ‡	870.0 (780.0-970.0)	900.0 (800.0-1000.0)	860.0 (770.0-950.0)	<0.001
Heart rate, beats/min ‡	69.0 (61.9-76.9)	66.7 (60.0-75.0)	69.8 (63.2-77.9)	<0.001

Values are shown before imputation and therefore not always add up to 100%.

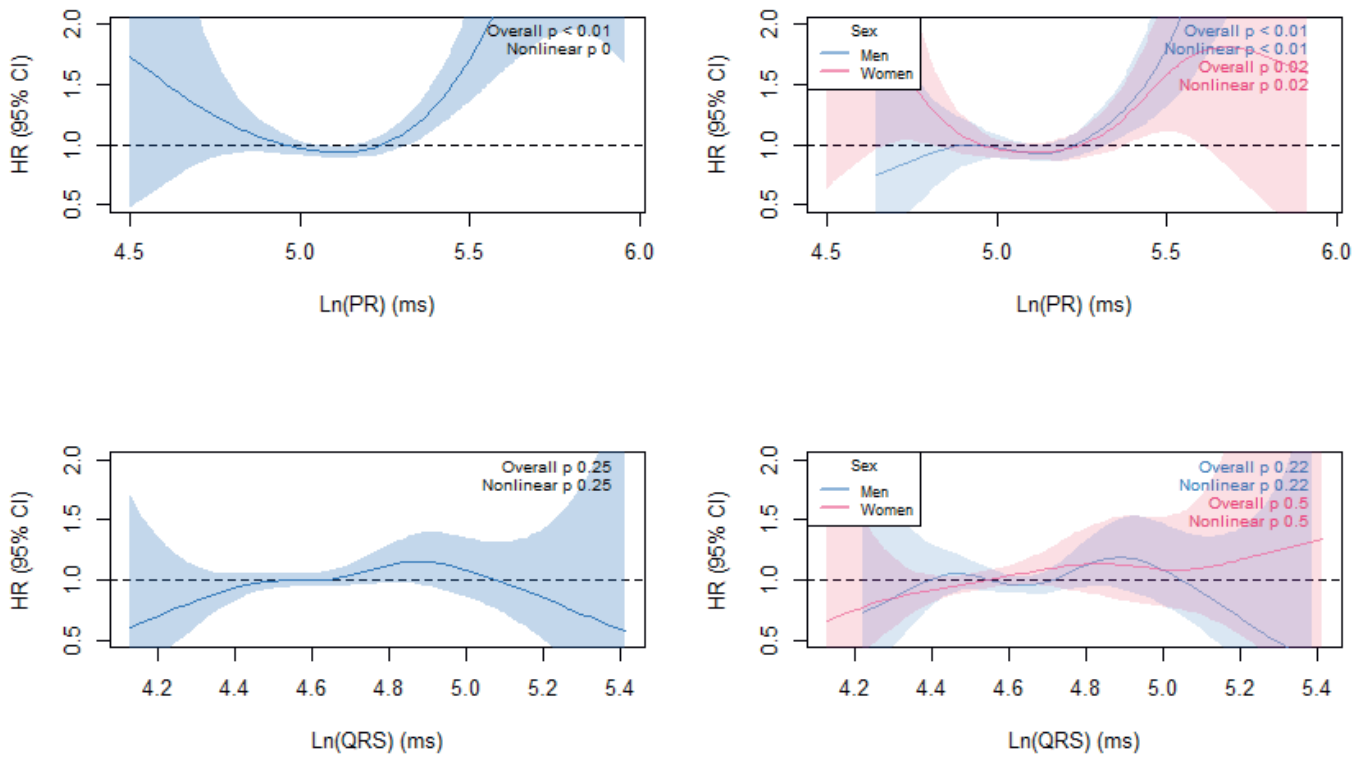
* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factors: to convert cholesterol to mg/dL divide values by 0.0259.

‡ Non-transformed median with interquartile range.

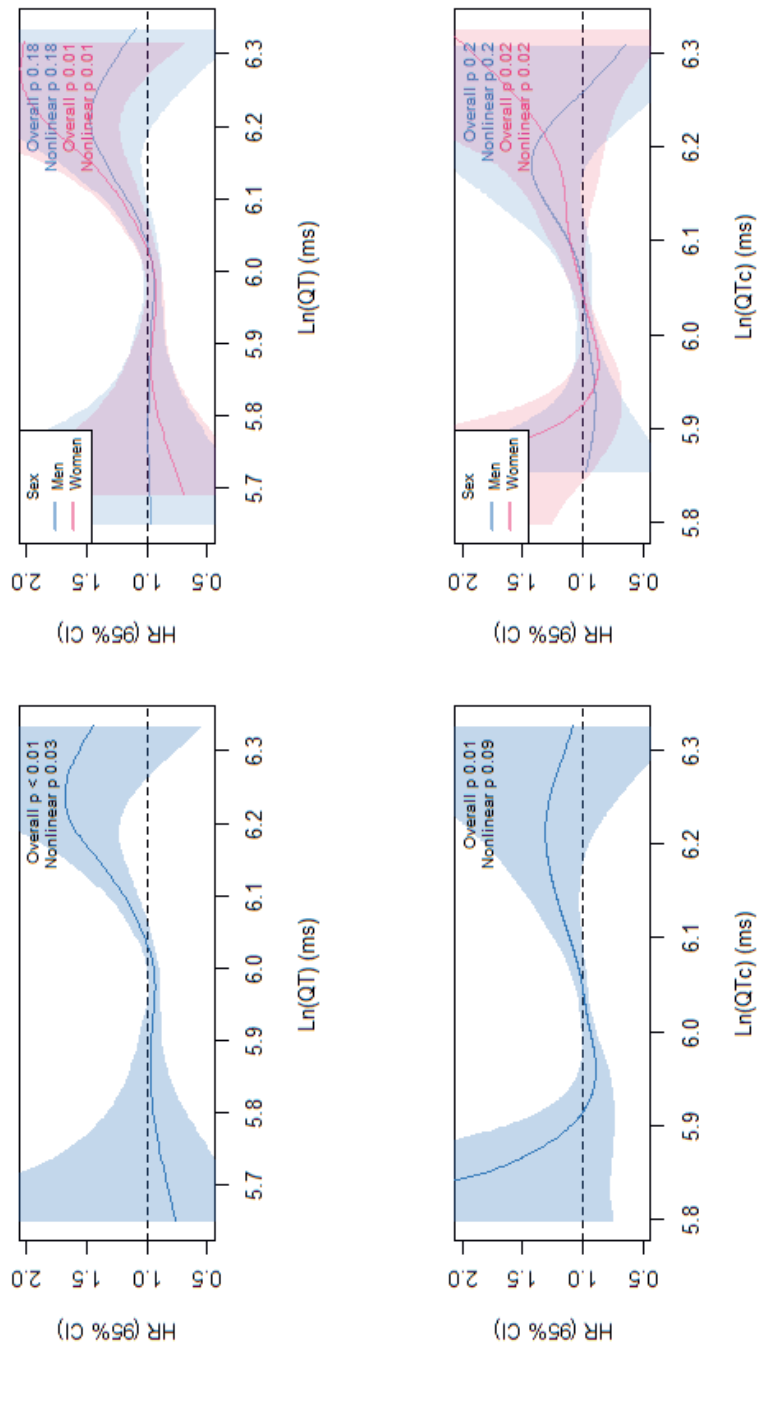
§ Statistical significance for continuous variables was tested using the Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) and for categorical variables was tested using the Chi-Square test.

Figure 1. Non-linear association between baseline measures of PR, QRS and the risk of new-onset atrial fibrillation



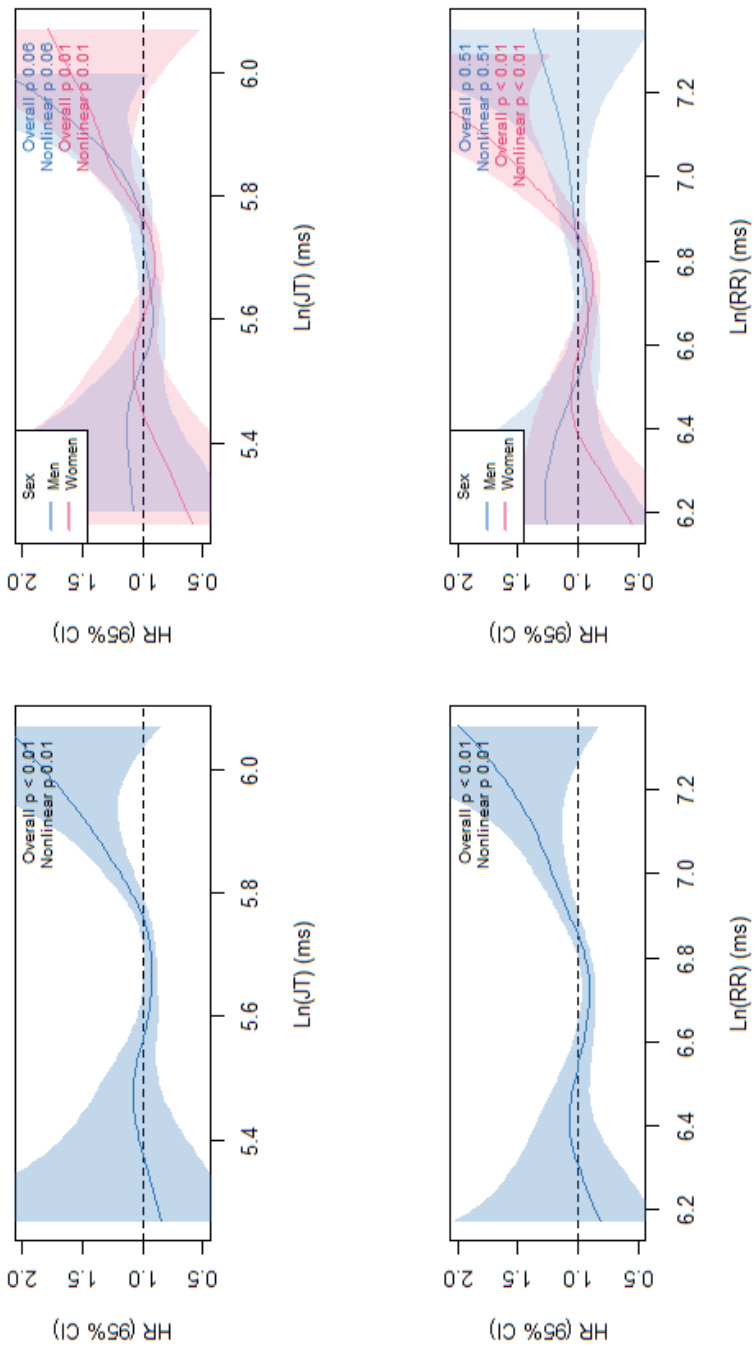
Abbreviations: CI, confidence interval; HR, hazard ratio.

Figure 2. Non-linear association between baseline measures of QT, QTc and the risk of new-onset atrial fibrillation



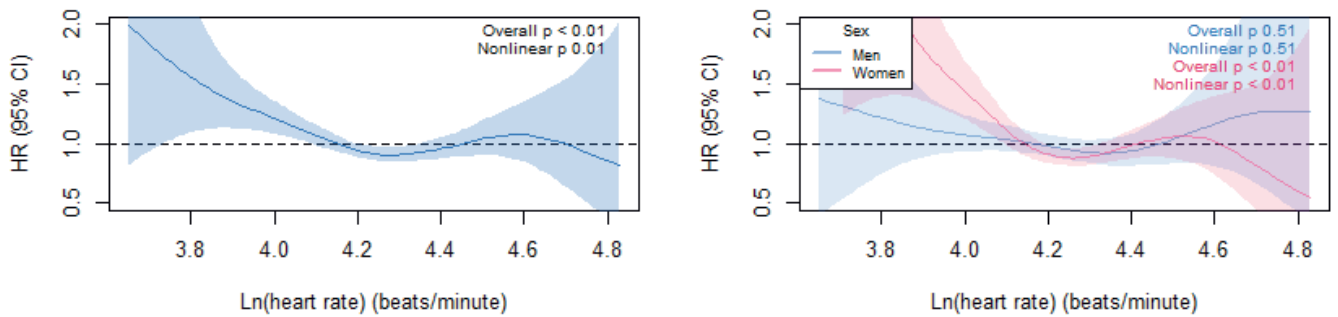
Abbreviations: CI, confidence interval; HR, hazard ratio.

Figure 3. Non-linear association between baseline measures of JT, RR and the risk of new-onset atrial fibrillation



Abbreviations: CI, confidence interval; HR, hazard ratio.

Figure 4. Non-linear association between baseline measures of heart rate and the risk of new-onset atrial fibrillation



Abbreviations: CI, confidence interval; HR, hazard ratio.

Table 2. Association between longitudinal measures of electrocardiographic parameters with the risk of new-onset atrial fibrillation in the total study population and stratified by sex

Electro-cardiographic measures	Total study population		Men		Women	
	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Joint models ‡						
PR §	**1.91 (1.34-2.91), p=0.0002**	**1.43 (1.02-2.04), p=0.0393**	**1.74 (1.12-2.64), p=0.0136**	1.48 (0.93-2.37), p=0.0986	1.58 (0.99-2.51), p=0.0555	1.21 (0.73-1.91), p=0.4282
QRS §	1.47 (0.98-2.13), p=0.0578	1.15 (0.83-1.61), p=0.4316	1.38 (0.90-2.11), p=0.1452	1.07 (0.68-1.72), p=0.7941	**1.73 (1.03-2.71), p=0.0353**	1.31 (0.85-1.99), p=0.2248
QT §	**2.32 (1.19-4.52), p=0.0135**	1.44 (0.73-2.80), p=0.2999	1.65 (0.73-3.67), p=0.2237	1.14 (0.49-2.64), p=0.7708	**2.57 (1.05-6.32), p=0.0389**	1.75 (0.73-4.01), p=0.2001
QTc §	**11.88 (5.24-27.39), p<0.0001**	**5.23 (2.18-12.45), p=0.0002**	**29.75 (10.16-86.91), p<0.0001**	**11.35 (3.76-34.78), p<0.0001**	**5.84 (1.93-17.61), p=0.0016**	2.81 (0.94-8.52), p=0.0647
JT §	1.07 (0.62-1.86), p=0.8208	0.97 (0.54-1.79), p=0.9047	0.78 (0.38-1.61), p=0.4931	0.71 (0.33-1.56), p=0.3774	1.13 (0.50-2.45), p=0.7509	0.93 (0.41-1.98), p=0.8707
RR §	1.07 (0.70-1.64), p=0.7341	1.01 (0.66-1.54), p=0.9591	**0.53 (0.32-0.87), p=0.0128**	**0.55 (0.32-0.94), p=0.0286**	1.59 (0.90-2.67), p=0.1032	1.34 (0.76-2.28), p=0.3133
Heart rate §	**1.68 (1.05-2.75), p=0.0279**	1.47 (0.88-2.57), p=0.1577	0.77 (0.44-1.35), p=0.3589	0.73 (0.36-1.34), p=0.3247	**2.45 (1.33-4.81), p=0.0032**	1.80 (0.99-3.27), p=0.0538

Abbreviations: CI, confidence interval; HR, hazard ratio. * Adjusted for age, sex (if applicable), and cohort. † Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication use. ‡ Association between longitudinal electrocardiographic parameters for up to 5 repeated measurements during follow-up with incident atrial fibrillation, assessed by joint models. § Hazard ratios represent 1 unit increase in ln(PR), ln(QRS), ln(QT), ln(QTc), ln(JT), ln(RR), and 1 unit decrease in ln(heart rate) with the risk of new-onset atrial fibrillation.
The associations with a p<0.05 are highlighted in **bold**.

DISCUSSION

This large population-based cohort study provides insight into the complex relationship between electrocardiographic parameters and new-onset AF. The associations of baseline electrocardiographic measures and risk of new-onset AF were mostly U- and N-shaped. Our joint model analyses showed that longitudinal measures of higher PR interval, and QTc interval were significantly associated with new-onset AF in the general population. In terms of the shape of the various associations, sex differences were mostly apparent for baseline PR, QT, QTc, RR interval, and heart rate in relationship to new-onset AF. Further, higher longitudinal measures of QTc interval, and RR interval among men, and none of the parameters among women, were significantly associated with new-onset AF. Our findings might imply that (different thresholds of) electrocardiographic parameters could translate to a differential risk among men and women, and that modulation of various electrocardiographic parameters might prevent AF in the general population, in particular in men.

The exact mechanistic insight that underlies the relationship between electrocardiographic parameters and AF is lacking. Shared underlying risk factors such as obesity, diabetes mellitus, coronary heart disease, and heart failure could influence the cardiac conduction system and are also known to play a role in AF development.(1) However, after extensive adjustment for shared cardiovascular risk factors in our study, the associations between electrocardiographic parameters and new-onset AF attenuated, but remained significant for PR interval and QTc interval. The PR interval represents the atrioventricular conduction and its possible interferences. PR interval prolongation (PR >200 ms or first-degree atrioventricular block) may arise from conduction disturbances within the atria, the atrioventricular (AV) node, His bundle, and/or at multiple sites which may be caused by structural remodeling.(12)This structural remodeling may be primary (idiopathic) or secondary to conditions such as aging, coronary heart disease, calcification, and inflammation.(12) It has been hypothesized that delayed ventricular repolarization, reflected by prolongation of the QT, QTc, and JT interval, could affect both the atria and ventricles leading to triggered arrhythmogenesis as a mechanism of AF.(13) Alternatively, prolonged ventricular repolarization may result in atrioventricular dyssynchrony which may cause left ventricular diastolic dysfunction and this could lead to increased atrial wall tension.(13) The elevated atrial pressure may then aggravate further remodeling of the left atrium and thereby produce a vulnerable substrate for AF.(13) The myocardial contractions , reflected by the RR interval, and heart rate, and its relationship with AF is also well established.(14) Heart rate regulation is a complex interaction between sympathetic activation and vagal withdrawal during physical exertion.(14) On one hand, a low heart rate is typically associated with a lower body mass index, increased exercise tolerance, reduced

morbidity, and mortality.(14) Nonetheless, a decreased heart rate during physical exertion might represent an altered reaction to physical activity due to prolonged vagal activity.(14) This prolonged vagal activity enhances acetylcholine-dependent potassium currents which reduce the action potential duration which may facilitate conduction abnormalities and hence development of AF.(14) On the other hand, a high heart rate is associated with hypertension, diabetes mellitus, coronary heart disease, and heart failure which are all involved in AF pathophysiology.(14) In addition, increased heart rate might be a marker of increased sympathetic tone which may reduce the atrial refractory period and thereby initiate AF.(14) The sinoatrial node is the pacemaker of the heart and it has been suggested that sinus node disease (SND) or sick sinus syndrome leads to AF through atrial extrasystoles, and conduction abnormalities.(15, 16) Atrial extrasystoles may arise more easily during the prolonged atrial cycle due to SND. These atrial extrasystoles are mostly followed by a compensatory pause. This pause may be prolonged, allowing atrial ectopic activity to occur, potentially causing AF.(16) Early premature beats that come from areas other than the sinoatrial node may result in a conduction block and re-entry, in turn imposing AF.(16) Taken all together, it seems reasonable that a combination of the aforementioned mechanistic pathways relate the cardiac conduction system, reflected by the electrocardiographic parameters, to AF.

The shape of the associations between baseline electrocardiographic measures and risk of new-onset AF were mostly U- and N-shaped. While the natural logarithmic transformation of the ECG parameters hampers direct clinical interpretation of our analyses, our findings underline that different values of electrocardiographic parameters might translate to a differential risk among men and women in the general population.

We also investigated the relation between new-onset AF and longitudinal measures of electrocardiographic parameters during a long follow-up time. Repeated measurements of these parameters may provide more insight and prognostic information than studies using only single baseline measurements.(2-4) Our findings extend previous evidence on the interplay of electrocardiographic parameters and AF, by simultaneously evaluating the repeated measurements, as well as sex differences.(2-4) Longitudinal measures of electrocardiographic parameters during follow-up were associated with an increased risk of incident AF. Furthermore, we observed more prominent associations among men than among women. In addition, the clinical implications of these results could be to avoid certain medication groups that potentially prolong PR and QTc interval as the present study indicates that this might negatively impact AF development over time. As we do not yet know which individuals should be screened for AF at a population-level.(1) This study does also provide some insight into which electrocardiographic parameters seem useful to tag individuals who are at a higher risk for AF development in the future. These tagged individuals could then be monitored more frequently by a physician or even

continuously with the upcoming use of wearable devices.

Electrocardiographic parameters are age- and sex-specific.(5, 17, 18) One potential explanation could be differences in cardiac size. Men, on average, have larger hearts which increase the depolarization time of cardiac tissue (increased PR interval and QRS duration).(5, 19) Another potential explanation could be differences in sex hormones. It has been shown that a sudden ovarian hormone withdrawal, induced by an oophorectomy, caused an increase in heart rate in women.(17) Additionally, estrogen replacement therapy for three months within the oophorectomized women restored the heart rate to a preoperative state.(17) This might explain why RR interval was only associated with incident AF in men, and not in women. It also has been demonstrated that differences in ion channel gene expression predispose to longer cardiac action potential duration in women.(20) Addition of sex hormones exacerbated these differences as higher levels of testosterone further led to shortened cardiac action potential duration in men, while higher levels of estrogen led to longer cardiac action potential duration in women which may put women at particular risk of AF.(20) Further, we hypothesize that competing risk of death is another potential explanation for the observed sex differences. Since AF is strongly associated with age, it might well be that men die from other (cardiovascular) diseases before the development of AF. This hypothesis was supported by our competing risk analyses which showed that QRS, QTc, JT, RR interval, and heart rate were significantly associated with mortality, especially among men. Nevertheless, we found a higher incidence of AF in men, than women, in our study.

The major strengths of the current study are its population-based nature, large sample size, meticulous adjudication of AF events, detailed information on cardiovascular risk factors, long follow-up time, multiple sensitivity analyses including complete case analyses, excluding prevalent and incident coronary heart disease prior to AF diagnosis, and the use of competing risk analyses to compute cause-specific hazards while taking mortality into account as a competing risk. The use of penalized cubic splines allowed us to examine the shape of the various associations and to assess any potential non-linearity. The availability of up to 5 repeated measurements for different electrocardiographic parameters during follow-up also enabled us to assess the longitudinal measures of electrocardiographic parameters in association with new-onset AF by using a joint modeling approach, providing more insight and information than a single baseline measurement. However, our study also has some limitations that should be considered. Distinction between paroxysmal, persistent, and permanent AF was not possible, because Holter monitoring has not been performed in this large population-based cohort. Also, we cannot rule out residual confounding despite our extensive adjustment for potential confounders. It is worth noting that some of the mentioned risk areas should be interpreted cautiously considering the sometimes large confidence intervals as presented in our figures. Finally, our findings may not be generalizable to younger

populations and other ethnicities, as our study included mainly older participants from European descent.

In conclusion, the associations of baseline electrocardiographic measures and risk of new-onset AF were mostly U- and N-shaped. Furthermore, longitudinal measures of PR interval and QTc interval were significantly associated with new-onset AF. Sex differences were most apparent with regard to the shape of the associations for baseline PR, QT, QTc, RR interval, and heart rate. Additionally, QTc interval and RR interval were significantly associated with new-onset AF among men, but not among women. These findings indicate that different levels of electrocardiographic parameters might translate to a differential risk among men and women, and that modulation of electrocardiographic parameters might prevent AF in the general population, in particular in men.

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SUPPLEMENTARY MATERIAL

Chapter 3.2 Electrocardiographic parameters and the risk of atrial fibrillation

Methods S1. Study population

Methods S2. Assessment of cardiovascular risk factors

Methods S3. Statistical analyses

Results S1. Sensitivity analyses

Table S1. Association between longitudinal measures of electrocardiographic parameters with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with non-imputed data

Table S2. Association between longitudinal measures of electrocardiographic parameters with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with exclusion of prevalent and incident coronary heart disease cases prior to incident atrial fibrillation

Table S3. Association between longitudinal measures of electrocardiographic parameters with the risk of mortality in the total study population and stratified by sex

Methods S1. Study population

The Rotterdam Study is a prospective population-based cohort study that aims to assess the occurrence and progression of risk factors for chronic diseases in middle-age and elderly persons. During 1990-1993, all inhabitants of the Ommoord district in the city of Rotterdam in The Netherlands aged ≥55 years were invited for the study. A total of 7,983 (78% of all invitees) agreed to participate (RS-I). In 2000, the cohort was extended with 3,011 participants who had become ≥55 years or had migrated into the research area (RS-II). In 2006, the cohort was again extended with 3,932 participants that were ≥45 years (RS-III). The overall response rate at baseline was 72%. Participants attended follow-up examinations every 3-5 years. Outcome data on morbidity and mortality were continuously collected through linkage with digital files from general practitioners in the study area.(7, 8)

Methods S2. Assessment of cardiovascular risk factors

All the participants responded to comprehensive questionnaires at baseline to assess their present health status, medical history, medication use, and lifestyle. Additionally, the participants were interviewed at home by trained interviewers, underwent more extensive clinical examinations, and laboratory assessments at the research center.(7, 8)

Standardized measurements of height (in m) and weight (in kg) were performed and body mass index (BMI) was calculated as weight divided by height squared. Serum total and high-density lipoprotein (HDL) cholesterol were measured with an automated enzymatic method. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressures were calculated as the mean of the 2 consecutive measurements. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or the use of antihypertensive drugs prescribed for hypertension.(11) Smoking status was derived from baseline questionnaires and was categorized into never, former, and current smokers. Diabetes mellitus (DM) was defined as fasting serum glucose levels ≥7.0 mmol/L (126 mg/dL) (or non-fasting serum glucose levels ≥11.1 mmol/L (200 mg/dL) if fasting samples were unavailable) or the use of antidiabetic therapy. The assessment and definition of coronary heart disease (CHD), and heart failure (HF) has been described in detail elsewhere.(11) Left ventricular hypertrophy (LVH) was diagnosed using MEANS with an algorithm that takes into account QRS voltages, with an age-dependent correction and repolarization. Medication use was derived from baseline questionnaires, pharmacy data and was categorized and defined according to the World Health Organization Anatomical Therapeutic Chemical (WHO ATC) classifications. In detail, cardiac medication, antihypertensive medication, use of beta blockers, use of calcium blockers, and lipid lowering medication were defined according to the WHO ATC categories c01, c02, c07, c08, and c10, respectively.

Methods S3. Statistical analyses

The baseline characteristics of the study population are presented as mean with standard deviation (SD) or number (n) with percentages as appropriate. The differences between men and women were examined by the Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) for continuous variables and Chi-Square test for categorical variables. The distribution of the different electrocardiographic parameters were skewed. Therefore, a natural logarithmic transformation was used to obtain a normal distribution.

Cox proportional hazard models with and without penalized cubic splines were used to investigate the shape of the association (for example linear, J-shaped or U-shaped) between baseline measures of electrocardiographic parameters with the risk of new-onset AF. Further, we conducted competing risk analyses using joint models to investigate the association between longitudinal measures of electrocardiographic parameters with the risk of new-onset AF with mortality as a competing event. Cause-specific hazard ratios (HRs) with their 95% confidence intervals (CIs) were calculated to quantify the associations. The proportional hazard assumptions were assessed using Schoenfeld residuals and were found to be satisfied.

Further, to investigate the association between repeated measures of the electrocardiogram over time with the risk of incident AF, joint modelling for longitudinal and time to event data were used. First, linear mixed effects models were used to analyze the longitudinal measures of electrocardiographic parameters and to account for the correlation of the repeated measures. The outcome of interest in each linear mixed effects model was either PR, QRS, QT, QTc, JT, RR interval, and heart rate with up to 5 repeated measurements during follow-up. Likelihood ratio tests were used to assess whether random slopes could be dropped from the model. Due to the relatively low number of repeated measurements per individual (range, 1-5), non-linear functions of time using splines were not used. The models investigating the longitudinal measures of PR, QRS, QT, QTc, JT, RR interval, and heart rate included a random intercept and slope, and an unstructured covariance matrix. Next, the estimated subject-specific trajectories from the mixed effects models for PR, QRS, QT, QTc, JT, RR interval, and heart rate were included in the Cox models as time-varying covariates under the joint modelling framework.

The analyses were conducted in the total study population and for men and women separately. Additionally, we presented the p of the sex interaction in the total study population from the joint model. Mixed models were adjusted for age, and sex (if applicable) while survival models were adjusted for age, sex (if applicable), and cohort (model 1), and additionally for cardiovascular risk factors including BMI, total cholesterol, HDL cholesterol, hypertension, smoking status, history of DM, history of CHD, history of HF, LVH on the ECG, use of cardiac medication, use of beta

blockers, use of calcium blockers, and use of lipid lowering medication (model 2). Time was measured in years after baseline. Missing values of any covariates were imputed under the assumption of missing at random and were imputed using Bayesian linear regression ("norm"), binary logistic regression ("logreg"), and a proportional odds model ("polyr") for continuous, binary, and ordered categorical covariates, respectively from the "mice" package in R. For imputation, all available data were used to generate 1 imputed dataset. Missing values for various covariates were as follows: BMI (2.4%), total cholesterol (11.1%), HDL cholesterol (11.2%), systolic blood pressure (1.4%), diastolic blood pressure (1.4%), smoking status (1.7%), history of CHD (2.3%), history of HF (0.1%), LVH on the ECG (4.1%), use of cardiac medication (9.8%), use of antihypertensive medication (1.6%), use of beta blockers (9.8%), use of calcium blockers (9.8%), and use of lipid lowering medication (9.8%).

As sensitivity analyses, we analyzed the associations using complete-case analyses based on non-imputed data. Moreover, we analyzed the associations after exclusion of participants with prevalent CHD and incident CHD (prior to incident AF) to evaluate if this would attenuate our original findings. Finally, we calculated the cause-specific HRs for mortality to assess the competing risk of mortality with incident AF.

Statistical significance was considered for the Cox proportional hazards models at two-tailed $p<0.05$ or for the Bayesian joint models with a tail probability of <0.05. The data management was done using IBM SPSS Statistics version 25.0 for Windows (IBM Corp, Armonk, New York). The statistical analyses were performed using the R package "pspline" and "JMbayes2" in R software (R 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

Results S1. Sensitivity analyses

Our sensitivity analyses indicated that the results after imputation did not differ substantially from the complete-case analyses (**Table S1**). Furthermore, excluding participants with prevalent and incident CHD (prior to incident AF) from the analyses did not change our original results noteworthy (**Table S2**). Finally, in our competing risk analyses QRS, QTc, JT, RR interval, and heart rate were all significantly associated with mortality in both model 1 and 2 which confirms that mortality is a potential competing risk for incident AF, especially among men (**Table S3**).

Table S1. Association between longitudinal measures of electrocardiographic parameters with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with non-imputed data

	Total study population		Men		Women	
Electro-cardiographic measures	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Joint models ‡						
PR §	**1.64 (1.11-2.43), p=0.0119**	1.21 (0.83-1.79), p=0.3296	1.42 (0.82-2.35), p=0.1958	1.20 (0.74-1.97), p=0.4822	1.43 (0.87-2.45), p=0.1652	1.03 (0.58-1.75), p=0.8958
QRS §	**1.81 (1.23-2.66), p=0.0009**	1.43 (0.97-2.12), p=0.0722	1.47 (0.92-2.35), p=0.1017	1.22 (0.73-1.97), p=0.4361	**2.09 (1.24-3.52), p=0.0053**	1.58 (0.92-2.63), p=0.1003
QT §	**2.33 (1.08-4.81), p=0.0294**	1.30 (0.61-2.85), p=0.5183	1.37 (0.54-3.45), p=0.5060	0.85 (0.34-2.15), p=0.7274	**3.03 (1.23-7.62), p=0.0157**	1.84 (0.69-4.94), p=0.2258
QTc §	**14.53 (5.73-35.89), p<0.0001**	**5.58 (2.22-14.51), p=0.0002**	**20.42 (5.95-69.17), p<0.0001**	**7.34 (2.09-25.49), p=0.0013**	**10.10 (2.80-36.44), p=0.0004**	**4.14 (1.13-15.38), p=0.0310**
JT §	1.02 (0.53-1.88), p=0.9396	0.85 (0.44-1.68), p=0.6535	0.68 (0.30-1.53), p=0.3558	0.57 (0.25-1.30), p=0.1864	1.02 (0.45-2.30), p=0.9769	0.84 (0.36-1.96), p=0.6798
RR §	1.07 (0.63-1.71), p=0.7697	0.87 (0.57-1.38), p=0.5395	**0.50 (0.29-0.84), p=0.0077**	**0.46 (0.26-0.79), p=0.0053**	1.39 (0.81-2.39), p=0.2408	1.12 (0.62-2.05), p=0.7195
Heart rate §	1.33 (0.80-2.23), p=0.2816	1.09 (0.65-1.74), p=0.7021	0.68 (0.36-1.30), p=0.2367	0.65 (0.34-1.23), p=0.1894	1.95 (0.97-4.05), p=0.0615	1.44 (0.71-2.93), p=0.3245

Abbreviations: CI, confidence interval; HR, hazard ratio.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication use.

‡ Association between longitudinal electrocardiographic parameters for up to 5 repeated measurements during follow-up with incident atrial fibrillation, assessed by joint models.

§ Hazard ratios represent 1 unit increase in ln(PR), ln(QRS), ln(QT), ln(QTc), ln(JT), ln(RR), and 1 unit decrease in ln(heart rate) with the risk of new-onset atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S2. Association between longitudinal measures of electrocardiographic parameters with the risk of new-onset atrial fibrillation in the total study population and stratified by sex with exclusion of prevalent and incident coronary heart disease cases prior to incident atrial fibrillation

	Total study population		Men		Women	
Electro-cardiographic measures	Cause-specific HR (95% CI)					
	Model 1 *	Model 2 †	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Joint models ‡						
PR §	**1.72 (1.14-2.51), p=0.0076**	1.33 (0.82-2.02), p=0.2503	1.21 (0.65-2.09), p=0.5333	1.04 (0.60-1.76), p=0.8808	**1.67 (1.01-2.95), p=0.0446**	1.30 (0.80-2.16), p=0.2946
QRS §	1.36 (0.92-2.01), p=0.1250	1.09 (0.73-1.63), p=0.6317	1.15 (0.63-1.90), p=0.5830	0.94 (0.55-1.59), p=0.8173	1.61 (0.95-2.63), p=0.0811	1.29 (0.80-2.19), p=0.3313
QT §	**2.21 (1.17-4.17), p=0.0145**	1.56 (0.80-3.03), p=0.1959	1.08 (0.40-3.15), p=0.8984	0.90 (0.33-2.40), p=0.8412	**2.63 (1.03-6.59), p=0.0439**	1.55 (0.64-3.73), p=0.3320
QTc §	**9.23 (3.75-22.64), p<0.0001**	**4.66 (1.84-11.73), p=0.0009**	**17.05 (4.52-63.46), p<0.0001**	**8.53 (2.21-32.43), p=0.0020**	**5.35 (1.65-17.31), p=0.0044**	2.81 (0.87-9.28), p=0.0860
JT §	1.25 (0.68-2.39), p=0.4968	1.03 (0.55-1.87), p=0.9228	0.80 (0.34-1.96), p=0.6089	0.69 (0.27-1.71), p=0.4206	1.44 (0.61-3.38), p=0.4229	1.17 (0.55-2.51), p=0.7010
RR §	1.19 (0.77-1.87), p=0.4487	1.13 (0.72-1.74), p=0.5855	**0.52 (0.29-0.95), p=0.0345**	**0.52 (0.31-0.88), p=0.0139**	1.54 (0.89-2.72), p=0.1241	1.28 (0.74-2.20), p=0.3758
Heart rate §	**1.71 (1.01-2.80), p=0.0448**	1.50 (0.83-2.62), p=0.1757	0.62 (0.31-1.23), p=0.1506	0.78 (0.43-1.51), p=0.4385	**2.57 (1.18-4.89), p=0.0208**	**1.95 (1.04-3.78), p=0.0362**

Abbreviations: CI, confidence interval; HR, hazard ratio.

* Adjusted for age, sex (if applicable), and cohort. † Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication use. ‡ Association between longitudinal electrocardiographic parameters for up to 5 repeated measurements during follow-up with incident atrial fibrillation, assessed by joint models. § Hazard ratios represent 1 unit increase in ln(PR), ln(QRS), ln(QT), ln(QTc), ln(JT), ln(RR), and 1 unit decrease in ln(heart rate) with the risk of new-onset atrial fibrillation.

The associations with a p<0.05 are highlighted in **bold**.

Table S3. Association between longitudinal measures of electrocardiographic parameters with the risk of mortality in the total study population and stratified by sex

	Total study population		Men		Women	
Electro-cardiographic measures	**Cause-specific HR (95% CI)**					
	Model 1 *	**Model 2 †**	**Model 1 ***	**Model 2 †**	**Model 1 ***	**Model 2 †**
Joint models ‡						
PR §	0.88 (0.70-1.10), p=0.2731	0.82 (0.66-1.03), p=0.0846	**0.67 (0.49-0.91), p=0.0087**	**0.64 (0.46-0.89), p=0.0062**	1.27 (0.94-1.70), p=0.1196	1.10 (0.81-1.50), p=0.5440
QRS §	**1.62 (1.31-1.99), p<0.0001**	**1.49 (1.22-1.81), p=7.41x10^{-05}**	**1.79 (1.35-2.37), p=5.19x10^{-05}**	**1.63 (1.22-2.18), p=0.0010**	**1.48 (1.13-1.93), p=0.0040**	**1.34 (1.03-1.75), p=0.0312**
QT §	1.52 (0.90-2.55), p=0.1159	1.01 (0.60-1.72), p=0.9614	1.36 (0.66-2.80), p=0.4041	1.01 (0.47-2.12), p=0.9901	1.95 (0.95-4.02), p=0.0682	1.14 (0.54-2.40), p=0.7225
QTc §	**11.91 (5.79-24.58), p<0.0001**	**5.34 (2.59-11.06), p=7.41x10^{-06}**	**32.22 (11.73-88.45), p<0.0001**	**13.74 (4.80-39.12), p<0.0001**	**5.31 (1.96-14.41), p=0.0010**	2.64 (0.96-7.23), p=0.0614
JT §	**0.60 (0.39-0.95), p=0.0277**	**0.49 (0.31-0.77), p=0.0021**	**0.51 (0.27-0.96), p=0.0354**	**0.49 (0.26-0.94), p=0.0310**	0.79 (0.42-1.47), p=0.4456	0.59 (0.31-1.12), p=0.1086
RR §	**0.49 (0.37-0.66), p<0.0001**	**0.44 (0.33-0.60), p<0.0001**	**0.32 (0.22-0.48), p<0.0001**	**0.34 (0.23-0.51), p<0.0001**	0.86 (0.57-1.28), p=0.4464	0.69 (0.45-1.06), p=0.0929
Heart rate §	**0.43 (0.31-0.58), p<0.0001**	**0.39 (0.28-0.54), p<0.0001**	**0.28 (0.19-0.43), p<0.0001**	**0.31 (0.20-1.47), p<0.0001**	0.75 (0.49-1.14), p=0.1727	**0.63 (0.41-0.98), p=0.0395**

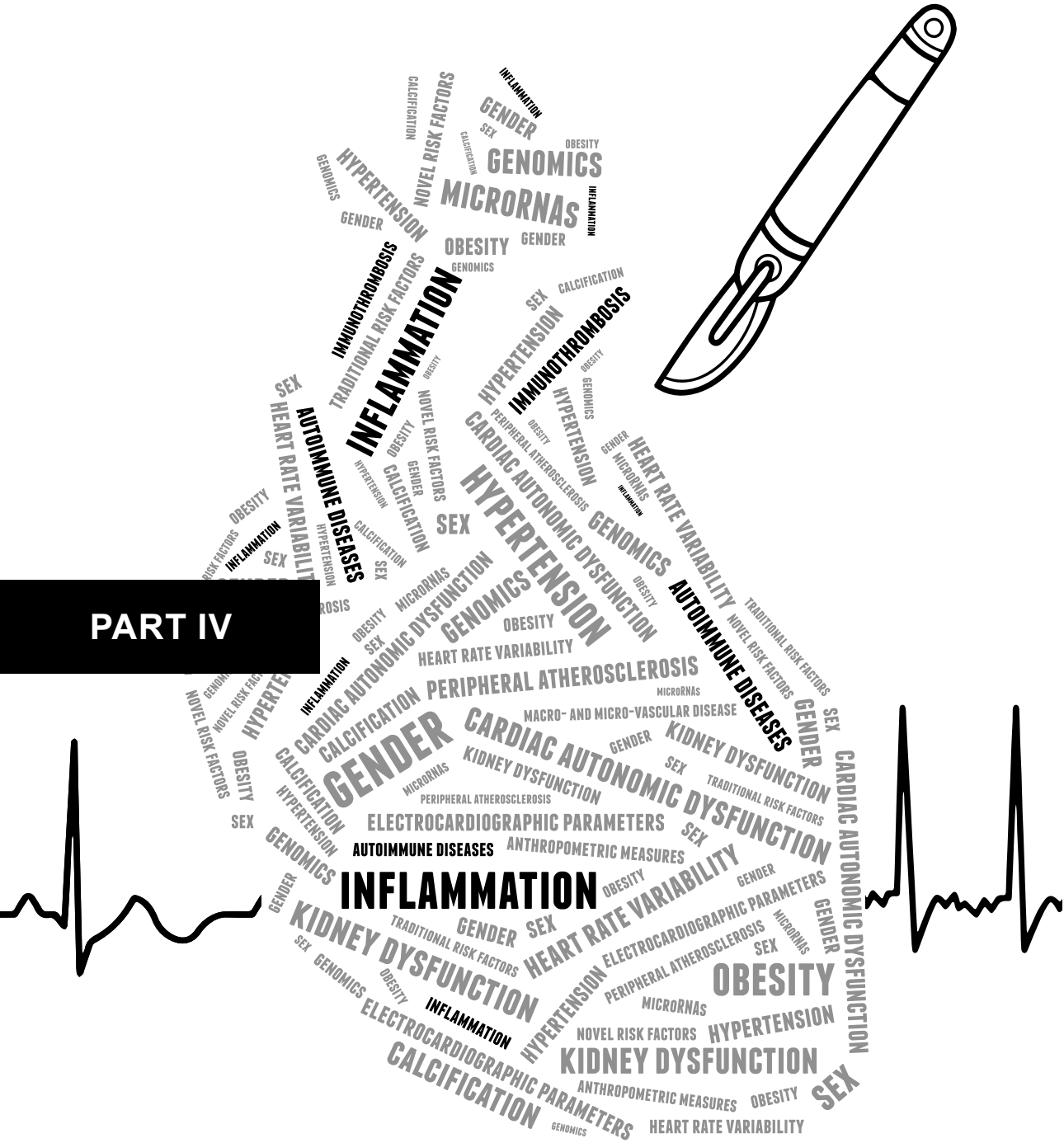
Abbreviations: CI, confidence interval; HR, hazard ratio.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, use of beta blockers, use of calcium blockers, and use of lipid lowering medication use.

‡ Association between longitudinal electrocardiographic parameters for up to 5 repeated measurements during follow-up with mortality, assessed by joint models.

§ Hazard ratios represent 1 unit increase in ln(PR), ln(QRS), ln(QT), ln(QTc), ln(JT), ln(RR), and 1 unit decrease in ln(heart rate) with the risk of mortality. The associations with a p<0.05 are highlighted in **bold**.


PART IV
INFLAMMATION
HYPERTENSION
GENOMICS
MICRORNAS
OBESITY
GENDER
SEX
CALCIFICATION
NOVEL RISK FACTORS
IMMUNOTHROMBOSIS
TRADITIONAL RISK FACTORS
AUTOIMMUNE DISEASES
HEART RATE VARIABILITY
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
KIDNEY DYSFUNCTION
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC MEASURES
MACRO- AND MICRO-VASCULAR DISEASE

Inflammation and the risk of atrial fibrillation


CHAPTER 4.1

Immunothrombosis and the risk of atrial fibrillation

Immunothrombosis and new-onset atrial fibrillation in the general population: the Rotterdam Study.

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ABSTRACT

Background
Atrial fibrillation (AF) is the most common age-related cardiac arrhythmia. The etiology underlying AF is still largely unknown. At the intersection of the innate immune system and hemostasis, immunothrombosis may be a possible cause of atrial remodeling, and therefore be an underlying cause of AF.

Methods
From 1990 to 2014, we followed participants aged 55 and over, free from AF at inclusion. Immunothrombosis factors fibrinogen, von Willebrand factor, ADAMTS13, and neutrophil extracellular traps (NETs) levels were measured at baseline. Participants were followed until either onset of AF, loss-to-follow-up, or reaching the end-date of 01-01-2014. Cox proportional hazards modelling was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), adjusted for cardiovascular risk factors.

Results
We followed 6,174 participants (mean age 69.1 years, 57.0% women) for a median follow-up time of 12.8 years. 364 men (13.7%, incidence rate 13.0 per 1,000 person-years) and 365 women (10.4%, incidence rate 8.9 per 1,000 person-years) developed AF. We found no significant association between markers of immunothrombosis and new-onset AF after adjusting for cardiovascular risk factors (HR 1.00 (95% CI 0.93-1.08) for fibrinogen, 1.04 (0.97-1.12) for von Willebrand factor, 1.00 (1.00-1.01) for ADAMTS13, and 1.01 (0.94-1.09) for NETs). In addition, we found no differences in associations between men and women.

Conclusions
We found no significant associations between markers of immunothrombosis and new-onset AF in the general population. Inflammation and immunothrombosis may be associated with AF through other cardiovascular risk factors or predisposing conditions of AF. Our findings challenge the added value of biomarkers in AF risk prediction.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia of clinical significance.(1) Despite the high prevalence, the etiology underlying AF is still largely unknown. Atrial remodeling is among the pathways promoting initiation and perpetuation of AF.(2) It is hypothesized that inflammation is one of the underlying conditions of atrial remodeling and AF.(3, 4)

Immunothrombosis refers to the complex participation of the innate immune system in the formation of intravascular thrombus through distinct cellular and molecular interactions.(5-7) This local coagulation can promote more inflammatory processes, initiating atrial remodeling through direct and indirect tissue damage.(3, 4) Fibrinogen, von Willebrand factor (vWF), and A Disintegrin and Metalloprotease with ThromboSpondin motif repeats 13 (ADAMTS13), a vWF-cleaving protease, are biomarkers that play key roles in coagulation and inflammatory pathways, and may therefore be associated with AF.(8-10) However, prospective research on this is scarce.

Activation of the innate immunity can cause neutrophils to release neutrophil extracellular traps (NETs).(5, 6, 11) Besides their important role in actively killing pathogens by releasing chromatin and DNA,(12) NETs also stimulate coagulation processes by recruiting and activating platelets, binding to tissue factor, and stimulating fibrinogen and vWF.(5, 7, 13, 14). This way, NETs are at the intersection between inflammation and thrombosis, both potentially major players in AF pathophysiology. However, the association of NETs and new-onset AF has not been investigated.

We aim to investigate the association between markers of immunothrombosis, including fibrinogen, vWF antigen (vWF:Ag), ADAMTS13, vWF:Ag/ADAMTS13 ratio, and NETs, with the risk of new-onset AF among community-dwelling men and women from the large population-based Rotterdam Study.

METHODS

Study design

Briefly, this study consists of men and women participating in the Rotterdam Study, an ongoing large, prospective population-based cohort study among inhabitants of Ommoord, a suburb in Rotterdam, the Netherlands.(15)

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Study population

A description of the study design and study population is available in **Methods S1**. We included 6174 participants free of AF at baseline who underwent blood sampling tests for fibrinogen, vWF:Ag, ADAMTS13, or MPO-DNA complex levels.

Assessment of markers of immunothrombosis

Fibrinogen levels were derived from the clotting curve of the prothrombin time assay, using Thromborel S (Behringwerke, Marburg, Germany) on the ACL 300 coagulation analyzer (Instrumentation Laboratory). vWF:Ag levels were measured with an in-house ELISA using polyclonal rabbit antihuman VWF antibodies and horseradish-peroxidase-conjugated antihuman VWF antibodies (DakoCytomation, Glostrup, Denmark) to catch and tag vWF. ADAMTS13 activity was measured in a kinetic assay using Fluorescence Resonance Energy Transfer Substrate VWF 73 (FRETS-VWF73), as is thoroughly described in the previous articles.(16, 17)

We determined NET levels by measuring MPO-DNA complexes with an ELISA as reported earlier.(18) We adjusted the commercial human cell death ELISA kit (Cell death detection ELISAPLUS, Roche Diagnostics Nederland B.V., Almere, The Netherlands). Briefly, as the capturing antibody, we used anti-MPO monoclonal antibody (clone 4A4, ABD Serotec). Patient plasma was added in combination with the peroxidase-labeled anti-DNA monoclonal antibody (from cell death detection ELISA kit; Roche). The absorbance at 405 nm wavelength was measured using Biotek Synergy HT plate reader with a reference filter of 490 nm. The values are expressed as milli-arbitrary units (mAU/mL).

Assessment of atrial fibrillation

AF was defined in accordance with the European Society of Cardiology (ESC) guidelines.(1) At study entry, prevalent AF and other diseases are assessed by an extensive interview and review of medical records. During the follow-up, participants are continuously monitored through a linkage of the study database with medical records of general practitioners and hospitals. The date of incident AF was defined as the date of the first occurrence of symptoms suggestive of AF with subsequent electrocardiogram (ECG) verification. At baseline and follow-up examinations, 10-second 12-lead ECGs were taken and stored digitally with an ACTA Gnosis IV ECG recorder (Esaote Biomedical, Florence Italy). All ECGs were analyzed using Modular ECG Analysis System (MEANS), a software system that has been described previously.(19) The ECGs diagnosed by MEANS as rhythm disorder were independently verified by 2 research physicians blind to the MEANS diagnosis. A cardiologist was consulted in case of disagreement. Events of AF were not included if these occurred during the process of dying, or in case of transient AF after cardiac surgery or myocardial infarction (MI). Participants were followed from the inclusion date until date of onset of AF, loss to follow-up, date of death, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of cardiovascular risk factors

We collected the data on body mass index (BMI), smoking status, alcohol use, renal function, differential blood count, hypertension, cardiac therapy, lipid reducing agents, prevalent coronary heart disease (CHD), heart failure (HF), and diabetes mellitus (DM). A complete description of the assessment of cardiovascular risk factors is available in **Methods S2**.

Statistical analyses

Baseline characteristics

Baseline characteristics were presented as counts and percentages or mean and standard deviation (SD), or median and interquartile range (IQR) in case of skewedness. Incidence rates are presented as events per 1,000 person-years. Differences between men and women were assessed through Student's T-test, Mann-Whitney U-test, and Chi-Square tests. Because of skewed distributions, values for fibrinogen, vWF:Ag and MPO-DNA complexes were transformed using the natural logarithm (Ln). Each marker was standardized to obtain hazard ratios (HRs) and 95% confidence intervals (CIs) per 1-SD increment. We determined the quartiles of fibrinogen, vWF:Ag, ADAMTS13, and MPO-DNA complexes. For fibrinogen, vWF:Ag, and MPO-DNA complexes the first quartiles were used as reference quartile. For ADAMTS13, the fourth quartile was used as reference. To examine the combination of vWF:Ag levels and ADAMTS13 activity on AF incidence, we combined vWF:Ag levels above or below the 75th percentile, and ADAMTS13 activity levels above and below the 25th percentile.

Cox proportional hazards models

Univariable and multivariable Cox proportional hazards regression analyses were performed. Models were adjusted for age, sex, and cohort (model 1), and additionally for cardiovascular risk factors including: current smoking status, alcohol use, estimated glomerular filtration rate (eGFR), hypertension, use of cardiac therapy, use of lipid reducing agents, history of DM, history of HF, and history of CHD (model 2). HRs and 95% CIs were calculated to quantify the associations. The proportional hazards assumptions were tested by Schoenfeld residual testing and found to be satisfied. Missing values of covariates (range 0.0-4.9%) were imputed under the assumption of missing at random. All available data were used to generate 5 imputed datasets. The results from each imputed dataset were combined to present single estimates. In addition, analyses were performed in men and women separately.

Statistical significance was considered at two-tailed $p \leq 0.05$. All analyses and data management were done with IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, New York, USA) and R: A language and environment for statistical computing, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

Baseline characteristics are presented in **Table 1**. We included 6,174 participants (mean age 69.1 ± 8.2 years), of whom 3,520 (57.0%) were women. Median blood levels for fibrinogen, vWF:Ag, and MPO-DNA complexes were 3.8 g/L, 1.19 IU/mL, and 53 mAU/mL, respectively. The mean plasma level for ADAMTS13 activity was 91.6% ± 17.7%.

Women were significantly older (mean age 69.6±8.4 years vs. 68.4±7.7 years) and had a higher mean BMI (27.3 ± 4.4 vs. 26.5 ± 3.7 kg/m^2), whereas men had significantly higher prevalence of DM (14.9% vs. 11.4%) and CHD (14.2% vs. 4.0%). Differential bloodwork showed significant differences for thrombocyte count (241.4 ± 55.7 vs. 269.4 ± 57.4x10^9/L), leucocyte count (7.0 ± 1.9 vs. 5.7 ± 1.9x10^9/L), lymphocyte count (2.6 ± 0.9 vs. 2.6 ± 1.0x10^9/L), lymphocyte percentage of total leucocytes (37.5 ± 7.5 vs. 39.4 ± 8.0%), and platelet to lymphocyte ratio (99.1 ± 32.3 vs. 111.6 ± 35.1) between men and women, respectively. Median fibrinogen (3.9 g/L (IQR 1.1) for men vs. 3.7 g/L (IQR 1.1) for women) and ADAMTS13 activity (94.8% ± 17.6% for men vs. 87.3% ± 16.9% for women) were significantly different between men and women (**Table S1**).

Atrial fibrillation incidence

During a median follow-up of 12.8 (IQR 5.6) years (69,093 person-years), 729 participants (364 men and 365 women) developed AF (incidence rate 10.6 per 1,000 person-years). Incidence rates were 13.0 per 1,000 person-years for men and 8.9 per 1,000 person-years for women. There were no significant associations between fibrinogen (HR (95% CI): 1.00 (0.93-1.08)), vWF:Ag (HR (95% CI): 1.03 (0.95-1.11)), ADAMTS13 (HR (95% CI): 1.00 (1.00-1.01)), vWF:Ag/ADAMTS13 ratio (HR (95% CI)): 1.00 (0.93-1.08)), or MPO-DNA complexes (HR (95% CI): 1.01 (0.94-1.09)) with new-onset AF after adjustments (**Table 2**).

Cox proportional hazards models

Univariable Cox proportional hazards regression showed a significant larger risk of new-onset AF with higher levels of vWF:Ag in both men (HR (95% CI): 1.19 (1.07-1.32)) and women (HR (95% CI): 1.14 (1.03-1.27)). After adjusting for cardiovascular risk factors, the associations attenuated (**Table S2**). For fibrinogen, ADAMTS13, and MPODNA complexes we found no associations in men or women.

Both univariable analysis and multivariable analysis showed no significant differences in risk between quartiles for fibrinogen or MPO-DNA complexes (**Figure 1**). We found a higher risk of new-onset AF with vWF:Ag levels in the highest quartile as compared to the lowest quartile (HR (95% CI): 1.37 (1.11-1.70)), and for

ADAMTS13 levels in the lowest quartile, as compared to the highest (HR (95% CI): 1.51 (1.23-1.86)) in univariable models. After adjustment for cardiovascular risk factors, the associations attenuated (**Figure 1**).

In sex-stratified analyses, highest vs. lowest vWF:Ag levels showed a significant larger AF risk in women (HR (95% CI): 1.55 (1.16-2.07)) in univariable analysis, but not in men. In contrast, the lowest vs. highest ADAMTS13 activity levels were associated with AF risk among men (HR (95% CI): 1.63 (1.20-2.22)) in univariable analysis, but not in women. After adjustment for cardiovascular risk factors, the associations were not statistically significant (**Figure S1**).

Combining vWF:Ag and ADAMTS13 levels, participants with vWF:Ag levels ≥1.61 IU/mL and ADAMTS13 activity ≤80.31% had a significantly higher risk of new-onset AF than participants with vWF:Ag <1.61 IU/mL and ADAMTS13 activity >80.31% (HR (95% CI): 1.47 (1.09-1.98)), albeit nonsignificant after adjustments (**Table 2**). Sex-stratified analyses showed a larger risk for AF with vWF:Ag levels <1.61 IU/mL and ADAMTS13 activity ≤80.31% (HR (95% CI): 1.55 (1.22-1.97)) in men in univariable analysis, but not in women. After adjustments, all associations attenuated (**Figure S1**).

Table 1. Baseline characteristics of the total study population

Baseline characteristics *	**Total study population n=6,174**
Age, years	69.1 ± 8.2
Body mass index, kg/m^2	27.0 ± 4.3
Current smoking, n (%)	1,247 (20.2)
Prevalent diabetes mellitus, n (%)	797 (12.9)
Prevalent coronary heart disease, n (%)	518 (8.4)
Prevalent heart failure, n (%)	163 (2.6)
Prevalent hypertension, n (%)	4,163 (67.4)
eGFR (ml/min per 1.73 m^2)	74.9 ± 15.7
Systolic blood pressure, mmHg,	143.3 ± 21.2
Diastolic blood pressure, mmHg,	76.8 ± 11.1
Antihypertensive medication, n (%)	2,172 (35.2)
Daily alcohol intake, g	5.0 (17.0)
Prevalent alcohol abuse, n (%) †	939 (15.2)
Use of cardiac therapy, n (%)	484 (7.8)
Lipid reducing agents, n (%)	812 (13.2)
Thrombocyte count, 10^9/L	257.4 ± 58.3
Leucocyte count, 10^9/L	6.8 ± 1.9
Lymphocyte count, 10^9/L	2.6 ± 1.0
Lymphocyte percentage of leucocytes, %	38.6 ± 7.8
Platelet to lymphocyte ratio	106.3 ± 34.5
Total cholesterol, mmol/L ‡	5.8 ± 1.0
High-density lipoprotein cholesterol, mmol/L ‡	1.4 ± 0.4
C-reactive protein, mg/L	1.7 (3.0)
Plasma fibrinogen, g/L	3.8 (1.1)
Plasma VWF:Ag, IU/mL	1.19 (0.66)
ADAMTS13 activity, %	91.6 ± 17.7
MPO-DNA complex, mAU/mL	53 (45)

Abbreviations: ADAMTS13, A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13; eGFR, estimated glomerular filtration rate; vWF:Ag, von Willebrand Factor antigen.

* Values are mean (standard deviation) for normally distributed continuous variables or median (interquartile range) for skewed continuous variables or number (percentages) for categorical variables.

† Alcohol abuse is defined as ≥ 4 alcoholic consumptions/day for men, and ≥ 2 for women.

‡ SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table 2. Association between markers of immunothrombosis and incident atrial fibrillation in the total study population

Immunothrombotic markers	Total study population	
	HR (95% CI)	
	Model 1 *	Model 2 †
Fibrinogen, g/L	1.02 (0.95-1.10)	1.00 (0.93-1.08)
vWF:Ag, IU/mL	1.05 (0.97-1.13)	1.03 (0.95-1.11)
ADAMTS13, %	1.00 (1.00-1.01)	1.00 (1.00-1.01)
vWF:Ag/ADAMTS13 ratio	1.02 (0.95-1.10)	1.01 (0.93-1.08)
MPO-DNA complex, mAU/mL	1.01 (0.94-1.09)	1.01 (0.94-1.09)

Abbreviations: ADAMTS13, A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13; CI, confidence interval; cIMT, HR, hazard ratio; vWF:Ag, von Willebrand Factor antigen.

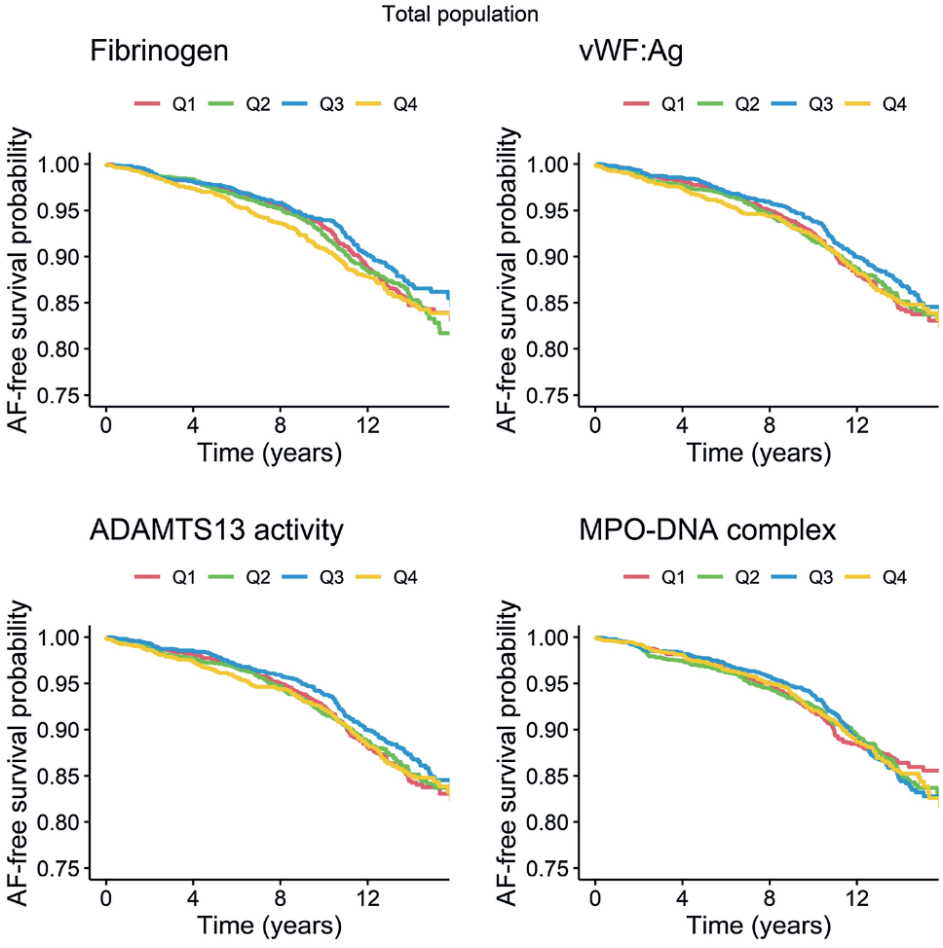
* Adjusted for age, sex, and cohort.

† Adjusted for age, sex, cohort, current smoking, alcohol use, renal function, hypertension, use of cardiac therapy, use of lipid reducing agents, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase of each immunothrombotic marker with the risk of new-onset atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

Figure 1. Association between markers of immunothrombosis and incident atrial fibrillation in the total study population per quartile



Abbreviations: AF, atrial fibrillation.

Adjusted for age, sex, cohort, current smoking, alcohol use, renal function, hypertension, use of cardiac therapy, use of lipid reducing agents, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

Quartiles fibrinogen: ≤3.30 g/L, 3.31-3.80 g/L, 3.81-4.40 g/L, and ≥4.41 g/L.

Quartiles vWF:Ag: ≤0.93 IU/mL, 0.94-1.20 IU/mL, 1.21-1.60 IU/mL, and ≥1.61 IU/mL.

Quartiles ADAMTS13: ≤80.31%, 80.32-91.00%, 91.01-101.75%, and ≥101.76%.

Quartiles MPO-DNA complex: ≤42 mAU/mL,42-53 mAU/mL, 54-87 mAU/mL, and 88 mAU/mL.

DISCUSSION

In this prospective population-based study, we examined the association between immunothrombosis and new-onset AF among men and women. Biomarkers related to inflammation and coagulation, including fibrinogen, vWF, ADAMTS13, vWF:Ag/ADAMTS13 ratio, and NETs were not independently associated with new-onset AF.

This is the first large prospective population-based cohort study to evaluate the link between NET formation and new-onset AF development. Previous studies suggested that markers of systemic and local inflammation are associated with AF development.(20, 21) While the exact pathways of the development of AF are still unknown, immunothrombosis is implicated in AF pathophysiology. NETs play an important role in immunothrombosis. During the process of NETosis, histones, antimicrobial proteins, and cell-free DNA are released from cells, especially neutrophils.(5, 6, 11-14) Through Toll-like receptors (TLR) 2, 4, and 9, these histones cause inflammation and eventually cell-death in endothelial and epithelial cells.(22) The histones, as well as the DNA, also directly activate local platelets, which in turn activate the coagulation cascade.(22) Through these processes NETs can cause tissue injury by directly killing endothelial cells and through local microvascular thrombosis. The local tissue damage caused by NETs, combined with the inflammatory effects related to immunothrombosis, can lead to structural and electrical remodeling of the atria.(2, 3, 23-28) This progressively impairs atrial conduction and promote reentry, giving rise to AF.(29, 30) However, the lack of significant associations in our study suggests that the potential impact of inflammation on AF development lies in other pathways than the above-described paths of immunothrombosis. Therefore, more research on the role of immunothrombosis in the development of AF is required.

Fibrinogen, vWF, and ADAMTS13 play important roles in both coagulation and inflammation, and have been reported as independent risk factors for cardiovascular disease.(9, 31-34) Higher levels of fibrinogen and vWF can lead to intravascular thrombosis, vascular damage, and thrombotic complications, whereas lower levels of ADAMTS13 result in decreased cleavage of large prothrombotic vWF multimers.(32) Nonetheless, we did not find any association between these risk markers with incident AF among women and men from the general population.

Similar to our findings, fibrinogen was not associated with incidence of AF within the Framingham Offspring Study(35) and the Malmö Preventive Study.(36) However, fibrinogen showed significant associations with incident AF in the Copenhagen City Heart Study, the ARIC study, and the Women's Health Study.(37-39) Higher levels of fibrinogen may indicate underlying inflammatory processes. Local inflammation

may cause local remodeling of the atria, eventually disrupting the conduction, and be a pathophysiological cause of new-onset AF.(40) However, the results regarding the association of fibrinogen with new-onset AF remain inconclusive. A possible explanation for these discrepancies could be the differences in study populations. The Copenhagen City Heart Study was based on hospitalized AF patients, and therefore possibly represent the most symptomatic and severe AF cases.(38) The ARIC study(37) and the Women's Healthy Study(39) studied younger cohorts. The latter also lacked periodical ECG screening for AF, thus participants with asymptomatic AF or less severe cases of AF may have been missed.

To our knowledge, we are also the first large prospective cohort study reporting on the combination of vWF and ADAMTS13 levels. The ARIC study(37) and the Framingham Offspring Study(41) have reported significant associations between vWF:Ag and AF. vWF is secreted by damaged endothelial cells and plays an active role in thrombogenesis by platelet aggregation.(42, 43) Thrombogenesis can cause further inflammation, cardiovascular complications, and oxidative stress, which can all be underlying causes of AF.(44-46) As ADAMTS13 degrades large, thrombogenic vWF-multimers into smaller and less thrombogenic molecules, an inverse association with AF is expected. A combination of higher levels for vWF:Ag and lower levels of ADAMTS13 may indicate underlying immunothrombosis. None of the previously mentioned studies looked at vWF:Ag and ADAMTS13 combined. The younger age of the participants in the ARIC study may partly explain differences between our results from the ones by ARIC investigators. Ko et al. used proteomics profiling to measure ADAMTS13 levels,(41) whereas we measured ADAMTS13 activity using the functional FRETS assay.(16, 17) These different methods might, at least partly, explain different results.

After adjusting for additional cardiovascular risk factors, the observed associations between markers of immunothrombosis and AF attenuated. Possibly, inflammation and immunothrombosis are associated with AF through other cardiovascular risk factors or predisposing conditions to AF, such as CHD or HF. As factors of inflammation and hemostasis were previously associated with cardiovascular disease,(31-34) the influence they have on AF initiation might be through these comorbidities. This way, the relation of inflammation and immunothrombosis with new-onset AF might be, partly, through other pathophysiological pathways than the direct effect of (local) inflammatory processes and atrial remodeling. In addition, immunothrombosis is a complex conjunction of the immune system and coagulation. Although we aimed to look at different aspects of immunothrombosis in this study, a connection between immunothrombosis and AF might be found through other pathways.

Recent studies have previously challenged the specificity and added value of various biomarkers in prediction of new-onset AF and AF complications.(47, 48) Most studies investigating the association of inflammatory biomarkers use specific patient populations. Therefore, elevated biomarkers may be representing the clinical situation or comorbidities of a patient, rather than actually having a causal relation with the investigated conditions. However, in a large, general population setting as in our current study, single biomarkers may not be specific enough to be of added value for AF prediction. Moreover, we carefully adjusted our analyses for relevant comorbidities and potential causes of confounding. This supports the notion that many biomarkers could often be a representation of the patient condition. While not investigated in this study, lack of specificity of biomarkers may also hold for the association of immunothrombotic biomarkers related to AF complications.(48)

Recent evidence suggests sex differences in AF pathophysiology.(49, 50) While the incidence of AF is lower in women, women with AF have an increased risk of developing cardiovascular complication and mortality.(49) Sex differences in atrial remodeling and electrophysiological function have been reported.(50) It is known that autoimmune diseases are more prevalent in women, and immunologic differences between men and women have been reported.(51, 52) Also, the role of inflammation in AF initiation may be different in men and women. However, in our study we found no evidence of sex differences in the associations of immunothrombosis with AF.

The large population-based study population and long follow-up are the main strengths of this study. Through extensive interviews by trained interviewers, periodical research center visits, linkage with GP records, and meticulous adjudication of the events by study physicians, AF events are carefully assessed and a range of risk factors are available. However, there are also limitations. Despite the meticulous assessment we are unable to distinguish between paroxysmal and long-lasting AF as Holter monitoring is not available. Additionally, as blood was sampled at baseline, no inferences regarding longitudinal changes in markers and the effect of these changes on AF risk could be made. As our study shows, the development of AF greatly relies on other cardiovascular risks and patient conditions. As biomarkers, as well as many other cardiovascular risk factors, are dynamic, we can expect the evolution of these risk factors as individuals grow older will differ. Future studies investigating these biomarkers, taking into account their dynamic nature through repeated measurements and regular reassessments, are warranted to increase our knowledge regarding the link between AF and immunothrombosis. While representative of the general Dutch population above 55 years old, these results may not apply to men and women of younger age or other ethnicities. Lastly, we determined NET levels by measuring MPO-DNA complexes through ELISA. The specificity of ELISA to accurately detect NETs is controversial, and these results should therefore be cautiously interpreted.(53)

In conclusion, fibrinogen, vWF:Ag, ADAMTS13, vWF:Ag/ADAMTS13, or NETs were not associated with the risk of new-onset AF in our large prospective population-based study. Our findings challenge the added value of biomarkers in AF prediction in a general population. Inflammation and immunothrombosis may be associated with AF through cardiovascular risk factors or other predisposing conditions to AF. Moreover, the impact of inflammation on new-onset AF could lie in other pathways than the examined immunothrombosis markers. Therefore, more prospective research towards markers of immunothrombosis in AF pathophysiology is warranted.

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SUPPLEMENTARY MATERIAL

Chapter 4.1 Immunothrombosis and the risk of atrial fibrillation

Methods S1. Study design and study population

Methods S2. Assessment of cardiovascular risk factors

Table S1. Baseline characteristics of the total study population stratified by sex

Table S2. Association between markers of immunothrombosis and the risk of new-onset atrial fibrillation stratified by sex

Figure S1. Association between markers of immunothrombosis and the risk of new-onset atrial fibrillation stratified by sex per quartile

Methods S1. Study design and study population

The study population consisted of participants of the Rotterdam Study (RS), an ongoing large, prospective population-based cohort study among adult inhabitants of Ommoord, a suburb in Rotterdam, the Netherlands.(15) The aim of the RS is to obtain an overview of chronic diseases in mid-life and late-life in regards to risk factors, prognosis, etiology and potential intervention targets, by collecting data on determinants and occurrence of cardiovascular, neurological, ophthalmologic, locomotor, and psychiatric diseases. In 1990 the first cohort (RS-I) was enrolled in the study, consisting of 7,983 out of 10,215 eligible individuals. Baseline data was collected between 1990 and 1993 (RS-I-1), with follow-up examinations and questionnaires every 3 to 5 years. A new cohort (RS-II) was included in 2000, consisting of 3,011 participants out of the 4,472 inhabitants who moved to Ommoord or turned 55 since the start of RS-I. In 2006, another cohort was initiated (RS-III), including 3,932 out of 6,057 inhabitants aged 45-54 years. By the end of 2008, the study contained 14,926 participants aged 45 or older, out of 20,744 total inhabitants of Ommoord (overall response 72%). Data on morbidity and mortality were continuously collected through linkage with digital files from general practitioners in the study area.(15)

For this current study, we included participants from the third examination round of the first cohort (RS-I-3) and the first examination round of the second cohort (RS-II-1). We excluded all individuals without informed consent for follow-up data collection (n=251) or with current or a history of AF (n=632). Out of 10,112 participants free of AF at baseline, 6,174 participants underwent blood sampling tests for fibrinogen, vWF antigen (vWF:Ag), ADAMTS13, or MPO-DNA complex blood levels and were included in this study.

The Rotterdam Study complies with the declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Methods S2. Assessment of cardiovascular risk factors

Assessment of cardiovascular risk factors Extensive baseline data were obtained from participants at inclusion of the study through physical examinations and questionnaires. Trained research assistants conducted interviews for relevant information (e.g. medication use, smoking status). Body mass index (BMI) was defined as the weight in kilograms, divided by the square of length in meters. Blood pressure was defined as the mean systolic and diastolic pressure of the right brachial artery after 2 consecutive measurements in sitting position. We defined hypertension as a systolic blood pressure of 140 mmHg or higher, or a diastolic blood pressure of 90mmHg or higher, or the use of antihypertensive medication. Alcohol use was measured in grams/day. Alcohol abuse was defined as ≥4 glasses or ≥40 grams/day for men, and ≥2 glasses or ≥20 grams/day for women. Data regarding coronary heart disease (CHD), heart failure (HF), and diabetes mellitus (DM) were obtained through the records of general practitioners and hospitals. Medication use was verified through the medical records of pharmacies. The use of cardiac therapy and lipid reducing agents was categorized and defined according to the World Health Organization Anatomical Therapeutic Chemical (WHO ATC) classifications. Renal function was defined as estimated glomerular filtration rate (eGFR), calculated based on the creatinine and Cystatin C values.

Table S1. Baseline characteristics of the total study population stratified by sex

Baseline characteristics *	Men n=2,654	Women n=3,520	p
Age, years	68.4 ± 7.7	69.6 ± 8.4	<0.001
Body mass index, kg/m^2	26.5 ± 3.7	27.3 ± 4.4	<0.001
Current smoking, n (%)	642 (24.2)	605 (17.2)	<0.001
Prevalent diabetes mellitus, n (%)	396 (14.9)	401 (11.4)	<0.001
Prevalent coronary heart disease, n (%)	377 (14.2)	141 (4.0)	<0.001
Prevalent heart failure, n (%)	81 (3.1)	82 (2.3)	0.048
Prevalent hypertension, n (%)	1,811 (68.2)	2,351 (66.8)	0.120
eGFR (ml/min per 1.73 m^2)	76.2 ± 15.9	74.0 ± 15.5	<0.001
Systolic blood pressure, mmHg,	143.9 ± 20.8	142.8 ± 21.4	0.035
Diastolic blood pressure, mmHg,	76.2 ± 15.9	75.6 ± 10.8	<0.001
Antihypertensive medication, n (%)	893 (33.6)	1,279 (36.3)	0.014
Daily alcohol intake, g	11.4 (20.9)	1.4 (10.1)	<0.001
Prevalent alcohol abuse, n (%) †	286 (10.8)	653 (18.6)	<0.001
Use of cardiac therapy, n (%)	226 (8.5)	258 (7.3)	0.047
Lipid reducing agents, n (%)	366 (13.8)	447 (12.7)	0.130
Thrombocyte count, 10^9/L	241.4 ± 55.7	269.4 ± 57.4	<0.001
Leucocyte count, 10^9/L	7.0 ± 1.9	6.7 ± 1.9	<0.001
Lymphocyte count, 10^9/L	2.6 ± 0.9	2.6 ± 1.0	0.528
Lymphocyte percentage of leucocytes, %	37.5 ± 7.5	39.4 ± 8.0	<0.001
Platelet to lymphocyte ratio	99.1 ± 32.3	111.6 ± 35.1	<0.001
Total cholesterol, mmol/L ‡	5.5 ± 1.0	6.0 ± 1.0	<0.001
High-density lipoprotein cholesterol, mmol/L ‡	1.2 ± 0.3	1.5 ± 0.4	<0.001
C-reactive protein, mg/L	1.7 (3.0)	1.8 (3.0)	0.040
Plasma fibrinogen, g/L	3.7 (1.1)	3.9 (1.1)	<0.001
Plasma VWF:Ag, IU/mL	1.20 (0.65)	1.18 (0.66)	0.120
ADAMTS13 activity, %	87.3 ± 16.9	94.8 ± 17.6	<0.001
MPO-DNA complex, mAU/mL	54 (47)	53 (43)	0.073

Abbreviations: ADAMTS13, A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13; eGFR, estimated glomerular filtration rate; vWF:Ag, von Willebrand Factor antigen.

* Values are mean (standard deviation) for normally distributed continuous variables or median (interquartile range) for skewed continuous variables or number (percentages) for categorical variables.

† Alcohol abuse is defined as ≥ 4 alcoholic consumptions/day for men, and ≥ 2 for women.

‡ SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S2. Association between markers of immunothrombosis and the risk of new-onset atrial fibrillation stratified by sex

Immunothrombotic markers	Men		Women	
	HR (95% CI)			
	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Fibrinogen, g/L	1.02 (0.92-1.13)	0.98 (0.88-1.09)	1.03 (0.92-1.16)	1.04 (0.93-1.17)
vWF:Ag, IU/mL	1.04 (0.93-1.16)	1.01 (0.91-1.13)	1.06 (0.95-1.18)	1.05 (0.94-1.17)
ADAMTS13, %	1.00 (0.99-1.00)	1.00 (0.99-1.00)	1.01 (1.00-1.01)	1.01 (1.00-1.01)
vWF:Ag/ADAMTS13 ratio	1.06 (0.96-1.17)	1.04 (0.94-1.15)	0.97 (0.86-1.09)	0.96 (0.85-1.08)
MPO-DNA complex, mAU/mL	0.98 (0.88-1.09)	0.98 (0.88-1.09)	1.04 (0.94-1.15)	1.04 (0.94-1.15)

Abbreviations: ADAMTS13, A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13; CI, confidence interval; cIMT, HR, hazard ratio; vWF:Ag, von Willebrand Factor antigen.

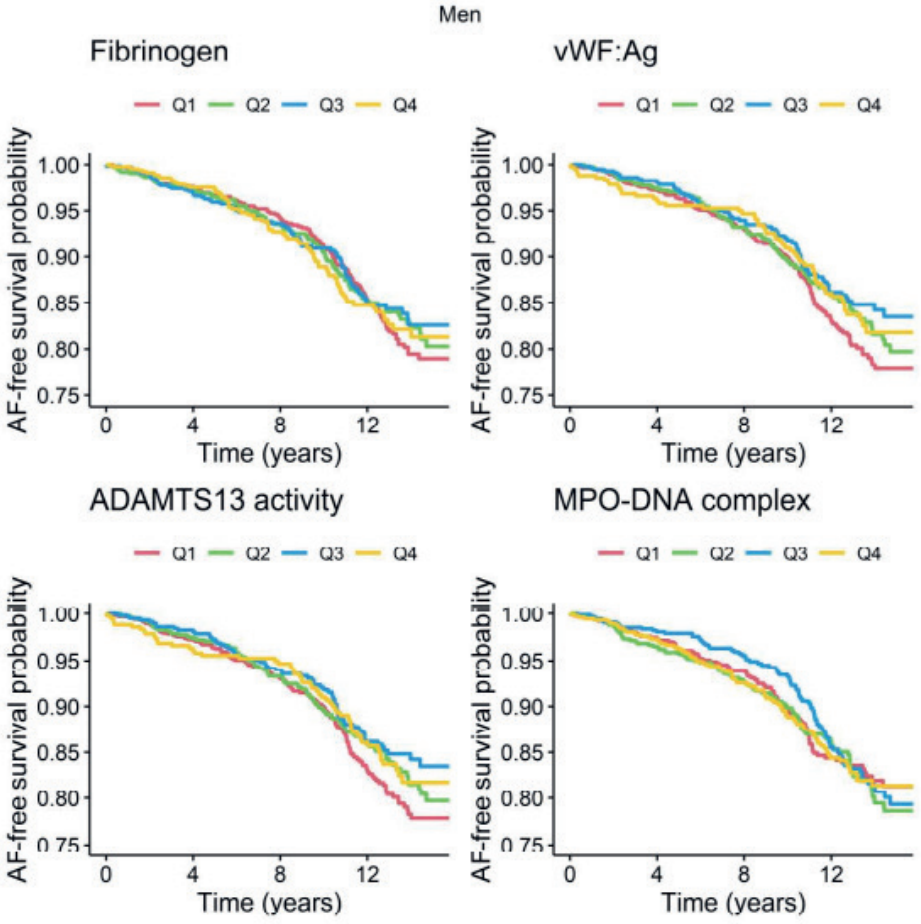
* Adjusted for age, sex, and cohort

† Adjusted for age, sex, cohort, current smoking, alcohol use, renal function, hypertension, use of cardiac therapy, use of lipid reducing agents, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase of each immunothrombotic marker with the risk of new-onset atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

Figure S1. Association between markers of immunothrombosis and the risk of new-onset atrial fibrillation stratified by sex per quartile



Abbreviations: AF, atrial fibrillation.

Adjusted for age, cohort, current smoking, alcohol use, renal function, hypertension, use of cardiac therapy, use of lipid reducing agents, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

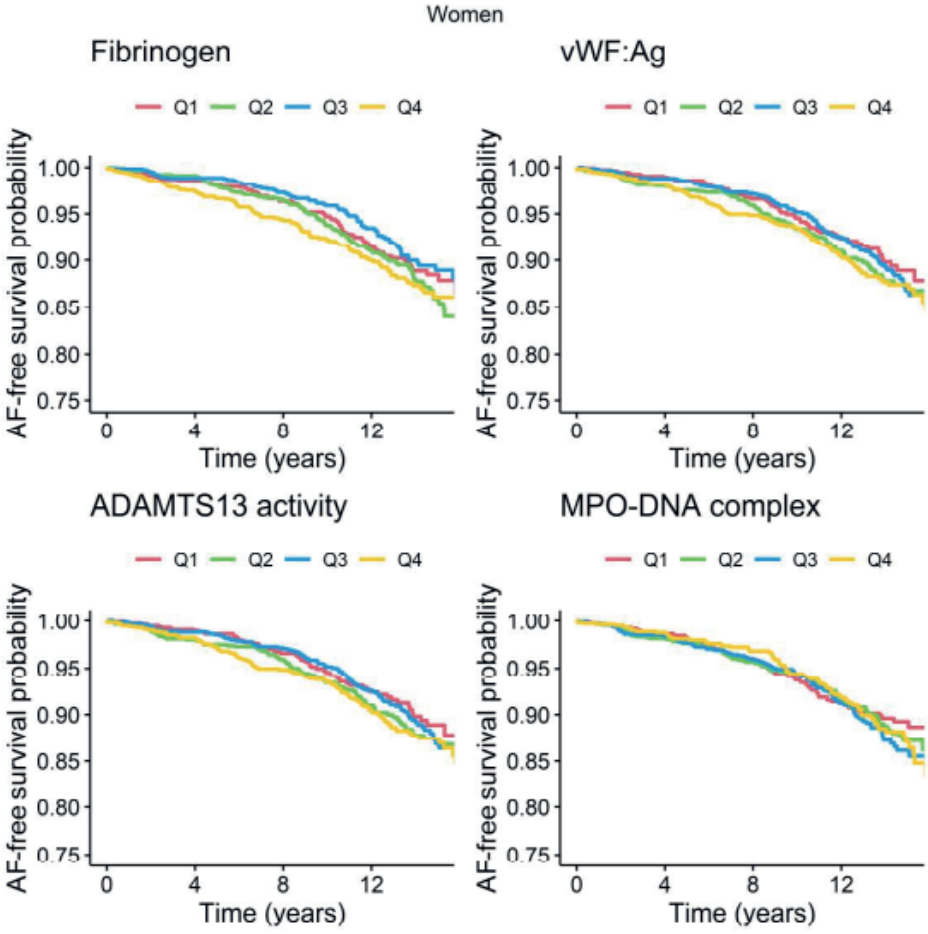
Quartiles fibrinogen: ≤3.30 g/L, 3.31-3.80 g/L, 3.81-4.40 g/L, and ≥4.41 g/L.

Quartiles vWF:Ag: ≤0.93 IU/mL, 0.94-1.20 IU/mL, 1.21-1.60 IU/mL, and ≥1.61 IU/mL.

Quartiles ADAMTS13: ≤80.31%, 80.32-91.00%, 91.01-101.75%, and ≥101.76%.

Quartiles MPO-DNA complex: ≤42 mAU/mL,42-53 mAU/mL, 54-87 mAU/mL, and 88 mAU/mL.

Figure S1. Association between markers of immunothrombosis and the risk of new-onset atrial fibrillation stratified by sex per quartile (continued)



Abbreviations: AF, atrial fibrillation.

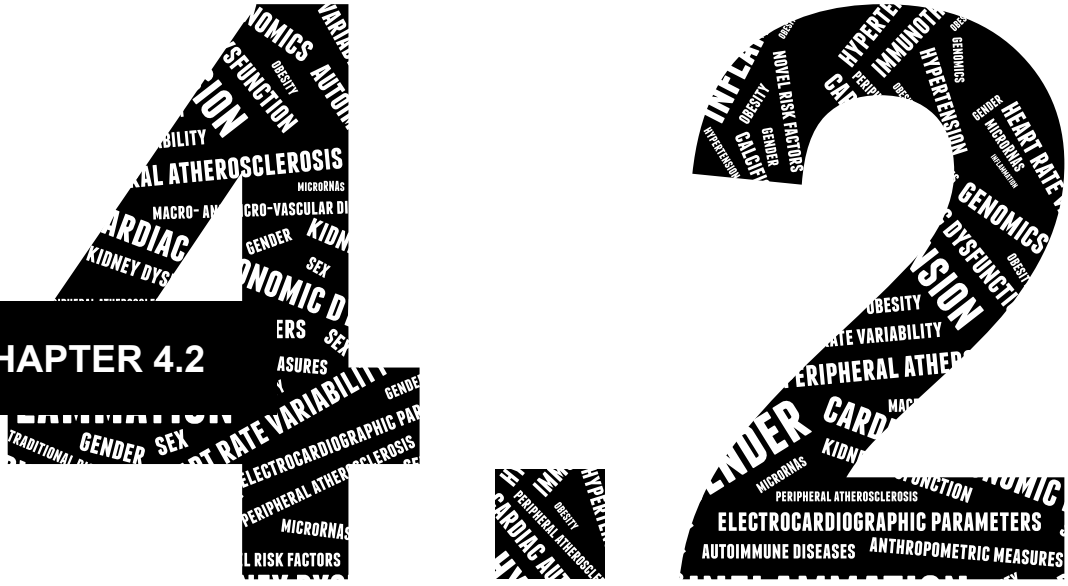
Adjusted for age, cohort, current smoking, alcohol use, renal function, hypertension, use of cardiac therapy, use of lipid reducing agents, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

Quartiles fibrinogen: ≤3.30 g/L, 3.31-3.80 g/L, 3.81-4.40 g/L, and ≥4.41 g/L.

Quartiles vWF:Ag: ≤0.93 IU/mL, 0.94-1.20 IU/mL, 1.21-1.60 IU/mL, and ≥1.61 IU/mL.

Quartiles ADAMTS13: ≤80.31%, 80.32-91.00%, 91.01-101.75%, and ≥101.76%.

Quartiles MPO-DNA complex: ≤42 mAU/mL,42-53 mAU/mL, 54-87 mAU/mL, and 88 mAU/mL.


CHAPTER 4.2
GENDER
SEX
RATE VARIABILITY
ELECTROCARDIOGRAPHIC PARAMETERS
AUTOIMMUNE DISEASES
ANTHROPOMETRIC MEASURES
PERIPHERAL ATHEROSCLEROSIS
MICRORNAS
OBESITY
GENOMICS
HEART RATE
HYPERTENSION
IMMUNOTHERAPY
NOVEL RISK FACTORS

Immunothrombotic markers in the literature and the prevalence and incidence of atrial fibrillation

The association of coagulation and atrial fibrillation: a systematic review and metaanalysis.

Tilly MJ, **Geurts S**, Pezzullo AM, Bramer WM, de Groot NMS, Ikram MA, Kavousi M, de Maat MPM.

Europace. 2022 Aug 9:euac130.

ABSTRACT

Background

While AF is suggested to induce a prothrombotic state, increasing thrombotic risk, it is also hypothesized that coagulation underlies AF onset. However, conclusive evidence is lacking. With this systematic review and meta-analysis we aimed to summarize and combine the evidence on the associations between coagulation factors with AF in both longitudinal and cross-sectional studies.

Methods

We systematically searched for longitudinal cohort and cross-sectional studies investigating AF and thrombosis. For longitudinal studies, pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. For cross-sectional studies, we determined pooled standardized mean differences (SMD) and 95% CIs.

Results

A total of 17 longitudinal and 44 cross-sectional studies were included. In longitudinal studies, we found significant associations between fibrinogen (HR 1.05, 95% CI 1.00-1.10), Plasminogen activator inhibitor 1 (PAI-1) (HR 1.06, 95% CI 1.00-1.12), and D-dimer (HR 1.10, 95% CI 1.02-1.19) and AF incidence. In cross-sectional studies, we found significantly increased levels of fibrinogen (SMD 0.47, 95% CI 0.20-0,74), von Willebrand factor (SMD 0.96, 95% CI 0.28-1.66), P-selectin (SMD 0.31, 95% CI 0.08-0.54), ß-thromboglobulin (SMD 0.82, 95% CI 0.61-1.04), Platelet Factor 4 (SMD 0.42, 95% CI 0.12-0.7), PAI-1 (1.73, 95% CI 0.26-3.19), and D-dimer (SMD 1.74, 95% CI 0.36-3.11) in AF patients, as opposed to controls.

Conclusions

These findings suggest that higher levels of coagulation factors are associated with prevalent and incident AF. These associations are most pronounced with prevalent AF in cross-sectional studies. Limited evidence from longitudinal studies suggests a prothrombotic state underlying AF development.

INTRODUCTION

With a lifetime risk of over 22% in men and women at the age of 55, atrial fibrillation (AF) is a highly prevalent disease, and expected to increase rapidly considering the aging of the population.(1, 2) This is especially relevant since AF patients are at an increased risk to develop stroke and heart failure, and have an increased risk of hospitalization and death.(3-6) Several risk factors for AF onset are already identified, including older age, male sex, and obesity.(7) While atrial remodeling is generally considered to be the underlying cause of AF, the exact pathways causing atrial remodeling are still largely unknown.(7-11) Previous studies suggest that a prothrombotic state is associated with prevalent AF, eventually leading to thrombotic events.(8) On the other hand, it is also suggested that coagulation underlies AF onset.(12-14) A possible mechanism underlying this association is immunothrombosis, as coagulation may increase local inflammation, stimulating atrial remodeling and eventually AF development.(15-17) However, studies investigating the relation of coagulation with incidence AF are scarce and contradicting. Additionally, studies investigating the association of coagulation and AF presence investigate a limited amount of biomarkers, and these studies generally have small sample sizes. More conclusive evidence is warranted on the association of coagulation and AF, to improve knowledge on AF risk, AF prevention, and AF management.

We aimed to investigate the role of various markers of coagulation in the development and presence of AF. For this, we performed a systematic review and meta-analysis, summarizing and pooling all available evidence from both longitudinal and cross-sectional studies.

METHODS

Data sources and study selection

The methods in this systematic review are described based on the Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) Checklist(18) and the Prima-S extension to the PRISMA Statement for Reporting Literature Searches in Systematic Reviews.(19) An exhaustive search strategy was developed by an experienced information specialist (WB) in Embase, optimized for sensitivity, and translated to other databases.(20) The search was carried out in Embase, Medline ALL via Ovid, Web of Science Core, and the Cochrane Central Register of Controlled Trial via Wiley. Additionally, the 200 most relevant references from Google Scholar were downloaded using Publish or Perish.(21) The original search was performed in February 2020, and last updated on January 26th 2022.(22) The search strategies for Embase and Medline used relevant thesaurus terms from

Emtree and Medical Subject Headings (MeSH), respectively. Titles and abstracts from all databases were searched for the established search terms. The search contained terms for 1) hemostasis and coagulation factors, 2) atrial fibrillation, and 3) prediction, disease association, or prevalence. The full search strategies of all databases are available in **Methods S1**. Non-English articles and animal-only articles were excluded from the search results. The reference lists of retrieved non-included relevant articles and of the included studies have been scanned for references missed by the search.(23) No authors or subject experts were contacted and we did not browse unindexed journals in the field.

The references were imported into EndNote and duplicates were removed by the medical librarian.(24) All titles and abstracts were independently screened by at least 2 reviewers (MJT, SG, or AMP) in EndNote.(25) Any discrepancies in the verdict were resolved by discussion with the third reviewer. The quality of all studies were scored based on the Newcastle-Ottawa criteria.(26)

Inclusion and exclusion criteria

All studies investigating one or more biomarkers of coagulation in humans with atrial fibrillation and controls in sinus rhythm were included. The outcome of interest was defined as non-valvular AF or overall AF. Eligible study types were cross-sectional studies and longitudinal studies. All reviews, meta-analyses, case reports, and non-original studies were excluded. Additionally, studies investigating the biomarkers of interest in an uninterpretable method (for example tertiles) and studies investigating patients in acute cardiovascular settings or after cardiac surgery were removed. Lastly, if no English full text was available, the studies were also excluded from this systematic review. To avoid individuals being counted multiple times in the meta-analyses, if multiple studies investigating the same biomarker were conducted in the same study populations, the largest study was included. Additionally, biomarkers only investigated in one study were not included in meta-analyses.

Statistical analyses

Coagulation and atrial fibrillation in longitudinal studies

For longitudinal studies, pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated with log-transformed HRs and 95% CIs using the generic inverse variance method. The most extensively adjusted model per study was included in the meta-analysis. Additionally, sensitivity analyses were performed by grouping the analyses into univariate models, simple multivariate models (adjusted for age, sex, race, and/or cohort), and complex multivariate analyses (additionally adjusted for cardiovascular risk factors).

Coagulation and atrial fibrillation in cross-sectional studies

For cross-sectional studies, means and standard deviation (SD) were extracted from the studies. If median and interquartile range (IQR) were reported in the studies, the SD was manually calculated by dividing the IQR with 1.35, as explained in the Cochrane handbook of systematic review.(27) Inverse variance weighting was used for pooling. Differences were reported as standardized mean difference (SMD) and 95% CIs. SMDs <0.2 are considered no effect, 0.2-0.5 as a small effect, 0.5-0.8 as an intermediate effect, and ≥0.8 as a large effect.

Due to the high probability of significant heterogeneity between the studies, random-effect models were used in the meta-analyses. Heterogeneity was assessed through I^2-statistics. If I^2 was above 75%, additional sensitivity analyses were performed to identify causes of heterogeneity. Publication bias was assessed visually through funnel plots. Statistical significance was considered at a two-tailed $p \leq 0.05$. Data management and statistical analyses were performed in IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, New York, USA) and R: A language and environment for statistical computing, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study selection

10,335 manuscripts were collected for title and abstract screening. 236 (2.3%) studies were eligible for full-paper reading. We excluded studies with a study design other than cohort and case-control studies (n=60), studies using populations in acute cardiovascular or surgical settings (n=34), studies without the biomarkers of interest (n=56), studies investigating other outcomes than non-valvular or overall AF as primary or secondary outcome (n=5), studies with uninterpretable data (for example tertiles) (n=7), and non-English full-text available studies (n=5). Through reference checking 2 additional studies were included. An overview of the study selection is depicted in **Figure 1**. Characteristics of the included studies are summarized in **Tables S1** and **S2**.

Coagulation and atrial fibrillation in longitudinal studies

Fibrinogen

9 population-based studies investigated the association of fibrinogen with incident AF, totalling 85,282 individuals (mean age 54.6 years, 60.0% women), of whom 4,164 (6.1%) developed AF.(13, 28-35) The median follow-up from this set of studies ranged from 3.6 to 25.0 years. Combining all 9 studies (**Figure 2**), our meta-analysis showed no significant association between fibrinogen and incident AF (HR 1.04, 95% CI 0.99-1.08). When combining the more extensively adjusted models, fibrinogen was minimally associated with incidence AF (HR 1.05, 95% CI 1.00-1.10).(13, 29-33, 35) In contrast, grouping the studies using simple multivariable analyses resulted in no significant association (HR 1.08, 95% CI 0.97-1.21).(29, 31, 32, 34, 35) Similarly, combining the 2 univariable analyses, no significant associations were found (HR 1.08, 95% CI 0.90-1.29).(28, 34)

Von Willebrand factor

2 studies (total n=21,032, AF events n=1,938, mean age 58.6 years, 55.6% women) investigated the relation between vWF and incidence AF. Pooled, we found no significant association (HR 1.10, 95% CI 0.98-1.23).(29, 35)

ADAMTS13

For a desintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) no statistical significant associations were found with incidence AF (HR 0.89, 95% CI 0.70-1.13), after combining the results of 2 studies (total n=8,059, AF events n=1,078, mean age 65.8 years, 56.3% women).(35, 36)

P-selectin

The association of P-selectin and incidence AF was investigated by 2 studies (total n=3,743, AF events n=265, mean age 59.9 years, 53.6% women).(34, 37) The pooled effect of these studies showed no significant association of P-selectin with AF (HR 1.10, 95% CI 0.87-1.18).

Plasminogen activator inhibitor 1 (PAI-1)

The pooled effect of 3 studies (total n=14,153, AF events n=1,197, mean age 60.1 years, 51.4% women) showed a significant association of PAI-1 and AF risk in complex multivariate models (HR 1.06, 95% CI 1.00-1.12).(33, 38, 39)

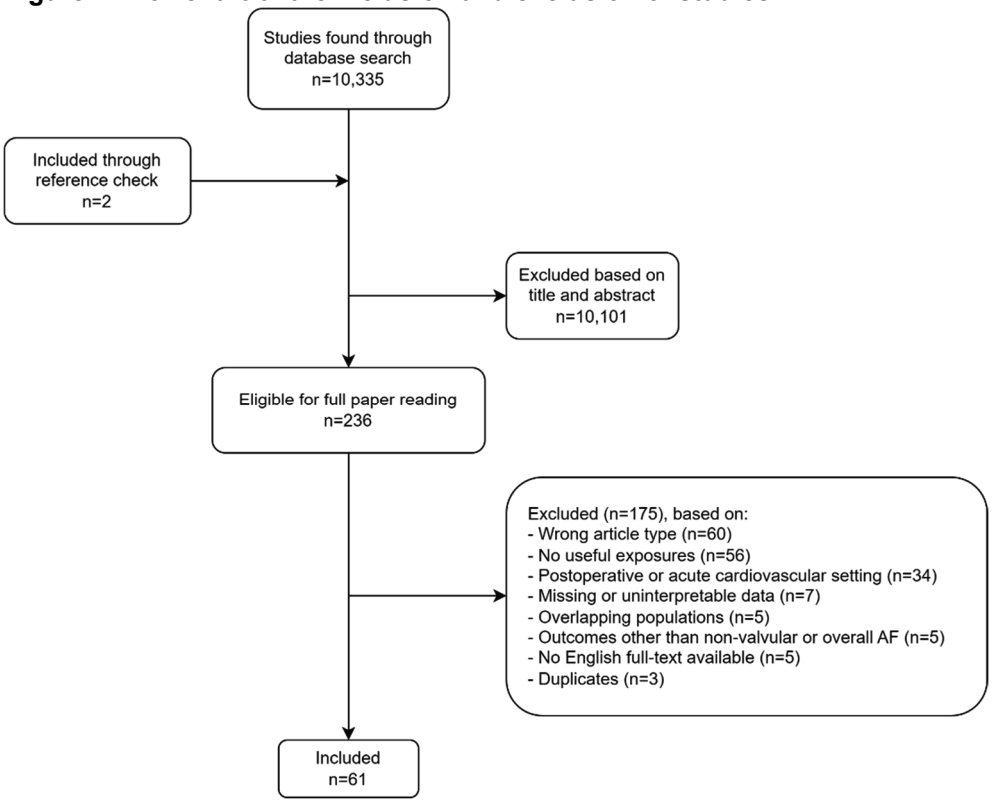
D-dimer

2 studies (total n=9,735, AF events n=1,084, mean age 60.2 years, 53.2% women) examined the association of D-dimer with new-onset AF in complex multivariate models.(33, 40) Pooled effect showed a significant association of D-dimer with AF incidence (HR 1.10, 95% CI 1.02-1.19).

Additional biomarkers

For β-thromboglobulin (HR 0.92, 95% CI 0.67-1.27) (41), platelet count (HR 0.90, 95% CI 0.73-1.10) (42), Factor VII (HR 0.94, 95% CI 0.88-1.00), Factor VIII (HR 1.17, 95% CI 1.10-1.23), Protein C (HR 1.05, 95% CI 0.98-1.05) (29) , tissue plasminogen activator (HR 1.03, 95% CI 0.97-1.10) (38), and soluble urokinase plasminogen activator receptor (HR 1.20, 95% CI 1.01-1.42) (43) only 1 study was available.

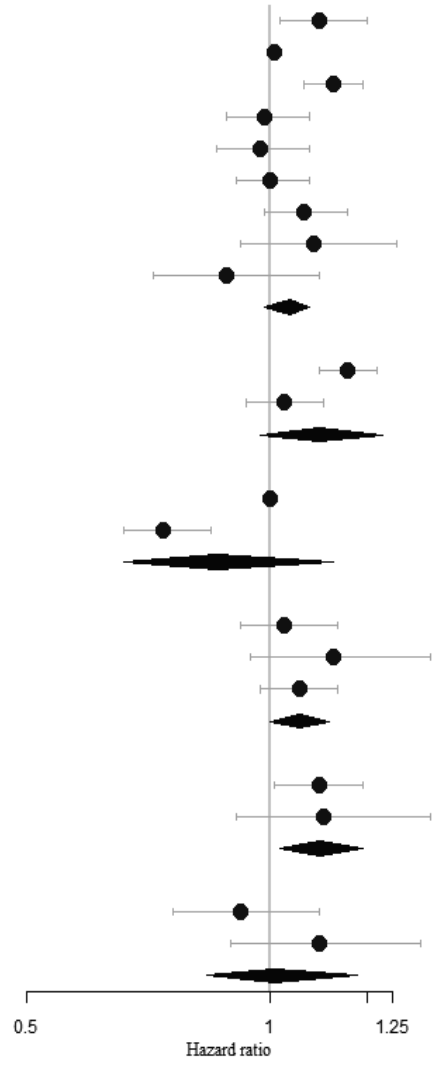
Figure 1. Flowchart of the inclusion and exclusion of studies



Abbreviations: AF, atrial fibrillation; n, number.

Figure 2. Forest plot of the associations between various coagulation biomarkers and incident AF in longitudinal studies

Author	Total N	AF N	HR
Fibrinogen			
Conen et al, 2010	24,734	747 (3.0%)	1.10
Kim et al, 2007	16,568	61 (0.4%)	1.00
Alonso et al, 2021	14,858	1,209 (8.1%)	1.13
Jang et al, 2021	6,602	859 (13.0%)	0.99
Nyrnes et al, 2021	6,315	566 (9.0%)	0.98
Tilly et al, 2021	6,174	729 (11.8%)	1.00
Eryd et al, 2011	6,031	667 (9.0%)	1.07
Schnabel et al, 2010	3,120	209 (6.7%)	1.09
Willeit et al, 2017	880	117 (13.3%)	0.91
Pooled	**85,282**	**5,164 (6.1%)**	**1.04**
vWF			
Alonso et al, 2012	14,858	1,209 (8.1%)	1.16
Tilly et al, 2021	6,174	729 (11.8%)	1.03
Pooled	**21,032**	**1,938 (9.2%)**	**1.10**
ADAMTS13			
Tilly et al, 2021	6,174	729 (11.8%)	1.00
Ko et al, 2019	1,885	349 (18.5%)	0.78
Pooled	**8,059**	**1,078 (13.4%)**	**0.89**
PAI-1			
Mulder et al, 2018	8,265	267 (3.2%)	1.03
Schnabel et al, 2010	3,120	209 (6.7%)	1.13
Dewland et al, 2015	2,768	721 (26.1%)	1.06
Pooled	**14,153**	**1,197 (8.5%)**	**1.06**
D-dimer			
Tattersall et al, 2020	6,615	875 (13.2%)	1.10
Schnabel et al, 2010	3,120	209 (6.7%)	1.11
Pooled	**9,735**	**1,084 (11.1%)**	**1.10**
P-Selectin			
Schnabel et al, 2009	2,863	148 (5.2%)	0.94
Willeit et al, 2017	880	117 (13.3%)	1.10
Pooled	**3,743**	**265 (7.1%)**	**1.01**



Abbreviations: ADAMTS13, a desintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; AF, atrial fibrillation; HR, hazard ratio; N, number; PAI-1, Plasminogen activator inhibitor 1. I^2 was 71.8% for fibrinogen, 83.9% for von Willebrand factor, 94.5% for ADAMTS13, 0.0% for PAI-1, 0.0% for D-dimer, and 40.4% for P-selectin.

Coagulation and atrial fibrillation in cross-sectional studies

Fibrinogen

The association of fibrinogen and AF was investigated in 15 cross-sectional studies (1,025 AF patients, 13,261 controls, mean age 56.4 years, 51.2% women).(44-58) Mean fibrinogen in AF patients ranged from 2.32 to 4.60 g/L, and from 2.26 to 4.08 g/L in controls, with higher fibrinogen levels in AF patients in 13 out of 15 studies. Pooled results showed higher fibrinogen values in AF patients (SMD 0.47, 95% CI 0.20-0.74) than in controls, as depicted in **Figure 3**.

Von Willebrand factor

VWF was assessed in 16 studies (1,439 AF patients, 4,723 controls, mean age 58.0 years, 46.3% women).(45, 48, 50, 51, 53, 54, 56, 59-67) vWF levels were higher in AF patients (mean 153.6%, range 116.5-210.3%) than in controls (mean 126.9%, range 89.0-153.2%). Pooled analysis showed that elevated vWF is significantly associated with AF (SMD 0.96, 95% CI 0.27-1.66). As visible in **Figure 3**, the results presented by Negreva et al.(64) were clear outliers. No explanation was found despite meticulous review of the studies. However, similar results were found in sensitivity analyses excluding this study.

P-Selectin

The association of P-selectin and AF was assessed in 10 studies (871 AF patients, 793 controls, mean age 69.2 years, 39.6% women).(48, 50-53, 67-71) Mean P-selectin was 84.7 ng/mL for AF patients (range 31.2-219 ng/mL), and 43.3 ng/mL (range 29.2-145 ng/mL) in the control group. Overall, we found that AF was associated with elevated P-selectin, as compared to individuals without AF (SMD 0.31, 95% CI 0.08-0.54).

β-thromboglobulin

7 studies investigated β-thromboglobulin in relation to AF (358 AF patients, 299 controls, mean age 63.3 years, 35.5% women).(52, 56, 58, 72-75). We found significantly higher values (SMD 0.82, 95% CI 0.61-1.04) for β-thromboglobulin in AF patients (mean 83.29 ng/mL, range 36.0-181.0) than in controls (mean 44.32 ng/mL, range 22.80-91.0 ng/mL)

Platelet factor 4

Platelet factor 4 was examined in 4 studies (192 AF patients, 216 controls, mean age range 61.2 years, insufficient data on sex).(56, 58, 72, 73) In all studies platelet factor levels were higher in AF patients (mean 17.8 ng/mL, range 3.9-20.9 ng/mL) compared to the controls (mean 11.5 ng/mL, range 2.6-15.3 ng/mL). Meta-analysis showed a significantly elevated platelet factor 4 levels in AF patients (SMD 0.42, 95% CI 0.12-0.71) as compared to controls.

Platelet count
14 studies examined platelet count in AF and controls (868 AF patients, 2,269 controls, mean age 62.6 years, 39.5% women).(50, 52, 56, 64, 68, 69, 71, 74, 76-81) The mean platelet count in AF patients was $235x10^9$/L (range $174-277x10^9$/L), and $225x10^9$ in controls (range $212-270x10^9$). Pooled, we found no significant difference between the 2 groups (SMD -0.41, 95% CI -1.42-0.59).

Mean platelet volume (MPV)
The relation of MPV with AF was investigated in 3 studies (264 AF patients, 202 controls, mean age 59.1 years, 41.0% women).(69, 77, 79) MPV was higher in AF patients (mean 8.9 fL, range 7.76-10.0 fL) than in controls (mean 8.0 fL, range 7.39-8.4 fL), but the pooled overall effect showed no significant difference (SMD 1.19, 95% CI -1.26-3.64).

Tissue factor
We included 2 studies with data on tissue factor (165 AF patients, 81 controls).(51, 65) The studies were similar in mean age (64.6 and 62.6 years), but differed in sex ratio (37.4% and 51.5% women). Despite comparable methodology, the studies differed largely in tissue factor values in both AF (115 vs. 750 pg/mL) and healthy control patients (95 vs. 455 pg/mL), whereas the SMDs were similar (0.33 and 0.27). The meta-analysis showed no significant differences between AF patients and controls (SMD 0.31, 95% CI -0.06-0.67).

Factor VIII
Factor VIII levels were measured in 104 AF patients and 3,211 controls in 2 studies (mean age 54.1 and 59.7 years, 53.2% and 49.5% women).(46, 64) Pooled, Factor VIII levels were higher in AF patients than in controls (136.3% vs. 133.6%), albeit not significantly (SMD 12.10, 95% CI -88.0-112.2).

Thrombin-antithrombin complex (TAT)
2 studies (151 AF patients, 29 controls, mean age 59.9 and 67.0 years, 23.3% and 46.0% women) assessed the association between TAT and AF.(58, 70) In AF patients the mean TAT values differed significantly (54.0 ± 237 and 6.7 ± 5.1 ng/L), whereas in control patients the mean TAT was comparable (2.7 ± 3.3 and 3.1 ± 1.9 ng/L). Pooled analysis showed no significant difference between AF and controls (SMD 0.42, 95% CI -2.91-3.75).

Thrombomodulin

Thrombomodulin levels were compared in 396 AF patients and 179 controls over 5 studies (mean age 66.2 years, 38.1% women) (53, 54, 62, 70, 82) Mean (range) levels were 40.5 ng/mL (11.8-52.2 ng/mL) and 35.3 ng/mL (5.9-44.0 ng/mL), respectively. Pooled analysis showed no significant differences between AF patients and controls (SMD 0.48, 95% CI -0.48-1.44).

Tissue plasminogen activator (TPA)

TPA was assessed in 8 studies (370 AF patients, 6,954 controls, mean age range 53.6-75.0 years, 17.5-55.4% women).(8, 45, 46, 54, 57, 68, 83, 84) Through pooled analysis we found no significant difference (SMD 2.42, 95% CI -2.02-6.85) in TPA value between AF (mean 11.43 ng/mL, range 2.31-20.37 ng/mL) and controls (mean 8.50 ng/mL, range 2.88-15.5 ng/mL). We performed a sensitivity analyses excluding the study by Negreva et al.(83), based on the distinctly higher values without a clear cause. However, similar to vWF, the pooled estimates did not change.

PAI-1

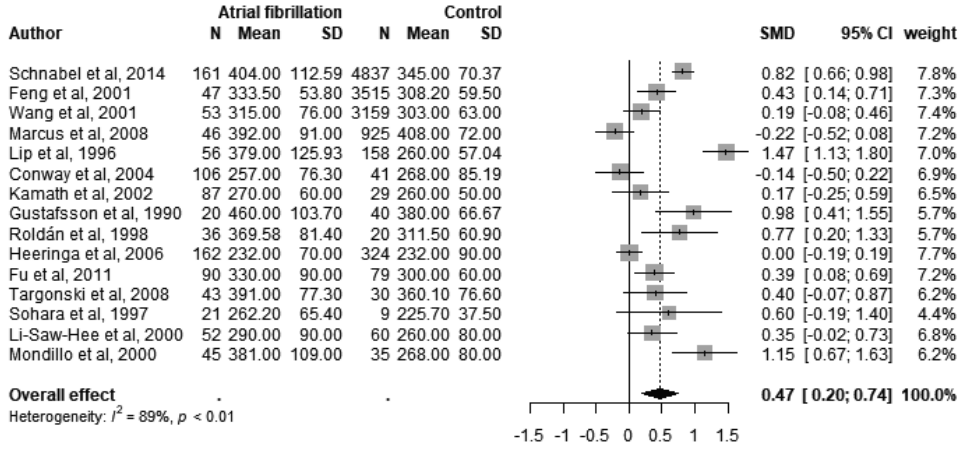
6 studies contained data on PAI-1 and AF (240 AF patients, 6,796 controls, mean age 55.5 years, 51.9% women).(45, 46, 54, 57, 84, 85) AF patients had higher PAI-1 levels (mean 23.6 ng/mL, range 15.2-42.8 ng/mL) than the control group (mean 19.6 ng/mL, range 5.4-22.9 ng/mL). Meta-analysis showed a large difference between PAI-1 in AF patients and controls (SMD 1.73, 95% CI 0.26-3.19).

D-dimer

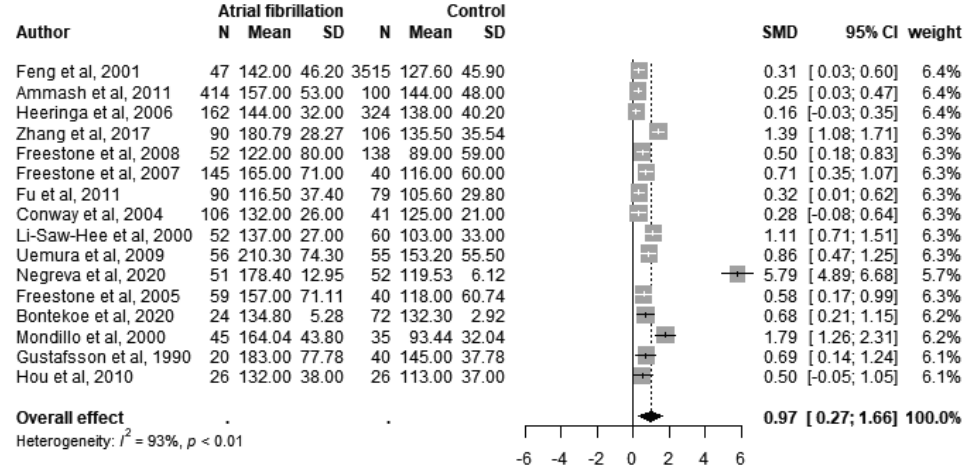
12 studies included D-dimer in the analyses (523 AF patients, 535 controls, mean age 64.7 years, 38.3% women).(49, 52, 54, 56-58, 67, 72, 74, 82, 83, 86) In AF patients the mean D-dimer was 0.47 µg/mL (range 0.13-1.15 µg/mL), as opposed to 0.16 µg/mL (range 0.01-0.59 µg/mL) in controls. AF patients had significantly higher D-dimer levels than controls in the meta-analysis (SMD 1.74, 95% CI 0.36-3.11). As visualized in **Figure 3**, Roldán et al.(57) presented a disparate result as compared to the other studies, without an obvious cause. The pooled result of the sensitivity analyses showed similar tendencies after excluding Roldán et al.(57)

Figure 3. Forest plots of the associations between various coagulation biomarkers and atrial fibrillation presence in cross-sectional studies

A: Fibrinogen



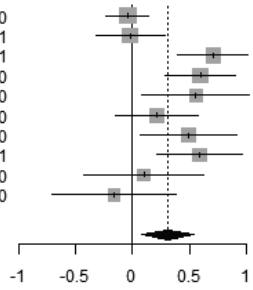
B: von Willebrand Factor



C: P-Selectin

Author	Atrial fibrillation N	Mean	SD	Control N	Mean	SD	SMD	95% CI	weight
Heeringa et al, 2006	162	31.30	10.10	324	31.80	13.10	-0.04	[-0.23; 0.15]	13.4%
Berge et al, 2013	63	31.20	11.04	126	31.40	9.11	-0.02	[-0.32; 0.28]	11.4%
Choudhury et al, 2007	121	59.00	31.11	65	40.00	14.81	0.71	[0.40; 1.02]	11.2%
Fu et al, 2011	90	33.40	7.40	79	29.20	6.50	0.60	[0.29; 0.91]	11.3%
Acevedo et al, 2012	130	219.00	141.00	20	145.00	29.00	0.56	[0.08; 1.03]	8.4%
Conway et al, 2004	106	54.00	17.78	41	50.00	20.00	0.22	[-0.15; 0.58]	10.3%
Kamath et al, 2002	87	39.00	10.00	29	34.00	10.00	0.50	[0.07; 0.92]	9.2%
Li-Saw-Hee et al, 2000	52	185.00	135.56	60	126.00	51.11	0.59	[0.21; 0.97]	10.0%
Kamath et al, 2002	34	37.00	10.00	23	36.00	9.00	0.10	[-0.43; 0.63]	7.6%
Hou et al, 2010	26	32.00	5.00	26	33.00	7.00	-0.16	[-0.71; 0.38]	7.3%
Overall effect	.			.			**0.31**	**[0.08; 0.54]**	**100.0%**

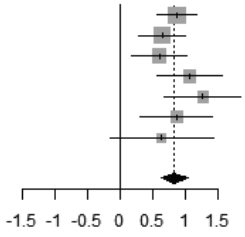
Heterogeneity: I^2 = 74%, p < 0.01



D: ß-thromboglobulin

Author	Atrial fibrillation N	Mean	SD	Control N	Mean	SD	SMD	95% CI	weight
Nozawa et al, 2004	78	72.60	35.70	110	44.30	30.30	0.86	[0.56; 1.17]	23.6%
Yamauchi et al, 1986	73	49.40	35.80	57	31.20	14.00	0.64	[0.28; 0.99]	20.0%
Kamath et al, 2002	87	87.00	44.44	29	63.00	22.96	0.59	[0.17; 1.02]	16.0%
Lip et al, 1996	51	181.00	88.89	26	91.00	74.81	1.05	[0.55; 1.56]	12.8%
Gustafsson et al, 1990	20	36.00	8.15	40	25.90	7.78	1.26	[0.68; 1.85]	10.2%
Minamino et al, 1999	28	79.70	57.26	28	41.60	24.67	0.85	[0.30; 1.40]	11.3%
Sohara et al, 1997	21	38.00	27.30	9	22.80	7.85	0.63	[-0.17; 1.43]	6.1%
Overall effect	.			.			**0.82**	**[0.61; 1.04]**	**100.0%**

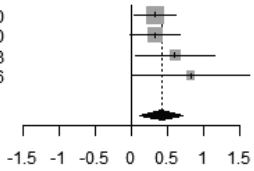
Heterogeneity: I^2 = 0%, p = 0.49



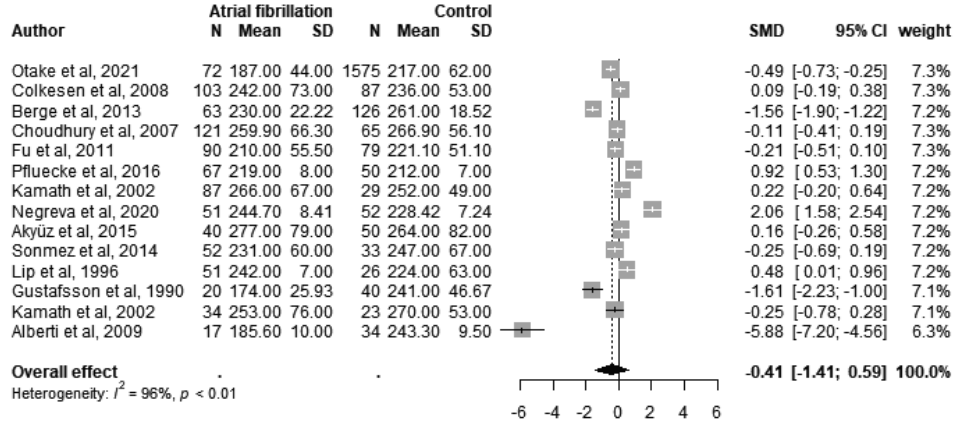
E: Platelet Factor 4

Author	Atrial fibrillation N	Mean	SD	Control N	Mean	SD	SMD	95% CI	weight
Nozawa et al, 2004	78	20.90	15.60	110	15.30	17.80	0.33	[0.04; 0.62]	41.0%
Yamauchi et al, 1986	73	18.60	27.20	57	11.60	8.20	0.33	[-0.02; 0.68]	33.2%
Gustafsson et al, 1990	20	3.90	3.19	40	2.60	1.33	0.60	[0.06; 1.15]	17.1%
Sohara et al, 1997	21	16.40	18.20	9	3.37	2.26	0.82	[0.01; 1.63]	8.7%
Overall effect	.			.			**0.42**	**[0.12; 0.71]**	**100.0%**

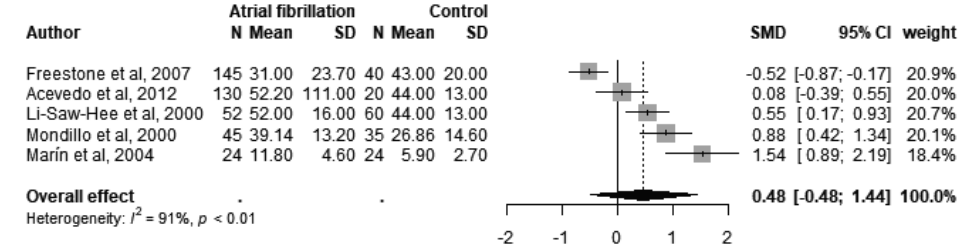
Heterogeneity: I^2 = 0%, p = 0.58



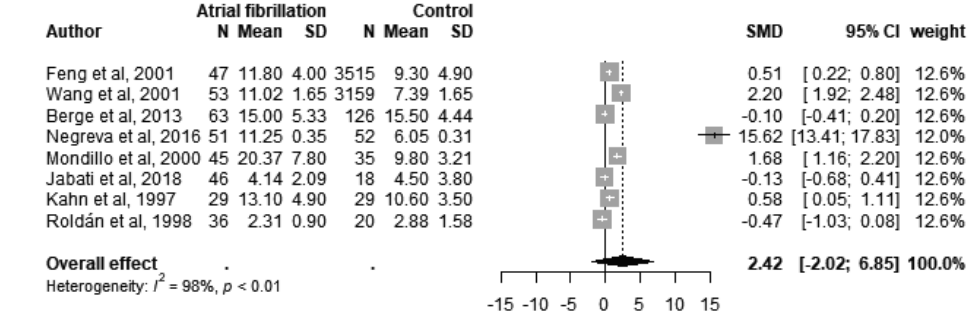
F: Platelet count



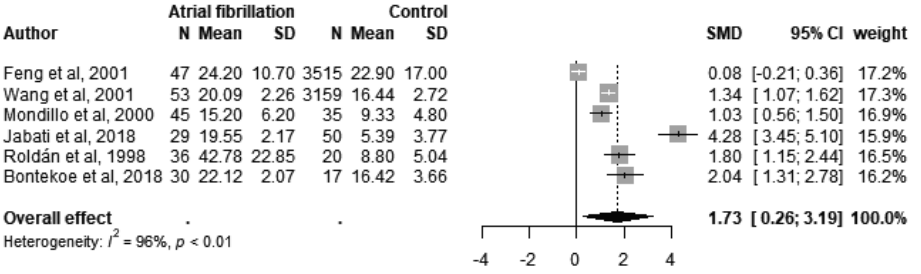
G: Thrombomodulin



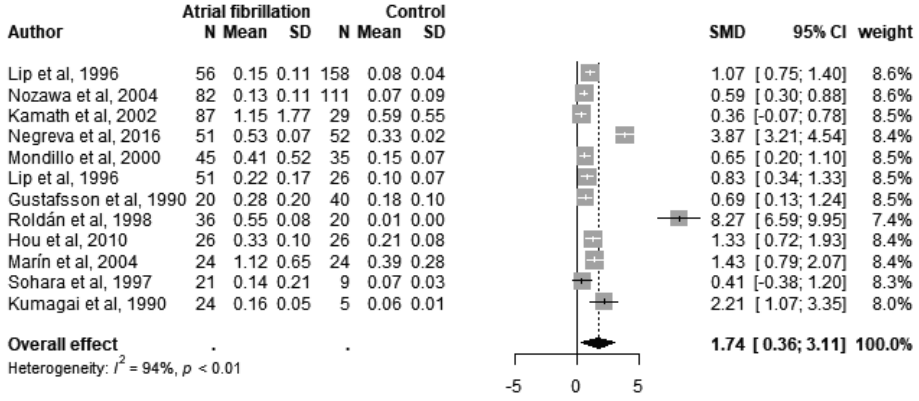
H: TPA



I: PAI-1



J: D-dimer



Abbreviations: CI, confidence interval; N, number; PAI-1, plasminogen activator inhibitor 1; SD, standard deviation; SMD, standardized mean difference; TAT, thrombin-antithrombin complex; TPA, tissue plasminogen activator.

DISCUSSION

With this systematic review and meta-analysis we aimed to identify markers of coagulation associated with AF incidence and AF prevalence. In longitudinal studies, we found significant associations between fibrinogen, PAI-1, and D-dimer levels at baseline with AF incidence. Additionally, in cross-sectional studies we found significant differences between AF patients and controls for fibrinogen, PAI-1, D-dimer, P-selectin, vWF, ß-thromboglobulin, and platelet factor 4. By investigating the association between coagulation and AF in longitudinal and cross-sectional studies separately, we strived to further clarify the direction in which coagulation and AF development and prognosis are dependent.

Our results give insight to the role of primary and secondary hemostasis, and fibrinolysis in the development of AF. Fibrinogen is an acute phase reactant that indicates inflammatory processes or tissue damage, and has been associated with ischemic heart disease in prior studies.(87, 88) We found a borderline significant association between fibrinogen and AF incidence combining studies with extensive multivariable models. However, after including models with less adjustments for confounding in the meta-analysis this association attenuated. One possibility for this is that there are discrepancies in the quality of study designs between studies with more robust statistical models, and studies only investigating the associations in univariable models. On one hand, this suggests that fibrinogen is associated with new-onset AF, but other cardiovascular risk factors such as BMI or comorbidities may attenuate this association. On the other hand, it is possible that underlying confounders, if not sufficiently corrected for, biased the association of fibrinogen with AF. PAI-1, a fibrinolysis inhibitor and established risk factor for atherosclerosis and thrombosis, was also significantly associated with AF incidence.(89) Increased PAI-1 causes decreased fibrinolysis, and thus a reduced degradation of thrombi. This could imply that coagulation, potentially promoted due to tissue damage or inflammation, is further sustained and perpetuated, as fibrinolysis is inhibited. PAI-1 is also associated with adiposity and inflammation, both of which may also contribute to AF development. To investigate the independent association between the role of PAI-1 in coagulation and AF, the studies investigating these associations all adjusted extensively for these potential confounders, such as BMI, sex, and medication use, mitigating the confounding role of adiposity and traditional cardiovascular risk factors. Lastly, we found a significant association of D-dimer, a marker of coagulation cascade activation, with incidence AF.(90) Increased D-dimer is associated with increased fibrin synthesis, and therefore increased coagulability. This implies that a hypercoagulable state underlies AF development, which is in line with the previously mentioned association between primary hemostasis and fibrinolysis. In contrast, vWF, ADAMTS13, and P-selectin were not significantly associated with AF. vWF and ADAMTS13 are inversely correlated with each other, and while a trend where

higher vWF and lower ADAMTS13 levels are associated with increased AF risk, the pooled HR was non-significant. For P-selectin, the 2 studies contradicted each other. While this contradicts our hypothesis of the role of coagulation in AF development, these results could be due to low power, as only 2 studies investigated these associations. More evidence from large, prospective cohort studies is warranted before conclusive conclusions can be made on the associations between AF and these single markers of coagulation.

The findings of this study suggest that coagulation is associated with AF development through multiple pathways of coagulation. A possible explanation for this is immunothrombosis, which comprises the complex interaction of the innate immune system and the coagulation system.(15-17) Prior studies found that markers of inflammation and endothelial damage, such as fibrinogen and vWF, underlie AF development and progression, but the exact pathophysiology remained unclear.(9, 90-93) Therefore, it is unsure if the association is causally related, or if these biomarkers are also related to another process, causally related to AF. The synergy between inflammation and coagulation, creating a hypercoagulable and hypofibrinolytic state, may result in structural and biochemical changes, eventually resulting in AF.

AF presence was also significantly associated with multiple biomarkers of primary and secondary hemostasis, and fibrinolysis. Distinctly higher levels of indicators of platelet activation (ß-thromboglobulin and platelet factor 4), platelet aggregation (von Willebrand factor and P-selectin), as well as the aforementioned markers underlying a hypercoagulable and hypofibrinolytic state, were found in AF patients, as compared to healthy controls. These findings corroborate that AF further propels the haemostatic balance towards a prothrombotic state, by promoting not only hypercoagulability, but also hypofibrinolysis.(57)

Given these results, one might state our study underwrites the often phrased *"*AF begets AF".(94) Immunothrombosis, as a potential underlying cause of increased coagulation, may stimulate AF development. Consequently, the restricted blood flow, atrial structural changes, and inflammatory processes related to AF further stimulate a prothrombotic state.(95-97) In other words, a hypercoagulable, inflammatory, and hypofibrinolytic state can promote AF development, which in turn further stimulates its own underlying pathophysiological pathways through a positive feedback loop.

However, despite our comprehensive search it is clear that longitudinal population-based cohort studies investigating the association of coagulation and AF are lacking. With only 2 studies investigating vWF, ADAMTS13, P-selectin, and D-dimer, there remains a possibility of publication bias in our meta-analyses. In the cross-sectional meta-analyses, asymmetry and outliers were visually assessed through funnel plots, especially for vWF, platelet count, TPA, PAI-1 and D-dimer (**Figures S1** and **S2**).

However, while funnel plots may assist in identifying publication bias, there have been increasing critiques on the added value.(98, 99) Due to the low number of studies and the potential publication bias as depicted in the funnel plots, the results of this meta-analysis, especially in the longitudinal studies, must be interpreted with caution, and more studies investigating the relation of coagulation and AF are warranted. Population-based research and early recognition and prevention or people at risk is critical in the modern day society. Especially in large, longitudinal population-based studies, replication, resulting in the uncovering of supporting or contradicting evidence, can be crucial in risk stratification, even more so in different populations.

Besides the lack of studies investigating the role of coagulation biomarkers in AF development and AF presence, it is possible that single biomarkers are not sensitive or specific enough to accurately predict AF risk. Especially in a large, population based study, subclinical elevation of single coagulation markers may not be strong enough to depict the elevated AF risk it is potentially associated with. However, combinations of biomarkers may give us a new insight. The International Society of Thrombosis and Haemostasis has previously developed a score to identify the degree of disseminated intravascular coagulation in individuals, using easy to test coagulation markers such as fibrinogen, D-dimer, and platelet count.(100) Our systematic review and meta-analysis suggests that other markers associated with primary and secondary hemostasis and fibrinolysis play a role in AF, and therefore could play a role in risk stratification and even AF prevention. We hope that our findings further stimulate researchers to assess these potential associations, opening the door to improved AF risk prediction, management, and prognosis. While we cannot reject the hypothesis of inflammation and coagulation preceding AF, coagulation is mostly increased after AF initiation. Additionally, the included studies were highly heterogeneous. While we carefully assessed the studies, and performed sensitivity analyses and step-by-step exclusion of potential causes, we were not able to significantly reduce the heterogeneity. To address this heterogeneity, we used random-effects models.

In conclusion, higher levels of coagulation factors are associated with prevalent and incident AF. These associations are most pronounced with prevalent AF in cross-sectional studies. Limited evidence from longitudinal studies suggests a prothrombotic state underlying AF development. These findings support the hypothesis of a prothrombotic and hypofibrinolytic state as both an underlying cause of AF, as well as further inducing atrial structural remodeling, in turn perpetuating AF.

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SUPPLEMENTARY MATERIAL

Chapter 4.2 Immunothrombotic markers and prevalence and incidence of atrial fibrillation

Methods S1. Search strategy

Database searched	via	Years of coverage	Records	Records after duplicates removed
Embase	Embase.com	1971 - Present	9,368	4,562
Medline ALL	Ovid	1946 - Present	5,251	5,245
Web of Science Core Collection *	Web of Knowledge	1975 - Present	3,272	209
Cochrane Central Register of Controlled Trials †	Wiley	1992 - Present	740	166
Other sources: Google Scholar			200	153
Total			**18,831**	**10,335**

* Science Citation Index Expanded (1975-present) ; Social Sciences Citation Index (1975-present) ; Arts & Humanities Citation Index (1975-present) ; Conference Proceedings Citation Index- Science (1990-present) ; Conference Proceedings Citation Index- Social Science & Humanities (1990-present) ; Emerging Sources Citation Index (2015-present)

† Manually deleted abstracts from trial registries

Embase.com (1971-)

('fibrinogen'/exp OR 'prothrombin'/exp OR 'thrombin antithrombin complex'/exp OR 'antithrombin III'/exp OR 'beta thromboglobulin'/exp OR 'thrombocyte factor 4'/exp OR 'thrombocyte activating factor'/exp OR 'von willebrand factor antigen'/de OR 'von willebrand factor'/de OR 'von Willebrand factor cleaving proteinase'/de OR 'D dimer'/de OR 'thrombin'/de OR 'blood clotting factor'/de OR 'blood clotting inhibitor'/de OR 'blood clotting factor 5'/de OR 'blood clotting factor 5 Leiden'/de OR 'blood clotting factor 5a'/de OR 'blood clotting factor 7'/de OR 'blood clotting factor 7a'/de OR 'blood clotting factor 8'/de OR 'blood clotting factor 8a'/de OR 'blood clotting factor 8c'/de OR 'blood clotting factor 9'/de OR 'blood clotting factor 9 complex'/de OR 'blood clotting factor 9a'/de OR 'blood clotting factor 11'/de OR 'blood clotting factor 11a'/de OR 'blood clotting factor 12'/de OR 'blood clotting factor 12a'/de OR 'blood clotting factor 13'/de OR 'blood clotting factor 13a'/de OR 'blood clotting factor 13b'/de OR 'fibrin'/de OR 'plasmin inhibitor'/exp OR 'platelet count'/de OR 'mean platelet volume'/de OR 'PADGEM protein'/de OR thromboplastin/de OR 'protein C'/de OR 'protein S'/de OR thrombomodulin/de OR 'tissue factor pathway inhibitor'/de OR 'plasminogen activator inhibitor'/exp OR 'plasminogen activator'/exp OR plasminogen/de OR 'thrombin activatable fibrinolysis inhibitor'/de OR carboxypeptidase/de OR 'fibrinopeptide A'/de Or fibrinopeptide/de OR (((hemostat* OR haemostat* OR coagul*) NEAR/3 (marker* OR abnormal*)) OR fibrinogen* OR prothrombin* OR thrombin* OR antithrombin-III OR thromboglobulin* OR 'clotting factor' OR factor-4 OR factor-IV OR factor-5* OR factor-Va OR factor-7 OR factor-VII* OR factor-8 OR factor-9 OR factor-IX* OR factor-11 OR factor-XI* OR factor-12* OR factor-13* OR ((thrombocyte* OR platelet*) NEAR/3 activat* NEAR/3 factor*) OR (willebrand NEAR/3 factor) OR adamts-13 OR adamts13 OR D-dimer* OR fibrin OR

antiplasmin* OR (plasmin NEAR/3 inhibitor*) OR ((platelet* OR thrombocyte*) NEAR/3 (count* OR volume*)) OR thromboplastin* OR (tissue* NEAR/3 factor*) OR protein-C OR autoprothrombin* OR protein-S OR Plasminogen* OR carboxypeptidas* OR fibrinopeptide* OR urokinas* OR Microparticle*):ab,ti) AND ('atrial fibrillation'/exp OR (((atrial* OR atrium*) NEAR/3 fibrillation*) OR PADGEM OR p-selectin*):ab,ti) AND ('prediction'/de OR 'predictive value'/de OR 'marker'/de OR 'biological marker'/de OR 'disease association'/de OR 'population'/de OR 'risk assessment'/de OR 'risk factor'/de OR 'prevalence'/de OR 'incidence'/de OR 'epidemiology'/de OR 'epidemiological data'/de OR 'population based case control study'/de OR 'cohort analysis'/de OR 'longitudinal study'/de OR 'prospective study'/de OR 'normal human'/de OR (predict* OR marker* OR biomarker* OR onset OR association* OR correlat* OR general-population OR risk* OR unknown OR prevalence OR incidence OR epidemiolog* OR population-based OR cohort* OR longitudinal* OR prospectiv* OR retrospectiv* OR healthy):Ab,ti) NOT ((animal/exp OR animal*:de OR nonhuman/de) NOT ('human'/exp)) NOT ([Conference Abstract]/lim) AND [English]/lim

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(Fibrinogen/ OR Prothrombin/ OR Antithrombin III/ OR beta-Thromboglobulin/ OR von Willebrand Factor/ OR Thrombin/ OR Blood Coagulation Factors/ OR exp Factor V/ OR exp Factor VII/ OR exp Factor VIII/ OR exp Factor IX/ OR exp Factor XI/ OR exp Factor XII/ OR exp Factor XIII/ OR Fibrin/ OR Antifibrinolytic Agents/ OR Platelet Count/ OR Mean Platelet Volume/ OR P-Selectin/ OR Thromboplastin/ OR Protein C/ OR Protein C Inhibitor/ OR Protein S/ OR Thrombomodulin/ OR exp Plasminogen Inactivators/ OR Plasminogen Activators/ OR Plasminogen/ OR exp Carboxypeptidases/ OR Fibrinopeptide A/ OR Fibrinopeptide B/ OR (((hemostat* OR haemostat* OR coagul*) ADJ3 (marker* OR abnormal*)) OR fibrinogen* OR prothrombin* OR thrombin* OR antithrombin-III OR thromboglobulin* OR clotting factor OR factor-4 OR factor-IV OR factor-5* OR factor-Va OR factor-7 OR factor-VII* OR factor-8 OR factor-9 OR factor-IX* OR factor-11 OR factor-XI* OR factor-12* OR factor-13* OR ((thrombocyte* OR platelet*) ADJ3 activat* ADJ3 factor*) OR (willebrand ADJ3 factor) OR adamts-13 OR adamts13 OR D-dimer* OR fibrin OR antiplasmin* OR (plasmin ADJ3 inhibitor*) OR ((platelet* OR thrombocyte*) ADJ3 (count* OR volume*)) OR thromboplastin* OR (tissue* ADJ3 factor*) OR protein-C OR autoprothrombin* OR protein-S OR Plasminogen* OR carboxypeptidas* OR fibrinopeptide* OR urokinas* OR Microparticle*).ab,ti.) AND (Atrial Fibrillation/ OR (((atrial* OR atrium*) ADJ3 fibrillation*) OR PADGEM OR p-selectin*).ab,ti.) AND (Predictive Value of Tests/ OR Biomarkers/ OR Population Groups/ OR Population/ OR Risk Assessment/ OR Risk Factors/ OR Prevalence/ OR Incidence/ OR Epidemiology/ OR Cohort Studies/ OR Longitudinal Studies/ OR Prospective Studies/ OR Healthy Volunteers/ OR (predict* OR marker* OR biomarker* OR onset OR association* OR correlat* OR general-population OR risk* OR unknown OR prevalence OR incidence OR epidemiolog* OR population-based OR cohort* OR

longitudinal* OR prospectiv* OR retrospectiv* OR healthy).ab,ti.) NOT ((animal/ OR animal*:de OR nonhuman/) NOT (human/)) NOT (news OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt. AND english.la.

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AB=(((((hemostat* OR haemostat* OR coagul*) NEAR/2 (marker* OR abnormal*)) OR fibrinogen* OR prothrombin* OR thrombin* OR antithrombin-III OR thromboglobulin* OR "clotting factor" OR factor-4 OR factor-IV OR factor-5* OR factor-Va OR factor-7 OR factor-VII* OR factor-8 OR factor-9 OR factor-IX* OR factor-11 OR factor-XI* OR factor-12* OR factor-13* OR ((thrombocyte* OR platelet*) NEAR/2 activat* NEAR/2 factor*) OR (willebrand NEAR/2 factor) OR adamts-13 OR adamts13 OR D-dimer* OR fibrin OR antiplasmin* OR (plasmin NEAR/2 inhibitor*) OR ((platelet* OR thrombocyte*) NEAR/2 (count* OR volume*)) OR thromboplastin* OR (tissue* NEAR/2 factor*) OR protein-C OR autoprothrombin* OR protein-S OR Plasminogen* OR carboxypeptidas* OR fibrinopeptide* OR urokinas* OR Microparticle*)) AND ((((atrial* OR atrium*) NEAR/2 fibrillation*) OR PADGEM OR p-selectin*)) AND ((predict* OR marker* OR biomarker* OR onset OR association* OR correlat* OR general-population OR risk* OR unknown OR prevalence OR incidence OR epidemiolog* OR population-based OR cohort* OR longitudinal* OR prospectiv* OR retrospectiv* OR healthy))) AND DT=(article) AND LA=(english)

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((((hemostat* OR haemostat* OR coagul*) NEAR/3 (marker* OR abnormal*)) OR fibrinogen* OR prothrombin* OR thrombin* OR antithrombin NEXT III OR thromboglobulin* OR 'clotting factor' OR factor NEXT 4 OR factor NEXT IV OR factor NEXT 5* OR factor NEXT Va OR factor NEXT 7 OR factor NEXT VII* OR factor NEXT 8 OR factor NEXT 9 OR factor NEXT IX* OR factor NEXT 11 OR factor NEXT XI* OR factor NEXT 12* OR factor NEXT 13* OR ((thrombocyte* OR platelet*) NEAR/3 activat* NEAR/3 factor*) OR (willebrand NEAR/3 factor) OR adamts NEXT 13 OR adamts13 OR D NEXT dimer* OR fibrin OR antiplasmin* OR (plasmin NEAR/3 inhibitor*) OR ((platelet* OR thrombocyte*) NEAR/3 (count* OR volume*)) OR thromboplastin* OR (tissue* NEAR/3 factor*) OR protein NEXT C OR autoprothrombin* OR protein NEXT S OR Plasminogen* OR carboxypeptidas* OR fibrinopeptide* OR urokinas* OR Microparticle*):ab,ti) AND ((((atrial* OR atrium*) NEAR/3 fibrillation*) OR PADGEM OR p NEXT selectin*):ab,ti) AND ((predict* OR marker* OR biomarker* OR onset OR association* OR correlat* OR general NEXT population OR risk* OR unknown OR prevalence OR incidence OR epidemiolog* OR population NEXT based OR cohort* OR longitudinal* OR prospectiv* OR retrospectiv* OR healthy):Ab,ti)

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"hemostatic|haemostatic|coagulation marker|markers" "atrial|atrium fibrillation" predict|prediction|onset|association|correlation|"general-population"|risk|prevalence|incidence|epidemiolog

Table S1. Study characteristics of included longitudinal studies

Author	Year	Cohort	Total n	AF events	Age	Women	Follow-up	Control source	Factors studied	Covariates †	NOS *
Alonso	2012	ARIC	14,858	1,209	54.2	8,183 (55.1)	16.8	General population	Fibrinogen vWF Factor VII Factor VIII Protein C	1, 2, 3, 4, 5, 7, 8, 11, 12, 13, 14, 15, 16, 18, 19	Good
Conen	2010	Women's health study	24,734	747	53.0	24,734 (100)	14.4	General population	Fibrinogen	1, 3, 4, 5, 7, 9, 11, 12, 13, 16	Good
Dewland	2015	Health ABC	2,768	721	73.0	1,435 (51.8)	10.9	General population	PAI-1	1, 2, 3, 4, 11, 12, 14, 16, 17, 19	Good
Ding	2017	NA	33,186	123	56.7	10,963 (33.0)	2.6	General population	Platelet count	1, 2, 4, 11, 12, 16	Good
Eryd	2011	Malmö preventive study	6,031	667	46.8	0 (0.0)	25.0	General population	Fibrinogen	1, 4, 5, 8, 11, 12, 13, 15, 16, 19	Good
Jang	2021	MESA	6,602	859	62.0	3,484 (52.8)	12.9	General population	Fibrinogen	1, 2, 3, 4, 5, 7, 8, 10, 11, 13, 14, 15	Good
Kim	2007	NA	16,568	61	49.0	5,883 (35.5)	3.6	General population	Fibrinogen	1, 12, 19	Fair
Ko	2019	Framingham Offspring Study	1,885	349	55.0	1,016 (53.9)	18.3	General population	ADAMTS13	1, 2, 4, 5, 6, 11, 15, 16, 18, 19	Good
Kubota	2018	ARIC	746	126	53.6	416 (55.8)	19.3	General population	ß-thromboglobulin	1, 2, 3, 4, 10, 11, 12, 16, 17, 18, 19	Good
Mulder	2018	PREVEND	8,265	267	49.0	4,145 (50.2)	9.7	General population	PAI-1 TPA	1, 2, 4, 11, 12, 15, 16, 18, 19	Good
Nyrnes	2012	Tromsø Study	6,315	566	60.0	3,222 (51.0)	10.9	General population	Fibrinogen	1, 2, 4, 5, 8, 11, 15, 16, 19	Good

Schnabel	2010	Framingham Offspring Study	3,120	209	58.4	1,692 (54.2)	9.7	General population	Fibrinogen D-dimer PAI-1	1, 2, 4, 5, 15, 18	Good
Schnabel	2009	Framingham Offspring Study	2,863	148	61.0	1,575 (55.0)	6.2	General population	P-selectin	1, 2, 4, 5, 11, 12, 15, 16, 18, 19	Good
Tattersall	2020	MESA	6,615	875	62.0	3,488 (52.7)	12.9	Asthma patients	D-dimer	1, 2, 3, 5, 11, 12, 15, 16	Good
Tilly	2021	Rotterdam Study	6,174	729	69.1	3,520 (57.0)	12.8	General population	Fibrinogen vWF ADAMTS13	1, 2, 10, 11, 12, 14, 15, 16, 17, 18, 19	Good
Westin	2018	NA	14,764	349	57.5	7,963 (53.9)	1.1	Acute medical patients	suPAR	1, 2	Fair
Willeit	2017	Bruneck Study	880	117	58.8	433 (49.2)	20.0	General population	Fibrinogen P-selectin	1, 2	Good

Abbreviations: ADAMTS13, A desintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; AF, atrial fibrillation; BMI, body mass index; MPV, mean platelet volume; N, number; NA, not available; NOS, Newcastle-Ottawa scale; PAI-1, plasminogen activator inhibitor 1; suPAR, soluble plasminogen activator receptor; TPA, tissue plasminogen activator; vWF, von Willebrand factor.

* If applicable. NOS 7-9: good, 4-6: fair, <4 poor.

† Covariates: age[1], sex[2], ethnicity[3], body mass index[4], systolic blood pressure[5], diastolic blood pressure[6], high-density lipoprotein cholesterol[7], total cholesterol[8], low-density lipoprotein cholesterol[9], renal function[10], smoking status[11], alcohol use[12], physical activity[13], lipid-lowering medication[14], blood pressure medication[15], prevalent diabetes mellitus[16], prevalent hypertension[17], prevalent heart failure[18], prevalent coronary heart disease[19]. Other less-frequently used covariates are not depicted.

Table S2. Study characteristics of included cross-sectional studies

Author	Year	AF n	Control n	Age	Women	AF subtype	Control source	Factors studied	NOS *
Acevedo	2012	130	20	67.0	46.0	Overall	Healthy controls	P-Selectin Thrombomodulin TAT	Fair
Akyüz	2015	90	40	62.4	27.8	Overall	OSAS patients	MPV Platelet count	Good
Alberti	2009	17	34	63.2	52.9	Overall	Healthy controls	Platelet count	Fair
Ammash	2011	414	100	80.5	12.5	Overall	Healthy controls	Platelet count	Fair
Berge	2013	63	126	75.0	29.1	Overall	General population	P-selectin Platelet count	Good
Bontekoe	2020	24	72	60.0	49.5	Overall	Kidney disease	vWF	Fair
Bontekoe	2018	30	17	60.0	49.4	Overall	Kidney disease	PAI-1	Fair
Choudhury	2007	121	65	62.0	28.0	Overall	Healthy controls	P-Selectin MPV Platelet count	Fair
Colkesen	2008	103	87	54.8	60.0	Paroxysmal AF	Healthy controls	MPV Platelet count	Fair
Conway	2004	106	41	68.4	37.4	Chronic AF	Healthy controls	Fibrinogen P-Selectin vWF Tissue Factor	Good
Feng	2001	47	3515	55.1	52.8	Overall	Healthy controls	Fibrinogen vWF PAI-1	Good

								TPA	
Freestone	2005	59	40	64.6	51.5	Chronic AF	Healthy controls	vWF Tissue factor	Good
Freestone	2007	145	40	65.5	46.5	Overall AF	Healthy controls	vWF Thrombomodulin	Good
Freestone	2008	52	138	68.8	NA	Chronic AF	Heart failure	vWF	Good
Fu	2011	90	79	54.4	36.1	Overall AF	Healthy controls	Fibrinogen P-selectin vWF Platelet count	Good
Gustafsson	1990	20	40	77.0	NA	Chronic AF	Healthy controls	Fibrinogen D-dimer vWf Platelet count ß-thromboglobulin Platelet factor 4	Good
Heeringa	2006	162	324	77.3	49.0	Overall AF	Healthy controls	Fibrinogen P-selectin vWF	Good
Hou	2010	26	26	65.3	42.3	Acute AF	Healthy controls	D-dimer P-selectin vWF	Good
Jabati	2018	46	18	53.6	NA	Overall AF	Healthy controls	PAI-1 TPA	Fair
Kahn	1997	29	29	69.4	NA	Overall AF	Healthy controls	TPA	Fair
Kamath	2002	87	29	65.0	42.2	Chronic AF	Healthy controls	Fibrinogen D-dimer P-Selectin	Fair

								Platelet count ß-thromboglobulin	
Kamath(2)	2002	34	23	73.4	57.9	Chronic AF	Healthy controls	P-selectin Platelet count	Good
Kumagai	1990	24	5	NA	NA	Chronic AF	Healthy controls	D-dimer	Good
Li-Saw-Hee	2000	52	60	66.9	22.3	Overall AF	Healthy controls	Fibrinogen P-selectin vWF Thrombomodulin	Fair
Lip	1996	56	158	60.4	43.9	Chronic AF	Healthy controls	Fibrinogen D-dimer	Good
Lip(2)	1996	51	26	71.2	35.1	Chronic AF	Healthy controls	D-dimer Platelet count ß-thromboglobulin	Fair
Marcus	2008	46	925	66.4	18.3	Overall	Coronary artery disease	Fibrinogen	Good
Marín	2004	24	24	66.4	18.3	Chronic AF	Healthy controls	D-dimer Thrombomodulin	Fair
Minamino	1999	28	28	64.0	28.6	Chronic AF	Healthy controls	ß-thromboglobulin	Good
Mondillo	2000	45	35	56.3	17.5	Chronic AF	Healthy controls	Fibrinogen D-dimer vWF PAI-1 TPA Thrombomodulin	Good
Negreva	2016	51	52	59.7	49.5	Paroxysmal AF	Healthy controls	D-dimer TPA	Good

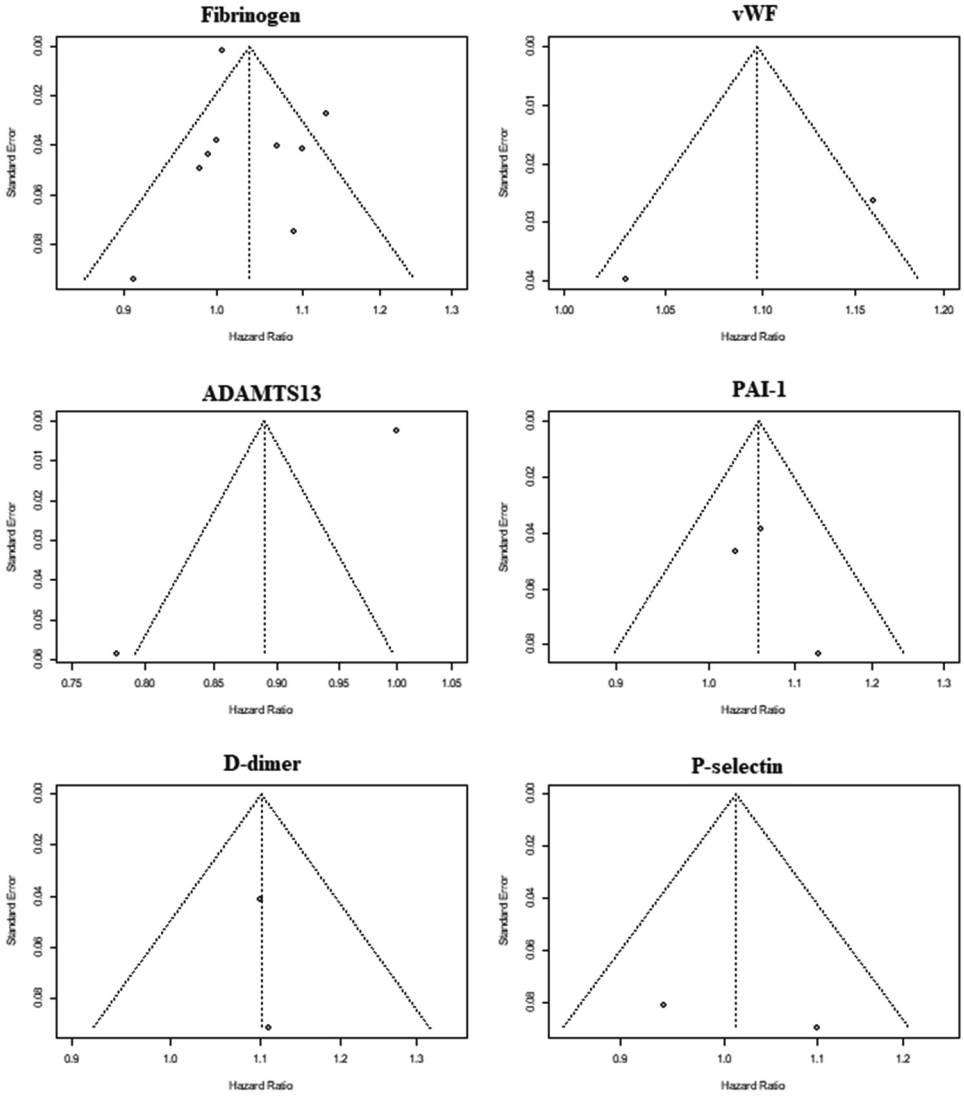
Negreva	2020	51	52	59.7	49.5	Paroxysmal AF	Healthy controls	vWF Platelet count Factor VIII	Good
Nozawa	2004	82	111	66.5	NA	Overall	Healthy controls	D-dimer ß-thromboglobulin Platelet factor 4	Fair
Otake	2021	72	1575	60.0	35.7	Overall	Diabetes	Platelet count	Good
Pfluecke	2016	67	50	81.0	66.7	Overall	Valvular heart disease	Platelet count	Fair
Roldán	1998	36	20	62.0	55.4	Chronic AF	Healthy controls	Fibrinogen D-dimer PAI-1 TPA	Good
Schnabel	2014	161	4,837	55.5	49.2	Overall	Healthy controls	Fibrinogen	Good
Sohara	1997	21	9	59.9	23.3	Paroxysmal AF	Healthy controls	Fibrinogen D-dimer ß-thromboglobulin Platelet factor 4 TAT	Fair
Sonmez	2014	52	33	70.0	63.5	Chronic AF	Healthy controls	Platelet count	Good
Targonski	2008	43	30	58.9	65.8	Chronic AF	Heart failure	Fibrinogen	Fair
Uemura	2009	56	55	67.9	33.3	Chronic AF	Healthy controls	vWF	Good
Wang	2001	53	3,159	54.1	53.2	Overall AF	Healthy controls	Fibrinogen PAI-1 TPA	Good

								Factor VIII	
Yamauchi	1986	73	57	46.7	NA	Overall AF	Healthy controls	ß-thromboglobulin Platelet factor 4	Fair
Zhang	2017	90	106	71.1	52.0	Overall AF	Heart failure	vWF	Fair

Abbreviations: AF, atrial fibrillation; MPV, mean platelet volume; N, number; NA, not available; NOS, Newcastle-Ottawa scale; OSAS, obstructive sleep apnea syndrome; PAI-1, plasminogen activator inhibitor 1; TAT, thrombin-antithrombin; TPA, tissue plasminogen activator; vWF, von Willebrand factor.

* If applicable. NOS 7-9: good, 4-6: fair, <4 poor.

Figure S1. Funnel plots for the association of coagulation biomarkers and incidence atrial fibrillation



Abbreviations: ADAMTS13, a desintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; PAI-1, plasminogen activator inhibitor 1; vWF, von Willebrand Factor.

Figure S2. Funnel plots for the association between coagulation biomarkers and atrial fibrillation presence

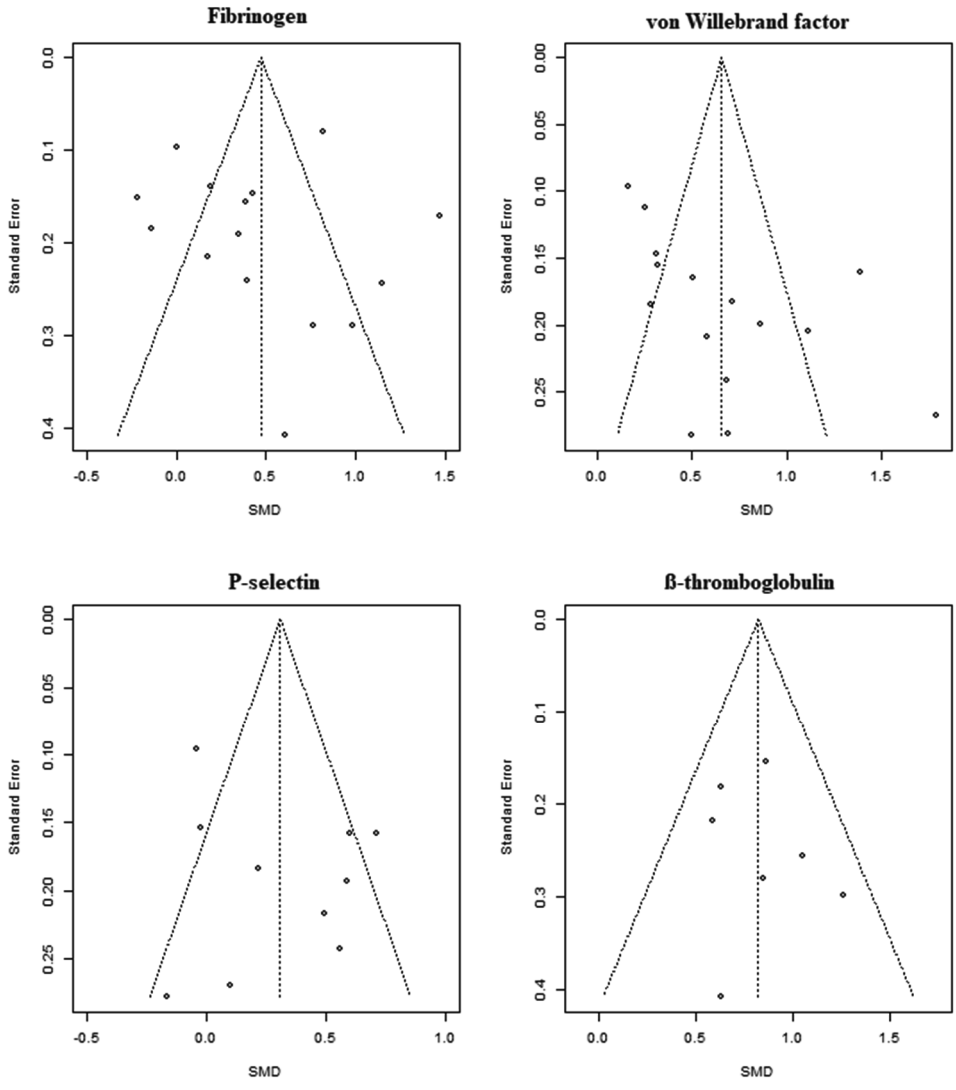


Figure S2. Funnel plots for the association between coagulation biomarkers and atrial fibrillation presence (continued)

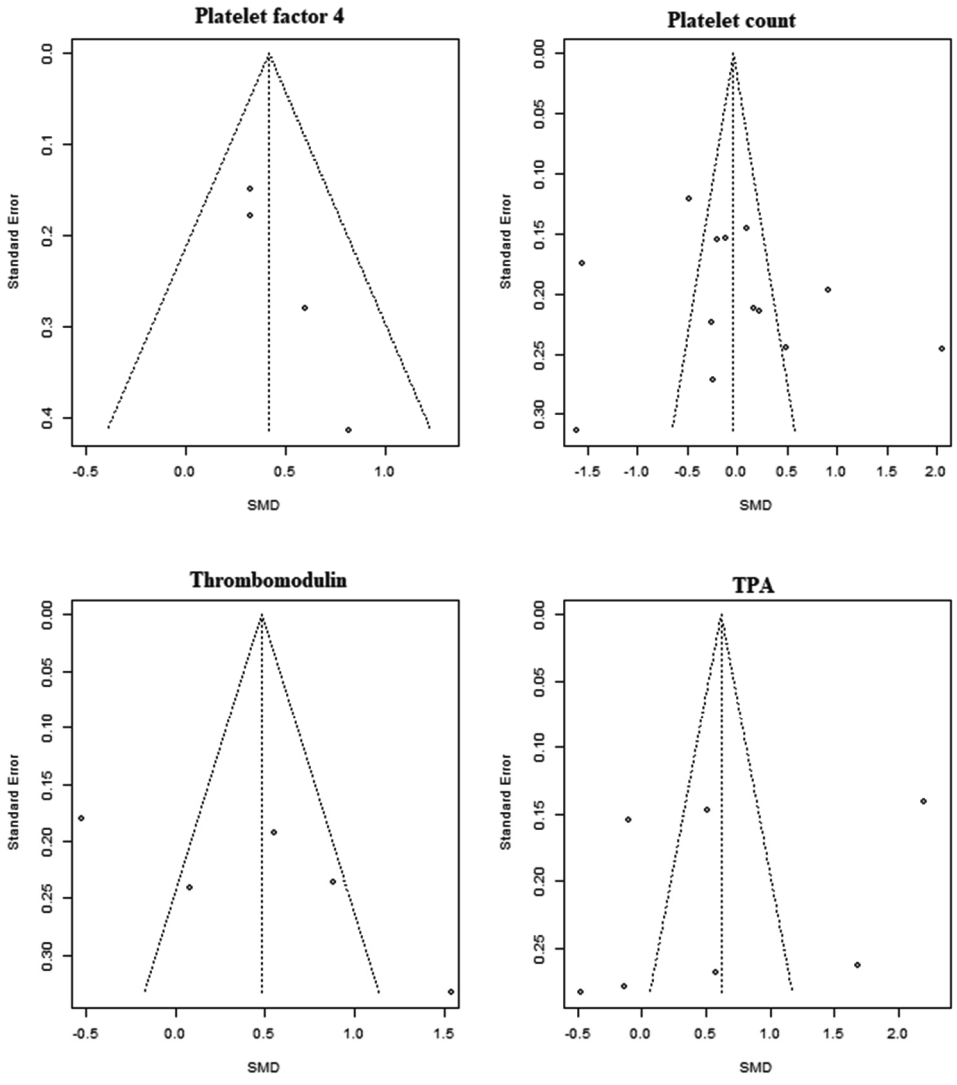
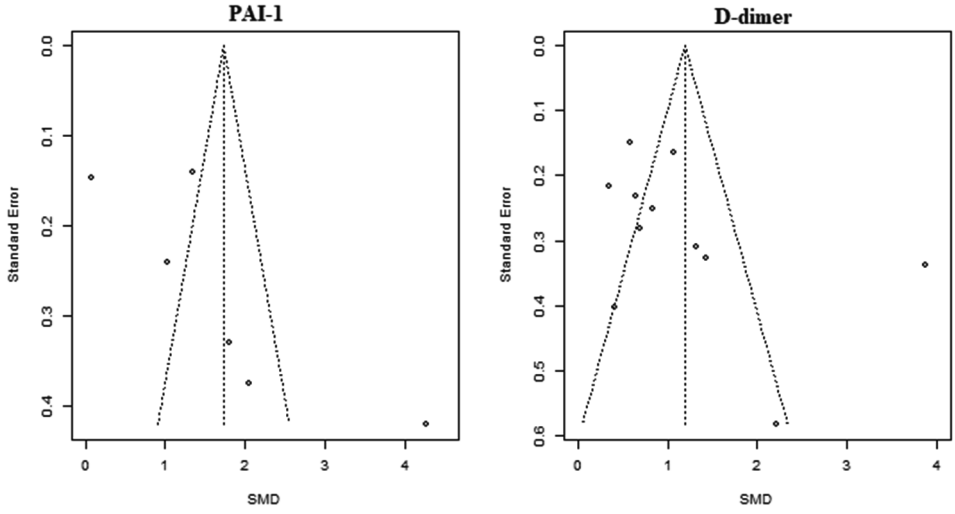
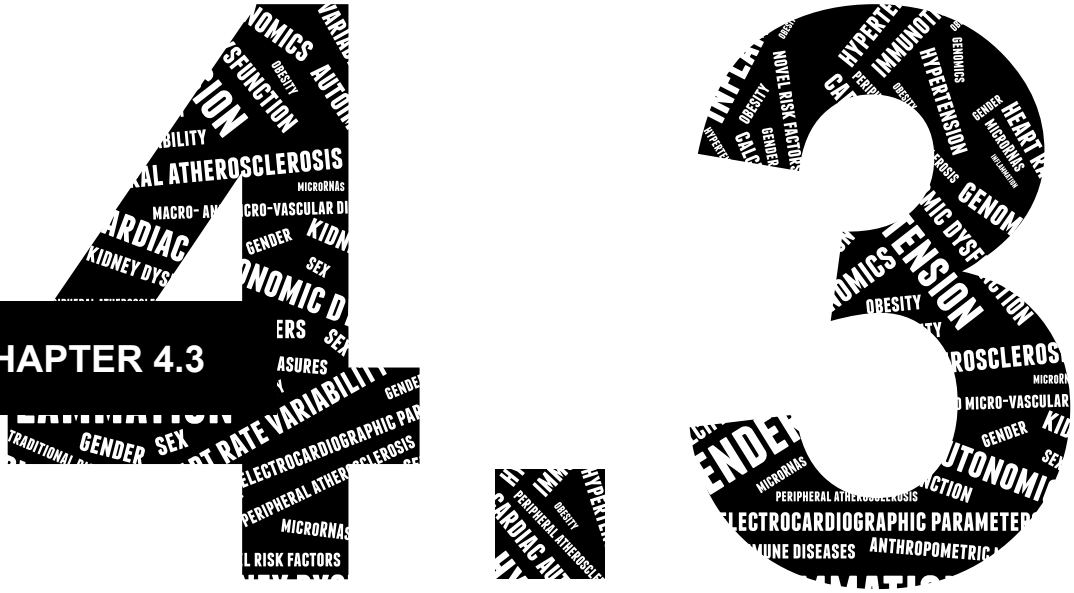


Figure S2. Funnel plots for the association between coagulation biomarkers and atrial fibrillation presence (continued)



Abbreviations: PAI-1, plasminogen activator inhibitor 1; SMD, standardized mean difference; TPA, Tissue plasminogen activator.
For von Willebrand Factor, Negreva et al (SMD 5.79) was excluded from the figure.
For Platelet count, Alberti et al (SMD -5.88) was excluded from the figure.
For Tissue plasminogen activator, Negreva et al (SMD 15.62) was excluded from the figure.
For D-dimer, Roldán et al (SMD 8.27) was excluded from the figure.


CHAPTER 4.3

Autoimmune diseases and the risk of atrial fibrillation

Autoimmune diseases and new-onset atrial fibrillation: a UK Biobank study.

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ABSTRACT

Background
The underlying mechanisms of atrial fibrillation (AF) are largely unknown. Inflammation may underlie atrial remodeling. Autoimmune diseases, related to increased systemic inflammation, may therefore be associated with new-onset AF.

Methods
Participants from the population-based UK Biobank were screened for rheumatic fever, gastrointestinal autoimmune diseases, autoimmune diseases targeting the musculoskeletal system and connective tissues, and neurological autoimmune diseases. Between 2006 and 2022, participants were followed for incident AF. Cox proportional hazards regression analyses were performed to calculate hazard ratios (HR) and 95% confidence intervals (CI) to quantify associations.

Results
494,072 participants free from AF were included (median age 58.0 years, 54.8% women). After a median of 12.8 years, 27,194 (5.5%) participants were diagnosed with new-onset AF. Rheumatic fever without heart involvement (HR, 95% CI: 1.47, 1.26-1.72), Crohn's disease (1.23, 1.05-1.45), ulcerative colitis (1.17, 1.06-1.31), rheumatoid arthritis (1.39, 1.28-1.51), polyarteritis nodosa (1.82, 1.04-3.09), systemic lupus erythematosus (1.82, 1.41-2.35), and systemic sclerosis (2.32, 1.57-3.44) were associated with a larger AF risk. In sex-stratified analyses, rheumatic fever without heart involvement, multiple sclerosis, Crohn's disease, seropositive rheumatoid arthritis, psoriatic and enteropathic arthropathies, systemic sclerosis and ankylosing spondylitis were associated with larger AF risk in women, whereas only men showed a larger AF risk associated with ulcerative colitis.

Conclusions
Various autoimmune diseases are associated with new-onset AF, more distinct in women. Our findings elaborate on the pathophysiological differences in autoimmunity and AF risk between men and women.

INTRODUCTION

Atrial fibrillation (AF) is a highly prevalent cardiac arrhythmia, associated with significant morbidity and mortality.(1, 2) While several AF risk factors have been identified, conclusive evidence on AF pathogenesis remains lacking.(3, 4)

Inflammation is suggested to be associated with AF development through structural and electrical remodeling of the atria.(5) Autoimmune diseases are accompanied by local or systemic inflammation, and may therefore be related to AF. A recent systematic review, indeed, suggested an association between rheumatoid arthritis and AF.(6) However, conclusive evidence on the relation between autoimmunity and AF is lacking. This is, at least partly, due to the low prevalence of autoimmune diseases, resulting in a lack of power, short follow-up periods, and the inability to investigate multiple autoimmune diseases.(7)

Accumulating evidence suggests differences in AF etiology and pathophysiology between men and women.(8) Nearly all autoimmune diseases are more prevalent in women.(9) While there could be various reasons for the higher prevalence, such as hormonal and genetic differences, an upcoming hypothesis is increased (re)activity of the innate immune system in women.(9) This could imply that, besides a higher prevalence of autoimmune diseases, the accompanying immune response may lead to more complications in women. However, evidence regarding sex differences in cardiovascular complications of autoimmune diseases, in particular AF, is lacking.

Using data of almost half a million participants of the UK Biobank, we aimed to identify the association between a range of autoimmune diseases, including diseases targeting the metabolic and gastrointestinal (GI) systems, the musculoskeletal system and connective tissues (MSK), and the nervous system, with AF incidence. Additionally, we aimed to identify potential differences in the role of inflammation in AF pathogenesis between men and women.

METHODS

Study design and study population

For this study we included participants from the UK Biobank. A detailed description of the aims of the study, study population, and methods of data collection has been published before.(10) In short, the UK Biobank is a population-based cohort study following over 500,000 inhabitants from the United Kingdom since 2006. Through questionnaires, interviews, recurrent visits to assessment centers, and linkage to the health records, a wide range of psychosocial, sociodemographic, physical, and genetic data was collected. Participants without informed consent for follow-up data collection, either at baseline or during the follow-up, were excluded from this study.

Assessment of autoimmune diseases and atrial fibrillation

Participants were continuously monitored for disease occurrences through linkages with health-related medical records, including primary care data, hospital inpatient data, death register records, and self-reported medical conditions. All diseases were recorded based on ICD-10 codes. Included autoimmune diseases and their corresponding ICD-10 codes were: I00 (rheumatic fever without mention of heart involvement), G35 (multiple sclerosis), G70 (myasthenia gravis), K50 (Crohn's disease), K51 (ulcerative colitis), M05 (seropositive rheumatoid arthritis), M06 (other rheumatoid arthritis), M07 (psoriatic and enteropathic arthropathies), M30 (polyarteritis nodosa), M32 (systemic lupus erythematosus; SLE), M33 (dermatopolymyositis), M34 (systemic sclerosis), M45 (ankylosing spondylitis), and M88 (Paget's disease). While events may rely on self-report only, previous studies used similar methodology, and reported robust validity within the UK Biobank(11). Cases were defined as participants with a report of any autoimmune disease at inclusion. AF was defined as I48. Participants with at least one reported AF event before the first assessment date were excluded (n=8,342).

Assessment of cardiovascular risk factors

Data on age, sex, ethnicity, use of lipid lowering medication, and use of blood pressure medication were collected through questionnaire and interviews at the assessment center. Furthermore, body mass index (BMI), defined as body weight divided by the square of height (kg/m^2), systolic blood pressure, diastolic blood pressure, total cholesterol (in mmol/L), high-density lipoprotein (HDL) cholesterol (in mmol/L), and serum glucose (in mmol/L) were measured at the first assessment center visit. If ICD-codes for hypertension (I10 or I15), heart failure (I50) or myocardial infarction (I21) were reported before the first assessment date, these were defined as prevalent cases.

Statistical analyses

Baseline characteristics

Characteristics of the study population were presented as mean and standard deviation (SD), median and interquartile range (IQR), or number (n) and percentages, as appropriate. Sex differences were calculated through Student's T-tests, Mann-Whitney U-tests, or Chi-Square test, as warranted by data and distribution type. Follow-up time was defined as the period between the first assessment center visit and first AF event, date of death, date of loss to follow-up, or February 1st 2022, whichever occurred first.

Cox proportional hazards models

Hazard ratios (HR) and 95% confidence intervals (CI) were calculated through Cox proportional hazards models using 2 models: adjusted for age at baseline, sex, and ethnicity (model 1), and additionally adjusted for BMI, total cholesterol, HDL-cholesterol, use of lipid lowering medication, use of blood pressure medication, smoking status, prevakebt hypertension, prevalent type 2 diabetes (T2DM), prevalent heart failure, and prevalent myocardial infarction (model 2). To identify potential sex differences, we performed all analyses for the total study population, included sex as an interaction term with the presence of various autoimmune diseases, and performed all analyses in men and women separately. Under the assumption of missing at random, missing data (range of missingness: 0.0-14.5%) were imputed ten times using fully conditional specification and predictive mean matching methods, by using all available data as predictors. Finally, complete-cases analyses were performed as sensitivity analyses. In total, 14,278 (2.9%) participants were lost to follow-up. Additionally, 27,705 (5.6%) participants died before AF onset, and were censored in the analyses.

Statistical significance was considered at two-tailed $p \leq 0.05$. Data management, imputation of the missing values, and statistical analyses were performed in R: a language and environment for statistical computing, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria), and IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, New York, USA).

RESULTS

Baseline characteristics

In total, we included 494,072 participants (median age 58.0, IQR 13.0), of whom 54.8% were women. Rheumatoid arthritis was the most prevalent autoimmune disease in our total study population (n=6,572, 1.3%). All autoimmune diseases but ulcerative colitis (0.8% vs. 0.9%), and ankylosing spondylitis (0.2% vs. 0.4%) were more common in women than in men (**Table 1**). As depicted in **Figure 1**, the majority of our population had only one type of autoimmune disease. In both men and women, solitary MSK autoimmune diseases were the most common, followed by GI disorders, neurological disorders, and rheumatic fever without heart involvement. Only GI and MSK disorders were coexistent in over one hundred individuals.

A significantly lower proportion of women used lipid lowering medication (12.4% vs. 22.0%) and blood pressure medication (17.3% vs. 23.7%). Women had lower prevalences at baseline of hypertension (8.7% vs. 11.2%), heart failure (0.2% vs. 3.8%), and acute myocardial infarction (0.8% vs. 3.8%), compared to men. All baseline characteristics are further depicted in **Table 1**.

Atrial fibrillation incidence

After a total follow-up time of 6,057,849 years (median 12.8 years, IQR 1.6), 16,804 men (7.5%) and 10,390 women (3.8%) developed new-onset AF. The incidence rates were 4.49 per 1000 person-years in the total study population, 6.25 per 1000 person-years in men, and 3.08 per 1000 person-years in women.

As depicted in **Table 2** and **Figure 2**, we found significant associations (HR, 95%CI) between the presence of rheumatic fever without heart involvement (1.47, 1.26-1.72.). Autoimmune disease affecting the digestive system, including Crohn's disease (1.23, 1.05-1.45) and ulcerative colitis (1.17, 1.05-1.31), were also significantly associated with incident AF. Autoimmune diseases targeting the MSK system also showed a larger risk for new-onset AF (1.35, 1.26-1.45). Looking at the specific autoimmune diseases, this association seemed to be mainly driven by rheumatic arthritis (1.39, 1.28-1.51), psoriatic and enteropathic arthropathies (1.38, 1.01-1.89), polyarteritis nodosa (1.79, 1.04-3.09), SLE (1.82, 1.41-2.35), and systemic sclerosis (2.32, 1.57-3.44). The other investigated diseases in this group, dermatopolymyositis, ankylosing spondylitis, and Paget's disease, showed trends towards higher risk of incident AF, but the results were not significant after adjusting for cardiovascular risk factors. For the autoimmune diseases targeting the nervous system (multiple sclerosis and myasthenia gravis), we found a significant association with incident AF when combining the diseases (1.23, 1.02-1.47), but not when investigating the conditions separately. To identify the role of sex in the associations between the autoimmune diseases and AF, we included interaction terms between

the autoimmune diseases and sex. These analyses showed a larger impact of rheumatic fever, all combined MSK diseases, rheumatoid arthritis, and ankylosing spondylitis on AF development in women. All other autoimmune disorders, excluding dermatopolymyositis, Paget's disease, and myasthenia gravis, showed similar trends, albeit not statistically significant.

As visible in **Figure 3** and **Table S1**, in men we found significant associations between GI autoimmune diseases (1.19, 1.05-1.35) due to ulcerative colitis (1.17, 1.01-1.35), and MSK disorders (1.18, 1.07-1.31) due to rheumatoid arthritis (1.25, 1.10-1.41) and SLE (1.81, 1.07-3.06) also showed significant associations with new-onset AF. In contrast to the total population, we also found a distinct association between the presence of myasthenia gravis and new-onset AF (1.61, 1.00-2.59). Other autoimmune diseases, including rheumatic fever without heart involvement, polyarteritis nodosa, Paget's disease, and neurological autoimmune diseases showed significant associations in univariable analyses, but these associations attenuated after adjusting for cardiovascular risk factors.

We found that more autoimmune diseases were significantly associated with new-onset AF in women (**Table S2**). We found significant associations for rheumatic fever without heart involvement (1.79, 1.45-2.20), Crohn's disease (1.35, 1.05-1.73), MSK (1.51, 1.38-1.66), due to rheumatoid arthritis (1.50, 1.35-1.67), psoriatic and enteropathic arthropathies (2.01, 1.32-3.05), SLE (1.79, 1.34-2.40), systemic sclerosis (2.51, 1.64-3.85), and ankylosing spondylitis (1.53, 1.13-2.07) and new-onset AF. In contrast to men, multiple sclerosis (1.37, 1.07-1.75) was significantly associated with new-onset AF in women.

Sensitivity analyses adjusting for the use of medication affecting the immune system showed similar results (**Table S3**).

Table 1. Baseline characteristics of the total study population and stratified by sex

Baseline characteristics *	Total study population n=494,072	Men n=223,268	Women n=270,804	p ¶
Age, years	58.0 ± 13	58.0 ± 13	57.0 ± 13	<0.001
Women, n (%)	270,804 (54.8)	NA	270,804 (100.0)	NA
Body mass index, kg/m^2	27.4 ± 4.8	27.8 ± 4.2	27.1 ± 5.2	<0.001
Smoking status				<0.001
Current, n (%)	52,347 (10.6)	28,133 (12.6)	24,214 (8.9)	
Former, n (%)	169,130 (34.2)	84,688 (37.9)	84,442 (31.2)	
Never, n (%)	269,712 (54.6)	109,051 (48.8)	160,661 (59.3)	
Diastolic blood pressure, mmHg	82.2 ± 10.7	84.0 ± 10.5	80.7 ± 10.6	<0.001
Systolic blood pressure, mmHg	139.7 ± 19.7	142.8 ± 18.5	137.2 ± 20.3	<0.001
Lipid lowering medication, n (%)	82,858 (16.8)	49,213 (22.0)	33,645 (12.4)	<0.001
Blood pressure medication, n (%)	99,685 (20.2)	52,897 (23.7)	46,788 (17.3)	<0.001
Total cholesterol, mmol/L †	5.7 ± 1.1	5.5 ± 1.2	5.9 ± 1.1	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.4 ± 0.5	1.2 ± 0.4	1.6 ± 0.5	<0.001
Glucose, mmol/L §	4.9 (0.7)	5.0 (0.8)	4.9 (0.7)	<0.001
Rheumatic fever without heart involvement, n (%)	1,472 (0.3)	568 (0.3)	904 (0.3)	<0.001
Rheumatic heart disease, n (%)	328 (0.1)	111 (0.0)	217 (0.1)	<0.001
Rheumatic fever with heart involvement, n (%)	18 (0.0)	6 (0.0)	12 (0.0)	0.312
Rheumatic chorea, n (%)	23 (0.0)	9 (0.0)	14 (0.0)	0.559
Rheumatic mitral valve disease, n (%)	141 (0.1)	44 (0.0)	97 (0.0)	<0.001
Rheumatic aortic valve disease, n (%)	40 (0.0)	16 (0.0)	24 (0.0)	0.510
Rheumatic tricuspid valve disease, n (%)	90 (0.0)	31 (0.0)	59 (0.0)	0.041
Other rheumatic valve disease, n (%)	36 (0.0)	7 (0.0)	29 (0.0)	0.002
Type 1 diabetes mellitus, n (%)	2,527 (0.5)	1,433 (0.6)	1,094 (0.4)	<0.001
Gastro-intestinal disorders, n (%)	5,655 (1.1)	2,649	3,006	0.012
Crohn's disease, n (%)	2,061 (0.4)	911 (0.4)	1,150 (0.4)	0.367

Ulcerative colitis, n (%)	4,067 (0.8)	1,967 (0.9)	2,100 (0.8)	<0.001
Musculoskeletal system and tissue disorders, n (%)	9,710 (2.0)	3,326 (1.5)	6,384 (2.4)	<0.001
Rheumatoid arthritis, n (%)	6,572 (1.3)	2,019 (0.9)	4,553 (1.7)	<0.001
Seropositive rheumatoid arthritis, n (%)	376 (0.1)	110 (0.0)	266 (0.1)	<0.001
Other rheumatoid arthritis, n (%)	6,546 (1.3)	2,008 (0.9)	4,538 (1.7)	<0.001
Psoriatic and enteropathic arthropathy, n (%)	395 (0.1)	188 (0.1)	207 (0.1)	0.337
Polyarteritis nodosa, n (%)	99 (0.0)	39 (0.0)	60 (0.0)	0.247
Systemic lupus erythematosus, n (%)	744 (0.2)	93 (0.0)	651 (0.2)	<0.001
Dermatopolymyositis, n (%)	152 (0.0)	57 (0.0)	95 (0.0)	0.057
Systemic sclerosis, n (%)	210 (0.0)	28 (0.0)	182 (0.1)	<0.001
Ankylosing spondylitis, n (%)	1,519 (0.3)	943 (0.4)	576 (0.2)	<0.001
Paget's disease, n (%)	392 (0.1)	77 (0.0)	315 (0.1)	<0.001
Neurological disorders, n (%)	2,208 (0.4)	619 (0.3)	1,589 (0.6)	<0.001
Multiple sclerosis, n (%)	1,983 (0.5)	521 (0.2)	1,462 (0.5)	<0.001
Myasthenia gravis, n (%)	230 (0.0)	99 (0.0)	131 (0.0)	0.513
Acute myocardial infarction, n (%)	10,520 (2.1)	8,405 (3.8)	2,115 (0.8)	<0.001
Coronary heart disease, n (%)	17,821 (3.6)	13,321 (6.0)	4,500 (1.7)	<0.001

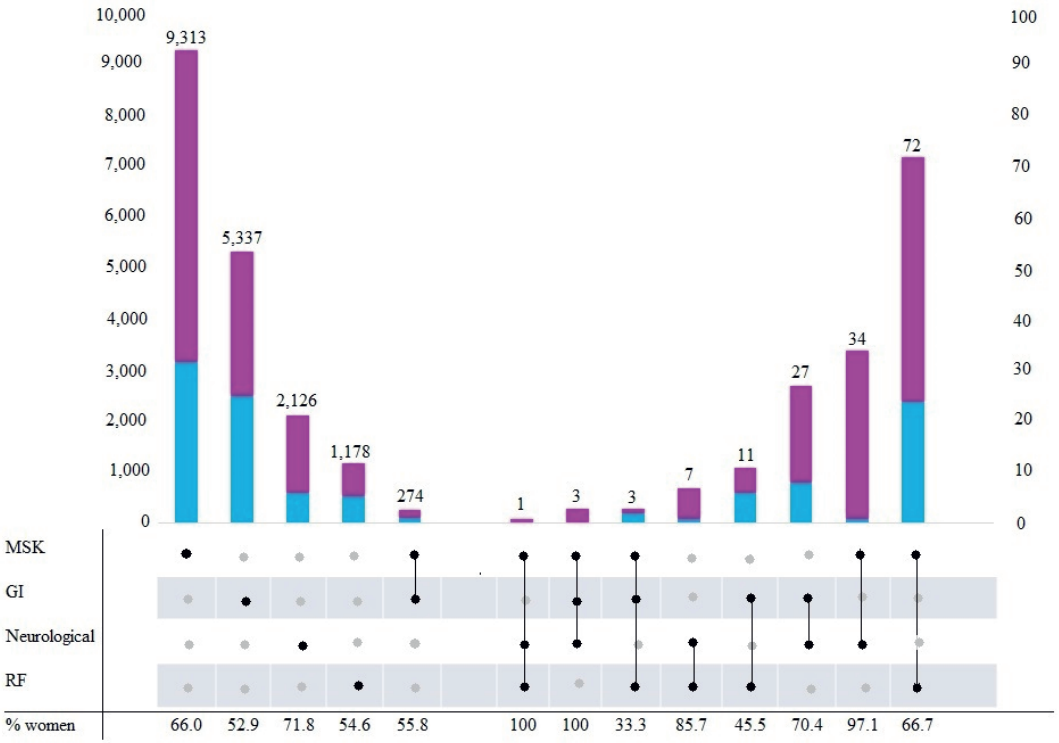
* Values are mean (standard deviation) for normally distributed continuous variables or median (interquartile range) for skewed continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

§ SI conversion factor: to convert glucose to mg/dL divide values by 18.

¶ Statistical significance for continuous variables was tested using the Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) and for categorical variables was tested using the Chi-Square test.

Figure 1. Prevalence of (the combination of) various autoimmune diseases



Abbreviations: GI, gastrointestinal disorders; MSK, musculoskeletal and connective tissue disorders; RF, rheumatic fever.
Depicted are the prevalences of single autoimmune diseases (one dot) as well as combinations of those autoimmune diseases (multiple dots) in individuals for women (pink) and men (blue). The left Y-axis depicts the number of individuals for MSK, GI, neurological, RF, and the combination of MSK and GI. On the right Y-axis, the numbers are depicted for the other combinations of diseases.
Rheumatic fever included rheumatic fever without heart involvement.
Neurological disorders included multiple sclerosis and myasthenia gravis.
Gastrointestinal disorders included Crohn's disease, and ulcerative colitis.
MSK disorders included rheumatoid arthritis, psoriatic and enteropathic arthropathies, systemic lupus erythematosus, dermatopolymyositis, systemic sclerosis, ankylosing spondylitis, and Paget's disease

Table 2. Cox proportional hazards analyses in the total study population

Autoimmune diseases	Total study population		
	HR (95% CI)		
	Univariable	Model 1 *	Model 2 †
Rheumatic fever without heart involvement	**2.03 (1.74-2.37)**	**1.50 (1.28-1.76)**	**1.46 (1.25-1.71)**
Rheumatic heart disease	**5.84 (4.73-7.21)**	**4.65 (3.76-5.73)**	**3.77 (3.05-4.65)**
Rheumatic fever with heart involvement	2.02 (0.50-8.06)	1.86 (0.470-7.44)	1.79 (0.45-7.15)
Rheumatic chorea	2.54 (0.82-7.86)	1.59 (0.51-4.94)	1.56 (0.50-4.82)
Rheumatic mitral valve disease	**7.70 (5.78-10.25)**	**6.37 (4.78-8.48)**	**4.93 (3.70-6.57)**
Rheumatic aortic valve disease	**7.40 (4.30-12.75)**	**5.17 (3.00-8.90)**	**3.96 (2.30-6.82)**
Rheumatic tricuspid valve disease	**4.56 (2.91-7.15)**	**3.58 (2.28-5.61)**	**2.76 (1.76-4.32)**
Other rheumatic valve disease	**7.67 (4.35-13.50)**	**6.60 (3.75-11.62)**	**6.75 (3.83-11.89)**
Type 1 diabetes mellitus	**2.79 (2.51-3.11)**	**2.44 (2.19-2.72)**	**1.41 (1.26-1.58)**
Gastro-intestinal diseases	**1.33 (1.21-1.47)**	**1.24 (1.12-1.37)**	**1.18 (1.07-1.31)**
Crohn's disease	**1.31 (1.11-1.54)**	**1.31 (1.11-1.54)**	**1.23 (1.04-1.44)**
Ulcerative colitis	**1.35 (1.21-1.52)**	**1.21 (1.08-1.36)**	**1.16 (1.04-1.31)**
All musculoskeletal diseases	**1.67 (1.56-1.78)**	**1.54 (1.44-1.65)**	**1.35 (1.26-1.45)**
All rheumatoid arthritis	**1.77 (1.64-1.92)**	**1.60 (1.48-1.73)**	**1.39 (1.28-1.51)**

Seropositive rheumatoid arthritis	**2.41 (1.80-3.23)**	**2.09 (1.56-2.80)**	**1.82 (1.36-2.44)**
Other rheumatoid arthritis	**1.77 (1.63-1.92)**	**1.59 (1.47-1.73)**	**1.39 (1.28-1.50)**
Psoriatic and enteropathic arthropathy	**1.87 (1.36-2.56)**	**1.89 (1.38-2.58)**	**1.40 (1.02-1.91)**
Polyarteritis nodosa	**2.58 (1.50-4.45)**	**2.39 (1.39-4.12)**	**1.83 (1.06-3.15)**
Systemic lupus erythematosus	**1.49 (1.15-1.92)**	**2.10 (1.63-2.71)**	**1.80 (1.39-2.32)**
Dermatopolymyositis	1.34 (0.74-2.42)	1.21 (0.67-2.18)	1.24 (0.69-2.23)
Systemic sclerosis	**2.34 (1.58-3.46)**	**2.52 (1.70-3.73)**	**2.31 (1.56-3.422)**
Ankylosing spondylitis	**1.47 (1.23-1.76)**	**1.24 (1.04-1.48)**	1.15 (0.96-1.38)
Paget's disease	1.16 (0.79-1.72)	1.36 (0.92-2.02)	1.24 (0.84-1.83)
Neurological diseases	0.99 (0.83-1.19)	**1.24 (1.04-1.49)**	**1.22 (1.02-1.47)**
Multiple sclerosis	0.90 (0.73-1.10)	1.19 (0.97-1.45)	1.19 (0.98-1.46)
Myasthenia gravis	**1.85 (1.22-2.81)**	**1.53 (1.01-2.33)**	1.34 (0.88-2.03)

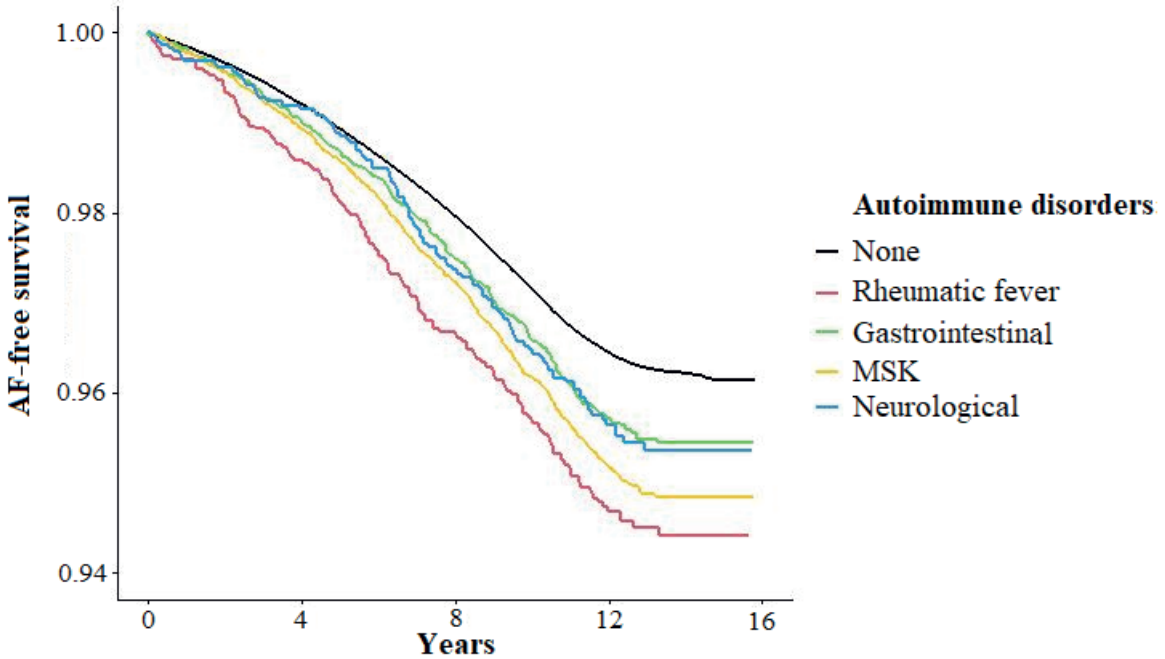
Abbreviations: CI, confidence interval; HR, hazard ratio.

* Adjusted for age, sex, and ethnicity.

† Adjusted for age, sex, ethnicity, body mass index, total cholesterol, high-density lipoprotein cholesterol, smoking status, history of hypertension, history of type 2 diabetes mellitus, history of heart failure, and history of acute myocardial infarction.

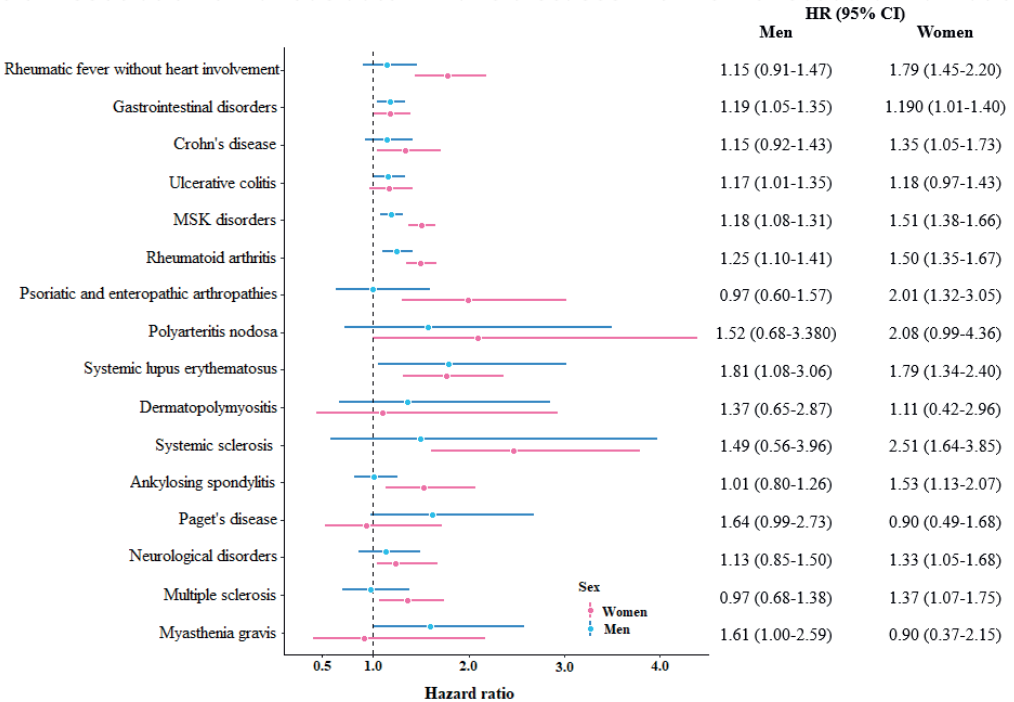
The associations with a $p<0.05$ are highlighted in **bold**.

Figure 2. The survival time free of atrial fibrillation per autoimmune disorder



Abbreviations: AF, atrial fibrillation; BMI, body mass index; HDL, high-density lipoprotein; MSK, musculoskeletal and connective tissue disorders.
Depicted is the probability of survival free of AF over time per autoimmune disorder group, adjusted for age, sex, ethnicity, BMI, total cholesterol, HDL-cholesterol, use of lipid lowering medication, use of blood pressure medication, smoking status, prevalent hypertension, prevalent type 2 diabetes, prevalent heart failure, and prevalent acute myocardial infarction.

Figure 3. Association of various autoimmune diseases with new-onset atrial fibrillation stratified by sex



Abbreviations: CI, confidence interval; HR, hazard ratio; MSK; musculoskeletal and connective tissue disorders.
Depicted are hazard ratios with their corresponding 95% confidence intervals from model 2.
Neurological disorders included multiple sclerosis and myasthenia gravis.
Gastrointestinal disorders included Crohn's disease, and ulcerative colitis.
MSK disorders included rheumatoid arthritis, psoriatic and enteropathic arthropathies, systemic lupus erythematosus, dermatopolymyositis, systemic sclerosis, ankylosing spondylitis, and Paget's disease.

DISCUSSION

In the present study we investigated the associations of multiple autoimmune diseases with new-onset AF in the population-based UK Biobank. We found significant associations between new-onset AF and rheumatic fever without heart involvement, GI (Crohn's disease and ulcerative colitis), and MSK (rheumatoid arthritis, psoriatic and enteropathic arthropathies, polyarteritis nodosa, SLE, and systemic sclerosis) autoimmune diseases, with AF incidence. Moreover, we found evidence of significant differences between men and women in these associations.

Recent evidence suggests a role for inflammation and the innate immune system in cardiovascular disease development, including myocardial infarction, cerebrovascular events, and heart failure.(12-15) However, evidence on the role of autoimmunity in AF development is scarce, at least partly due to the rarity of these disorders. In our present study, we identified a broad range of autoimmune diseases associated with incident AF in the general population.

We found that Crohn's disease was significantly associated with AF in women, whereas ulcerative colitis was associated with AF solely in men. A systematic review of the limited available data suggested an association between GI disorders and AF presence.(16) However, due to a lack of paucity of data, no robust conclusions could be made, and none of the studies evaluated potential sex differences. Our findings support the hypothesis that estrogen receptors have a paradoxical effect on GI inflammation, resulting in more severe colitis in men, but more ileitis in women.(17, 18)

Rheumatoid arthritis is the most common autoimmune disease afflicting the MSK, mostly prevalent in women, and is suggested to increase the risk of cardiovascular disease, including AF.(6, 14, 15, 19, 20) Our results are in line with the relatively large amount of evidence available on the relation between rheumatoid arthritis and AF. However, we were the first to investigate the risk in men and women separately in a large population. We found that the larger AF risk was mainly observed in women. Sex hormones may, at least partly, be the explanation for this. In patients with rheumatoid arthritis, elevated estrogen levels are found in the synovial fluid of the afflicted joints.(21) Additionally, a decrease in disease activity was observed in patients undergoing androgen replacement therapy.(21) However, while the mediating role of estrogen in autoimmune diseases may be part of the explanation, much remains unknown on the histopathology that causes these sex differences. This is further supported by the significant interactions we found between sex and autoimmune diseases targeting the MSK system.

In a similar way, psoriatic and enteropathic arthropathies, systemic sclerosis, and

ankylosing spondylitis were associated with a higher AF risk in women only. While cardiac arrhythmias are relatively common in systemic sclerosis, data on the association between these diseases and AF is lacking.(22) A Korean study found that individuals with ankylosing spondylitis had a 28% higher AF risk.(23) We found a similar risk in the total population, attributed mainly to women. While this could be explained by a significant diagnostic delay and less targeted treatment in women, our findings support the notion that women with ankylosing spondylitis have a higher disease activity, and more extra-articular manifestations.(24) In the same Korean population, patients with SLE had a doubled AF risk.(25) We found a 82% larger risk, equal for men and women. SLE is a heterogeneous disease, targeting mostly the joints and hematological system in women, and the skin and internal organs in men.(26) These different characterizations and localizations may imply different causes of atrial fibrosis and remodeling, resulting in a higher AF risk for both sexes.

We found a significantly higher AF risk in women, but not in men, in relation to multiple sclerosis. This contradicts a Swedish study, which found a lower AF risk in both men and women with multiple sclerosis.(27) One explanation could be the lower number of events in our study. However, another explanation could be that, while we extensively adjusted for cardiovascular risk factors, Jadidi et al.(27) only adjusted for age and country of birth.

Our results may, at least partly, be influenced by medication use. Certain autoimmune diseases can be treated with strong anti-inflammatory drugs, or even chemotherapy.(28) The medication itself may give rise to AF through different pathways.(29) While we performed sensitivity analyses adjusting for the use of numerous medications affecting the immune system, residual confounding due to medication use can not be ruled out. Similarly, autoimmune diseases may lead to lifestyle changes, such as lower physical activity due to joint pains, or an unhealthy diet due to GI diseases. While we adjusted for factors suggesting an unhealthy lifestyle, such as BMI, cholesterol, hypertension, and smoking status, other factors may underlie the associations. Shared genetic basis may reflect another potential underlying factor. It may be that the genetic preposition for the development of autoimmune disease is also associated with AF development. Future research, including Mendelian randomization studies, may shed light on this.

The strengths in our present study are the large population-based study population of almost 500,000 individuals, the median of over 12 years follow-up time, the continuous and careful assessment of both physical and social data, and the continuous linkage with multiple registries to confirm disease occurrences. In addition, we used robust statistical models to adjust for confounding, and used both multiple imputation and complete-case analyses to confirm our findings. However, our study also has some limitations. Due to the rarity of autoimmune disorders, some of our diseases of interest were low in prevalence. Moreover, due to the nature of

data assessment in the UK Biobank, we were constricted to using ICD-10 codes. Using the ICD-10 codes carries various limitations, including the inability to investigate specific diseases, but rather the groups of autoimmune diseases. Moreover, this could have also influenced the relatively low number of participants with prevalent hypertension (9.8%), while 20.2% used blood pressure medication. Medication may be started due to comorbidity or prevention, while the ICD-10 diagnosis for hypertension is not registered. Also, due to this coding, we were unable to distinguish between the various AF patterns and were restricted to overall AF. As clinical trials in patients and are often expensive and time consuming, we hope that our results open the door for future patient cohorts to further investigate differences in AF characteristics and prognosis between AF patients with and without autoimmune disorders. As AF can have an asymptomatic presentation, it is possible that AF patients free of symptoms, or who avoided visiting a healthcare professional, were missed. However, this would be similar to a real-world situation, in which individuals from the general population could also remain undiagnosed. Additionally, the UK Biobank comprises of mostly men and women from European heritage and of middle-to-older age. As all participants are volunteering to participate, healthy-volunteer bias is also a possibility. Lastly, in comparison to all other included autoimmune diseases, of which the vast majority is documented within the UK Biobank due to hospital or primary care registries, rheumatic fever was registered mainly due to self-report only. Recall bias or ambiguity in the questionnaires may have inflated the actual numbers of rheumatic fever events, as the prevalence within this population is higher than expected. Therefore, the results from this study may not be directly generalized to other populations.

In conclusion, autoimmune diseases are significantly associated with the risk of new-onset AF in this prospective population-based study, comprising almost half a million participants. Our findings further elaborate on and contribute to the current knowledge of the pathophysiological differences in autoimmunity between men and women. This implies that various autoimmune diseases may modulate the propensity to develop AF, particularly in women. This information could also guide future preventive strategies. However, evidence on the role of autoimmunity in AF development is still scarce. Further evidence is required to support clinical translation of our findings.

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SUPPLEMENTARY MATERIAL

Chapter 4.3 Autoimmune diseases and the risk of atrial fibrillation

Table S1. Cox proportional hazards analyses in men

Table S2. Cox proportional hazards analyses in women

Table S1. Cox proportional hazards analyses in men

Autoimmune diseases	Men			
	HR (95% CI)			
	Univariable	Model 1 *	Model 2 (imputed) †	Model 2 (complete-cases) ‡
Rheumatic fever without heart involvement	**1.60 (1.26-2.04)**	1.16 (0.91-1.47)	1.15 (0.90-1.46)	1.20 (0.93-1.55)
Rheumatic heart disease	**4.65 (3.29-6.58)**	**3.43 (2.42-4.85)**	**2.82 (1.99-3.98)**	**2.58 (1.76-3.80)**
Rheumatic fever with heart involvement	2.27 (0.32-16.06)	2.19 (0.31-15.57)	1.89 (0.26- 13.39)	1.89 (0.27-13.38)
Rheumatic chorea	1.52 (0.21-10.71)	0.96 (0.14-6.82)	1.00 (0.14-7.10)	1.00 (0.14-7.13)
Rheumatic mitral valve disease	**8.15 (5.25-12.63)**	**6.40 (4.12-9.92)**	**4.96 (3.20-7.70)**	**5.03 (3.12- 8.10)**
Rheumatic aortic valve disease	**4.50 (1.69-12.00)**	**2.71 (1.02-7.20)**	2.16 (0.81-5.72)	2.24 (0.72-6.94)
Rheumatic tricuspid valve disease	2.38 (0.99-5.73)	1.64 (0.68- 3.93)	1.30 (0.54-3.13)	0.79 (0.26-2.46)
Other rheumatic valve disease	2.00 (0.28-14.21)	1.95 (0.28-13.86)	1.69 (0.24-11.97)	1.91 (0.27-13.57)
Type 1 diabetes mellitus	**2.41 (2.11-2.75)**	**2.21 (1.94-2.53)**	**1.34 (1.17-1.54)**	**1.27 (1.09- 1.48)**
Gastro-intestinal diseases	**1.31 (1.15-1.48)**	**1.23 (1.09-1.40)**	**1.18 (1.04-1.34)**	**1.25 (1.10-1.42)**
Crohn's disease	1.21 (0.87-1.51)	1.21 (0.97-1.50)	1.14 (0.92-1.42)	1.21 (0.97-1.52)
Ulcerative colitis	**1.32 (1.14-1.52)**	**1.213 (1.05-1.40)**	**1.16 (1.01-1.34)**	**1.22 (1.05-1.42)**
All musculoskeletal diseases	**1.60 (1.45-1.77)**	**1.35 (1.22-1.49)**	**1.19 (1.07-1.31)**	**1.16 (1.03-1.29)**

All rheumatoid arthritis	**1.81 (1.60-2.05)**	**1.44 (1.27-1.63)**	**1.25 (1.10-1.41)**	**1.19 (1.04-1.37)**
Seropositive rheumatoid arthritis	**2.09 (1.24-3.53)**	1.56 (0.82-2.63)	1.37 (0.81-2.32)	1.11 (0.58-2.13)
Other rheumatoid arthritis	**1.80 (1.59-2.03)**	**1.43 (1.26-1.62)**	**1.24 (1.09-1.40)**	**1.19 (1.03-1.36)**
Psoriatic and enteropathic arthropathy	1.22 (0.76-1.96)	1.32 (0.82-2.13)	0.99 (0.62-1.60)	0.89 (0.52-1.54)
Polyarteritis nodosa	**2.32 (1.04-5.16)**	2.07 (0.93-4.60)	1.57 (0.71-3.50)	1.42 (0.59-3.41)
Systemic lupus erythematosus	**2.22 (1.32-3.75)**	**2.13 (1.26-3.60)**	**1.79 (1.06-3.02)**	**1.98 (1.12-3.49)**
Dermatopolymyositis	1.73 (0.82-3.62)	1.33 (0.63-2.79)	1.36 (0.65-2.85)	1.47 (0.66-3.28)
Systemic sclerosis	2.16 (0.81-5.75)	2.02 (0.76-5.38)	1.49 (0.56-3.98)	1.19 (0.38-3.68)
Ankylosing spondylitis	1.12 (0.89-1.39)	1.07 (0.85-1.33)	1.01 (0.81-1.26)	0.99 (0.77-1.26)
Paget's disease	**2.74 (1.65-4.55)**	**1.840(1.11-3.05)**	1.62 (0.98-2.69)	**1.80 (1.07-3.04)**
Neurological diseases	1.08 (0.81-1.43)	1.16 (0.87-1.54)	1.13 (0.85-1.50)	1.06 (0.76-1.47)
Multiple sclerosis	0.82 (0.57-1.16)	0.95 (0.67-1.35)	0.97 (0.68-1.38)	0.80 (0.52-1.25)
Myasthenia gravis	**2.56 (1.59-4.12)**	**1.90 (1.18-3.06)**	**1.60 (0.99-2.58)**	**1.74 (1.06-2.83)**

Abbreviations: CI, confidence interval; DM, diabetes mellitus; GI, gastrointestinal; HR, hazard ratio; NA, not available.

* Adjusted for age, and ethnicity. † Adjusted for age, ethnicity, body mass index, total cholesterol, high-density lipoprotein cholesterol, smoking status, prevalent hypertension, history of type 2 diabetes mellitus, history of heart failure, and history of acute myocardial infarction. ‡ Cases dropped in complete-case analyses: n=19,976 (8.9%).

The associations with a $p<0.05$ are highlighted in **bold**.

Table S2. Cox proportional hazards analyses in women

Autoimmune diseases	Women			
	HR (95% CI)			
	Univariable	Model 1 *	Model 2 (imputed) †	Model 2 (complete-cases) ‡
Rheumatic fever without heart involvement	**2.78 (2.26-3.42)**	**1.91 (1.55-2.34)**	**1.78 (1.44-2.18)**	**1.68 (1.33-2.12)**
Rheumatic heart disease	**8.08 (6.20-10.53)**	**5.84 (4.48-7.62)**	**4.51 (3.45-5.90)**	**4.50 (3.38-6.07)**
Rheumatic fever with heart involvement	2.16 (0.31-15.32)	1.59 (0.22-11.27)	1.66 (0.23-11.78)	Error
Rheumatic chorea	**4.12 (1.03-16.47)**	2.37 (0.59-9.48)	2.09 (0.52-8.37)	1.72 (0.24-2.20)
Rheumatic mitral valve disease	**9.08 (6.22-13.25)**	**6.26 (4.29-9.13)**	**4.64 (3.17-6.81)**	**4.44 (2.88-6.86)**
Rheumatic aortic valve disease	**11.81 (6.14-22.70)**	**8.98 (4.67-17.26)**	**6.31 (3.28-12.14)**	**7.32 (3.66-14.65)**
Rheumatic tricuspid valve disease	**7.69 (4.55-12.99)**	**6.26 (3.71-10.57)**	**4.46 (2.64-7.54)**	**4.43 (2.51-7.82)**
Other rheumatic valve disease	**13.41 (7.42-24.22)**	**8.24 (4.56-14.88)**	**8.96 (4.95-16.22)**	**10.33 (5.55-19.23)**
Type 1 diabetes mellitus	**3.06 (2.55-3.67)**	**3.09 (2.57-3.71)**	**1.57 (1.30-1.91)**	**1.61 (1.30-2.01)**
Gastro-intestinal diseases	**1.34 (1.14-1.57)**	**1.25 (1.06-1.47)**	**1.18 (1.01-1.39)**	**1.21 (1.02-1.44)**
Crohn's disease	**1.48 (1.16-1.90)**	**1.47 (1.15-1.88)**	**1.34 (1.05-1.72)**	**1.36 (1.04-1.78)**
Ulcerative colitis	**1.33 (1.10-1.62)**	**1.22 (1.01-1.47)**	1.17 (0.97-1.42)	1.19 (0.97-1.47)
All musculoskeletal diseases	**2.03 (1.85-2.23)**	**1.74 (1.59-1.91)**	**1.51 (1.37-1.65)**	**1.47 (1.32-1.63)**

All rheumatoid arthritis	**2.14 (1.92-2.38)**	**1.73 (1.56-1.93)**	**1.50 (1.34-1.66)**	**1.46 (1.29-1.64)**
Seropositive rheumatoid arthritis	**3.26 (2.29-4.64)**	**2.45 (1.72-3.48)**	**2.09 (1.47-2.97)**	**2.09 (1.41-3.10)**
Other rheumatoid arthritis	**2.14 (1.92-2.38)**	**1.74 (1.56-1.93)**	**1.49 (1.34-1.66)**	**1.45 (1.29-1.64)**
Psoriatic and enteropathic arthropathy	**2.95 (1.94-4.48)**	**2.79 (1.83-4.23)**	**1.99 (1.31-3.02)**	**1.75 (1.06-2.91)**
Polyarteritis nodosa	**3.22 (1.54-6.77)**	**2.74 (1.30-5.74)**	2.09 (1.00-4.39)	**2.40 (1.08-5.34)**
Systemic lupus erythematosus	**1.87 (1.39-2.50)**	**2.10 (1.57-2.82)**	**1.77 (1.32-2.37)**	**1.64 (1.19-2.28)**
Dermatopolymyositis	1.10 (0.41-2.93)	1.06 (0.40-2.83)	1.10 (0.41-2.94)	0.36 (0.05-2.58)
Systemic sclerosis	**3.28 (2.14-5.03)**	**2.62 (1.71-4.01)**	**2.47 (1.61-3.79)**	**2.48 (1.54-4.00)**
Ankylosing spondylitis	**1.95 (1.44-2.65)**	**1.74 (1.29-2.36)**	**1.53 (1.13-2.08)**	**1.57 (1.14-2.18)**
Paget's disease	0.83 (0.45-1.54)	1.00 (0.54-1.87)	0.92 (0.50-1.72)	0.98 (0.49-1.95)
Neurological diseases	1.18 (0.93-1.49)	**1.32 (1.05-1.67)**	**1.32 (1.05-1.68)**	**1.32 (1.02-1.72)**
Multiple sclerosis	1.19 (0.93-1.52)	**1.36 (1.06-1.73)**	**1.36 (1.07-1.74)**	**1.35 (1.02-1.77)**
Myasthenia gravis	1.02 (0.42-2.45)	0.95 (0.39-2.27)	0.90 (0.38-2.17)	1.06 (0.44-2.54)

Abbreviations: CI, confidence interval; DM, diabetes mellitus; GI, gastrointestinal; HR, hazard ratio; NA, not available.

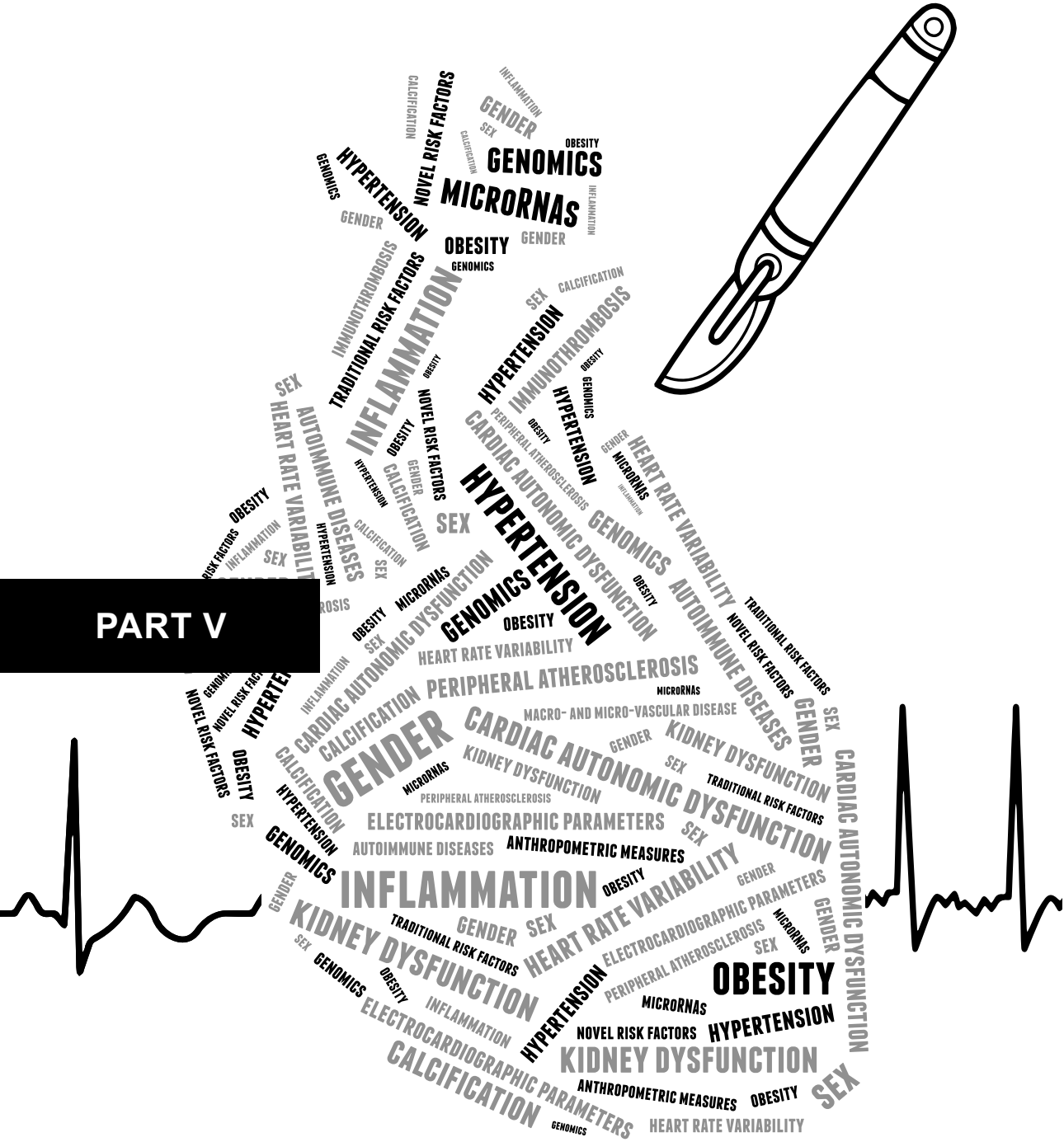
* Adjusted for age, and ethnicity.

† Adjusted for age, ethnicity, body mass index, total cholesterol, high-density lipoprotein cholesterol, smoking status, prevalent hypertension, history of type 2 diabetes mellitus, history of heart failure, and history of acute myocardial infarction.

‡ Cases dropped in complete-case analyses: n=23,059 (8.5%).

The associations with a $p<0.05$ are highlighted in **bold**.

PART V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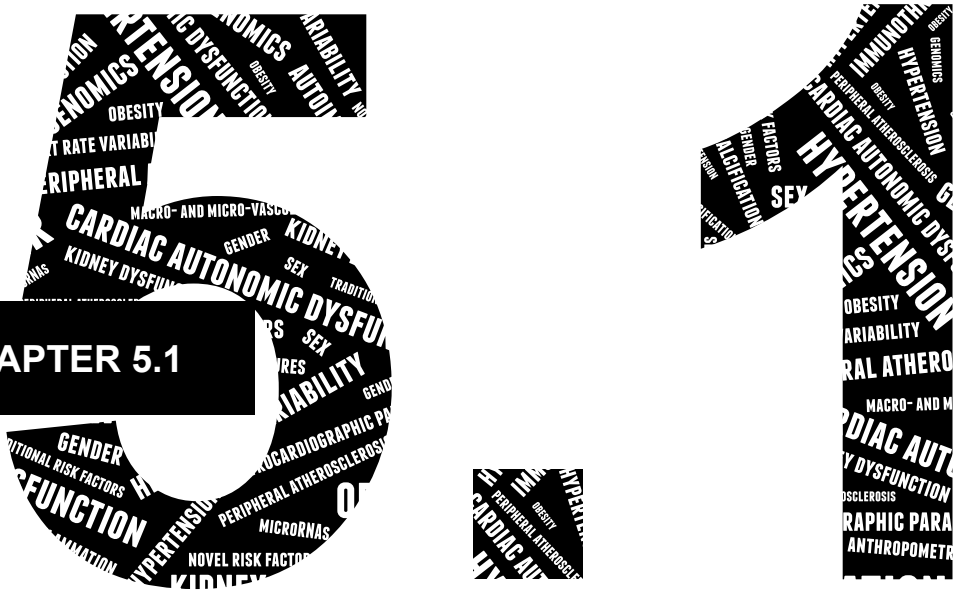
IMMUNOTHROMBOSIS
HEART RATE VARIABILITY
AUTOIMMUNE DISEASES
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
KIDNEY DYSFUNCTION
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC MEASURES
MACRO- AND MICRO-VASCULAR DISEASE

Traditional and novel risk factors for atrial fibrillation


CARDIAC AUTONOMIC DYSFUNCTION
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MACRO- AND MICRO-VASCULAR
KIDNEY DYSFUNCTION
NOVEL RISK FACTORS
MICRORNAS
ANTHROPOMETRIC PARAMETERS
IMMUNOTHROMBOSIS
GENOMICS

CHAPTER 5.1

Anthropometric measures and the risk of atrial fibrillation

Longitudinal anthropometric measures and risk of new-onset atrial fibrillation among community-dwelling men and women.

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ABSTRACT

Background
The sex-specific evolution of various anthropometric measures and the association of their longitudinal trajectories with new-onset atrial fibrillation (AF) are unknown.

Methods
Among 5,266 men and 7,218 women free of AF at baseline from the prospective population-based Rotterdam Study, each anthropometric measure was measured 1-5 times from 1989 to 2014. Anthropometric measures were standardized to obtain hazard ratios per 1 standard deviation (SD) increase to enable comparison. Joint models were used to assess the association between longitudinal trajectories of anthropometric measures with incident AF. Use of the joint models is a preferred method for simultaneous analyses of repeated measurements and survival data for conferring less biased estimates. Models were adjusted for traditional cardiovascular risk factors.

Results
Mean (SD) age was 63.9 (8.9) years for men and 64.9 (9.8) years for women. Median follow-up time was 10.5 years. Longitudinal evolution of weight, height, waist circumference, hip circumference and body mass index were associated with an increased risk of new-onset AF in both men and women. In joint models, larger height in men (hazard ratio (95% credible interval), per 1-SD, 1.27 (1.17-1.38)) and weight in women (1.24 (1.16-1.34)) showed the largest associations with AF. In joint models, waist-to-hip ratio was significantly associated with incident AF only in women (1.10 (1.03-1.18)).

Conclusions
Considering the entire longitudinal trajectories in joint models, anthropometric measures were positively associated with an increased risk for new-onset AF among men and women in the general population. Increase in measure of central obesity showed a stronger association with increased risk of AF onset among women compared with men.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia with significant health burden and socioeconomic impact.(1) Worldwide prevalence of AF was estimated around 46.3 million individuals in 2016.(2) Notably, recent evidence suggests differences in epidemiology and risk factors of AF between men and women.(3, 4) Thus, a better understanding of modifiable AF risk factors among men and women is imperative to improve personalized prevention of AF.

Obesity is a well-established risk factor for incident AF.(5, 6) The risk for development of AF is estimated to increase by 49% in obese subjects compared with non-obese subjects.(7, 8) Various anthropometric measures have been shown to be significantly associated with a higher risk of AF.(9-12) The available evidence, however, has primarily focused on a single baseline assessment of anthropometric measures in association with the risk of AF. Anthropometric measures tend to change over time. Therefore, solely focusing on baseline assessment of anthropometric measures fails to account for changes in anthropometric measurements over time that may affect the risk. Moreover, comprehensive assessment of the association between various anthropometric measures and incident AF among men and women is sparse.

Compared with the traditional time varying covariate Cox model, joint modelling can infer more accurate estimates in the association between an observed longitudinal measure of a marker and the hazard of an event by simultaneously modelling the profile of the marker and the time to event data.(13-15) Taking advantage of the joint modelling approach, we aimed to investigate the evolution of several anthropometric measures over time and furthermore to assess their associations with new-onset AF among men and women from the large population-based Rotterdam Study.

METHODS

Study design

The present study was conducted within the framework of the Rotterdam Study.(16) During 1990-1993, 7,983 participants of Ommoord district in the city of Rotterdam in The Netherlands aged ≥55 years were recruited in the first cohort (RS-I). In 2000, the cohort was extended with 3,011 participants who had become ≥55 years or had migrated into the research area (RS-II). In 2006, the cohort was again extended with 3,932 participants that were ≥45 years (RS-III). The overall response rate at baseline was 72%. Participants attended followed-up examinations every 3-4 years.

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians. Detailed description of the Rotterdam Study may be found in **Methods S1**.

Study population

For this study, we included participants from the first examination of the original cohort and the extended cohorts (n=14,926). The first measurement of anthropometrics for each participant was used as the baseline. Participants with prevalent AF at baseline (n=574), no informed consent for follow-up data collection (n=305), or no available data for anthropometric measures (n=1,563) were excluded. After exclusions, 12,484 participants were included.

Assessment of anthropometric measures

Height and weight were measured with the participants standing without shoes and heavy outer garments. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Waist circumference (WC) was measured at the level midway between the lower rib margin and the iliac crest. Hip circumference (HC) was measured as the distance around the largest part of hips. Waist-to-hip ratio (WHR) was calculated by dividing WC by HC. For every participant, at least 1 anthropometric measure was assessed between 1-5 times during the follow-up period. Therefore, the number of participants included for analysis for different anthropometric measures could slightly vary. Height and weight were measured at 5 visits in RS-I (RS-I-1 till RS-I-5), 3 visits in RS-II (RS-II-1 till RS-II-3) and 2 visits in RS-III (RS-III-1 and RS-III-2). WC and HC were measured at 4 visits in RS-I (RS-I-1, RS-I-3, RS-I-4 and RS-I-5), 3 visits in RS-II (RS-II-1 till RS-II-3), and 2 visits in RS-III (RS-III-1 and RS-III-2).

Assessment of atrial fibrillation

Methods on event adjudication for AF have been described in detail previously.(17) In short, to assess AF at baseline and follow-up examinations, a 10-second 12-lead electrocardiogram (ECG) was used with an ACTA Gnosis IV ECG recorder (Esaote Biomedical, Florence Italy). The ECG records were stored digitally and analyzed with the Modular ECG Analysis System (MEANS). Subsequently, 2 research physicians validated the diagnosis of AF. Additional follow-up data was obtained from medical files of participating general practitioners, hospitals, outpatient clinics, national registration of all hospital discharge diagnoses and follow-up examinations at the research center. The date of incident AF was defined as the date of the first occurrence of symptoms suggestive of AF with subsequent ECG verification obtained from the medical records. Participants were followed from the date of enrolment until the date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of cardiovascular risk factors

Methods for assessment of cardiovascular risk factors are detailed in **Methods S2**.

Statistical analyses

Baseline characteristics

Characteristics of the participants are presented as mean with standard deviation (SD) or number (n) with percentages as appropriate. Differences between men and women were examined by Student's T-test for continuous variables and Chi-Square test for categorical variables. Each anthropometric measure was standardized to obtain hazard ratios (HRs) per 1-SD increase to enable comparison.

Cox proportional hazards models

Traditional Cox proportional hazards regression analysis with delayed entry and age as a time scale was performed to investigate the relationship between anthropometric measures at baseline and incident AF.(18) HRs with 95% confidence intervals (CIs) were calculated to quantify the associations. We first used each anthropometric measure as a continuous variable to assess the HR for incident AF. For continuous exposure variables, an examination of the shape of relation with incident AF was performed using natural cubic splines. No deviation from linearity was found. Then, each anthropometric measure was categorized in deciles with the first decile as a reference to plot the graphical relationship, and p values for trend were derived. The proportional hazard assumptions were tested by Schoenfeld Residual tests and were found to be satisfied.

Joint models

Next, linear mixed-effects models were fitted for the changes in anthropometric measures over time and to account for the correlation of repeated measurements. Time was represented by age in years, and only age and sex were treated as fixed effects in all models (**Tables S1-S6**). Each model included random intercept and slope and an unstructured covariance matrix. Natural cubic splines of age with 2 or 3 knots were used to check the non-linear changes of each anthropometric measure over time. Likelihood-ratio tests were used to choose the best model. Then, we used final linear mixed-effect models to plot the evolution of each anthropometric measure with age among men and women.

Furthermore, to investigate the association between the longitudinal anthropometric measurements and the risk of incident AF, we used joint models for longitudinal and time to event data. The joint models were fitted in R using package “JMbayes” which fitted joint models under a Bayesian approach using Markov chain Monte Carlo (MCMC) algorithms,(19) and HRs with 95% CIs were calculated. We checked for an interaction between sex and each anthropometric measure by running the models in the total study population and adding an interaction term for sex in the joint models.

All analyses were performed in men and women separately. Survival models were adjusted for baseline age and cohort (model 1) and additionally for baseline cardiovascular risk factors including total and high-density lipoprotein (HDL) cholesterol, systolic blood pressure (SBP), history of diabetes mellitus (DM), history of coronary heart disease (CHD), history of heart failure (HF), smoking status, use of lipid lowering medication, antihypertensive medication and cardiac medication (model 2). In an alternative analysis, models were adjusted for all risk factors as time-varying covariates instead of baseline values. Correlation analyses were used to determine possible multicollinearity between the covariates. Missing values in covariates were imputed under the assumption of missing at random using multiple imputation.(20) For multiple imputation, all available data were used to generate 5 imputed data sets for traditional Cox proportional hazards regression analysis. For the joint model analysis, one randomly chosen dataset was used, as the “JMbayes” R package could not handle the pooled results.(21)

Sensitivity analyses

In sensitivity analysis, we stratified participants by BMI categories as BMI<25, 25≤BMI<30, and BMI≥30 kg/m^2. We also stratified participants by baseline age (at the age of 65 years). In addition, we performed the analysis only among participants of whom complete data were available.

Statistical significance was considered at two-tailed $p<0.05$. The analyses were done using R software (R 4.0.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

Baseline characteristics for men and women are shown in **Table 1**. Of 12,484 participants for the present study, 5,266 (42.2%) were men.

Atrial fibrillation incidence

During a median follow-up of 10.5 years (interquartile range (IQR), 6.4-15.4), 630 (12.0%) men and 692 (9.6%) women experienced new-onset AF (incidence rate of 11.2 per 1,000 person-years among men and 8.1 per 1,000 person-years among women).

Cox proportional hazards models

Table S7 shows HR with 95% CI for incident AF per 1-SD increase in baseline measures of anthropometric parameters among men and women based on traditional Cox models. In general, joint model analyses yielded slightly larger HRs for all anthropometric measures compared to traditional Cox regression model, with the exception of height. **Figure 1** presents the multivariable adjusted HRs (95% CIs) per decile of each anthropometric measure. As shown, positive associations with risk of new-onset AF were seen for weight, height, BMI, WC, and HC in men and women (p-for-trend<0.001). WHR showed a significant positive association with incident AF in women (p-for-trend=0.02), but not in men (p-for-trend=0.20). No non-linearity was observed in any of the associations.

Joint models

Figure 2 shows the evolution of anthropometric parameters among men and women. For weight, both men and women underwent a slight increase before the age of 62 years in men and 67 years in women, followed by rapid decrease in the rest of their lifetime. For height, a significant decrease was observed among men and women after 62 years. Significantly rapid increases in BMI were observed until 62 years of age after which BMI tended to remain stable among men and women. No significant interaction between sex and age was found in evolution of WC. Both men and women showed a similar pattern in evolution of WC as an increase before 67 years of age and a stable pattern thereafter. For HC, men showed a significant decrease until 62 years of age, whereas women showed a gradual decrease over the entire included age range with a slight fluctuation. WHR underwent a significant rapid increase among men before the age of 70, and a gradual increase among women before the age of 72 followed by a stable trend.

Table 2 shows HRs with 95% CIs for incident AF per 1-SD increase in anthropometric measures among men and women based on the joint models. The largest multivariate-adjusted HR (95% CI) was height in men: 1.30 (1.20-1.41), followed by 1.29 (1.19-1.40) for weight, 1.23 (1.13-1.32) for HC, 1.13 (1.04-1.22) for WC and 1.12 (1.03-1.21) for BMI. In women, the largest HR (95% CI) was 1.24 (1.16-1.34) for weight, and then 1.21 (1.12-1.30) for WC, 1.18 (1.10-1.27) for BMI, 1.17 (1.09-1.25) for HC and 1.12 (1.04-1.21) for height. Larger WHR was significantly associated with increased risk of AF in women (1.10 (1.03-1.18)), but not in men (0.98 (0.90-1.06)). The p values were significant for interaction between sex and height (p=0.006) and sex and WHR (p=0.049).

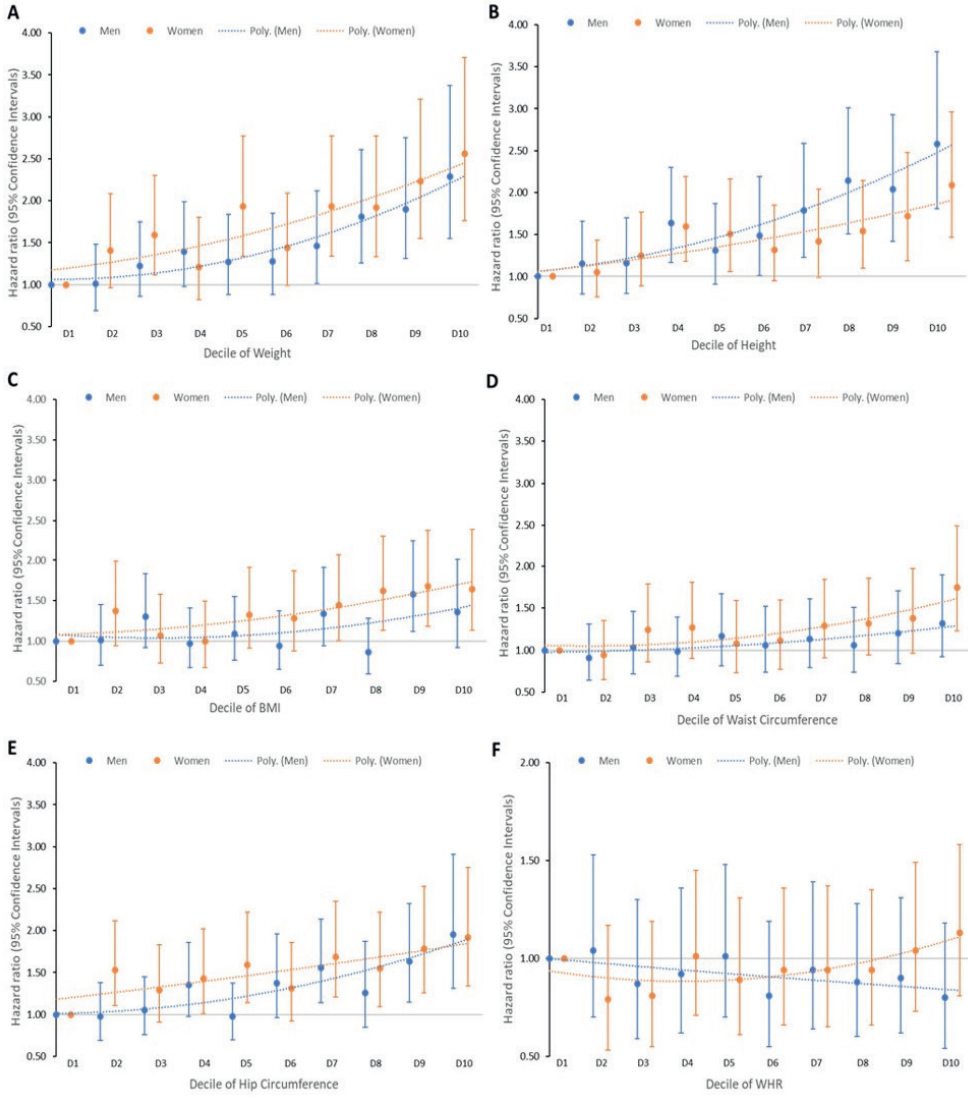
Table S8 shows results of joint models adjusted for all potential confounders as time-varying covariates which were not different from our main analyses for both men and women.

Table 1. Baseline characteristics of the study population

Baseline characteristics *	**Men n=5,266**	**Women n=7,218**	**p** ‡
Age, years	63.87 ± 8.86	64.94 ± 9.80	<0.001
Weight, kg	82.36 ± 12.82	71.56 ± 12.74	<0.001
Height, cm	175.86 ± 7.03	162.30 ± 6.68	<0.001
Body mass index, kg/m^2	26.59 ± 3.52	27.16 ± 4.50	<0.001
Waist circumference, cm	96.63 ± 10.45	88.71 ± 11.77	<0.001
Hip circumference, cm	101.60 ± 7.55	103.42 ± 9.52	<0.001
Waist-to-hip ratio	0.95 ± 0.07	0.86 ± 0.08	<0.001
Total cholesterol, mmol/L †	5.89 ± 1.18	6.36 ± 1.24	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.23 ± 0.33	1.49 ± 0.40	<0.001
Systolic blood pressure, mmHg	139.08 ± 20.69	137.61 ± 22.06	<0.001
Diastolic blood pressure, mmHg	78.64 ± 11.88	76.55 ± 11.70	<0.001
Smoking status			<0.001
Never, n (%)	748 (14.2)	3,270 (45.3)	
Former, n (%)	2,981 (56.6)	2,469 (34.2)	
Current, n (%)	1,532 (29.1)	1,487 (20.6)	
Antihypertensive medication, n (%)	1,448 (27.5)	2,129 (29.5)	0.02
Lipid lowering medication, n (%)	337 (6.4)	361 (5.3)	0.02
Cardiac medication, n (%)	611 (11.6)	650 (9.0)	<0.001
History of coronary heart disease, n (%)	558 (10.6)	209 (2.9)	<0.001
History of heart failure, n (%)	74 (1.4)	108 (1.5)	0.75
History of diabetes mellitus, n (%)	579 (11.0)	606 (8.4)	<0.001

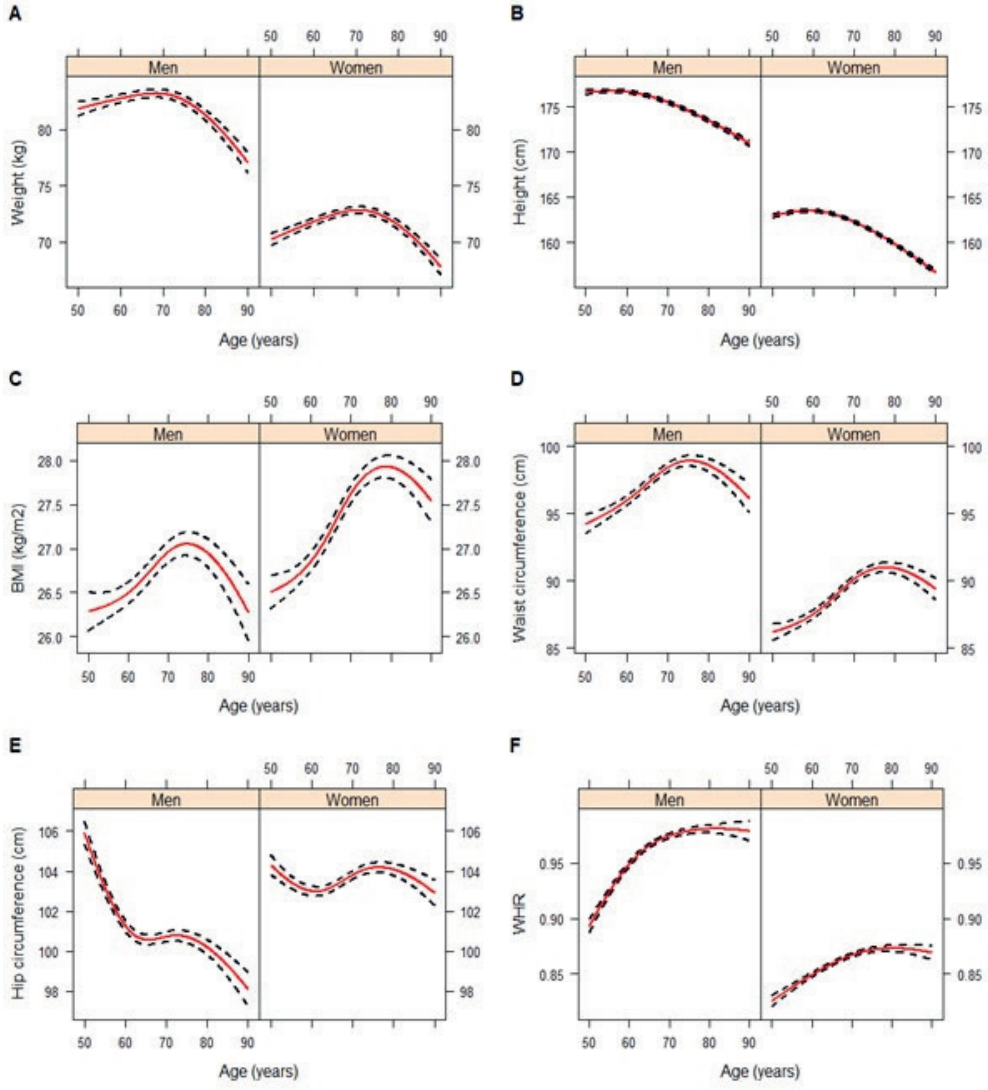
* Values are mean (standard deviation) for normally distributed continuous variables or median (interquartile range) for skewed continuous variables or number (percentages) for categorical variables.
† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.
‡ Statistical significance for continuous variables was tested using the Student's T-test and for categorical variables was tested using the Chi-Square test.

Figure 1. Multivariate-adjusted hazard ratios with 95% confidence intervals for incident atrial fibrillation by deciles of each anthropometric measure among men and women



Dashed lines fitted by quadratic polynomial. Corresponding decile values of anthropometric measures for men and women can be found in **Table S12**. Model adjusted for age, cohort, high-density lipoprotein, total cholesterol, smoking status, systolic blood pressure, use of antihypertensive medication, use of lipid lowering medication, use of cardiac medication, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

Figure 2. Evolution of anthropometric parameters among men and women



Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio.
Dashed lines represent the 95% confidence intervals.

Table 2. Association between longitudinal anthropometric measures with incident atrial fibrillation among men and women

Anthropometric measures	Men		Women	
	HR (95% CI)			
	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Weight ‡	**1.33 (1.22-1.43)**	**1.29 (1.19-1.40)**	**1.32 (1.23-1.41)**	**1.24 (1.16-1.34)**
Height ‡ §	**1.27 (1.17-1.38)**	**1.30 (1.20-1.41)**	**1.12 (1.04-1.21)**	**1.12 (1.04-1.21)**
BMI ‡	**1.18 (1.09-1.27)**	**1.12 (1.03-1.21)**	**1.27 (1.19-1.36)**	**1.18 (1.10-1.27)**
WC ‡	**1.19 (1.09-1.28)**	**1.13 (1.04-1.22)**	**1.26 (1.17-1.36)**	**1.21 (1.12-1.30)**
HC ‡	**1.25 (1.15-1.34)**	**1.23 (1.13-1.32)**	**1.25 (1.17-1.34)**	**1.17 (1.09-1.25)**
WHR ‡	1.06 (0.98-1.14)	0.98 (0.90-1.06)	**1.14 (1.06-1.22)**	**1.10 (1.03-1.18)**

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for age, and cohort.

† Adjusted for age, cohort, high-density lipoprotein, total cholesterol, smoking status, systolic blood pressure, use of Antihypertensive medication, use of lipid lowering medication, use of cardiac medication, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase in the corresponding anthropometric measure with the risk of new-onset atrial fibrillation.

§ $p<0.05$ for sex interaction.

The associations with a $p<0.05$ are highlighted in **bold**.

Sensitivity analyses

Sensitivity analyses in joint models that included only participants for whom complete data were available yielded similar results (**Table S9**). In addition, after stratification by BMI categories, the association between longitudinal BMI measures and incident AF was only significant among overweight individuals (25≤BMI<30) (HRs (95% CI) per 1-SD larger BMI: 1.43 (1.20-1.72) in men and 1.68 (1.36-2.09) in women) (**Table S10**). We also performed joint models among men and women in subgroups stratified by baseline age above or below 65 years (**Table S11**). Similar results were observed in women in 2 age subgroups. However, increased weight, BMI, WC, HC, and WHR in men showed larger associations with incident AF in younger group (<65 years old) than in older group (≥65 years old) (p-for-all interactions<0.05).

DISCUSSION

In this large prospective population-based cohort study, longitudinal trajectories of anthropometric measures were significantly associated with new-onset AF among both men and women. In joint models that allow for both individual-level and cohort-level trajectories, height in men and weight in women showed the strongest associations with new-onset AF. Increase in measure of central obesity showed a stronger association with incident AF among women as compared with men.

Our study extends previous evidence by assessing the longitudinal evolution of anthropometric measures among men and women and correlating the longitudinal trajectories with AF development during a long follow-up. Although several previous studies have investigated the associations between baseline measure of anthropometric parameters and AF, it is reasonable to imagine that changes of anthropometric parameters during follow-up may alter the impact of anthropometric parameters on AF development. Indeed, Feng et al. recently reported that obesity earlier in life and BMI changes exerted cumulative effects on AF development in the HUNT study.(12) Thus, studies using only baseline measures discard the information on variations of anthropometric parameters during follow-up, in particular during longer follow-up terms. To our knowledge, our study is the first to assess the longitudinal anthropometric measures and risks of incident AF. Taking into account the entire longitudinal trajectories of anthropometric measures in joint models, higher values of anthropometric measures over time were significantly associated with new-onset AF. After adjustment for cardiovascular risk factors, the associations did not change. These findings are in line with previous studies,(22, 23) and further support the hypothesis that traditional cardiovascular risk factors might not play a substantial role in the association between anthropometric measures and AF in both men and women. In fact, obesity is an established independent risk factor for cardiovascular conditions underlying AF.(5, 6) Increased BMI is strongly related to ventricular remodeling, impaired left ventricular relaxation, and elevated left ventricular diastolic filling pressure.(24) Obesity has been shown to be associated with hypoxia of the expanding adipose tissue and resulting in adipose fibrosis and the production of adipocytokines that further contributes to generating epicardial fat and myocardium damage.(25, 26) It seems likely that the combination of the aforementioned mechanisms represents the association between anthropometric measures and AF.

Notably, our results underline the significant sex differences in associations between height and AF. In this study, women had a lower mean height than men, and results of longitudinal models suggested that women had a more rapid shrinkage of height with aging compared to men. Thus, both may partly explain the lower risk of height for AF among women. Moreover, this study found that height serves as the predominant risk factor for new-onset AF in men. Several mechanisms have been

proposed to explain the relationship between height and AF. Left atrial volume is significantly associated with height even after adjustment for age and sex.(9, 27) As large left atrial size is highly associated with AF,(25) tall stature may contribute to AF development, at least partly, induced by large left atrial size. Besides, taller height has been suggested to be associated with reduced PR interval and QRS duration among healthy individuals.(28) These findings suggest an underlying pathway from height-induced electrophysiological dysfunction of the heart to AF occurrence. Our results showed a strong positive association between height and incident AF among both men and women. Although height is an unmodifiable risk factor, our study could underscore the potential value for AF prevention in an older population by screening AF among taller individuals.

We also found a robust sex difference in the association between WHR and AF. WHR is a specific measurement for body fat distribution that can be used to denote central fat accumulation.(29) To our knowledge, 3 previous studies have assessed sex differences in the relationship between anthropometric measures at baseline and incident AF.(10, 22, 23) Contrary to our results, significant associations between WHR and AF were previously found in men, but not in women.(10, 22) Of note, population differences between the current study and previous reports should be taken into consideration. First, our participants were older than the population in the aforementioned studies, and predisposing comorbidities with aging, may have a large impact on AF development. Men had higher AF risks due to unhealthy lifestyles and a higher prevalence of baseline cardiovascular disease than women.(3) This could have diluted the association between WHR and AF in men. Second, women in this study had larger values of baseline WHR than the previous studies. This implies more abdominal fat and thus a larger risk for AF among women in our population.(29) Moreover, WHR and central fat have previously been associated with poorer cardiac mechanisms including worse global longitudinal and diastolic strain rate of the heart, as well as ventricular concentric remodeling and this may increase AF susceptibility.(30, 31) Commonly, men are more prone to have a central fat distribution than women. However, postmenopausal women tend to accumulate more abdominal fat, because of the lack of estrogens.(32) With aging, there is a clear shift from primarily subcutaneous adipose tissue to central fat accumulation in both men and women.(33) Taken together, these data suggest that at older ages, women are more susceptible to the hazardous impact of central fat in AF development than men.

Strengths of our study include its prospective design, the long follow-up time with meticulous adjudication of AF events, and availability of a broad range of confounders and possible intermediate risk factors of AF. Particularly, our study is the first study that has examined sex differences in the longitudinal anthropometric measures and their associations with risk of new-onset AF. Compared to a single measurement, use of joint modeling and repeated anthropometric measurements

over time allows for assessment of varying effects of exposure and therefore provides more information for unbiased assessment of AF risks.(15) However, we acknowledge several limitations within this study. First, most of our participants were Caucasian and older adults, limiting the generalizability of our findings to other ethnicities and younger populations. Second, given the observational study design, and although, we adjusted for many potential confounders for AF, we cannot rule out the possibility of residual or unmeasured confounding. Third, because AF may be paroxysmal and asymptomatic, we might have underestimated the true number of AF cases in our study population. However, it is estimated that more than 75% of AF cases among the European population are permanent or persistent AF and most paroxysmal AF cases end as the permanent form.(34) In addition, the prevalence of AF in the Rotterdam Study is around 4% which is in line with the global estimate of AF prevalence.(35) Moreover, the possible underestimation of AF prevalence could not affect the direction of our results, as the resulting misclassification most likely happened independent of the exposures stats.

In conclusion, we found robust associations between evolution of anthropometric measures and risk of new-onset AF. Our results from a joint modelling approach highlight height as a predominant risk factor for new-onset AF in men and weight as the predominant risk factor in women. Moreover, increased central obesity showed a stronger association with incident AF among women as compared with men. The findings also underscore the importance of sex-specific approaches for screening and monitoring of anthropometric measures for AF prevention.

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SUPPLEMENTARY MATERIAL

Methods S1. Study design

The Rotterdam Study is a prospective population-based cohort study that aims to assess the occurrence and progression of risk factors for chronic diseases in middle-aged and elderly persons. During 1990-1993, all inhabitants of Ommoord district in the city of Rotterdam in The Netherlands aged ≥ 55 years were invited for the study. A total of 7,983 (78% of all invitees) agreed to participate (RS-I). In 2000, the cohort was extended with 3011 participants who had become ≥ 55 years or had migrated into the research area (RS-II). In 2006, the cohort was again extended with 3,932 participants that were ≥45 years (RS-III). In total, the Rotterdam Study comprised 14,926 subjects aged 45 years or over by the end of 2008. The overall response rate at baseline was 72%. Participants attended followed-up examinations every 3-6 years. Outcome data on morbidity and mortality were continuously collected through linkage with digital files from general practitioners in the study area.

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Methods S2. Assessment of cardiovascular risk factors

All participants responded to comprehensive computerized questionnaires at baseline about their current health status, medical history, medication, and lifestyle. They were interviewed at home by trained interviewers, and underwent more extensive clinical examination and laboratory assessments at the research center.

Serum total and high-density lipoprotein (HDL) cholesterol were measured with an automated enzymatic method. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressures were calculated as the mean of the 2 consecutive measurements. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or use of antihypertensive medication prescribed for hypertension.(17) Diabetes mellitus (DM) was defined as fasting serum glucose levels ≥126 mg/dL (7.0 mmol/L) (or non-fasting serum glucose levels ≥200 mg/dL (11.1 mmol/L) if fasting samples were unavailable) or the use of antidiabetic therapy. Smoking information derived from baseline questionnaires was categorized into current, former and never smokers. The assessment and definition

of prevalent coronary heart disease (CHD) and heart failure (HF) has been described in detail previously.(17) Information on lipid-lowering and cardiac medication use was derived from baseline questionnaires and pharmacy data. Cardiac medication was defined as use of digoxin, nitrates or antiarrhythmic drugs.(17)

Table S1. Longitudinal changes of weight in mixed effects model

	Value	SE	SD	2.5%	97.5%	p
Men						
Intercept	0.447	0.005	0.031	0.390	0.516	<0.001
ns(age, 3) 1	0.217	0.002	0.015	0.187	0.246	<0.001
ns(age, 3) 2	-0.154	0.020	0.074	-0.301	-0.025	<0.01
ns(age, 3) 3	-0.563	0.003	0.032	-0.629	-0.504	<0.001
Women						
Intercept	-0.172	0.006	0.031	-0.234	-0.116	<0.001
ns(age, 3) 1	0.301	0.004	0.018	0.263	0.333	<0.001
ns(age, 3) 2	-0.037	0.007	0.055	-0.152	0.065	0.52
ns(age, 3) 3	-0.699	0.004	0.036	-0.775	-0.633	<0.001

Abbreviations: SE, standard error; SD, standard deviation; ns, natural spline.
The knots of natural spline of age were 62.3 and 70.7 years for men; and 62.7 and 72.2 years for women.
P<0.05 for sex interaction.

Table S2. Longitudinal changes of height in mixed effects model

	Value	SE	SD	2.5%	97.5%	p
Men						
Intercept	0.951	0.012	0.032	0.884	0.998	<0.001
ns(age, 3) 1	-0.183	0.002	0.010	-0.204	-0.164	<0.001
ns(age, 3) 2	-0.440	0.008	0.046	-0.531	-0.361	<0.001
ns(age, 3) 3	-0.764	0.003	0.024	-0.807	-0.715	<0.001
Women						
Intercept	-0.496	0.013	0.034	-0.555	-0.443	<0.001
ns(age, 3) 1	-0.231	0.002	0.010	-0.247	-0.210	<0.001
ns(age, 3) 2	-0.458	0.003	0.027	-0.512	-0.408	<0.001
ns(age, 3) 3	-1.028	0.004	0.028	-1.086	-0.978	<0.001

Abbreviations: SE, standard error; SD, standard deviation; ns, natural spline.
The knots of natural spline of age were 62.3 and 70.7 years for men; and 62.7 and 72.2 years for women.
P<0.05 for sex interaction.

Table S3. Longitudinal changes of body mass index in mixed effects model

	Value	SE	SD	2.5%	97.5%	p
Men						
Intercept	-0.019	0.008	0.045	-0.095	0.071	0.72
ns(age, 3) 1	0.422	0.002	0.019	0.382	0.458	<0.001
ns(age, 3) 2	0.271	0.007	0.064	0.147	0.408	<0.001
ns(age, 3) 3	0.071	0.008	0.053	-0.034	0.174	0.21
Women						
Intercept	-0.221	0.006	0.035	-0.290	-0.157	<0.001
ns(age, 3) 1	0.520	0.002	0.016	0.490	0.551	<0.001
ns(age, 3) 2	0.391	0.006	0.055	0.292	0.505	<0.001
ns(age, 3) 3	0.112	0.003	0.037	0.048	0.189	<0.001

Abbreviations: SE, standard error; SD, standard deviation; ns, natural spline.
The knots of natural spline of age were 62.3 and 70.6 years for men; and 62.8 and 72.2 years for women.
P<0.05 for sex interaction.

Table S4. Longitudinal changes of waist circumference in mixed effects model

	Value	SE	SD	2.5%	97.5%	p
Men						
Intercept	0.001	0.008	0.034	-0.065	0.061	0.95
ns(age, 3) 1	-0.351	0.005	0.039	-0.424	-0.274	<0.001
ns(age, 3) 2	0.668	0.002	0.023	0.626	0.716	<0.001
ns(age, 3) 3	0.469	0.007	0.075	0.331	0.625	0.65
Women						
Intercept	-0.285	0.005	0.033	-0.352	-0.226	<0.001
ns(age, 3) 1	0.601	0.004	0.023	0.556	0.652	<0.001
ns(age, 3) 2	0.310	0.010	0.071	0.161	0.442	<0.001
ns(age, 3) 3	-0.026	0.007	0.049	-0.128	0.060	0.60

Abbreviations: SE, standard error; SD, standard deviation; ns, natural spline.
The knots of natural spline of age were 62.0 and 70.7 years for men; and 62.4 and 72.2 years for women.
No significant sex interaction.

Table S5. Longitudinal changes of hip circumference in mixed effects model

	Value	SE	SD	2.5%	97.5%	p
Men						
Intercept	0.770	0.005	0.042	0.686	0.847	<0.001
ns(age, 3) 1	-0.338	0.003	0.026	-0.392	-0.290	<0.001
ns(age, 3) 2	-1.950	0.009	0.089	-2.123	-1.758	<0.001
ns(age, 3) 3	-0.767	0.009	0.059	-0.886	-0.655	<0.001
Women						
Intercept	0.238	0.006	0.037	0.174	0.317	<0.001
ns(age, 3) 1	0.242	0.005	0.024	0.192	0.287	<0.001
ns(age, 3) 2	-0.497	0.022	0.100	-0.706	-0.316	<0.001
ns(age, 3) 3	-0.178	0.006	0.053	-0.286	-0.081	<0.001

Abbreviations: SE, standard error; SD, standard deviation; ns, natural spline.
The knots of natural spline of age were 62.0 and 70.7 years for men; and 62.4 and 72.2 years for women.
P<0.05 for sex interaction.

Table S6. Longitudinal changes of waist-to-hip-ratio in mixed effects model

	Value	SE	SD	2.5%	97.5%	p
Men						
Intercept	-0.472	0.017	0.054	-0.583	-0.372	<0.001
ns(age, 3) 1	1.044	0.008	0.036	0.986	1.124	<0.001
ns(age, 3) 2	2.281	0.016	0.097	2.107	2.461	<0.001
ns(age, 3) 3	0.847	0.011	0.061	0.728	0.961	<0.001
Women						
Intercept	-0.865	0.005	0.037	-0.936	-0.789	<0.001
ns(age, 3) 1	0.558	0.002	0.022	0.516	0.599	<0.001
ns(age, 3) 2	0.855	0.006	0.071	0.713	0.994	<0.001
ns(age, 3) 3	0.181	0.004	0.047	0.089	0.272	<0.001

Abbreviations: SE, standard error; SD, standard deviation; ns, natural spline.
The knots of natural spline of age were 62.0 and 70.7 years for men; and 62.4 and 72.2 years for women.
P<0.05 for sex interaction.

Table S7. Association between baseline anthropometric measures with incident atrial fibrillation among men and women

Anthropometric measures	Men		Women	
	HR (95% CI)			
	Model 1 *	Model 2 †	Model 1 *	Model 2 †
Weight ‡	**1.35 (1.23-1.47)**	**1.29 (1.18-1.42)**	**1.34 (1.24-1.44)**	**1.24 (1.14-1.34)**
Height ‡	**1.28 (1.18-1.39)**	**1.31 (1.20-1.42)**	**1.16 (1.07-1.27)**	**1.16 (1.07-1.26)**
BMI ‡	**1.17 (1.07-1.28)**	**1.11 (1.00-1.21)**	**1.26 (1.17-1.36)**	**1.16 (1.07-1.26)**
WC ‡	**1.18 (1.09-1.28)**	**1.11 (1.02-1.22)**	**1.27 (1.17-1.37)**	**1.19 (1.09-1.29)**
HC ‡	**1.23 (1.13-1.35)**	**1.21 (1.10-1.33)**	**1.23 (1.14-1.33)**	**1.15 (1.06-1.24)**
WHR ‡	1.05 (0.96-1.14)	0.97 (0.89-1.08) §	**1.12 (1.05-1.20)**	**1.09 (1.01-1.17)** §

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for age, and cohort.

† Adjusted for age, cohort, high-density lipoprotein, total cholesterol, smoking status, systolic blood pressure, use of antihypertensive medication, use of lipid lowering medication, use of cardiac medication, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase in the corresponding baseline anthropometric measure with the risk of new-onset atrial fibrillation.

§ $p<0.05$ for sex interaction.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S8. Association between longitudinal anthropometric measures with incident atrial fibrillation among men and women, taking into account the cardiovascular risk factors as time-varying covariates

Anthropometric measures *	Men	Women
	HR (95% CI)	
Weight ‡	**1.37 (1.25-1.49)**	**1.21 (1.12-1.31)**
Height ‡	**1.36 (1.24-1.49)**	**1.15 (1.05-1.25)**
BMI ‡	**1.18 (1.09-1.28)**	**1.14 (1.04-1.24)**
WC ‡	**1.20 (1.10-1.32)**	**1.18 (1.08-1.29)**
HC ‡	**1.28 (1.18-1.39)**	**1.21 (1.11-1.31)**
WHR ‡	1.00 (0.91-1.09)	1.09 (1.00-1.20)

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for age, cohort, high-density lipoprotein, total cholesterol, smoking status, systolic blood pressure, use of antihypertensive medication, use of lipid lowering medication, use of cardiac medication, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase in the corresponding baseline anthropometric measure with the risk of new-onset atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S9. Association between longitudinal anthropometric measures with incident atrial fibrillation among men and women with non-imputed data

Anthropometric measures *	Men	Women
	HR (95% CI)	
Weight ‡	**1.34 (1.21-1.46)**	**1.30 (1.20-1.41)**
Height ‡	**1.46 (1.29-1.65)**	**1.19 (1.05-1.34)**
BMI ‡	**1.17 (1.06-1.29)**	**1.18 (1.10-1.27)**
WC ‡	**1.15 (1.04-1.27)**	**1.21 (1.11-1.32)**
HC ‡	**1.30 (1.17-1.42)**	**1.18 (1.09-1.26)**
WHR ‡	1.02 (0.91-1.15)	1.11 (0.99-1.20)

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for age, cohort, high-density lipoprotein, total cholesterol, smoking status, systolic blood pressure, use of antihypertensive medication, use of lipid lowering medication, use of cardiac medication, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase in the corresponding baseline anthropometric measure with the risk of new-onset atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S10. Association between longitudinal anthropometric measures over time with incident AF among men and women stratified by body mass

Anthropometric measures *	Men	Women
	HR (95% CI)	
Weight ‡		
BMI<25	**1.44 (1.21-1.72)**	**1.42 (1.15-1.74)**
25≤BMI<30	**1.55 (1.33-1.77)**	**1.55 (1.32-1.83)**
BMI≥30	**1.38 (1.11-1.72)**	**1.19 (1.02-1.39)**
Height ‡		
BMI<25	**1.34 (1.17-1.53)**	**1.15 (1.01-1.34)**
25≤BMI<30	**1.30 (1.15-1.46)**	**1.16 (1.03-1.30)**
BMI≥30	**1.46 (1.16-1.83)**	1.12 (0.97-1.30)
BMI ‡		
BMI<25	1.16 (0.92-1.46) §	1.24 (0.95-1.61) §
25≤BMI<30	**1.43 (1.20-1.72)** §	**1.68 (1.36-2.09)** §
BMI≥30	1.06 (0.82-1.37) §	1.16 (0.97-1.37) §
WC ‡		
BMI<25	1.19 (0.99-1.43)	**1.22 (1.01-1.49)**
25≤BMI<30	**1.23 (1.05-1.43)**	**1.17 (1.03-1.37)**
BMI≥30	1.26 (0.95-1.67)	**1.24 (1.05-1.47)**
HC ‡		
BMI<25	**1.32 (1.11-1.56)**	**1.39 (1.11-1.73)**
25≤BMI<30	**1.32 (1.17-1.49)**	**1.26 (1.07-1.47)**
BMI≥30	1.20 (0.97-1.50)	1.06 (0.93-1.20)
WHR ‡ §		
BMI<25	1.03 (0.90-1.20)	1.05 (0.90-1.21)
25≤BMI<30	0.92 (0.81-1.05)	1.03 (0.91-1.16)
BMI≥30	1.01 (0.78-1.31)	1.13 (0.98-1.29)

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for age, cohort, high-density lipoprotein, total cholesterol, smoking status, systolic blood pressure, use of antihypertensive medication, use of lipid lowering medication, use of cardiac medication, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase in the corresponding baseline anthropometric measure with the risk of new-onset atrial fibrillation.

§ $p<0.05$ for sex interaction.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S11. Association between longitudinal anthropometric measures with incident atrial fibrillation among men and women stratified by baseline age

Anthropometric measures *	Men		Women	
	HR (95% CI)			
	<65 years	≥65 years	<65 years	≥65 years
Weight ‡	**1.42 (1.28-1.58)** §	**1.15 (1.02-1.30)** §	**1.22 (1.09, 1.36)**	**1.26 (1.14, 1.39)**
Height ‡	**1.28 (1.14-1.43)**	**1.30 (1.16-1.45)**	1.11 (0.98, 1.26)	**1.14 (1.03, 1.26)**
BMI ‡	**1.26 (1.13-1.39)** §	0.99 (0.88-1.11) §	**1.19 (1.06, 1.32)**	**1.19 (1.09, 1.30)**
WC ‡	**1.33 (1.18-1.49)** §	0.97 (0.86-1.09) §	**1.20 (1.06, 1.36)**	**1.10 (1.03, 1.18)**
HC ‡	**1.37 (1.21-1.54)** §	1.11 (0.99-1.24) §	**1.19 (1.06, 1.34)**	**1.14 (1.04, 1.25)**
WHR ‡	1.13 (0.97-1.26) §	0.89 (0.80-1.00) §	1.11 (0.97, 1.26)	1.06 (0.99, 1.13)

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for age, cohort, high-density lipoprotein, total cholesterol, smoking status, systolic blood pressure, use of antihypertensive medication, use of lipid lowering medication, use of cardiac medication, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

‡ Hazard ratios represent 1-SD increase in the corresponding baseline anthropometric measure with the risk of new-onset atrial fibrillation.

§ $p<0.05$ for sex interaction.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S12. Decile values of baseline anthropometric measures among men and women

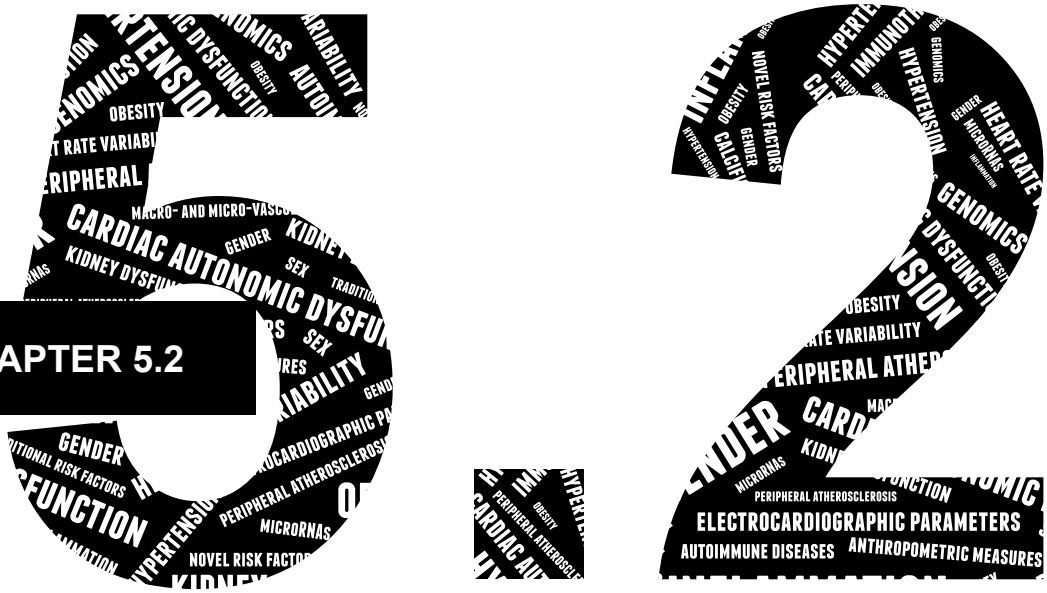
Anthropometric measures *	Decile values				
	D1 (reference)	D2	D3	D4	D5
Weight ‡					
Men	≤67.8	67.8-72.3	72.3-75.5	75.5-78.3	78.3-81.0
Women	≤57.2	57.2-61.3	61.3-64.5	64.5-67.4	67.4-70.1
Height ‡					
Men	≤167.0	167.0-170.0	170.0-172.0	172.0-174.0	174.0-176.0
Women	≤153.8	153.8-157.0	157.0-159.0	159.0-161.0	161.0-162.5
BMI ‡					
Men	≤22.5	22.5-23.8	23.8-24.7	24.7-25.5	25.5-26.3
Women	≤22.14	22.1-23.5	23.5-24.6	24.6-25.6	25.6-26.5
WC ‡					
Men	≤84.45	84.4-88.2	88.2-91.0	91.0-94.0	94.0-96.0
Women	≤74.50	74.5-79.0	79.0-82.0	82.0-85.0	85.0-87.6
HC ‡					
Men	≤93.0	93.0-95.9	95.9-97.5	97.5-99.2	99.2-101.0
Women	≤93.0	93.0-96.0	96.0-98.0	98.0-100.1	100.1-102.3
WHR ‡					
Men	≤0.86	0.86-0.89	0.89-0.92	0.92-0.93	0.93-0.95
Women	≤0.76	0.76-0.79	0.79-0.81	0.81-0.83	0.83-0.85

Abbreviations: D, Decile; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

Table S12. Decile values of baseline anthropometric measures among men and women (continued)

Anthropometric measures *	Decile values				
	D6	D7	D8	D9	D10
Weight ‡					
Men	81.0-84.2	84.2-87.5	87.5-91.8	91.8-98.5	98.5-153.2
Women	70.1-73.0	73.0-76.4	76.4-80.5	80.5-87.8	87.8-158.6
Height ‡					
Men	176.0-177.5	177.5-179.4	179.4-181.8	181.8-184.8	184.8-210.0
Women	162.5-164.0	164.0-165.8	165.8-168.0	168.0-170.6	170.6-191.5
BMI ‡					
Men	26.3-27.1	27.1-28.0	28.0-29.1	29.1-30.8	30.8-51.5
Women	26.5-27.7	27.7-28.9	28.9-30.5	30.5-32.9	32.9-56.9
WC ‡					
Men	96.0-98.8	98.8-101.0	101.0-104.6	104.6-109.6	109.6-198.6
Women	87.6-90.8	90.8-94.0	94.0-98.0	98.0-104.1	104.1-160.0
HC ‡					
Men	101.0-102.8	102.8-105.0	105.0-107.2	107.2-111.0	111.0-180.0
Women	102.3-104.5	104.5-107.0	107.0-110.0	110.0-115.5	115.5-161.8
WHR ‡					
Men	0.95-0.97	0.97-0.99	0.99-1.01	1.01-1.04	1.04-2.02
Women	0.85-0.87	0.87-0.89	0.89-0.92	0.92-0.96	0.96-1.54

Abbreviations: D, Decile; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.


CHAPTER 5.2
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
ELECTROCARDIOGRAPHIC PARAMETERS
AUTOIMMUNE DISEASES
ANTHROPOMETRIC MEASURES
HEART RATE VARIABILITY
NOVEL RISK FACTORS
MICRORNAS
OBESITY
GENDER
SEX
GENOMICS
HYPERTENSION

Trajectories of obesity-related measures and blood pressure and the risk of atrial fibrillation

Sex-specific anthropometric and blood pressure trajectories and risk of incident atrial fibrillation: the Rotterdam Study.

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ABSTRACT

Background
The sex-specific longitudinal trajectories of various obesity-related measures and blood pressure at the population level and the impact of these trajectories on new-onset atrial fibrillation (AF) are unclear.

Methods
Participants with ≥2 repeated assessments for various risk factors from the population-based Rotterdam Study were included. Latent class linear mixed models were fitted to identify the potential classes. Cox proportional hazards models were used to assess the association between risk factors' trajectories and the risk of new-onset AF, with the most favourable trajectory as reference.

Results
Among 7,367 participants (mean baseline age: 73 years, 58.8% women), after a median follow-up time of 8.9 years (interquartile range (IQR), 5.3-10.4), 769 (11.4%) participants developed new-onset AF. After adjustments for cardiovascular risk factors, persistent-increasing body mass index (BMI) trajectory carried a higher risk for AF (hazard ratio, 95% confidence interval: (1.39, 1.05-1.85) in men and (1.60; 1.19-2.15) in women), compared with the lower-and-stable BMI trajectory. Trajectories of elevated-and-stable waist circumference (WC) in women (1.53, 1.09-2.15) and elevated-and-stable hip circumference (HC) in men (1.83, 1.11-3.03) were associated with incident AF. For systolic blood pressure (SBP), the initially hypertensive trajectory carried the largest risk for AF among women (1.79, 1.21-2.65) and men (1.82, 1.13-2.95). Diastolic blood pressure trajectories were significantly associated with AF risk among women but not among men.

Conclusions
Longitudinal trajectories of weight, BMI, WC, HC, and SBP were associated with new-onset AF in both men and women. Diastolic blood pressure trajectories were additionally associated with AF in women. Our results highlight the importance of assessing long-term exposure to risk factors for AF prevention among men and women.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia.(1, 2) The prevalence of AF is increasing with advancing age,(1) and the lifetime risk of developing AF is estimated as 1 in 3 individuals at an index age of 55 years among the European ancestry.(3) Recent evidence also suggests sex differences in AF epidemiology, pathophysiology, and prognosis.(4-6)

The pathophysiological mechanisms underlying AF development are known to be complex. The mechanistic pathways involved in AF initiation and perpetuation include electrical and structural remodelling induced by chronic inflammation, hemodynamic changes, and nervous system dysregulations, which are intricately linked to obesity and high blood pressure.(7, 8) Obesity and hypertension are well-established risk factors for AF. The population-attributable risks of elevated body mass index (BMI) and hypertension for incident AF have been reported to be 18.6% and 14.2% for women and 18.0% and 13.7% for men, respectively.(9) Baseline single assessment of BMI or blood pressure has widely been associated with incident AF in several population-based epidemiological studies.(10-12) Also, the long-term changes and variations in weight and blood pressure, assessed using the mean of repeated measures, have been reported to associate with AF onset.(13, 14) However, the impact of various longitudinal patterns of obesity or hypertension at the population level on AF risks remains largely unknown. Assessing the longitudinal patterns of risk factors reflects the cumulative exposure of risk factors and may provide additional information for AF development. Previous population-based research on trajectories of BMI or blood pressure in association with AF risk has been performed on relatively young populations or has not addressed the potentially differential impacts among women and men.(15-17) As obesity and hypertension represent 2 major modifiable AF risk factors, a knowledge of their potentially sex-specific, longitudinal patterns over time may facilitate AF prevention strategies.

Using data from the large prospective population-based Rotterdam Study, we aimed at exploring sex-specific longitudinal trajectories of obesity-related measures and blood pressure at the population level and their associations with the risk of new-onset AF.

METHODS

Study design

The current study was performed within the framework of the Rotterdam Study.(18) The Rotterdam Study is a prospective population-based cohort study, which aims to assess the occurrence and determinants of age-related diseases in the general population. Between 1990 and 1993, all inhabitants of Ommoord district in the city of Rotterdam in The Netherlands aged ≥55 years were invited for the study. A total of 7,983 (78% of all invitees) agreed to participate (RS-I). In 2000, the cohort was extended with 3,011 participants who had become ≥55 years or had migrated into the research area (RS-II). The overall response rate at baseline was 72%. Participants of the Rotterdam Study have been re-visited as the study research center every 3-6 years.

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Study population

For the current study, we included participants with ≥2 repeated measurements for one or more assessed risk factors between 1989 and 2005. After recruitment, participants in RS-I were examined up to 4 times until 2005 (RS-I-1, RS-I-2, RS-I-3, and RS-I-4) and participants in RS-II were examined up to 2 times until 2005 (RS-II-1 and RS-II-2). From 7,983 individuals with ≥2 repeated measurements, participants with prevalent AF at baseline were excluded (n=637). Finally, 7,346 participants were included in the analyses. Since the availability of various risk factor measurements differs per participant, the number of participants included in the analyses for each risk factor varied from 7,189 for systolic blood pressure (SBP) to 5,540 for waist-to-hip ratio (WHR) (**Figure S1**).

Assessment of anthropometric measures and blood pressure

Height and weight were measured with the participants standing without shoes and heavy outer garments. Body mass index was calculated as weight divided by height squared (kg/m^2). Waist circumference (WC) was measured at the level midway

between the lower rib margin and the iliac crest. Hip circumference (HC) was measured as the distance around the largest part of the hips. WHR was calculated by dividing WC by HC. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. SBP and diastolic blood pressure (DBP) were calculated as the mean of the 2 consecutive measurements. Pulse pressure (PP) was calculated as the difference between SBP and DBP. Height, weight, and blood pressure were measured at 4 visits in RS-I (RS-I-1 till RS-I-4) and 2 visits in RS-II (RS-II-1 and RS-II-2). WC and HC were measured at 3 visits in RS-I (RS-I-1, RS-I-3, and RS-I-4) and 2 visits in RS-II (RS-II-1 and RS-II-2).

Assessment of atrial fibrillation

Methods on event adjudication for prevalent and incident AF have been described previously.(19) The definition of AF was in accordance with the guidelines of the European Society of Cardiology (ESC).(1) Ascertainment of AF at baseline and follow-up examinations in our study have been based on clinical information from the medical records for all participants of the Rotterdam Study. Within the Rotterdam Study, data on medical history and medication use are continuously being collected through multiple sources including a baseline home interview, a physical examination at our research center, the pharmacy prescription records, the Nationwide Medical Registry of all primary and secondary hospital discharge diagnosis, and screening of general practitioner's records. In addition, a resting 10-second 12-lead electrocardiogram (ECG) used with an ACTA Gnosis IV ECG recorder (Esaote Biomedical, Florence, Italy) is obtained from all participants at every visit of the Rotterdam Study to verify AF. The ECG records were stored digitally and analyzed with the Modular ECG Analysis System (MEANS). Subsequently, the AF outcomes are adjudicated independently by 2 research physicians. In case of disagreement, a senior cardiologist is consulted. The date of incident AF was defined as the date of the first occurrence of symptoms suggestive of AF with subsequent ECG verification obtained from the medical records. For the current study, we set the baseline for follow-up at the last examination of each participant for obesity-related and blood pressure measurements. Participants were followed until the date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first.

Assessment of cardiovascular risk factors

Methods for assessment of cardiovascular risk factors are detailed in **Methods S1**.

Statistical analyses

Baseline characteristics

All analyses were performed among men and women separately. Sex-specific means (standard deviations) and number (percentages) were calculated to describe the baseline characteristics of the study population.

Latent class trajectory models

Latent class trajectory analysis (with the 'lcmm' package in R)(20) was used to determine clusters of participants who followed similar trajectories of each risk factor over the given exposure period (from 1989 to 2005). The latent class trajectory model assumes that each participant belongs to one of several latent classes, and that the repeated measurements of participants in the same latent class follow a linear mixed-effects model. The trajectories are allowed to vary between latent classes, and the number of latent classes and their sizes in the population are estimated from the data. In our analyses, each assessed risk factor was considered as a dependent variable and time was represented by age in years. Each model included a random intercept. Age and cohorts were added in all models as independent variables. In addition, the use of blood pressure-lowering medication (as a dichotomous (yes/no) variable) was added in models to better discriminate the trajectories of SBP, DBP, and PP. Natural cubic splines of age with up to 4 knots in the fixed effect part were used to identify the potential non-linearity of assessed risk factors over time. The number of trajectory groups was limited to 5. The Bayesian information criterion was used to assess the fit of the model and choose the best model.(20) The latent class trajectory model calculates a posterior probability of membership in each latent class for each participant. Each participant is assigned to the class for which his/her posterior probability is the highest. To ensure that all obtained classes are of clinically meaningful size, we imposed the condition that each class should include at least 5% of the participants by ignoring the results of models with classes <5%.(21) Then, class-specific predictions were calculated to plot the class-specific trajectory for each risk factor in men and women separately.

Cox proportional hazards models

After determining the trajectories of each risk factor among men and women, Cox proportional hazards models were used to estimate the associations between a certain trajectory group of risk factors and the risk of new-onset AF. We set the baseline at the last examination of risk factors for each participant. Therefore, the follow-up period for the Cox proportional hazards analyses was from 2002 to 2014. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for the trajectory groups, with the potentially most favourable trajectory as the reference. Models were adjusted for baseline age and cohorts (model 1) and additionally for HDL cholesterol, total cholesterol, smoking status, use of lipid lowering medication, cardiac medication, and history of diabetes mellitus (DM), heart failure (HF), coronary heart disease (CHD), and hypertension (for SBP, DBP, and PP, we adjusted for antihypertensive medication instead of hypertension to avoid multicollinearity) (model 2). We additionally adjusted for height in models including weight and for BMI in models including SBP, DBP, and PP. The proportional hazards assumptions were tested using scaled Schoenfeld residuals and were satisfied.

Sensitivity analyses

In sensitivity analyses, we additionally performed competing risk analyses, taking death as a competing event into account. Moreover, we repeated all analyses among participants free of cancers, HF, and CHD at baseline to rule out the potential impact of chronic diseases. Also, age-stratified analysis (at 70 years) was conducted to assess potential effect modifications by baseline age. Finally, we constructed an additional model adjusting for the baseline measure of each risk factor in the corresponding Cox models to determine whether the trajectory for each risk factor provided additional predictive information for AF than a single baseline measurement.

The complete-case analyses were performed in the latent class linear mixed models. Missing values in covariates in the Cox model (each of all ≤5%) were imputed under the assumption of missing at random using multiple imputations with a fully conditional specification using package “mice”.(22) For multiple imputations, all available data were used to generate 5 imputed data sets. Statistical significance was considered at two-tailed $p<0.05$. Meanwhile, to account for potential multiple comparison for our 5 main exposures (BMI, WC, HC, DBP, and SBP), Bonferroni adjustment for multiple testing ($p<0.01$) was reported as well. The analyses were done using R software (R 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

Table 1 shows the baseline characteristics for 7,189 participants, including 2,967 men (41.3%) and 4,222 women (58.7%). Men were more often current smokers. Women were older and had lower weight, WC, and WHR, but higher BMI and HC, compared with men. Higher levels of total cholesterol and HDL cholesterol, but lower proportion of lipid lowering medication, were observed in women, in contrast to men. The prevalence of baseline CHD and DM was lower, but the prevalence of hypertension was higher among women.

Atrial fibrillation incidence

The exposure period spanned over 16 years (from 1989 to 2005) during which up to 4 repeated measurements of weight, BMI, SBP, DBP, and PP; and up to 3 of WC, HC, and WHR were available (**Table S1**). The follow-up period for incident AF started at the last examination of risk factors for each participant. During a median follow-up of 8.9 (interquartile range (IQR), 5.3-10.4) years, 357 (12.4%) men and 412 (10.4%) women experienced new-onset AF. The incidence rate was 16.5 per 1,000 person-years for men and 11.7 per 1,000 person-years for women.

Latent class trajectory models

Figures 1-5 and **Figures S1-S3** depict the sex-specific longitudinal patterns of each risk factor and the associated HRs with 95% CIs for new-onset AF. In men, 3 trajectories were identified for weight, BMI, WC, HC, WHR, DBP, and PP, and 5 trajectories were identified for SBP. In women, 2 trajectories were identified for WHR; 3 for weight, BMI, WC, HC, and PP; 4 for DBP; and 5 for SBP.

Cox proportional hazards models

Evolution of various body mass index and weight trajectories

As shown in **Figure 1**, after adjustments for cardiovascular risk factors (model 2), persistent-increasing BMI (class 2) carried higher AF risks (HR (95% CI): 1.38 (1.06-1.79) for men and 1.70 (1.35-2.13) for women). In addition, an increased risk of AF was observed in women with long-term exposure to obesity (class 3), albeit not statistically significant. In general, trends of weight trajectories were similar to the trends of BMI trajectories among men and women (**Figure S1**). No significant associations were observed between weight trajectories and incident AF among men. In women, persistently increasing weight conferred higher risks for incident AF (HR (95% CI): 1.83 (1.36-2.46)). Baseline characteristics of men and women in various trajectories of BMI and weight can be found in **Tables S2** and **S3** for BMI and **Tables S4** and **S5** for weight, respectively.

Evolution of various waist circumference and hip circumference trajectories

As shown in **Figure 2**, results from the multivariate Cox model (model 2) showed that the persistently increasing patterns of WC and HC (class 2) conferred higher risks for incident AF (HRs (95% CI): 1.35 (1.003-1.83) and 1.46 (1.01-2.12) in men, 1.38 (1.06-1.80) and 1.54 (1.05-2.26) in women). Compared with the reference group (class 1), elevated-and-stable WC pattern (class 3) was associated with incident AF in women (1.53 (1.09-2.15)). Elevated-and-stable HC pattern (class 3) was associated with incident AF in men (1.83 (1.11-3.03)) and in women (1.37 (1.01-1.85)). Baseline characteristics of men and women in various trajectories of WC and HC can be found in **Tables S6** and **S7** for WC and **Tables S8** and **S9** for HC, respectively.

Results of WHR trajectories and AF are shown in **Figure S2**.

Table 1. Baseline characteristics of the total study population stratified by sex

Baseline characteristics *	**Men n=2,967**	**Women n=4,222**	**p** ‡
Age, years	72.3 ± 7.5	73.7 ± 8.3	<0.001
Weight, kg	81.1 ± 12.1	71.2 ± 12.5	<0.001
Body mass index, kg/m^2	26.7 ± 3.5	27.6 ± 4.5	<0.001
Systolic blood pressure, mmHg	146.8 ± 20.3	149.4 ± 21.6	<0.001
Diastolic blood pressure, mmHg	79.6 ± 11.4	78.0 ± 11.1	<0.001
Pulse pressure, mmHg	67.2 ± 17.3	71.4 ± 18.5	<0.001
Waist circumference, cm	98.1 ± 10.1	89.9 ± 11.3	<0.001
Hip circumference, cm	100.7 ± 6.6	103.7 ± 9.4	<0.001
Waist-to-hip ratio	0.97 ± 0.07	0.87 ± 0.08	<0.001
Total cholesterol, mmol/L †	5.36 ± 0.95	5.90 ± 1.00	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.30 ± 0.34	1.55 ± 0.41	<0.001
Hypertension, n (%)	2,266 (76.4)	3,338 (79.1)	<0.010
Coronary heart disease, n (%)	489 (16.5)	227 (5.4)	<0.001
Heart failure, n (%)	160 (5.4)	185 (4.4)	0.060
Diabetes mellitus, n (%)	419 (14.1)	482 (11.4)	<0.001
Smoking status			<0.001
Never, n (%)	311 (10.5)	1,972 (46.7)	
Former, n (%)	2,003 (67.5)	1,619 (38.3)	
Current, n (%)	653 (22.0)	631 (14.9)	
Lipid lowering medication, n (%)	586 (19.8)	710 (16.8)	<0.010
Cardiac medication, n (%)	325 (11.0)	431 (10.2)	0.330
Antihypertensive medication, n (%)	1,260 (42.5)	1,888 (44.7)	0.060

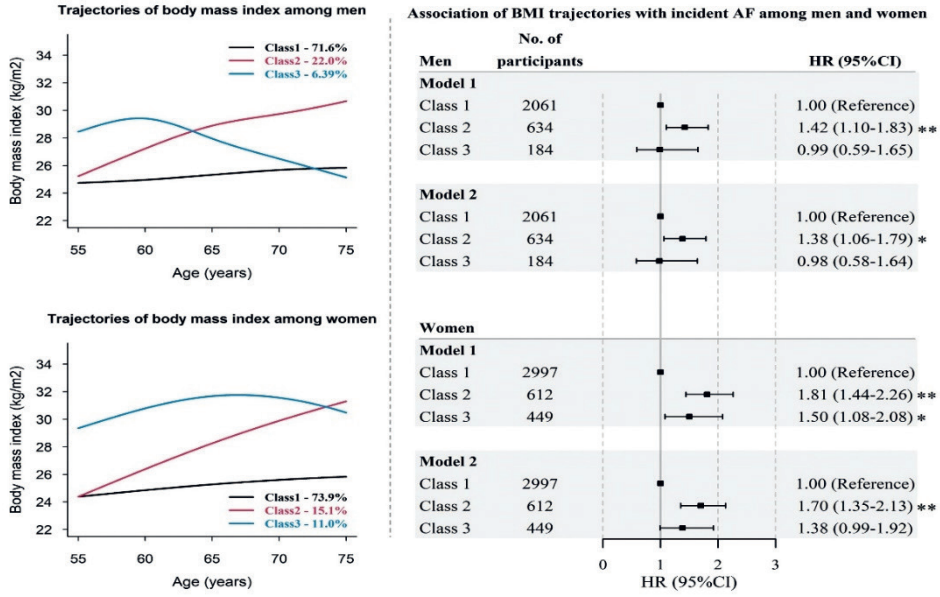
Since different number of participants were included in the analyses for various risk factors, Table 1 shows the baseline characteristics for men and women in the final analyses for SBP as an example. The baseline characteristics for men and women included in the analyses for other risk factors are presented in Tables **S14-S20**.

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

‡ Statistical significance for continuous variables was tested using the Student's T-test and for categorical variables was tested using the Chi-Square test.

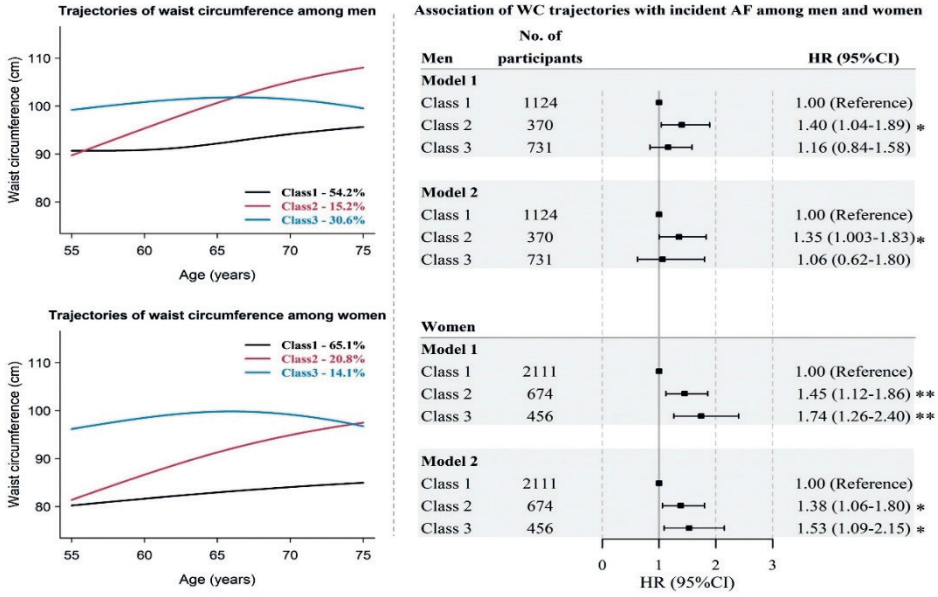
Figure 1. Various body mass index trajectories and their associated risks for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of HDL-cholesterol, total cholesterol, smoking, use of serum lipid reducing agents, use of cardiac medication and history of hypertension, diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable BMI trajectory; Class 2 refers to a "persistent-increasing" BMI trajectory; Class 3 refers to a "persistent-decreasing" BMI trajectory in men or an "elevated-and-decreasing" BMI trajectory in women.
* P <0.05. ** P <0.01.

Abbreviations: AF, atrial fibrillation; BMI, body mass index; HR, hazard ratio; CI, confidence interval.

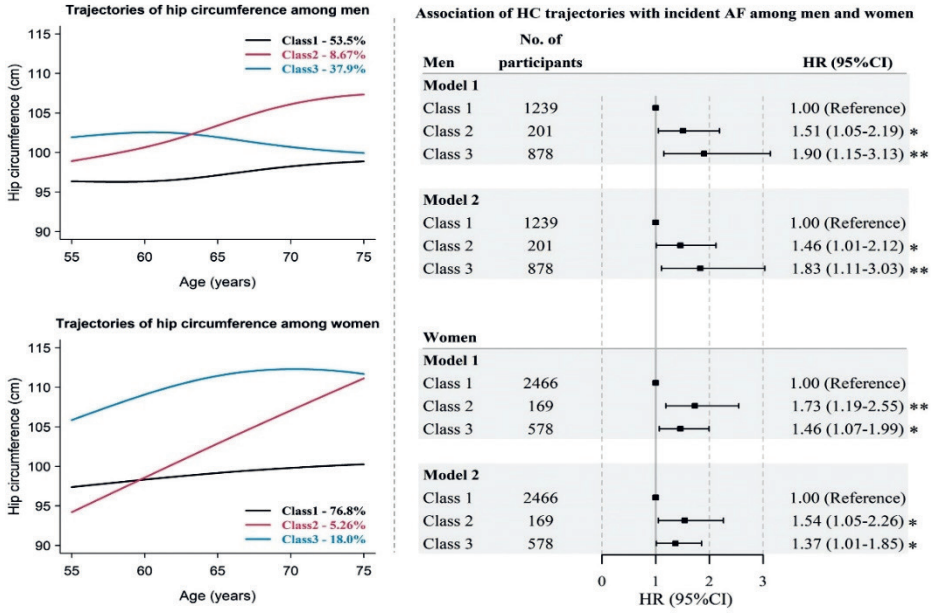
Figure 2. Various waist circumference trajectories and their associated risks for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of HDL-cholesterol, total cholesterol, smoking, use of serum lipid reducing agents, use of cardiac medication and history of hypertension, diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable WC trajectory; Class 2 refers to a "persistent-increasing" WC trajectory; Class 3 refers to an "elevated-and-stable" WC trajectory. * P <0.05. ** P <0.01.

Abbreviations: AF, atrial fibrillation; WC, waist circumference; HR, hazard ratio; CI, confidence interval.

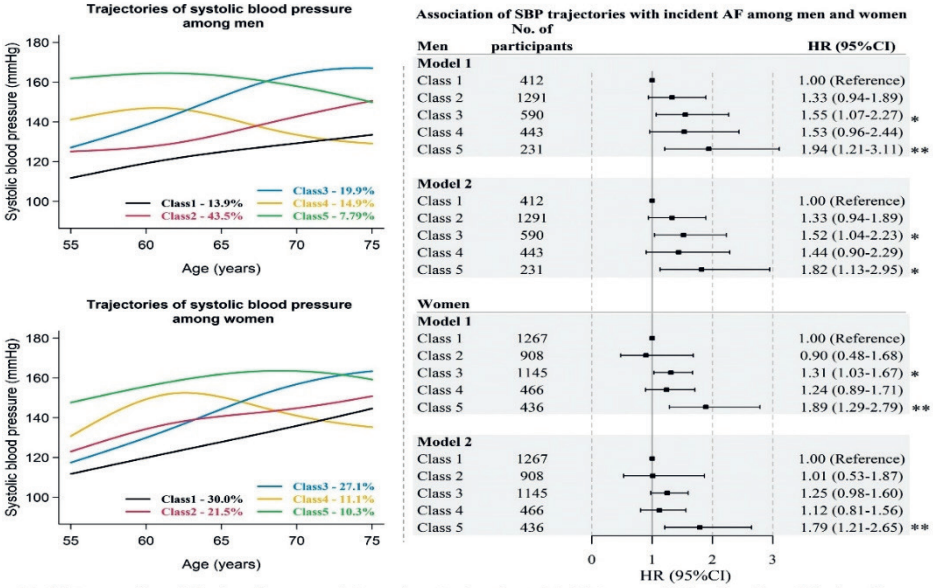
Figure 3. Various hip circumference trajectories and their associated risks for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of HDL-cholesterol, total cholesterol, smoking, use of serum lipid reducing agents, use of cardiac medication and history of hypertension, diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable HC trajectory; Class 2 refers to a "persistent-increasing" HC trajectory; Class 3 refers to an "elevated-and-stable" HC trajectory. * P <0.05. ** P <0.01.

Abbreviations: AF, atrial fibrillation; HC, hip circumference; HR, hazard ratio; CI, confidence interval.

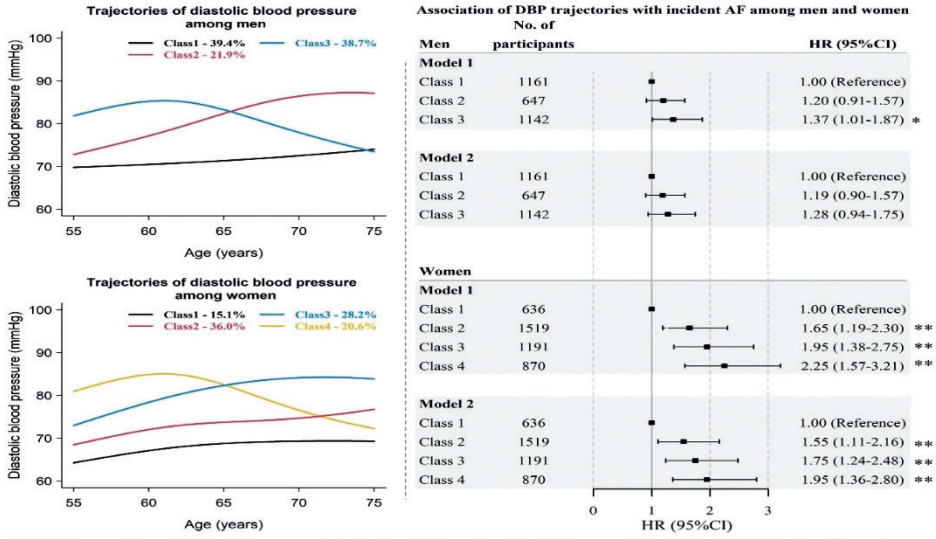
Figure 4. Various systolic blood pressure trajectories and their associated risks for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of BMI, HDL-cholesterol, total cholesterol, smoking, use of blood pressure lowing medication, use of serum lipid reducing agents, use of cardiac medication and history of diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable SBP trajectory; Class 2 refers to a "slightly persistent-increasing" SBP trajectory; Class 3 refers to a "steeply persistent-increasing" SBP trajectory; Class 4 refers to a "hypertensive-and-decreasing" SBP trajectory; Class 5 refers to a "severe hypertensive-and-decreasing" SBP trajectory. * P <0.05. ** P <0.01.

Abbreviations: AF, atrial fibrillation; SBP, systolic blood pressure; HR, hazard ratio; CI, confidence interval.

Figure 5. Various diastolic blood pressure trajectories and their associated risks for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of BMI, HDL-cholesterol, total cholesterol, smoking, use of blood pressure lowing medication, use of serum lipid reducing agents, use of cardiac medication and history of diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable DBP trajectory; Class 2 refers to a "persistent-increasing" DBP trajectory in men or a "slightly elevated" DBP trajectory in women; Class 3 refers to an "elevated-and-decreasing" DBP trajectory in men or a "persistent-increasing" DBP trajectory in women; Class 4 refers to a "hypertensive-and-decreasing" DBP trajectory in women. * P <0.05. ** P <0.01.

Abbreviations: AF, atrial fibrillation; DBP, diastolic blood pressure; HR, hazard ratio; CI, confidence interval.

Evolution of various blood pressure trajectories

Around 75% of the study population had a persistent increase in SBP after the age of 55 years, as depicted in **Figure 4** (classes 1-3). Interestingly, only the substantially increasing SBP pattern (class 3) was significantly associated with incident AF in men (1.52 (1.04-2.23)) and borderline associated with incident AF in women (1.25 (0.98-1.60)) after multivariate adjustments (model 2). Around 14.9% men and 11.1% women developed a fluctuating SBP pattern over time (Class 4). No significant associations with incident AF were found for this pattern. Participants within the persistent-hypertensive pattern (Class 5) experienced the largest risk of new-onset AF as 1.82 (1.13-2.95) in men and 1.79 (1.21-2.65) in women. Baseline characteristics of men and women in various trajectories of SBP can be found in **Tables S10** and **S11**.

For DBP patterns (**Figure 5**), in multivariate adjusted models (model 2), we did not observe significant associations between DBP patterns and incident AF in men. However, in women, the risk of new-onset AF increased gradually from the lowest to the highest DBP trajectory with the HRs (95% CI) of 1.55 (1.11-2.16), 1.75 (1.24-2.48), and 1.95 (1.36-2.80), respectively. Baseline characteristics of men and women in various trajectories of DBP and weight can be found in **Tables S12** and **S13**.

Figure S3 shows the associations between various PP trajectories and new-onset AF among men and women.

Sensitivity analyses

After taking into account death as competing event in the analyses, associations between various risk factors' trajectories and AF did not change (**Table S21**). Moreover, similar results were observed after excluding those with severe diseases at baseline (**Table S22**). In age-stratified analysis (**Table S23**), the associations between risk factor trajectories and AF risk were generally consistent among older participants (age >70 years old) and younger participants (age ≤70 years old), no significant age interaction was found (P for all interactions > 0.05). In the additional model adjusted for baseline values of each risk factor (**Table S24**), associations between various SBP and DBP trajectories with AF did not substantially change and remained significant. For anthropometric measures, the association between a persistent-increasing BMI trajectory (class 2) with AF in men was attenuated. Various HC and WC trajectories were no longer statistically significantly associated with AF among men and women, with exception of a significantly higher AF risk in elevated-and-stable HC trajectory in men (class 3; 1.76 (1.06-2.94)).

DISCUSSION

Our study showed that various longitudinal trajectories of obesity and blood pressure at the population level were associated with new-onset AF in men and women. The main findings can be summarized as: (i) increasing trajectories of weight and BMI conferred the highest risks for incident AF in both men and women. Weight loss among overweight individuals might reduce the risk for incident AF. (ii) Fat accumulation around the waist and hip over time was strongly associated with incident AF in men and women. The association between elevated-and-stable WC and incident AF was more pronounced in women. In contrast, the association between elevated-and-stable HC and AF was more evident in men. (iii) For both men and women, trajectories initially starting from hypertensive ranges carried the highest risk for incident AF. The increased AF risks were parallel to the initial values of DBP at the start of the trajectories in women. Various DBP trajectories in men did not confer higher risks for incident AF.

To our knowledge, our study is the first to comprehensively explore the sex-specific longitudinal patterns of obesity-related measures in association with incident AF at the population level. We demonstrated that increasing BMI over time conferred 40% and 70% increased risks of AF in men and women, respectively. A previous investigation among 10,559 men and women (combined) from the Atherosclerosis Risk in Communities (ARIC) study reported that among 5 BMI trajectories participants with high BMI during exposure period had a higher risk for AF.(16) Of note, the 5 BMI trajectories in the ARIC study were parallel, implying a small within-subject variation during the exposure period. We provide sex-specific evidence in the associations with AF and also identify a BMI trajectory with decreasing trend in our population. Participants in the current study had large exposure period to risk factors (up to 16 years) and tended to be older at baseline (mean baseline age >72 years). It is well known that body weight on average tends to decrease among the elderly after 60 years of age.(23) Weight loss by bariatric surgery has also been suggested to have a beneficial impact on incident AF.(24) Our results showed a steeper and more evident decline in weight and BMI in men than in women, and further confirmed the evidence that the risk of incident AF decreased among participants with a long-term decreasing BMI pattern, even if they had suffered from obesity at younger age, compared with those with persistently increasing BMI pattern.

Potential mechanisms of involvement of obesity in AF development are intricately linked to the impact of obesity on cardiac structure, inflammation, hemodynamics, and nervous system.(7, 25) Excessive fat is related to increases in blood volume and cardiac output but not in heart rate.26 Therefore, augmentation of cardiac output caused by obesity may result in left ventricular enlargement and hypertrophy.(26,

27) Meanwhile, obesity can trigger AF by inducing chronic systemic inflammation with cytokine release and activating the connective tissue growth factor and endothelin-1 which may disrupt atrial conduction and increase atrial fibrosis.(28-30)

We found that various trajectories of WC and HC were independently associated with new-onset AF in men and women. The persistently increasing patterns of WC or HC carried around 40% larger risks for incident AF in men and women. Previous studies have reported positive associations between baseline HC and WC with incident AF.(31, 32) Besides, providing novel evidence on longitudinal changes of HC and WC over time in association with new-onset AF among men and women, our study also showed that elevated WC from younger ages conferred higher risk for incident AF in women, whereas long-term exposure to elevated HC was associated with new-onset AF in men. Results suggested a sex-specific cumulative impact of WC and HC on AF occurrence. In fact, men are more predisposed to “apple” shape that is characterized by fat accumulation around the waist which is intricately regulated by the male reproductive hormone testosterone.(33) Thus, long-term exposure to excessive fat around the hip in men may reflect disturbances in sex hormone balance which have been considered as an independent risk factor for incident AF and may further interact with obesity to augment the risk.(33) In contrast, an adequate level of estrogen is vital to keep the “pear”-shaped fat distribution characterized by fat accumulation around the hip among women.33 Meanwhile, evidence suggests that estrogen exerts an important protective impact on AF in women by extending atrial conduction time, action potential duration, and the atrial effective refractory period.(34) Thus, long-term exposure to excessive fat around the waist in women implies the loss of estrogen and the loss of its protective impact on cardiovascular health. However, further studies to investigate the underlying mechanisms of such sex differences are warranted.

Our results showed that longitudinal SBP patterns were associated with incident AF among men and women, in line with the former results from the Tromsø Study.(17) Compared with the reference group, participants who had been affected by severe hypertension (>160 mmHg) at younger age, despite a decline thereafter (Class 5), experienced the highest risk for incident AF. Besides, a rapid increase in SBP with aging (class 3) was associated with incident AF. In contrast, a moderate growth of SBP (class 2) or an elevated-and-restricted SBP trajectory (Class 4) was not independently associated with incident AF. Taken together, these findings highlight the importance of long-term exposure to elevated SBP in the development of AF. Indeed, high SBP values and long-term burden of hypertension are widely known as risk factors for AF occurrence,(35, 36) which is also genetically confirmed by Mendelian randomization studies.(37) The underlying pathophysiological mechanisms of the relationship between high blood pressure and AF are not fully understood, but various factors could contribute to this link. First, high blood pressure accelerates the structural remodeling process of the left atrium and gives rise to

inflammatory changes, fibrosis, and atrial hypertrophy.(8) Second, long-term hypertension with increased afterload in the left ventricle contributes to ventricular muscle thickening and subsequent development of left ventricular hypertrophy.(8) Finally, hypertension may interact with autonomic nervous system dysregulations and further facilitate development of the arrhythmia.(38)

We also observed overt sex differences in the associated AF risk with the DBP trajectories. Significant associations with incident AF were found only in women but not in men. Several factors need to be taken into consideration while interpreting the observed associations for DBP. Firstly, our population comprised middle-aged and elderly participants. Unlike SBP, DBP tends to decrease with advancing age and after 55 years.(39) Indeed, most of our population showed low DBP values (<90 mmHg) throughout the whole exposure period and thus the elderly might be less susceptible to the lower mean DBP. Secondly, the reference DBP pattern showed stable DBP values of around 70 mmHg in men and around 65 mmHg in women. This could result in a smaller difference between the reference group and the other groups in men, leading to the attenuation of the associations. Thirdly, the AF-associated risk of hypertension in women may increase after menopause, as the protective impact of estrogens against cardiovascular disease vanishes.(40) Finally, a previous study among 863 old patients with hypertension has reported that hypertensive women exhibited higher prevalence of left ventricular hypertrophy, which is a significant risk factor for AF development.(41) Taken together, though further research regarding the potential mechanisms is warranted, our findings suggest a stronger association between various DBP trajectories and AF incident in women, compared with men. Moreover, the AF associated risk in women seems proportional to the initial DBP values at the start of the trajectories, regardless of subsequent variations. For example, women in Class 4 experienced the largest AF risks. This group was exposed to the highest DBP at the age of 55 years, followed by obvious decline after 65 years of age. This could suggest a lag time for the impact of high blood pressure on incident AF, highlighting the importance of therapeutic interventions that control blood pressure from younger ages to have an optimal impact on AF prevention and to avoid a high residual risk for AF development at older ages.

The strengths of our study include its prospective design, the long follow-up time, and adequate adjustment for a broad range of confounders and possible intermediate risk factors of AF. Besides, AF events were meticulously adjudicated, validated, and confirmed on ECG. Moreover, rather than investigating only single baseline measurement of risk factors, availability of repeated measurements over a long exposure period allowed for the assessment of the cumulative impact of risk factors on AF development. However, certain limitations merit consideration. First, the majority of our participants was older adults, with a minimum age of 55 years at the initial exposure period, and of European ancestry, limiting the generalizability of our findings to younger populations and other ethnicities. Second, because of the

observational study design, we cannot rule out the possibility of residual or unmeasured confounding. Third, our latent class models to identify the trajectories were modelled separately by sex. This might hamper drawing robust conclusions on whether the relationship between risk factor trajectories and AF differed by sex. Fourth, multiple comparisons might be a problem due to a set of exposures. However, after taking the multiple comparisons into consideration using a Bonferroni adjusted $p<0.01$, the results did not challenge our main conclusions. Finally, since AF may be paroxysmal and asymptomatic, we might have underestimated the true number of AF cases in our study population. However, the prevalence of AF in the Rotterdam Study is ~4% which is in line with the global estimate of AF prevalence.(1) Moreover, the possible underestimation of AF prevalence could not affect the direction of our results, as the resulting misclassification most likely happened independent of the exposures status.

In conclusion, we report significant associations between various longitudinal trajectories of obesity and blood pressure with new-onset AF among men and women from the general population, highlighting the importance of screening and monitoring of long-term exposure to risk factors for AF prevention. Potential sex differences were suggested in the association of WC, HC, and DBP trajectories with incident AF. Our results also emphasize the importance of preventing hypertension and implementing blood pressure-lowering interventions among hypertensive individuals as early as possible to optimize AF prevention.

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SUPPLEMENTARY MATERIAL

Chapter 5.2 Trajectories of obesity-related measures and blood pressure and the risk of atrial fibrillation

Methods S1. Assessment of cardiovascular risk factors

Table S1. Number of repeated measurements for each risk factor during exposure period

Table S2. Baseline characteristics for various trajectories of body mass index in men

Table S3. Baseline characteristics for various trajectories of body mass index in women

Table S4. Baseline characteristics for various trajectories of weight in men

Table S5. Baseline characteristics for various trajectories of weight in women

Table S6. Baseline characteristics for various trajectories of waist circumference in men

Table S7. Baseline characteristics for various trajectories of waist circumference in women

Table S8. Baseline characteristics for various trajectories of hip circumference in men

Table S9. Baseline characteristics for various trajectories of hip circumference in women

Table S10. Baseline characteristics for various trajectories of systolic blood pressure in men

Table S11. Baseline characteristics for various trajectories of systolic blood pressure in women

Table S12. Baseline characteristics for various trajectories of diastolic blood pressure in men

Figure S2. Various waist-to-hip ratio trajectories and their associated risks for new-onset atrial fibrillation among men and women

Figure S3. Various pulse pressure trajectories and their associated risks for new-onset atrial fibrillation among men and women

Methods S1. Assessment of cardiovascular risk factors

All participants responded to comprehensive computerized questionnaires at baseline about their current health status, medical history, medication use, and lifestyle. Participants were interviewed at home by trained interviewers and underwent more extensive clinical examination and laboratory assessments at the research center. Serum total and high-density lipoprotein (HDL) cholesterol were measured with an automated enzymatic method. Hypertension was defined as a SBP of ≥140 mmHg or a DBP ≥90 mmHg or use of antihypertensive medication prescribed for hypertension (ATC-codes C02, C03, C07, C08, and C09).(19) Diabetes mellitus (DM) was defined as fasting serum glucose levels ≥7.0 mmol/L (126 mg/dL) (or non-fasting serum glucose levels ≥11.1 mmol/L (200 mg/dL) fasting samples were unavailable) or the use of antidiabetic therapy (ATC-code A10). Smoking information derived from baseline questionnaires was categorized into current, former, and never smokers. Data on alcohol consumption were collected as part of a dietary interview and expressed in ethanol intake per day in grams. The assessment and definition of prevalent coronary heart disease (CHD) and heart failure (HF) has been described in detail previously.(19) Information on lipid lowering medication (ATC-code C10) and cardiac medication use was derived from baseline questionnaires and pharmacy data. Cardiac medication was defined as usage nitrates (ATC-code C01DA) or antiarrhythmic drugs (ATC-code C01B) and digoxin (ATC-code C01AA05).

Table S1. Number of repeated measurements for each risk factor during the exposure period

Risk factors *	Numbers or measurements			
	2	**3**	**4**	**Total**
Men (%)				
Weight	1,411 (48.9)	510 (17.7)	964 (33.4)	2,885
BMI	1,401 (48.7)	511 (17.7)	967 (33.6)	2,879
WC	1,306 (56.2)	1,019 (43.8)	-	2,325
HC	1,306 (56.3)	1,012 (43.7)	-	2,318
WHR	1,297 (56.1)	1,017 (43.9)	-	2,314
SBP	1,440 (48.5)	495 (16.7)	1,032 (34.8)	2,967
DBP	1,425 (48.3)	493 (16.7)	1,032 (35.0)	2,950
PP	1,426 (48.4)	493 (16.7)	1,027 (34.9)	2,946
Women (%)				
Weight	1,954 (48.0)	742 (18.2)	1,375 (33.8)	4,071
BMI	1,940 (47.8)	739 (18.2)	1,377 (33.9)	4,056
WC	1,777 (54.8)	1,463 (45.2)	-	3,240
HC	1,756 (54.7)	1,457 (45.3)	-	3,213
WHR	1,757 (54.5)	1,469 (45.5)	-	3,226
SBP	2,003 (47.4)	726 (17.2)	1,493 (35.4)	4,222
DBP	1,999 (47.4)	724 (17.2)	1,493 (35.4)	4,216
PP	2,004 (47.5)	721 (17.1)	1,492 (35.4)	4,217

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Values are number (percentages) for categorical variables.

Table S2. Baseline characteristics for various trajectories of body mass index in men

Baseline characteristics *	Trajectories of body mass index in men			
	Class 1 n=2,061	Class 2 n=634	Class 3 n=184	p
Age, years	73.33 (7.21)	69.27 (6.24)	69.45 (8.01)	<0.001
Weight, kg	77.10 (9.11)	91.05 (9.16)	85.77 (14.32)	<0.001
Body mass index, kg/m^2	25.46 (2.39)	29.97 (2.19)	28.15 (4.36)	<0.001
Systolic blood pressure, mmHg	147.64 (21.51)	148.29 (19.25)	142.75 (22.67)	<0.010
Diastolic blood pressure, mmHg	78.67 (11.50)	82.88 (10.70)	79.82 (13.30)	<0.001
Pulse pressure, mmHg	68.93 (17.98)	65.38 (16.18)	62.96 (18.21)	<0.001
Waist circumference, cm	94.93 (8.24)	105.94 (7.86)	101.26 (10.24)	<0.001
Hip circumference, cm	98.90 (5.49)	105.22 (5.25)	103.22 (7.89)	<0.001
Waist-to-hip ratio	0.96 (0.07)	1.01 (0.06)	0.98 (0.07)	<0.001
Total cholesterol, mmol/L †	5.38 (0.93)	5.40 (0.98)	5.13 (1.01)	<0.010
High-density lipoprotein cholesterol, mmol/L †	1.32 (0.35)	1.21 (0.28)	1.25 (0.35)	<0.001
Hypertension, n (%)	1556 (75.5)	510 (80.4)	137 (74.5)	0.030
Coronary heart disease, n (%)	350 (17.0)	95 (15.0)	30 (16.3)	0.490
Heart failure, n (%)	98 (4.8)	35 (5.5)	13 (7.1)	0.330
Diabetes mellitus, n (%)	242 (11.7)	102 (16.1)	50 (27.2)	<0.001
Smoking status				0.001
Never, n (%)	204 (9.9)	72 (11.4)	18 (9.8)	
Former, n (%)	1,363 (66.1)	461 (72.7)	123 (66.8)	
Current, n (%)	494 (24.0)	101 (15.9)	43 (23.4)	
Lipid lowering medication, n (%)	348 (16.9)	162 (25.6)	43 (23.4)	<0.001
Cardiac medication, n (%)	204 (9.9)	50 (7.9)	22 (12.0)	0.17

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S3. Baseline characteristics for various trajectories of body mass index in women

Baseline characteristics *	Trajectories of body mass index in women			
	Class 1 n=2,995	Class 2 n=612	Class 3 n=449	p
Age, years	73.52 (8.21)	75.28 (6.22)	70.15 (7.94)	<0.001
Weight, kg	66.30 (8.72)	81.76 (8.57)	82.92 (12.72)	<0.001
Body mass index, kg/m^2	25.64 (2.83)	32.07 (2.63)	31.98 (3.90)	<0.001
Systolic blood pressure, mmHg	149.65 (22.67)	153.83 (21.31)	149.87 (21.02)	<0.001
Diastolic blood pressure, mmHg	77.59 (11.00)	79.68 (11.34)	80.50 (10.83)	<0.001
Pulse pressure, mmHg	72.09 (19.38)	74.14 (17.75)	69.35 (18.49)	<0.001
Waist circumference, cm	85.84 (8.92)	99.49 (8.97)	99.10 (9.82)	<0.001
Hip circumference, cm	100.26 (6.76)	111.34 (8.16)	111.05 (8.94)	<0.001
Waist-to-hip ratio	0.86 (0.08)	0.90 (0.08)	0.89 (0.07)	<0.001
Total cholesterol, mmol/L †	5.96 (0.98)	5.86 (0.97)	5.80 (0.95)	0.001
High-density lipoprotein cholesterol, mmol/L †	1.59 (0.40)	1.46 (0.40)	1.44 (0.38)	<0.001
Hypertension, n (%)	2,263 (75.6)	547 (89.4)	378 (84.2)	<0.001
Coronary heart disease, n (%)	143 (4.8)	39 (6.4)	28 (6.2)	0.150
Heart failure, n (%)	93 (3.1)	41 (6.7)	25 (5.6)	<0.001
Diabetes mellitus, n (%)	273 (9.1)	84 (13.7)	93 (20.7)	<0.001
Smoking status				<0.001
Never, n (%)	1,444 (48.2)	274 (44.8)	189 (42.1)	
Former, n (%)	1,065 (35.6)	265 (43.3)	193 (43.0)	
Current, n (%)	486 (16.2)	73 (11.9)	67 (14.9)	
Lipid lowering medication, n (%)	470 (15.7)	113 (18.5)	81 (18.0)	0.140
Cardiac medication, n (%)	221 (7.4)	65 (10.6)	49 (10.9)	<0.010

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S4. Baseline characteristics for various trajectories of weight in men

Baseline characteristics *	Trajectories of weight in men			
	Class 1 n=1,790	Class 2 n=430	Class 3 n=665	p
Age, years	73.54 (7.17)	71.37 (6.74)	69.14 (6.93)	<0.001
Weight, kg	74.61 (7.43)	89.92 (7.23)	91.13 (8.67)	<0.001
Body mass index, kg/m^2	25.53 (2.74)	29.25 (2.98)	28.02 (3.06)	<0.001
Systolic blood pressure, mmHg	147.60 (21.96)	150.20 (19.26)	144.06 (19.37)	<0.001
Diastolic blood pressure, mmHg	78.51 (11.70)	82.93 (11.10)	80.55 (10.97)	<0.001
Pulse pressure, mmHg	69.07 (18.10)	67.28 (16.43)	63.49 (16.57)	<0.001
Waist circumference, cm	94.39 (8.21)	105.06 (8.57)	102.91 (8.40)	<0.001
Hip circumference, cm	98.13 (5.47)	104.97 (5.42)	104.50 (5.26)	<0.001
Waist-to-hip ratio	0.96 (0.07)	1.00 (0.07)	0.98 (0.07)	<0.001
Total cholesterol, mmol/L †	5.38 (0.95)	5.38 (0.91)	5.33 (0.97)	0.440
High-density lipoprotein cholesterol, mmol/L †	1.33 (0.35)	1.22 (0.29)	1.25 (0.32)	<0.001
Hypertension, n (%)	1,349 (75.4)	354 (82.3)	500 (75.2)	<0.010
Coronary heart disease, n (%)	307 (17.2)	71 (16.5)	99 (14.9)	0.410
Heart failure, n (%)	95 (5.3)	17 (4.0)	34 (5.1)	0.520
Diabetes mellitus, n (%)	230 (12.8)	58 (13.5)	113 (17.0)	0.030
Smoking status				<0.010
Never, n (%)	176 (9.8)	39 (9.1)	81 (12.2)	
Former, n (%)	1,182 (66.0)	324 (75.3)	444 (66.8)	
Current, n (%)	432 (24.1)	67 (15.6)	140 (21.1)	
Lipid lowering medication, n (%)	313 (17.5)	110 (25.6)	142 (21.4)	<0.001
Cardiac medication, n (%)	177 (9.9)	42 (9.8)	54 (8.1)	0.400

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S5. Baseline characteristics for various trajectories of weight in women

Baseline characteristics *	Trajectories of weight in women			
	Class 1 n=3,393	Class 2 n=269	Class 3 n=409	p
Age, years	73.68 (8.06)	74.56 (5.93)	70.56 (8.21)	<0.001
Weight, kg	67.72 (9.10)	87.55 (7.72)	82.47 (13.73)	<0.001
Body mass index, kg/m^2	26.47 (3.44)	33.20 (3.41)	31.12 (4.78)	<0.001
Systolic blood pressure, mmHg	149.99 (22.57)	154.45 (21.14)	149.80 (20.38)	<0.010
Diastolic blood pressure, mmHg	77.82 (11.10)	79.93 (10.63)	80.11 (10.91)	<0.001
Pulse pressure, mmHg	72.16 (19.19)	74.52 (17.95)	69.66 (18.03)	<0.010
Waist circumference, cm	87.37 (9.65)	102.25 (8.67)	97.79 (10.89)	<0.001
Hip circumference, cm	101.39 (7.35)	114.40 (7.78)	110.58 (9.50)	<0.001
Waist-to-hip ratio	0.86 (0.08)	0.90 (0.08)	0.89 (0.08)	<0.001
Total cholesterol, mmol/L †	5.96 (0.98)	5.81 (0.95)	5.81 (0.99)	<0.010
High-density lipoprotein cholesterol, mmol/L †	1.56 (0.40)	1.41 (0.33)	1.47 (0.42)	<0.001
Hypertension, n (%)	2618 (77.2)	242 (90.0)	344 (84.1)	<0.001
Coronary heart disease, n (%)	171 (5.0)	21 (7.8)	18 (4.4)	0.110
Heart failure, n (%)	124 (3.7)	19 (7.1)	20 (4.9)	0.020
Diabetes mellitus, n (%)	333 (9.8)	44 (16.4)	81 (19.8)	<0.001
Smoking status				<0.001
Never, n (%)	1,619 (47.7)	101 (37.5)	183 (44.7)	
Former, n (%)	1,237 (36.5)	134 (49.8)	166 (40.6)	
Current, n (%)	537 (15.8)	34 (12.6)	60 (14.7)	
Lipid lowering medication, n (%)	560 (16.5)	59 (21.9)	78 (19.1)	0.040
Cardiac medication, n (%)	274 (8.1)	31 (11.5)	40 (9.8)	0.090

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S6. Baseline characteristics for various trajectories of waist circumference in men

Baseline characteristics *	Trajectories of waist circumference in men			
	Class 1 n=1,224	Class 2 n=370	Class 3 n=731	p
Age, years	74.07 (6.56)	73.96 (5.29)	67.90 (7.20)	<0.001
Weight, kg	75.99 (8.78)	91.58 (9.70)	86.13 (10.69)	<0.001
Body mass index, kg/m^2	25.32 (2.51)	29.99 (2.78)	28.09 (3.03)	<0.001
Systolic blood pressure, mmHg	147.58 (20.78)	151.23 (19.79)	144.41 (19.32)	<0.001
Diastolic blood pressure, mmHg	78.33 (11.09)	80.58 (11.61)	81.75 (10.91)	<0.001
Pulse pressure, mmHg	69.26 (17.87)	70.60 (16.34)	62.63 (16.50)	<0.001
Waist circumference, cm	93.63 (7.58)	109.78 (5.88)	100.96 (8.18)	<0.001
Hip circumference, cm	98.34 (5.39)	105.87 (6.35)	102.77 (5.55)	<0.001
Waist-to-hip ratio	0.95 (0.07)	1.04 (0.06)	0.98 (0.06)	<0.001
Total cholesterol, mmol/L †	5.35 (0.93)	5.37 (0.97)	5.35 (0.97)	0.970
High-density lipoprotein cholesterol, mmol/L †	1.34 (0.36)	1.20 (0.27)	1.26 (0.32)	<0.001
Hypertension, n (%)	932 (76.1)	321 (86.8)	540 (73.9)	<0.001
Coronary heart disease, n (%)	222 (18.1)	81 (21.9)	80 (10.9)	<0.001
Heart failure, n (%)	66 (5.4)	25 (6.8)	27 (3.7)	0.070
Diabetes mellitus, n (%)	156 (12.7)	84 (22.7)	127 (17.4)	<0.001
Smoking status				<0.001
Never, n (%)	123 (10.0)	21 (5.7)	116 (15.9)	
Former, n (%)	838 (68.5)	297 (80.3)	477 (65.3)	
Current, n (%)	263 (21.5)	52 (14.1)	138 (18.9)	
Lipid lowering medication, n (%)	227 (18.5)	98 (26.5)	178 (24.4)	<0.001
Cardiac medication, n (%)	143 (11.7)	51 (13.8)	51 (7.0)	<0.001

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S7. Baseline characteristics for various trajectories of waist circumference in women

Baseline characteristics *	Trajectories of waist circumference in women			
	Class 1 n=2,110	Class 2 n=674	Class 3 n=456	p
Age, years	72.97 (7.87)	75.68 (6.63)	69.25 (7.04)	<0.001
Weight, kg	65.81 (8.56)	79.66 (9.82)	84.23 (11.89)	<0.001
Body mass index, kg/m^2	25.58 (3.02)	30.92 (3.54)	31.86 (4.24)	<0.001
Systolic blood pressure, mmHg	149.66 (22.22)	154.48 (22.30)	149.79 (20.57)	<0.001
Diastolic blood pressure, mmHg	77.88 (10.94)	78.90 (11.72)	80.20 (9.93)	<0.001
Pulse pressure, mmHg	71.79 (18.89)	75.60 (19.22)	69.60 (18.02)	<0.001
Waist circumference, cm	83.55 (7.18)	100.52 (6.43)	100.23 (8.59)	<0.001
Hip circumference, cm	100.31 (6.63)	109.15 (8.66)	111.36 (9.26)	<0.001
Waist-to-hip ratio	0.83 (0.06)	0.92 (0.08)	0.90 (0.07)	<0.001
Total cholesterol, mmol/L †	5.94 (0.97)	5.87 (1.01)	5.80 (0.94)	0.010
High-density lipoprotein cholesterol, mmol/L †	1.61 (0.41)	1.43 (0.36)	1.46 (0.37)	<0.001
Hypertension, n (%)	1,588 (75.3)	601 (89.2)	375 (82.2)	<0.001
Coronary heart disease, n (%)	107 (5.1)	49 (7.3)	18 (3.9)	0.030
Heart failure, n (%)	60 (2.8)	46 (6.8)	16 (3.5)	<0.001
Diabetes mellitus, n (%)	182 (8.6)	128 (19.0)	91 (20.0)	<0.001
Smoking status				0.020
Never, n (%)	979 (46.4)	312 (46.3)	180 (39.5)	
Former, n (%)	817 (38.7)	280 (41.5)	210 (46.1)	
Current, n (%)	314 (14.9)	82 (12.2)	66 (14.5)	
Lipid lowering medication, n (%)	398 (18.9)	120 (17.8)	104 (22.8)	0.090
Cardiac medication, n (%)	169 (8.0)	84 (12.5)	30 (6.6)	<0.001

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S8. Baseline characteristics for various trajectories of hip circumference in men

Baseline characteristics *	Trajectories of hip circumference in men			
	Class 1 n=1,239	Class 2 n=201	Class 3 n=878	p
Age, years	74.98 (6.23)	74.47 (5.67)	67.55 (6.34)	<0.001
Weight, kg	77.15 (9.31)	94.91 (8.63)	84.66 (11.05)	<0.001
Body mass index, kg/m^2	25.82 (2.83)	30.63 (2.83)	27.55 (3.12)	<0.001
Systolic blood pressure, mmHg	149.12 (21.15)	149.75 (19.30)	143.70 (18.89)	<0.001
Diastolic blood pressure, mmHg	78.63 (11.36)	80.33 (11.56)	81.26 (10.81)	<0.001
Pulse pressure, mmHg	70.49 (17.85)	69.42 (16.47)	62.45 (16.21)	<0.001
Waist circumference, cm	95.97 (8.87)	109.82 (7.33)	99.36 (9.35)	<0.001
Hip circumference, cm	98.51 (4.76)	110.03 (3.70)	101.91 (5.43)	<0.001
Waist-to-hip ratio	0.97 (0.07)	1.00 (0.06)	0.97 (0.07)	<0.001
Total cholesterol, mmol/L †	5.33 (0.93)	5.36 (0.96)	5.40 (0.97)	0.270
High-density lipoprotein cholesterol, mmol/L †	1.31 (0.35)	1.20 (0.30)	1.29 (0.33)	<0.001
Hypertension, n (%)	976 (78.8)	177 (88.1)	636 (72.4)	<0.001
Coronary heart disease, n (%)	245 (19.8)	34 (16.9)	100 (11.4)	<0.001
Heart failure, n (%)	77 (6.2)	13 (6.5)	25 (2.8)	<0.010
Diabetes mellitus, n (%)	190 (15.3)	41 (20.4)	133 (15.1)	0.160
Smoking status				<0.001
Never, n (%)	105 (8.5)	12 (6.0)	143 (16.3)	
Former, n (%)	882 (71.2)	158 (78.6)	563 (64.1)	
Current, n (%)	252 (20.3)	31 (15.4)	172 (19.6)	
Lipid lowering medication, n (%)	236 (19.0)	49 (24.4)	217 (24.7)	<0.010
Cardiac medication, n (%)	160 (12.9)	24 (11.9)	60 (6.8)	<0.001

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S9. Baseline characteristics for various trajectories of hip circumference in women

Baseline characteristics *	Trajectories of hip circumference in women			
	Class 1 n=2,466	Class 2 n=169	Class 3 n=578	p
Age, years	73.65 (7.72)	76.97 (5.43)	69.23 (7.16)	<0.001
Weight, kg	66.87 (8.92)	83.49 (8.03)	85.22 (9.94)	<0.001
Body mass index, kg/m^2	26.07 (3.23)	32.34 (3.18)	32.13 (3.79)	<0.001
Systolic blood pressure, mmHg	150.46 (22.42)	157.63 (22.97)	149.75 (20.18)	<0.001
Diastolic blood pressure, mmHg	77.81 (10.98)	79.46 (12.20)	80.58 (10.47)	<0.001
Pulse pressure, mmHg	72.65 (19.09)	78.17 (19.13)	69.22 (17.72)	<0.001
Waist circumference, cm	86.33 (9.40)	100.21 (9.23)	99.13 (9.83)	<0.001
Hip circumference, cm	100.31 (5.94)	115.51 (5.01)	113.46 (6.22)	<0.001
Waist-to-hip ratio	0.86 (0.08)	0.87 (0.07)	0.87 (0.08)	<0.010
Total cholesterol, mmol/L †	5.93 (0.98)	5.84 (0.99)	5.80 (0.96)	0.010
High-density lipoprotein cholesterol, mmol/L †	1.57 (0.40)	1.49 (0.40)	1.51 (0.39)	<0.001
Hypertension, n (%)	1,916 (77.7)	155 (91.7)	471 (81.5)	<0.001
Coronary heart disease, n (%)	129 (5.2)	14 (8.3)	27 (4.7)	0.180
Heart failure, n (%)	83 (3.4)	13 (7.7)	25 (4.3)	0.010
Diabetes mellitus, n (%)	280 (11.4)	33 (19.5)	87 (15.1)	<0.010
Smoking status				<0.010
Never, n (%)	1,142 (46.3)	82 (48.5)	238 (41.2)	
Former, n (%)	950 (38.5)	65 (38.5)	276 (47.8)	
Current, n (%)	374 (15.2)	22 (13.0)	64 (11.1)	
Lipid lowering medication, n (%)	481 (19.5)	34 (20.1)	104 (18.0)	0.680
Cardiac medication, n (%)	217 (8.8)	24 (14.2)	44 (7.6)	0.030

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table S10. Baseline characteristics for various trajectories of systolic blood pressure in men

Baseline characteristics *	Trajectories of systolic blood pressure in men					
	Class 1 n=412	Class 2 n=1,291	Class 3 n=590	Class 4 n=443	Class 5 n=231	p
Age, years	76.87 (6.70)	72.11 (7.24)	73.31 (6.46)	68.59 (7.68)	70.23 (7.49)	<0.001
Weight, kg	76.48 (10.35)	80.92 (11.76)	80.77 (11.36)	84.73 (12.51)	83.91 (14.11)	<0.001
Body mass index, kg/m^2	25.60 (3.14)	26.54 (3.28)	26.81 (3.30)	27.74 (3.77)	27.79 (3.96)	<0.001
Systolic blood pressure, mmHg	129.67 (13.95)	143.78 (16.86)	165.57 (17.64)	140.02 (14.48)	158.71 (21.35)	<0.001
Diastolic blood pressure, mmHg	71.92 (9.91)	78.77 (9.60)	84.62 (11.75)	80.29 (11.54)	83.18 (14.00)	<0.001
Pulse pressure, mmHg	57.74 (12.93)	65.01 (16.62)	80.95 (16.06)	59.74 (11.19)	75.53 (15.72)	<0.001
Waist circumference, cm	95.66 (9.40)	97.56 (9.95)	99.04 (9.96)	99.69 (10.24)	100.77 (11.16)	<0.001
Hip circumference, cm	98.82 (5.95)	100.74 (6.51)	100.80 (6.85)	102.07 (6.44)	102.06 (8.48)	<0.001
Waist-to-hip ratio	0.97 (0.07)	0.97 (0.07)	0.98 (0.08)	0.98 (0.07)	0.99 (0.07)	<0.001
Total cholesterol, mmol/L †	5.32 (0.92)	5.41 (0.96)	5.33 (0.94)	5.32 (0.95)	5.33 (0.99)	0.150
High-density lipoprotein cholesterol, mmol/L †	1.32 (0.36)	1.30 (0.35)	1.27 (0.36)	1.31 (0.33)	1.26 (0.37)	0.030
Hypertension, n (%)	179 (43.4)	908 (70.3)	582 (98.6)	373 (84.2)	222 (96.1)	<0.001
Coronary heart disease, n (%)	82 (19.9)	192 (14.9)	116 (19.7)	75 (16.9)	24 (10.4)	<0.010
Heart failure, n (%)	30 (7.3)	59 (4.6)	37 (6.3)	16 (3.6)	18 (7.8)	0.030
Diabetes mellitus, n (%)	41 (10.0)	148 (11.5)	102 (17.3)	72 (16.3)	54 (23.4)	<0.001
Smoking status						<0.001
Never, n (%)	30 (7.3)	137 (10.6)	47 (8.0)	68 (15.3)	28 (12.1)	
Former, n (%)	276 (67.0)	849 (65.8)	418 (70.8)	299 (67.5)	160 (69.3)	
Current, n (%)	106 (25.7)	305 (23.6)	125 (21.2)	76 (17.2)	43 (18.6)	
Lipid lowering medication, n (%)	47 (11.4)	224 (17.4)	148 (25.1)	123 (27.8)	52 (22.5)	<0.001
Cardiac medication, n (%)	47 (11.4)	127 (9.8)	75 (12.7)	65 (14.7)	25 (10.8)	0.060
Antihypertensive medication, n (%)	47 (11.4)	224 (17.4)	148 (25.1)	123 (27.8)	52 (22.5)	<0.001

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S11. Baseline characteristics for various trajectories of systolic blood pressure in women

Baseline characteristics *	Trajectories of systolic blood pressure in women					
	Class 1 n=1,267	Class 2 n=908	Class 3 n=1,145	Class 4 n=466	Class 5 n=436	p
Age, years	76.41 (7.12)	67.39 (6.82)	75.55 (7.33)	77.31 (8.90)	70.76 (8.23)	<0.001
Weight, kg	68.76 (11.84)	72.29 (12.33)	71.62 (12.42)	71.44 (13.18)	74.97 (13.42)	<0.001
Body mass index, kg/m^2	26.79 (4.26)	27.47 (4.37)	27.94 (4.47)	27.99 (4.63)	28.88 (4.69)	<0.001
Systolic blood pressure, mmHg	143.41 (20.21)	140.82 (16.26)	162.38 (18.23)	137.60 (19.51)	163.64 (21.50)	<0.001
Diastolic blood pressure, mmHg	75.36 (9.80)	77.98 (9.20)	80.95 (11.08)	73.35 (12.17)	83.14 (12.46)	<0.001
Pulse pressure, mmHg	68.05 (17.70)	62.84 (14.84)	81.43 (17.33)	64.25 (15.39)	80.51 (17.59)	<0.001
Waist circumference, cm	88.32 (11.06)	88.56 (11.07)	91.36 (11.31)	92.01 (11.30)	92.67 (11.93)	<0.001
Hip circumference, cm	102.21 (9.15)	104.16 (8.65)	103.94 (9.61)	103.75 (9.71)	105.94 (9.88)	<0.001
Waist-to-hip ratio	0.86 (0.09)	0.85 (0.07)	0.88 (0.08)	0.89 (0.09)	0.87 (0.07)	<0.001
Total cholesterol, mmol/L †	5.90 (0.99)	5.97 (0.98)	5.90 (0.96)	5.89 (1.06)	5.78 (0.95)	0.02
High-density lipoprotein cholesterol, mmol/L †	1.57 (0.41)	1.61 (0.41)	1.50 (0.38)	1.48 (0.41)	1.49 (0.42)	<0.001
Hypertension, n (%)	853 (67.3)	572 (63.0)	1,106 (96.6)	381 (81.8)	426 (97.7)	<0.001
Coronary heart disease, n (%)	70 (5.5)	22 (2.4)	59 (5.2)	48 (10.3)	28 (6.4)	<0.001
Heart failure, n (%)	59 (4.7)	8 (0.9)	54 (4.7)	41 (8.8)	23 (5.3)	<0.001
Diabetes mellitus, n (%)	102 (8.1)	86 (9.5)	145 (12.7)	60 (12.9)	86 (19.7)	<0.001
Smoking status						<0.001
Never, n (%)	619 (48.9)	350 (38.5)	569 (49.7)	239 (51.3)	195 (44.7)	
Former, n (%)	452 (35.7)	399 (43.9)	420 (36.7)	163 (35.0)	174 (39.9)	
Current, n (%)	196 (15.5)	159 (17.5)	156 (13.6)	64 (13.7)	67 (15.4)	
Lipid lowering medication, n (%)	153 (12.1)	165 (18.2)	189 (16.5)	67 (14.4)	117 (26.8)	<0.001
Cardiac medication, n (%)	137 (10.8)	40 (4.4)	120 (10.5)	94 (20.2)	48 (11.0)	<0.001
Antihypertensive medication, n (%)	399 (31.5)	238 (26.2)	630 (55.0)	298 (63.9)	323 (74.1)	<0.001

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S12. Baseline characteristics for various trajectories of diastolic blood pressure in men

Baseline characteristics *	Trajectories of diastolic blood pressure in men			
	Class 1 n=1,161	Class 2 n=647	Class 3 n=1,142	p
Age, years	74.80 (6.77)	73.13 (6.48)	69.54 (7.69)	<0.001
Weight, kg	78.03 (11.25)	82.10 (12.11)	83.20 (12.19)	<0.001
Body mass index, kg/m^2	25.92 (3.22)	26.94 (3.45)	27.42 (3.52)	<0.001
Systolic blood pressure, mmHg	142.91 (20.54)	158.44 (20.71)	144.55 (18.63)	<0.001
Diastolic blood pressure, mmHg	73.83 (9.02)	88.62 (8.90)	79.98 (10.64)	<0.001
Pulse pressure, mmHg	69.09 (17.93)	69.82 (17.69)	64.57 (16.97)	<0.001
Waist circumference, cm	96.27 (10.03)	98.83 (10.25)	99.49 (10.08)	<0.001
Hip circumference, cm	99.26 (6.31)	101.47 (6.87)	101.55 (6.77)	<0.001
Waist-to-hip ratio	0.97 (0.08)	0.97 (0.07)	0.98 (0.07)	0.003
Total cholesterol, mmol/L †	5.32 (0.97)	5.45 (0.92)	5.37 (0.95)	0.012
High-density lipoprotein cholesterol, mmol/L †	1.34 (0.37)	1.28 (0.35)	1.28 (0.32)	<0.001
Hypertension, n (%)	778 (67.0)	585 (90.4)	893 (78.2)	<0.001
Coronary heart disease, n (%)	233 (20.1)	85 (13.1)	173 (15.1)	<0.001
Heart failure, n (%)	69 (5.9)	32 (4.9)	59 (5.2)	0.593
Diabetes mellitus, n (%)	152 (13.1)	80 (12.4)	176 (15.4)	0.129
Smoking status				<0.001
Never, n (%)	76 (6.5)	67 (10.4)	167 (14.6)	
Former, n (%)	783 (67.4)	459 (70.9)	757 (66.3)	
Current, n (%)	302 (26.0)	121 (18.7)	218 (19.1)	
Lipid lowering medication, n (%)	196 (16.9)	111 (17.2)	269 (23.6)	<0.001
Cardiac medication, n (%)	131 (11.3)	55 (8.5)	119 (10.4)	0.175
Antihypertensive medication, n (%)	587 (51.4)	287 (44.4)	385 (33.2)	<0.001

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S13. Baseline characteristics for various trajectories of diastolic blood pressure in women

Baseline characteristics *	Trajectories of diastolic blood pressure in women				
	Class 1 n=636	Class 2 n=1,519	Class 3 n=1,191	Class 4 n=870	p
Age, years	78.23 (7.35)	73.96 (7.97)	72.13 (7.72)	72.63 (9.23)	<0.001
Weight, kg	66.36 (11.08)	69.91 (11.75)	72.92 (12.77)	74.43 (12.99)	<0.001
Body mass index, kg/m^2	26.09 (3.91)	27.10 (4.32)	28.14 (4.59)	28.72 (4.46)	<0.001
Systolic blood pressure, mmHg	139.55 (20.36)	148.83 (22.47)	156.44 (21.35)	150.35 (20.56)	<0.001
Diastolic blood pressure, mmHg	66.95 (7.30)	77.17 (8.75)	85.17 (8.58)	77.50 (11.18)	<0.001
Pulse pressure, mmHg	72.59 (19.52)	71.66 (18.97)	71.28 (18.59)	72.85 (19.22)	0.210
Waist circumference, cm	87.00 (11.21)	89.04 (10.99)	90.80 (11.67)	92.91 (11.09)	<0.001
Hip circumference, cm	100.95 (7.98)	102.72 (9.31)	104.63 (9.52)	105.60 (9.12)	<0.001
Waist-to-hip ratio	0.86 (0.09)	0.87 (0.08)	0.87 (0.08)	0.88 (0.08)	<0.001
Total cholesterol, mmol/L †	5.85 (0.94)	5.90 (1.02)	5.96 (0.98)	5.76 (0.94)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.57 (0.40)	1.55 (0.40)	1.57 (0.41)	1.48 (0.40)	<0.001
Hypertension, n (%)	384 (60.4)	1,106 (72.8)	1,035 (86.9)	810 (93.1)	<0.001
Coronary heart disease, n (%)	47 (7.4)	73 (4.8)	48 (4.0)	56 (6.4)	<0.010
Heart failure, n (%)	38 (6.0)	57 (3.8)	40 (3.4)	47 (5.4)	0.020
Diabetes mellitus, n (%)	64 (10.1)	164 (10.8)	112 (9.4)	135 (15.5)	<0.001
Smoking status					0.053
Never, n (%)	312 (49.1)	696 (45.8)	561 (47.1)	405 (46.6)	
Former, n (%)	225 (35.4)	562 (37.0)	460 (38.6)	354 (40.7)	
Current, n (%)	99 (15.6)	261 (17.2)	170 (14.3)	111 (12.8)	
Lipid lowering medication, n (%)	92 (14.5)	239 (15.7)	176 (14.8)	187 (21.5)	<0.001
Cardiac medication, n (%)	65 (10.2)	119 (7.8)	71 (6.0)	112 (12.9)	<0.001
Antihypertensive medication, n (%)	167 (26.3)	522 (34.4)	535 (44.9)	660 (75.9)	<0.001

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S14. Baseline characteristics for men and women in the analyses for weight

Baseline characteristics *	Men n=2,885	Women n=4,071	p
Age, years	72.20 (7.29)	73.43 (8.01)	<0.001
Weight, kg	80.70 (10.96)	70.51 (11.48)	<0.001
Body mass index, kg/m^2	26.66 (3.22)	27.38 (4.16)	<0.001
Systolic blood pressure, mmHg	147.26 (21.08)	150.29 (22.31)	<0.001
Diastolic blood pressure, mmHg	79.68 (11.57)	78.20 (11.07)	<0.001
Pulse pressure, mmHg	67.61 (17.71)	72.10 (19.02)	<0.001
Waist circumference, cm	97.89 (9.57)	89.62 (10.86)	<0.001
Hip circumference, cm	100.60 (6.19)	103.18 (8.61)	<0.001
Waist-to-hip ratio	0.97 (0.07)	0.87 (0.08)	<0.001
Total cholesterol, mmol/L †	5.37 (0.94)	5.93 (0.98)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.29 (0.34)	1.54 (0.40)	<0.001
Hypertension, n (%)	2,205 (76.4)	3,203 (78.7)	0.030
Coronary heart disease, n (%)	477 (16.5)	210 (5.2)	<0.001
Heart failure, n (%)	146 (5.1)	164 (4.0)	0.046
Diabetes mellitus, n (%)	403 (14.0)	453 (11.1)	<0.001
Smoking status			<0.001
Never, n (%)	294 (10.2)	1,911 (46.9)	
Former, n (%)	1,960 (67.9)	1,523 (37.4)	
Current, n (%)	631 (21.9)	637 (15.6)	
Lipid lowering medication, n (%)	563 (19.5)	662 (16.3)	<0.001
Cardiac medication, n (%)	285 (9.9)	341 (8.4)	0.030

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S15. Baseline characteristics for men and women in the analyses for body mass index

Baseline characteristics *	**Men n=2,879**	**Women n=4,056**	**p**
Age, years	72.19 (7.29)	73.42 (8.01)	<0.001
Weight, kg	80.73 (11.20)	70.47 (11.59)	<0.001
Body mass index, kg/m^2	26.62 (3.16)	27.31 (4.06)	<0.001
Systolic blood pressure, mmHg	147.47 (21.14)	150.30 (22.34)	<0.001
Diastolic blood pressure, mmHg	79.67 (11.58)	78.23 (11.08)	<0.001
Pulse pressure, mmHg	67.76 (17.72)	72.10 (19.08)	<0.001
Waist circumference, cm	97.76 (9.49)	89.37 (10.80)	<0.001
Hip circumference, cm	100.57 (6.23)	103.13 (8.71)	<0.001
Waist-to-hip ratio	0.97 (0.07)	0.87 (0.08)	<0.001
Total cholesterol, mmol/L †	5.37 (0.95)	5.93 (0.98)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.29 (0.34)	1.55 (0.40)	<0.001
Hypertension, n (%)	2,203 (76.5)	3,188 (78.6)	0.040
Coronary heart disease, n (%)	475 (16.5)	210 (5.2)	<0.001
Heart failure, n (%)	146 (5.1)	159 (3.9)	0.030
Diabetes mellitus, n (%)	394 (13.7)	450 (11.1)	<0.001
Smoking status			<0.001
Never, n (%)	294 (10.2)	1,907 (47.0)	
Former, n (%)	1,947 (67.6)	1,523 (37.5)	
Current, n (%)	638 (22.2)	626 (15.4)	
Lipid lowering medication, n (%)	553 (19.2)	664 (16.4)	<0.010
Cardiac medication, n (%)	276 (9.6)	335 (8.3)	0.060

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S16. Baseline characteristics for men and women in the analyses for waist circumference

Baseline characteristics *	**Men n=2,325**	**Women n=3,240**	**p**
Age, years	72.11 (7.18)	73.01 (7.74)	<0.001
Weight, kg	81.66 (11.42)	71.29 (12.05)	<0.001
Body mass index, kg/m^2	26.93 (3.27)	27.57 (4.31)	<0.001
Systolic blood pressure, mmHg	147.16 (20.29)	150.68 (22.09)	<0.001
Diastolic blood pressure, mmHg	79.76 (11.22)	78.42 (11.00)	<0.001
Pulse pressure, mmHg	67.39 (17.51)	72.27 (18.93)	<0.001
Waist circumference, cm	98.50 (9.56)	89.43 (10.82)	<0.001
Hip circumference, cm	100.93 (6.31)	103.70 (8.84)	<0.001
Waist-to-hip ratio	0.98 (0.07)	0.86 (0.08)	<0.001
Total cholesterol, mmol/L †	5.36 (0.95)	5.90 (0.97)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.29 (0.34)	1.55 (0.40)	<0.001
Hypertension, n (%)	1,793 (77.1)	2,564 (79.1)	0.080
Coronary heart disease, n (%)	383 (16.5)	174 (5.4)	<0.001
Heart failure, n (%)	118 (5.1)	122 (3.8)	0.020
Diabetes mellitus, n (%)	367 (15.8)	401 (12.4)	<0.001
Smoking status			<0.001
Never, n (%)	260 (11.2)	1,471 (45.4)	
Former, n (%)	1,612 (69.3)	1,307 (40.3)	
Current, n (%)	453 (19.5)	462 (14.3)	
Lipid lowering medication, n (%)	503 (21.6)	622 (19.2)	0.030
Cardiac medication, n (%)	245 (10.5)	283 (8.7)	0.03

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S17. Baseline characteristics for men and women in the analyses for hip circumference

Baseline characteristics *	**Men n=2,318**	**Women n=3,213**	**p**
Age, years	72.12 (7.18)	73.03 (7.76)	<0.001
Weight, kg	81.54 (11.33)	71.05 (11.83)	<0.001
Body mass index, kg/m^2	26.89 (3.26)	27.49 (4.22)	<0.001
Systolic blood pressure, mmHg	147.12 (20.33)	150.71 (22.12)	<0.001
Diastolic blood pressure, mmHg	79.77 (11.23)	78.39 (11.01)	<0.001
Pulse pressure, mmHg	67.35 (17.55)	72.32 (18.95)	<0.001
Waist circumference, cm	98.46 (9.72)	89.36 (10.95)	<0.001
Hip circumference, cm	100.80 (5.92)	103.48 (8.28)	<0.001
Waist-to-hip ratio	0.98 (0.07)	0.86 (0.08)	<0.001
Total cholesterol, mmol/L †	5.36 (0.95)	5.90 (0.98)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.29 (0.34)	1.55 (0.40)	<0.001
Hypertension, n (%)	1,789 (77.2)	2,542 (79.1)	0.09
Coronary heart disease, n (%)	379 (16.4)	170 (5.3)	<0.001
Heart failure, n (%)	115 (5.0)	121 (3.8)	0.04
Diabetes mellitus, n (%)	364 (15.7)	400 (12.4)	<0.001
Smoking status			<0.001
Never, n (%)	260 (11.2)	1,462 (45.5)	
Former, n (%)	1,603 (69.2)	1,291 (40.2)	
Current, n (%)	455 (19.6)	460 (14.3)	
Lipid lowering medication, n (%)	502 (21.7)	619 (19.3)	0.03
Cardiac medication, n (%)	244 (10.5)	285 (8.9)	0.04

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S18. Baseline characteristics for men and women in the analyses for waist-to-hip ratio

Baseline characteristics *	Men n=2,314	Women n=3,226	p
Age, years	72.10 (7.17)	72.91 (7.75)	<0.001
Weight, kg	81.84 (11.89)	71.75 (12.89)	<0.001
Body mass index, kg/m^2	27.00 (3.44)	27.71 (4.56)	<0.001
Systolic blood pressure, mmHg	147.30 (20.30)	150.75 (22.01)	<0.001
Diastolic blood pressure, mmHg	79.82 (11.21)	78.50 (10.97)	<0.001
Pulse pressure, mmHg	67.47 (17.55)	72.25 (18.88)	<0.001
Waist circumference, cm	98.65 (9.82)	89.63 (11.35)	<0.001
Hip circumference, cm	101.09 (6.45)	104.12 (9.20)	<0.001
Waist-to-hip ratio	0.98 (0.07)	0.86 (0.07)	<0.001
Total cholesterol, mmol/L †	5.37 (0.95)	5.89 (0.98)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.29 (0.34)	1.55 (0.40)	<0.001
Hypertension, n (%)	1,786 (77.2)	2,559 (79.3)	0.060
Coronary heart disease, n (%)	379 (16.4)	171 (5.3)	<0.001
Heart failure, n (%)	113 (4.9)	117 (3.6)	0.030
Diabetes mellitus, n (%)	366 (15.8)	411 (12.7)	<0.001
Smoking status			<0.001
Never, n (%)	261 (11.3)	1,457 (45.2)	
Former, n (%)	1,599 (69.1)	1,318 (40.9)	
Current, n (%)	454 (19.6)	451 (14.0)	
Lipid lowering medication, n (%)	496 (21.4)	619 (19.2)	0.040
Cardiac medication, n (%)	238 (10.3)	277 (8.6)	0.040

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S19. Baseline characteristics for men and women in the analyses for diastolic blood pressure

Baseline characteristics *	**Men n=2,950**	**Women n=4,216**	**p**
Age, years	72.40 (7.46)	73.81 (8.33)	<0.001
Weight, kg	81.01 (12.05)	71.10 (12.50)	<0.001
Body mass index, kg/m^2	26.72 (3.45)	27.58 (4.46)	<0.001
Systolic blood pressure, mmHg	146.95 (20.78)	149.90 (22.11)	<0.001
Diastolic blood pressure, mmHg	79.45 (11.14)	77.96 (10.74)	<0.001
Pulse pressure, mmHg	67.50 (17.66)	71.94 (19.00)	<0.001
Waist circumference, cm	98.16 (10.16)	89.88 (11.37)	<0.001
Hip circumference, cm	100.73 (6.61)	103.58 (9.31)	<0.001
Waist-to-hip ratio	0.97 (0.07)	0.87 (0.08)	<0.001
Total cholesterol, mmol/L †	5.37 (0.95)	5.91 (1.00)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.30 (0.35)	1.54 (0.40)	<0.001
Hypertension, n (%)	2,256 (76.5)	3,335 (79.1)	<0.010
Coronary heart disease, n (%)	491 (16.6)	224 (5.3)	<0.001
Heart failure, n (%)	160 (5.4)	182 (4.3)	0.040
Diabetes mellitus, n (%)	406 (13.8)	486 (11.5)	<0.010
Smoking status			<0.001
Never, n (%)	315 (10.7)	1,968 (46.7)	
Former, n (%)	1,983 (67.2)	1,612 (38.2)	
Current, n (%)	652 (22.1)	636 (15.1)	
Lipid lowering medication, n (%)	578 (19.6)	699 (16.6)	<0.001
Cardiac medication, n (%)	330 (11.2)	383 (9.1)	<0.010
Antihypertensive medication	1259 (42.7)	1884 (44.7)	0.100

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S20. Baseline characteristics for men and women in the analyses for pulse pressure

Baseline characteristics *	Men n=2,946	Women n=4,217	p
Age, years	72.27 (7.45)	73.68 (8.34)	<0.001
Weight, kg	81.10 (12.05)	71.17 (12.49)	<0.001
Body mass index, kg/m^2	26.75 (3.46)	27.60 (4.48)	<0.001
Systolic blood pressure, mmHg	146.55 (20.40)	149.42 (21.72)	<0.001
Diastolic blood pressure, mmHg	79.69 (11.55)	78.22 (11.17)	<0.001
Pulse pressure, mmHg	66.86 (16.95)	71.20 (18.15)	<0.001
Waist circumference, cm	98.03 (10.06)	89.94 (11.38)	<0.001
Hip circumference, cm	100.77 (6.59)	103.57 (9.27)	<0.001
Waist-to-hip ratio	0.97 (0.07)	0.87 (0.08)	<0.001
Total cholesterol, mmol/L †	5.37 (0.95)	5.88 (0.99)	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.30 (0.34)	1.55 (0.41)	<0.001
Hypertension, n (%)	2,246 (76.2)	3,333 (79.0)	<0.010
Coronary heart disease, n (%)	485 (16.5)	230 (5.5)	<0.001
Heart failure, n (%)	158 (5.4)	181 (4.3)	0.040
Diabetes mellitus, n (%)	411 (14.0)	477 (11.3)	<0.001
Smoking status			<0.001
Never, n (%)	309 (10.5)	1,952 (46.3)	
Former, n (%)	1,980 (67.2)	1,621 (38.4)	
Current, n (%)	657 (22.3)	644 (15.3)	
Lipid lowering medication, n (%)	590 (20.0)	729 (17.3)	<0.010
Cardiac medication, n (%)	331 (11.2)	401 (9.5)	0.020
Antihypertensive medication	1,247 (42.3)	1,887 (44.7)	0.045

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

Table S21. Association between various risk factors' trajectories and new-onset atrial fibrillation among men and women, after taking death as a competing event into account

Risk factors *	Trajectories of various risk factors				
	Class 1	Class 2	Class 3	Class 4	Class 5
Men					
Weight	1.00 (reference)	1.47 (1.11-1.96)	1.25 (0.96-1.63)	-	-
BMI	1.00 (reference)	1.40 (1.07-1.83)	0.82 (0.49-1.37)	-	-
WC	1.00 (reference)	1.36 (1.01-1.85)	0.93 (0.58-1.49)	-	-
HC	1.00 (reference)	1.48 (1.02-2.14)	1.89 (1.17-3.07)	-	-
WHR	1.00 (reference)	1.37 (1.02-1.83)	0.81 (0.52-1.28)	-	-
SBP	1.00 (reference)	1.33 (0.93-1.88)	1.47 (1.01-2.15)	1.33 (0.84-2.10)	1.73 (1.07-2.79)
DBP	1.00 (reference)	1.16 (0.84-1.59)	1.25 (0.95-1.64)	-	-
PP	1.00 (reference)	1.34 (1.07-1.69)	1.29 (0.81-2.05)	-	-
Women					
Weight	1.00 (reference)	2.01 (1.50-2.70)	1.07 (0.76-1.52)	-	-
BMI	1.00 (reference)	1.88 (1.50-2.37)	1.21 (0.86-1.72)	-	-
WC	1.00 (reference)	1.47 (1.12-1.92)	1.58 (1.12-2.23)	-	-
HC	1.00 (reference)	1.72 (1.18-2.53)	1.32 (0.97-2.53)	-	-
WHR	1.00 (reference)	1.07 (0.84-1.36)	-	-	-
SBP	1.00 (reference)	0.78 (0.42-1.46)	1.21 (0.95-1.54)	1.01 (0.72-1.42)	1.50 (1.01-2.23)
DBP	1.00 (reference)	1.63 (1.16-2.27)	1.90 (1.34-2.67)	1.96 (1.37-2.81)	-
PP	1.00 (reference)	1.31 (1.05-1.64)	1.72 (1.31-2.26)	-	-

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for baseline age, cohort, body mass index (if applicable), high-density lipoprotein cholesterol, total cholesterol, smoking status, use of lipid lowering medication, use of cardiac medication, history of hypertension, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

Table S22. Association between various trajectories of risk factors and new-onset atrial fibrillation among participants without cancers, coronary heart disease and heart failure

Risk factors *	Trajectories of various risk factors				
	Class 1	Class 2	Class 3	Class 4	Class 5
Men					
Weight	1.00 (reference)	1.32 (0.94-1.87)	1.14 (0.78-1.67)	-	-
BMI	1.00 (reference)	1.69 (1.22-2.30)	0.89 (0.43-1.86)	-	-
WC	1.00 (reference)	1.35 (0.99-1.83)	1.06 (0.62-1.81)	-	-
HC	1.00 (reference)	1.56 (0.98-2.49)	2.05 (1.08-3.88)	-	-
WHR	1.00 (reference)	1.38 (0.96-1.99)	0.66 (0.36-1.19)	-	-
SBP	1.00 (reference)	1.51 (0.92-2.47)	1.68 (0.90-3.16)	1.35 (0.86-2.12)	1.80 (0.98-3.30)
DBP	1.00 (reference)	0.94 (0.66-1.35)	1.32 (0.87-1.99)	-	-
PP	1.00 (reference)	1.45 (0.81-2.59)	1.39 (1.03-1.87)	-	-
Women					
Weight	1.00 (reference)	1.96 (1.40-2.75)	1.28 (0.86-1.89)	-	-
BMI	1.00 (reference)	1.58 (1.21-2.06)	1.26 (0.85-1.87)	-	-
WC	1.00 (reference)	1.37 (1.05-1.79)	1.51 (1.08-2.12)	-	-
HC	1.00 (reference)	1.66 (1.08-2.55)	1.22 (0.84-1.77)	-	-
WHR	1.00 (reference)	1.12 (0.86-1.47)	-	-	-
SBP	1.00 (reference)	1.04 (0.48-2.22)	1.37 (1.03-1.81)	1.29 (0.82-1.83)	1.89 (1.19-2.98)
DBP	1.00 (reference)	1.64 (1.12-2.41)	1.79 (1.19-2.70)	2.15 (1.41-3.28)	-
PP	1.00 (reference)	1.34 (1.04-1.74)	2.24 (1.66-3.03)		-

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for baseline age, cohort, body mass index (if applicable), high-density lipoprotein cholesterol, total cholesterol, smoking status, use of lipid lowering medication, use of cardiac medication, history of hypertension, and history of diabetes mellitus.

Table S23. Age-stratified associations between various trajectories of risk factors and new-onset atrial fibrillation

Risk factors *	Trajectories of various risk factors					
	Men			Women		
	≤70 years	>70 years	p for interaction	≤70 years	>70 years	p for interaction
Weight			0.58			0.77
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	1.27 (0.73-1.79)	1.52 (1.08-2.14)	-	1.76 (0.81-2.84)	1.85 (1.34-2.53)	-
Class 3	1.14 (0.74-1.80)	1.32 (0.93-1.87)	-	1.82 (1.05-3.16)	1.88 (1.25-2.28)	-
BMI			0.10			0.48
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	1.40 (0.90-2.18)	1.27 (0.91-1.78)	-	1.02 (0.50-1.09)	1.78 (1.39-2.28)	-
Class 3	0.82 (0.37-1.82)	0.86 (0.42-1.78)	-	1.51 (0.87-2.61)	1.24 (0.80-1.91)	-
WC			0.40			0.42
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	1.59 (0.80-3.16)	1.35 (0.95-1.90)	-	1.37 (0.65-2.88)	1.36 (1.02-1.81)	-
Class 3	1.03 (0.45-2.30)	0.95 (0.45-2.03)	-	2.03 (1.16-3.53)	1.34 (0.86-2.10)	-
HC			0.08			0.74
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	2.11 (0.81-5.51)	1.29 (0.83-2.00)	-	0.74 (0.10-5.45)	1.59 (1.07-2.23)	-
Class 3	2.45 (1.14-5.28)	1.69 (0.92-3.12)	-	1.31 (0.77-2.23)	1.30 (0.88-1.92)	-
WHR			0.99			0.98
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	1.16 (0.59-2.29)	1.41 (1.02-1.94)	-	1.06 (0.64-1.78)	1.08 (0.82-1.42)	-
Class 3	0.75 (0.42-1.35)	0.79 (0.36-1.72)	-	-	-	-
SBP			0.41			0.85
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	0.96 (0.39-2.34)	1.44 (0.98-2.13)	-	0.92 (0.29-2.89)	1.13 (0.78-1.64)	-
Class 3	1.10 (0.43-2.78)	1.69 (1.10-2.61)	-	1.45 (0.66-3.19)	1.31 (1.00-1.71)	-

Class 4	1.10 (0.41-2.96)	1.52 (0.82-2.81)	-	2.38 (1.00-5.68)	1.40 (0.55-3.56)	-
Class 5	1.25 (0.45-3.43)	2.02 (1.08-3.79)	-	2.66 (1.03-6.84)	1.62 (1.01-2.59)	-
DBP			0.66			0.17
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	0.88 (0.48-1.62)	1.30 (0.94-1.79)	-	2.59 (0.60-11.2)	1.56 (1.09-2.23)	-
Class 3	0.86 (0.47-1.57)	1.39 (0.96-2.01)	-	3.02 (0.69-13.1)	1.72 (1.15-2.57)	-
Class 4	-	-	-	5.55 (1.27-24.1)	1.82 (1.25-2.65)	-
PP			0.06			0.25
Class 1	1.00 (reference)	1.00 (reference)	-	1.00 (reference)	1.00 (reference)	-
Class 2	1.14 (0.72-1.80)	2.10 (1.14-3.85)	-	1.74 (1.02-2.95)	1.23 (0.96-1.58)	-
Class 3	0.85 (0.39-1.86)	1.48 (1.12-1.96)	-	3.22 (1.89-5.50)	1.71 (1.23-2.37)	-

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for baseline age, cohort, body mass index (if applicable), high-density lipoprotein cholesterol, total cholesterol, smoking status, use of lipid lowering medication, use of cardiac medication, history of hypertension, history of diabetes mellitus, history of heart failure, and history of coronary heart disease.

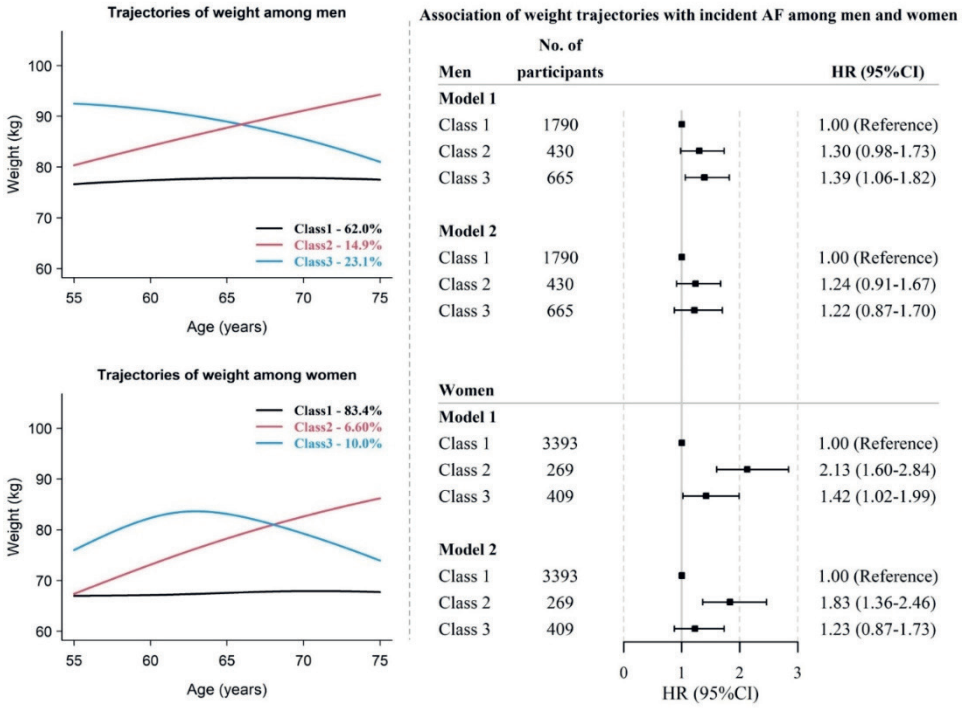
Table S24. Association between various risk factors' trajectories and new-onset atrial fibrillation among men and women, further adjustment for baseline values corresponding risk factor

Risk factors *	Trajectories of various risk factors				
	Class 1	Class 2	Class 3	Class 4	Class 5
Men					
Weight	1.00 (reference)	0.98 (0.68-1.40)	0.96 (0.67-1.37)	-	-
BMI	1.00 (reference)	1.07 (0.78-1.49)	0.87 (0.51-1.47)	-	-
WC	1.00 (reference)	1.04 (0.71-1.54)	0.83 (0.47-1.45)	-	-
HC	1.00 (reference)	1.19 (0.76-1.87)	1.76 (1.06-2.94)	-	-
WHR	1.00 (reference)	1.24 (0.84-1.85)	0.72 (0.42-1.22)	-	-
SBP	1.00 (reference)	1.34 (0.93-1.94)	1.55 (0.99-2.42)	1.44 (0.90-2.32)	1.84 (1.10-3.09)
DBP	1.00 (reference)	1.21 (0.88-1.65)	1.26 (0.92-1.71)	-	-
PP	1.00 (reference)	1.63 (1.21-2.19)	1.53 (0.94-2.47)	-	-
Women					
Weight	1.00 (reference)	1.35 (0.95-1.92)	0.99 (0.68-1.43)	-	-
BMI	1.00 (reference)	1.39 (1.01-1.90)	1.06 (0.72-1.57)		-
WC	1.00 (reference)	1.06 (0.74-1.54)	1.20 (0.79-1.80)	-	-
HC	1.00 (reference)	1.01 (0.63-1.61)	0.85 (0.57-1.26)	-	-
WHR	1.00 (reference)	1.03 (0.76-1.40)	-	-	-
SBP	1.00 (reference)	0.98 (0.52-1.84)	1.19 (0.56-1.66)	1.08 (0.83-1.40)	1.53 (1.02-2.30)
DBP	1.00 (reference)	1.48 (1.04-2.10)	1.63 (1.09-2.42)	1.89 (1.30-2.74)	-
PP	1.00 (reference)	1.16 (0.90-1.50)	1.77 (1.33-2.36)	-	-

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

* Adjusted for baseline age, cohort, body mass index (if applicable), high-density lipoprotein cholesterol, total cholesterol, smoking status, use of lipid lowering medication, use of cardiac medication, history of hypertension, history of diabetes mellitus, history of heart failure, history of coronary heart disease, and baseline values of corresponding risk factor.

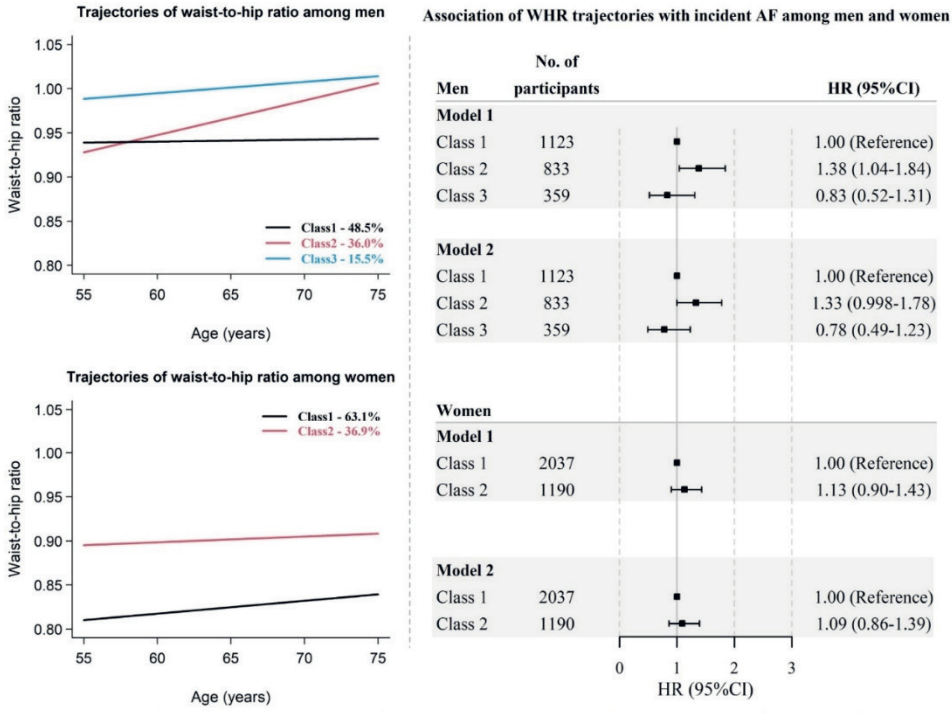
Figure S1. Various weight trajectories and their associated risk for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of HDL-cholesterol, total cholesterol, smoking, use of serum lipid reducing agents, use of cardiac medication and history of hypertension, diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable weight trajectory; Class 2 refers to a "persistent-increasing" weight trajectory; Class 3 refers to a "persistent-decreasing" weight trajectory in men or an "elevated-and-decreasing" weight trajectory in women.

Abbreviations: AF, atrial fibrillation; BMI, body mass index; HDL, high-density lipoprotein.

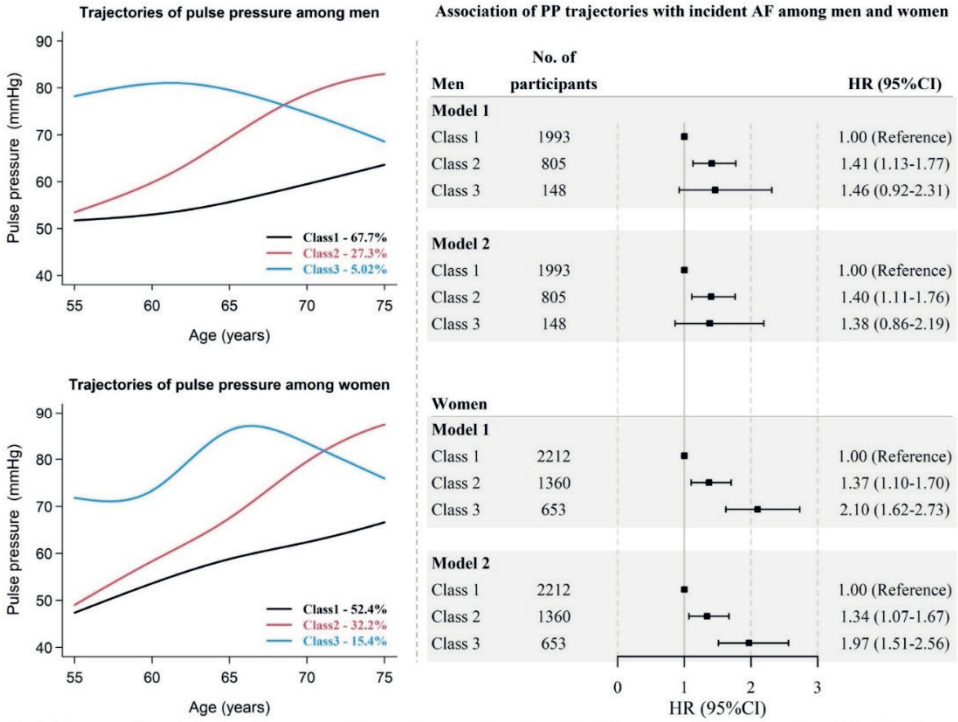
Figure S2. Various waist-to-hip ratio trajectories and their associated risks for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of HDL-cholesterol, total cholesterol, smoking, use of serum lipid reducing agents, use of cardiac medication and history of hypertension, diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable WHR trajectory; Class 2 refers to a "persistent-increasing" WHR trajectory in men and an "elevated-and-stable" WHR trajectory in women; Class 3 refers to an "elevated-and-stable" WHR trajectory in men.

Abbreviations: AF, atrial fibrillation; WHR, waist-to-hip ratio; HDL, high-density lipoprotein.

Figure S3. Various pulse pressure trajectories and their associated risks for new-onset atrial fibrillation among men and women



Model 1 was adjusted for baseline age and Rotterdam Study cohort. Model 2 was additionally adjusted for baseline values of BMI, HDL-cholesterol, total cholesterol, smoking, use of blood pressure lowing medication, use of serum lipid reducing agents, use of cardiac medication and history of diabetes mellitus, heart failure and coronary heart disease.
Class 1 refers to the most favorable PP trajectory; Class 2 refers to a "persistent-increasing" PP trajectory; Class 3 refers to an "elevated-and-decreasing" PP trajectory.

Abbreviations: AF, atrial fibrillation; PP, pulse pressure; HDL, high-density lipoprotein.


CHAPTER 5.3
5
3
CARDIAC AUTONOMIC DYSFUNCTION
HYPERTENSION
OBESITY
GENDER
SEX
KIDNEY DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
MICRORNAS
NOVEL RISK FACTORS
IMMUNOTHERAPY
GENOMICS
HEART
MICRONAS
MACRO- AND MICRO-VASCULAR
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC

Antihypertensive drugs for the prevention of atrial fibrillation

Antihypertensive drugs for the prevention of atrial fibrillation: a drug target Mendelian randomization study.

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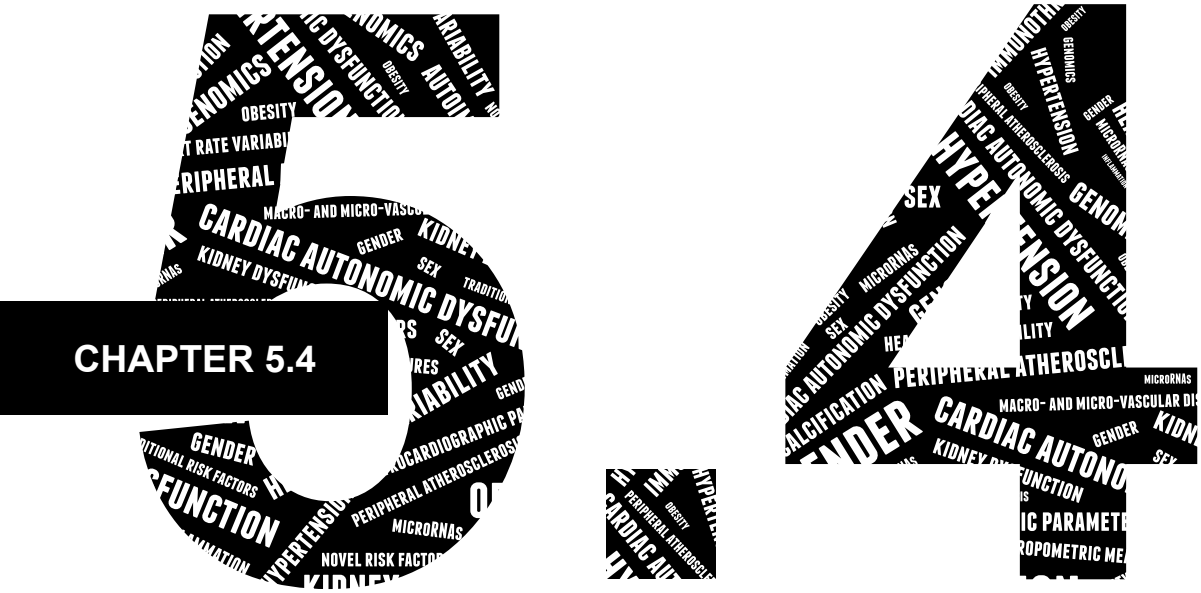
CHAPTER 5.4

Circulatory microRNAs in plasma and the risk of atrial fibrillation

Circulatory microRNAs in plasma and atrial fibrillation in the general population: the Rotterdam Study.

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ABSTRACT

Background

MicroRNAs (miRNAs), small non-coding RNAs regulating gene expression, have been shown to play an important role in cardiovascular disease. However, limited population-based data regarding the relationship between circulatory miRNAs in plasma and atrial fibrillation (AF) exist. Moreover, it remains unclear if the relationship differs by sex. We therefore aimed to determine the (sex-specific) association between plasma circulatory miRNAs and AF at the population level.

Methods

Plasma levels of miRNAs were measured using a targeted next-generation sequencing method in 1,999 participants from the population-based Rotterdam Study. Logistic regression and Cox proportional hazards models were used to assess the associations of 591 well-expressed miRNAs with the prevalence and incidence of AF. Models were adjusted for cardiovascular risk factors. We further examined the link between predicted target genes of the identified miRNAs.

Results

The mean age was 71.7 years (57.1% women), 98 participants (58 men and 40 women) had prevalent AF at baseline. Moreover, 196 participants (96 men and 100 women) developed AF during a median follow-up of 9.0 years. After adjusting for multiple testing, miR-4798-3p was significantly associated with the odds of prevalent AF among men (odds ratio, 95% confidence interval, 0.39, 0.24-0.66, p=0.000248). No miRNAs were significantly associated with incident AF. MiR-4798-3p could potentially regulate the expression of a number of AF-related genes, including genes involved in calcium and potassium handling in myocytes, protection of cells against oxidative stress, and cardiac fibrosis.

Conclusions

Plasma levels of miR-4798-3p were significantly associated with the odds of prevalent AF among men. Several target genes in relation to AF pathophysiology could potentially be regulated by miR-4798-3p that warrant further investigations in future experimental studies.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide.(1, 2) The prevalence of AF is expected to increase steeply in the coming decades due to aging of the population.(1, 2, 3) Despite the identification of risk factors for AF.(4-7) and improvement in its management, AF still confers a high morbidity and mortality risk.(1, 2, 7) Furthermore, recent evidence suggests that sex differences in AF pathophysiology and prognosis exist.(8) Women with AF are older at diagnosis, have a higher prevalence of hypertension, valvular heart disease, and have an increased risk of stroke, myocardial infarction, and mortality in comparison to men.(8)

MicroRNAs (miRNAs) are a class of small non-coding RNAs that post-transcriptionally regulate gene expression by complementary binding to target transcripts. Dysregulation of miRNA function could affect pathology of diseases.(9) Extensive studies have also shown the potential of miRNAs to be used as disease biomarkers, as their expression remains stable after drawing blood and they are easily accessible in different types of body fluid.(10) Over the past years, the role of miRNAs in various cardiovascular diseases has received a major interest.(11) MiRNAs have been suggested, among others, as key regulators of electrical remodeling,(12) structural remodeling,(13) autonomic nerve remodeling,(14) calcium handling abnormalities,(15) and inflammation(16) of the heart. These functions suggest a role for miRNAs in AF pathophysiology.

Previous studies have identified plasma levels of several miRNAs to be associated with AF.(17-25) However, most of these studies were limited to cross-sectional analysis, a subgroup of AF patients, or hypothesis-driven by studying only subsets of specific miRNAs.(18) To date, limited data exist on the association between circulatory miRNAs in plasma and AF in the general population.(20, 24) Furthermore, research regarding sex differences in the associations of miRNAs with AF is sparse.

In this study, we aimed to investigate the association between circulatory miRNAs in plasma with prevalent and incident AF in the general population using data from the prospective population-based Rotterdam Study to gain more insight into AF pathophysiology. Additionally, we evaluated if the association differs by sex. We further retrieved the predicted target genes of identified miRNAs and examined if any of these target genes have been associated with AF pathophysiology by previous literature. Moreover, we provided an extensive literature review of the previously reported circulatory miRNAs in blood/plasma in association with AF and we provided a detailed review per type of miRNA and the corresponding study characteristics. Subsequently, we did a look up of our findings and compared them with the findings of the association between previously reported circulatory miRNAs with prevalent and incident AF in an attempt to replicate our findings and previous evidence. Finally,

we also sought to investigate in silico whether the identified miRNAs are expressed in the heart in an attempt to further unravel the potential underling mechanism that relates the identified miRNAs to AF pathophysiology.

METHODS

Study design

This study was embedded in the Rotterdam Study.(26, 27) In short, the Rotterdam Study is a prospective population-based cohort study that investigates the occurrence and progression of risk factors for chronic diseases in middle-age and elderly persons. The design of the Rotterdam Study is explained in detail in the **Methods S1**.(26, 27)

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Study population

For the present study, we included 1,000 participants from the fourth visit of RS-I (RS-I-4) and 1,000 participants from the second visit of RS-II (RS-II-2) for whom miRNA expression data were obtained (n=2000). These visits took place between 2002 and 2005 and we considered this as the baseline of our study. From these 2,000 randomly chosen participants, one participant was excluded, due to insufficient baseline data on AF for the cross-sectional study with prevalent AF. For the longitudinal study with incident AF, we additionally excluded prevalent AF cases (n=98). A total of 1,999 participants were included in the current study.

Assessment of circulatory microRNAs in plasma

Methods on plasma miRNA level measurement have been described previously.(28) In short, the expression levels of 2083 mature human miRNAs (HTG Molecular Diagnostics, Tuscon, AZ, USA) and using the Illumina NextSeq 500 sequencer (Illumina, San Diego, CA, USA) were measured. Out of 2,083 measured miRNAs, 591 miRNAs were well-expressed in plasma.(28) The assessment of miRNAs is

further explained in the **Methods S2**.

Assessment of atrial fibrillation

AF was defined in accordance with the European Society of Cardiology (ESC) guidelines.(7) Methods on event adjudication for prevalent and incident AF have been described previously.(3) In short, a 10 s 12-lead electrocardiogram (ECG) was used to assess AF at baseline and additional follow-up information was obtained from medical files and follow-up examinations at the research center. All participants were followed from the date of enrolment in the Rotterdam Study until the date of onset of AF, date of death, loss to follow-up, or to the end of data collection on January 1st 2014, whichever came first. The assessment of AF is further explained in detail in the **Methods S3**.(3, 26, 29)

Assessment of cardiovascular risk factors

The cardiovascular risk factors included in the study were body mass index (BMI), total cholesterol, high-density lipoprotein (HDL) cholesterol, hypertension, smoking status, history of diabetes mellitus (DM), history of coronary heart disease (CHD), history of heart failure (HF), left ventricular hypertrophy (LVH) on the ECG, use of cardiac medication, and use of lipid lowering medication. Methods for measurements of cardiovascular risk factors are explained in detail in the **Methods S4**.(26, 27)

Statistical analyses

Baseline characteristics

Participant characteristics at study entry are presented as mean with standard deviation (SD) or number (n) with percentages as appropriate. Group differences between men and women were examined by Student's T-test for continuous variables and Chi-Square test for categorical variables.

Logistic regression and Cox proportional hazards models

Logistic regression and Cox proportional hazards models were used to assess the association between plasma miRNAs at baseline with prevalent and incident AF, respectively (**Figure 1**). Odds Ratios (ORs) and hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to quantify the associations. An examination of the shape of relation with AF was performed using natural cubic splines for continuous variables, and no deviation from linearity was found. No influential values were observed when using Cook's distance and no multicollinearity among the variables was observed using a variance inflation factors threshold of <5. The proportional hazards assumptions were tested by Schoenfeld tests and were found to be satisfied. Additionally, we examined the interaction of miRNAs and sex before subsequently stratifying our analyses.

Analyses were performed in the total study population and for men and women separately. All models were adjusted for age, sex (if applicable), and cohort (model

1) and additionally for cardiovascular risk factors including BMI, total cholesterol, HDL cholesterol, hypertension, smoking status, history of DM, history of CHD, history of HF, LVH on the ECG, use of cardiac medication, and use of lipid lowering medication (model 2). Missing values of variables were imputed under the assumption of missing at random using multiple imputation. For multiple imputation, all available data were used to generate an imputed dataset.

The p threshold was corrected for multiple testing based on the eigenvalues of the correlation matrix from all the miRNAs. This adapted method was proposed by Li(30) and is based on a method which was introduced by Cheverud(31) to adjust correlated tests as if they were independent, according to an “effective number” of independent tests, as there is evidence that miRNAs are clustered together or may be co-expressed.(32) This means that the miRNAs are thereby correlated with each other and by adopting this p correction proposed by Li(30) we take this correlation into account when adjusting. Based on the aforementioned method from Li,(30) the significance p cutoff was set at 0.000352 based on 142 identified independent tests (0.05/142).

The data management and analyses were performed using IBM SPSS Statistics version 25.0 for Windows (IBM Corp., Armonk, NY, USA) and R software (R 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).(33)

Assessment of predictive target genes

We retrieved a list of predicted targets genes of identified miRNAs associated with AF using the 3 commonly used target prediction databases: miRDB,(34, 35) TargetScan,(36) and miRTarBase.(37). Furthermore, we assessed if any of these predicted target genes have been associated previously with AF by a systematic review and a genome-wide association study(18, 38) and we assessed if any of these genes are potentially involved in AF pathophysiology by electrical and/or structural remodeling of the heart (**Figure 1**).

Literature review

We searched the literature (PubMed) to identify studies that reported on circulatory miRNAs in blood/plasma in association with AF. Subsequently, we tested the association of these previously reported circulatory miRNAs with prevalent and incident AF in our study in an attempt to replicate our findings and previous findings (**Figure 1**).

In silico analyses

We also sought to investigate whether the identified miRNAs are expressed in the heart in an attempt to further understand the potential underlying mechanism that might link the identified miRNAs to AF pathophysiology. In addition, we retrieved the miRNA host genes as proxy for the identified miRNAs to evaluate their expression

in the heart using the Human Protein Atlas (**Figure 1**).(39) The idea behind this is that intragenic miRNAs and their host genes are likely to be co-expressed.(40) Furthermore, the genomic location of the identified miRNAs was obtained using miRIAD.(41)

RESULTS

Statistical analyses

Baseline characteristics

A total of 1,999 participants, 858 men (42.9%) and 1,141 women (57.1%), were eligible for the analyses of miRNAs associated with prevalent AF. The baseline characteristics for the study sample are depicted in **Table 1**. For the longitudinal analyses of incident AF, after exclusion of prevalent AF cases, 1,901 individuals, 800 men (42.1%) and 1,101 women (57.9%), were included. Characteristics of this study population are presented in **Table S1**. Compared to men, women were slightly older, more often hypertensive and never smokers. DM, CHD, HF, and LVH on the ECG were less prevalent among women. Women used cardiac medication and lipid lowering medication less frequently than men.

Logistic regression

At baseline, 98 cases (4.9%) of prevalent AF were identified, from which 58 cases (6.8%) were in men and 40 cases (3.5%) were in women. Logistic regression showed that 47 miRNAs in the total study population, 45 miRNAs in men, and 31 miRNAs in women were nominally significantly ($p<0.05$) associated with prevalent AF after adjustment for age and cardiovascular risk factors (model 2). See **Table S2** for an overview of the nominally significantly associated miRNAs. For one unit increase in miR-4798-3p plasma levels at baseline, the odds for prevalent AF in the total study population was OR, 95% CI, 0.64, 0.44-0.97, p=0.028033 (model 1). After adjusting for cardiovascular risk factors, the odds did not attenuate OR, 95% CI, 0.63, 0.42-0.99, p=0.034433 (model 2). The odds for prevalent AF were lower in men than in women. After adjustment for cardiovascular risk factors, ORs, 95% CIs were 0.39, 0.24-0.66, p=0.000248 in men and 1.84, 0.76-4.97, p=0.203587 in women (model 2). However, after adjusting for multiple testing (0.05/142=0.000352), only miR-4798-3p, remained statistically significantly associated with prevalent AF among men (**Table 2**). The interaction term between miRNA-4798-3p and sex in relation to the odds of prevalent AF in the total study population using logistic regression was significant (p=0.004730). This significant sex interaction further highlights our observed sex differences for miR-4798-3p. **Figure 2** illustrates the nominally significant miRNAs associated with prevalent AF among men described by a volcano plot.

Atrial fibrillation incidence
During a median follow-up of 9.0 years (interquartile range (IQR), 7.7-10.3), 196 incident AF cases (10.3%) (96 in men and 100 in women) occurred. The incidence rate of AF was 12.5 per 1,000 person-years in the total study population (15.2 per 1,000 person-years in men, 10.7 per 1,000 person-years in women).

Cox proportional hazards models
Cox proportional hazards models showed that a total of 17 miRNAs in the total study population, 26 miRNAs in men, and 13 miRNAs in women were nominally significant in association with incident AF (model 2), but none of them remained statistically significant after adjustment for multiple testing. **Table S3** shows a complete list of the nominally significantly associated miRNAs with incident AF.

There was little overlap in similarity between the effect estimates of the miRNAs among the prevalent AF cases when we compared them to the effect estimates in the incident AF sample and vice versa. This was also the case when we compared the effect estimates of miR-4798-3p for the association with prevalent AF among men with the effect estimates of miR-4798-3p for incident AF among men (OR, 95% CI, 0.39, 0.24-0.66, p=0.000248 vs. HR, 95% CI, 1.10, 0.68-1.77, p=0.704529) (model 2).

Predictive target genes
We additionally examined the predicted target genes of miR-4798-3p using 3 miRNA target prediction databases: miRDB.(34, 35) TargetScan,(36) and miRTarBase.(37). To reduce error, we only retained predicted target genes if they were identified by at least 2 out of the 3 databases that were used.(42) Among predicted target genes of miR-4798-3p are CACNB2,(43) KCNN3,(44) SIRT1,(45) and STAT3(46, 47) that are suggested to be involved in electrical and/or structural remodeling of the heart. **Table S4** depicts the genes, that were among the predicted target genes of miR-4798-3p, and that have been previously associated with AF by a systematic review and a genome-wide association study.(18, 38) In addition, the potential remodeling mechanisms of the heart for these genes are also provided.(48)

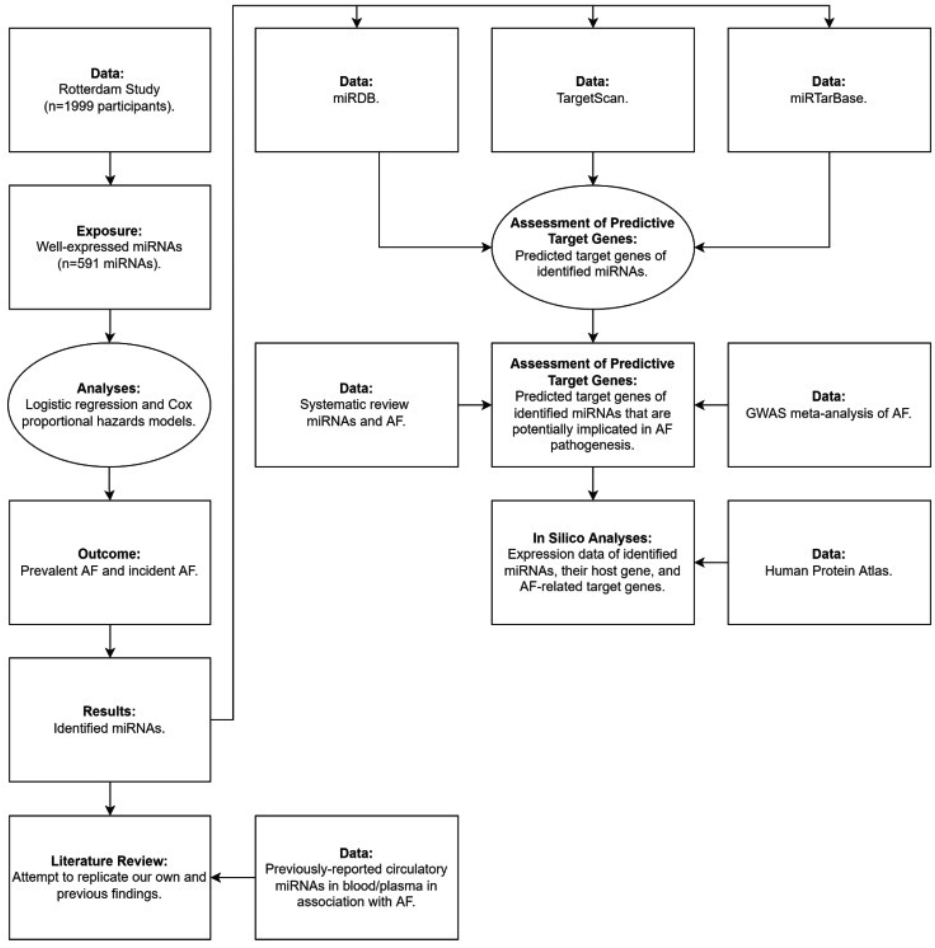
Literature review
Additionally, we provided an extensive literature review of circulatory miRNAs in blood/plasma in association with AF that have been reported in the literature before (**Table S5**). In this review, we provided detailed information per type of miRNA and the corresponding study characteristics including study design, study population, baseline characteristics, reported effect estimates, the statistical models, and adjustments. Furthermore, we did a look-up for these AF-associated miRNAs in our results in an attempt to replicate our results and previous results. The effect estimates and p values for the association of these previously reported miRNAs with prevalent and incident AF in our data are reported in **Tables S6** and **S7**, respectively.

For the prevalent AF analyses, we were able to compare 39 miRNAs. Among these 39 miRNAs, the direction of the effect estimates reported in the literature were in line with our results for 18 miRNAs. The reported effect estimate in the literature that was most similar to our findings was the effect estimate for miR-20a-5p (literature-reported OR, 95% CI, 1.36, 1.14-1.61, p=0.001,(24), while we found an OR, 95% CI, 1.30, 0.68-2.58, p=0.435846). Moreover, for the incident AF analyses, we were able to compare 10 miRNAs from the literature with our results. The direction of the effect was similar for 4 miRNAs and the miRNA with the most similar effect estimate was miR-193a-5p with a literature-reported HR, 95% CI, 0.87, 0.77-0.98, p=0.024,(20) while we found a HR, 95% CI, 0.93, 0.67-1.28, p=0.640964.

In silico analyses

Finally, we explored whether miR-4798-3p was expressed in the heart. We were unable to find any information regarding its expression levels within the heart.(39) Alternatively, we evaluated the expression of its host gene as a proxy for miR-4798-3p.(40) MiR-4798-3p is located within an intron of the protein-coding gene SORCS2.(41) SORCS2 is especially found within the central nervous system, and it is well-expressed within the brain and to a lesser degree within the heart.(39) Moreover, the expression levels of AF-associated target genes of miR-4798-3p (**Table S4**) were also detected in various degrees within the heart.(39)

Figure 1. Flow chart for the conducted analyses and search strategy



Abbreviations: AF, atrial fibrillation; GWAS, genome-wide association study; miRNAs, microRNAs.

Table 1. Baseline characteristics of the total study population and stratified by sex

Baseline characteristics *	Total study population n=1,999	Men n=858	Women n=1,141	p ‡
Age, years	71.7 ± 7.6	71.4 ± 7.3	71.9 ± 7.8	0.116
Women, n (%)	1141 (57.1)	NA	1141 (100)	
Body mass index, kg/m^2	27.7 ± 4.1	27.6 ± 3.4	27.7 ± 4.6	0.382
Total cholesterol, mmol/L †	5.6 ± 1.0	5.3 ± 1.0	5.9 ± 1.0	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.4 ± 0.4	1.0 ± 0.3	1.6 ± 0.4	<0.001
Hypertension, n (%)	1558 (77.9)	654 (76.2)	904 (79.2)	0.109
Smoking status				<0.001
Never, n (%)	599 (30.0)	117 (13.6)	482 (42.2)	
Former, n (%)	1094 (54.7)	592 (69.0)	502 (44.0)	
Current, n (%)	306 (15.3)	149 (17.4)	157 (13.8)	
History of diabetes mellitus, n (%)	268 (13.4)	145 (16.9)	123 (10.8)	<0.001
History of coronary heart disease, n (%)	213 (10.7)	145 (16.9)	68 (6.0)	<0.001
History of heart failure, n (%)	101 (5.1)	50 (5.8)	51 (4.5)	0.170
Left ventricular hypertrophy, n (%)	108 (5.4)	62 (7.2)	46 (4.0)	0.002
Cardiac medication, n (%)	210 (10.5)	102 (11.9)	108 (9.5)	0.108
Lipid lowering medication, n (%)	450 (22.5)	208 (24.2)	242 (21.2)	0.080

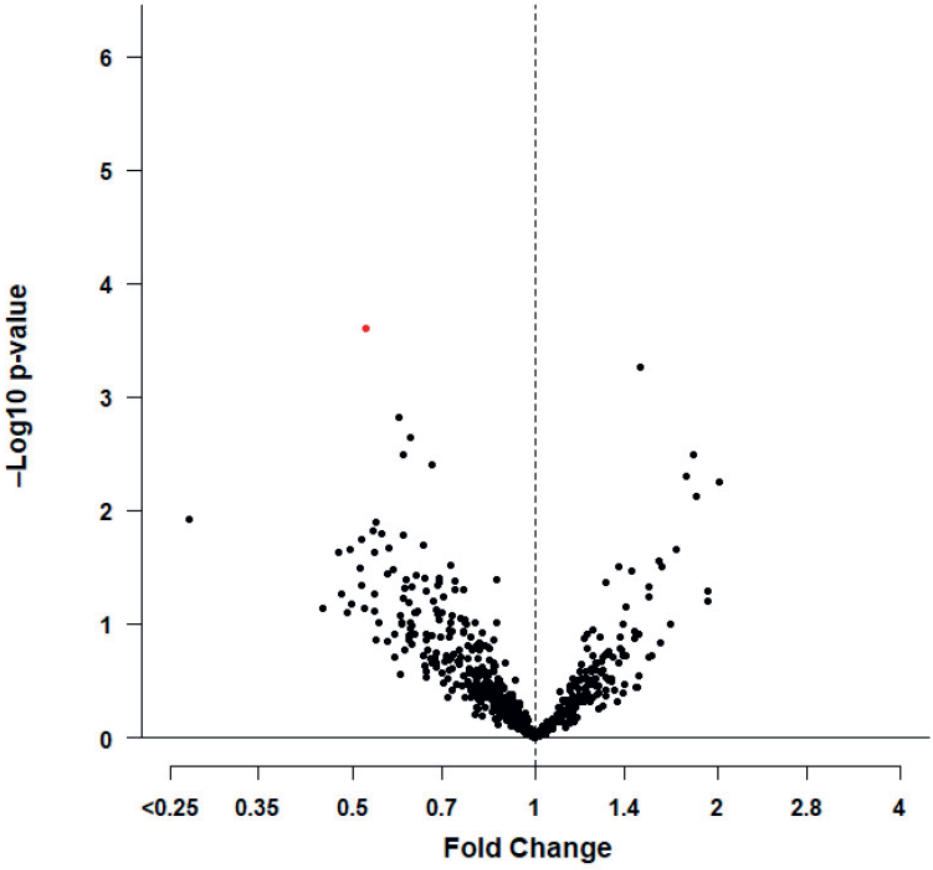
Abbreviations: n, number; NA, not applicable.

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

‡ Statistical significance for continuous variables was tested using the Student's T-test and for categorical variables was tested using the Chi-Square test.

Figure 2. Volcano plot of nominally significant microRNAs in association with prevalent atrial fibrillation among men



Abbreviations: AF, atrial fibrillation; GWAS, genome-wide association study; miRNAs, microRNAs. The y-axis represents the negative log of the p on the y-axis and the x-axis represents the log of the fold change for prevalent AF. The black dots indicate the associations of the nominally significant miRNAs. The red dot indicates the significant association after correction for multiple testing of miR-4798-3p.

Table 2. Association between miR-4798-3p with the odds of prevalent atrial fibrillation in the total study population and stratified by sex

	OR (95% CI)			
	Model 1 *	**p**	**Model 2** †	**p**
Total study population				
miR-4798-3p ‡	**0.64 (0.44-0.97)**	**0.028033**	**0.63 (0.42-0.99)**	**0.034433**
Men				
miR-4798-3p ‡	**0.42 (0.27-0.69)**	**0.000254**	**0.39 (0.24-0.66)**	**0.000248**
Women				
miR-4798-3p ‡	1.53 (0.71-3.70)	0.311964	1.84 (0.76-4.97)	0.203587

Abbreviations: CI, confidence interval; OR, odds ratio.

* Adjusted for age, sex (if applicable), and cohort.

† Adjusted for age, sex (if applicable), cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

‡ Odds ratios represent 1 unit increase of miR-4798-3p plasma levels with the odds of prevalent atrial fibrillation.

The associations with a $p<0.05$ are highlighted in **bold**.

DISCUSSION

In this prospective population-based study, we conducted a systematic analysis of 591 circulatory miRNAs well-expressed in plasma with the odds and the risk of AF in the general population and for men and women separately. We found that plasma levels of miR-4798-3p were significantly associated with the odds of prevalent AF among men after extensive adjustment for potential confounders and correcting for multiple testing. Several predicted target genes of miR-4798-3p have been associated previously with AF in a systematic review(18) and data from a recent genome-wide association study on AF.(38) These target genes are potentially involved in electrical and/or structural remodeling of the heart and thereby may mediate the effect of miR-4798-3p in AF pathophysiology. Future experimental studies are warranted to investigate the potential (sex-specific) role of this miRNA in molecular pathways underlying AF. We also provided an extensive and detailed literature review of the previously reported miRNAs linked to AF and compared these literature-reported associations with the associations observed in our study in attempt to replicate our results and previous studies. Lastly, we investigated in silico if miRNA-4798-3p and its AF-associated target genes are expressed within heart.

Plasma levels of miR-4798-3p were significantly associated with the odds of prevalent AF in our study. The exact pathology behind the associations of many miRNAs with cardiovascular diseases is not completely understood. In general, miRNAs are involved in every biological pathway through regulating expression of target genes/transcripts. However, no other studies have identified circulatory miR-4798-3p in plasma as a potential risk factor or biomarker for atrial fibrillation. MiR-4798-3p has a predicted number of more than 50 target genes that it may regulate.(34-37) Various genes which are potentially regulated by this miRNA are involved in calcium and potassium handling in myocytes (CACNB2, KCNN3), in protection of cells against oxidative stress (SIRT1), and in regulating cardiac fibrosis (STAT3). These aforementioned mechanisms are linked to electrical and/or structural remodeling of the heart which are associated with AF pathophysiology.(8, 12, 18, 38, 49) The host gene SORCS2 is profoundly expressed within the central nervous system and may thereby potentially exert an effect on AF vulnerability, as an effect on the extensive network of vagal ganglionated plexi is known to affect AF risk.(50). However, if these circulating levels of miRNA in plasma by themselves cause AF, or if the circulating levels of miRNAs are merely a reflection of an underlying pathology that may lead to the pathogenesis of AF, is not clear. In addition, it is beyond the scope of this investigation to elucidate on the pathophysiologic implications of a host gene and putative target genes. Future experimental studies are warranted to investigate the interaction between miR-4798-3p, its host gene, its target genes, and their relation to AF. These future experimental studies could then further aid in early AF diagnosis, risk stratification, therapeutic

monitoring, or identification of potential interesting pharmaceutical drug targets among men. The limited overlap between miRNAs associated with prevalent and incident AF might suggest that miRNAs may indeed be a reflection of underlying pathology that is associated with prevalent AF instead of that miRNAs may cause incident AF over time. The potential discrepancy between cell-specific expression of miRNAs and circulatory (cell-free) miRNAs in plasma makes it more difficult to disentangle this pathophysiology. However, it has been shown that circulatory miRNAs constitute a way of cell-to-cell communication, and miRNAs are released to extracellular matrix and blood by exosome from the diseased tissue/cells. Moreover, the duration of time that is involved in the release of miRNA in plasma (by a pathological event), and the effect that it may have, is still elusive. Nevertheless, miR-4798-3p could still be a potentially useful plasma biomarker for AF prediction or prognosis.

Sex differences in AF pathophysiology are increasingly gaining interest.(8) The association of miR-4798-3p with prevalent AF in our study was only significant among men. This difference could be explained by the different target genes of miR-4798-3p and their potential sex-specific effects. For example, KCNN3 and CACNB2 regulate L-type calcium channels and may thereby influence QT intervals of the heart.(51) Women have different and longer QT-intervals than men,(8) and a long QT-interval has been associated with AF initiation.(52) SIRT1 is known to be upregulated in patients with CHD, possibly as a potential compensatory mechanism to counteract the adverse effects of oxidative stress caused by CHD.(53, 54) CHD is more prevalent among men than in women(8) and is implicated in AF pathophysiology.(4-8) STAT3 is involved in cardiac fibrosis and previous research has shown that women with AF have more atrial fibrosis than men.(8) Although these effects could be sex-specific, further exploration is warranted to examine the exact underlying molecular mechanisms that might explain these sex differences.

To the best of our knowledge, the Framingham Heart Study is the only population-based cohort study that has previously investigated the association between miRNAs and AF at the population level. McManus et al.(20) identified one miRNA that was significantly associated with prevalent AF (miR-328) in the Framingham Heart Study, while they did not find any significant miRNAs that were associated with incident AF. Vaze et al.(24) identified 6 miRNAs that were significantly associated with incident AF in the Framingham Heart Study, including 4 also significantly associated with prevalent AF (miR-106b, miR-26a-5p, miR-484, and miR-20a-5p). We could not replicate our findings and the findings from McManus et al.(20) and Vaze et al.(24) or the results from the studies assessed during the literature review. These differences may be due to the fact that we measured circulatory miRNA levels in plasma instead of whole blood(55) as in the Framingham Heart Study, differences in miRNA expression profiling,(56) differences in adjusting for confounders, differences in correcting for multiple testing, and differential expression of miRNAs

related to the type and phase of AF. It is worth noting that an internationally adopted standardized method to evaluate miRNA expression could potentially improve future miRNA studies (for example plasma vs. blood or circulatory vs. tissue). As such, a standardization could then improve the comparability between future miRNA studies, and this would also benefit any potential clinical applications of miRNA-based therapies in the future. However, our findings extend the aforementioned studies by examining 591 (instead of 253-339) miRNAs, a longer follow-up time, and more extensive adjustment for potential confounding. Additionally, we also examined potential sex differences in the associations between miRNAs and AF in our study population, and we thereby also add to the emerging evidence that circulating miRNAs play a critical role in the pathophysiology of AF that may potentially be sex-specific.

Major strengths of our study include its population-based nature, large sample size, precise adjudication of prevalent and incident AF, detailed information on cardiovascular risk factors, a long follow-up time, including a well-expressed set of 591 miRNAs, extensive adjustment for potential confounders, the examination of potential sex differences between miRNAs and AF, our detailed literature review, and in silico analyses to further understand the potential underlying mechanisms. Nonetheless, there are some limitations. We could not distinguish between paroxysmal, persistent, long-standing persistent, and permanent AF, as Holter monitoring is not available in this large population-based cohort study. Although, we extensively adjusted for confounders, residual confounding cannot be entirely ruled out. Furthermore, our study population included mainly elderly participants from European descent and our results may therefore not be generalizable to younger populations or other ethnicities.

In this large population-based cohort study we assessed 591 well-expressed circulatory miRNAs in plasma in relation to prevalent and incident AF. We found that plasma levels of miR-4798-3p were significantly associated with the odds of prevalent AF among men. Several target genes in relation to AF pathophysiology could potentially be regulated by miR-4798-3p that warrant further investigations in future experimental studies.

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SUPPLEMENTARY MATERIAL

Chapter 5.4 Circulatory microRNAs in plasma and the risk of atrial fibrillation

Methods S1. Study design and study population

Methods S2. Assessment of circulatory microRNAs in plasma

Methods S3. Assessment of atrial fibrillation

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Table S4. Predicted target genes of miR-4798-3p that have been previously associated with atrial fibrillation and their potential remodeling mechanism

Table S5. Circulatory microRNAs reported in previous literature in association with atrial fibrillation

Table S6. Association of previously-reported microRNAs with the odds of prevalent atrial fibrillation in our total study population and stratified by sex

Table S7. Association of previously-reported microRNAs with the risk of incident atrial fibrillation in our total study population and stratified by sex

Methods S1. Study design and study population

The Rotterdam Study is a prospective population-based cohort study that investigates the occurrence and progression of risk factors for chronic diseases in middle-age and elderly persons.(26, 27) The Rotterdam study started in 1990 and all inhabitants aged ≥55 years of Ommoord district in the city of Rotterdam in The Netherlands were invited. A total of 7983 (78% of all invitees) inhabitants participated (RS-I). The cohort was extended in 2000 with 3,011 participants who were ≥55 years or who had migrated to Ommoord (RS-II). The cohort was again extended in 2006 with 3,932 participants that were ≥45 years (RS-III). The overall response rate was 72% at baseline. All participants were re-examined every 3-6 years at the research center. Data on morbidity and mortality were continuously collected through linkage with medical files from general practitioners in the study area.(26, 27)

For the present study, we included 1,000 participants from the fourth visit of RS-I (RS-I-4) and 1,000 participants from the second visit of RS-II (RS-II-2) for whom miRNA expression data was obtained (n=2,000). These visits took place between 2002 and 2005 which we considered as the baseline of our study. From these 2,000 randomly chosen participants, 1 participant was excluded, because of insufficient baseline data on AF for the cross-sectional study. For the longitudinal study with incident AF, we additionally excluded prevalent AF cases (n=98). A total of 1,999 participants were included in the current study.

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl/trials) and into the WHO International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/) under shared catalogue number NL6645/NTR6831. All participants provided written informed consent to participate, prior to inclusion, in the study and to have their information obtained from treating physicians.

Methods S2. Assessment of circulatory microRNAs in plasma

Plasma miRNA levels were obtained between 2002 and 2005 in 1,000 randomly selected participants from the fourth visit of RS-I (RS-I-4) and 1,000 randomly selected participants from the second visit of RS-II (RS-II-2). Plasma miRNA levels were determined using the HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA), which measures the expression levels of 2,083 mature human miRNAs (HTG Molecular Diagnostics, Tuscon, AZ, USA) and using the Illumina NextSeq 500 sequencer (Illumina, San Diego, CA, USA). The quantification of miRNA expression was based on counts per million. As standardization and adjustment for total reads

within each sample a Log2 transformation of counts per million was used. MiRNAs with a Log2 counts per million <1.0 were indicated as not expressed. A lower limit of quantification was used to select well-expressed miRNAs. Well-expressed miRNAs in plasma were those miRNAs with >50% values above the lower limit of quantification. Out of 2,083 measured miRNAs, 591 miRNAs were well-expressed in plasma.(28)

Methods S3. Assessment of atrial fibrillation

AF was defined in accordance with the European Society of Cardiology (ESC) guidelines.(7) A 10-second 12-lead electrocardiogram (ECG) was used to assess AF at baseline and follow-up examinations with an ACTA Gnosis IV ECG recorder (Esaote; Biomedica, Florence Italy). The ECG records were stored digitally, and analyzed with Modular ECG Analysis system (MEANS).(29) The ECG records diagnosed by MEANS as rhythm disorder were independently verified by 2 research physicians blinded to the MEANS diagnosis.(3, 26) A cardiologist was consulted in case of disagreement between the research physicians. Events of AF were not included if these occurred during the process of dying, or in case of transient AF after cardiac surgery or myocardial infarction.(3, 26) Additional follow-up information was obtained from medical files of participating general practitioners, outpatient clinics, hospitals, a national registration of all hospitals discharge diagnoses and follow-up examinations at the research center. The date of incident AF was defined as the date of the first occurrence of symptoms suggestive of AF with subsequent electrocardiogram (ECG) verification obtained from the medical records. All participants were followed from the date of enrolment in the Rotterdam Study until the date of onset of AF, date of death, loss to follow-up, or to January 1st, 2014, whichever occurred first.

Methods S4. Assessment of cardiovascular risk factors

Information on current health status, medical history, medication, and life style was obtained using computerized questionnaires. Participants were interviewed at home by trained interviewers, and underwent clinical examination and laboratory blood sampling at the research center.(26, 27)

Body mass index (BMI) was calculated as weight (in kg) divided by height (in m) squared. Serum total and high-density lipoprotein (HDL) cholesterol were measured with an automated enzymatic method. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressures were calculated as the mean of the 2 consecutive measurements. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or use of antihypertensive drugs prescribed for hypertension.(3) Smoking information derived from baseline questionnaires was categorized into never, former, and current smokers. Diabetes mellitus (DM) was defined as fasting serum glucose levels ≥7.0

mmol/L (126 mg/dL) (or non-fasting serum glucose levels ≥11.1 mmol/L (200 mg/dL) if fasting samples were unavailable) or the use of antidiabetic therapy. The assessment and definition of history of coronary heart disease (CHD), and heart failure (HF) has been described in detail previously.(26) Left ventricular hypertrophy (LVH) on electrocardiogram (ECG) was diagnosed using the MEANS program with an algorithm that takes into account QRS voltages, with an age-dependent correction and repolarization. Medication use was derived from baseline questionnaires, pharmacy data and was categorized and defined according to the World Health Organization Anatomical Therapeutic Chemical (WHO ATC) classifications. More specifically, cardiac medication, antihypertensive medication, and lipid lowering medication were defined according to the WHO ATC categories c01, c02, and c10 respectively.

Table S1. Baseline characteristics of the total study population and stratified by sex

Baseline characteristics *	Total study population n=1,901	Men n=800	Women n=1,101	p ‡
Age, years	71.4 ± 7.5	71.0 ± 7.1	71.7 ± 7.7	0.022
Women, n (%)	1101 (57.9)	NA	1101 (100)	
Body mass index, kg/m^2	27.7 ± 4.1	27.6 ± 3.4	27.7 ± 4.6	0.582
Total serum cholesterol, mmol/L †	5.7 ± 1.0	5.4 ± 1.0	5.9 ± 1.0	<0.001
High-density lipoprotein cholesterol, mmol/L †	1.4 ± 0.4	1.3 ± 0.3	1.6 ± 0.4	<0.001
Hypertension, n (%)	1469 (77.3)	604 (75.5)	865 (78.6)	0.115
Smoking status				<0.001
Never, n (%)	572 (30.1)	112 (14.0)	460 (41.7)	
Former, n (%)	1031 (54.2)	545 (68.1)	486 (44.1)	
Current, n (%)	298 (15.7)	143 (17.9)	155 (14.1)	
History of diabetes mellitus, n (%)	248 (13.0)	129 (16.1)	119 (10.8)	0.001
History of coronary heart disease, n (%)	188 (9.9)	121 (15.1)	67 (6.1)	<0.001
History of heart failure, n (%)	83 (4.4)	37 (4.6)	46 (4.2)	0.638
Left ventricular hypertrophy, n (%)	95 (5.0)	50 (6.3)	45 (4.1)	0.033
Cardiac medication, n (%)	168 (8.8)	76 (9.5)	92 (8.4)	0.100
Lipid lowering medication, n (%)	419 (22.0)	191 (23.9)	228 (20.7)	0.386

Abbreviations: n, number; NA, not applicable.

The table presents baseline characteristics of the total study population for the analyses of incident atrial fibrillation

* Values are mean (standard deviation) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

‡ Statistical significance for continuous variables was tested using the Student's T-test (normal distribution) or the Mann Whitney U-test (skewed distribution) and for categorical variables was tested using the Chi-Square test.

Table S2. MicroRNAs nominally associated with the odds of prevalent atrial fibrillation in the total study population and stratified by sex

Total study population			Men			Women		
miRNA	**OR (95% CI) ***	**p**	**miRNA**	**OR (95% CI) ***	**p**	**miRNA**	**OR (95% CI) ***	**p**
miR-122-5p	1.42 (1.12-1.78)	0.002967	miR-4798-3p	0.39 (0.24-0.66)	0.000248	miR-1273h-5p	2.55 (1.36-4.89)	0.004270
miR-8078	1.54 (1.16-2.06)	0.003384	miR-122-5p	1.77 (1.28-2.46)	0.000539	miR-6788-3p	0.55 (0.37-0.86)	0.004616
miR-194-5p	1.82 (1.21-2.74)	0.004300	miR-4798-5p	0.47 (0.30-0.77)	0.001531	miR-1273d	3.06 (1.45-6.82)	0.004675
miR-1322	2.01 (1.24-3.32)	0.005358	miR-605-3p	0.50 (0.32-0.80)	0.002291	miR-1304-3p	5.56 (1.74-18.97)	0.004847
miR-378e	0.49 (0.30-0.83)	0.006478	miR-4784	0.48 (0.30-0.82)	0.003185	miR-548ay-5p	4.81 (1.65-15.27)	0.005846
miR-1254	1.77 (1.17-2.69)	0.007442	miR-192-5p	2.38 (1.35-4.29)	0.003233	miR-8078	1.97 (1.21-3.32)	0.008302
miR-574-3p	1.99 (1.19-3.31)	0.008025	miR-4721	0.57 (0.39-0.86)	0.003958	miR-1322	3.04 (1.36-7.10)	0.008354
miR-4721	0.66 (0.50-0.92)	0.008546	miR-194-5p	2.29 (1.29-4.11)	0.005029	miR-1231	0.53 (0.33-0.87)	0.009599
miR-6852-5p	0.54 (0.34-0.88)	0.009831	miR-4512	2.74 (1.37-5.70)	0.005538	miR-574-3p	2.94 (1.24-6.98)	0.014333
miR-4798-5p	0.60 (0.42-0.91)	0.010017	miR-6747-3p	2.41 (1.30-4.68)	0.007422	miR-5585-3p	2.36 (1.22-4.84)	0.014432
miR-1909-3p	1.51 (1.10-2.07)	0.010265	miR-6794-5p	0.15 (0.03-0.67)	0.012026	miR-6797-5p	0.64 (0.45-0.95)	0.017631
miR-204-3p	0.36 (0.16-0.80)	0.011161	miR-2116-5p	0.42 (0.22-0.89)	0.012516	miR-6500-3p	1.89 (1.15-3.37)	0.020219
miR-1285-5p	1.58 (1.11-2.27)	0.012415	miR-125a-5p	0.41 (0.21-0.87)	0.015047	miR-1285-5p	2.06 (1.14-3.89)	0.020352
miR-1273d	1.71 (1.13-2.65)	0.013537	miR-3124-3p	0.43 (0.22-0.88)	0.016097	miR-1254	2.30 (1.13-4.71)	0.021772
miR-4512	1.84 (1.16-3.03)	0.013550	miR-3667-5p	0.48 (0.28-0.92)	0.016510	miR-1273e	2.06 (1.13-3.88)	0.022274
miR-5585-3p	1.63 (1.11-2.43)	0.014015	miR-4723-3p	0.39 (0.18-0.89)	0.017808	miR-378e	0.43 (0.21-0.94)	0.024289
miR-192-5p	1.64 (1.11-2.45)	0.014723	miR-335-5p	0.54 (0.32-0.91)	0.020447	miR-566	2.73 (1.19-6.91)	0.025762
miR-1304-3p	2.22 (1.17-4.30)	0.016146	miR-10b-5p	0.45 (0.23-0.93)	0.021481	miR-4421	2.09 (1.14-4.16)	0.026404
miR-1255b2-3p	1.54 (1.08-2.18)	0.017186	miR-3912-5p	0.36 (0.16-0.91)	0.022020	miR-1255b2-3p	1.95 (1.08-3.51)	0.026521
miR-4784	0.61 (0.41-0.95)	0.019058	miR-4734	2.16 (1.08-4.07)	0.022058	miR-1273a	2.23 (1.13-4.68)	0.026830
miR-616-3p	1.62 (1.10-2.48)	0.019789	miR-6715b-3p	0.42 (0.20-0.91)	0.023262	miR-213p	0.47 (0.25-0.94)	0.027541
miR-4421	1.54 (1.08-2.23)	0.019957	miR-30a-5p	0.34 (0.14-0.89)	0.023364	miR-1273c	2.49 (1.10-5.65)	0.028319
miR-1273h-5p	1.54 (1.07-2.25)	0.020961	miR-3687	1.98 (1.05-3.54)	0.027593	miR-1269b	2.10 (1.13-4.25)	0.028337
miR-29c-5p	0.69 (0.51-0.96)	0.021375	miR-4713-3p	0.63 (0.42-0.98)	0.030730	miR-548d-5p	2.18 (1.08-4.50)	0.031964
miR-3687	1.67 (1.06-2.58)	0.022307	miR-1909-3p	1.58 (1.05-2.41)	0.030829	miR-3674	2.31 (1.09-5.07)	0.032957
miR-141-3p	0.51 (0.29-0.93)	0.022368	miR-100-5p	1.99 (1.09-4.00)	0.031450	miR-3135a	2.26 (1.06-4.83)	0.035470
miR-6794-5p	0.28 (0.09-0.85)	0.023037	miR-670-3p	0.38 (0.16-0.95)	0.032241	miR-4539	2.03 (1.05-3.98)	0.037016
miR-6788-3p	0.70 (0.52-0.97)	0.023570	miR-920	0.46 (0.23-0.96)	0.032879	miR-616-3p	2.19 (1.10-4.89)	0.039789

miR-3674	1.63 (1.07-2.51)	0.024082	miR-4695-5p	1.70 (1.01-2.74)	0.034056	miR-765	0.57 (0.34-1.02)	0.041683
miR-566	1.74 (1.08-2.87)	0.026028	miR-133a-5p	0.45 (0.22-0.99)	0.036036	miR-1539	2.02 (1.12-4.30)	0.041994
miR-765	0.64 (0.43-0.96)	0.027041	miR-6761-5p	0.52 (0.29-0.99)	0.037263	miR-4459	2.55 (0.98-6.29)	0.048123
miR-4753-5p	0.49 (0.27-0.95)	0.027462	miR-4543p	0.59 (0.36-0.99)	0.039182			
miR-5684	1.41 (1.05-1.97)	0.029441	miR-4319	0.55 (0.32-1.01)	0.039455			
miR-1273e	1.49 (1.04-2.15)	0.030004	miR-1365p	0.81 (0.66-1.00)	0.040095			
miR-7106-5p	0.71 (0.52-0.98)	0.032349	miR-193a-5p	0.49 (0.25-0.96)	0.040877			
miR-4798-3p	0.63 (0.42-0.99)	0.034433	miR-362-5p	0.64 (0.43-1.00)	0.041871			
miR-6877-3p	2.12 (1.06-4.31)	0.034941	miR-6791-5p	1.48 (1.01-2.15)	0.043547			
miR-6716-3p	0.57 (0.34-0.99)	0.035978	miR-155-5p	0.59 (0.36-1.00)	0.043632			
miR-4426	0.76 (0.59-0.99)	0.036714	miR-548ay-5p	0.58 (0.36-1.04)	0.045788			
miR-3667-5p	0.63 (0.42-1.01)	0.038123	miR-2116-3p	0.39 (0.16-1.05)	0.045877			
miR-1228-3p	1.93 (1.04-3.66)	0.041108	miR-196b-3p	0.51 (0.26-1.00)	0.047484			
miR-1303	1.58 (1.02-2.47)	0.041760	miR-4722-3p	1.86 (1.00-3.39)	0.047545			
miR-1273c	1.62 (1.01-2.57)	0.042953	miR-217	0.49 (0.25-1.03)	0.048351			
miR-6887-5p	0.71 (0.51-0.99)	0.043152	let-7d-3p	0.49 (0.24-1.00)	0.048457			
miR-1269b	1.44 (1.03-2.08)	0.043577	miR-6887-5p	0.64 (0.41-1.00)	0.049277			
miR-6747-3p	1.53 (1.02-2.36)	0.047232						
miR-30a-5p	0.48 (0.23-1.02)	0.049055						

Abbreviations: CI, confidence interval; OR, odds ratio.

* Adjusted for age, cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Table S3. MicroRNAs nominally associated with the risk of incident atrial fibrillation in the total study population and stratified by sex

Total study population			Men			Women		
miRNA	**HR (95% CI)** *	**p**	**miRNA**	**HR (95% CI)** *	**p**	**miRNA**	**HR (95% CI)** *	**p**
miR-197-3p	0.58 (0.40-0.84)	0.003531	miR-378e	0.43 (0.26-0.71)	0.001137	miR-197-3p	0.47 (0.29-0.79)	0.003940
miR-548w	1.38 (1.10-1.74)	0.006042	miR-6769b-3p	4.22 (1.51-11.74)	0.005900	miR-181b-5p	0.56 (0.37-0.84)	0.005111
miR-6765-5p	1.54 (1.08-2.20)	0.017530	miR-1290	0.67 (0.49-0.90)	0.008000	miR-185-3p	0.78 (0.64-0.94)	0.010826
miR-6894-5p	0.75 (0.59-0.97)	0.025632	miR-7111-3p	0.69 (0.52-0.91)	0.008684	miR-181d-5p	0.81 (0.68-0.96)	0.017475
miR-548b-5p	1.24 (1.02-1.51)	0.033402	miR-5006-5p	2.61 (1.24-5.49)	0.011750	miR-548w	1.53 (1.08-2.18)	0.018272
miR-6126	1.17 (1.01-1.36)	0.033909	miR-4270	0.58 (0.38-0.89)	0.013263	miR-561-5p	1.98 (1.11-3.53)	0.019960
miR-3197	0.89 (0.79-0.99)	0.036662	miR-4758-5p	0.61 (0.40-0.92)	0.019000	miR-6765-5p	1.82 (1.06-3.12)	0.028780
miR-324-3p	0.70 (0.49-0.98)	0.038061	miR-532-5p	0.65 (0.46-0.93)	0.019790	miR-3689a-3p	0.81 (0.67-0.98)	0.031271
miR-1307-5p	1.32 (1.01-1.73)	0.039834	miR-378a-3p	0.53 (0.31-0.91)	0.021570	miR-204-3p	2.50 (1.08-5.77)	0.032405
miR-17-3p	0.69 (0.48-0.99)	0.041655	miR-6500-3p	0.80 (0.66-0.97)	0.023000	miR-125a-5p	0.55 (0.31-0.97)	0.038775
miR-6715b-3p	1.57 (1.02-2.43)	0.041864	miR-7978	1.74 (1.07-2.81)	0.024209	miR-4676-3p	1.54 (1.02-2.33)	0.041641
miR-1287-5p	1.23 (1.01-1.51)	0.042095	miR-4279	2.20 (1.09-4.44)	0.027113	miR-3169	1.41 (1.01-1.96)	0.042371
miR-4800-5p	0.73 (0.54-0.99)	0.044090	miR-6892-3p	1.82 (1.07-3.10)	0.027160	miR-324-3p	0.60 (0.36-0.99)	0.043760
miR-4522	1.32 (1.01-1.72)	0.044800	miR-3157-3p	2.39 (1.08-5.29)	0.031678			
miR-658	1.40 (1.01-1.94)	0.044996	miR-2115-3p	3.15 (1.10-9.02)	0.032931			
miR-4449	0.72 (0.52-0.99)	0.045036	miR-299-5p	1.54 (1.03-2.29)	0.034224			
miR-93-5p	0.71 (0.51-0.99)	0.046484	miR-4646-3p	0.75 (0.57-0.98)	0.035213			
			miR-149-3p	1.42 (1.02-1.98)	0.035383			
			miR-122-5p	0.75 (0.57-0.98)	0.036170			
			miR-2276-3p	1.62 (1.03-2.55)	0.037520			
			miR-4644	1.30 (1.02-1.66)	0.037586			
			miR-658	1.64 (1.03-2.62)	0.038120			
			miR-6742-5p	2.05 (1.04-4.06)	0.039417			
			miR-4309	2.05 (1.01-4.16)	0.046510			
			miR-1287-5p	1.34 (1.00-1.79)	0.046800			
			miR-1229-3p	0.81 (0.66-1.00)	0.047938			

Abbreviations: CI, confidence interval; HR, hazard ratio.

* Adjusted for age, cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering

medication.

Table S4. Predicted target genes of microR-4798-3p that have been previously associated with atrial fibrillation and their potential remodeling mechanism

Predicted target gene	Target gene name	Remodeling mechanism
CACNB2	Calcium channel, voltage-dependent, beta 2 subunit.(43)	Electrical.
KCNN3	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3.(44)	Electrical.
SIRT1	Sirtuin 1.(45)	Structural.
STAT3	Signal transducer and activator of transcription 3.(46, 47)	Structural.
SLIT3	Slit homolog 3.(48)	Structural.
NAV2	Neuron navigator 2.(48)	Structural.
MYOCD	Myocardin.(48)	Structural.
PHLDA1	Pleckstrin homology-like domain, family A, member 1.(38)	Structural.
REEP1	Receptor accessory protein 1.(38)	Structural.
FAM13B	Family with sequence similarity 13, member B.(38)	Structural.
ATXN1	Ataxin 1-like.(38)	Structural.
UST	Uronyl-2-sulfotransferase.(38)	Structural.
CDK6	Cyclin-dependent kinase 6.(38)	Structural.
CAV2	Caveolin 2.(38)	Structural.
XPO7	Exportin 7.(38)	Structural.
FBXO32	F-box protein 32.(38)	Structural.
FUT11	Fucosyltransferase 11.(38)	Structural.
ASAH1	N-acylsphingosine amidohydrolase 1.(38)	Structural.
ORMDL3	ORM1-like 3.(38)	Structural.
WNT3	Wingless-type MMTV integration site family, member 3.(38)	Structural.
USP36	Ubiquitin specific peptidase 36.(38)	Structural.

Table S5. Circulatory microRNAs reported in previous literature in association with atrial fibrillation

miRNA	Study design	Study population	Characteristics	Reported effect estimates	Model and adjustments
let-7b-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.48, 95% CI: 0.38-0.61 ($p=3.6x10^{-10}$) Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
let-7b-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.27, 95% CI: 1.02-1.59 (p=0.034).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
let-7c-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.47, 95% CI: 0.37-0.59 ($p=2.5x10^{-10}$)	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent

		treatment and that underwent ablation if needed (no AF vs. AF).		Bonferroni p=0.0006 (0.05/86 miRNAs).	heart failure, and myocardial infarction.
let-7d-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.29, 95% CI: 1.04-1.6 (p=0.023).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
RNU48-a2	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	1.10-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 1.29, 95% CI: 1.08-1.53 (p=0.005) Model 2: OR: 1.28, 95% CI: 1.03-1.59 (p=0.027).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive

				Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-1	Prospective patient-based cohort study.(23)	Patients that underwent on-pump CABG without history of AF were monitored for POAF.	n=42 patients (16.7% POAF), mean age 65.0 ± 1.3 years, 26.2% women. Follow-up to 7 days after surgery by continuous ECG monitoring.	No difference in plasma concentrations between POAF vs. SR (p=NS).	NA.
miR-1	Cross-sectional Patient-based case-control study.(22)	Patients that underwent ablation for AF were matched with WPW patients as controls.	n=40 patients (75.0% AF), mean age 63.7 ± 2.3 years, 30.0% women.	No difference in plasma concentrations between AF vs. SR (p=NS).	NA.
miR-10b-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.49, 95% CI: 0.39-0.62 ($p=1.9x10^{-9}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.

miR-15	Cross-sectional Patient-based case-control study.(16)	Patients from outpatient clinic (no AF vs. AF no HF vs. no AF with HF and vs. AF, HF).	n=95 patients (34.7% AF), mean age 66.4 ± 1.7 years, 18.9% women.	No difference in plasma concentrations between AF vs. SR (p=NS). Plasma concentrations were not associated with prevalent AF. Beta: 1.9, 95% CI: -1.3 - 5.1 (p=0.23).	Multivariable linear regression. Model: age, sex, coronary artery disease, hypertension, diabetes mellitus, hypercholesterolemia, use of angiotensin converting enzyme inhibitor or AT1-receptor blocker, aldosterone antagonists, and/or statins.
miR-15b-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.31, 95% CI: 1.08-1.59 (p=0.007).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.

miR-17-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.24, 95% CI: 1.03-1.5 (p=0.023).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-19a-3p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.18, 95% CI: 1-1.39 (p=0.048).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.

miR-20a-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.36, 95% CI: 1.14-1.61 (p=0.001).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-20a-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with incident AF. HR: 0.05, 95% CI: 0.05-0.05 (p=0.05).	Multivariable Cox regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.

miR-21	Cross-sectional Patient-based case-control study.(16)	Patients from outpatient clinic (no AF vs. AF no HF vs. no AF with HF and vs. AF, HF).	n=95 patients (34.7% AF), mean age 66.4 ± 1.7 years, 18.9% women.	61% decreased plasma concentrations in AF vs. SR (p=0.041). Plasma concentrations associated with prevalent AF. Beta: -3.7, 95% CI: -7.3 - -0.2 (p=0.041).	Multivariable linear regression. Model: age, sex, coronary artery disease, hypertension, diabetes mellitus, hypercholesterolemia, use of angiotensin converting enzyme inhibitor or AT1-receptor blocker, aldosterone antagonists, and/or statins.
miR-21-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	2.1-fold decreased plasma concentrations in AF vs. SR (p=<0.05). Plasma concentrations associated with prevalent AF. OR: 0.51, 95% CI: 0.41-0.63 ($p=10.0x10^{-10}$) Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction
miR-23b-3p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.28, 95% CI: 1.02-1.6 (p=0.032).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.

					Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-24-3p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.45, 95% CI: 0.36-0.58 ($p=1.5x10^{-10}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-26a	Cross-sectional Patient-based case-control study.(22)	Patients that underwent ablation for AF were matched with no AF controls.	n=40 patients (75.0% AF), mean age 63.7 ± 2.3 years, 30.0% women.	No difference in plasma concentrations between AF vs. SR (p=NS).	NA.
miR-26a-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.28, 95% CI: 1.07-1.54 (p=0.007).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147

					(0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-26a-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with incident AF. HR: 1.16, 95% CI: 1.02-1.32 (p=0.026).	Multivariable Cox regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-27b-3p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.23, 95% CI: 1.01-1.51 (p=0.041).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147

					(0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-28-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	1.09-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 1.15, 95% CI: 1.05-1.26 (p=0.002) Model 2: OR: 1.08, 95% CI: 0.98-1.19 (p=0.14). Bonferroni p=2.0×10−4 (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-29a-3p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.47, 95% CI: 0.37-0.60 ($p=2.5x10^{-10}$) Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.

		ablation if needed (no AF vs. AF).			
miR-29b	Cross-sectional Patient-based case-control study.(16)	Patients from outpatient clinic (no AF vs. AF no HF vs. no AF with HF and vs. AF, HF).	n=95 patients (34.7% AF), mean age 66.4 ± 1.7 years, 18.9% women.	54% decreased plasma concentrations in AF vs. SR (p=<0.001). Plasma concentrations associated with prevalent AF. Beta: -10.5, 95% CI: -17.9 - -3.1 (p=0.007).	Multivariable linear regression. Model: age, sex, coronary artery disease, hypertension, diabetes mellitus, hypercholesterolemia, use of angiotensin converting enzyme inhibitor or AT1-receptor blocker, aldosterone antagonists, and/or statins.
miR-29b-2-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.99-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.84, 95% CI: 0.73-0.97 (p=0.02) Model 2: OR: 0.83, 95% CI: 0.72-0.97 (p=0.016). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure,

					myocardial infarction, and diabetes mellitus.
miR-30a-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.25, 95% CI: 1.05-1.49 (p=0.013).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-30c-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.41, 95% CI: 0.32-0.54 ($p=8.0x10^{-11}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-31-3p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years,	0.98-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations	Multivariable logistic regression. Model 1: age, sex, miR-processing.

			56.0% women. Median follow-up 5.4 years.	associated with prevalent AF. Model 1: OR: 0.69, 95% CI: 0.54-0.89 (p=0.004) Model 2: OR: 0.69, 95% CI: 0.54-0.90 (p=0.005). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-93-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.28, 95% CI: 1.06-1.55 (p=0.011).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-99b-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.64, 95% CI: 0.53-0.77 ($p=1.980\times10^{-6}$).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent

		treatment and that underwent ablation if needed (no AF vs. AF).		Bonferroni p=0.0006 (0.05/86 miRNAs).	heart failure, and myocardial infarction.
miR-99b-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	1.07-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 1.12, 95% CI: 1.04-1.20 (p=0.003) Model 2: OR: 1.05, 95% CI: 0.96-1.16 (p=0.27). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-100-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.42, 95% CI: 0.33-0.54 ($p=4.5x10^{-12}$) Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-106b-5p	Prospective population-	Participants who were enrolled in	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean	Plasma concentrations associated with prevalent AF.	Multivariable logistic regression. Model: age, sex, current smoking,

	based cohort study.(24)	Framingham Heart Study.	age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	OR: 1.36, 95% CI: 1.13-1.63 (p=0.001).	diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-106b-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with incident AF. HR: 1.16, 95% CI: 1.02-1.33 (p=0.029).	Multivariable Cox regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.

miR-122-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.55, 95% CI: 0.46-0.67 ($p=6.3x10^{-10}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-125a-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.47, 95% CI: 0.38-0.58 ($p=5.0x10^{-12}$) Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-125b-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.50, 95% CI: 0.41-0.62 ($p=5.7x10^{-11}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction
miR-126	Cross-sectional Patient-based case-control study.(25)	Patients admitted to the department of Cardiology (no AF vs. AF no HF vs. no AF with HF and vs. AF, HF).	n=135 patients (52.6% AF), mean age 63.9 ± 15.1 years, 48.1% women.	3.4-fold decreased plasma concentrations in AF vs. SR (p=<0.01).	NA.

miR-126-3p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.45, 95% CI: 0.35-0.57 ($p=8.0x10^{-11}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-126-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.32, 95% CI: 1.1-1.58 (p=0.003).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-133a	Prospective patient-based cohort study.(23)	Patients that underwent on-pump CABG without history of AF were monitored for POAF.	n=42 patients (16.7% POAF), mean age 65.0 ± 1.3 years, 26.2% women. Follow-up to 7 days after surgery by continuous ECG monitoring.	No difference in plasma concentrations between POAF vs. SR (p=NS).	NA.

miR-133a	Cross-sectional Patient-based case-control study.(16)	Patients from outpatient clinic (no AF vs. AF no HF vs. no AF with HF and vs. AF, HF).	n=95 patients (34.7% AF), mean age 66.4 ± 1.7 years, 18.9% women.	No difference in plasma concentrations between AF vs. SR (p=NS). Plasma concentrations were not associated with prevalent AF. Beta: -0.86, 95% CI: -1.88 - 0.15 (p=0.09).	Multivariable linear regression. Model: age, sex, coronary artery disease, hypertension, diabetes mellitus, hypercholesterolemia, use of angiotensin converting enzyme inhibitor or AT1-receptor blocker, aldosterone antagonists, and/or statins.
miR-133a	Cross-sectional Patient-based case-control study.(22)	Patients that underwent ablation for AF were matched with WPW patients as controls.	n=40 patients (75.0% AF), mean age 63.7 ± 2.3 years, 30.0% women.	No difference in plasma concentrations between AF vs. SR (p=NS).	NA.
miR-134	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.97-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.86, 95% CI: 0.76-0.97 (p=0.013).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current

				Model 2: OR: 0.85, 95% CI: 0.75-0.96 (p=0.009). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-140-3p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.31, 95% CI: 1.05-1.63 (p=0.015).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-146a-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.38, 95% CI: 0.29-0.50 ($p=1.3x10^{-11}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.

miR-148b-3p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.46, 95% CI: 0.36-0.59 ($p=5.9x10^{-10}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-150-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	2.3-fold decreased plasma concentrations in AF vs. SR (p=<0.05). Plasma concentrations associated with prevalent AF. OR: 0.51, 95% CI: 0.41-0.63 ($p=1.7x10^{-10}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction
miR-150-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	1.10-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 1.29, 95% CI: 1.11-1.50 (p=0.001). Model 2: OR: 1.24, 95% CI: 1.04-1.49 (p=0.018).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive

				Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-150-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.21, 95% CI: 1.04-1.41 (p=0.013).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.

miR-151a-3p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.99-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.87, 95% CI: 0.78-0.98 (p=0.019). Model 2: OR: 0.88, 95% CI: 0.78-0.99 (p=0.031). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-152	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.97-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.87, 95% CI: 0.79-0.95 (p=0.001). Model 2: OR: 0.89, 95% CI: 0.81-0.98 (p=0.015). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.

miR-182-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.94-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 0.91, 95% CI: 0.85-0.97 (p=0.003). Model 2: OR: 0.93, 95% CI: 0.87-1.00 (p=0.04). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-186-5p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.28, 95% CI: 1.02-1.61 (p=0.340).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.

miR-193a-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.98-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.87, 95% CI: 0.77-0.98 (p=0.018). Model 2: OR: 0.87, 95% CI: 0.77-0.98 (p=0.024). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-196b-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.97-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 0.84, 95% CI: 0.75-0.95 (p=0.005). Model 2: OR: 0.83, 95% CI: 0.73-0.94 (p=0.004). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.

miR-199a-3p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.26, 95% CI: 1.04-1.53 (p=0.019).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-200c-3p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.96-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.88, 95% CI: 0.80-0.98 (p=0.017). Model 2: OR: 0.89, 95% CI: 0.80-0.98 (p=0.022). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.

miR-221-3p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.49, 95% CI: 0.39-0.61 ($p=2.3x10^{-10}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-221-3p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.99-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.86, 95% CI: 0.76-0.96 (p=0.01). Model 2: OR: 0.85, 95% CI: 0.76-0.97 (p=0.012). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.

miR-223-3p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.48, 95% CI: 0.39-0.60 ($p=5.9x10^{-11}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-324-3p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with incident AF. HR: 1.19, 95% CI: 1.03-1.36 (p=0.016).	Multivariable Cox regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-328	Cross-sectional Patient-based case-control study.(22)	Patients that underwent ablation for AF were matched with WPW patients as controls.	n=40 patients (75.0% AF), mean age 63.7 ± 2.3 years, 30.0% women.	No difference in plasma concentrations between AF vs. SR (p=NS).	NA.

miR-328	Cross-sectional patient-based case-control study.(19)	Patients that underwent ablation for AF were age-sex matched with no AF controls.	n=200 patients (50.0% AF), mean age 53.1 ± 12.2 years, 28.0% women.	37% decreased pre-operative plasma concentrations in AF vs. SR (p=0.217).	NA.
miR-328	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	1.13-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 1.21, 95% CI: 1.09-1.33 (p=0.00018). Model 2: OR: 1.14, 95% CI: 1.02-1.28 (p=0.017). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-331-3p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	1.09-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 1.18, 95% CI: 1.06-1.31 (p=0.002).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current

				Model 2: OR: 1.14, 95% CI: 1.01-1.28 (p=0.035). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-339-5p	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	1.04-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with prevalent AF. Model 1: OR: 1.13, 95% CI: 1.04-1.23 (p=0.006). Model 2: OR: 1.10, 95% CI: 1.00-1.21 (p=0.05). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-342-3p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.31, 95% CI: 0.23-0.43 ($p=2.4x10^{-12}$) Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.

miR-363-3p	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with incident AF. HR: 1.17, 95% CI: 1.01-1.36 (p=0.043).	Multivariable Cox regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-375	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. Model: OR: 0.36, 95% CI: 0.26-0.50 ($p=2.7x10^{-10}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-375	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.99-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.83, 95%	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood

				CI: 0.72-0.96 (p=0.001). Model 2: OR: 0.82, 95% CI: 0.71-0.95 (p=0.007). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-409-3p	Cross-sectional patient-based case-control study.(19)	Patients that underwent ablation for AF were age-sex matched with no AF controls.	n=200 patients (50.0% AF), mean age 53.1 ± 12.2 years, 28.0% women.	54% decreased pre-operative plasma concentrations in AF vs. SR (p=<0.001). Plasma concentrations associated with prevalent AF. OR: 1.50, 95% CI: 1.02-2.22 (p=0.040).	Multivariable logistic regression. Model: age, gender, hypertension.
miR-411-5p	Prospective patient-based cohort study.(21)	Patients enrolled in the miRhythm Study who were evaluated for treatment and that underwent ablation if needed (no AF vs. AF).	n=211 patients (52.6% AF), mean age 58.6 ± 11.9 years, 41.7% women.	Plasma concentrations associated with prevalent AF. OR: 0.42, 95% CI: 0.31-0.56 ($p=4.3x10^{-9}$). Bonferroni p=0.0006 (0.05/86 miRNAs).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction.
miR-432	Cross-sectional patient-based case-control study.(19)	Patients that underwent ablation for AF were age-sex matched with no AF controls.	n=200 patients (50.0% AF), mean age 53.1 ± 12.2 years, 28.0% women.	68% decreased pre-operative plasma concentrations in AF vs. SR (p=<0.001). Plasma concentrations associated with prevalent	Multivariable logistic regression. Model: age, gender, hypertension.

				AF. OR: 1.63, 95% CI: 1.09-2.43 (p=0.018).	
miR-483-5p	Prospective patient-based cohort study.(17)	Patients that underwent on-pump CABG without history of AF were monitored for POAF.	n=34 patients (38.2% POAF), mean age 61.5 ± 11.8 years, 29.4% women. Follow-up from time of surgery to hospital discharge by continuous ECG monitoring.	Increased pre-operative plasma concentrations in AF vs. SR (p=0.0137).	NA.
miR-484	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4% women. Median follow-up 8.6 years.	Plasma concentrations associated with prevalent AF. OR: 1.3, 95% CI: 1.07-1.58 (p=0.007).	Multivariable logistic regression. Model: age, sex, current smoking, diabetes, prevalent heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-484	Prospective population-based cohort study.(24)	Participants who were enrolled in Framingham Heart Study.	n=1,788 participants (4.7% prevalent AF and 8.8% incident AF), mean age 66 ± 9 years, 54.4%	Plasma concentrations associated with incident AF. HR: 1.15, 95% CI: 1-1.32	Multivariable Cox regression. Model: age, sex, current smoking, diabetes, prevalent

			women. Median follow-up 8.6 years.	(p=0.049).	heart failure, and myocardial infarction. Bonferroni p=0.000147 (0.05/340 miRNAs). Model and Bonferroni correction were used in step 1 of the analyses to identify 73 microRNAs and these 73 miRNAs were used for the second analysis.
miR-590	Cross-sectional Patient-based case-control study.(22)	Patients that underwent ablation for AF were matched with WPW patients as controls.	n=40 patients (75.0% AF), mean age 63.7 ± 2.3 years, 30.0% women.	No difference in plasma concentrations between AF vs. SR (p=NS).	NA.
miR-720	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.99-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.84, 95% CI: 0.73-0.97 (p=0.02). Model 2: OR: 0.83, 95% CI: 0.72-0.97 (p=0.016).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use,

				Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	prevalent heart failure, myocardial infarction, and diabetes mellitus.
miR-1274b	Prospective population-based cohort study.(20)	Participants who were enrolled in Framingham Heart Study.	n=2,445 participants (6.2% prevalent AF and 6.6% incident AF), mean age 66.3 ± 8.9 years, 56.0% women. Median follow-up 5.4 years.	0.94-fold increased plasma concentrations in AF vs. SR (p=NR). Plasma concentrations associated with incident AF. Model 1: OR: 0.81, 95% CI: 0.69-0.96 (p=0.013). Model 2: OR: 0.83, 95% CI: 0.71-0.99 (p=0.033). Bonferroni $p=2.0\times10^{-4}$ (0.05/253 miRNAs).	Multivariable logistic regression. Model 1: age, sex, miR-processing. Model 2: age, sex, miR-processing, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, antihypertensive medication use, prevalent heart failure, myocardial infarction, and diabetes mellitus.

Abbreviations: AF, atrial fibrillation; CABG, coronary artery bypass graft; CHD, coronary heart disease; CI, confidence interval; HF, heart failure; HR, hazard ratio; n, number; NA, not available; NR, not reported; NS, not significant; OR, odds ratio; POAF, postoperative atrial fibrillation; SR, sinus rhythm.

Table S6. Association of previously-reported microRNAs with the odds of prevalent atrial fibrillation in our total study population and stratified by sex

Total study population			Men			Women		
miRNA	**OR (95% CI) ***	**p**	**miRNA**	**OR (95% CI) ***	**p**	**miRNA**	**OR (95% CI) ***	**p**
let-7b-5p	0.98 (0.51-1.87)	0.941	let-7b-5p	0.82 (0.35-1.99)	0.658	let-7b-5p	1.06 (0.36-3.18)	0.918
let-7c-5p	0.85 (0.41-1.82)	0.672	let-7c-5p	0.95 (0.35-2.79)	0.921	let-7c-5p	0.58 (0.20-1.92)	0.359
let-7d-5p	0.90 (0.51-1.67)	0.741	let-7d-5p	0.69 (0.34-1.48)	0.317	let-7d-5p	1.27 (0.46-3.54)	0.652
RNU48-a2	NA	NA	RNU48-a2	NA	NA	RNU48-a2	NA	NA
miR-1	NA	NA	miR-1	NA	NA	miR-1	NA	NA
miR-10b-5p	0.71 (0.43-1.27)	0.222	miR-10b-5p	0.45 (0.23-0.93)	0.022	miR-10b-5p	1.14 (0.47-3.20)	0.797
miR-15	NA	NA	miR-15	NA	NA	miR-15	NA	NA
miR-15b-5p	1.05 (0.62-1.80)	0.848	miR-15b-5p	0.96 (0.50-1.93)	0.914	miR-15b-5p	1.22 (0.50-2.97)	0.657
miR-17-5p	1.04 (0.56-1.95)	0.903	miR-17-5p	0.82 (0.38-1.82)	0.623	miR-17-5p	1.49 (0.52-4.17)	0.455
miR-19a-3p	0.85 (0.51-1.38)	0.514	miR-19a-3p	0.86 (0.45-1.64)	0.643	miR-19a-3p	0.80 (0.34-1.75)	0.592
miR-20a-5p	1.30 (0.68-2.58)	0.436	miR-20a-5p	0.98 (0.45-2.27)	0.958	miR-20a-5p	2.20 (0.69-7.18)	0.187
miR-21	NA	NA	miR-21	NA	NA	miR-21	NA	NA
miR-21-5p	1.06 (0.69-1.63)	0.783	miR-21-5p	1.18 (0.66-2.12)	0.575	miR-21-5p	0.92 (0.46-1.80)	0.806
miR-23b-3p	0.82 (0.47-1.43)	0.486	miR-23b-3p	0.86 (0.42-1.81)	0.687	miR-23b-3p	0.75 (0.30-1.86)	0.540
miR-24-3p	1.04 (0.60-1.79)	0.881	miR-24-3p	1.22 (0.59-2.58)	0.591	miR-24-3p	0.81 (0.33-1.90)	0.643
miR-26a	NA	NA	miR-26a	NA	NA	miR-26a	NA	NA
miR-26a-5p	0.78 (0.48-1.27)	0.311	miR-26a-5p	0.70 (0.38-1.31)	0.248	miR-26a-5p	0.87 (0.36-2.04)	0.744
miR-27b-3p	0.84 (0.48-1.49)	0.534	miR-27b-3p	0.74 (0.37-1.58)	0.411	miR-27b-3p	0.97 (0.37-2.52)	0.944
miR-28-5p	0.91 (0.57-1.49)	0.711	miR-28-5p	1.14 (0.61-2.20)	0.690	miR-28-5p	0.78 (0.35-1.76)	0.550
miR-29a-3p	0.67 (0.35-1.29)	0.227	miR-29a-3p	0.57 (0.24-1.43)	0.220	miR-29a-3p	0.60 (0.20-1.75)	0.368
miR-29b	NA	NA	miR-29b	NA	NA	miR-29b	NA	NA
miR-29b-2-5p	NA	NA	miR-29b-2-5p	NA	NA	miR-29b-2-5p	NA	NA
miR-30a-5p	0.48 (0.23-1.02)	0.049	miR-30a-5p	0.34 (0.14-0.89)	0.023	miR-30a-5p	0.58 (0.17-2.25)	0.418
miR-30c-5p	0.70 (0.37-1.36)	0.272	miR-30c-5p	0.55 (0.26-1.25)	0.140	miR-30c-5p	0.97 (0.31-3.37)	0.964
miR-31-3p	NA	NA	miR-31-3p	NA	NA	miR-31-3p	NA	NA

miR-93-5p	0.85 (0.53-1.36)	0.505	miR-93-5p	0.80 (0.42-1.48)	0.476	miR-93-5p	0.99 (0.44-2.15)	0.976
miR-99b-5p	0.99 (0.55-1.82)	0.971	miR-99b-5p	0.62 (0.28-1.42)	0.252	miR-99b-5p	1.93 (0.75-5.39)	0.189
miR-100-5p	1.50 (0.94-2.47)	0.092	miR-100-5p	1.99 (1.09-4.00)	0.032	miR-100-5p	0.88 (0.41-2.04)	0.748
miR-106b-5p	1.09 (0.61-1.95)	0.776	miR-106b-5p	0.93 (0.45-1.97)	0.843	miR-106b-5p	1.35 (0.51-3.50)	0.543
miR-122-5p	1.42 (1.12-1.78)	0.003	miR-122-5p	1.77 (1.28-2.46)	0.001	miR-122-5p	1.07 (0.71-1.59)	0.731
miR-125a-5p	0.80 (0.45-1.50)	0.478	miR-125a-5p	0.41 (0.21-0.87)	0.015	miR-125a-5p	2.07 (0.71-6.39)	0.194
miR-125b-5p	1.55 (0.96-2.61)	0.080	miR-125b-5p	1.59 (0.87-3.13)	0.129	miR-125b-5p	1.60 (0.64-4.11)	0.323
miR-126	NA	NA	miR-126	NA	NA	miR-126	NA	NA
miR-126-3p	0.78 (0.49-1.27)	0.294	miR-126-3p	0.74 (0.40-1.44)	0.354	miR-126-3p	0.69 (0.32-1.59)	0.372
miR-126-5p	0.85 (0.59-1.26)	0.401	miR-126-5p	0.75 (0.47-1.23)	0.239	miR-126-5p	0.96 (0.50-1.96)	0.905
miR-133a	NA	NA	miR-133a	NA	NA	miR-133a	NA	NA
miR-134	NA	NA	miR-134	NA	NA	miR-134	NA	NA
miR-140-3p	NA	NA	miR-140-3p	NA	NA	miR-140-3p	NA	NA
miR-146a-5p	0.89 (0.68-1.15)	0.369	miR-146a-5p	0.88 (0.61-1.25)	0.470	miR-146a-5p	0.89 (0.59-1.33)	0.572
miR-148b-3p	0.95 (0.64-1.42)	0.791	miR-148b-3p	0.84 (0.49-1.46)	0.528	miR-148b-3p	1.09 (0.57-2.10)	0.797
miR-150-5p	1.02 (0.63-1.65)	0.948	miR-150-5p	0.88 (0.46-1.76)	0.706	miR-150-5p	1.16 (0.55-2.52)	0.699
miR-151a-3p	0.80 (0.49-1.32)	0.381	miR-151a-3p	0.65 (0.33-1.29)	0.214	miR-151a-3p	0.93 (0.43-2.01)	0.850
miR-152	NA	NA	miR-152	NA	NA	miR-152	NA	NA
miR-182-5p	NA	NA	miR-182-5p	NA	NA	miR-182-5p	NA	NA
miR-186-5p	0.60 (0.32-1.17)	0.114	miR-186-5p	0.58 (0.26-1.43)	0.202	miR-186-5p	0.49 (0.17-1.51)	0.197
miR-193a-5p	0.64 (0.39-1.03)	0.066	miR-193a-5p	0.49 (0.25-0.96)	0.041	miR-193a-5p	0.76 (0.35-1.68)	0.494
miR-196b-5p	NA	NA	miR-196b-5p	NA	NA	miR-196b-5p	NA	NA
miR-199a-3p	0.78 (0.50-1.20)	0.259	miR-199a-3p	0.66 (0.36-1.19)	0.169	miR-199a-3p	0.96 (0.49-1.88)	0.915
miR-200c-3p	NA	NA	miR-200c-3p	NA	NA	miR-200c-3p	NA	NA
miR-221-3p	0.86 (0.59-1.25)	0.446	miR-221-3p	0.82 (0.49-1.38)	0.468	miR-221-3p	0.90 (0.49-1.55)	0.705
miR-223-3p	0.84 (0.57-1.23)	0.371	miR-223-3p	0.77 (0.46-1.29)	0.314	miR-223-3p	0.99 (0.53-1.86)	0.971
miR-324-3p	0.88 (0.53-1.43)	0.602	miR-324-3p	1.08 (0.57-2.03)	0.810	miR-324-3p	0.61 (0.26-1.43)	0.263
miR-328	NA	NA	miR-328	NA	NA	miR-328	NA	NA
miR-331-3p	0.85 (0.53-1.40)	0.515	miR-331-3p	0.83 (0.45-1.59)	0.571	miR-331-3p	0.99 (0.44-2.30)	0.982

miR-339-5p	0.78 (0.47-1.29)	0.342	miR-339-5p	0.80 (0.39-1.61)	0.537	miR-339-5p	0.83 (0.37-1.78)	0.644
miR-342-3p	1.07 (0.58-2.01)	0.827	miR-342-3p	0.67 (0.30-1.58)	0.350	miR-342-3p	1.67 (0.63-4.32)	0.296
miR-363-3p	0.86 (0.53-1.45)	0.548	miR-363-3p	0.62 (0.35-1.19)	0.131	miR-363-3p	1.27 (0.53-3.46)	0.615
miR-375	0.88 (0.72-1.08)	0.206	miR-375	0.85 (0.66-1.10)	0.222	miR-375	0.92 (0.65-1.34)	0.658
miR-409-3p	0.92 (0.77-1.12)	0.401	miR-409-3p	0.92 (0.73-1.18)	0.498	miR-409-3p	0.91 (0.68-1.26)	0.568
miR-411-5p	NA	NA	miR-411-5p	NA	NA	miR-411-5p	NA	NA
miR-432	NA	NA	miR-432	NA	NA	miR-432	NA	NA
miR-483-5p	NA	NA	miR-483-5p	NA	NA	miR-483-5p	NA	NA
miR-484	0.90 (0.53-1.51)	0.697	miR-484	0.83 (0.41-1.63)	0.605	miR-484	0.99 (0.41-2.27)	0.988
miR-590	NA	NA	miR-590	NA	NA	miR-590	NA	NA
miR-720	NA	NA	miR-720	NA	NA	miR-720	NA	NA
miR-1274b	NA	NA	miR-1274b	NA	NA	miR-1274b	NA	NA

Abbreviations: CI, confidence interval; n, number; NA, not available; OR, odds ratio.

***** Adjusted for age, cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

Table S7. Association of previously-reported microRNAs with the risk of incident atrial fibrillation in our total study population and stratified by sex

Total study population			Men			Women		
miRNA	**HR (95% CI)** *	**p**	**miRNA**	**HR (95% CI)** *	**p**	**miRNA**	**HR (95% CI)** *	**p**
let-7b-5p	0.82 (0.53-1.25)	0.353	let-7b-5p	0.97 (0.53-1.78)	0.916	let-7b-5p	0.63 (0.34-1.17)	0.145
let-7c-5p	0.89 (0.54-1.47)	0.641	let-7c-5p	0.97 (0.47-2.01)	0.943	let-7c-5p	0.74 (0.36-1.50)	0.396
let-7d-5p	0.93 (0.62-1.39)	0.704	let-7d-5p	0.93 (0.52-1.66)	0.812	let-7d-5p	0.90 (0.50-1.62)	0.736
RNU48-a2	NA	NA	RNU48-a2	NA	NA	RNU48-a2	NA	NA
miR-1	NA	NA	miR-1	NA	NA	miR-1	NA	NA
miR-10b-5p	0.85 (0.58-1.24)	0.406	miR-10b-5p	0.85 (0.48-1.51)	0.579	miR-10b-5p	0.81 (0.48-1.37)	0.432
miR-15	NA	NA	miR-15	NA	NA	miR-15	NA	NA
miR-15b-5p	1.02 (0.71-1.47)	0.903	miR-15b-5p	0.95 (0.57-1.57)	0.842	miR-15b-5p	1.10 (0.64-1.87)	0.738
miR-17-5p	0.76 (0.50-1.15)	0.193	miR-17-5p	0.71 (0.40-1.26)	0.239	miR-17-5p	0.79 (0.43-1.46)	0.454
miR-19a-3p	0.80 (0.57-1.12)	0.193	miR-19a-3p	0.67 (0.41-1.12)	0.125	miR-19a-3p	0.94 (0.59-1.50)	0.790
miR-20a-5p	0.94 (0.60-1.46)	0.772	miR-20a-5p	0.92 (0.49-1.71)	0.780	miR-20a-5p	0.88 (0.46-1.68)	0.691
miR-21	NA	NA	miR-21	NA	NA	miR-21	NA	NA
miR-21-5p	1.11 (0.83-1.48)	0.478	miR-21-5p	1.16 (0.76-1.76)	0.496	miR-21-5p	1.12 (0.76-1.66)	0.571
miR-23b-3p	0.88 (0.61-1.28)	0.512	miR-23b-3p	1.05 (0.62-1.79)	0.862	miR-23b-3p	0.73 (0.43-1.25)	0.252
miR-24-3p	0.85 (0.59-1.23)	0.390	miR-24-3p	0.94 (0.55-1.60)	0.813	miR-24-3p	0.76 (0.44-1.29)	0.302
miR-26a	NA	NA	miR-26a	NA	NA	miR-26a	NA	NA
miR-26a-5p	0.90 (0.64-1.27)	0.545	miR-26a-5p	0.99 (0.61-1.61)	0.961	miR-26a-5p	0.83 (0.50-1.36)	0.451
miR-27b-3p	0.79 (0.52-1.17)	0.238	miR-27b-3p	0.95 (0.52-1.78)	0.908	miR-27b-3p	0.62 (0.36-1.09)	0.096
miR-28-5p	1.05 (0.76-1.46)	0.763	miR-28-5p	1.25 (0.79-1.98)	0.343	miR-28-5p	0.90 (0.56-1.44)	0.656
miR-29a-3p	0.82 (0.52-1.29)	0.388	miR-29a-3p	0.98 (0.50-1.92)	0.955	miR-29a-3p	0.66 (0.34-1.26)	0.207
miR-29b	NA	NA	miR-29b	NA	NA	miR-29b	NA	NA
miR-29b-2-5p	NA	NA	miR-29b-2-5p	NA	NA	miR-29b-2-5p	NA	NA
miR-30a-5p	0.79 (0.45-1.40)	0.425	miR-30a-5p	1.13 (0.49-2.62)	0.771	miR-30a-5p	0.51 (0.23-1.12)	0.094
miR-30c-5p	0.84 (0.53-1.35)	0.477	miR-30c-5p	0.83 (0.44-1.58)	0.571	miR-30c-5p	0.83 (0.42-1.66)	0.599
miR-31-3p	NA	NA	miR-31-3p	NA	NA	miR-31-3p	NA	NA

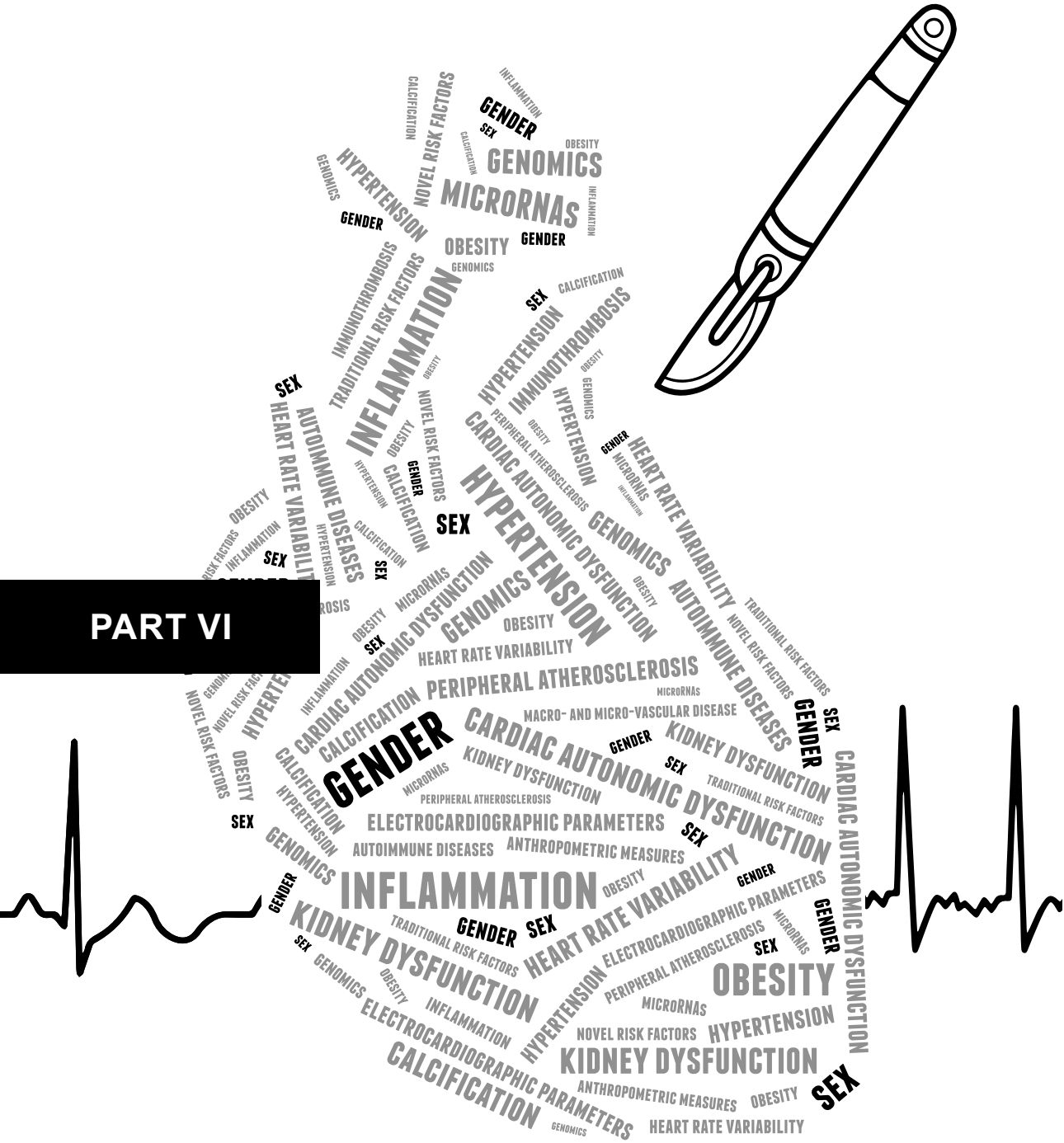
miR-93-5p	0.71 (0.51-1.00)	0.047	miR-93-5p	0.65 (0.40-1.04)	0.073	miR-93-5p	0.74 (0.46-1.20)	0.223
miR-99b-5p	0.81 (0.55-1.19)	0.284	miR-99b-5p	0.87 (0.47-1.63)	0.670	miR-99b-5p	0.73 (0.44-1.21)	0.218
miR-100-5p	0.87 (0.63-1.22)	0.427	miR-100-5p	0.99 (0.59-1.63)	0.958	miR-100-5p	0.78 (0.50-1.20)	0.251
miR-106b-5p	0.83 (0.55-1.23)	0.346	miR-106b-5p	0.77 (0.45-1.34)	0.358	miR-106b-5p	0.83 (0.46-1.50)	0.532
miR-122-5p	0.91 (0.76-1.09)	0.304	miR-122-5p	0.75 (0.57-0.98)	0.036	miR-122-5p	1.07 (0.84-1.37)	0.587
miR-125a-5p	0.70 (0.47-1.03)	0.071	miR-125a-5p	0.81 (0.46-1.45)	0.487	miR-125a-5p	0.55 (0.31-0.97)	0.039
miR-125b-5p	0.91 (0.63-1.31)	0.599	miR-125b-5p	1.04 (0.59-1.82)	0.905	miR-125b-5p	0.76 (0.46-1.24)	0.274
miR-126	NA	NA	miR-126	NA	NA	miR-126	NA	NA
miR-126-3p	0.77 (0.55-1.08)	0.124	miR-126-3p	0.87 (0.0.53-1.43)	0.587	miR-126-3p	0.65 (0.40-1.05)	0.078
miR-126-5p	0.89 (0.68-1.16)	0.369	miR-126-5p	0.88 (0.61-1.27)	0.486	miR-126-5p	0.91 (0.61-1.34)	0.618
miR-133a	NA	NA	miR-133a	NA	NA	miR-133a	NA	NA
miR-134	NA	NA	miR-134	NA	NA	miR-134	NA	NA
miR-140-3p	NA	NA	miR-140-3p	NA	NA	miR-140-3p	NA	NA
miR-146a-5p	0.94 (0.79-1.12)	0.493	miR-146a-5p	0.96 (0.74-1.24)	0.740	miR-146a-5p	0.92 (0.73-1.17)	0.516
miR-148b-3p	0.93 (0.71-1.22)	0.607	miR-148b-3p	0.92 (0.62-1.38)	0.696	miR-148b-3p	0.92 (0.64-1.33)	0.653
miR-150-5p	0.98 (0.71-1.36)	0.922	miR-150-5p	1.13 (0.70-1.82)	0.631	miR-150-5p	0.87 (0.55-1.36)	0.534
miR-151a-3p	0.80 (0.57-1.11)	0.178	miR-151a-3p	0.95 (0.58-1.57)	0.847	miR-151a-3p	0.68 (0.44-1.06)	0.091
miR-152	NA	NA	miR-152	NA	NA	miR-152	NA	NA
miR-182-5p	NA	NA	miR-182-5p	NA	NA	miR-182-5p	NA	NA
miR-186-5p	0.95 (0.59-1.54)	0.839	miR-186-5p	1.09 (0.53-2.23)	0.822	miR-186-5p	0.85 (0.43-1.66)	0.628
miR-193a-5p	0.93 (0.67-1.28)	0.641	miR-193a-5p	0.70 (0.45-1.10)	0.120	miR-193a-5p	1.16 (0.73-1.86)	0.533
miR-196b-5p	NA	NA	miR-196b-5p	NA	NA	miR-196b-5p	NA	NA
miR-199a-3p	0.99 (0.73-1.33)	0.919	miR-199a-3p	1.04 (0.67-1.62)	0.852	miR-199a-3p	0.96 (0.63-1.45)	0.832
miR-200c-3p	NA	NA	miR-200c-3p	NA	NA	miR-200c-3p	NA	NA
miR-221-3p	0.93 (0.73-1.19)	0.569	miR-221-3p	1.04 (0.72-1.51)	0.833	miR-221-3p	0.84 (0.60-1.18)	0.309
miR-223-3p	0.95 (0.73-1.24)	0.704	miR-223-3p	1.01 (0.69-1.46)	0.979	miR-223-3p	0.92 (0.63-1.33)	0.645
miR-324-3p	0.70 (0.50-0.98)	0.038	miR-324-3p	0.73 (0.45-1.18)	0.197	miR-324-3p	0.60 (0.36-0.99)	0.044
miR-328	NA	NA	miR-328	NA	NA	miR-328	NA	NA
miR-331-3p	0.96 (0.69-1.33)	0.805	miR-331-3p	1.25 (0.78-2.02)	0.360	miR-331-3p	0.75 (0.47-1.18)	0.215

miR-339-5p	1.06 (0.77-1.47)	0.719	miR-339-5p	1.07 (0.67-1.70)	0.778	miR-339-5p	1.02 (0.65-1.59)	0.943
miR-342-3p	0.95 (0.63-1.44)	0.809	miR-342-3p	1.02 (0.53-1.96)	0.958	miR-342-3p	0.83 (0.48-1.45)	0.521
miR-363-3p	0.80 (0.56-1.13)	0.198	miR-363-3p	0.75 (0.46-1.22)	0.243	miR-363-3p	0.79 (0.48-1.30)	0.355
miR-375	0.94 (0.81-1.08)	0.376	miR-375	0.84 (0.70-1.01)	0.061	miR-375	1.07 (0.86-1.32)	0.549
miR-409-3p	0.93 (0.82-1.06)	0.263	miR-409-3p	0.92 (0.76-1.12)	0.392	miR-409-3p	0.91 (0.76-1.08)	0.260
miR-411-5p	NA	NA	miR-411-5p	NA	NA	miR-411-5p	NA	NA
miR-432	NA	NA	miR-432	NA	NA	miR-432	NA	NA
miR-483-5p	NA	NA	miR-483-5p	NA	NA	miR-483-5p	NA	NA
miR-484	0.78 (0.53-1.13)	0.192	miR-484	0.78 (0.45-1.35)	0.373	miR-484	0.73 (0.44-1.21)	0.218
miR-590	NA	NA	miR-590	NA	NA	miR-590	NA	NA
miR-720	NA	NA	miR-720	NA	NA	miR-720	NA	NA
miR-1274b	NA	NA	miR-1274b	NA	NA	miR-1274b	NA	NA

Abbreviations: CI, confidence interval; HR, hazard ratio; n, number; NA, not available.

***** Adjusted for age, cohort, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, smoking status, history of diabetes, history of coronary heart disease, history of heart failure, left ventricular hypertrophy on the electrocardiogram, use of cardiac medication, and use of lipid lowering medication.

PART VI


GENDER
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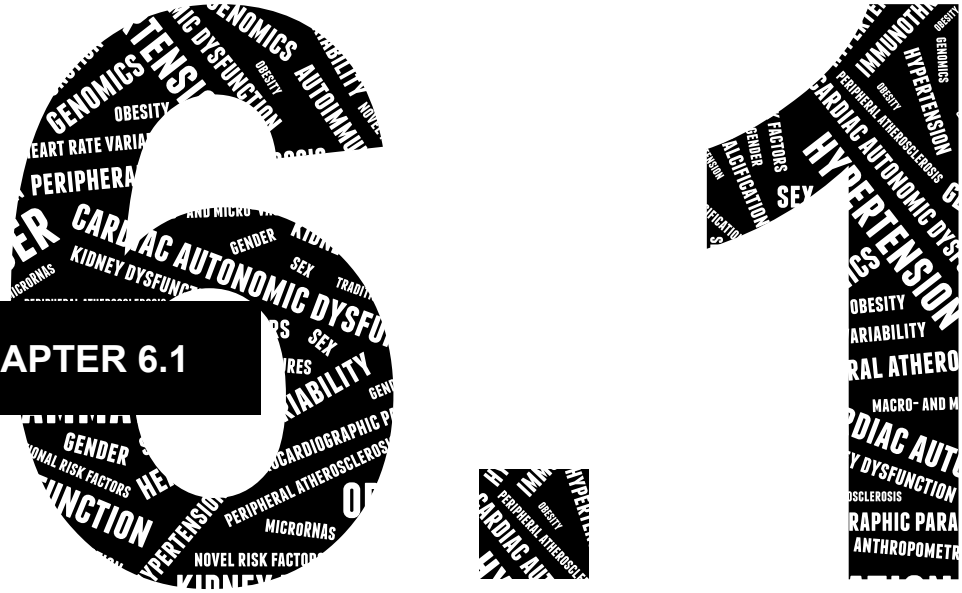
NOVEL RISK FACTORS
CALCIFICATION
IMMUNOTHROMBOSIS
TRADITIONAL RISK FACTORS
INFLAMMATION
HEART RATE VARIABILITY
AUTOIMMUNE DISEASES
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
MACRO- AND MICRO-VASCULAR DISEASE
KIDNEY DYSFUNCTION
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC MEASURES

Sex and gender implications and the risk of atrial fibrillation


CHAPTER 6.1

Women-specific risk factors and the risk of atrial fibrillation

Association between sex-specific risk factors and risk of new-onset atrial fibrillation among women.

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ABSTRACT

Background

Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide with differences in epidemiology and pathophysiological processes for women vs. men and a poorer prognosis for women. Further investigation of sex-specific risk factors associated with AF development in women is warranted.

Methods

This population-based cohort study obtained data from the 2006 to 2010 UK Biobank study, a cohort of more than 500 000 participants aged 40 to 69 years. 235,191 women without AF and history of hysterectomy and/or bilateral oophorectomy at baseline were included. Women-specific risk factors, including age at menarche, irregular menstrual cycle, menopause status, age at menopause, years after menopause, age at first birth, number of live births, and total reproductive years were also included. Cox proportional hazards models, adjusted for cardiovascular risk factors, were used to investigate the linear and potential non-linear associations between sex-specific risk factors and the risk of new-onset AF in women.

Results

The mean (standard deviation) age was 55.7 (8.1) years. During a median follow-up period of 11.6 years, 4,629 (2%) women experienced new-onset AF. In multivariable-adjusted models, having irregular menstrual cycle was significantly associated with higher AF risk (hazard ratio (HR); 95% confidence interval (95% CI): 1.34; 1.01-1.79). Both early menarche (7-11 years) and late menarche (13-18 years) were significantly associated with AF incidence (HR; 95% CI: 1.10; 1.00-1.21 and 1.08; 1.00-1.17, respectively). Early menopause (age 35-44 years) and delayed menopause (≥60 years) were significantly associated with higher risk of AF (HR; 95% CI: 1.24; 1.10-1.39 and 1.34; 1.01-1.78, respectively). Compared with women with 1-2 live births, those with 0 live births (HR; 95% CI: 1.13; 1.04-1.24), or 7 or more live births (HR; 95% CI: 1.67; 1.03-2.70) both had significantly higher AF risk.

Conclusions

Results of this study suggest that irregular menstrual cycles, nulliparity, and multiparity were associated with higher risk of new-onset AF among women. The results highlight the importance of taking into account the reproductive history of women in devising screening strategies for AF prevention.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide and carries a large morbidity and mortality risk (1). Evidence suggests differences in pathophysiological processes of AF between men and women and an association of AF with a poor prognosis among women.(1, 2) Such findings warrant additional research into the sex-specific risk factors in the development of AF.

Sex hormones may play a key role in cardiovascular health.(3) The suggested benefits of estrogen for cholesterol metabolism and endothelial function diminish as women age.(4) This age-related decline in estrogen levels, particularly after menopause, has been associated with a higher risk of cardiovascular disease (CVD).(5)

The pathophysiological processes of AF are known to be complex and multifaceted. An electrophysiological dysfunction within the heart including a disordered refractory period and action potential duration is thought as one of the most important factors in initiating AF.(2) Despite the lack of direct evidence, estrogen may confer an advantage in AF by extending atrial conduction time, action potential duration, and the atrial effective refractory period.(6) Thus, we speculated that reproductive lifespan function is potentially associated with AF development in women, induced by the long-lasting changes in estrogen levels related to aging.

Although associations of menopausal age and reproductive lifespan with incident AF have been reported,(7-9) a comprehensive evaluation of the potential association of a wide range of range of reproductive lifespan factors with AF development is sparse. In the present study, we aimed to investigate the linear and potential non-linear associations between sex-specific risk factors and the risk of new-onset AF among a large population of women in the UK Biobank study.

METHODS

Study design

Data were obtained from the UK Biobank database. The UK Biobank is a large, prospective population-based cohort study in the UK that recruited more than 500,000 participants aged 40 to 69 years in 2006 to 2010.(10) These participants provided medical history, health behavior, physical measures, and biological samples at the time of enrolment. The UK Biobank received ethics approval from the North West Multi-Centre Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland. All participants provided

written informed consent before inclusion in the UK Biobank, and any participant who withdrew from the study was removed from the present cohort study. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Study population

In the current study, 273,382 women at study enrolment were assessed for inclusion. Participants with prevalent AF at baseline or with only self-reported incident AF during follow-up were excluded (n=2,673). Furthermore, participants with a history of hysterectomy and/or bilateral oophorectomy were also excluded (n=35,351). Finally, there were 235,191 participants included in the final analysis. The number of participants in each analysis for the various sex-specific risk factors varied because of missing values per specific risk factor.

Assessment of the women-specific risk factors

Reproductive history was ascertained at the baseline study visit by participant self-report.(9) Potential sex-specific risk factors included in the current study were age at menarche, irregular menstrual cycle (yes or no), menopause status (yes or no), age at menopause, years after menopause (calculated as baseline age minus menopausal age), age at first live birth, years after last birth (calculated as baseline age minus age at last birth), history of spontaneous miscarriages (yes or no), history of stillbirths (yes or no), number of live births, and total reproductive years (calculated as menopausal age minus menarcheal age).(11, 12)

Assessment of atrial fibrillation

AF was assessed using the hospital admission, primary care, and/or death registry data linked to the UK Biobank.(10) Onset of AF was defined by the use of International Statistical Classification of Diseases and Related Health Problems, Tenth Revision code I48. Follow-up ended on October 3, 2020. Participants were censored at the end of follow-up, the date of incident AF, date of death, or loss to follow-up whichever occurred first.

Assessment of cardiovascular risk factors

Assessment of potential confounders at baseline has been described previously.(10) The details are provided in the **Methods S1**.

Statistical analyses

Cox proportional hazards models

Multivariable Cox proportional hazards analyses were used to quantify associations between each risk factor and incident AF. All risk factors were first treated as continuous variables in the Cox proportional hazards models. In model 1, we adjusted the analyses for baseline age only. In model 2, we also adjusted for cardiovascular risk factors such as race and ethnicity (which were self-identified by

participants and included Asian, Black, White, mixed, and other [ie, all other potential racial and ethnic groups]), educational level, body mass index (BMI), total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus (DM), history of coronary heart disease (CHD), history of heart failure (HF), history of stroke, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

Natural cubic splines

Furthermore, we added natural cubic splines with up to 5 knots to the corresponding multivariable-adjusted Cox proportional hazards regression models for each risk factor to ascertain the potential non-linear associations of factors with incident AF. The Akaike information criterion, an estimator of how well a model fits the data, was used to compare the various models and choose the best model. We then recorded the cutoff value of each risk factor if non-linearity was found. The cutoff value was used to group participants and construct the categorical variables, which were subsequently used in the Cox proportional hazards regression models to quantify the non-linear associations.

Sensitivity analyses

In sensitivity analyses, we repeated all of the analyses among participants without CVD (including CHD, HF, and stroke) at baseline to ascertain the presence of any residual confounding despite the extensive adjustments. Furthermore, we stratified analyses by BMI (calculated as weight in kilograms divided by height in meters squared) categories: (1) underweight: BMI lower than 18.5; (2) healthy weight: BMI between 18.5 and lower than 25; (3) overweight: BMI between 25 and lower than 30; (4) obese: BMI of 30 or higher. To investigate the role of sex hormones in the linear association between sex-specific risk factors and AF, we also adjusted model 2 for the serum concentrations of testosterone and sex hormone binding globulin instead of estradiol because estradiol was present in a small proportion of the participants (6.2%, 14,588) in the UK Biobank. Moreover, given that women without live birth may have infertility induced by hormonal imbalance or may have had pregnancy loss, we performed a sensitivity analysis by further adjusting for sex hormone levels and a subgroup analysis among women without stillbirth, spontaneous miscarriage, or termination. In addition, we recognized that several of the assessed risk factors inherently captured the aging process. For instance, postmenopausal women are expected to be older than premenopausal women. Therefore, age-stratified analysis with 5-year age groups was conducted to limit the residual confounding of age.

Missing values in covariates were imputed under the assumption of missing at random using the multiple imputation with fully conditional specification by means of R package “mice”. Values of HDL-cholesterol were missing in 15.3% participants.

Missing values of all other co-variables were ≤8.0%. For multiple imputation, all available data were used to generate 5 imputed datasets and the pooled results were reported. In sensitivity analyses, a complete case analysis was carried out.

Statistical significance was considered at two-tailed $p<0.05$. The analyses were done using R software (R 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

A total of 235,191 women free of AF (mean age 55.7 ± 8.1 years) at baseline were included in the study. The self-reported baseline characteristics are shown in **Table 1**. The median follow-up period was 11.6 years (interquartile range (IQR), 10.9-12.3 years) during which 4,629 (2.0%) women experienced new-onset AF.

Cox proportional hazards models

Table 2 describes the associations between various women-specific risk factors and the risk of new-onset AF among participants. In the age-adjusted model (model 1), significant associations were established between most assessed risk factors and incident AF with the exception of menopause status (HR (95% CI): 1.04 (0.90-1.20)), history of spontaneous miscarriage (HR (95% CI): 1.05 (0.98-1.11)), and history of stillbirth (HR (95% CI): 1.17 (1.00-1.38)). After additional adjustments for other potential confounders (model 2), the associations between age at menarche and number of live births with AF were attenuated and were no longer statistically significant.

In model 2, women with a history of irregular menstrual cycles had a higher risk of incident AF compared with women with regular menstrual cycles (HR (95% CI): 1.34 (1.01-1.79)). A greater number of years after last birth was associated with higher AF risk (HR (95% CI): 1.06 (1.02-1.10) per 5-year increase in years after last birth). Older age at menopause was beneficial for incident AF (HR (95% CI): 0.95 (0.92-0.98) per 5-year increase in menopausal age), whereas a greater number of years after menopause was detrimental to the association with new-onset AF (HR (95% CI): 1.05 (1.02-1.09) per 5-year increase in years after menopause). Older age at first live birth was associated with a lower risk of incident AF (HR (95% CI): 0.92 (0.88-0.96) per 5-year increase in age at first live birth). Moreover, longer reproductive lifespan, reflecting the period between menarche and menopause, was associated with lower risk of incident AF (HR (95% CI): 0.96 (0.93-0.99) per 5-year increase in total reproductive years).

Natural cubic splines

Figure 1 depicts the non-linear association between sex-specific risk factors and AF development. A N-shaped association was found between age at menarche and incident AF (p for non-linearity=0.25). Experiencing menarche earlier between ages 7-11 years (HR (95% CI): 1.10 (1.00-1.21)), or later between ages 13-18 years (HR (95% CI): 1.08 (1.00-1.17)) were significantly associated with a higher risk of incident AF compared to menarche age at 12 years (**Table 3**, **Figure 1A**). Moreover, an U-shaped association was identified between menopausal age with the risk of new-onset AF. Experiencing menopause at approximaetely 52 years of age was significantly associated with the lowest risk for incident AF (**Figure 1B**). Early menopause (<35 years of age: HR (95% CI): 2.25 (1.48-3.34) and 35-44 years of age: HR (95% CI): 1.24 (1.10-1.39) and late menopause at ≥60 years (HR (95% CI): 1.34 (1.10-1.78)) were significantly associated with a higher risk for incident AF (**Table 3**).

Figure 1C illustrates a J-shaped association between number of live births and incident AF. The lowest risk of AF was observed among women with 1-2 live births. Compared with women who had 1-2 live births, those with none had a higher risk of incident AF (HR (95% CI): 1.13 (1.04-1.24)). Risk of AF was also higher among women with 4-6 live births (HR (95% CI): 1.12 (1.01-1.24)) and substantially higher among women with ≥7 live births (HR (95% CI): 1.67 (1.03-2.70)).

We also observed an U-shaped association between reproductive lifespan and the risk of new-onset AF (**Figure 1D**). In **Table 3**, short reproductive lifespans of <20 years (HR (95% CI): 1.74 (1.07-2.86)) and 21-30 years (HR (95% CI): 1.23 (1.10-1.38)) were markedly associated with higher AF risks. In contrast, a reproductive lifespan of ≥41 years was not significantly associated with AF (HR (95% CI): 1.03 (0.95-1.11)).

Sensitivity analyses

After excluding women with baseline prevalent CHD, HF and stroke from the analyses, the associations between various women-specific risk factors and incident AF remained statistically significant and were slightly stronger than our original results (**Table S1**). Moreover, we found a significant interaction between menopause status and incident AF across BMI groups (p for interaction<.001) (**Table S2**). Menopause status was significantly associated with incident AF only among normal-weight women (HR (95% CI): 1.39 (1.05-1.84)). In addition, further adjustment for blood levels of sex hormones did not substantially change the associations between each women-specific risk factor and AF (**Table S3**). Finally, age-stratified analyses suggested significant interactions between having irregular menstrual cycle and baseline age (p for interaction=0.03). In addition, a potentially stronger association was indicated between menopausal age and AF among younger women, though the interaction was not statistically significant (p for interaction=0.08). Also, borderline significant interactions were observed between menopause status (p for interaction=0.09) and years after menopause (p for

interaction=0.10) with age (**Table S4**).

The complete case analysis showed generally similar directions compared with the results after multiple imputation. However, no association was found between menarcheal age and AF (**Table S1**). After excluding women with baseline prevalent CHD, heart failure, and stroke, the associations between various sex-specific risk factors and incident AF remained significant and were similar to the original results (**Table S2**). For example, history of irregular menstrual cycle was significantly associated with higher AF risk (HR (95% CI): 1.34 (1.01-1.79)). Moreover, we found a significant interaction (p for interaction<0.001) between menopause status and incident AF across BMI categories (**Table S3**). Menopause status was associated with incident AF only among those in the healthy weight BMI group (HR (95% CI): 1.39 (1.05-1.84)). Further adjustment for blood levels of sex hormones did not substantially change the associations between each risk factor and AF (**Table S4**). Specifically, compared with women with 1-2 live births, nulliparity was associated with a higher AF risk (HR (95% CI): 1.10 (1.01-1.19), p=0.03).

Age-stratified analyses suggested significant interactions between history of irregular menstrual cycle and baseline age (age ≤45 years: HR (95% CI): 0.76 (0.36-1.61), age 46-50 years (HR (95% CI): 1.30 (0.83-2.03)), and age 51-55 years (HR (95% CI): 2.37 (1.40-4.02), p for interaction=0.03)). In addition, a potential association was found between menopausal age and AF among younger women, although the interaction was not statistically significant. Borderline significant interactions were also observed between age and menopause status (age ≤45 years (HR (95% CI): 2.95 (1.26-6.89), age 46-50 years (HR (95% CI): 1.18 (0.79-1.77), age 51-55 years (HR, (95% CI): 1.35 (1.00-1.82), age 56-60 years (HR (95% CI): 1.21 (0.62-2.34), age 61-65 years (HR (95% CI): 0.79 (0.47-1.31), and age >65 years (HR (95% CI): 0.96 (0.64-1.45), p for interaction =0.09)), and years after menopause: age 46-50 years (HR (95% CI): 1.51 1.08-2.11), age 51-55 years (HR (95% CI): 1.14 (0.98-1.34), age 56-60 years (HR (95% CI): 1.13 1.04-1.23), age 61-65 years (HR (95% CI): 1.00 (0.94-1.06), and age >65 years (HR (95% CI): 1.05 (0.99-1.11), p for interaction=0.18)) (**Table S5**).

To evaluate the additional value of the reproductive lifespan beyond menopausal age, we adjusted for menopausal age in the association between total reproductive years and new-onset AF; this association remained, although it was slightly attenuated (age 21-30 years (HR (95% CI): 1.19 (1.01-1.40), p=0.04) (**Table S6**). After excluding women with a history of pregnancy loss, an association was found between number of live births and AF (0 live births (HR (95% CI): 1.15 (1.06-1.27), 3 live births (HR (95% CI): 1.14 (1.04-1.25), and 4-6 live births (HR (95% CI): 1.16 (1.01-1.34)) (**Table S7**). Further adjustment for the Townsend index did not change the significance. Compared with women who had 1-2 live births, those with 0 live births had a higher risk of incident AF (HR (95% CI): 1.09 (1.00-1.18)). Risk of AF was also higher among women with 3 live births (HR (95% CI): 1.08 (1.00-1.16) and among women with 4 to 6 live births (HR (95% CI): 1.13 (1.01-1.26)) (**Table S7**).

Table 1. Baseline characteristics of the total study population

Baseline characteristics *	Total study population n=235,191
Age, years	55.7 ± 8.1
Weight, kg	71.1 ± 14.0
Body mass index, kg/m^2	26.9 ± 5.1
Systolic blood pressure, mmHg	136.5 ± 20.2
Diastolic blood pressure, mmHg	80.5 ± 10.5
Total cholesterol, mmol/L †	5.86 ± 1.12
High-density lipoprotein cholesterol, mmol/L †	1.60 ± 0.38
Total oestradiol, pmol/L	402.8 (268.5, 643.8)
Total testosterone, nmol/L	1.03 (0.73, 1.39)
Sex hormone binding globulin, nmol/L	62.0 ± 30.4
Ethnicity	
White, n (%)	222,577 (94.6)
Mixed, n (%)	1,650 (0.7)
Asian, n (%)	3,964 (1.7)
Black, n (%)	3,905 (1.7)
Other, n (%)	3,095 (1.3)
Education, university or college (%)	36,938 (15.7)
Smoking status	
Never, n (%)	141,240 (60.1)
Former, n (%)	72,888 (31.0)
Current, n (%)	21,063 (9.0)
Diabetes mellitus, n (%)	7,549 (3.2)
Heart failure, n (%)	384 (0.2)
Coronary heart disease, n (%)	5,916 (2.5)
Stroke, n (%)	2,430 (1.0)
Antihypertensive medication, n (%)	44,798 (19.0)
Lipid lowering medication, n (%)	51,795 (22.0)
Oral contraceptive, n (%)	51,238 (21.8)
Hormone replacement therapy, n (%)	76,438 (32.6)
Age at menarche, years	12.9 ± 1.6
History of irregular menstrual cycle, n (%)	10,804 (22.5)
Menopause status, yes (%)	143,067 (69.2)
Age at menopause, years	50.2 ± 4.4
Years after menopause	9.6 ± 6.3
Age at first birth, years	25.6 ± 4.7
History of spontaenous miscarriages, n (%)	46,972 (23.5)
History of stillbirth, n (%)	5,655 (2.4)
Number of live births	
0, n (%)	45,641 (19.5)
1, n (%)	31,838 (13.6)
2, n (%)	102,185 (43.6)
≥3, n (%)	54,867 (23.3)
Total reproductive years	37.3 ± 4.7

Values are mean (standard deviation) or median (interquartile range) for continuous variables or number (percentages) for categorical variables.

† SI conversion factor: to convert cholesterol to mg/dL divide values by 0.0259.

Table 2. Linear association between women-specific risk factors with the risk of new-onset atrial fibrillation

Women-specific risk factors	Total study population	
	HR (95% CI)	
	Model 1 *	Model 2 †
Age at menarche (n=227,319) ‡	**0.98 (0.96-0.99), p=0.01**	1.00 (0.98-1.02), p=0.99
Irregular menstrual cycle, yes/no (n=58,843)	**1.36 (1.02-1.81), p=0.04**	**1.34 (1.01-1.79), p=0.04**
Menopause, yes/no (n=206,886)	1.04 (0.90-1.20), p=0.63	1.14 (0.98-1.32), p=0.09
Age at menopause (n=134,419) §	**0.94 (0.90-0.97), p<0.001**	**0.95 (0.92-0.98), p<0.01**
Years after menopause (n=134,419) §	**1.07 (1.03-1.11), p<0.001**	**1.05 (1.02-1.09), p<0.01**
Age at first live birth (n=156,773) §	**0.86 (0.82-0.89), p<0.001**	**0.92 (0.88-0.96), p<0.001**
Years after last birth (n=156,527)	**1.09 (1.05-1.13), p<0.001**	**1.06 (1.02-1.10), p<0.01**
Spontaneous miscarriages, yes/no (n=230,587)	1.05 (0.98-1.13), p=0.20	1.04 (0.97-1.11), p=0.32
Stillbirth, yes/no (n=230,953)	1.05 (0.98-1.11), p=0.17	1.03 (0.97-1.10), p=0.28
Number of live births (n=234,531)	**1.03 (1.01-1.05), p=0.03**	1.01 (0.98-1.03), p=0.57
Reproductive years (n=131,449) § ¶	**0.96 (0.93-0.99), p=0.02**	**0.96 (0.93-0.99), p=0.02**

Abbreviations: CI; confidence interval, HR; hazard ratio.

* Adjusted for for baseline age.

† Adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

‡ Hazard ratios represent 1 unit increase in age at menarche with the risk of new-onset atrial fibrillation.

§ Hazard ratios represent 5 unit increase in age at menopause, years after menopause, age at first live birth, reproductive years with the risk of new-onset atrial fibrillation.

¶ Reproductive years was defined as the difference between menopausal age and menarcheal age. The associations with a p<0.05 are highlighted in **bold**.

Table 3. Non-linear association between women-specific risk factors with the risk of new-onset atrial fibrillation

Women-specific risk factors	Total study population	
	HR (95% CI)	
	Model 1 *	Model 2 †
Age at menarche		
7-11 years old (n=44,309) ‡	**1.18 (1.07-1.29), p<0.001**	**1.10 (1.00-1.21), p=0.04**
12 years old (n=43,314)	1.00 (reference)	1.00 (reference)
13-18 years old (n=139,696) ‡	1.04 (0.96-1.13), p=0.34	1.08 (1.00-1.17), p=0.05
Age at menopause		
<35 years old (n=494) ‡	**2.48 (1.63-3.78), p<0.001**	**2.25 (1.48-3.43), p<0.001**
35-44 years old (n=12,074) ‡	**1.33 (1.19-1.49), p<0.001**	**1.24 (1.10-1.39), p<0.001**
45-49 years old (n=32,042) ‡	1.09 (1.00-1.83), p=0.06	1.07 (0.98-1.17), p=0.12
50-54 years old (n=68,206)	1.00 (reference)	1.00 (reference)
55-59 years old (n=20,665) ‡	1.06 (0.97-1.17), p=0.20	1.04 (0.95-1.14), p=0.39
≥60 years old (n=938) ‡	**1.39 (1.05-1.84), p=0.02**	**1.34 (1.01-1.78), p=0.04**
Number of live births		
0 (n=45,641) ‡	1.09 (1.00-1.19), p=0.05	**1.13 (1.04-1.24), p<0.01**
1-2 (n=134,023)	1.00 (reference)	1.00 (reference)
3 (n=40,695) ‡	**1.11 (1.02-1.20), p=0.01**	1.08 (1.00-1.16), p=0.05
4-6 (n=13,784) ‡	**1.23 (1.10-1.37), p<0.001**	**1.12 (1.01-1.24), p=0.04**
≥7 (n=388) ‡	**1.87 (1.18-2.98), p<0.001**	**1.67 (1.03-2.70), p=0.03**
Reproductive years §		
≤20 years (n=462) ‡	**1.87 (1.14-3.06), p=0.01**	**1.74 (1.07-2.86), p=0.03**
21-30 years (n=10,863) ‡	**1.31 (1.16-1.47), p<0.0001**	**1.23 (1.10-1.38), p<0.001**
31-40 years (n=88,122)	1.00 (reference)	1.00 (reference)
41-50 years (n=31,939) ‡	1.07 (0.99-1.16), p=0.09	1.03 (0.95-1.11), p=0.45

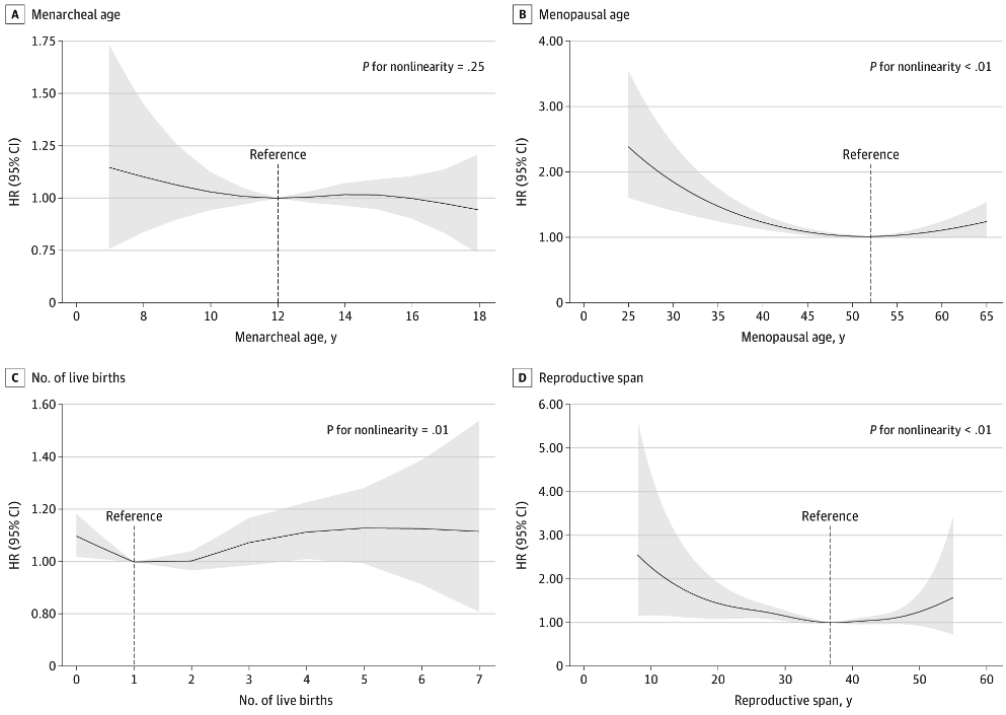
* Adjusted for for baseline age.

† Adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, use of lipid lowering medication.

‡ Hazard ratios in relation to their reference category with the risk of new-onset atrial fibrillation.

§ Reproductive years was defined as the difference between menopausal age and menarcheal age. The associations with a p<0.05 are highlighted in **bold**.

Figure 1. Non-linear association between women-specific risk factors and the risk of new-onset atrial fibrillation



Model was adjusted for baseline age, race and ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, use of lipid lowering medication.

DISCUSSION

This study found independent significant linear associations between risk of new-onset AF and the risk factors of age at menopause, years after menopause, total reproductive years, history of irregular menstrual cycle, number of live births, age at first live birth, and years after last birth. Significant non-linear associations were also found between risk of new-onset AF and age at menopause, total reproductive years, and number of live births.

To our knowledge, this study was the first to report an independent association between irregularity in menstrual cycle and new-onset AF and thereby add to previous evidence. Epidemiological studies have reported that irregular cycles might be associated with the development of CHD and CHD mortality.(13, 14) Meanwhile, the findings of a study of 40 Indian women suggested that menstrual cycle irregularity was associated with glucose intolerance and insulin resistance.(15) Irregular cycles that are commonly induced by sex-hormone disorders were considered to be an independent risk factor for cardiometabolic disorders.(16) In addition, the observed association in the present study was significant only among older women, suggesting that loss of estrogen with aging might mediate the association between history of irregular menstrual cycle and new-onset AF.(5, 6) However, further research to investigate the underlying pathophysiological mechanisms between irregular cycles and AF are warranted.

Although menopause status was not associated with new-onset AF in the present study, a greater number of years after menopause was associated with a higher AF risk among postmenopausal women. Moreover, the risk of AF was significantly increased among women who had experienced menopause at 44 years or younger or at 60 years or older. These results reflected a U-shaped association, with the lowest AF-associated risk found for the menopausal ages of 45 to 59 years, and thus were complementary to results of a previous study conducted within the UK Biobank that showed linear associations between menopausal age and incident AF among women with natural menopause or surgical menopause.(9) However, analyses of the Framingham Heart Study of 1,809 women (median follow-up of 20.5 years) and the Women's Health Study (WHS) of 30,034 women (median follow-up of 10 years) did not report an association between categorical menopausal age and new-onset AF.(17, 18) Participants in the Framingham Heart Study (mean age, 70 years) were much older than those in the current study. Advancing age was the most important risk factor for incident AF, leading to a sharp increase in AF incidence after 70 years of age.(1) Thus, the association between menopausal age and AF could have been masked by both the limited sample size and the older population in the Framingham Heart Study. In the present study, results of the sensitivity analyses suggested an association between menopausal age and AF among younger women. Moreover,

the reference group in the WHS was arbitrarily set to women with menopausal age older than 54 years. In this study, we found that menopausal age of 60 years or older was associated with a substantially higher risk of AF. Therefore, not taking into account the non-linearity in the WHS was probably a factor in the diluted association between menopausal age and AF. We extended the previous studies by assessing the potential non-linear associations and identifying an appropriate reference group with the lowest risk for AF.

We found a J-shaped association between the number of live births and incident AF. Compared with women with 1-2 live births, those with 0 or with more than 4 live births had a higher risk of AF development. To our knowledge, only 1 study from the WHS found that women with more than 3 pregnancies vs. 1 pregnancy had a substantially higher AF risk, whereas women without pregnancy did not have a higher AF risk.(19) Population heterogeneity should be assessed to interpret such a discrepancy. The participants in the WHS were healthier than those in the UK Biobank. Thus, in the present study, the higher risk of AF among women who had 0 live births might be partially attributed to a poorer health status and a larger burden of comorbidities. Nevertheless, the sensitivity analyses of women without CVD at baseline showed similar results, suggesting that the burden of comorbidity did not fully account for the observed association. Compared with women without children, primiparous women experience a series of changes in vascular function during pregnancy, and these changes are normally beneficial to accommodate maternal and fetal needs.(20) On one hand, research has indicated that a normal pregnancy might contribute to reduced arterial stiffness and elevated vascular compliance in primiparas.(21) On the other hand, pregnancy may be associated with abnormal hemodynamic changes in the cardiovascular system, which further result in cardiac hypertrophy, valvular disease, and CHD.(22) Evidence shows that at least 0.2% to 0.4% of all pregnancies are complicated by these cardiovascular pathologies.(23) Herein, multiparity was associated with increased risk of pathological changes within the cardiovascular system after pregnancy. Overall, the findings of this study underscored the higher AF risk among both nulliparous and multiparous women.

In addition, the present study found associations between various factors of reproductive history and incident AF. Associations of age at first live birth and years after last birth with incident AF were other novel findings of this study. Evidence has shown that women with an early first pregnancy are at greater risk of poor general health and worse physical functioning.(24) Similar to a prospective Korean study, this study confirmed the inverse association between the number of years in the reproductive lifespan and incident AF.(8)

This study has several strengths. Among these strengths are the large sample size, prospective study design, long-term follow-up, and extensive adjustment for a broad range of confounders. This study also has several limitations. First, given that

participants were predominantly of European ancestry, the results may not be generalizable to women of other ethnic backgrounds. Second, various exposures and covariates were self-reported and thus may be subject to recall bias, which is inherent to the use of self-administered questionnaires. Third, because of the observational study design, we cannot rule out the possibility of residual or unmeasured confounding. Specifically, the association between the number of live births and cardiovascular health might be, to some extent, altered by possible residual confounding by socioeconomic and cultural factors.(25) However, the observed association remained statistically significant after further adjustment for the Townsend index. Fourth, previous studies(26) have suggested an increased risk of CVD among women who had experienced adverse pregnancy outcomes such as preeclampsia, preterm delivery, and gestational diabetes, which were not covered in the present study because of the low prevalence of adverse pregnancy outcomes at baseline. Fifth, given that AF may be paroxysmal and asymptomatic, we might have underestimated the true number of AF cases in this study population. Furthermore, the use of International Statistical Classification of Diseases and Related Health Problems, Tenth Revision code I48 to detect the onset of AF was tied to health care utilization and not perfectly accurate and was thus at a potential risk of misclassification.(27)

To conclude, this large population-based cohort study found that sex-specific risk factors were significantly associated with the risk of new-onset AF among women. The AF risk was elevated among women with early or delayed menopause. In addition, women with irregular menstrual cycles had a greater risk for AF onset. Both nulliparity and multiparity were associated with greater risk of incident AF compared with having 1-2 live births. These results underscored the importance of taking into account the reproductive history of women when developing tailored screening strategies for AF prevention in women.

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SUPPLEMENTARY MATERIAL

Chapter 6.1 Women-specific risk factors and the risk of atrial fibrillation

Methods S1. Assessment of cardiovascular risk factors

In brief, age, sex, ethnicity (recoded to 5 groups: White, Asian, Black, mixed, and other), educational level (recoded to 2 groups: university/college, and other), alcohol intake frequency (recoded to 3 groups: never, 1-6 times per week, and every day), smoking status (never, former, and current), and medication use of blood pressure lowering, lipid lowering, oral contraceptive, and hormone replacement therapy were obtained from the touchscreen questionnaires. Blood pressure was automatically measured twice during an interview using an Omron 705 IT electronic blood pressure monitor. Height (in meters) and weight (in kilogram) were measured in the assessment centers, and body mass index (BMI) was calculated as weight in kilograms divided by weight in meters squared. Prevalent cardiometabolic disorders at baseline, including coronary heart disease (CHD; ICD-10 codes: I20-22,24,25), AF (ICD-10 codes: I48), stroke (ICD codes: I60-64), heart failure (HF; ICD-10 codes: I50) and diabetes mellitus ((DM) ICD-10 codes: E10-14) was defined by ICD-10 codes on basis of the hospital admission and/or primary care. All self-reported cases of prevalent cardiometabolic disorders at baseline were set to missing.

Table S1. Complete case analysis

Women-specific risk factors *	Total study population	
	HR (95% CI)	p
Age at menarche (n=219,415)	1.00 (0.97-1.02)	0.72
7-11 years old ‡	1.05 (0.92-1.19)	0.50
12 years old	1.00 (reference)	-
13-18 years old ‡	1.03 (0.92-1.15)	0.60
Irregular menstrual cycle, yes/no (n=38,677)	1.39 (0.96-2.02)	0.08
Menopause, yes/no (n=126,540)	1.20 (0.98-1.48)	0.08
Age at menopause (n=80,626)	**0.94 (0.90-0.99)**	**0.02**
<35 years old ‡	**2.92 (1.75-4.86)**	**<0.001**
35-44 years old ‡	**1.24 (1.06-1.44)**	**<0.001**
45-49 years old ‡	0.99 (0.88-1.11)	0.83
50-54 years old	1.00 (reference)	-
55-59 years old ‡	0.98 (0.86-1.11)	0.76
≥60 years old ‡	**1.49 (1.04-2.14)**	**0.03**
Years after menopause (n=80,626)	**1.06 (1.01-1.11)**	**0.02**
Age at first live birth (n=95,253)	**0.82 (0.86-0.97)**	**<0.01**
Stillbirth, yes/no (n=222,941)	1.10 (0.87-1.40)	0.40
Number of live births (n=141,783)	1.00 (0.97-1.04)	0.85
0 ‡	**1.15 (1.03-1.29)**	**0.01**
1-2	1.00 (reference)	-
3 ‡	1.06 (0.95-1.17)	0.29
4-6 ‡	**1.17 (1.01-1.35)**	**0.04**
≥7 ‡	**2.33 (1.34-4.03)**	**<0.01**
Reproductive years (n=78,852) §	0.95 (0.91-1.00)	0.05
≤20 years ‡	**2.43 (1.34-4.41)**	**<0.01**
21-30 years ‡	**1.22 (1.04-1.43)**	**0.01**
31-40 years	1.00 (reference)	-
41-50 years ‡	1.02 (0.91-1.13)	0.78

Abbreviations: CI; confidence interval, HR; hazard ratio.

* Adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

† Hazard ratios represent 1 unit increase in age at menarche with the risk of new-onset atrial fibrillation.

‡ Hazard ratios represent 5 unit increase in age at menopause, years after menopause, age at first live birth, reproductive years with the risk of new-onset atrial fibrillation.

§ Reproductive years was defined as the difference between menopausal age and menarcheal age. The associations with a p<0.05 are highlighted in **bold**.

Table S2. Association between women-specific risk factors with the risk of new-onset atrial fibrillation among participants free of cardiovascular disease at baseline

Women-specific risk factors *	Total study population	
	HR (95% CI)	**p**
Age at menarche (n=219,415) †	0.99 (0.97-1.01)	0.45
Irregular menstrual cycle, yes/no (n=58,230)	**1.36 (1.01-1.83)**	**0.04**
Menopause, yes/no (n=200,043)	1.14 (0.98-1.33)	0.09
Age at menopause (n=128,726) ‡	**0.94 (0.90-0.97)**	**<0.01**
Years after menopause (n=128,726) ‡	**1.07 (1.03-1.11)**	**<0.01**
Age at first live birth (n=150,894) ‡	**0.91 (0.87-0.96)**	**<0.01**
Stillbirth, yes/no (n=222,941)	1.01 (0.95-1.09)	0.68
Number of live births (n=226,384)	0.99 (0.97-1.02)	0.85
Reproductive years (n=125,859) ‡ §	**0.95 (0.91-0.98)**	**<0.01**

Abbreviations: CI; confidence interval, HR; hazard ratio.

* Adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

† Hazard ratios represent 1 unit increase in age at menarche with the risk of new-onset atrial fibrillation.

‡ Hazard ratios represent 5 unit increase in age at menopause, years after menopause, age at first live birth, reproductive years with the risk of new-onset atrial fibrillation.

§ Reproductive years was defined as the difference between menopausal age and menarcheal age. The associations with a p<0.05 are highlighted in **bold**.

Table S3. Association between various women-specific risk factors with the risk of new-onset atrial fibrillation stratified by body mass index

Women-specific risk factors *	BMI categories				
	BMI<18.5	18.5≤BMI<25	25≤BMI<30	BMI≥30	p for interaction
Age at menarche (n=227,319) †	0.93 (0.74-1.18)	0.98 (0.95-1.02)	1.02 (0.98-1.05)	1.00 (0.97-1.03)	0.90
Irregular menstrual cycle, yes/no (n=58,843)	-	0.97 (0.55-1.71)	1.62 (0.94-2.79)	1.45 (0.94-2.23)	0.51
Menopause, yes/no (n=206,886)	1.49 (0.15-14.6)	**1.39 (1.05-1.84)**	1.15 (0.88-1.49)	0.96 (0.76-1.22)	<0.001
Age at menopause (n=134,419) ‡	0.87 (0.57-1.33)	0.92 (0.86-0.99)	0.96 (0.90-1.02)	0.97 (0.91-1.03)	0.19
Years after menopause (n=134,419) ‡	1.15 (0.75-1.75)	1.09 (1.02-1.17)	1.04 (0.98-1.11)	1.03 (0.97-1.09)	0.13
Age at first live birth (n=156,773) ‡	0.77 (0.40-1.46)	0.95 (0.87-1.03)	0.89 (0.83-0.96)	0.96 (0.89-1.03)	0.60
Stillbirth, yes/no (n=230,953)	0.85 (0.34-2.16)	0.99 (0.88-1.11)	1.00 (0.90-1.12)	1.11 (1.00-1.23)	0.07
Number of live births (n=234,531)	1.06 (0.76-1.46)	0.97 (0.92-1.02)	1.02 (0.98-1.07)	1.03 (0.99-1.07)	0.21
Reproductive years (n=131,449) ‡ §	0.92 (0.61-1.39)	0.95 (0.88-1.01)	0.96 (0.91-1.01)	0.98 (0.93-1.04)	0.24

Abbreviations: BMI, body mass index.

* Model was adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

† Hazard ratios represent 1 unit increase in age at menarche with the risk of new-onset atrial fibrillation.

‡ Hazard ratios represent 5 unit increase in age at menopause, years after menopause, age at first live birth, reproductive years with the risk of new-onset atrial fibrillation.

§ Reproductive years was defined as the difference between menopausal age and menarcheal age.

The associations with a p<0.05 are highlighted in **bold**.

Table S4. Association between women-specific risk factors with the risk of new-onset atrial fibrillation additionally adjusted for sex hormones

Women-specific risk factors *	Total study population	
	HR (95% CI)	**p**
Age at menarche (n=227,319) †	1.01 (0.98-1.03)	0.54
Irregular menstrual cycle, yes/no (n=58,843)	**1.41 (1.02-1.96)**	**0.04**
Menopause, yes/no (n=206,886)	1.14 (0.96-1.36)	0.09
Age at menopause (n=134,419) ‡	0.96 (0.92-1.00)	0.06
Years after menopause (n=134,419) ‡	1.04 (1.00-1.09)	0.07
Age at first live birth (n=156,773) ‡	**0.92 (0.87-0.97)**	**<0.01**
Stillbirth, yes/no (n=230,953)	1.06 (0.98-1.14)	0.16
Number of live births (n=234,531)	1.02 (0.99-1.05)	0.25
Reproductive years (n=131,449) ‡ §	0.96 (0.92-1.00)	0.07

Abbreviations: CI; confidence interval, HR; hazard ratio.

* Adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

† Hazard ratios represent 1 unit increase in age at menarche with the risk of new-onset atrial fibrillation.

‡ Hazard ratios represent 5 unit increase in age at menopause, years after menopause, age at first live birth, reproductive years with the risk of new-onset atrial fibrillation.

§ Reproductive years was defined as the difference between menopausal age and menarcheal age. The associations with a p<0.05 are highlighted in **bold**.

Table S5. Association between various women-specific risk factors with the risk of new-onset atrial fibrillation stratified by baseline age

Women-specific risk factors *	Baseline age categories			
	≤45 years	**46-50 years**	**51-55 years**	**55-65 years**
Age at menarche (n=227,319) †	1.04 (0.92-1.18)	0.97 (0.88-1.06)	(0.95-1.07)	0.99 (0.94-1.03)
Irregular menstrual cycle, yes/no (n=58,843)	0.76 (0.36-1.61)	1.30 (0.83-2.03)	**2.37 (1.40-4.02)**	-
Menopause, yes/no (n=206,886)	2.95 (1.26-6.89)	1.18 (0.79-1.77)	1.35 (1.00-1.82)	1.21 (0.62-2.34)
Age at menopause (n=134,419) ‡	-	0.66 (0.47-0.92)	0.88 (0.75-1.03)	0.88 (0.81-0.97)
Years after menopause (n=134,419) ‡	-	1.51 (1.08-2.11)	1.14 (0.98-1.34)	1.13 (1.04-1.23)
Age at first live birth (n=156,773) ‡	0.88 (0.65-1.19)	0.91 (0.75-1.11)	0.81 (0.71-0.93)	0.92 (0.83-1.02)
Stillbirth, yes/no (n=230,953)	0.92 (0.60-1.40)	1.12 (0.83-1.52)	1.17 (0.95-1.43)	0.95 (0.81-1.11)
Number of live births (n=234,531)	0.93 (0.78-1.10)	1.1 (0.98-1.23)	1.02 (0.94-1.10)	0.98 (0.92-1.04)
Reproductive years (n=131,449) ‡ §	-	0.70 (0.50-0.99)	0.91 (0.78-1.06)	0.91 (0.83-0.99)

Abbreviations: BMI, body mass index.

* Model was adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

† Hazard ratios represent 1 unit increase in age at menarche with the risk of new-onset atrial fibrillation.

‡ Hazard ratios represent 5 unit increase in age at menopause, years after menopause, age at first live birth, reproductive years with the risk of new-onset atrial fibrillation.

§ Reproductive years was defined as the difference between menopausal age and menarcheal age.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S5. Association between various women-specific risk factors with the risk of new-onset atrial fibrillation stratified by baseline age (continued)

Women-specific risk factors *	Baseline age categories		
	61-65 years	**>65 years**	**p for interaction**
Age at menarche (n=227,319) †	(0.97-1.04)	1.00 (0.97-1.03)	0.31
Irregular menstrual cycle, yes/no (n=58,843)	-	-	0.03
Menopause, yes/no (n=206,886)	0.79 (0.47-1.31)	0.96 (0.64-1.45)	0.09
Age at menopause (n=134,419) ‡	1.00 (0.94-1.06)	0.96 (0.90-1.01)	0.08
Years after menopause (n=134,419) ‡	1.00 (0.94-1.06)	1.05 (0.99-1.11)	0.18
Age at first live birth (n=156,773) ‡	0.99 (0.92-1.06)	0.89 (0.82-0.96)	0.05
Stillbirth, yes/no (n=230,953)	0.96 (0.86-1.07)	1.13 (1.01-1.26)	0.61
Number of live births (n=234,531)	0.98 (0.94-1.03)	1.04 (1.00-1.08)	0.91
Reproductive years (n=131,449) ‡ §	1.00 (0.95-1.06)	0.96 (0.91-1.02)	0.21

Abbreviations: BMI, body mass index.

* Model was adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, use of lipid lowering medication, use of hormone replacement therapy (if applicable), and use of contraceptive medication (if applicable).

† Hazard ratios represent 1 unit increase in age at menarche with the risk of new-onset atrial fibrillation.

‡ Hazard ratios represent 5 unit increase in age at menopause, years after menopause, age at first live birth, reproductive years with the risk of new-onset atrial fibrillation.

§ Reproductive years was defined as the difference between menopausal age and menarcheal age.

The associations with a $p<0.05$ are highlighted in **bold**.

Table S6. Sensitivity analysis: association between categorical reproductive lifespan and new-onset atrial fibrillation after further adjustment for menopausal age

Women-specific risk factors *	Total study population	
	HR (95% CI)	**p**
Reproductive years (n=149,462) †		
≤20 years	1.62 (0.92-2.82)	0.09
21-30 years	**1.19 (1.01-1.40)**	**0.04**
31-40 years	1.00 (reference)	-
41-50 years	1.06 (0.95-1.18)	0.28

Abbreviations: CI; confidence interval, HR; hazard ratio.

* Adjusted for baseline age, ethnicity, educational level, body mass index categories, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, use of antihypertensive medication, use of lipid lowering medication, and menopausal age.

† Hazard ratios represent 5 unit increase in reproductive years with the risk of new-onset atrial fibrillation. The associations with a p<0.05 are highlighted in **bold**.

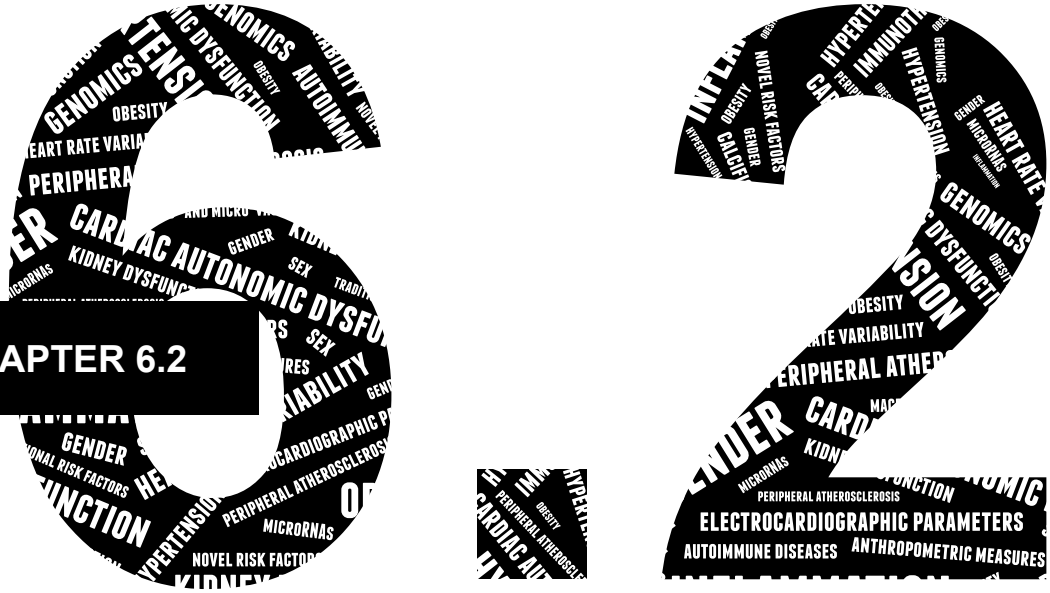
Table S7. Sensitivity analysis: association between categorical number of live births and new-onset atrial fibrillation

Women-specific risk factors	Total study population	
	HR (95% CI)	
	Model 1 *	Model 2 †
Number of live births		
0	**1.15 (1.06-1.27**	**1.09 (1.00-1.18)**
1-2	1.00 (reference)	1.00 (reference)
3	**1.14 (1.04-1.25)**	**1.08 (1.00-1.16)**
4-6	**1.16 (1.01-1.34)**	**1.13 (1.01-1.26)**
≥7	0.91 (0.38-2.18)	1.56 (0.98-2.49)

Abbreviations: CI; confidence interval, HR; hazard ratio.

* Model 1 was derived from a sub-sample excluding women with ever stillbirth, spontaneous miscarriage, or termination. Model was adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, and use of lipid lowering medication.

† Model 2 was additionally adjusted for baseline age, ethnicity, educational level, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, smoking status, history of diabetes mellitus, history of coronary heart disease, history of heart failure, history of stroke, use of antihypertensive medication, use of lipid lowering medication, and Townsend index.


CHAPTER 6.2
GENOMICS
OBESITY
GENDER
SEX
KIDNEY DYSFUNCTION
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
MICRORNAS
NOVEL RISK FACTORS
HYPERTENSION
HEART RATE VARIABILITY
IMMUNOTHERAPY
ELECTROCARDIOGRAPHIC PARAMETERS
AUTOIMMUNE DISEASES
ANTHROPOMETRIC MEASURES

Sex- and gender-specific prediction of atrial fibrillation

Perspectives on sex- and gender-specific prediction of new-onset atrial fibrillation by leveraging big data.

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ABSTRACT

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, has a large impact on quality of life and is associated with increased risk of hospitalization, morbidity, and mortality. Over the past 2 decades advances regarding the clinical epidemiology and management of AF have been established. Moreover, sex differences in the prevalence, incidence, prediction, pathophysiology, and prognosis of AF have been identified. Nevertheless, AF remains to be a complex and heterogeneous disorder and a comprehensive sex- and gender-specific approach to predict new-onset AF is lacking. The exponential growth in various sources of big data such as electrocardiograms, electronic health records, and wearable devices, carries the potential to improve AF risk prediction. Leveraging these big data sources by artificial intelligence (AI)-enabled approaches, in particular in a sex- and gender-specific manner, could lead to substantial advancements in AF prediction and ultimately prevention. We highlight the current status, premise, and potential of big data to improve sex- and gender-specific prediction of new-onset AF.

INTRODUCTION

Atrial fibrillation (AF), the most common cardiac arrhythmia, markedly increases the risk of hospitalization, morbidity, and mortality.(1-3) Over the last two decades advances regarding the clinical epidemiology and management of AF have been established.(1-3) Recent evidence indicates that both sex and gender play a role in the development and progression of cardiovascular disease, drug reactions, and healthcare utilization.(4-7) While sex refers to the biology; including chromosomes, gene expression, hormone levels, and their function, gender comprises the socio-cultural attributes; including socially constructed roles, behaviors, expressions, and identities.(4-7) In the field of AF, however, sex and gender implications remain understudied.(2, 8-10) Notably, the age-adjusted prevalence, incidence, and lifetime risk of AF are higher in men than in women.(1-3) More specifically, it is suggested that several risk factors (hypertension, smoking, alcohol intake, obesity, history of diabetes mellitus, history of myocardial infarction or history of heart failure), carry a differential impact on AF risk in men and women.(1, 8-11) It has also been suggested that AF-related adverse outcomes and response to various treatment modalities differ between men and women.(1-3, 8-11) Nevertheless, AF remains to be a complex and heterogeneous disorder and a comprehensive sex- and gender-specific approach to predict new-onset AF is lacking.

The past decade has witnessed an exponential growth in recorded data in the healthcare sector. The massive amount of recorded information, i.e. big data, has turned to a topic of special interest, because of its great potential. Leverage of big data, using artificial intelligence (AI)-enabled approaches, provides an opportunity to further improve prediction of AF.(12, 13) Use of various sources of big data such as electrocardiograms (ECGs), electronic health records (EHRs), and wearable devices, in particular in a sex- and gender-specific approach, could lead to substantial advancements in AF prediction and ultimately prevention. Here, we review the current status and highlight the premise and potential of these data sources to improve sex- and gender-specific prediction of new-onset AF.

LEVERAGING BIG DATA FOR PREDICTION OF NEW-ONSET ATRIAL FIBRILLATION

Over the past decade, several AF risk prediction scores have been developed and validated using more traditional research methods.(14–22) These prediction scores predominantly use traditional cardiovascular risk factors such as age, sex, race, height, weight, hypertension, diabetes mellitus, coronary heart disease, and heart failure which are readily obtainable clinical variables.(14–22) In general, these scores have a moderate to good performance (C-statistic ranging between 0.65-

0.78).(14–22) Over the past years, there has been an exponential interest in using various big data sources to further improve the AF risk prediction beyond the traditional AF risk factors.(23, 24) Specifically, multiple studies have employed AI-enabled algorithms to evaluate new-onset AF prediction by leveraging various big data modalities including the clinical data, ECGs, EHRs, and wearable devices.(23-42) Some of these studies showed that AI-enabled AF prediction models performed similar to or better than established traditional AF prediction models.(25, 27-30). Furthermore, targeted AF screening using a machine learning (ML) risk prediction algorithm showed the potential to enhance AF screening and to improve the cost-effectiveness of AF screening through an efficient use of limited healthcare resources.(31)

A variety of studies have highlighted the potential predictive capacity of AI to assess the risk of new-onset AF from a 12-lead ECG with acceptable to excellent performance (area under the receiver operating characteristic curve ranging between 0.70-0.90).(32, 33) The potential of the ECG to predict AF might be explained by the fact that the AF substrate is caused by electrical and structural remodeling of the heart.(2) AI-enabled algorithms for ECG assessment could mark the very early stages of remodeling, not yet being detected by the cardiologist using routine measures, to predict new-onset AF. However, previous studies mainly predicted new-onset AF risk within a short time period (<1 year).(33) Thereby, their value for primary prevention is limited, as the time window may not be long enough to intervene in individuals at high AF risk. In a recent study,(32) a convolutional neural network for 10-second ECG measures was trained to infer 5-year risk of new-onset AF. The investigators concluded that their method had similar predictive utility as the widely accepted clinical risk factor model: the Cohorts for Heart and Aging Research in Genomic Epidemiology AF score (CHARGE-AF).(14) Yet, the combination of both (clinical risk factors and ECG) provided the greatest predictive accuracy with good discrimination and calibration. These findings underscore the capacity of AI-enabled approaches to improve AF prediction in an inexpensive, non-invasive, widely available, and point-of-care testing manner.

2 studies utilized EHRs to develop AF prediction models.(41, 42) EHRs contain real-time, patient-centered data that are instantly available to patients and authorized healthcare providers. The increasing digitalization of healthcare systems makes EHRs more and more widely available, making them particularly useful to predict new-onset AF. A recent study developed an AF prediction model for a 6-month time period using 200 most common EHR features of 2,252,219 individuals. However, this ML approach did not substantially perform better than a logistic regression model using traditional AF risk factors.(41) Another study developed a model to predict new-onset AF over a 2-year time period with good discrimination (C-statistic of 0.81) using a 10-variable model compromised of covariates commonly available in the EHRs of 53,552 subjects. This study, however, did not compare the developed

prediction model to previous validated AF risk prediction models.(42) Another study evaluated the improvement in 5-year AF risk prediction when adding novel variables identified by ML to the CHARGE-AF enriched score.(27) Although this study was not conducted using EHRs, it did include clinical, serological, echocardiographic, and cardiac imaging information that are increasingly becoming more available within EHRs. This method, however, did not significantly improve AF prediction in comparison to the CHARGE-AF enriched score.(27)

The emergence of wearable devices constitutes another major source to improve AF prediction and management.(23)
Wearable devices often use photoplethysmography or ballistocardiography to monitor an individual's cardiac rhythm. Other forms of wearable devices include cardiac implantable electronic or patch-based ECG devices that have proven to be useful in selected patient populations to detect AF or assess the risk of stroke in AF patients beyond the CHA_2DS_2-VASc score.(43, 44) Such wearable devices are simple to apply and enable real-time continuous monitoring of the heart. This makes the use of wearable devices promising as one could use this electrical information to predict new-onset AF. Nonetheless, the use of wearable devices and AI algorithms is currently mainly limited to AF detection rather than new-onset AF prediction.

PREMISE OF BIG DATA IN PREDICTION OF NEW-ONSET ATRIAL FIBRILLATION

Leveraging big data by AI-enabled approaches could offer great opportunities to improve AF prediction. First, AI methods could be used to overcome statistical issues that are potentially challenging in traditional approaches. In particular, for complex diseases such as AF, simultaneous use of hundreds of quantitative biomarkers may lead to problems such as multi-collinearity, non-linearity, complex interactions, and the possibility of over-fitting.(45-47) AI methods, including random (survival) forest method, a non-parametric ML decision tree-based approach, have been proposed to overcome such challenges.(45, 47) Second, AI approaches allow for data mining purposes to automatically extract more valuable information from unstructured and complex datasets to improve AF prediction. Ultimately, use of AI would allow combination of various extensive, annotated data libraries into multidimensional datasets which include genotyping, imaging, clinical, and other subphenotypic information. These multidimensional datasets could be used to identify different AF subphenotypes which then could be utilized to improve AF prediction, prevention, and management in a potentially sex and gender-specific manner. Particularly, the inclusion of multilayered high-throughput omics data in such datasets seems promising, given the vast contribution of genetic studies (genome-wide association studies, experimental and in silico candidate gene studies, and Mendelian randomization studies) to advance AF pathophysiology. Recently, a data-driven

cluster analysis of 9,749 AF patients, using 60 clinical characteristics, led to identification of 4 cluster AF phenotypes.(48) The 4 AF phenotypes were: AF with limited risk factors, younger AF patients with comorbid behavioral disorders, AF patients with tachycardia-bradycardia with device implantation due to sinus node dysfunction, and AF with atherosclerotic vascular disease.(48) Another cluster analysis of 2,458 AF patients, using 46 variables, identified 3 cluster AF phenotypes including younger paroxysmal AF, persistent/permanent AF with left atrium enlargement, and atherosclerotic comorbid AF in elderly.(49) Another study used hierarchical clustering analyses to identify distinct phenotypes of primary mitral regurgitation which is also considered a heterogeneous disease, as it is the case with AF.(50) As such, AI applications could further aid in improving subphenotypic AF classifications to further unravel the complexity and heterogeneity of AF. Based on data-driven approaches, rather than hypothesis-driven approaches with a-priori assumptions, leverage of big data with AI methods can also identify and prioritize AF biomarkers within the realm of AF risk prediction.

POTENTIAL OF BIG DATA FOR SEX-SPECIFIC PREDICTION OF NEW-ONSET ATRIAL FIBRILLATION

While big data sources such as ECGs, EHRs, and wearable devices could improve AF prediction and management, their potential for a sex- and gender-specific approach to AF needs further attention.

In particular, recent electrophysiological evidence highlights sex differences with regard to cardiac cellular electrophysiology and their translation to the ECG parameters. It is well documented that sex hormones affect the action potential morphology and cellular electrophysiology through their influence on ion channel function and current densities. Specifically, men have a shorter action potential duration, have a more prominent phase 1 repolarization, and shorter phase 3 repolarization than women.(51) It is hypothesized that primarily inward depolarizing L-type Ca^{2+} current and outward repolarizing K^{+} currents modified by sex hormones are responsible for these sex differences in action potential morphology. Moreover, women have a higher heart rate, lower heart rate variability, shorter and taller P waves, shorter PR interval, shorter and smaller QRS complexes, longer QT and QTc interval, longer JT interval, wider and smaller T waves, smaller J point, and smaller ST segment in comparison to men.(51) From a clinical perspective, the longer QTc interval in women makes women more prone to drug-induced QTc prolongation which may result in torsades de pointes.(51) The higher J point and ST segment in men may explain the higher prevalence of J wave syndromes (Brugada syndrome, and early repolarization syndrome) in men.(51) Next to these sex hormones' modulations on a cellular level, it is thought that the smaller size of the women's heart, at least partially, explains some of the documented ECG sex-differences.

Noteworthy, these sex differences in sinus rhythm generally persist when men and women develop AF.(52)

EHRs hold real-time, patient-centered data from men and women that reflect sexual and biological differences such as obesity and hypertension, among others. However, lack of precise and inclusive documentation of gender, the socio-culturally constructed characteristics of men and women, in EHRs is notable.(7, 53) Although, gender documentation may be incomplete in the narrow sense (no actual documentation of i.e. cis-, trans-, or non-binary gender identity), EHRs provide more information in a broad sense on socio-cultural habits, thereby representing gender. More specifically, EHRs could provide information on socio-culturally determined characteristics, roles or habits; including but not limited to socio-economic status, physical and social behaviors that influence e.g. physical activity, social interaction, medication use and adherence to medication, and healthcare use.(5-7) Such information could shed more light on gender-related factors that may impact AF. With regards to wearable devices, previous evidence has shown a higher burden of atrial and ventricular arrhythmia in women using a wearable cardioverter-defibrillator, compared to men.(54) This is in line with the evidence underpinning the existence of sex-specific ECG features, as mentioned earlier. In addition, similar to EHRs, wearable devices may give more insight into gender-related behavioral habits including physical activity, working hours, sleep, caloric and fluid intake, and other social activities. The latter variables have indeed been suggested to be of added value when modeling sex and gender differences in various health domains, although data to support this claim within the AF field is lacking.(55)

The sex-related dimensions in ECGs, and gender-related features in EHRs and wearable devices, as discussed earlier, are recognizable and should yet be fully explored to improve AF prediction and management. AI-enabled algorithms may be able to detect more sex-specific characteristics and gender-related behavioral patterns within these big data sources that might not be apparent on a more macroscopic level or by using traditional statistical methods. This may eventually improve our understanding of sex- and gender-related differences in AF.

DISCUSSION

This review highlighted the current status, premise, and potential of big data to improve prediction of new-onset AF in a sex- and gender-specific manner. While leveraging big data using AI-enabled algorithms offers major opportunities to further advance AF prediction, adoption of a sex- and gender-specific approach is still lacking.

Ample challenges remain before AI-enabled algorithms can be adopted for prediction, prevention, and management of AF. First, the interpretability (transparency and explainability) of AI and exact definition of how all the different methods work is yet difficult.(56) This so called “black box” is dependent on the type of AI algorithms that is being used (in particularly deep learning). Application of such algorithms warrants careful and thorough examination of the methods before their implementation, because an algorithm that is intransparent and/or unexplainable may lead to erroneous conclusions that could potentially harm a patient. Second, validation and calibration of AI-enabled algorithms for AF prediction while using external data sources are essential before such algorithms could be widely adopted, implemented, and used within the AF field. Third, the classification codes of clinical variables, drugs, and diseases in different countries and hospitals are different. This leads to challenges with regard to data extraction and harmonization. Careful standardization to harmonize the data, derived from multiple sources, and to integrate all the data modalities within a multidimensional dataset is warranted. Fourth, various ethical issues such as privacy, transparency, informed consent, and trust should be taken care of, and the potential for criminal and malicious use and contested ownership of data should be carefully considered.(57) Lastly, rigorosity of AI-enabled algorithms depends on the objectivity, quality, and size of the data used to train them. False, low quality, non-representative study sample, and/or missing data will result in invalid models, while also limiting the generalizability of such models.(58) This latter limitation is in particular of concern, as it further magnifies the sex and gender inequalities that already exist within research.

Large, diverse, and multidimensional data sources carry the potential to improve AF prediction and management. Yet, various challenges remain before AI-enabled algorithms can be adopted and implemented. Enhancement of personalized and precision medicine in AF warrants taking into account the complexity of sex and gender dimensions in big data sources and methods, while also overcoming the challenges that currently accompany the use of AI-enabled algorithms.

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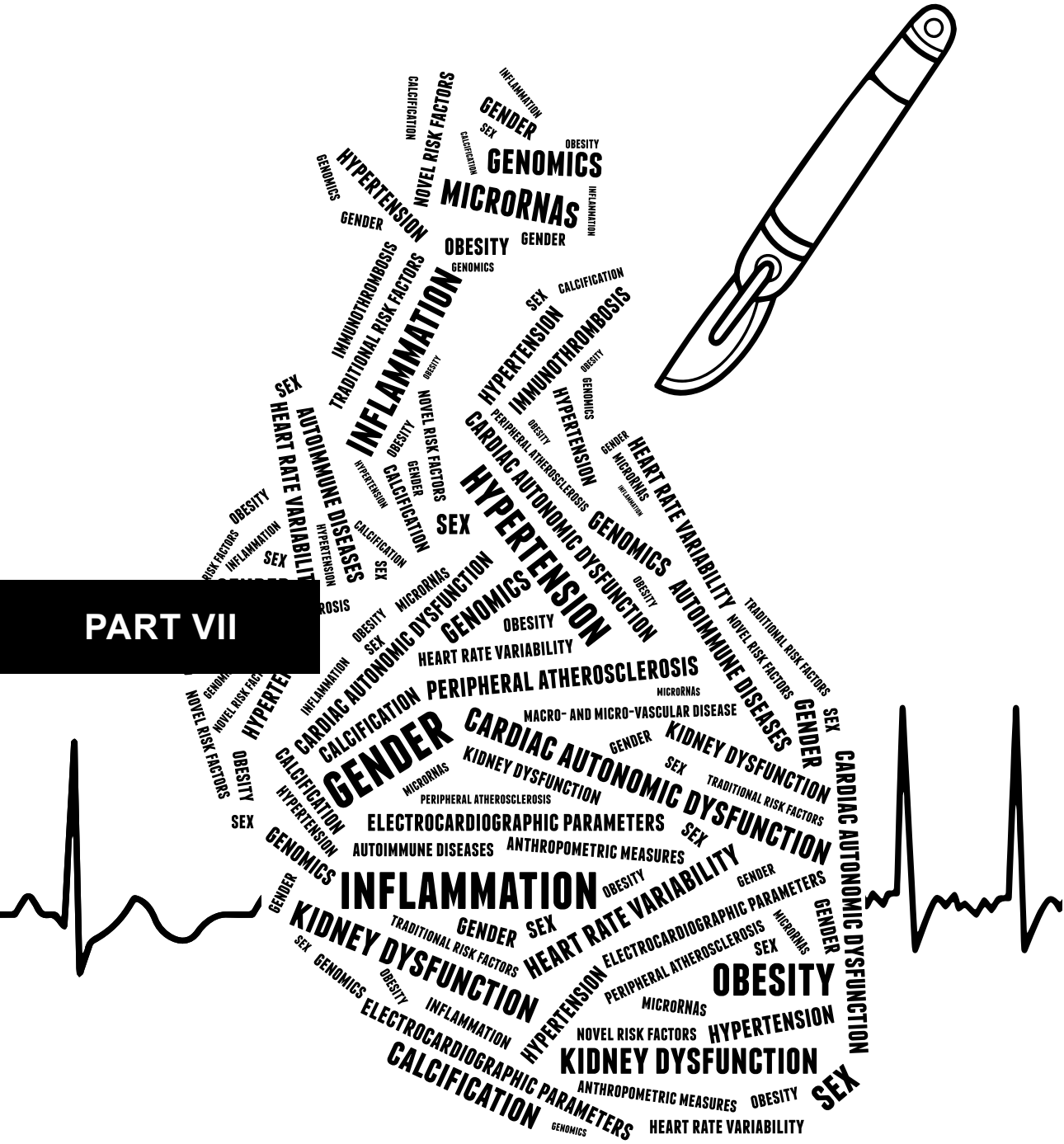
PART VII
INFLAMMATION
HYPERTENSION
GENOMICS
MICRORNAS
OBESITY
GENDER
SEX
CALCIFICATION
NOVEL RISK FACTORS
IMMUNOTHROMBOSIS
TRADITIONAL RISK FACTORS
HEART RATE VARIABILITY
AUTOIMMUNE DISEASES
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
KIDNEY DYSFUNCTION
ELECTROCARDIOGRAPHIC PARAMETERS
ANTHROPOMETRIC MEASURES
MACRO- AND MICRO-VASCULAR DISEASE

General discussion and summary


CHAPTER 7.1

General discussion

General discussion

In this thesis, the aim was to increase our knowledge on the etiology of atrial fibrillation through studying various risk factors and pathways. In particular, the association between macro- and micro-vascular disease (**Part II**), cardiac autonomic dysfunction (**Part III**), inflammation (**Part IV**), (shape of) trajectories of traditional risk factors and novel risk factors (**Part V**) has been studied in relationship with atrial fibrillation. I further addressed some sex- and gender-specific considerations in atrial fibrillation research (**Part VI**). More specifically, regarding macro- and micro-vascular disease, I highlighted the impact of coronary- and extra-coronary arteriosclerotic calcification, peripheral atherosclerosis, and kidney function on the risk of atrial fibrillation. Moreover, I studied how cardiac autonomic dysfunction, represented by heart rate variability, and electrocardiographic parameters, modulated atrial fibrillation risk. Also, immunothrombosis and autoimmune diseases, both proxies of inflammation, and their roles in atrial fibrillation were assessed. Furthermore, traditional and novel risk factors such as anthropometric measures and trajectories of obesity-related measures and blood pressure, and microRNAs and their influence on atrial fibrillation risk were examined. In this general discussion, the main findings of this thesis are briefly summarized. Subsequently, methodological considerations, potential clinical implications, and future directions are addressed.

Main findings

Part II Macro- and micro-vascular disease and the risk of atrial fibrillation

In **Part II**, we investigated macro- and micro-vascular disease and the risk of atrial fibrillation. In **Chapter 2.1**, we aimed to investigate the (sex-specific) association between arteriosclerotic calcification, a proxy of macro-vascular disease more proximally to the heart, and atrial fibrillation. Arteriosclerotic calcification, mirrored by volumes of coronary artery calcification, aortic arch calcification, extra- and intracranial carotid artery calcification, vertebrobasilar artery calcification, and the aortic valve calcification was quantified using CT-examinations in 2,259 participants free of atrial fibrillation from the Rotterdam Study. We observed that higher coronary artery calcification volume in the general population, especially in men, and higher aortic arch calcification volume in women were significantly associated with an increased risk of new-onset atrial fibrillation. These findings imply that interventions to lower arteriosclerotic calcification, in particular coronary artery calcification, carry a potential preventive effect on atrial fibrillation in the general population, especially among men. As **Chapter 2.1** pointed out the impact of macro-vascular disease, more proximally to the heart, on atrial fibrillation risk we widen this view in **Chapter 2.2** by examining the (sex-specific) link between subclinical measures of peripheral atherosclerosis, a proxy of macro-vascular disease more distally to the heart, and atrial fibrillation risk. Peripheral atherosclerosis, represented by carotid intima-media thickness, carotid plaque, and ankle-brachial index, was assessed in 12,840

individuals free of atrial fibrillation from the Rotterdam Study. We found that larger carotid and lower extremity peripheral atherosclerosis burden, both baseline and longitudinal measurements, were significantly associated with an increased risk of new-onset atrial fibrillation, especially in women. Our results suggest that treatment to reduce subclinical peripheral atherosclerosis carries a potential for prevention of atrial fibrillation in the general population, especially in women. After determining the role of proximal and distal macro-vascular disease in atrial fibrillation pathophysiology, we move forward in **Chapter 2.3** with examining the bidirectional association between kidney function, a surrogate of micro-vascular disease, and atrial fibrillation. This was investigated in 9,228 Rotterdam Study participants and our results indicated that kidney function, in particular estimated glomerular filtration rate (eGFR) based on cystatin C, and atrial fibrillation are significantly bidirectionally associated. In **Chapter 2.4**, we aimed to confirm our findings from the Rotterdam Study by employing a MR approach. We retrieved genetic variants from multiple GWAS that were associated with kidney function, reflected by eGFR based on creatinine, blood urea nitrogen, chronic kidney disease (stage ≥G3), eGFR based on cystatin C, urine albumin-to-creatinine ratio, and microalbuminuria, and atrial fibrillation. Our findings supported a significant bidirectional causal association between kidney function and atrial fibrillation. These findings from the previous two subchapters imply that kidney dysfunction and atrial fibrillation are correlated and therapeutic targets aiming one condition carry the potential to prevent/treat both conditions in the general population. Collectively, **Part II** highlights the importance of macro- and micro-vascular disease in atrial fibrillation pathogenesis and suggests that prevention and reduction of macro- and micro-vascular disease might prevent atrial fibrillation development. As **Part II** merely addresses more structural atrial fibrillation risk factors such as macro- and micro-vascular disease, we continue in **Part III** with cardiac autonomic dysfunction as a more electrical atrial fibrillation risk factor.

Part III Cardiac autonomic dysfunction and the risk of atrial fibrillation

In **Part III**, we examined cardiac autonomic dysfunction and the risk of atrial fibrillation. In **Chapter 3.1**, the link between heart rate variability, a surrogate of cardiac autonomic dysfunction, with atrial fibrillation was examined in 12,334 participants free of atrial fibrillation from the Rotterdam Study. Heart rate variability was reflected by the standard deviation of normal RR-intervals (SDNN), SDNN corrected for heart rate (SDNNc), RR-interval differences (RMSSD), RMSSD corrected for heart rate (RMSSDc), and heart rate. Additionally, we used genetic variants for heart rate variability, and atrial fibrillation obtained from multiple GWAS to conduct a MR analysis. We observed that longitudinal measurements of uncorrected heart rate variability were significantly associated with increased new-onset atrial fibrillation risk, in particular in women. Subsequently, our MR analysis supported the causal relation between uncorrected measures of heart rate variability with atrial fibrillation. Overall, our study suggests that measures to modulate heart

rate variability might prevent atrial fibrillation in the general population, in particular in women. In **Chapter 3.2**, our aim was to determine the (shape of the) association and sex differences between electrocardiographic parameters, as other surrogates of cardiac autonomic dysfunction, and atrial fibrillation in 12,212 individuals from the Rotterdam Study that were free of atrial fibrillation at baseline. Electrocardiographic parameters included PR, QRS, QT, QT corrected for heart rate (QTc), JT, RR interval, and heart rate. The shape of the association between baseline electrocardiographic measures and risk of new-onset atrial fibrillation were mostly U- and N-shaped. Longitudinal electrocardiographic measures of PR, and QTc interval were significantly associated with an increased new-onset atrial fibrillation risk, in particular among men. This implies that different thresholds of electrocardiographic parameters might translate to a differential risk among men and women and that treatment options targeting electrocardiographic parameters might prevent atrial fibrillation in the general population, in particular in men. Taken together, **Part III** stresses the contribution of cardiac autonomic dysfunction to atrial fibrillation risk. **Part II** and **Part III** merely address some of the etiologic questions regarding structural and electrical atrial fibrillation risk factors. We therefore in **Part IV** attempt to complement **Part II** and **Part III** by answering etiologic research regarding inflammatory atrial fibrillation risk factors.

Part IV Inflammation and the risk of atrial fibrillation

In **Part IV**, the aim was to investigate the role of inflammation in atrial fibrillation risk. In **Chapter 4.1,** the association between immunothrombosis, a potential novel atrial fibrillation risk factor at the intersection of the innate immune system and hemostasis, and atrial fibrillation was evaluated in 6,174 Rotterdam Study participants free of atrial fibrillation at baseline. Factors of immunothrombosis included fibrinogen, von Willebrand factor, ADAMTS13, and neutrophil extracellular traps. No significant association between markers of immunothrombosis and new-onset atrial fibrillation in the general population was observed. Therefore, immunothrombosis may be associated with atrial fibrillation through other cardiovascular risk factors or conditions that may predispose to atrial fibrillation. In **Chapter 4.2**, we systematically reviewed the literature on the interaction of immunothrombotic markers and atrial fibrillation, and performed a meta-analysis. This systematic review and meta-analysis showed that atrial fibrillation is significantly associated with higher levels of immunothrombosis. The associations were most pronounced in the cross-sectional analyses while limited longitudinal studies were available that investigated if immunothrombosis underlie atrial fibrillation initiation. This suggests the notion that immunothrombosis, in particular a prothrombotic and hypofibrinolytic state, could be promoted by atrial fibrillation. Further, it also underlines the hypothesis that “atrial fibrillation begets atrial fibrillation” as the prothrombotic state is suggested as an underlying mechanism of atrial fibrillation maintenance, and progression. Next to immunothrombosis, we also studied whether autoimmune diseases, representing dysregulation of the immune system, were implicated in atrial fibrillation initiation.

Therefore in **Chapter 4.3**, the association between autoimmune diseases and the risk of atrial fibrillation were examined in 494,072 UK Biobank participants free of atrial fibrillation at baseline. Autoimmune diseases such as rheumatic fever, rheumatic heart disease, type 1 diabetes mellitus, multiple sclerosis, myasthenia gravis, Crohn's disease, ulcerative colitis, rheumatoid arthritis, psoriatic and enteropathic arthropathies, polyarteritis nodosa, systemic lupus erythematosus, dermatopolymyositis, systemic sclerosis, ankylosing spondylitis, and Paget's disease were included. Significant associations between rheumatic fever without heart involvement, type 1 diabetes mellitus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, polyarteritis nodosa, systemic lupus erhythematosus, and systemic sclerosis and new-onset atrial fibrillation were observed, most distinctly in women. These findings elaborate on the pathophysiological differences in autoimmune diseases and atrial fibrillation risk between men and women. In total, **Part IV** underpins the role of inflammation in atrial fibrillation risk. Yet **Part II**, **Part III**, and **Part IV** do not cover the whole spectrum of possible atrial fibrillation risk factors. Therefore, in **Part V** we again attempt to complement **Part II**, **Part III**, and **Part IV** by answering etiologic research questions regarding risk factors that cannot be classified under the aforementioned parts by addressing some traditional and novel atrial fibrillation risk factors.

Part V Traditional and novel risk factors for atrial fibrillation

In **Part V**, we aimed to further expand our knowledge about atrial fibrillation by applying novel methods for investigating the (shape of) trajectories of traditional risk factors and the risk of atrial fibrillation and identifying potential novel atrial fibrillation risk factors. In **Chapter 5.1**, the relationship between anthropometric measures, traditional atrial fibrillation risk factors, and atrial fibrillation in 12,484 free of atrial fibrillation individuals from the Rotterdam study was investigated. The anthropometric measures in this study included weight, height, waist circumference, hip circumference, waist-to-hip ratio, and body mass index. Longitudinal measures of anthropometrics were significantly associated with an increased risk of new-onset atrial fibrillation. Increased height in men and increased weight in women showed the largest associations with atrial fibrillation. Further, an increase in central obesity (waist-to-hip ratio) showed a more pronounced association with higher risk for new-onset atrial fibrillation in women, as compared to men. This highlights height in men and weight, and in particular central obesity (waist-to-hip ratio), in women as predominant risk factors for new-onset atrial fibrillation. These findings stress the importance of a sex-specific approach for screening and monitoring of anthropometrics in an attempt to prevent atrial fibrillation. **Chapter 5.2** extends the knowledge on traditional risk factors by assessing the link between trajectories of obesity-related measures and blood pressure and atrial fibrillation. In this subchapter 7,367 individuals free of atrial fibrillation from the Rotterdam Study were examined. Repeated measurements of weight, body mass index, hip circumference, waist circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure,

and pulse pressure were analyzed using latent class linear mixed models to identify the potential trajectory classes and assess their impact on the risk for new-onset atrial fibrillation. Longitudinal trajectories of weight, body mass index, hip circumference, waist circumference, waist-to-hip ratio, and systolic blood pressure were significantly associated with an increased risk of new-onset atrial fibrillation in both men and women. Diastolic blood pressure trajectories were additionally significantly associated with increased new-onset atrial fibrillation risk in women. Findings suggested sex differences regarding the associations between waist circumference, hip circumference, and diastolic blood pressure trajectories and the occurrence of atrial fibrillation. This highlights the importance of assessing long-term exposure to modifiable traditional risk factors such as obesity, and hypertension for atrial fibrillation prevention strategies among men and women. As trajectories of hypertension indeed were identified as traditional risk factor for atrial fibrillation, we carried this idea forward in **Chapter 5.3**. In this subchapter we evaluated the effect of antihypertensive drugs by applying a drug target MR study approach to study the potential preventive capacity of antihypertensive drugs on atrial fibrillation prevention. Additionally, by conducting a drug target MR approach we tried to avoid the potential limitations of clinical studies. We used well-validated published genetic variants that mirror the action of 12 antihypertensive drug classes including alpha-adrenoceptor blockers, adrenergic neuron blockers, angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, beta-adrenoceptor blockers, centrally acting antihypertensive drugs, calcium channel blockers, loop diuretics, potassium-sparing diuretics and mineralocorticoid receptor antagonists, renin inhibitors, thiazides and related diuretic agents, and vasodilators via their corresponding gene and protein targets, and we estimated their downstream effect for atrial fibrillation prevention via systolic blood pressure using two-sample MR analyses. Drug target MR analyses supported the significant preventive causal effects of lowering systolic blood pressure per 10 mmHg via alpha-adrenoceptor blockers, beta-adrenoceptor blockers, calcium channel blockers, vasodilators, and all 12 antihypertensive drug classes combined on atrial fibrillation risk. These results indicate that lowering systolic blood pressure via genetic proxies of various antihypertensive drugs seems promising for atrial fibrillation prevention. These findings may guide future clinical trials and have implications for repurposing antihypertensive drugs in atrial fibrillation prevention. After discussing traditional atrial fibrillation risk factors by employing novel methods in subchapters **Chapter 5.1**, **Chapter 5.2**, and **Chapter 5.3**, we carry on in the following subchapters with identifying novel atrial fibrillation risk factors. Lastly, in **Chapter 5.4**, we assessed the association between 591 well-expressed circulatory microRNAs in plasma, as a potential novel atrial fibrillation risk factor, and atrial fibrillation in 1,999 participants from the Rotterdam Study. We further examined the link between predicted target genes of identified microRNAs and reviewed the literature to get an overview of microRNAs and atrial fibrillation. We found that plasma levels of miR-4798-3p were significantly associated with the odds of prevalent atrial fibrillation in men. In addition, miR-4798-3p may potentially regulate

the expression of a number of atrial fibrillation-related genes including genes involved in calcium and potassium handling in myocytes, protection of cells against oxidative stress, and cardiac fibrosis. This is promising, as this might explain why miR-4798-3p is implicated in atrial fibrillation pathophysiology, but future experimental studies are warranted for confirmation. Altogether, the findings in **Part V** show that various traditional and novel atrial fibrillation risk factors modulate atrial fibrillation risk. Although sex differences have also been evaluated throughout this thesis, we proceed with the implications for sex and gender in atrial fibrillation research in **Part VI**.

Part VI Sex and gender implications and the risk of atrial fibrillation

In **Part VI**, the implications of sex and gender in atrial fibrillation are discussed. In **Chapter 6.1**, the relationship between women-specific risk factors and atrial fibrillation is investigated in 235,191 women free of atrial fibrillation from the UK Biobank. Women-specific risk factors consisted of age at menarche, irregular menstrual cycle, menopause status, age at menopause, years after menopause, age at first birth, history of stillbirth, number of live birth, and total reproductive years. Early or delayed menopause, early or delayed menarche, or irregular menstrual cycles were significantly associated with an increased new-onset atrial fibrillation risk. Also, both nulliparity and multiparity, compared to having 1-2 children, were significantly associated with an increased new-onset atrial fibrillation risk. Our findings underpin that the knowledge regarding the reproductive history of women is of added value in devising screening strategies for the prevention of atrial fibrillation. In **Chapter 6.2**, we described the current status and future directions of sex- and gender-specific atrial fibrillation prediction by leveraging big data. atrial fibrillation remains to be a complex and heterogeneous disorder and atrial fibrillation prediction models are not yet conclusive, despite the advances over the last two decades regarding epidemiology, prediction, pathophysiology, and treatment of atrial fibrillation. While leveraging big data using artificial intelligence-enabled algorithms offers major opportunities to advance the scientific research field of atrial fibrillation, adoption of a sex- and gender-specific approach is still lacking. The opportunities with clinical data, multidimensional datasets, electrocardiograms (ECGs), electronic health records, and wearable devices are profound. However, ample obstacles remain in the way before artificial intelligence-enabled algorithms can be adopted for prediction, prevention, and management of atrial fibrillation. These obstacles include interpretability (transparency and explainability), classification of clinical variables, drugs, and diseases, ethical issues, and the objectivity, quality, and size of the data used to train the various algorithms. To further advance sex- and gender-specific prediction, management, and ultimately prevention of atrial fibrillation, researchers using artificial intelligence need to properly design, conduct, and analyze future research while taking sex and gender into account. All things considered in **Part VI,** the implications of sex in atrial fibrillation should be evident.

Methodological considerations

This research was embedded within the epidemiological framework of the Rotterdam Study, the UK Biobank, and multiple GWAS. Methodological considerations that are tied to the individual studies in this thesis have been described in the each respective subchapter. Here, we present more general methodological considerations, as one should bear in mind numerous more general considerations when evaluating the implications and conclusions obtained from the studies included in this thesis or from any observational population-based cohort study in general. In particular, prior knowledge, validity (absence of biases), and precision (power) should be considered. I will address several methodological considerations such as causality, internal validity, external validity, and precision in the following sections.

Causality

The concept of causality was already touched upon by Plato (428 BCE-348 BCE) in the earliest Greek literature as Plato said: “Everything that becomes or changes must do so owing to some cause; for nothing can come to be without a cause”. Naturally, this concept about causality further evolved over time. More recently, Austin Hill (1897-1991) defined causality according to 9 epidemiologic criteria: strength, consistency, specificity, temporality, dose response relationship, biological plausibility, coherence, experiment, and analogy which were also called Hill’s criteria. Nowadays, causal inference is continuously evolving, yet Hill’s criteria still provide a useful guideline to investigate causality.

We will briefly touch upon Hill’s criteria and use them to comment on and discuss the studies included in this thesis where possible. These comments are not exhaustive, but rather indicative. First, the larger the strength or effect size the more likely an association is to be causal, although a small effect size does not mean that there is no causality, i.e. especially the genetic variants used in MR analyses are known to have very small effect sizes on diseases. Second, consistency refers to finding consistent results obtained by different study populations at different times and places, i.e. especially within GWAS replication is a common practice. Third, specificity means that the exposure only causes one disease which is a challenging one, in particular with respect to atrial fibrillation being a multifactorial or complex disease. Fourth, the longitudinal study design of prospective cohort studies such as the Rotterdam Study and UK Biobank is particularly helpful when evaluating the temporality of an association, because the assessment of the exposure is performed prior to the outcome of interest occurrence which largely limits certain biases such as differential misclassification of exposure, recall bias, and reverse causality. In addition, the longitudinal nature of the Rotterdam Study allowed me to include repeated measurements of various exposures for several studies included in this thesis. Fifth, next to the temporality, analyzing longitudinal repeated measurements may also further support Hill’s strength criterion. Evaluation of longitudinal repeated measurements captures the time-varying nature of a given exposure on the outcome

of interest, thus further strengthening the dose response relationship criterion. Sixth, all studies in this thesis were based on biological plausibility which refers to a biological hypothesis that relates an exposure with an outcome. This plausibility is based on the current available knowledge, although complete underlying knowledge regarding the causal framework is likely to be impossible. Seventh, using data from multiple data sources could strengthen the coherence criterion. Next to our main reason to use MR analyses to support causality of observational studies. We also included MR analyses using genomic data where possible to complement our initial analyses using non-genomic data to use data from different sources. Eighth, although no formal experiment with investigator initiated experiments were used in this thesis, we performed natural experiments using observational data. More specifically, one could compare MR with randomized clinical trials (RCTs), because the random allocation of alleles at conception is comparable to the randomization that happens in RCTs. Where the effect allele represents the medical intervention and the other allele the control group. Lastly, analogy means that more hypotheses can be generated from the studied association. These hypotheses that could be derived from this thesis and any research in general are of most interest for future research.

Proving causality is formally speaking reserved for RCTs, but conducting RCTs is not always possible due to ethical, logistic, and financial considerations. In addition, RCTs have other limitations such as non-adherence, loss to follow-up, and blinding. When this is the case, observational studies can provide the first steps towards inferring causality. While proving causality from observational studies may be challenging, ample of examples are available that prove the contrary. Famous examples that celebrate the major epidemiological successes from observational studies include smoking and lung cancer, and the Broad Street pump and cholera scenario described by John Snow.

Internal validity

One should critically review the accuracy of a study when appraising epidemiological research. Generally speaking, accuracy consists of precision, and validity. While precision is about the reproducibility of a study which is mainly handled by an adequate sample size, validity is about the correctness of a study and can be divided into internal and external validity. Three sources of error or bias that are collapsed within internal validity are selection bias, information bias, and confounding. The determinants of internal validity will be reviewed first and the determinants of external validity will be discussed later.

First, selection bias is the type of bias that occurs when an association between exposure and outcome is different for individuals under investigation in comparison to the individuals that theoretically could have been eligible to participate in that same study. This happens when exposure, outcome or both are related to the probability

to participate in a study or remain under follow-up. It is well known that participants of a population-based study are on average healthier than participants that do not wish to participate, the so called healthy volunteer or healthy worker effect. This could have been the case in this thesis as participants were only included if they were willing and were able to visit the research centers for the wide range of measurements that were conducted. One can imagine that participants that did not visit the research centers were on average older and had more comorbidities. Thus, individuals with a higher risk of atrial fibrillation or comorbidities due to atrial fibrillation may have not been included in our studies. Given the relative high response rate of 72% at baseline in the Rotterdam Study it seems unlikely that at the inclusion stage this would have caused a real concern.(1) The exceptionally low response rate at baseline of 5.5% from the UK Biobank may be of concern.(2, 3) In other words, findings derived from such studies may be of limited value to reliably describe the prevalence and incidence of various diseases. However, studies that use this kind of data to answer causal research questions provide reliable results. Additionally, excluding older and more diseased individuals at baseline may have caused selection bias to some degree, although it probably generated underestimated and more conservative effect estimates than the currently reported effect estimates in this thesis.

Selection bias regarding remaining under follow-up is often a matter of competing risk which refers to dependent or informative censoring whereas loss to follow-up by moving abroad refers to independent or non-informative censoring. Noteworthy, the loss to follow-up is very low in the Rotterdam Study due to a wide variety of measures to ensure completeness of our follow-up data. Competing risk is the concept of that the occurrence of an event (the competing event) precludes the onset of the outcome of interest. From an etiologic perspective, which was the aim of most studies included in this thesis, it seems intuitive to incorporate competing risk analyses. In our case, the occurrence of mortality precludes the onset of atrial fibrillation. Therefore, we tried to address this informative censoring by conducting competing risk analyses where we considered mortality, before the onset of atrial fibrillation, as a competing event. Conducting such analyses is important to obtain cause-specific associations which could then inform and guide future studies with targeted interventions that aim to reduce atrial fibrillation risk. Of note, ignoring competing events can lead to estimates of cumulative incidence and risk that are biased upwards.(4-6)

Second, information bias is the error that occurs when information is inaccurately measured. Often, it is also named misclassification bias which can be differential (non-random) or non-differential (random). The former one is mainly problematic, because it may lead to under- or overestimated effect estimates. It happens when the measurement error in the exposure is related to confounder, outcome, or both and may also apply the other way around. The latter one generally nullifies any observed effect and occurs when there is random error in the measurement that is

unrelated to confounder, outcome, or both. Despite that most data were derived from standardized lab, physical examination, CT, and ECG measurements, this does not rule out potential misclassification bias of the exposures that were investigated in this thesis. However, any misclassification bias that could have occurred in the exposures of interest is likely to be non-differential (random) due to biological variation rather than differential (non-random). This biological variation is more intuitive for lab, physical examination, and ECG measurements such as eGFR, blood pressure, and PR interval and may be exaggerated in analyses that rely on adjustments based on single baseline measurements. Therefore, we tried to reduce this potential misclassification bias due to random biological variation by analyzing repeated measurements using joint models, if repeated measurements were available. Furthermore, heart rate variability and electrocardiographic parameters were based on 10-second ECGs, while heart rate variability guidelines recommend that heart rate variability measures are based on preferably 5-minute or 24-hour ECG recordings.(7) Such recommendations regarding the ECG duration are not necessarily made for evaluating electrocardiographic parameters with the exception of assessing heart rate in arrhythmic disorders such as atrial fibrillation.(8) Nevertheless, 10-second ECGs are routinely assessed in healthcare settings, are cheaper, faster, and thereby more patient friendly than longer ECG recordings. In particular, heart rate variability measures from 10-second ECGs have already been associated with left ventricular function,(9) heart failure,(9, 10) cardiac- (11) and all-cause mortality.(12) It is also worth noting that other studies that investigated the reliability of 10-second ECGs in comparison to 5-minute ECGs to assess heart rate variability showed that 10-second ECGs are also a reliable tool for heart rate variability risk assessment, in particular within population-based studies.(13, 14)

On one hand, one could argue whether the assessment of atrial fibrillation, our outcome, within the Rotterdam Study and UK Biobank was adequate, because of our inability to distinguish between paroxysmal, persistent, long-term persistent, and permanent atrial fibrillation as Holter monitoring has not been done in these (very) large population-based cohort studies. Nonetheless, the follow-up data were continuously collected through linkage with digital files from general practitioners in the study area, hospitals, outpatient clinics, national registration of all hospitals discharge diagnoses, and follow-up examinations at the research center within the Rotterdam Study. Within the UK Biobank a comparable methodology was used as medical records using hospital admission, primary care and/or death registry data linked to the UK Biobank were used to assess atrial fibrillation. We can indeed not rule that paroxysmal atrial fibrillation, at baseline and during follow-up, could have occurred and gone unnoticed. In particular, as we relied on 10-second ECGs to assess atrial fibrillation prevalence and incidence. However, our atrial fibrillation diagnosis is in accordance with the latest European Society of Cardiology ESC guidelines which state that a 12-lead ECG or 1-lead ECG tracing of at least 30 seconds is required to diagnose atrial fibrillation.(15) In addition, the prevalence of

atrial fibrillation in the Rotterdam Study is around 4% at baseline and around 1% at baseline in the UK Biobank which are more or less in line with the estimated atrial fibrillation prevalence of 2-4% in adults.(15) About missing participants who developed atrial fibrillation during follow-up and that were not flagged as incident atrial fibrillation cases within this thesis, this would mean that our observed associations and effect estimates are an underestimation of the true underlying associations between the studied exposures and atrial fibrillation. This means that our reported results are potentially more conservative than the reality. On the other hand, potential misclassification of atrial fibrillation is inherent to the nature of the disease and it is hypothesized that atrial fibrillation first occurs paroxysmally and gradually progresses to permanent atrial fibrillation over time.(16) If this is indeed the case then this would limit the added value to differentiate between paroxysmal, persistent, long-term persistent, and permanent atrial fibrillation to begin with.

Third, confounding refers to a shared cause between exposure and outcome which provides a spurious relationship between exposure and outcome. This shared underlying cause or factor is independent of exposure and outcome. Confounding is in particular a threat in observational studies due to the lack of exposure assignment randomization. The Rotterdam Study and the UK Biobank are both well known for their deep phenotyping, and in-depth assessment of a whole range of health and disease determinants. This broad availability of determinants allowed us to extensively adjust for a wide variety of factors which we considered as confounders in our studies. Adjusting for confounders is a necessity to unravel the mechanisms underlying atrial fibrillation, because atrial fibrillation is a multifactorial polygenetic arrhythmia that is closely intertwined with concomitant cardiovascular diseases and shared traditional cardiovascular risk factors. Therefore, we adjusted for other cardiovascular diseases such as coronary heart disease, heart failure and cardiovascular risk factors such as obesity, hypertension, smoking, dyslipidemia, and among others. Despite our best intentions to extensively adjust for a variety of confounders based on the principles of confounder selection called the “disjunctive cause criterion” proposed by VanderWeele,(17) we cannot entirely rule out residual confounding or unmeasured confounding. We also cannot rule out that we could have adjusted for potential mediators and/or potential colliders in our analyses, although we tried to avoid this with our knowledge about the potential biology at play. A mediator is an intermediate third variable that is on the causal pathway from the exposure to the outcome.(18) Mediation analysis is applied when one wants to examine to which extent the effect of the exposure is explained (or not), by one or multiple mediators. By applying mediation analysis one can decompose the total effect of the exposure on an outcome which is the sum of the direct effect, the effect of the exposure on the outcome without the mediator(s), and the indirect effect, the effect of the exposure on the outcome with the mediator(s).(18) A collider is a common third variable that is influenced by both the exposure and outcome.(19) When one controls for this third variable by design or analysis one would introduce

collider bias.(19) This in contrast to confounding bias which is introduced when an exposure and outcome share a common cause that one is not controlling for. For illustrative purposes, we highlight one association from the studies embedded in this thesis to describe potential concerns when one is to assess an association. Although, the real biology is far more complex, let us assume the following oversimplified association. Where arteriosclerotic calcification is the exposure, atrial fibrillation the outcome and coronary heart disease the potential confounder, mediator and/or collider. It could very well be that in this scenario coronary heart disease may be a confounder, mediator and/or collider. As coronary heart disease is associated with both arteriosclerotic calcification and atrial fibrillation. If one assumes that coronary heart disease is on the causal pathway from arteriosclerotic calcification to atrial fibrillation this would make coronary heart disease a mediator (i.e. coronary heart disease is an intermediate variable on the causal pathway from arteriosclerotic calcification to atrial fibrillation), if not it would be a confounder (i.e. coronary heart disease is a common cause of both arteriosclerotic calcification and atrial fibrillation). Regarding the possibility of coronary heart disease being a collider, this would mean that both arteriosclerotic calcification and atrial fibrillation cause coronary heart disease (i.e. coronary heart disease is a common effect of both arteriosclerotic calcification and atrial fibrillation). We are unable to provide a definitive answer to the question whether coronary heart disease is a confounder, mediator and/or collider in this association. Although we would think it is more likely that coronary heart disease is a confounder and/or mediator in this association. Furthermore, we used the following empirical approach in the absence of completely understanding the underlying biology (“oracle knowledge”) that could aid in this illustrated example.(20) We tried to tackle this by using multiple models in our analyses where model 1 was adjusted for unmodifiable risk factors such as age, sex (and study cohort) and model 2 for additional (modifiable) cardiovascular risk factors to evaluate the association of interest. In short and probably oversimplified, when one adjusts for a confounder or mediator one attenuates the association between the exposure and outcome and when adjusting for a collider one induces an association between the exposure and outcome.(18, 19) In our analyses, we almost always observed a reduced effect estimate from the exposure on the outcome when we additionally adjusted for cardiovascular risk factors (model 2) in comparison to our adjustment for age, sex, and cohort (model 1) which could narrow the three possibilities down to two: confounder and/or mediator. Although, on one hand, one should not adjust for a mediator as it provides more attenuated effect estimates. On the other hand, it might be better to minimize potential confounding bias by a potential confounder at the expense of being more conservative in estimating effect estimates by unrightfully adjusting for a potential mediator.

Aforesaid, observational studies are prone to residual confounding and reverse causality and therefore cannot formally speaking support causality between an exposure and outcome.(21) MR has emerged as a reliable genetic research method

to leverage genetic variation to overcome some of the limitations of observational studies and to estimate causality.(21, 22) MR uses genetic proxies of risk factors that are not prone to these biases, because of the random distribution of genetic variants at conception. MR also extends traditional or non-genetic observational research by calculating risk estimates that represent a life time subjection to a certain exposure where traditional observational studies estimate a risk estimate of an exposure with a certain or limited follow-up time, e.g. 10-years risk. In addition, MR is a valid and reliable research method to assess causality of associations which are not possible or feasible to be investigated with RCTs due to limitations such as being unethical, unpractical and/or very expensive. Nonetheless, three assumptions should be fulfilled for MR analyses to provide valid causal estimates. The first assumption is that the genetic variant is strongly associated with the exposure of interest. The second assumption is that the genetic variant only affects the outcome through its effect on the exposure of interest. The third assumption is that the genetic variant is not associated with any confounders of the exposure-outcome relationship. Selecting genome-wide significant genetic variants and selecting genetic variants with a F-statistic >10 satisfies the first assumption. Yet, it is impossible to formally test if the second and third assumptions have been fulfilled. It is up to us the researchers to determine, with our in-depth knowledge about the underlying biology, if it is reasonable to assume that the latter two assumptions have been satisfied. This means that we cannot rule out unobserved horizontal pleiotropy for the studies included in this thesis, although we tried to address horizontal pleiotropy through current best practices for MR analyses by conducting various sensitivity analyses. Despite these limitations of MR, we consider MR to be a reliable tool to strengthen and complement our traditional longitudinal analyses throughout this thesis and to leverage genetic variation to infer causality.

External validity

External validity, also called generalizability, refers to the idea if the findings of a study in a particular population are also applicable to another population. In other words, if the results from the Rotterdam Study and UK Biobank would also translate to another population within Europe or to the rest of the world. Before we can answer such a question we need to consider the following. Participants from the Rotterdam Study and UK Biobank are mainly of middle- and older age. Also, all individuals are living in the same Ommoord suburb within the city of Rotterdam for the Rotterdam Study, and within England, Scotland, and Wales for the UK Biobank. Further, both study populations are mostly of European descent.

On one hand, one could argue that this creates a homogeneous study population, strengthening the observed effect estimates and eliminating noise from unmeasured confounders that could cloud the results. On the other hand, this homogeneity limits the generalizability and suggests that the findings might not apply to younger individuals and/or individuals from other ethnicities. The same can be said for the

participants from the UK Biobank. While this is not necessarily an issue, because the aims of the Rotterdam Study and UK Biobank are to study European middle-aged and elderly individuals. It is something to keep in mind when interpreting, discussing or extrapolating our results. Evidence from other population-based cohorts across the globe are therefore warranted to extend the findings from the Rotterdam Study and UK Biobank in an attempt to further advance our knowledge regarding atrial fibrillation in other source populations. In essence, there should be a well-considered tradeoff between internal and external validity when one is designing a study. However, the study results should be valid (i.e. internal validity) to start with.

Precision

Most data used in this thesis has been drawn from (very) large population-based cohort studies such as the Rotterdam Study, the UK Biobank, and various large-scale GWAS. The (very) large sample sizes of these cohort studies provided us with a greater probability (power) to obtain statistically true effect estimates in our analyses. These large sample sizes stand in contrast to the average sample size of <50 patients that is mostly observed in clinical studies. In addition, the (very) large sample size empowered us to stratify most of our analyses for various (clinically) relevant factors, e.g. sex, and obesity status. This stratification informed us about the potential effect modification (interaction) of these factors for the observed association between our exposures and atrial fibrillation.

Implications and future directions

In the past years the scientific knowledge regarding the epidemiology, prediction, pathophysiology, and treatment of atrial fibrillation has been propelled. Yet, the prevalence, incidence, and life time risk of atrial fibrillation render atrial fibrillation as one of the important cardiovascular diseases of the 21st century with a high morbidity, mortality, and significant healthcare burden. This motivates the need to further unravel traditional and novel risk factors of atrial fibrillation to better understand, predict, and ultimately prevent this cardiovascular epidemic. Next, I will review the potential implications of this thesis and touch upon future directions for atrial fibrillation research.

Part II Macro- and micro-vascular disease and the risk of atrial fibrillation

Macro-vascular disease such as arteriosclerotic calcification, and peripheral vascular disease may lead to ischemia of the atria, atrial fibrosis, and subsequently increase the propensity to develop atrial fibrillation.(23, 24) In addition, atrial, and ventricular remodeling of the heart may occur by the increased cardiac afterload through arterial stiffness.(25-29) It has also been suggested that inflammation, endothelial dysfunction, and platelet-mediated thrombosis have a contributing role in the underlying mechanisms that link (peripheral) atherosclerosis with atrial fibrillation.(29-32) Micro-vascular disease mirrored by kidney dysfunction may trigger

atrial fibrillation through the renin-angiotensin-aldosterone system,(33-40) hypertension,(37, 38) left ventricular hypertrophy,(37) inflammation,(37, 41-43) and by inducing various cardiovascular diseases such as coronary heart disease, and heart failure.(38, 44, 45) The results from **Chapter 2** indicated that (sub)clinical measures of macro- and micro-vascular disease represented by arteriosclerotic calcification, carotid atherosclerosis, lower extremity atherosclerosis, and kidney dysfunction in the general population have a strong relationship with atrial fibrillation development.

Some potential clinical implications that could be carried forward from these findings are that appropriate management of (sub)clinical macro- and micro-vascular disease might prevent atrial fibrillation. Potential specific therapies to prevent and treat macro- and micro-vascular disease include treatment for hypercholesterolemia, hypertension, promoting a healthy lifestyle, and smoking cessation.(46) Additionally, medication such as statins are associated with a slower progression of coronary artery calcification, with increased plaque calcification, and reduction of high-risk plaque features.(47, 48) Of note, emerging evidence is being generated that show even more favorable effects of statins on coronary artery calcification when statins are being combined with PCSK9 inhibitors.(49) Future RCTs could support the findings in this thesis by evaluating atrial fibrillation as an outcome when performing vascular disease-targeted interventions. Moreover, in this thesis we used total arteriosclerotic calcification volumes, carotid intima-media thickness, carotid plaque, ankle-brachial index, estimated glomerular filtration rate, among others, as proxies for macro- and micro-vascular disease from mostly single baseline measurements. It would be interesting to conduct more quantitative repeated measurements of these proxies at a nationwide population-level or patient-level to assess their role in atrial fibrillation etiology and progression. Future research could benefit from including repeated CT or even MRI measurements of macro- and micro-vascular disease and assessing the slope, cumulative exposure to these risk factors, and trajectories over time with regards to the risk of new-onset atrial fibrillation. As repeated measurements may provide more information than a single baseline measurement and could be leveraged to assess the impact from the yearly increment of vascular disease and the cumulative risk exposure during an extended time period on new-onset atrial fibrillation. It is suggested that total calcium scores, higher calcium score categories, and a calcium score of 0 are of most potential predictive value for coronary artery disease.(50) This method does not take into account the degree of potential stenosis and the specific branch or region that may be the culprit lesion. Interesting work from the clinic with regard to coronary artery stenosis and new-onset atrial fibrillation after 1 year has already been conducted. In this study, patients with coronary artery disease during cardiac catheterization were recruited between 2007 and 2013 and underwent quantitative measurements of stenosis severity for the sinoatrial nodal artery, atrioventricular nodal artery, right intermediate atrial artery, right coronary, left circumflex, and left anterior descending proximal to the takeoff for

each atrial level artery.(51) This study showed that in patients with obstructive coronary artery disease, stenosis of the atrioventricular nodal artery, right intermediate atrial artery, as well as a higher burden of coronary artery disease within all coronary arteries that vascularize the atria were associated with a higher odds of new-onset atrial fibrillation at 1 year follow-up.(51) Adopting such an artery- or site-specific approach using non-invasive imaging modalities (CT and/or MRI) could help determine the exact impact from a specific coronary artery or a specific vascular region on atrial fibrillation pathogenesis in a population- or patient-based setting. Moreover, screening for coronary artery calcification or calcium scoring is probably the most widely studied population-based screening modality for cardiovascular disease.(52) It has been shown that it may even improve a patient's adherence to medication and lifestyle.(52) Future research could also aid in determining if such a population-based screening strategy for total coronary artery calcium or artery- or site-specific calcification may also help to prevent atrial fibrillation. Nonetheless, I hypothesize that early screening for (sub)clinical macro- and micro-vascular disease and appropriate and timely management of vascular disease may be of benefit to reduce the cumulative risk exposure to vascular disease and eventually reduce and ultimately prevent the (residual) risk to develop atrial fibrillation.

Part III Cardiac autonomic dysfunction and the risk of atrial fibrillation

Cardiac autonomic dysfunction mirrored by heart rate variability, and electrocardiographic parameters may induce atrial fibrillation through a blend of underlying mechanisms such as atrial enlargement,(53) inflammation,(54) atrial extrasystoles, conduction abnormalities,(55, 56) and sinus node ischemia.(55) In **Chapter 3**, we showed that cardiac autonomic dysfunction, measured non-invasively and easily measured with an ECG, is related to future risk of atrial fibrillation.

This implies for the clinic that information from the ECG could be used to identify and manage cardiac autonomic dysfunction in an attempt to influence and prevent atrial fibrillation development. Potential specific therapies to cardiac autonomic dysfunction management probably include promoting a heart healthy lifestyle. With regards to electrocardiographic parameters it may guide clinicians to strain away from medication that prolong PR and QTc interval to prevent their drug-induced proarrhythmic effects that may contribute to atrial fibrillation, if possible.(57, 58) Examples include antiarrhythmic drugs (mostly class II, and IV for PR interval prolongation and class I, and III for QTc interval prolongation), antihistamines, antidepressants, antibiotics, antifungal drugs, antipsychotics, and among others.(57, 58) Future RCTs could support the findings in this thesis by evaluating atrial fibrillation as an outcome when performing interventions that may target cardiac autonomic dysfunction. Future research could profit from including repeated ECG measurements of cardiac autonomic dysfunction and assessing the slope, cumulative exposure to these risk factors, and trajectories over time regarding new-onset atrial fibrillation risk to gain more knowledge about atrial fibrillation and to guide

future preventive interventions. Future studies could also show that population-based ECG screening may prove to be helpful to mitigate new-onset atrial fibrillation. Of note, large-scale studies to evaluate the effect of atrial fibrillation screening are already ongoing.(59) A major opportunity lies here for artificial intelligence to deal with the huge amount of data that accompanies such screening strategies and to detect subtle electrocardiographic changes that may be of importance that may be missed by the human eye. Particularly, artificial intelligence may also be of great interest to use all information from the ECG (amplitude, morphology, duration, heart axis, and among others) that could be derived from an ECG rather than primarily focusing on a few selected electrocardiographic parameters such as PR interval, and QTc interval to better understand atrial fibrillation pathogenesis. Noteworthy, longer and continuous ECG recordings can also be more easily handled and managed using artificial intelligence to conduct research with all the smartwatches, and other wearable devices that are nowadays widely available to the consumer. Nevertheless, research using this kind of wearable devices is at its infancy.

Part IV Inflammation and the risk of atrial fibrillation

Inflammation proxied by immunothrombosis may cause local tissue damage and this local tissue damage together with other inflammatory effects may then lead to electrical and structural remodeling of the atria, and thereby contribute to atrial fibrillation initiation.(60-62) Similarly, autoimmune diseases may also contribute to atrial fibrillation development by inflammatory processes that negatively impact atrial electrical and structural remodeling.(63-66) In **Chapter 4**, we demonstrated that immunothrombosis and autoimmune diseases, both proxies of inflammation, are correlated with atrial fibrillation risk.

The potential clinical utilities that could be taken away from this chapter are threefold. First, serum markers of immunothrombosis seem to be of limited value regarding atrial fibrillation etiology, Second, it confirmed the notion that “atrial fibrillation begets atrial fibrillation” as the prothrombotic state is suggested to be implicated in atrial fibrillation maintenance and progression. This would mean for a clinician to be aware that immunothrombosis may be maintained and augmented by atrial fibrillation. Third, it implies that various autoimmune diseases may influence predisposition to atrial fibrillation. This suggests that treatment of autoimmune diseases could prevent atrial fibrillation onset. Yet the questions remains if the mere presence of autoimmune diseases or the inflammatory burden initiated by these autoimmune diseases solely impact atrial fibrillation risk or if both factors contribute to atrial fibrillation development. Additionally it would be insightful to examine if the diseases and the inflammatory burden caused by these diseases act in an additive or multiplicative manner. Although, minimizing the inflammatory burden by tightly controlling the disease activity feels intuitive, evidence about this topic to prevent arrhythmias is scarce. Interestingly, both systemic and local inflammation have been linked to atrial fibrillation pathogenesis.(41, 42) Yet, others questions remain such

as if one should target the location (systematic and/or local) or the timing (acute and/or chronic) of the inflammation or both in an attempt to prevent atrial fibrillation. Future RCTs and experimental studies could hopefully further elaborate on this matter and provide more insight in preventive therapies.

Part V Traditional and novel risk factors for atrial fibrillation

Traditional and novel risk factors such as the whole spectrum of anthropometric measures, trajectories of obesity-related measures and blood pressure, as well as microRNAs may predispose to atrial fibrillation through numerous mechanisms. Obesity is closely related with ventricular remodeling, impaired left ventricular relaxation, increased left ventricular diastolic filling pressure, and atrial fibrillation.(67) Obesity is further linked to adipose fibrosis, production of adipocytokines, increased epicardial fat, and myocardium damage.(68, 69) Especially, the latter two are known to be implicated in the development of atrial fibrillation.(15, 70) Hypertension promotes atrial remodeling by inflammation, atrial fibrosis, and atrial hypertrophy.(71) The increased cardiac afterload in the left ventricle caused by hypertension also contributes to left ventricular hypertrophy.(71) Dysregulation of the autonomic nervous system which may trigger atrial fibrillation is also noted.(72) MicroRNAs are important regulators of electrical remodeling,(73) structural remodeling,(74) autonomic nerve remodeling,(75) calcium handling abnormalities,(76) and inflammation (77) of the heart and thereby implicated in atrial fibrillation pathophysiology. **Chapter 5** indicated that these (modifiable) atrial fibrillation risk factors are of value for future studies on preventive interventions.

Clinical considerations from this chapter are that identifying and targeting traditional and novel modifiable atrial fibrillation risk factors may help reduce the burden of atrial fibrillation. However, as with any results from observational studies, future experimental studies are warranted to confirm the preventive capacity of the investigated risk factors on preventing the disease of interest. Next, to RCTs, causal mediation analyses, and MR analyses may identify and confirm more modifiable atrial fibrillation risk factors. As the effect of separate risk factors on atrial fibrillation have been extensively studied. I hypothesize that a more holistic approach to studying (concomitant) atrial fibrillation risk factors could benefit the scientific atrial fibrillation field given the complexity and heterogeneity of atrial fibrillation.

Artificial intelligence could aid in identifying and improving atrial fibrillation classification on the basis of atrial fibrillation (sub)type. Promising work has already been done regarding this topic as a recent data-driven cluster analysis of 9,749 atrial fibrillation patients, used 60 clinical characteristics, to identify four cluster atrial fibrillation phenotypes.(78) The four identified atrial fibrillation phenotypes included atrial fibrillation with limited risk factors, younger atrial fibrillation patients with comorbid behavioral disorders, atrial fibrillation patients with tachycardia-bradycardia with device implantation due to sinus node dysfunction, and atrial

fibrillation with atherosclerotic vascular disease.(78) Another cluster analysis of 2,458 atrial fibrillation patients, used 46 variables, and identified three cluster atrial fibrillation phenotypes such as younger paroxysmal atrial fibrillation, persistent/permanent atrial fibrillation with left atrium enlargement, and atherosclerotic comorbid atrial fibrillation in elderly.(79) I think that future applications of artificial intelligence will further aid in improving (sub)phenotypic atrial fibrillation classifications to further unravel the complexity and heterogeneity of atrial fibrillation. Additionally, such data-driven approaches, rather than hypothesis driven approaches with a-priori assumptions, can leverage big data with artificial intelligence methods to identify and prioritize atrial fibrillation biomarkers within the realm of atrial fibrillation risk prediction. I also think that understudied external exposures such as social determinants, geographic residential environment, and neighborhood-specific risk factors (i.e. air pollution) will gain more and more interest the upcoming years within atrial fibrillation research as many unanswered questions remain regarding such exposures.

Part VI Sex and gender implications and the risk of atrial fibrillation

The implications of sex- and gender in the prediction, pathophysiology, and prognosis of atrial fibrillation are increasingly gaining interest. **Chapter 6** underpinned the importance of sex and gender implications in atrial fibrillation research and clinical practice when designing and conducting preventive strategies for atrial fibrillation. Recent evidence suggested that risk factors (hypertension, smoking, alcohol intake, obesity, history of diabetes mellitus, history of myocardial infarction or history of heart failure) carry a differential influence on atrial fibrillation risk in men and women implying that some risk factors might be sex-specific.(61, 80-83) Also, it has been suggested that atrial fibrillation-related adverse outcomes and treatment responses differ between men and women.(15, 61, 80-84) Despite this knowledge that sex is a strong effect modifier in atrial fibrillation pathophysiology, prognosis, and response to treatment modalities, sex and gender is often not considered systematically within atrial fibrillation research and in clinical decision making. With the exception of female sex which is a well-recognized independent risk factor for atrial fibrillation-related stroke within the clinic. Various stepping stones are warranted within atrial fibrillation research before standard application of sex- and gender-specific research can become common practice.

First, increasing overall awareness of sex and gender is essential and warranted within atrial fibrillation research. After all, men are not big women, and women are not small men. In other words, research that is performed in mostly men may not be applicable to women and vice versa. This awareness is probably hindered by the common belief that cardiovascular disease is a men’s disease, because men present themselves earlier with cardiovascular disease and die earlier while women present themselves on average 10 years later and live longer with cardiovascular disease in comparison to men.

Second, although gender documentation is often incomplete or missing in the narrow sense (i.e. no actual documentation of cis-, trans-gender, or non-binary gender identity). Nevertheless, behavioral habits may also represent gender in a broad sense and could thereby be used as a proxy for gender. Such information could shed more light on gender-specific modulation of atrial fibrillation. Of note, the same can be said for awareness about ethnicities as most atrial fibrillation research is focused on Europeans.

Genomics

Genes are at the root of a disease and thereby provide more insight into the etiology of a disease. Thus far, 160 independent risk variants at 111 loci are genome-wide significantly associated with atrial fibrillation and thereby explain 4.6% of the genetic variance in atrial fibrillation.(85) This heritability and increasing genetic variance that is being identified by the continuously increasing sample sizes of GWAS explains the major interest in genomics that has been gained in the past years to identify more and more atrial fibrillation-related genetic variants. Genetic variance in genes encoding for ion-channels, transcription factors, and myocardial structural integrity may lead to increased cardiac automaticity and re-entry activity which may be implicated in atrial fibrillation pathogenesis.(85) Our MR studies in various chapters (**Chapter 2.4**, **Chapter 3.1**, and **Chapter 5.3**) used genomics to complement our other analyses, where possible, and to answer etiologic research questions. Future GWAS with larger sample sizes could aid in identifying more genetic variants that are associated with the risk factors described in this thesis and atrial fibrillation which then could be exploited by MR studies to determine if genetic predisposition to certain risk factors increases one's susceptibility to atrial fibrillation.

The complexity of atrial fibrillation at least partly explains why currently no effective preventive treatments exist. To date, clinical evidence regarding the effects of non-antiarrhythmic drugs for atrial fibrillation prevention are inconclusive and/or lacking.(86, 87) However, future GWAS could identify more and more genetic variants for atrial fibrillation that could be taken forward in pathway and functional enrichment analyses to get a better understanding of atrial fibrillation etiology. Particularly, I hypothesize that large-scale GWAS conducted in men and women separately are essential to identify genetic variants that might be sex-specific. Such studies might help us to elucidate the underlying biology among men and women. Moreover, future GWAS that include multiple ethnicities are required to close the remaining knowledge gaps. Especially, because most genomic research is primarily focused on European ancestry. Additionally, I suggest that future drug target MR studies could be of great value to guide and design future RCTs to examine the effect of (new) drugs to prevent atrial fibrillation (i.e. the drug ability of atrial fibrillation). As drug target MR studies exploit drug target-related genetic variants that mimic the down-stream effects of drugs, thereby providing a valid and reliable alternative to RCTs to evaluate the effects of existing and/or new drugs to prevent disease.(88,

89) RCTs that evaluate (new) drugs are challenging, costly, often only include high-risk patients, have a short duration of follow-up, and have other limitations such as non-adherence, loss to follow-up, and blinding. Moreover, I suggest that there is still a substantial portion of “missing heritability”,(90) as suggested by the aforementioned explained heritability, that can be uncovered from gene-gene interactions (i.e. two genetic variants may have small individual effects, but may have a large interaction effect), gene-environment interactions (i.e. the influence of a genotype on disease risk is different in individuals with different environmental exposures), and high-throughput sequencing in future genomic atrial fibrillation studies. The latter one is a particularly promising fast-developing field within genomics, also for population-based studies as the genetic sequencing technologies have evolved rapidly and the prices for whole genome sequencing have dropped substantially.(91) High-throughput sequencing includes techniques such as whole-genome sequencing that enable high-resolution genomic analyses of the whole genome (both coding and non-coding variants). The main advantage of this technique is that one can analyze genetic variants with much lower frequencies and large(r) effect sizes on disease risk in contrary to GWAS which are mainly limited to analyzing common variants with small(ler) effect sizes on disease risk. Such studies will further unravel the different causal pathways that underlie atrial fibrillation. Knowing these different pathways is vital for treatment differentiation as atrial fibrillation is a heterogeneous disease.

Concluding remarks

In conclusion, this thesis aimed to dissect the etiology of atrial fibrillation using a population perspective on risk factors and sex differences. In this **Chapter 7.1**, we discussed the main findings of this thesis, in the light of relevant methodological considerations, and provided recommendations for future research. We showed that vascular-, cardiac autonomic-, inflammatory-, traditional-, novel-, and sex-specific risk factors play a role in the pathophysiology of atrial fibrillation in the general population. While the pathogenesis of atrial fibrillation remains to be complex and incompletely understood, the studies included in this thesis have added to the emerging evidence of atrial fibrillation risk factors’ identification which could aid the development of future preventive strategies. To advance our understanding regarding the underlying pathophysiology of atrial fibrillation, this complex and polygenetic arrhythmia needs to be further dissected layer by layer by combining observational and experimental studies.

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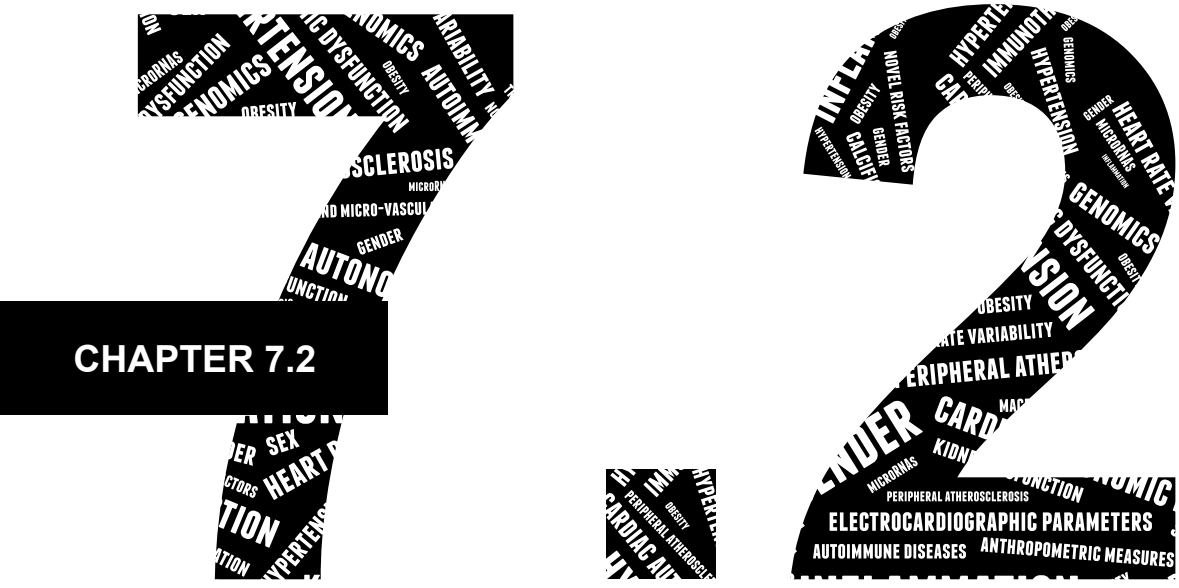
CHAPTER 7.2
HYPERTENSION
GENOMICS
DYSFUNCTION
OBESITY
GENDER
SEX
HEART RATE
MICRORNAS
NOVEL RISK FACTORS
PERIPHERAL ATHEROSCLEROSIS
ELECTROCARDIOGRAPHIC PARAMETERS
AUTOIMMUNE DISEASES
ANTHROPOMETRIC MEASURES

Summary

Summary

The human heart (myocardium) is a fascinating and powerful fist-sized muscular organ that pumps around blood through the cardiovascular system. The heart is located in the middle compartment of the chest (thorax) called the mediastinum. The heart is anatomically divided into four chambers: two upper chambers called the left and right atria and two lower chambers called the left and right ventricle. At rest the heart beats around 60-80 times per minute whereas during physical exertion it may beat up to 180-200 times per minute, depending on the metabolic needs of the body. The pump function and its frequency are further regulated by the sinoatrial node, located in the upper right atrium, which is the orchestrator of the cardiac rhythm. In a physiological setting, electrical activity is initiated by pacemaker cells in the sinoatrial node. These very specialized cells generate an electrical current that travels in series from the sinoatrial node to the atrioventricular node to the bundle of His and ends at the Purkinje fibers located in the ventricles. This travelling electrical current causes the atria and ventricles to contract sequentially which makes blood flow from the atria to the ventricles to the cardiovascular system. In atrial fibrillation, this normal cardiac rhythm is disturbed by chaotic electrical activity in the atria. This chaotic atrial electrical activity or excitation leads to unproductive atrial contraction and may subsequently cause an irregularly irregular ventricular excitation and contraction. Atrial fibrillation is the most common cardiac arrhythmia worldwide and its prevalence has reached epidemic proportions due to aging of the general population and the increasing prevalence of atrial fibrillation risk factors such as obesity, hypertension, diabetes mellitus, coronary heart disease, and heart failure. In the past decades, the scientific community propelled the knowledge regarding the epidemiology, prediction, pathophysiology, and treatment of atrial fibrillation. Despite this global scientific effort in the past decades to better understand this complex and polygenetic disorder, the atrial fibrillation etiology remains unclear. As one can imagine this complexity further complicates the prediction, prevention, and management of atrial fibrillation. Generating new evidence is therefore warranted to reduce the disease burden of atrial fibrillation. Therefore, we mainly investigated the etiology (cause of a disease) of atrial fibrillation in participants from the Rotterdam Study, the UK Biobank, and several large-scale genomic consortia.

In **Part I**, we introduced atrial fibrillation, the aim, and the topics that underlie this thesis.

In **Part II**, we described macro- and micro-vascular disease as a risk factor for atrial fibrillation. In **Chapter 2.1**, we investigated the (sex-specific) association between arteriosclerotic calcification and atrial fibrillation. We found that higher coronary artery calcification volume in the general population, especially in men, and higher aortic arch calcification volume in women were significantly associated with an

increased risk of new-onset atrial fibrillation. This underlines that interventions to lower arteriosclerotic calcification, specifically coronary artery calcification, might prevent atrial fibrillation in the general population, in particular in men. In **Chapter 2.2**, we examined the (sex-specific) association between peripheral atherosclerosis and atrial fibrillation. We showed that higher carotid atherosclerosis, and lower extremity atherosclerosis were significantly associated with an increased risk of new-onset atrial fibrillation, especially in women. This suggests that treatment to reduce (sub)clinical peripheral atherosclerosis carries a preventive capacity for atrial fibrillation in the general population, especially in women. In **Chapter 2.3**, we evaluated the bidirectional association between kidney function and atrial fibrillation. We observed that kidney function, in particular estimated glomerular filtration rate based on cystatin C, and atrial fibrillation are significantly bidirectionally associated. In **Chapter 2.4**, we examined the bidirectional causality between kidney function and atrial fibrillation by using Mendelian randomization. Our results supported the significant bidirectional causality between kidney function and atrial fibrillation. Overall, the findings from the latter two subchapters imply that kidney dysfunction and atrial fibrillation may form therapeutic targets to prevent both conditions in the general population.

In **Part III**, we assessed cardiac autonomic dysfunction as a risk factor for atrial fibrillation. In **Chapter 3.1**, we studied the (sex-specific) association between heart rate variability and atrial fibrillation. We observed that heart rate variability was significantly associated with increased new-onset atrial fibrillation risk, in particular in women. Subsequently, our Mendelian randomization also supported the causality of the association between heart rate variability with atrial fibrillation. This suggests that modulation of heart rate variability might prevent atrial fibrillation in the general population, in particular in women. In **Chapter 3.2**, we determined the (shape of the) association and sex differences between electrocardiographic parameters and atrial fibrillation. We observed that the shape of association between baseline electrocardiographic measures and their risk of new-onset atrial fibrillation were generally mostly U- and N-shaped. Further, we reported that longitudinal measures of higher PR, and higher QTc interval were significantly associated with an increased new-onset atrial fibrillation risk, more specifically in men. This means that different thresholds of electrocardiographic parameters might translate to a differential risk among men and women and that therapies that target electrocardiographic parameters might prevent atrial fibrillation in the general population, in particular in men.

In **Part IV**, we reported on inflammation as a risk factor for atrial fibrillation. In **Chapter 4.1**, we described the association between immunothrombosis and atrial fibrillation. No significant associations between markers of immunothrombosis and new-onset atrial fibrillation in the general population were observed. Therefore, we hypothesize that immunothrombosis may be associated with atrial fibrillation through

other cardiovascular risk factors or conditions that may lead to atrial fibrillation. In **Chapter 4.2**, we systematically reviewed, and meta-analyzed the literature on the association between immunothrombotic markers and atrial fibrillation. We found that atrial fibrillation is significantly associated with higher levels of immunothrombosis. The associations were most pronounced in the cross-sectional analyses whereas limited longitudinal studies were available. This supports the hypothesis that immunothrombosis is promoted by atrial fibrillation and that "atrial fibrillation begets atrial fibrillation", as the prothrombotic state is considered as an underlying mechanism of atrial fibrillation. In **Chapter 4.3**, we studied autoimmune diseases and their influence on atrial fibrillation risk. Various autoimmune diseases such as rheumatic fever, rheumatic heart disease, type 1 diabetes mellitus, multiple sclerosis, myasthenia gravis, Crohn's disease, ulcerative colitis, rheumatoid arthritis, psoriatic and enteropathic arthropathies, polyarteritis nodosa, systemic lupus erythematosus, dermatopolymyositis, systemic sclerosis, ankylosing spondylitis, and Paget's disease were studied. Significant associations between rheumatic fever without heart involvement, type 1 diabetes mellitus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, polyarteritis nodosa, systemic lupus erhythematosus, and systemic sclerosis and new-onset atrial fibrillation risk were found. These associations were mostly prominent in women. These results highlight the (potential sex-specific) impact of different autoimmune diseases on atrial fibrillation risk.

In **Part V**, we investigated anthropometric measures, trajectories of obesity-related measures and blood pressure, and microRNAs as traditional and novel risk factors for atrial fibrillation. In **Chapter 5.1**, we described the association between anthropometric measures and atrial fibrillation. We reported that measures of anthropometrics were significantly associated with an increased risk of new-onset atrial fibrillation. Increased height in men and increased weight, and central obesity (waist-to-hip ratio) in women showed the largest associations with atrial fibrillation risk. This underpins that height in men and weight, and central obesity (waist-to-hip ratio) in women are risk factors for new-onset atrial fibrillation risk. This stresses that a sex-specific approach for screening and monitoring of anthropometrics might aid in atrial fibrillation prevention. In **Chapter 5.2**, we evaluated the association between trajectories of obesity-related measures and blood pressure and atrial fibrillation. Longitudinal trajectories of obesity-related measures and blood pressure were significantly associated with new-onset atrial fibrillation risk. Sex differences in the associations between waist circumference, hip circumference, and diastolic blood pressure trajectories and new-onset atrial fibrillation risk were also observed. This highlights the importance to assess the long-term exposure to modifiable risk factors such as obesity, and hypertension for future atrial fibrillation preventive strategies. In **Chapter 5.3**, we assessed the effect of antihypertensive (high blood pressure) drugs by conducting a drug target Mendelian randomization study. Well-validated published genetic variants were used as genetic proxies of 12 antihypertensive drug classes including alpha-adrenoceptor blockers, adrenergic neuron blockers,

angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, beta-adrenoceptor blockers, centrally acting antihypertensive drugs, calcium channel blockers, loop diuretics, potassium-sparing diuretics and mineralocorticoid receptor antagonists, renin inhibitors, thiazides and related diuretic agents, and vasodilators via their corresponding gene and protein target, and we determined their impact on atrial fibrillation prevention through their downstream effect which is to lower systolic blood pressure. Significant preventive causal effects of lowering systolic blood pressure per 10 mmHg via alpha-adrenoceptor blockers, beta-adrenoceptor blockers, calcium channel blockers, vasodilators, and all 12 antihypertensive drug classes combined on atrial fibrillation risk, were observed in our drug target Mendelian randomization analyses. This is promising as it indicates that lowering systolic blood pressure using certain antihypertensive drugs carries a potential to prevent atrial fibrillation. These findings may guide future clinical trials and have implications for repurposing antihypertensive drugs in atrial fibrillation prevention. In **chapter 5.4**, we studied the association between microRNAs and atrial fibrillation. We observed that higher plasma levels of MiR-4798-3p were significantly associated with the odds of prevalent atrial fibrillation in men. Furthermore, miR-4798-3p may potentially regulate the expression of a number of atrial fibrillation-related genes including genes involved in calcium and potassium handling in myocytes, protection of cells against oxidative stress, and cardiac fibrosis. This might explain why miR-4798-3p is involved in atrial fibrillation pathophysiology.

In **Part VI**, we discussed the implications of sex and gender in atrial fibrillation research. In **Chapter 6.1**, we determined the association between women-specific risk factors and atrial fibrillation. We showed that early or delayed menopause, early or delayed menarche, or irregular menstrual cycles were significantly associated with increased new-onset atrial fibrillation risk. Further, both nulliparity and multiparity, compared to having 1-2 children, were significantly associated with an increased new-onset atrial fibrillation risk. This highlights that the reproductive history of women is of added value while designing preventive strategies for atrial fibrillation. In **Chapter 6.2**, we described the current status, future directions, and potential of sex- and gender-specific atrial fibrillation prediction by leveraging big data. atrial fibrillation is a complex and polygenetic arrhythmia and its prediction models are not yet conclusive despite the advances over the last two decades regarding the epidemiology, prediction, pathophysiology, and treatment of atrial fibrillation. The possibilities with clinical data, multidimensional datasets, electrocardiograms, electronic health records, and wearable devices are apparent. However, ample challenges remain before artificial intelligence-enabled algorithms can be adopted for sex- and gender-specific prediction, prevention, and management of atrial fibrillation.

Lastly, in **Part VII**, we discussed and summarized the main findings of the studies embedded in this thesis. Further, we addressed methodological considerations, reviewed potential implications of this thesis, and pointed out potential future directions. In summary, we aimed to dissect the etiology of atrial fibrillation using a population perspective on various risk factors and sex differences. We found that vascular-, cardiac autonomic-, inflammatory-, traditional-, novel-, and sex-specific risk factors are implicated in the etiology of atrial fibrillation. Future research may further dissect this complex and polygenetic arrhythmia layer by layer by combining observational and experimental studies to eventually expose the underlying biology of atrial fibrillation.


CHAPTER 7.3

Samenvatting

Samenvatting

Het menselijk hart (myocard) is een fascinerend en krachtig vuistgroot gespierd orgaan dat bloed rond pompt door het cardiovasculaire systeem. Het hart is gelokaliseerd in het midden compartiment van de borstkas (thorax) genaamd het mediastinum. Het hart is anatomisch verdeeld in vier kamers: twee bovenste kamers genaamd het linker en rechter atrium en twee onderste kamers genaamd het linker en rechter ventrikel. Tijdens rust klopt het hart ongeveer 60-80 keer per minuut terwijl het tijdens fysieke inspanning tot 180-200 keer per minuut kan kloppen afhankelijk van de metabole behoeften van het lichaam. De pomp functie en de frequentie worden verder gereguleerd door de sinoatriale knoop, gelokaliseerd bovenin het rechter atrium, die de orkestleider van het cardiale ritme is. In een fysiologische situatie wordt elektrische activiteit geïnitieerd door de pacemaker cellen in de sinoatriale knoop. Deze erg gespecialiseerde cellen genereren een elektrische stroom die in serie reist van de sinoatriale knoop naar de atrioventriculare knoop naar de bundel van His en eindigt in de Purkinje vezels gelegen in de ventrikels. Deze reizende elektrische stroom zorgt ervoor dat de atria en ventrikels contraheren in sequentie wat maakt dat bloed stroomt van de atria naar de ventrikels naar het cardiovasculaire systeem. Bij atriumfibrilleren is het normale cardiale ritme verstoord door chaotische elektrische activiteit of excitatie in de atria. Deze chaotische elektrische activiteit leidt tot een onproductieve atriale contractie en kan hierop volgend zorgen voor een onregelmatige ventriculaire excitatie en contractie. Atriumfibrilleren is wereldwijd de meest voorkomende cardiale aritmie en de prevalentie ervan heeft epidemische proporties bereikt door vergrijzing van de algemene populatie en de toenemende prevalentie van atriumfibrilleren risicofactoren zoals obesitas, hypertensie, diabetes mellitus, coronaire hartziekte, en hartfalen. De wetenschappelijke gemeenschap heeft de afgelopen decennia de kennis omtrent de epidemiologie, predictie, pathofysiologie, en behandeling van atriumfibrilleren voortgestuwd. Ondanks deze wereldwijde wetenschappelijke inzet de afgelopen decennia om deze complexe en polygenetische ziekte beter te begrijpen, blijft de etiologie van atriumfibrilleren vooralsnog onduidelijk. Men kan zich voorstellen dat deze complexiteit de predictie, preventie, en management van atriumfibrilleren verder compliceert. Het genereren van nieuw bewijs is daarom noodzakelijk om de ziektelast van atriumfibrilleren te kunnen reduceren. We onderzochten daarom met name de etiologie (de ontstaanswijze van een ziekte) van atriumfibrilleren in deelnemers van de ERGO studie (The Rotterdam Study), de UK Biobank, en meerdere grootschalige genomische consortia.

In **Deel I**, introduceerden we atriumfibrilleren, het doel, en de onderwerpen die aan deze thesis ten grondslag liggen.

In **Deel II**, beschreven we macro- en micro-vasculaire ziekten als een risicofactor voor atriumfibrilleren. In **Hoofdstuk 2.1**, onderzochten we de (sekse-specifieke) associatie tussen arteriosclerotische calcificatie en atriumfibrilleren. We vonden dat een hoger coronair arterieel calcificatie volume in de algemene populatie, vooral in mannen, en een hoger aortaboog calcificatie volume in vrouwen significant waren geassocieerd met een toegenomen risico op atriumfibrilleren incidentie. Dit onderstreept dat interventies om arteriosclerotische calcificatie te verlagen, met name coronaire arteriële calcificatie, atriumfibrilleren zou kunnen voorkomen in de algemene populatie, in het bijzonder bij mannen. In **Hoofdstuk 2.2**, onderzochten we de (sekse-specifieke) associatie tussen perifere atherosclerose en atriumfibrilleren. We toonden aan dat meer atherosclerose van de carotiden, en meer atherosclerose van de onderste extremiteiten significant waren geassocieerd met een toegenomen risico op atriumfibrilleren incidentie, in het bijzonder bij vrouwen. Dit suggereert dat de behandeling om (sub)klinische perifere atherosclerose te verminderen een preventieve capaciteit voor atriumfibrilleren heeft in de algemene populatie, met name bij vrouwen. In **Hoofdstuk 2.3**, evalueerden we de bidirectionele associatie tussen nierfunctie en atriumfibrilleren. We observeerden dat nierfunctie, met name de geschatte glomerulaire filtratiesnelheid gebaseerd op cystatine C, en atriumfibrilleren significant bidirectioneel waren geassocieerd. In **Hoofdstuk 2.4**, examineerden we de bidirectionele causaliteit tussen nierfunctie en atriumfibrilleren door Mendeliaanse randomisatie te gebruiken. Onze resultaten ondersteunden de significant bidirectionele causaliteit tussen nierfunctie en atriumfibrilleren. Overall, de bevindingen van de laatste twee subhoofdstukken impliceren dat nierinsufficiëntie en atriumfibrilleren mogelijk therapeutische doelen vormen om beide aandoeningen in de algemene populatie te voorkomen.

In **Deel III**, beoordeelden we cardiale autonome dysfunctie als een risicofactor voor atriumfibrilleren. In **Hoofdstuk 3.1**, bestudeerden we de (sekse-specifieke) associatie tussen hartslag variabiliteit en atriumfibrilleren. We observeerden dat hartslag variabiliteit significant was geassocieerd met een toegenomen risico op atriumfibrilleren incidentie, in het bijzonder bij vrouwen. Hierop volgend, onze bevindingen uit de Mendeliaanse randomisatie ondersteunden ook de causaliteit tussen de associatie van hartslag variabiliteit en atriumfibrilleren. Dit suggereert dat modulatie van hartslag variabiliteit atriumfibrilleren zou kunnen voorkomen in de algemene populatie, met name bij vrouwen. In **Hoofdstuk 3.2**, bepaalden we de (de vorm van de) associatie en sekse verschillen tussen elektrocardiografische parameters en atriumfibrilleren. We observeerden dat de vorm van de associatie tussen baseline elektrocardiografische metingen en hun risico op incident atriumfibrilleren over het algemeen met name U- en N-vormig waren. Verder rapporteerden we dat longitudinale metingen van een hoger PR, en hoger QTc interval significant waren geassocieerd met een toegenomen risico op atriumfibrilleren, met name bij mannen. Dit betekent dat verschillende drempelwaarden van elektrocardiografische parameters zich tot een differentieel risico onder mannen en vrouwen zou kunnen vertalen en dat therapieën die gericht

zijn op elektrocardiografische parameters mogelijk atriumfibrilleren zou kunnen voorkomen in de algemene populatie, met name bij mannen.

In **Deel IV**, rapporteerden we over inflammatie als een risicofactor voor atriumfibrilleren. In **Hoofdstuk 4.1**, beschreven we de associatie tussen immunotrombose en atriumfibrilleren. Er werden geen significante associaties tussen markers van immunotrombose en atriumfibrilleren incidentie geobserveerd in de algemene populatie. We veronderstelden daarom dat immunotrombose mogelijk met atriumfibrilleren is geassocieerd door andere cardiovasculaire risicofactoren of aandoeningen die mogelijk tot atriumfibrilleren kunnen leiden. In **Hoofdstuk 4.2**, beoordelen we systematisch, en meta-analyseerden we de literatuur over de associatie tussen immunotrombotische markers en atriumfibrilleren. We vonden dat atriumfibrilleren significant is geassocieerd met hogere levels van immunotrombose. De associaties waren het meest uitgesproken in de cross-sectionele analyses terwijl er slechts beperkte longitudinale studies beschikbaar waren. Dit ondersteunt de hypothese dat immunotrombose wordt gepromoot door atriumfibrilleren en het idee dat “atriumfibrilleren verwekt atriumfibrilleren” doordat deze protrombotische staat wordt overwogen als een onderliggend mechanisme voor atriumfibrilleren. In **Hoofdstuk 4.3**, bestudeerden we autoimmuun ziektes en hun invloed op atriumfibrilleren risico. Verschillende autoimmuun ziektes zoals reumatische koorts, reumatische hartziekte, type 1 diabetes mellitus, multiple sclerose, myasthenia gravis, ziekte van Crohn, colitis ulcerosa, reumatoïde artritis, psoriatische en enteropathische artropathieën, polyarteritis nodosa, systemische lupus erhythematodes, dermatopolymyositis, systemische sclerose, spondylitis ankylopoetica, en ziekte van Paget werden bestudeerd. Significante associaties tussen reumatische koorts zonder betrokkenheid van het hart, type 1 diabetes mellitus, ziekte van Crohn, colitis ulcerosa, reumatoïde artritis, polyarteritis nodosa, systemische lupus erhythematodes, en systemische sclerose en incident atriumfibrilleren werden gevonden. Deze associaties waren het meest prominent in vrouwen. Deze resultaten belichten de (potentiële sekse-specifieke) impact van verschillende autoimmuun ziektes op het risico van atriumfibrilleren.

In **Deel V**, onderzochten we antropometrische metingen, trajecten van obesitas-gerelateerde metingen en bloeddruk, en microRNAs als traditionele en nieuwe risicofactoren voor atriumfibrilleren. In **Hoofdstuk 5.1**, beschreven we de associatie tussen antropometrische metingen en atriumfibrilleren. We rapporteerden dat metingen van antropometrie significant waren geassocieerd met een toegenomen risico op atriumfibrilleren incidentie. Een toegenomen lichaamslengte in mannen en een toegenomen gewicht, en centrale obesitas (middel-heup ratio) in vrouwen toonden de grootste associaties met het risico op atriumfibrilleren. Dit onderstreept dat lichaamslengte in mannen en gewicht, en centrale obesitas (middel-heup ratio) in vrouwen risicofactoren voor incident atriumfibrilleren zijn. Dit benadrukt dat een sekse-specifieke aanpak voor screening en monitoring van antropometrie zou kunnen helpen bij de preventie van atriumfibrilleren. In **Hoofdstuk 5.2**, evalueerden we de associatie tussen trajecten van obesitas-gerelateerde metingen en bloeddruk

en atriumfibrilleren. Longitudinale trajecten van obesitas-gerelateerde metingen en bloeddruk waren significant geassocieerd met het risico op incident atriumfibrilleren. Sekse verschillen in de associaties tussen middelomtrek-, heupomtrek-, en diastolische bloeddruk- trajecten en het risico op incident atriumfibrilleren werden ook geobserveerd. Dit benadrukt het belang om lange termijn blootstelling aan modificeerbare risicofactoren zoals obesitas, en hypertensie te beoordelen voor toekomstige preventieve strategieën voor atriumfibrilleren. In **Hoofdstuk 5.3**, beoordeelden we het effect van antihypertensiva (te hoge bloeddruk medicatie) door het verrichten van een medicatie gerichte Mendeliaanse randomisatie studie. Goed gevalideerde gepubliceerde genetische varianten werden als genetische proxies van 12 klassen antihypertensiva gebruikt waaronder alfa-blokkers, adrenerge neuron blokkers, ACE-remmers, angiotensine-II receptor blokkers, beta-blokkers, centraal aangrijpende antihypertensiva, calcium kanaal blokkers, lisdiuretica, kaliumsparende diuretica en minerale corticoïd receptor antagonisten, renine remmers, thiazides en gerelateerde diuretica, en vasodilatoren via hun corresponderende gen en eiwit doelwit. Vervolgens bepaalden we hun impact op het voorkomen van atriumfibrilleren door hun beoogde effect welke het verlagen van de systolische bloeddruk is. Significante preventieve causale effecten van het verlagen van de systolische bloeddruk per 10 mmHg via alfa-blokkers, beta-blokkers, calcium kanaal blokkers, vasodilatoren, en alle 12 antihypertensiva klassen gecombineerd op het risico van atriumfibrilleren werden geobserveerd in onze medicatie gerichte Mendeliaanse randomisatie analyses. Dit is veelbelovend en indiceert dat bepaalde klassen antihypertensiva potentie hebben in het voorkomen van atriumfibrilleren. Deze bevindingen kunnen mogelijk toekomstige klinische trials sturen en implicaties hebben voor het herbestemmen van antihypertensiva in het voorkomen van atriumfibrilleren. In **Hoofdstuk 5.4**, bestudeerden we de associatie tussen microRNAs en atriumfibrilleren. We observeerden dat hogere plasma levels van miR-4798-3p significant waren geassocieerd met de odds op prevalent atriumfibrilleren in mannen. Verder zou miR-4798-3p mogelijk de expressie van een aantal atriumfibrilleren-gerelateerde genen reguleren zoals genen die betrokken zijn bij het hanteren van calcium en kalium in myocyten, protectie van cellen tegen oxidatieve stress, en cardiale fibrose. Dit verklaart mogelijk waarom miR-4798-3p is betrokken in atriumfibrilleren pathofysiologie.

In **Deel VI**, discussieerden we de implicaties van sekse en gender in atriumfibrilleren onderzoek. In **Hoofdstuk 6.1**, bepaalden we de associatie tussen vrouw-specifieke risicofactoren en atriumfibrilleren. We toonden aan dat vroege en verlate menopauze, vroege en verlate menarche, of onregelmatige menstruele cycli significant waren geassocieerd met een toegenomen risico op atriumfibrilleren incidentie. Verder, zowel nulliparitеit als multiparitеit, vergeleken met het hebben van 1-2 kinderen, waren significant geassocieerd met het toegenomen risico op atriumfibrilleren incidentie. Dit benadrukt dat de reproductieve historie van vrouwen van toegevoegde waarde is bij het ontwerpen van preventieve strategieën voor atriumfibrilleren. In **Hoofdstuk 6.2**, beschreven we de huidige status, toekomstige directie, en potentie van sekse- en gender-specifieke predictie van atriumfibrilleren

door gebruik te maken van big data. atriumfibrilleren is een complexe en polygenetische aritmie en zijn predictiemodellen zijn nog niet beslissend ondanks de vooruitgangen de afgelopen twee decennia met betrekking tot de epidemiologie, predictie, pathofysiologie, en behandeling van atriumfibrilleren. De mogelijkheden met klinische data, multidimensionale datasets, elektrocardiogrammen, elektronische patiëntendossiers, en draagbare apparaten zijn duidelijk. Echter, er blijven genoeg uitdagingen alvorens algoritmen met behulp van artificiële intelligentie kunnen worden geadopteerd voor een sekse- en gender-specifieke predictie, preventie, en management van atriumfibrilleren.

Als laatste, in **Deel VII**, discussieerden en vatten we de voornaamste bevindingen samen van de studies ingebed in deze thesis. Verder, adresseerden we methodologische consideraties, beoordeelden we potentiële implicaties van deze thesis, en wezen we op potentiële toekomstige richtingen voor onderzoek. Samengevat, beoogden we de etiologie van atriumfibrilleren te ontleden door gebruik te maken van een populatie perspectief op verscheidene risicofactoren en sekse verschillen. We vonden dat vasculaire-, cardiaal autonome-, inflammatoire-, traditionele-, nieuwe-, en sekse-specifieke risicofactoren zijn geïmpliceerd in de etiologie van atriumfibrilleren. Toekomstig onderzoek zou deze complexe en polygenetische aritmie laag voor laag verder kunnen ontleden door het combineren van observationele en experimentele studies om uiteindelijk de onderliggende biologie van atriumfibrilleren bloot te leggen.

APPENDICES


HYPERTENSION
GENDER
SEX
CARDIAC AUTONOMIC DYSFUNCTION
CALCIFICATION
KIDNEY DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
MICRORNAS
OBESITY

Dankwoord
PhD portfolio
List of publications
About the author

Dankwoord

Dankwoord

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PhD portfolio

PhD portfolio

Name of PhD student:	S. (Sven) Geurts
Department:	Epidemiology, Erasmus Medical Center, University Medical Center, Rotterdam
Research school:	Netherlands Institute for Health Sciences (NIHES)
PhD Period:	December 2018 - May 2022
Promotor:	prof. dr. M.A. (Arfan) Ikram
Co-promotor:	dr. M. (Maryam) Kavousi

	Year	ECTS
Courses		
Master of Science in Health Sciences, Genetic and Molecular Epidemiology (cum laude), Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands		
Core curriculum		
Study design	2019	4.3
Biostatistical Methods I: Basic Principles	2019	5.7
Biostatistical Methods II: Classical Regression Models	2019	4.3
Introduction to Medical Writing	2020	2.0
Scientific Integrity MSc	2019	0.6
Intervision	2020	0.4
Leadership and Teamwork	2019	0.3
Master Research	2019	33.0
Specialization		
Principles of Research in Medicine and Epidemiology	2019	0.7
Principles of Genetic Epidemiology	2019	0.7
Advances in Genomics Research	2019	0.4
Genomics in Molecular Medicine	2019	1.4
Erasmus Summer Lectures	2019	0.4
Genome-wide Association Studies	2019	0.7
Human Epigenomics	2019	0.7
Genetic Epidemiologic Research Methods	2019	5.1
SNPs and Human Diseases	2019	1.4
Linux for Scientists	2019	0.6
Advances in Genome-Wide Association Studies	2020	1.4
An Introduction to the Analysis of Next-Generation	2020	1.4

Sequencing Data		
Mendelian Randomization	2020	0.9
Elective courses		
Competing Risks and Multi-state Models	2020	0.9
Repeated Measurements in Clinical Studies	2020	1.7
Cardiovascular Epidemiology	2020	0.9
Causal Inference	2020	1.4
Extra-curricular courses		
The Basic Introduction Course on SPSS	2019	1.0
The Basic Course on R	2019	1.8
Causal Mediation Analysis	2020	1.4
Fundamentals of Medical Decision Making	2021	0.7
Joint Models for Longitudinal and Survival Data	2021	0.7
Data Science in Epidemiology	2021	0.7

Seminars, symposia, and workshops

Departmental Epidemiology Seminars	2018-2022	4.0
Journal Club Seminars	2018-2022	2.0
2020 Meetings	2018-2022	2.0
Cardiometabolic Epidemiology Meetings	2018-2022	2.0
e-Xpert ECG: Beoordelen van Ritmes	2018	0.5
e-Xpert ECG: 3D Coronair Anatomie	2019	0.5
Assessing Comorbidities in Atrial Fibrillation Patients	2021	0.3
COEUR De Patiënt Centraal in Onderzoek	2020	0.5
COEUR Atrial Fibrillation: From Cardiac Morphology to Electrophysiology	2020	0.5
Scientific Integrity PhD	2020	0.3
COEUR Congenital Cardiology	2021	0.5
Science Communication Animated Presentation	2021	0.5
Omgaan met Groepen voor Tutoren	2020	0.3
Digital PhD Day	2021	0.2
Thrombosis and COVID-19	2021	0.5
Pulmonary Hypertension	2021	0.5
Heart Failure	2021	0.5
Cardiovascular Clinical Epidemiology	2021	0.5
Diversity and Inclusion	2021	0.5
Data Management Plan	2022	0.5
Hybrid ablation: A New Standard of Care in Long Standing Persistent Atrial Fibrillation	2022	0.3

Conferences

Health Sciences Research Day, Rotterdam, The Netherlands	2019	0.5
European Society of Cardiology (ESC) Congress, online [a]	2020	1.7
European Society of Cardiology (ESC) Preventive Cardiology Congress, online [a]	2021	1.5
European Atherosclerosis Society (EAS) Congress, online [a, b]	2021	1.7
European Society of Cardiology (ESC) Congress, online [a]	2021	1.7
European Heart Rhythm Association (EHRA) Congress, Copenhagen, Denmark [c]	2022	1.7
European Society of Cardiology (ESC) Preventive Cardiology Congress, online [c]	2022	1.7
Biomedical Science PhD Day, Rotterdam, The Netherlands [a]	2022	1.0

Teaching

Tutorship Medicine bachelor students Erasmus MC	2021	1.5

Supervision

Cathrine Brunborg (MSc student). Atherosclerosis and the risk of new-onset atrial fibrillation in the general population: the Rotterdam Study	2019-2020	2.0
Angelo Pezzullo (MSc student). Dietary patterns and risk of new-onset atrial fibrillation among men and women from the general population: the Rotterdam Study	2020-2021	2.0

Other

Peer review for scientific journals	2019-	3.0
Coordinator Medical Doctor Team (“uitschrijfartsen”)	2018-2021	3.0
Medical Duties at Research Center (“uitschrijfdiensten”)	2018-2021	5.0
Coordinator Event Adjudication and Cardiometabolic Data Management	2018-2022	3.0
Event Adjudication (Atrial Fibrillation, Carotic Desobstruction, Coronary Heart Disease, Myocardial Infarction, Percutaneous Coronary Intervention, Coronary Artery Bypass Grafting, Heart Failure, Heart Rate Variability, and Cardiovascular Mortality)	2018-2022	20.0
Student Chapter Master of Science in Health Sciences	2020	1.0
Student Panel Visitation Master of Science in Health Sciences	2020	1.0
PhD Panel Visitation Department of Epidemiology	2020	1.0

Rotterdam Study COVID-19 Research Entering Questionnaires	2020	5.0
Rotterdam Study COVID-19 Research Methods and Data Cleaning	2020	5.0
COVID@HOME Data Collection and Logistics	2020-2021	5.0
Total ECTS	**2018-2022**	**164**

[a] Poster presentation.

[b] Young Investigator Fellowship.

[c] Oral presentation.

1 European Credit Transfer and Accumulation System (ECTS) equals a workload of 28 hours.

List of publications

List of publications

Geurts S, Bos MM, van der Toorn JE, Stricker BHC, Ghanbari M, Kors JA, Deckers JW, Ikram MA, Bos D, Kavousi M. Arteriosclerotic calcification and atrial fibrillation in the general population: a longitudinal and Mendelian randomization study. Submitted.

Geurts S, Brunborg C, Papageorgiou G, Ikram MA, Kavousi M. Subclinical measures of peripheral atherosclerosis and the risk of new-onset atrial fibrillation in the general population: the Rotterdam Study. J Am Heart Assoc. 2022 Jan 4;11(1):e023967.

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About the author

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Sven Geurts was born on 18 March 1993 in Tiel, The Netherlands. He graduated in 2011 from high school Koningin Wilhelmina College in Culemborg. At his first attempt to study Medicine he was not enrolled. Therefore, he decided to attend the Royal Dutch Naval College in Den Helder to become a Marine Corps officer while studying Military Business Science. In 2012 he was thankfully enrolled as a Medical student at the University of Utrecht and he did quit his Military career to pursue his initial career and dream to become a surgeon. He graduated in 2018 from Medicine and during his (research) internships his fascination and great interest in cardiothoracic surgery and cardiovascular research became clear. Directly after his Medicine graduation he therefore decided to become proficient in doing thorough research. He applied for a PhD vacancy to investigate the etiology of atrial fibrillation in the general population and to become an epidemiologist at the department of Epidemiology in the Erasmus MC, University Medical Center Rotterdam. During his PhD he also started a second Master in (Genetic and Molecular) Epidemiology in 2019 from which he graduated cum laude in 2020. When he completed his PhD trajectory in 2022 he applied for a resident position (not in training/ANIOS) at the department of Cardiothoracic Surgery in the Erasmus MC, University Medical Center Rotterdam. There he will continue his aspiration and dream to hopefully become a cardiothoracic surgeon one day.


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CALCIFICATION
IMMUNOTHROMBOSIS
TRADITIONAL RISK FACTORS
HEART RATE VARIABILITY
AUTOIMMUNE DISEASES
CARDIAC AUTONOMIC DYSFUNCTION
PERIPHERAL ATHEROSCLEROSIS
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