Genetic Determinants of Atrial Fibrillation

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Genetic Determinants of Atrial Fibrillation

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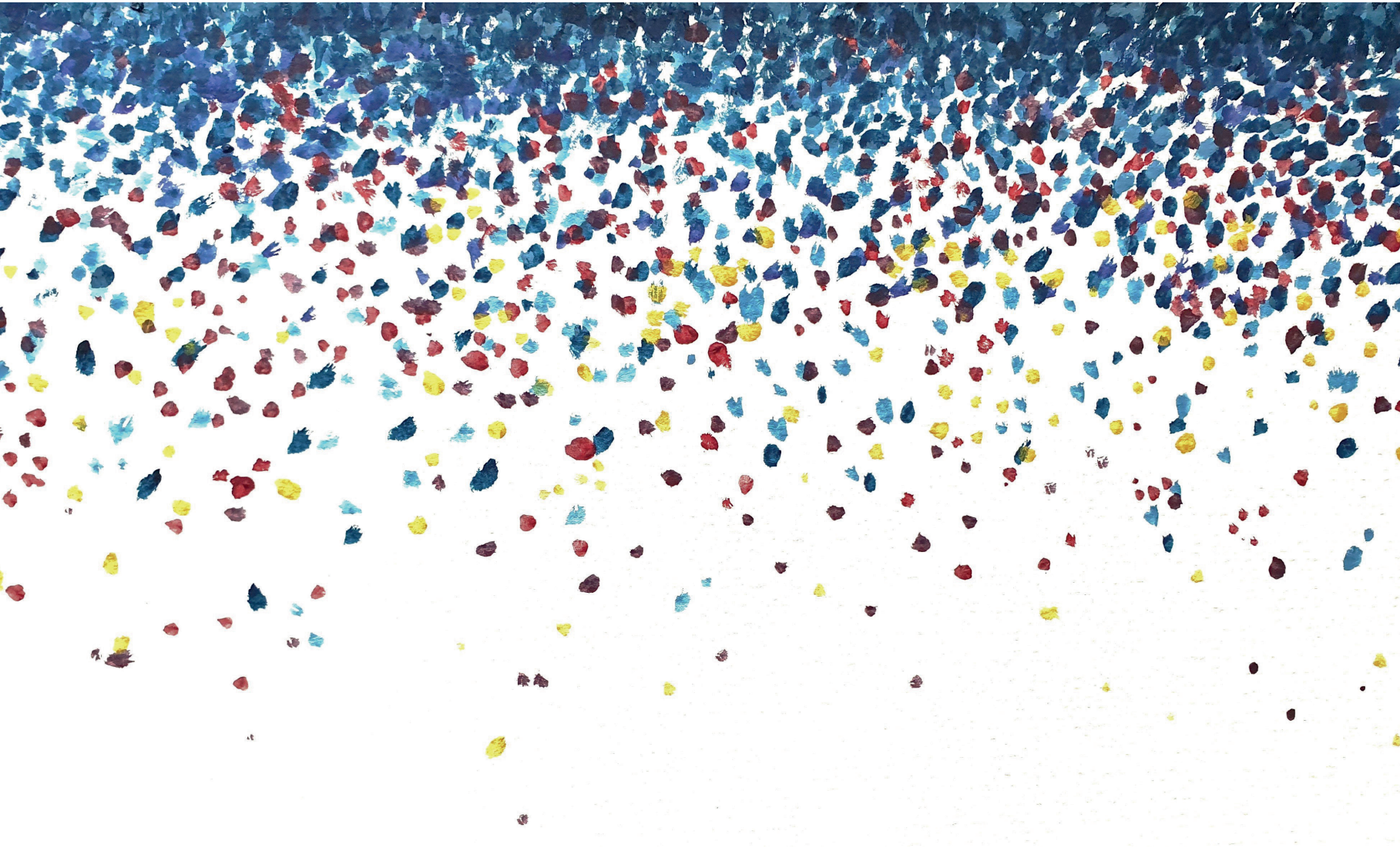

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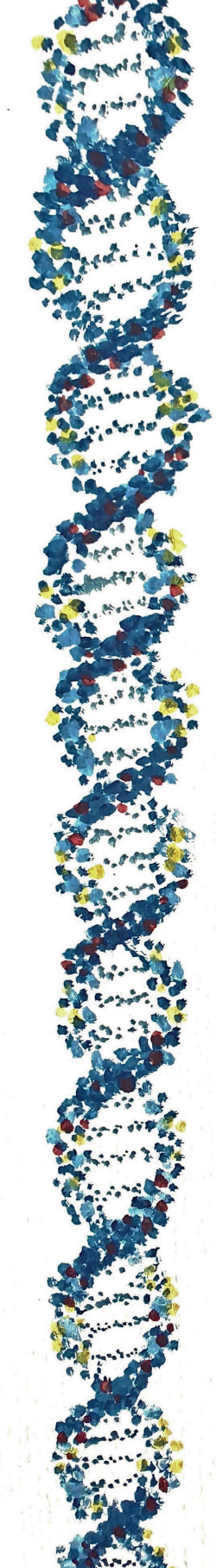
PART I

Genetics and determinants of incident atrial fibrillation



Introduction

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Atrial fibrillation genetics update: towards clinical implementation

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CHAPTER 1

General Introduction

Efforts are made to understand irregular heartbeats since ancient times. One of the first descriptions of atrial fibrillation (AF) probably descends from the emperor physician Huang Ti in China throughout the 26th century BC; "When the pulse is irregular and tremulous and the beats occur at intervals, then the impulse of life fades".[1] Individuals with AF may experience as if 'life fades' and report symptoms like palpitations, shortness of breath, dizziness and exhaustion.[2,3] The symptoms of AF may impair quality of life and can lead to an increased risk of hospitalization.[4,5]

Contemporary epidemiological data shows that the incidence and prevalence of AF have increased over the past decades,[6-8] which may have influenced the increase in lifetime risk that we see in AF (37.0% among individuals aged >55 years),[9,10] compared with prior estimates (men 23.8%, women 22.2% at age 55 years in the Rotterdam study; men 25.9%, women 23.2% at age 50 years in the Framingham Heart Study).[11,12] In Europe, the prevalence of AF will most likely increase in the coming years based on the expected growth and ageing of the population.[13,14] Among others, advancing medical technology contributes to the expected increase of AF prevalence. Screening and detection of AF improved and individuals are now able to survive conditions that were previously poorly treatable (e.g. myocardial infarction) that may result in the development of AF.

The publication of electrical activity during AF on an electrocardiogram (ECG) by Sir William Einthoven (1860 – 1927) led to the internationally accepted use of ECG features of absent P waves and irregular R-R intervals to determine AF.[15] The absent P waves reflect uncoordinated electrical impulses that lead to ineffective atrial contractions. This results in irregular ventricular contractions and thus R-R intervals on the ECG.[2] AF recurrence and increasing duration of AF episodes may occur after the initial ECG detection of AF. Atrial fibrillation episodes may present as paroxysmal AF, persistent AF or permanent AF. In the current European guidelines, paroxysmal AF is classified as self-limiting AF episodes not longer than 7 days.[2] Persistent AF is non-self-limiting and the AF episode lasts longer than 7 days. Anti-arrhythmic drugs, electrocardioversion or AF ablation can reverse the persistent AF episode to sinus rhythm. Permanent AF is a non-self-limiting AF episode, for which it is accepted by the treating physician to sustain AF, conditional on a lenient heart rate control.[16] In patients, AF may change from paroxysmal to persistent, or to permanent AF, suggesting disease progression.

AF is associated with an increased risk of heart failure, dementia, stroke and death.[2,13] AF progression from selfterminating to non-selfterminating, is associated with an increased risk of major adverse cardiovascular events, e.g. coronary artery disease, myocardial infarction, unstable angina pectoris, ischemic stroke or transient ischemic attack (TIA), and mortality.[17-21] One of the first steps to prevent these detrimental consequences of AF involves identification of individuals at risk of incident AF. Observational studies have identified male sex and advancing age as factors that increase the risk of incident AF, but also modifiable clinical risk factors have been recognized, such as hypertension, diabetes and high body mass index (BMI).[22-24] Pre-existing cardiovascular diseases such as heart failure, previous myocardial infarction or stroke, increase the risk of AF even more.[8,22] More than half of the incidence of AF could be attributed to these established risk factors.[25] For instance, hypertension has been shown to increase risk of AF by 40% in women and 50% in men; diabetes increases the risk by 60% in women and 40% in men, whereas a 10-year increase in age doubles the risk for AF.[25,26] Treatment of AF risk factors can reduce the risk of developing AF. In an attempt to reduce the risk of AF development, management of modifiable clinical risk factors is recommended.[2,27]

After AF development, the AF guidelines recommend the assessment of patients according to structured characterization of AF. The proposed 4S-AF scheme (Stroke risk, Symptom severity, Severity of AF burden, Substrate severity) facilitates communication among physicians and treatment decision making.[2] The four domains enforce evaluating stroke risk with the CHA_2DS_2-VASC risk score, symptom severity with the European Heart Rhythm Association symptom score, severity of AF burden by defining the temporal pattern of AF in combination with the total AF burden and the substrate severity with imaging in combination with cardiovascular comorbidities and risk factors. Subsequently, the implementation of the Atrial fibrillation Better Care (ABC) pathway is recommended to reduce cardiovascular events, symptoms and death in AF patients.[2] In this management strategy, anticoagulation reduces the risk of thromboembolic events; rate and rhythm control improves symptoms and quality of life; and cardiovascular and comorbidity control reduces the risk of AF burden, symptom severity and mortality.[2]

Despite all knowledge of AF risk factors, a large proportion of AF risk remains unexplained (**Figure 1**). Multiple causative factors and pathophysiological pathways may be involved in the initiation and progression of AF, and may differ between persons and in time. Identifying causal risk factors is important to improve current risk assessment and to understand of mechanisms underlying AF.

In addition to clinical risk factors, major advances are made in the identification of a genetic component to the development of AF. Advances in genetic analyses of the last decades led to an incredible increase of genetic information about AF. The genetic background of individuals at risk of AF and disease progression may increase understanding of AF. Additionally, primary and secondary prevention of AF may be improved.

▼**Figure 1.** Conceptual model of risk factors leading to atrial fibrillation.

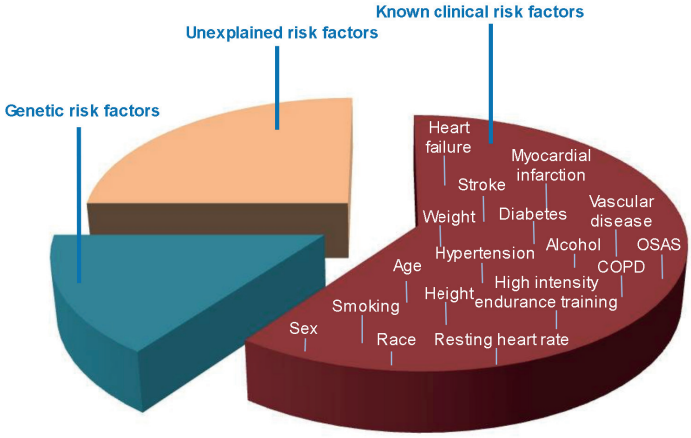

Figure 1 *illustrates that more than half of the risk of AF is attributable to known clinical risk factors. Still a large proportion of AF risk remains unexplained. Genetic risk factors may be part of the unexplained risk of AF. Last decades this genetic contribution is explored. Abbreviations: COPD = Chronic obstructive pulmonary disease, OSAS = Obstructive sleep apnea.*

Familial Clustering of AF

Familial clustering of AF led to the understanding that AF was heritable.[28-30] One of the first descriptions of familial aggregation of AF was published in 1943, describing three brothers diagnosed with AF who had experienced irregular heart rate since childhood.[31] More recently, several studies have described familial clustering of AF in larger populations.[28,29,32-35] In 2004, Fox et al. showed that one-third of the individuals diagnosed with AF in the Framingham Heart Study had at least one parent also diagnosed with AF, providing evidence that parental AF increased the risk of AF in offspring, in the general population.[28] Short after this publication, the Icelandic nation-wide genealogic community-based data were used in an analysis of more than 5,000 individuals. An important finding was that the AF risk ratio

declined exponentially when the proportion of identically shared alleles by descent diminished in relatives, confirming the importance of genetics in relation to AF risk. Similarly, a Danish twin study showed that having a co-twin with AF increased an individual's risk of AF and that the risk was doubled in monozygotic twins, compared to dizygotic twins.[29] In 2010, the Framingham Heart Study confirmed that familial AF, particularly when the onset was before or at the age of 65 years, slightly improved prediction of new-onset AF beyond clinical risk factors.[30] Moreover, the risk of AF increased with a growing number of affected first-degree relatives, while having an affected sibling conferred a similar magnitude of risk as parental AF. Together, the observations suggest that familial clustering of AF may be genetically caused.

Genetic Mapping of AF

Segregation Analyses and Candidate Gene Sequencing

Historically, segregation analyses and candidate gene sequencing were used to identify genotype–phenotype correlations, involving families or populations with high prevalence of AF. Segregation analysis is used to identify genetic markers co-segregating with the AF phenotype, utilizing the linkage phenomenon. Because of genetic recombination, genetic markers that are located in close proximity are more likely to co-segregate through pedigrees than genetic markers located far apart. In 2003, a gain-of-function mutation in KCNQ1 was identified through segregation analysis, making it the first AF-associated gene.[36] Candidate gene sequencing involves sequencing of candidate genes, based on our knowledge of their biological function, and thus an *a priori* hypothesis that increased or decreased function of the resulting proteins may lead to AF. Although many genetic variants, largely related to ion channels, have been associated with AF using segregation analysis and candidate gene sequencing, there are several limitations to these methods. The limited sample size in most of these studies and the low frequency of the genetic variants discovered have made them underpowered. The recent introduction of large reference databases of exomes and genomes has shown that many genetic variants, previously related to AF and other cardiac arrhythmia disorders through candidate gene sequencing, are present at a much higher frequency in the population than what we would expect of a disease-causing variant.[37-40]

Genome-Wide Association Studies on AF

The new millennium brought along the genome-wide association study (GWAS) approach, which was novel in its unbiased nature and its focus on common rather than rare genetic variants. One of the first GWAS was performed in 2002 by Ozaki et al. who identified a candidate locus associated with myocardial infarction[41]. This achievement initiated an accelerated search for disease-associated loci through GWAS. The first large GWAS for AF, including 550 AF cases, was performed on Iceland in 2007,[42] identifying a genetic locus associated with AF on chromosome 4q25. The most significant variant was located in an intergenic region, with the nearest gene, *PITX2*, 150 kilobases (kb) away. Pitx2c is a homeodomain transcription factor involved in embryonic development, essential in the formation of the atria, the sinus node, and left-right heart asymmetry.[42,43] **Figure 1** shows an overview of GWAS performed for AF to date. The association between the 4q25 locus and AF was replicated in two independent studies in 2009,[44,45] and since then, *PITX2* has continued to be the most significant genetic locus in AF GWAS. Gudbjartsson et al. expanded their GWAS to include 2,385 individuals with AF, and identified the *ZFHX3* locus in 2009.[46] In the same period, Benjamin et al. showed a similar association between the *ZFHX3* locus and AF in a European ancestry population.[47] The *ZFHX3* gene encodes a transcription factor enriched in cardiac tissue,[43,47] and there is evidence showing that interaction between ZFHX3 and Pitx2c or TBX5, both transcription factors active in embryogenic cardiogenesis, may increase the risk of AF significantly.[48,49]

In 2010, the *KCNN3* gene, encoding a calcium-gated potassium channel involved in atrial repolarization, was identified as the third AF-associated locus in a lone AF population by Ellinor et al. Two years later, the first GWAS meta-analysis from the international Atrial Fibrillation Genetics (AFGen) Consortium, including ~6,500 AF cases, revealed six new genetic loci for AF.[50] The most significant novel association was found for the *PRRX1* locus, which encodes a transcription factor highly expressed in the developing heart. Further studies on *PRRX1* have shown that loss of *PRRX1* expression results in shortening of the atrial action potential duration and may, thus, promote AF.[51] From 2007 to 2017, there was a steady increase in the identification of novel GWAS loci for AF, and a total of 14 new loci were identified in this period.[42,46,47,50,52,53]

▼**Figure 2.** An overview of genetic loci identified through GWAS performed for AF.

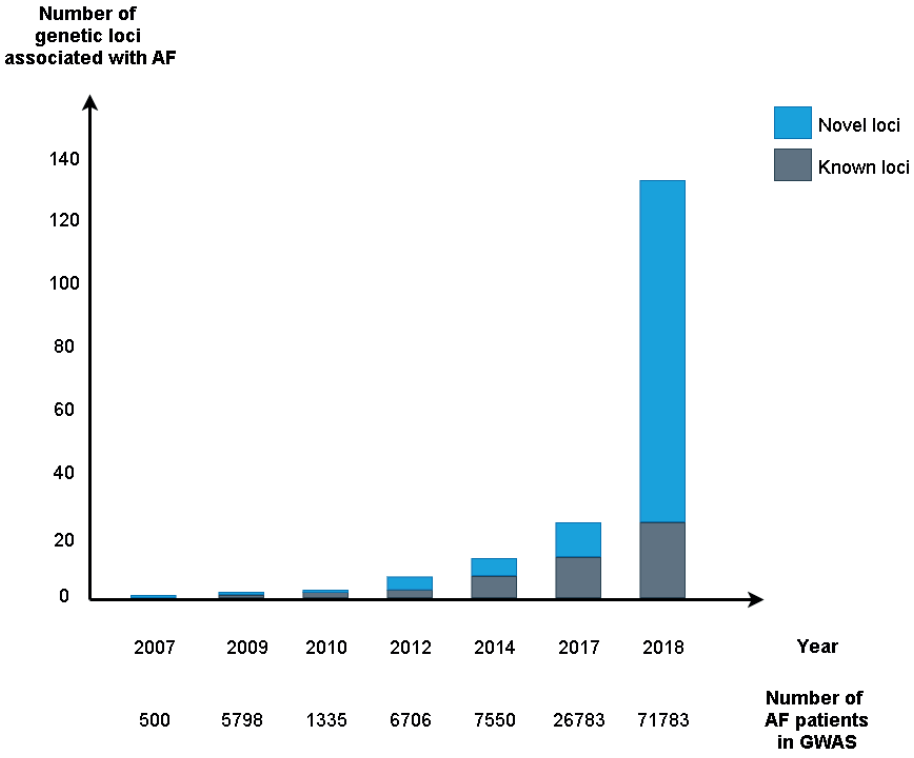

Figure 2 illustrates the number of identified loci for AF. The X axis represents which years the different GWAS for AF was performed. Under each year, the total number of cases included in all GWAS for AF the current year is listed, illustrating the relationship between the increasing sample sizes and the increasing number of AF-associated loci identified. The Y axes represent the number of genetic loci associated with AF. The blue parts of the columns represent genetic loci that have not previously been reported in relation to AF. The grey parts of the columns represent previously reported genetic loci associated with AF, making the total of each column reflect the total number of AF-associated loci at a given time. Abbreviations: AF= atrial fibrillation, GWAS = genome-wide association study.

In 2017, Christophersen et al. identified 12 new loci associated with AF in a multi ancestry GWAS meta-analysis from the AFGen Consortium, nearly doubling the number of AF risk loci. Their study included European, Asian, and African-American ancestry groups.[54] An intriguing finding was the *TTN* locus, which encodes titin, the largest protein in the human body and an essential building block in the sarcomeres of striated muscle tissue. Titin is highly expressed in the human heart, in both atria and ventricles. Hence, titin dysfunction can alter the cardiomyocyte structure.[55] A second biologically interesting discovery was the gene *KCNN2*, encoding the

calcium-dependent potassium channel subunit SK2. SK2 forms a complex with SK3, which is encoded by the gene *KCNN3,* previously associated with AF in an early AF GWAS, as described above.[53] *SK2* blockers are under development as a new potential treatment against AF.[56–59] GWAS in Korean and Japanese populations identified the same year six loci, of which the loci *KCND3, PPFIA4, HAND2*, and *NEBL* were specific to East-Asian AF patients.[60]

In 2018, a GWAS including 6,337 cases and 61,607 referents, identified one novel risk locus on chromosome 1p3Q2, with *DMRTA* and *CDKN2C* as suggested functional genes, potentially involved in structural remodeling of cardiac tissue as a substrate for AF.[61] Later in 2018, there was a major leap in the progression of AF GWAS when a large GWAS meta-analysis from the AFGen Consortium revealed 97 AF risk loci, of which 92 represented common variants and 70 were novel findings.[62] The study included over half a million individuals of combined ancestry and approximately 65,000 AF cases. Another GWAS meta-analysis of the same magnitude was published the same year, including ~1 million individuals, of which ~60,000 were AF cases,[63] identifying 111 AF-associated loci. A preliminary meta-analysis combining non-overlapping participants from these two largest GWAS performed, including ~93,000 AF cases, resulted in ~134 genetic loci associated with AF.[62] **Figure 3** shows the main biological pathways implicated in AF pathophysiology by GWAS and high-throughput sequencing studies.

High-Throughput Sequencing in AF

During the last decades, genetic sequencing technology has evolved rapidly, and with that, the price for sequencing a whole genome has dropped dramatically.[64] High-throughput sequencing describes a variety of techniques, including whole-exome and -genome sequencing, that enable high-resolution genetic analysis of all-coding and non-coding regions of our genome. The advantage of high-throughput sequencing is the ability to analyze genetic variants with lower frequencies, which may have large effects on disease risk, as opposed to GWAS, which is mainly suitable for analysis of common variants that confer small effects on disease risk.

▼**Figure 3.** Main biological pathways of AF implicated by GWAS and high-throughput sequencing studies.

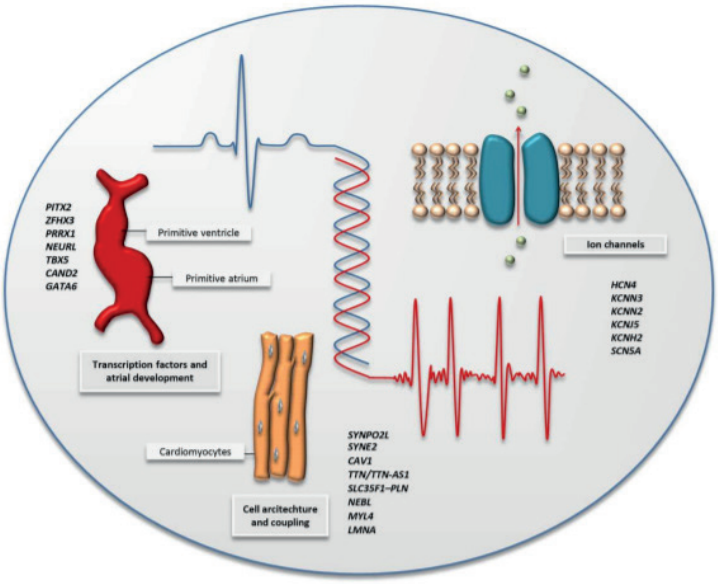

Figure 3 illustrates the main biological pathways implicated by AF-associated variants identified by GWAS and high-throughput sequencing; cardiac transcription factors and embryonic cardiogenesis, the architecture of the cardiac cells, and ion channel function. A selection of genes associated with AF through GWAS and high-throughput sequencing is listed for each pathway.

High-Throughput Sequencing in Family Studies

One of the first reports of high-throughput sequencing in AF was performed in 2014, where 39 very rare variants [minor allele frequency (MAF) <0.04%] were identified through whole-exome sequencing in six families with aggregation of AF.[65] The potentially pathogenic variants, with a range of 7 to 15 shared variants per family, underscored the complexity of the genetics of AF and suggested that non-coding regions may be more important in the search for causal genetic variants. However, the lack of sequencing of healthy family members leaves us uncertain whether the identified genetic variants truly segregate with the disease. The same year, five novel rare variants were identified performing high-throughput sequencing of nine AF-associated genes in 20 parent–offspring trios.[66] One of the variants was located in the 5' untranslated region of the *PITX2* gene, downregulating expression of Pitx2c in atrial myocytes. The remaining variants were exonic nonsense mutations in the genes *SYNE2, ZFXH3*, and *KCNN3*, and

thus represent each of the three main pathological pathways for AF development suggested by prior GWAS. Whereas, mechanisms for AF development previously have been focused at cardiac electrical function, there are several genetic findings that now also point to structural changes in the atrium as a cause of the arrhythmia. Orr et al. highlighted one such association describing a family with aggregation of early-onset AF, cardiac conduction disease, and atrial myopathy.[67] Through whole-exome sequencing, they identified a novel genetic variant in *MYL4* shared by all five affected relatives. *MYL4* is expressed in adult atria and embryonic muscle tissue, and it has been shown that loss-of-function variants in *MYL4* can lead to atrial cardiomyopathy.[68] Another genetic variant related to structural changes in the heart, a non-sense mutation in the *LMNA* gene (c.G1494A, p.Trp498Ter), was identified through whole-exome sequencing and Sanger sequencing in a four-generation AF family from northern China.[69] *LMNA* encodes the nuclear membrane proteins Lamin A and Lamin C, and pathological variants are known to cause a broad variety of inherited diseases referred to as laminopathies, including myopathies in skeletal muscle with a dystrophy-like picture, cardiomyopathy, and conduction system disease.[70-72]

In 2017, Lieve *et al.* performed a gene panel testing in a two-generation Dutch family, with a phenotype presenting with ventricular arrhythmias and early-onset AF.[73] They found a gain-of-function variant in *SCN5A*, which encodes the alpha subunit of the main cardiac sodium channel, and with this, drew attention back to cardiac electrical function as a biological pathway for AF. The functional effect of the variant seemed to increase channel availability and current duration. *SCN5A* has previously been shown to be one of the most important genes for overall atrial conduction.[54] The same year, Tucker et al. identified a gain-of-function variant in *GATA6* through whole-exome sequencing in two families with early-onset AF; one of the families also displayed atrioventricular septal defects.[74] *GATA6* encodes a cardiac transcription factor important to cardiac morphogenesis, and loss-of-function variants have previously been reported to be associated with congenital cardiac defects.[75,76] In addition, loss-of-function variants in *GATA6* have been associated with lone AF through candidate gene studies.[77,78]

As described, the gene *TTN* has been associated with AF in two independent GWAS meta-analyses,[54,61] and results from recent high-throughput sequencing studies support this association.[74,79] Ahlberg et al. performed whole-exome sequencing

in 24 families with aggregation of AF and discovered that the frequency of titin-truncating variants (*TTNtv*) in cardiac transcripts was significantly higher in the individuals with AF compared to an unaffected referent group (16.7 vs. 0.5%, $P = 1.76 \times 10^{-6}$).[79] The association was replicated in 399 individuals with early-onset AF (AF before age 40), showing an increased frequency of *TTNtv* in cases compared to referents (odds ratio [OR] = 36.8, 95%, confidence interval [CI] = 5.0–4692.5, $P = 4.13 \times 10^{-6}$). They further examined the effect of *TTNtv* on atrial development in a zebrafish model, which revealed disorganized sarcomeres and atrial fibrosis in adult heterozygous fish, and electrocardiogram (ECG) analysis showed prolongation of the PR interval, which is a known risk factor for AF.[80]

High-Throughput Sequencing in Population-Based Studies

Large-scale high-throughput sequencing is merely in the initial phase when it comes to AF in the general population. In 2015, Gudbjartsson et al. performed large-scale whole-genome sequencing in 2,636 individuals on Iceland.[81] They found a recessive frameshift mutation in *MYL4* (c.234delC, MAF = 0.65%), increasing the risk for early-onset AF by 110-fold (recessive OR = 110.3, $P = 5.2 \times 10^{-10}$). Eight homozygous carriers of the *MYL4* variant were identified. All eight carriers had been diagnosed with early-onset AF. The need for large sample sizes in order to have sufficient power to detect significant associations in high-throughput sequencing studies was demonstrated by Lubitz et al. who performed whole-exome sequencing in 1,734 individuals with AF and 9,423 referents, with no significant associations detected.[82] Recently, Choi et al. reported results from whole-genome sequencing in almost 2,781 individuals with early-onset AF and 4,959 referents of European ancestry.[83] They found an association between a rare (MAF = 0.1%) loss-of-function variant in *TTN* and early-onset AF and showed that the probability of being a carrier of the variant increased conversely with age at AF onset. As previously described, *TTN* encodes the large protein titin, which forms a crucial component in the sarcomeres of muscle tissue. An association between *TTN* variants and dilated cardiomyopathy (DCM) is well-established,[84,85] and patients with familial or early-onset DCM are routinely screened for variants in the gene.[86] The recent discoveries indicate that *TTNtv* variants may contribute to AF without a clear DCM phenotype.[79,83]

The population-based studies describing a role for *TTN* and *MYL4* in the genetic background for AF are in line with the findings from the family-based studies by Orr and

Ahlberg, described above. The results suggest that a proportion of AF-related genes or genetic regions may be involved in structural cardiac abnormalities, in addition to the well-known electrical abnormalities, identified for several ion channel genes, and support the hypothesis that some types of AF may be considered to be atrial cardiomyopathies.[87] The obvious fact that AF, being a heterogeneous disease, displays different causal mechanisms, is important for differentiating treatment. In the future, we may routinely be identifying underlying causes of AF through genetic testing, before choice of treatment.

Mendelian Randomization Studies—Causal Effects of Genetic Variants

In a polygenic and heterogeneous disease, such as AF, it is difficult to prove causality. Causality can only be established when confounding factors are eliminated in the analyses. Mendelian randomization is a statistical method with similarities of a randomized control trial and is currently used as a powerful control for reverse causation and confounding (**Figure 4**). Although the exact biological traits of genetic variants are not revealed by Mendelian randomization analyses, the results of a Mendelian randomization analyses may infer causal associations that can support AF risk management. The use of genetic variants of AF-related risk factors may clarify the complexity between AF and the environmental factors that interact with AF.

A causal relation between BMI and incident AF has been suggested by observational data,[22,88] until recently confirmed by Chatterjee et al. using Mendelian randomization,[89] supporting prevention of obesity as a therapeutic target to reduce incident AF. However, BMI covers weight and height but does not define the components of body mass, e.g., distinction between fat mass and muscle mass. In an attempt to understand how body mass plays a causal role in AF, Tikkanen et al. evaluated the relation between fat mass and fat-free mass, and incident AF, using multivariate Mendelian randomization.[90] During a follow-up period of 6.1 years, 10,852 incident AF cases occurred in the UK biobank population of 502,619 individuals (54% women, mean age = 56.5 ± 8.1 years). The multivariate Mendelian randomization showed that the effects of fat mass and fat-free mass were independently of each other associated with incident AF. Moreover, fat mass showed stronger causal associations in women, whereas fat-free mass displayed a similar association across sexes. Reductions in fat mass and fat-free mass likely follow different biological pathways, and both reduce AF risk independently.

▼**Figure 4.** Concept of Mendelian randomization.

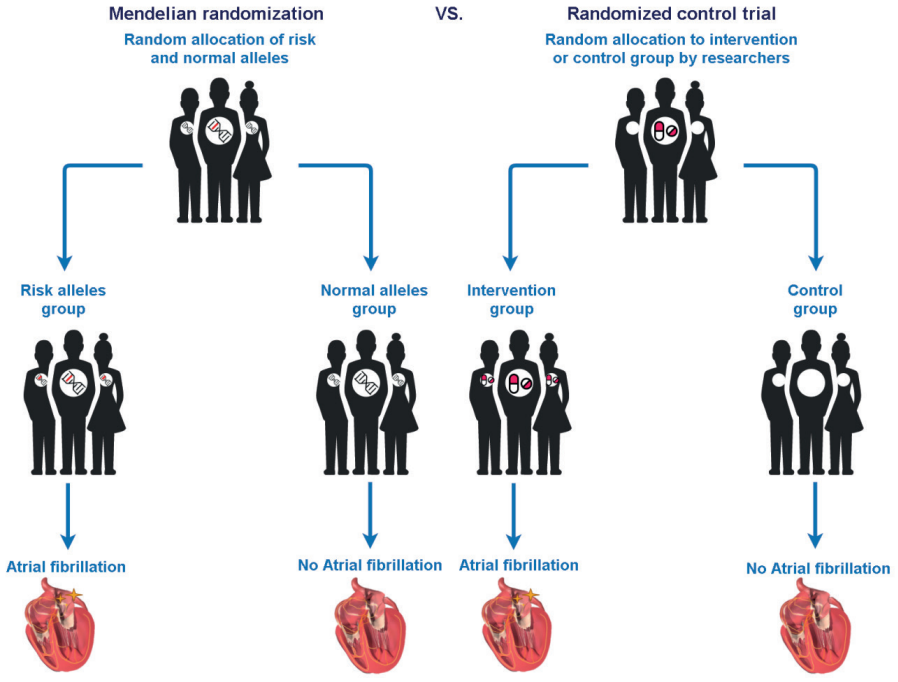

Figure 4. *Mendelian randomization and Randomized control trials are compared. Both methods compare two random groups (e.g. risk allele vs. no risk allele or intervention vs. no intervention) that develop the outcome variable (e.g. AF or no AF). Mendelian randomization assumes that two random individuals (parents) have transferred a random genetic composition to an individual that may or may not develop AF. Risk alleles with known association to an AF risk factor are used as instrumental variables to examine the causal effect of the AF risk factor on the disease. Included risk alleles are (1) strongly related with the exposure (e.g. the AF risk factor of interest), (2) genetic variants have no significant association with confounding factors (pleiotropy) and (3) genetic variants are not associated with the outcome (e.g. AF).*

Another anthropometric trait, height, is a well-known risk factor for AF.[91] More than 400 genetic loci have been associated with height,[92-96] of which selected variants have also been associated with AF.[97] Taller individuals have larger atria and more frequent premature atrial contractions. These are both strong determinants of AF and can influence the cardiac conduction system, reflected in PR interval and QRS duration on ECG.[98-102]. Kofler et al. investigated 2,149 individuals [54% women, median age = 37 (30 – 40) years], with a median height of 1.71 m, of the Genetic and Phenotypic Determinants of Blood Pressure and other Cardiovascular Risk Factors study.[103] The Mendelian randomization showed a significant association between height and both PR interval and QRS duration. The authors propose that genetically

determined body size has impact on the cardiac conduction system and thus may lead to increased risk of AF. However, the association may not be generalizable to all populations. The genetic variants associated with height discovered in data of the GIANT consortium and replication studies have been suggested to be biased by population stratification.[104,105] Berg et al. highlights the challenge that comes with correcting for population stratification in GWAS of polygenic traits and distinguishing differences in polygenic scores between populations.

Hyperthyroidism increases the risk of AF, and hypothyroidism is associated with a reduced risk of AF. The increased risk or AF persists despite treatment against hyperthyroidism.[106,107] In a Mendelian randomization of 55,114 AF cases and nearly half a million referents, supporting evidence for a causal pituitary–thyroid–cardiac axis was found. Low thyrotropin and an increased ratio of triiodothyronine: free thyroxine was genetically associated with AF. However, due to the lack of genetic instruments, especially for triiodothyronine, and the lack of association with increased thyroxine, a link between a specific agent of the thyroid and AF can still not be addressed.[108] Although Mendelian randomization seems a good tool for identifying causality, knowledge gaps and relatively small sample sizes can still prevent the discovery of true associations between AF risk factors and AF.

AF Genetic Risk Prediction

Genetic Risk Interacts with Lifestyle

As described above, most known genetic variants associated with AF have small effect sizes, increasing the risk for AF in the range 0.1–20.0%, except for the 4q25 locus, for which an effect size up to ~48% has been estimated.[62] Said et al. investigated the association of combined health behavior and genetic risk group in cardiovascular disease, including AF, in a cohort of nearly 340,000 individuals included from the UK Biobank.[109] They showed that both high genetic risk and poor behavioral lifestyle are associated with increased risk of new onset of cardiovascular disease yet there was no interaction effect observed between genetic risk profile and lifestyle. Genetic composition and lifestyle had a logon additive effect on risk for cardiovascular disease, and the relative effect of poor lifestyle was comparable between genetic risk groups. Said et al. point to the fact that everyone benefits from lifestyle intervention, but due to the logon additive effect of genetic risk and lifestyle, high genetic risk may be a suitable selection criterium for intervention.

Polygenic Risk Scores and Risk of Incident AF

Since common genetic variants confer small effects of risk of AF, polygenic risk scores can be constructed, summing the weighted risk of each genetic variant. Such a score can be used to evaluate the overall association of all known genetic variants with a specific phenotype. However, unlike a biomarker that represents an underlying biological pathway, the underlying biological pathways of genetic variants included in a polygenic risk score are unknown.

Lubitz et al. have evaluated the association between several polygenic risk scores and incident AF.[110] Because of the unknown true significance of each genetic variant, they used several significance thresholds and built risk scores with varying numbers (from 11 to 719) of included genetic variants. They found that polygenic risk scores were associated with AF beyond established clinical risk factors, underscoring the important contribution of genetic variants in the identification of individuals at risk for AF. The polygenic nature, the ongoing discovery of genetic variants associated with AF, and the unknown true significance of each genetic variant in the development of AF have motivated ongoing adjustments to the polygenic risk scores.[110,111] In a recent paper led by Lubitz, polygenic and clinical risk scores were combined to estimate the lifetime risk of AF in the Framingham Heart Study.[9] More than 5,000 individuals were analyzed. Both the polygenic risk score, including approximately a thousand genetic variants, and the clinical risk score contributed to the lifetime risk of AF. In individuals with low clinical risk for AF, the lifetime risk was doubled when moving from low to high polygenic risk [22.3% (95 CI, 15.4–29.1%) vs. 43.6% (95% CI, 35.6–51.6)], and the effect was similar in individuals with high clinical risk. Studies on polygenic risk scores for AF show that an estimation of genetic predisposition to AF is feasible with GWAS data and that polygenic risk scores can be utilized in lifetime risk models; however, regardless of the inherited predisposition to AF, the arrhythmia develops at an older age when individuals have a low burden of clinical risk factors. Modifiable risk factor management as for hypertension, obesity, smoking, and obstructive sleep apnea is still important to reduce AF risk. Future studies should determine to what extent AF risk factors can compete with polygenic AF risk.

Polygenic AF and Risk of Stroke

Ten years ago, cardioembolic stroke was described to be associated with the two strongest AF risk loci (chromosome 4q25 and 16q22).[46,112] In the prospective Malmö Diet and Cancer Study, incident ischemic stroke was associated with an AF genetic risk score containing 12 AF risk variants. From the 27,471 individuals, 2,160 individuals developed AF, and 1,495 individuals developed stroke. The AF genetic risk score was associated with incident AF, but also with incident ischemic stroke. The individuals in the top AF genetic risk score quintile had a 2-fold increased risk of incident AF after adjusting for clinical risk factors, and 23% increased risk of ischemic stroke.[112] Recently, in a separate analysis, Lubitz et al. described an association between increased polygenic risk for AF and stroke in both individuals with and without established AF.[110] Moreover, genetic risk for AF was strongly associated with cardioembolic stroke, suggesting that an elevated polygenic risk score may serve as a surrogate for thromboembolism from AF.

The risk of stroke increases when AF is present, but AF genetic risk was also associated with cardioembolic stroke in the absence of a diagnosis of AF.[113,114] The authors hypothesize that AF genetic risk is a clinically relevant marker for subclinical or previously undiagnosed AF. If individuals with subclinical or undiagnosed AF can be identified using a polygenic risk score for AF, this may be used to prevent stroke. Thus, polygenic risk scores for AF may be clinically useful in primary and secondary prevention of AF-related stroke.

Polygenic AF and Risk of Heart Failure

Heart failure may cause and be caused by AF. In a 38-year follow-up in the Framingham Heart study, heart failure was the strongest determinant of AF.[115] Increasing age, non-ischemic etiology of heart failure, and a New York Heart Association (NYHA) class greater than II has been associated with the coexistence of AF.[116] In the last 10 years, five GWAS have been conducted for heart failure. In aggregate, six genetic loci and five candidate genes were identified in 10,468 cases and 33,029 referents.[117-121] The GWAS that have been reported for heart failure have had limited statistical power. The diverse phenotype of heart failure complicates collecting genetic data of large numbers with similar phenotypes and currently relatively small numbers of individuals were analyzed for GWAS compared to the half a million individuals that led to the discovery of 134 genetic variants for AF.

Although heart failure and AF are strongly associated, only one shared genetic locus has been discovered. The ubiquitin–proteasome system enzyme 3 (*USP3*) gene is one of the novel genetic AF loci recently identified. *USP3* produces a protease that breaks down and modulates many intracellular proteins and eliminates damaged and misfolded proteins and regulates cellular processes as apoptosis. In cardiomyocytes, *USP* regulates voltage-gated membrane channels, such as sodium and potassium channels, and cell surface receptors. Cardiac signal transduction pathways are regulated by *USP* through regulating proapoptotic factors.[122,123] The onset and progression of hypertrophic cardiomyopathy are possibly linked to low concentrations or dysfunction of this protease.[122,124] The *USP*-regulated cardiac signal transduction pathways may play a mechanistic role in AF, but further studies are warranted.

Recently, a polygenic risk score for AF summing 97 genetic variants was associated with AF prevalence in 3759 heart failure patients of the BIOlogy Study to Tailored Treatment in Chronic Heart Failure study.[125] The CHARGE-AF risk model classified AF prevalence in heart failure patients significantly better when the polygenic risk score of AF was added to the model (respectively area under the curve (AUC) 0.699 (95% CI 0.682 – 0.716) vs AUC 0.721 (95% CI 0.704 0 0.737)). Additionally, the study showed that the currently known genetic variants of AF explained less of the AF variance in heart failure patients than in the general population (respectively 23% versus 42%). Unidentified genetic risk factors, gene-environment interactions, pleiotropy and the high burden of concomitant risk factors in heart failure patients may explain the lower genetic contribution to AF.[126] The heterogeneity of heart failure complicates the identification of the genetic susceptibility and phenotype of heart failure. There is a large number of variety that contributes to heart failure which can easily lead to different heart failure phenotypes. Investigating genetic information of AF, heart failure and heart failure related risk factors may reveal more of the missing heritability.

Aims of this Thesis

This thesis focuses on genetics and determinants of AF. We aim to converge clinical information and genetic information of AF risk factors to elucidate some of the complexity underlying AF.

In **part I** of this thesis, genetics and clinical determinants of incident AF are studied. One of the major AF risk factors is advancing age. The genetic markers of biological ageing are telomeres, DNA-protein complexes at the ends of chromosomes. Telomeres are essential in preventing DNA degradation. In **chapter 2** the association between telomere length and the development of AF is investigated. Another risk factor of incident AF is resting heart rate. Epidemiological data showed an association between both higher and lower resting heart rate and incident AF. However, causality of this non-linear association is not confirmed. Genetic variants that determine resting heart rate may also impact the incidence of AF. In **chapter 3** the non-linear association between genetically-determined heart rate and incident AF is assessed for causality with a Mendelian randomization. In **part II** genetics and determinants of AF after initial diagnosis, which entails profiling AF types, AF progression and changing dimensions of the heart, are studied. In **chapter 4** we studied the clinical, biomarker, and genetic profiles of individuals with different types of recurrences of AF (without 2-year recurrence, self-terminating AF and non-self-terminating AF). Previously, BMI was associated with progression of AF. In **chapter 5** we assessed causal entities of BMI and the risk of AF progression in men and women separately. In **chapter 6** we evaluated with Mendelian randomization analyses whether the association between AF and left atrial size and function is causal. Finally, in **chapter 7** the results of reported studies and their relevance are discussed and future perspectives are given.

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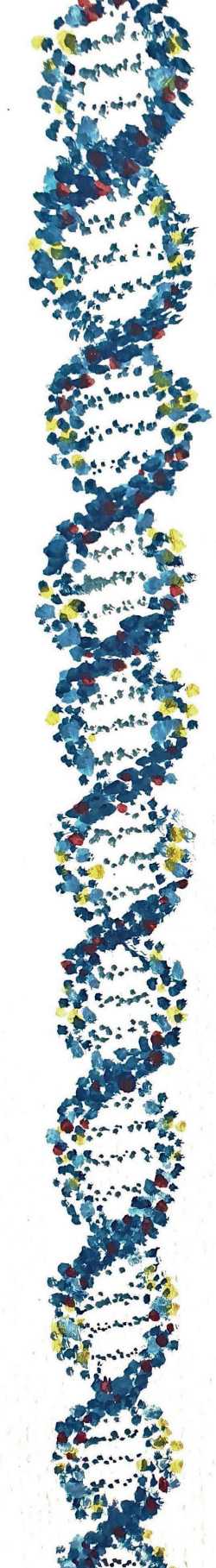

Telomere length and incident atrial fibrillation – data of the PREVEND cohort

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CHAPTER 2

Abstract

Background. The incidence of atrial fibrillation (AF) increases with age. Telomere length is considered a marker of biological ageing. We investigated the association between leukocyte telomere length and incident AF in the Dutch Prevention of Renal and Vascular End-stage Disease (PREVEND) study.

Methods. We included 7775 individuals without prevalent AF, and with leukocyte telomere length measured. Mean telomere length was determined by a monochrome multiplex quantitative polymerase chain reaction-based assay.

Results. Mean age of our cohort was 49±13 years, and 50% were men. During a mean follow-up of 11.4±2.9 years incident AF was detected in 367 (4.7%) individuals. Telomere length was shorter in individuals developing incident AF compared to those without AF (p=0.013). Incident AF was inversely related to the telomere length. In the quartile with the longest telomere length 68 (3.5%) individuals developed AF, in the shortest telomere length quartile 100 (5.1%) individuals (p=0.032). Telomere length was associated with incident AF in the second shortest telomere length quartile using the longest telomere length quartile as reference (hazard ratio 1.64; 95% CI 1.02-2.66; p=0.043). After including age or AF risk factors, the relation between telomere length and incident AF was no longer significant. We found a significant interaction of age, male sex, systolic blood pressure, BMI, heart failure, and myocardial infarction with telomere length for the association with incident AF.

Conclusions. We found that shorter leukocyte telomere length is not independently associated with incident AF in a community-based cohort.

Introduction

Advancing age is one of the major risk factors for atrial fibrillation (AF). The prevalence of AF increases with advancing age, to approximately 8% in those older than 80 years.[1-3] Approximately 70% of the individuals with AF are 65 to 80 years of age.[4,5] At all ages, AF is more frequently present in men than in women.[6-8] However, the exact reasons behind the influence of age on AF are not completely understood.

Telomere length shortens with advancing age and is considered to be a marker of biological aging.[9] Telomeres are DNA-protein complexes located at the ends of chromosomes, and are essential structures preventing DNA degradation. The ability of protection and maintenance of chromosomal stability becomes increasingly limited as a result of repeated cell division. When cell proliferation, senescence, or apoptosis occurs, this results in loss of telomere length.[10-12] Shortening of telomere length is observed in an inconsistent linear decline throughout life, and is considered to be a marker for biological aging.[9] Telomere length shortening is associated with cardiovascular diseases, including atherosclerosis, left ventricular hypertrophy and heart failure.[12]Interestingly, AF is more common in men than in women, and men have shorter telomere lengths than women.[13] These observations may suggest a relation between telomere length and incident AF. However, in previous studies no relation has been found.[14] We hypothesize that shorter telomere length is associated with incident AF, as mechanism underlying the observed association between age and sex with incident AF. We studied the association of telomere length and incident AF in healthy individuals included in the community-based Dutch Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort.

Methods

Population. The association of telomere length and incident AF was examined in the PREVEND study, a community-based cohort, founded in 1997 in Groningen, the Netherlands. A detailed description of PREVEND has been published. In total, 8592 individuals were included in the PREVEND study. AF assessment has been described previously.[3] In brief, at each three-year interval study visit ECGs, blood and urine samples were collected. In addition, ECGs made at hospital visits and hospital admission between the study visits were screened for AF. We excluded individuals with no telomere length information (n=518), individuals without ECG data (n=225),

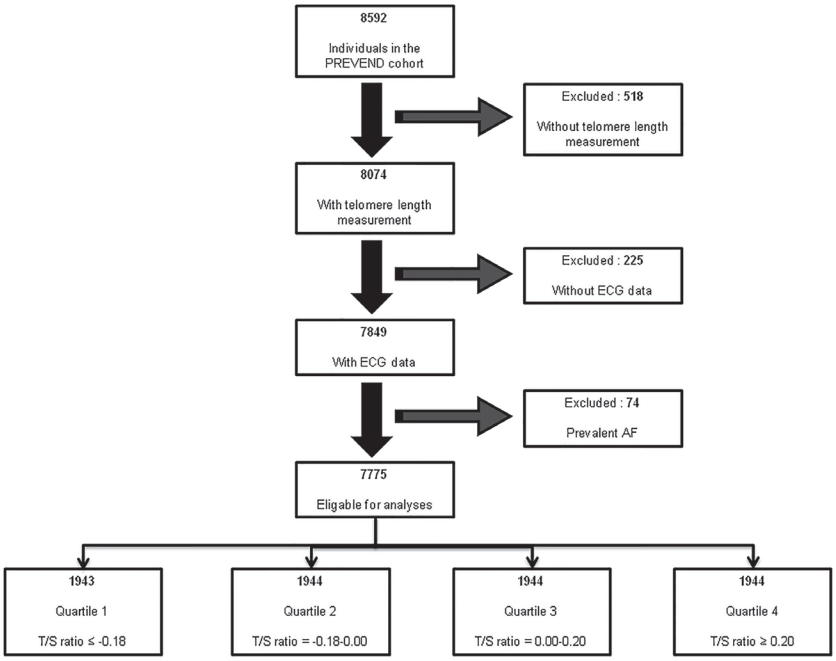
and individuals with prevalent AF (n=74) from analysis (**Figure 1**). The medical Ethics Committee of the University Medical Center Groningen approved the PREVEND study, and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Covariate definitions. Systolic and diastolic blood pressure was calculated using an automatic Dinamap XL Model 9300 series device as the mean of the last two measurements of the two visits. Hypertension was defined as self-reported use of anti-hypertensive treatment, or as systolic blood pressure >140 mmHg, and diastolic blood pressure >90 mmHg. Antihypertensive treatment was defined as angiotensin receptor blockers, angiotensin converting enzyme inhibitors, calcium antagonists or diuretics. Body mass index (BMI) was defined as the ratio of weight to height squared (kg/m^2), and obesity as a BMI >30 kg/m^2. A fasting plasma glucose ≥7.0 mmol/L (126 mg/dL), or a non-fasting plasma glucose ≥11.1 mmol/L, or use of anti-diabetic treatment was appointed as diabetes mellitus. Total serum cholesterol of >6.5 mmol/L (251 mg/dl) or a serum cholesterol of >5.0 mmol/L (193 mg/dL) if a history of myocardial infarction was present or use of lipid lowering treatment was defined as hypercholesterolemia. Nicotine use in the last 5 years was described as smoking. Myocardial infarction or stroke was defined as participant-reported hospitalization for at least 3 days for this condition. Previously, an expert panel ascertained heart failure in detail.[15] The glomular filtration rate (eGFR) was measured using the simplified modification of diet formula. Measurements of highly sensitive C-reactive protein (highly sensitive CRP) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) were described previously.[16,17]

Follow-up. Duration of follow-up was defined as the time between the baseline screening visit to first AF, the last contact date (end of the third PREVEND follow-up visit), or death. The latest last contact date was December 31, 2010.

Leukocyte telomere length measurement. DNA was drawn from full blood using a standard DNA extraction kit (Qiamp, Qiagen, Venlo, the Netherlands). Mean leukocyte telomere length was determined from blood taken at the inclusion date using the monochrome multiplex quantitative polymerase chain method as described previously.[18,19] DNA samples were stored in a -80°C freezer, and no defrosting cycles were done before analyses. Methods of measurements, the reference gene and primer sequences have been described in detail before.[18] All

▼**Figure 1.** Exclusions for present study.



DNA samples were assayed in triplicate. The samples were measured on different plates but in the same well position. In brief, 7 concentrations of a reference DNA sample of DNA concentrations were prepared by serial dilution and analyzed in triplicate in 384-well plates. As no-template control two wells received water, two wells received a human control sample, and, as a positive/max control, two wells were loaded with DNA of a human leukemia cell line with extreme long telomeres. The primers used for analyzes were; telomere, telg, ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT and telc, TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA, to generate a short, fixed-length product. The S albumin primers were albu: CGGCGGCGGGC G G C G C G G G C T G G G C G G a a a t g c t g c a c a g a a t c c t t g , a l b d : GCCCGGCCCGCCGCGCCCGTCCCGCCGgaaaagcatggtcgcctgtt.

Finally, in the PCR consisted of the following concentrations: 1U Titanium Taq DNA polymerase with the provided Titanium Taq PCR buffer, 0.75xSYBR Green I (Sigma),

0.2 mM of each dNTP, 1 mM DTT, 1M betaine, 900nM of each telomere primers (Telg and Telc), 900nM of each albumin (Albu and Albd). The intra-assay coefficient of variation was 2.0% (T), 1.85% (S) and 4.5% (T/S ratio). Samples with a coefficient variation > 10% were re-run, when after re-run a coefficient of variation remained ≥ 10% the samples were omitted from statistical analyses.

Statistical analyses. Characteristics of individuals are expressed as mean ± standard deviation, and median (interquartile range) for continuous variables and numbers (%) for categorical variables. For descriptive analysis, the fisher exact was used or Chi-square test for categorical data, the t-test or the Wilcoxon rank-test for continuous variables, depending on the normality of the data. For comparisons of more than two groups the Kruskal Wallis test or one way Anova test was used, depending on the normality of the data. A p-value of <0.05 was considered statistically significant for these tests. Leukocyte telomere length was expressed as a T/S, calculated by dividing the telomere (T) expression by the expression of a reference gene (S). Eventually, the leukocyte telomere length was calculated as (T/S ratio average - mean [T/S ratio average]) divided by standard deviation [T/S ratio average]. Since T/S ratios were skewed, ratios were logarithmically transformed and centered around 0. Logarithmically-transformed T/S ratio was discretized into quartiles. Time-to-event analyses using Cox proportional hazards models evaluate the association of T/S ratios and incident AF. In model 1, we performed unadjusted analyses, in model 2, we adjusted for age, in model 3 we adjusted for sex, in model 4 we adjusted for sex, BMI, antihypertensive treatment, myocardial infarction, stroke, heart failure, and PR-interval. As secondary analyses, Cox regression analyses were used to study the interactions between AF risk factors and telomere length for the association with incident AF (effect modification). Additionally, stratified Cox regression was performed for age categories and for men and women separately. A p-value of <0.05 was considered statistically significant.

Results

Individual characteristics. The 7775 included individuals had a mean age of 48.9±12.6 years, and 50% were men. During a mean follow-up of 11.4±2.9 years, 367 individuals developed incident AF (4.7%). Of the individuals with incident AF, 69% were men, and the mean age was 61.5±9.0 years (**Table 1**). Individuals that developed AF were older, more often men, and had more cardiovascular risk factors

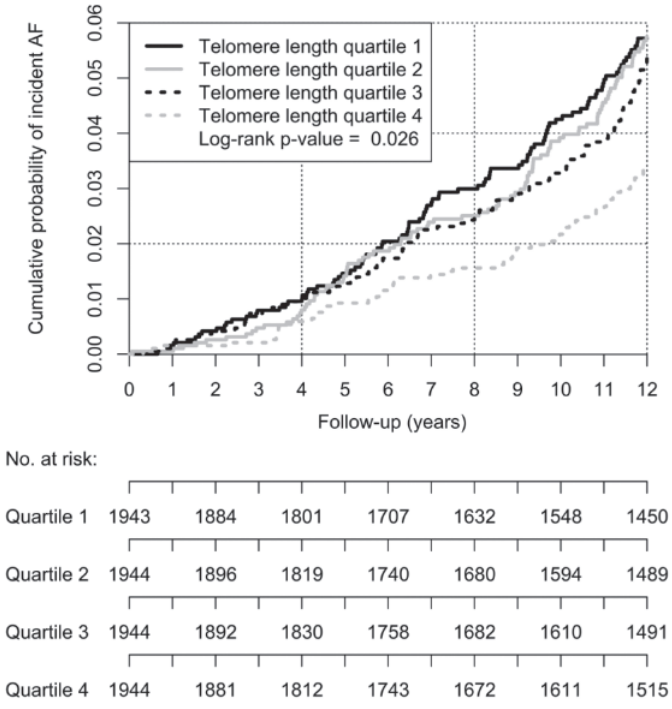
Table 1. Baseline characteristics.

Clinical profile	Total (n=7775)	Incident AF (n=367)	No AF (n=7408)	P-value
Telomere length	0.00 (-0.18-0.20)	-0.03 (-0.21-0.13)	0.00 (-0.18-0.21)	0.013
Age-years	49±13	62±9	48±12	<0.001
Male sex	3872 (50%)	254 (69%)	3618 (49%)	<0.001
BMI-kg/m2	25.6 (23.1-28.4)	27.5 (25.2-29.9)	25.5 (23.1-28.3)	<0.001
Obesity	1210 (16%)	90 (25%)	1120 (15%)	<0.001
Systolic blood pressure in mmHg	129±20	143±22	128±20	<0.001
Diastolic blood pressure in mmHg	74±10	79±9	74±10	<0.001
Heart rate-bpm	69±10	67±10	69±10	<0.001
Hypertension	2093 (27%)	198(54%)	1895 (26%)	<0.001
Heart failure	17 (0.2%)	6 (1.6%)	11 (0.1%)	0.002
Diabetes mellitus	285 (4%)	35 (10%)	250 (3%)	<0.001
Smoking	3442 (45%)	143 (39%)	3299 (45%)	0.040
Hypercholesterolemia	333 (4%)	42 (12%)	291 (4%)	<0.001
Glucose lowering treatment	107 (2%)	11 (3%)	96 (2%)	0.020
Lipid lowering treatment	314 (5%)	41 (13%)	273 (4%)	<0.001
Myocardial infarction	228 (3%)	50 (14%)	178 (2%)	<0.001
Stroke	54 (0.7%)	8 (2.2%)	46 (0.6%)	0.003
Antihypertensive treatment	1036 (16%)	131 (41%)	905 (15%)	<0.001
PR-interval-ms	158 (143-172)	168 (153-187)	158 (143-172)	<0.001
Biomarker profile	**Total (n=7775)**	**Incident AF (n=367)**	**No AF (n=7408)**	**P-value**
eGFR-ml/min/1.73m2	80.9±14.5	76.6±15.7	81.1±14.4	<0.001
Creatinine-umol/L	82.0 (73.0-92.0)	88.0 (77.0-98.0)	82.0 (73.0-91.0)	<0.001
NTpro-BNP-ng/L	37.3 (16.6-72.6)	86.3 (40.8-195.5)	35.7 (16.0-69.0)	<0.001
hs-CRP -mg/L	1.27 (0.55-2.94)	1.97 (0.94-3.66)	1.25 (0.54-2.89)	<0.001
Glucose-mmol/L	4.9±1.2	5.4±1.7	4.9±1.2	<0.001

Data is expressed as mean ± standard deviation, median (interquartile range) or numbers (%). Telomere lengths are divided in quartiles. Abbreviations: AF = atrial fibrillation, BMI = Body Mass Index, eGFR = estimate glomerular filtration rate, hs-CRP = highly sensitive C-reactive protein, NT pro-BNP = N-terminal prohormone of brain natriuretic peptide.

and diseases, except for smoking, which was less common in those with incident AF (39% versus 45%, p=0.04). The most common cardiovascular risk factor in those with incident AF was hypertension (54%), obesity (25%), hypercholesterolemia (12%), and diabetes (10%).

▼**Figure 2.** Cumulative incidence curve of incident AF, by quartiles of leukocyte telomere length.



Quartile 1 represents the shortest telomere length group. Quartile 4 represents the longest telomere length group.

Telomere length and risk of atrial fibrillation. Telomere length was shorter in individuals developing incident AF compared to those without AF (p=0.013). When discretizing individuals into quartiles based on the telomere length, the number of individuals with incident AF was inversely related to the telomere length. In the longest telomere length quartile 68 (3.5%) individuals developed incident AF, where 100 (5.1%) individuals developed AF in the shortest telomere length quartile (p=0.032) (**Figure 2, Supplementary Table 1**). Telomere length was associated

with incident AF in the second shortest telomere length quartile using the longest telomere length quartile as reference (hazard ratio 1.64; 95% confidence interval 1.02-2.66; p=0.043). After age-adjusted analysis the relation between short telomere length and incident AF was no longer significant. In additional regression models, adjustments for sex and cardiovascular risk factors were performed, but in none of the models a significant association was found (**Table 2**). We performed secondary analyses with age as the time-variable, which led to similar findings as our primary analyses.

Effect modification of AF risk factors by telomere length. As secondary analyses, we studied effect modification of age, sex and other conventional AF risk factors. For most AF risk factors, we found a significant interaction with telomere length for the association with incident AF (**Supplementary Table 2**). For age, systolic blood pressure, BMI and heart failure the interaction with telomere length was >1, meaning that the presence of the risk factor weakens or counteracts the association between shorter telomere length and incident AF, or, equivalently that decrease in telomere length weakens or counteracts the association between incident AF and the risk factor. For male sex and stroke, the interaction with telomere length was <1, meaning that the presence of the risk factor strengthens the association between shorter telomere length and incident AF, or, equivalently that decrease in telomere length strengthens the association between incident AF and the risk factor. For myocardial infarction, and antihypertensive treatment no effect modification was found.

In addition, we performed stratified analyses in three age categories <50 years, 50–65 years, and >65 years. In the age category >65 years, the third shortest telomere length quartile was associated with AF (hazard ratio 2.70; 95% confidence interval 1.09-6.66, p-value=0.031). No association between telomere length and incident AF was found in any of the other age categories or for one of the other telomere length quartiles (**Supplementary Table 3**). In the analysis stratified for sex, the second shortest telomere length quartile in men was associated with AF (hazard ratio 2.06; 95% confidence interval 1.13-3.76, p-value=0.018). No association between telomere length and incident AF was found for women (**Supplementary Table 4**).

Table 2. Telomere length in quartiles and risk of incident AF.

	Telomere quartiles	Hazard Ratio (95% Confidence Interval)	P-value
Unadjusted model	1. (≤ -0.18)	1.43(0.85-2.39)	0.181
	2. (-0.18-0.00)	1.64(1.02-2.66)	0.043
	3. (0.00-0.20)	1.32(0.83-2.09)	0.245
	4. (≥ 0.20)	1(reference)	
Age-adjusted model	1. (≤ -0.18)	0.69(0.44-1.10)	0.123
	2. (-0.18-0.00)	1.03(0.67-1.60)	0.882
	3. (0.00-0.20)	1.05(0.68-1.64)	0.820
	4. (≥ 0.20)	1(reference)	
Sex-adjusted model	1. (≤ -0.18)	1.38(0.82-2.32)	0.221
	2. (-0.18-0.00)	1.61(0.99-2.59)	0.053
	3. (0.00-0.20)	1.31(0.82-2.07)	0.258
	4. (≥ 0.20)	1(reference)	
Model adjusted for sex, BMI, antihypertensive treatment, myocardial infarction, stroke, heart failure, PR-interval	1. (≤ -0.18)	0.84(0.50-1.42)	0.517
	2. (-0.18-0.00)	1.19(0.72-1.96)	0.490
	3. (0.00-0.20)	1.19(0.71-1.98)	0.509
	4. (≥ 0.20)	1(reference)	

Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference (quartile 4). The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0. Abbreviations: AF = Atrial Fibrillation, BMI = Body Mass Index, CI = Confidence Interval, HR = Hazard ratio.

Discussion

One of the main risk factors of AF is advancing age. We therefore hypothesized an association between shorter telomere length and AF. However, the observed association between leukocyte telomere length and AF diminished when age was included in the model. Effect modification by most conventional risk factors was present, suggestive for a role of telomere length via AF risk factors in development of AF, although more research is needed.

Telomere length, biological ageing and incident atrial fibrillation. Biological ageing is marked by shortening of telomere length, and short telomere length is associated with several age-related cardiovascular diseases, including atherosclerosis, left

ventricular hypertrophy and heart failure.[12] Telomere length has been suggested to be susceptible to age-related diseases.[20] Our finding that telomere length is not independently associated with AF is consistent with an analysis of the Cardiovascular Health Study investigators, who also, found no association between telomere length and incident AF.[14] The Cardiovascular Health Study analysis was restricted to individuals >65 years in the United States, and sample size was modest (1639 individuals). We included 7775 individuals with a wide age range between 29 and 74 years. Nevertheless, results of both studies were consistent. Reasons for the absence of an independent relation between telomere length and incident AF can be the following. First, telomere length was measured in leukocytes, and this may not be fully representative of telomere length in atrial cells, although several previous studies found a positive relation between telomere length in leukocytes and AF-related cardiovascular diseases.[14,21-24] Differentiated cardiomyocytes undergo limited cell division after embryogenesis, and telomere length shortening in left atrial cardiomyocytes and dividing leukocytes may be different. However, telomere biology in left atrial cardiomyocytes is thought to play a role in the initiation of AF.[14] Premature apoptosis of cardiomyocytes resulting from loss of telomere length may contribute to fibrous replacement, and subsequently facilitate electrical chaotic activity (re-entry) that occurs in AF.[25-27] Secondly, other risk factors may contribute more than telomere length to the association of ageing and AF. Chronological ageing has been proven a risk factor of incident AF after adjustments for presence of cardiovascular risk factors and diseases, in numerous community-based cohorts.[3,14,28,29] With ageing stiffening of the left ventricle, and subsequent more diastolic dysfunction, atrial enlargement may occur, which may set the stage for AF.[30,31] Also sinus node dysfunction, and increase in premature atrial beats with ageing may be other mechanisms increasing the susceptibility for AF.[32-35] Thirdly, since we found modification by most conventional risk factors of the relation between telomere length and incident AF, it is possible that telomere length is not directly and independently related to development of AF, but via AF risk factors. Whether the presence of an AF risk factor itself influences the relation between telomere length and incident AF, or whether telomere length influences the relation between an AF risk factor and incident AF cannot be determined on present data. The further clarify the importance of effect modification of most AF risk factors by telomere length more studies are warranted, that compare patients with the same risk factors but different telomere lengths. Finally, other not-measured or subclinical risk factors and diseases potentially present in individuals at

risk for AF may have influenced the association between ageing and development of AF (residual confounding).

Strengths and limitations. Strengths of our analysis are the well-characterized large cohort, and the prospective design. The study also had several limitations. First, AF ascertainment is insensitive to asymptomatic paroxysms of AF, so asymptomatic AF may have been overlooked. And no information on temporal patterns of AF was available. Secondly, undetected age-related (cardiovascular) diseases may have influenced the results (residual confounding). Thirdly, telomere length was measured in leukocytes, and not in atrial cardiomyocytes. Fourthly, telomere length is measured at one time-point, and telomere length declines throughout life and the rate of telomere length attrition could affect the incidence of AF.[9] Fifthly, long term storage may had an impact on telomere length, but the time frame of data collection was relatively small in our study, thus large differences in telomere length between sampling dates seem unlikely. All DNA samples were stored at -80°C, and no defrosting cycles were done before analyses. Lastly, results cannot be extended to other ethnicities, telomere lengths are longer in Afro-Americans for example, since the majority of individuals included were of European ancestry.

Future perspectives. Despite our study results, telomere biology may be of importance in the development of AF-risk factors and AF-associated cardiovascular outcomes, such as stroke, heart failure, death, and the progression of AF. Effect modification by AF-risk factors was present, suggestive for a role of telomere length via AF risk factors in development of AF, although more research is needed. Furthermore, in a cross-sectional analysis of patients with and without a history of AF included in the Intermountain Heart Collaborative Study differences were found in telomere lengths according to paroxysmal, persistent and permanent AF.[36] So**,** the progression of AF may be associated with a higher rate of shortening of telomeres. Further investigation is needed to study telomere biology in the setting of AF.

Conclusions

Shorter leukocyte telomere length is not associated with incident AF in the community-based cohort of PREVEND. Effect modification by most conventional risk factors was present, suggestive for a role of telomere length via AF risk factors in development of AF, although more research is needed.

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SUPPLEMENTARY DATA

Supporting information legend

Supplementary Table 1. Baseline characteristics according to quartiles. Data is expressed as mean ± standard deviation, median (interquartile range) or numbers (%). Telomere lengths are divided in quartiles. Abbreviations: AF = Atrial Fibrillation, BMI = Body Mass Index, eGFR = estimate Glomerular Filtration rate, hs-CRP = highly sensitive C-Reactive Protein, NT pro-BNP = N-terminal prohormone of brain natriuretic peptide.

Supplementary Table 2. Association of telomere length and incident AF, modified by AF risk factor.[a,b] No significant interaction means that no effect modification was present for that AF risk factor. An interaction hazard ratio <1 means that the presence of the risk factor strengthens the association between shorter telomere length and incident AF, or, equivalently that a decrease in telomere length strengthens the association between incident AF and the risk factor. Similarly, an interaction hazard ratio >1 means that the presence of the risk factor weakens or counteracts the association between shorter telomere length and incident AF, or, equivalently that an increase in telomere length intensifies the association between incident AF and the risk factor. Abbreviation: BMI = Body Mass Index. [a] Model includes AF risk factor, telomere length and the interaction-term of both. [b] All continues covariates were centered around their means.

Supplementary Table 3. Cox regression models by age categories. Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference. The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0. Abbreviations: AF = Atrial Fibrillation, CI = Confidence Interval, HR = Hazard ratio.

Supplementary Table 4. Cox regression models by sex. Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference. The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0. Abbreviations: AF = Atrial Fibrillation, CI = Confidence Interval, HR = Hazard ratio.

Supplementary Table 1. Baseline characteristics according to quartiles.

Clinical profile	1st quartile (≤ -0.18) (n=1943)	2nd quartile (-0.18 - 0.00) (n=1944)	3rd quartile (0.00 - 0.20) (n=1944)	4th quartile (≥ 0.20) (n=1944)	
Telomere length	-0.31 (-0.40--0.25)	-0.09 (-0.14--0.04)	0.09 (0.05-0.14)	0.36 (0.27-0.49)	<0.001
AF	100 (5%)	101 (5%)	98 (5%)	68 (3%)	0.032
Age-years	52±13	49 ±12	48±12	46±12	<0.001
Male sex	1038 (53%)	1001 (52%)	942 (49%)	891 (46%)	<0.001
BMI-kg/m^2	26.3 (23.7-28.9)	25.6 (23.2-28.6)	25.4 (23.1-28.3)	25.1 (22.7-27.7)	<0.001
Obesity	361 (19%)	317 (16%)	299 (16%)	233 (12%)	<0.001
Systolic blood pressure-mmHg	133±21	129±20	128±20	126±19	<0.001
Diastolic blood pressure-mmHg	75±10	74±10	74±10	73±10	<0.001
Heart rate-bpm	70±10	69±10	69±10	69±10	0.002
Hypertension	658 (34%)	532 (27%)	496 (26%)	407 (21%)	<0.001
Heart failure	6 (0.3%)	5 (0.3%)	6 (0.3%)	0 (0.0%)	0.109
Diabetes mellitus	106(6%)	65 (3%)	68 (4%	46 (2%)	<0.001
Smoking	911 (47%)	874 (45%)	839 (43%)	818 (42%)	0.018
Hypercholesterolemia	107 (6%)	103 (6%)	70 (4%)	53 (3%)	<0.001
Glucose lowering treatment	42 (3%)	24 (1%)	23 (1%)	18 (1%)	0.011
Lipid lowering treatment	110 (7%)	95 (6%)	58 (4%)	51 (3%)	<0.001
Myocardial infarction	85 (4.5%)	66 (3.5%)	48 (2.5%)	29 (1.5%)	<0.001
Stroke	16 (0.8%)	13 (0.7%)	15 (0.8%)	10 (0.5%)	0.649
Antihypertensive treatment	331 (20%)	301 (19%)	221 (14%)	183 (12%)	<0.001
PR-interval-ms	160 (147-173)	158 (143-173)	157 (143-172)	157 (143-170)	<0.001

Biomarker profile	1st quartile (≤ -0.18) (n=1943)	2nd quartile (-0.18 - 0.00) (n=1944)	3rd quartile (0.00 - 0.20) (n=1944)	4th quartile (≥ 0.20) (n=1944)	P-value for trend
eGFR-ml/min/1.73m^2	79.2±14.9	80.8±14.8	81.1±14.1	82.4±14.1	<0.001
Creatinine-umol/L	84.0 (75.0-93.0)	83.0 (74.0-92.0)	82.0 (73.0-91.0)	80.0 (73.0-90.0)	<0.001
NTpro-BNP-ng/L	40.1 (18.0-80.8)	36.5 (16.3-71.0)	38.5 (16.6-72.9)	34.2 (15.6-66.7)	<0.001
hs-CRP-mg/L	1.63 (0.72-3.58)	1.28 (0.56-3.06)	1.18 (0.54-2.73)	1.05 (0.48-2.49)	<0.001
Glucose-mmol/L	5.1±1.4	4.9±1.1	4.9±1.2	4.7±1.0	<0.001

Data is expressed as mean ± standard deviation, median (interquartile range) or numbers (%). Telomere lengths are divided in quartiles. Abbreviations: AF=atrial fibrillation, BMI=body mass index, eGFR=estimate glomerular filtration rate, hs-CRP=highly sensitive C-reactive protein , NT pro-BNP=N-terminal prohormone of brain natriuretic peptide.

Supplementary Table 2. Cox regression models by age categories.

	Telomere quartiles	HR(95% CI)	P-value
Unadjusted model (individuals aged <50 years)	**1. (≤ -0.18)**	1.44(0.31-6.76)	0.645
	2. (-0.18-0.00)	1.29(0.37-4.47)	0.690
	3. (0.00-0.20)	0.95(0.28-3.26)	0.940
	4. (≥ 0.20)	1	
Unadjusted model (individuals aged 50-65 years)	**1. (≤ -0.18)**	0.66(0.34-1.29)	0.227
	2. (-0.18-0.00)	0.69(0.35-1.37)	0.294
	3. (0.00-0.20)	0.62(0.32-1.19)	0.148
	4. (≥ 0.20)	1	
Unadjusted model (individuals aged >65 years)	**1. (≤ -0.18)**	1.21(0.41-3.61)	0.727
	2. (-0.18-0.00)	2.52(0.94-6.74)	0.065
	3. (0.00-0.20)	2.70(1.09-6.66)	0.031
	4. (≥ 0.20)	1	

Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference. The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0. Abbreviations: AF = atrial fibrillation, CI = confidence interval, HR = Hazard ratio.

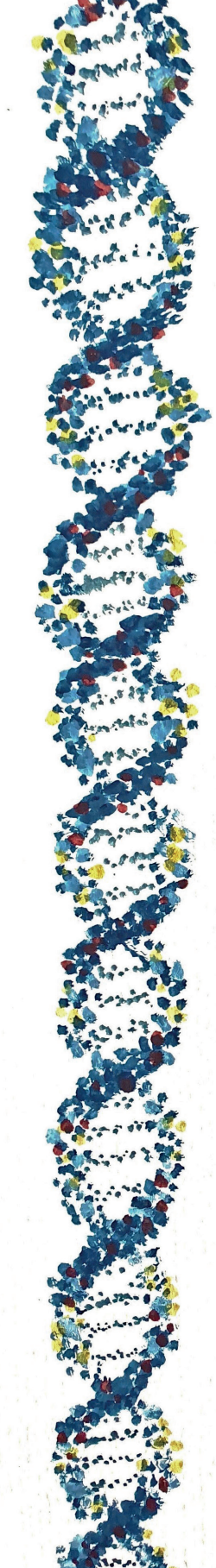
Supplementary Table 3. Cox regression models by sex.

	Telomere quartiles	HR(95% CI)	P-value
Unadjusted model (women)	**1. (≤ -0.18)**	1.02(0.40-2.60)	0.961
	2. (-0.18-0.00)	1.14(0.51-2.53)	0.746
	3. (0.00-0.20)	1.14(0.55-2.39)	0.719
	4. (≥ 0.20)	1	
Unadjusted model (men)	**1. (≤ -0.18)**	1.69(0.90-3.15)	0.101
	2. (-0.18-0.00)	2.06(1.13-3.76)	0.018
	3. (0.00-0.20)	1.52(0.84-2.76)	0.169
	4. (≥ 0.20)	1	

Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference. The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0. Abbreviations: AF = atrial fibrillation, CI = confidence interval, HR = Hazard ratio.

Supplementary Table 4. Interaction of AF risk factors and incident AF with telomere length.

AF risk factors	**HR(CI)**	**p-value**
Age	1.48(1.01-1.07)	0.010
Male sex	0.39(0.20-0.76)	0.005
Heart failure	461.39(3.10-68649.82)	0.016
Stroke	0.00(0.00-0.05)	0.000
Biomarker profile	**HR(CI)**	**p-value**
NTpro-BNP-ng/L	0.31(1.00-1.00)	0.000



Resting heart rate and incident atrial fibrillation: a stratified Mendelian randomization in the AFGen Consortium

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Submitted

CHAPTER 3

Abstract

Background. Both elevated and low resting heart rates are associated with atrial fibrillation (AF), suggesting a U-shaped relationship. However, evidence for a U-shaped causal association between genetically-determined resting heart rate and incident AF is limited.

Method and results. Seven cohorts of the AFGen consortium contributed data to this meta-analysis. All participants were of European ancestry and had known AF status, genotype information, and a heart rate measurement from a baseline electrocardiogram (ECG). Three strata of instrumental variable-free resting heart rate were used to assess possible non-linear associations between genetically-determined resting heart rate and the logarithm of the incident AF hazard rate: <65; 65-75; and >75 beats per minute (bpm). Mendelian randomization analyses using a weighted resting heart rate polygenic risk score were performed for each stratum.

We studied 38,981 individuals (mean age 59±10 years, 54% women) with a mean resting heart rate of 67±11 bpm. During a mean follow-up of 13±5 years, 4,779 (12%) individuals developed AF. A U-shaped association between the resting heart rate and the incident AF-hazard ratio was observed. Genetically-determined resting heart rate was inversely associated with incident AF for instrumental variable-free resting heart rates below 65 bpm (hazard ratio for genetically determined resting heart rate, 0.96; 95% confidence interval, 0.94–0.99; p=0.01). Genetically determined resting heart rate was not associated with incident AF in the other two strata.

Conclusions. For resting heart rates below 65 bpm, our results support an inverse causal association between genetically-determined resting heart rate and incident AF.

Introduction

Resting heart rate is a known predictor of several cardiovascular conditions, such as myocardial infarction, heart failure, and stroke.[1-4] For some conditions, including heart failure, lower heart rates can be associated with event reduction, providing evidence that heart rate may be a modifiable, causal risk factor -- and not just a risk marker or a reflection of comorbidities.[5-7] Heart rate control is also an effective strategy for improving symptoms and reducing the risk of adverse cardiovascular events related to atrial fibrillation (AF).[8] Several epidemiological studies have suggested that resting heart rate is a risk factor for development of AF. However, the shape of the association is unclear. While only lower heart rates were associated with an increased risk of incident AF in some studies,[9-11] other studies found that an elevated resting heart rate were also associated.[12] A U-shaped association has been reported.[13,14]

The pathways underlying the association between resting heart rate and incident AF are not fully understood. Resting heart rate is regulated by complex interactions of biological systems, including the autonomic nervous system. Some of the genetic loci associated with resting heart rate are related to arrhythmia susceptibility.[1,15] Recently, a Mendelian randomization analysis was performed in an attempt to determine if genetic loci that affect resting heart rate are causal to AF. The study inferred an inverse causal linear association between resting heart rate and incident AF in 367,703 individuals (including 13,538 AF cases) in the UK Biobank.[16] However, U-shaped associations were not investigated, because Mendelian randomization analyses allow analyses allow testing of only linear associations.

Mendelian randomization can be viewed as randomized controlled trials, with genotypes randomly assigned at birth. Therefore, an association between genetic variants that determine resting heart rate and incident AF implies causality, assuming that genetically-determined resting heart rate is not directly associated with possible confounding factors.

Evidence for a U-shaped causal association between genetically-determined resting heart rate and incident AF may provide insight into how AF is initiated, and may lead to improved AF risk assessment. We divided resting heart rate into three strata to observe potential directional changes of the linear associations involved in Mendelian randomization analyses. The approach allowed us to investigate evidence for a U-shaped causal association.

Methods

Study population. The meta-analysis was performed in seven cohorts of the AFGen consortium: the Atherosclerosis Risk in Communities (ARIC) study; the Framingham Heart Study (FHS); the Multi-Ethnic Study of Atherosclerosis (MESA); the Prevention of Renal and Vascular End-stage Disease (PREVEND) study; the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) study; the Rotterdam Study (RS-I and RS-II); and the Study of Health in Pomerania (SHIP). Detailed cohort descriptions are referenced in the **Supplementary Notes**. Participants were of European ancestry, with known AF status and a resting heart rate measured at baseline with a 10-second 12-lead electrocardiogram (ECG). Individuals with prevalent AF at baseline were excluded. We did not exclude individuals with negative dromotropic medication or pacemakers. Additionally, genome-wide genotyping was performed for the common genetic variants used in the resting heart rate polygenic risk score (PRS) (**Supplementary Table 1**). Written informed consent by all participants and approval of the ethical review boards of all participating cohorts were provided.

Ascertainment of atrial fibrillation. AF was assessed by each cohort, as referenced in the **supplementary notes**. The AF status of participants was established by evidence on follow-up ECG recordings and/or by diagnostic codes for AF [International Statistical Classification of Diseases and Related Health Problems (ICD)].

Assessment of resting heart rate and comorbidities. Resting heart rate was measured as a continuous covariate by a resting ECG recorded during baseline evaluations. Information was collected on recognized AF risk factors, such as hypertension, diabetes, heart failure, myocardial infarction and body mass index (BMI). Age was set as age at the time of the baseline ECG. Follow-up duration was defined as the time between the baseline ECG and diagnosis of incident AF or censoring. Detailed information is described for each cohort in the **Supplementary Notes**.

Genotyping and genetic instrument. Genotyping methodologies within the AFGen consortium were described previously.[17] A weighted PRS was calculated for each participant, incorporating genetic variants previously associated with resting heart rate.[1] Specifically, the PRS was the sum of the dose of each effect allele multiplied by the allele effect size (beta coefficient) (listed in **Supplementary Table 1**). The heart rate PRS was used as the genetic instrument in the Mendelian randomization.

Pleiotropy was assessed by adjusting for heart rate in the regression analyses of resting heart rate PRS and incident AF.

Statistical analysis. Mendelian randomization analyses were stratified for instrumental variable-free resting heart rate. The instrumental variable-free heart rate is the heart rate without the effect of the heart rate PRS.[18] First, multivariable-adjusted Cox proportional hazard regressions of resting heart rate as a risk factor for incident AF were performed in each cohort. However, no time to event was available for one of the cohorts (SHIP), with the exception of SHIP, for which logistic regression was performed due to the absence of time to event information. The odds ratio from SHIP was used as an approximation of the hazard ratio and meta-analyzed with the other hazard ratios.

Heart rate was included in the models of the regressions using linear and quadratic terms together with age, sex, eigen vector, and center if appropriate. From the beta-coefficients of the linear and quadratic terms, the hazard ratio (HR) and resting heart rate were derived for each cohort. Meta-analysis of the beta-coefficients of the linear and quadratic terms were used to derive a meta-analyzed value of the HR and resting heart rate.

The association between genetically-determined resting heart rate and incident AF was investigated by performing Mendelian randomization studies in three strata, with approximately equal numbers in the three groups. The instrumental variable free heart rate distribution is used to stratify heart rate and avoid an association between heart rate PRS and incident AF based on the association between the heart rate PRS and heart rate.[18] The genetic variants were tested for pleiotropic effects by performing a regression between resting heart rate PRS and incident AF, adjusted for heart rate. A significant result suggests pleiotropy. We used the ratio method of Mendelian randomization. Specifically, the causal beta is estimated as the ratio of (1) a beta-coefficient representing the association between resting heart rate PRS and incident AF to (2) a beta coefficient representing the association between the heart rate PRS and resting heart rate. The first beta coefficient (1) was estimated by meta-analyzing the beta coefficients of the regression analyses of resting heart rate PRS and incident AF. The second beta coefficient (2) was estimated by meta-analyzing the beta coefficients of the regression analyses of the heart rate PRS and resting heart rate. The resulting beta coefficients, when exponentiated,

express the causal hazard ratio of AF per bpm increase in resting heart rate (**Figure 1**). The standard error of the three causal beta coefficients was calculated by use of Taylor series expansion.[19] Bonferroni corrected statistical significance was defined as $p < 0.017$ based on testing three strata. All analyses were performed using the R package (version 3.1.6; R Foundation for Statistical Computing, Vienna, Austria). Meta-analyses were performed with the function metagen () of the meta R package. Forest plots were created with the forest () function of the metaphor R package. Cox regressions were performed with the coxph function () of the survival R package.

Study population. The meta-analysis was performed in seven cohorts of the AFGen consortium: the Atherosclerosis Risk in Communities (ARIC) study; the Framingham Heart Study (FHS); the Multi-Ethnic Study of Atherosclerosis (MESA); the Prevention of Renal and Vascular End-stage Disease (PREVEND) study; the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) study; the Rotterdam Study (RS-I and RS-II); and the Study of Health in Pomerania (SHIP). Detailed cohort descriptions are referenced in the **Supplementary Notes**. Participants were of European ancestry, with known AF status and a resting heart rate measured at baseline with a 10-second 12-lead electrocardiogram (ECG). Individuals with prevalent AF at baseline were excluded. We did not exclude individuals with negative dromotropic medication or pacemakers. Additionally, genome-wide genotyping was performed for the common genetic variants used in the resting heart rate polygenic risk score (PRS) (**Supplementary Table 1**). Written informed consent by all participants and approval of the ethical review boards of all participating cohorts were provided.

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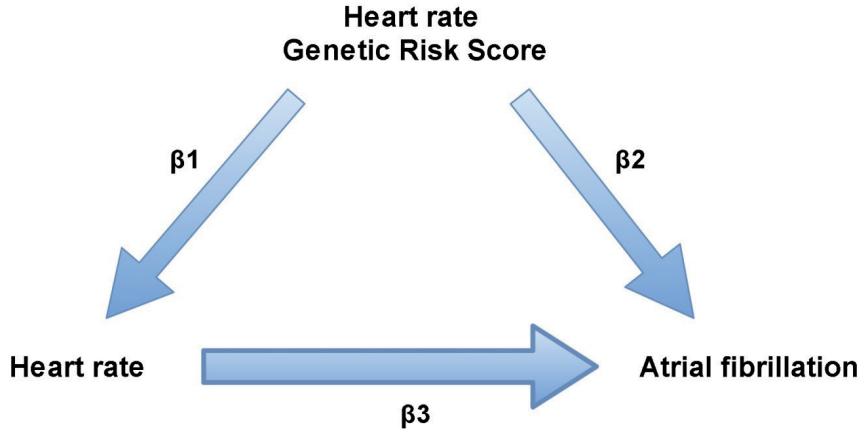
Statistical analysis. Mendelian randomization analyses were stratified for instrumental variable-free resting heart rate. The instrumental variable-free heart rate is the heart rate without the effect of the heart rate PRS.[18] First, multivariable-adjusted Cox proportional hazard regressions of resting heart rate as a risk factor for incident AF were performed in each cohort. However, no time to event was available for one of the cohorts (SHIP), with the exception of SHIP, for which logistic regression was performed due to the absence of time to event information. The odds ratio from SHIP was used as an approximation of the hazard ratio and meta-analyzed with the other hazard ratios.

Heart rate was included in the models of the regressions using linear and quadratic terms together with age, sex, eigen vector, and center if appropriate. From the beta-coefficients of the linear and quadratic terms, the hazard ratio (HR) and resting heart rate were derived for each cohort. Meta-analysis of the beta-coefficients of the linear and quadratic terms were used to derive a meta-analyzed value of the HR and resting heart rate.

The association between genetically-determined resting heart rate and incident AF was investigated by performing Mendelian randomization studies in three strata, with approximately equal numbers in the three groups. The instrumental variable free heart rate distribution is used to stratify heart rate and avoid an association between heart rate PRS and incident AF based on the association between the heart rate PRS and heart rate.[18] The genetic variants were tested for pleiotropic effects by performing a regression between resting heart rate PRS and incident AF, adjusted for heart rate. A significant result suggests pleiotropy. We used the ratio method of Mendelian randomization. Specifically, the causal beta is estimated

▼**Figure 1.** Visualization of the ratio estimator.



First, regression analyses of resting heart rate PRS and instrumental variable-free resting heart rate (β_1) and regression analyses of resting heart rate PRS and incident AF (β_2) are calculated using the following formula's; $\beta_1 = \frac{\Delta Resting\ heart\ rate}{\Delta Resting\ heart\ rate\ PRS}$ and $\beta_2 = \frac{\Delta\ log\ hazard\ rate\ of\ Incident\ AF}{\Delta Resting\ heart\ rate\ PRS}$. Beta coefficients of three resting heart rate strata were calculated separately. Subsequently, $\beta_3 = \frac{\beta 2}{\beta 1}$ was calculated for each stratum. The hazard ratio (e β_3) of each stratum indicates the causal association between resting heart rate and incident AF. Abbreviations: PRS = Polygenic risk score, HR = Hazard ratio.

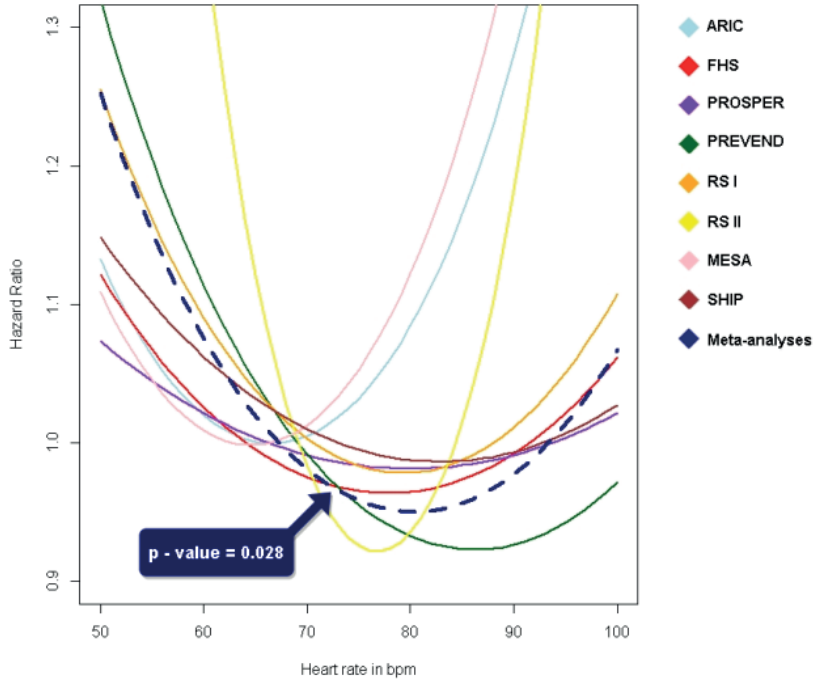
as the ratio of (1) a beta-coefficient representing the association between resting heart rate PRS and incident AF to (2) a beta coefficient representing the association between the heart rate PRS and resting heart rate. The first beta coefficient (1) was estimated by meta-analyzing the beta coefficients of the regression analyses of resting heart rate PRS and incident AF. The second beta coefficient (2) was estimated by meta-analyzing the beta coefficients of the regression analyses of the heart rate PRS and resting heart rate. The resulting beta coefficients, when exponentiated, express the causal hazard ratio of AF per bpm increase in resting heart rate (**Figure 1**). The standard error of the three causal beta coefficients was calculated by use of Taylor series expansion.[19] Bonferroni corrected statistical significance was defined as $p < 0.017$ based on testing three strata. All analyses were performed using the R package (version 3.1.6; R Foundation for Statistical Computing, Vienna, Austria). Meta-analyses were performed with the function metagen () of the meta R package. Forest plots were created with the forest () function of the metaphor R package. Cox regressions were performed with the coxph function () of the survival R package.

Results

Population. A total of 38,981 individuals were studied (mean age 59±10 years, 54% women). The mean resting heart rate was 67±11 bpm on baseline ECGs. A total of 4,779 (12%) individuals developed AF during a mean follow-up period of 13±5 years. **Table 1** shows baseline characteristics of participating cohorts.

Resting Heart rate and Atrial fibrillation. Observed associations between resting heart rate and incident AF are plotted in **Figure 2**. The meta-analyzed association between resting heart rate and incident AF showed a U-shaped curve (p = 0.028). Thus, both lower and higher resting heart rates were positively associated with incident AF.

▼**Figure 2.** U-shaped association between resting heart rate and incident AF in seven cohorts of the AFGen consortium.



A regression analysis using a quadratic term was performed per cohort to explore a non-linear association. The meta-analysed quadratic term of the association between resting heart rate and incident AF is significant (p-value = 0.028).

Table 1. Characteristics of participating cohorts.

Characteristics	ARIC (n=8994)	FHS (n=7829)	MESA (n=2489)	PREVEND (n=3501)	PROSPER (n=5244)	RS I (n=5043)	RS II (n=1987)	SHIP (n=3894)
Ethnicity	European Ancestry	European Ancestry	European Ancestry	European Ancestry	European Ancestry	European Ancestry	European Ancestry	European Ancestry
Age in years	54 ± 6	53 ± 16	63 ± 10	49 ± 12	75 ± 3	68 ± 9	65 ± 8	49 ± 16
Male	4214 (47)	3553 (45)	1183 (48)	1804 (52)	2524 (48)	2021 (40)	899 (45)	1908 (49)
Resting heart rate in bpm	67 ± 10	64 ± 11	63 ± 10	69 ± 10	66 ± 12	71 ± 12	69 ± 11	73 ± 12
Hypertension	2393 (27)	2567 (33)	957 (38)	972 (28)	3257 (62)	1802 (36)	794 (40)	2017 (52)
Diabetes	763 (9)	489 (7)	146 (9)	133 (4)	544 (10)	517 (10)	213 (11)	413 (11)
BMI in kg/m^2	27.0 ± 4.9	27.3 ± 5.4	27.7 ± 5.1	26.2 ± 4.3	26.8 ± 4.2	26.4 ± 3.9	27.3 ± 4.1	27.3 ± 4.8
Heart failure	309 (3)	85 (1)	0 (0)	6 (0.2)	0 (0)	126 (3)	22 (1)	305 (8)
Myocardial infarction	413 (5)	186 (2)	0 (0)	86 (3)	708 (14)	255 (5)	73 (4)	102 (3)
Incident AF	1817 (20)	757 (10)	448 (7)	169 (5)	505 (10)	818 (16)	171 (9)	94 (2)
Follow-up duration in years	22 ± 7	11 ± 4	12 ± 4	11 ± 3	3 ± 1	15 ± 8	12 ± 4	NA*

*Values are mean ± standard deviation or N (%). Abbreviations: AF = atrial fibrillation, ARIC = Atherosclerosis Risk in Communities study, BMI = body mass index, bpm = beats per minute, ECG = electrocardiogram, FHS = Framingham Heart Study, MESA= Multi-Ethnic Study of Atherosclerosis, PREVEND = Prevention of Renal and Vascular End-stage Disease study, PROSPER = PROspective Study of Pravastatin in the Elderly at Risk study, RS = Rotterdam Study, SHIP = Study of Health in Pomerania. * Since no time to event (event = incident AF) was available, linear regressions analyses were performed for the SHIP cohort*

Resting heart rate and atrial fibrillation: causal inference using the ratio estimator. The heart rate PRS was associated with resting heart rate in all strata (< 65 bpm, p < 0.001; 65-75 bpm, p < 0.001; >75 bpm, p < 0.001) (**Supplementary Figures 1a, 1b, 1c**). The association between the heart rate PRS and incident AF was not significant (<65 bpm, p = 0.027; 65-75 bpm, p = 0.017; >75 bpm, p = 0.293) (**Supplementary Figures 2a, 2b, 2c**). Pleiotropy was assessed by performing regression analyses of resting heart rate PRS and incident AF, adjusted for resting heart rate (<65 bpm, p= 0.052; 65-75 bpm, p = 0.082; >75 bpm, p = 0.778) (**Supplementary Figures 3a, 3b, 3c**). Thus, the effect of the resting heart rate PRS on incident AF may be completely due to resting heart rate. Moreover, absence of pleiotropy was confirmed when the three p values of **Supplementary Figures 3** were combined using Fishers method (p = 0.076).

Causal effects, estimated by the ratio method of Mendelian randomization (**Figure 1**), showed an inverse relation between resting heart rate and incident AF (HR per 5 bpm, 0.82; 95% confidence interval (CI), 0.73–0.95; p = 0.010) for heart rates <65 bpm). The other strata of instrumental variable-free resting heart rates were not causally associated with incident AF (65-75 bpm, HR 0.82, 95% CI 0.59 – 1.05, p = 0.13; >75 bpm, HR 1.16, 95% CI 0.95 – 1.34, p = 0.15) (**Table 2**).

Table 2. Causal inference using instrumental variable analysis.

Stratum	Hazard ratio (per 5 bpm increase)	Interval		P-value
Instrumental variable-free resting heart rate < 65 bpm	0.82	0.73	0.95	0.010
Instrumental variable-free resting heart rate ≥ 65 or < 75 bpm	0.82	0.59	1.05	0.133
Instrumental variable-free resting heart rate ≥ 75 bpm	1.16	0.95	1.34	0.150

Discussion

A causal association between resting heart rate and incident AF was investigated using Mendelian randomization analyses. Separate Mendelian randomization analyses were performed on three resting heart rate strata, because Mendelian randomization analyzes only linear associations. Results support an inverse causal

association between genetically-determined resting heart rate and incident AF, for resting heart rates below 65 bpm. Despite suggestions of a U-shaped association between resting heart rate and incident AF, evidence for a causal U-shaped association could not be confirmed in our study.

Our study confirms that lower resting heart rates are associated with an increased risk of incident AF, as some studies demonstrated.[9-11] For example, in the Troms Study, individuals with resting heart rates below 50 bpm had significantly increased risks of incident AF, compared to individuals with resting heart rates above 60 bpm.[9] Moreover, individuals exposed to years of endurance training are known to have a low resting heart and are reported to have increased risks of AF.[20-23]

The mechanisms by which low resting heart rate may rates predispose to AF are poorly understood. On one hand, low resting heart rate is often associated with good exercise capacity and reduced morbidity and mortality.[2] However, low resting heart rates may indicate increased vagal tone. Enhanced vagal tone potentially creates conditions favoring excitability and atrial re-entry -- via reduced effective refractory periods within the atria -- leading to AF.[20,24-26] Our results suggest that low resting heart rate is causal to AF. Future studies may investigate whether low heart rates lead to changes in vagal tone, triggering AF.

An increased resting heart rate has also been proposed to be a marker for increased risks for AF.[12,13] Higher resting heart rates are associated with sympathetic overactivity and subclinical left ventricular dysfunction. Enhanced sympathetic tone may promote automaticity, reduce the atrial effective refractory period, and increase left atrial pressure via left ventricular dysfunction. These effects may induce AF.[27-29] However, our results provided causal evidence for only a reverse association between resting heart rate and AF, for resting heart rates below 65 bpm. In our data, prior evidence of an association between high resting heart rate and incident AF could not be translated to a causal interaction. Resting heart rates above 65 bpm may potentially be driven by other (sub)clinical comorbidities that increase sympathetic tone and are causal to incident AF. Although the results seem to show a U shaped trend, our data lack genetic support for a causal association between resting heart rates above 65 bpm and incident AF. Further research is needed to confirm.

Resting heart rate may be a useful component for understanding the complex mechanisms underlying the initiation of AF. Our Mendelian randomization study suggests a role for resting heart rate in the initiation of AF. However, the extent to which the epidemiological data of the U-shaped association reflect a causal association should be explored further. Resting heart rate is easily measured in clinical practice and may be utilized for future AF risk assessments.

Strengths and limitations. Our study is one of the largest studies to specifically investigate causal evidence for the observed non-linear association between resting heart rate and incident AF. Moreover, the Mendelian randomization design is less susceptible to confounding, reverse causation, and selection bias, compared to observational analyses. However, several limitations should be discussed. First, although the 10-second ECG is a standard method for measuring resting heart rate, unusual circumstances in combination with the short measurement time (10 seconds) can distort the resting heart rate. Second, asymptomatic AF may have gone undetected in some individuals, leading to incorrect AF status (i.e., false negative classification). But in view of the large number of participants, the effect of false-negative classification results may be limited. Third, genetic variants associated with heart rate may have biased the heart rate PRS through (unknown) associations with AF or AF related risk factors. However, pleiotropic effects of heart rate were reduced to an absolute minimum by adjusting the association between the heart rate PRS and incident AF for heart rate. Fifth, information on negative dromotropic medication or pacemaker rhythm was not available for all participants. Individuals with higher heart rates may be more likely to be treated with heart rate lowering medications, which also reduces the risk of AF. Although participants with negative dromotropic medication or pacemaker rhythm were not excluded, the heart rate was still strongly associated with the constructed heart rate PRS (**Supplementary Figure 2**). The data of PROSPER showed a weaker association between heart rate and heart rate PRS, which may be due to the higher mean age of the population. Exclusion of individuals with myocardial infarction did not result in different data. However, the association between resting heart rate and heart rate PRS of all cohorts together was still significant. Finally, the generalizability of our findings beyond European ancestry and the age range in the cohorts is uncertain.

Conclusions

In seven cohorts of the AFGen consortium, genetically-determined resting heart rate was inversely associated with incident AF for resting heart rates below 65 bpm. Evidence for a causal U-shaped association could not be confirmed. Our results may suggest that low resting heart rate is not only a risk marker for AF, but may also cause incident AF. Further research is needed to confirm and to elucidate the mechanisms underlying the association of low resting heart rate with AF.

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SUPPLEMETARY DATA

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Table 1. Genetic variants used for Mendelian randomization.

Variant	Non-coded Allele	Coded Allele	Beta-coefficient
rs151041685	G	T	1.061
rs182770070	A	T	0,94
rs1320761	C	T	0,902
rs422068	T	C	0,731
rs11183443	T	C	0,676
rs41317993	G	A	0,63
rs17881696	G	A	0,578
rs3951016	T	A	0,52
rs11320420	GA	G	0,427
rs12889267	A	G	0,416
rs117159291	A	C	0,404
rs1994135	T	C	0,4
rs174536; (proxy of rs11320420 - R2=0,96)	A	C	0,399
rs73158705	A	G	0,393
rs272564	A	C	0,351
rs62172372	A	G	0,337
rs3915499	G	A	0,303
rs867400	T	C	0,298
rs12501032	C	G	0,288
rs1483890	A	G	0,284
rs236349	A	G	0,281
rs11920570	G	A	0,268
rs3749237	G	A	0,258
rs11454451	C	CT	0,256
rs12576326	A	G	0,253
rs4608502	T	C	0,249
rs16974196	G	A	0,244
rs2744375	A	T	0,24
rs17265513	T	C	0,24

Variant	Non-coded Allele	Coded Allele	Beta-coefficient
rs62144050	T	C	0,225
rs10880689; (proxy of rs11183443 - R2=0,92)	A	G	0,208
rs1549118	C	T	0,2
rs4900069	A	C	0,2
rs2283847	C	T	-0,174
rs12941356	A	G	-0,181
rs1425518	C	T	-0,182
rs1592560	A	C	-0,183
rs17494056	A	C	-0,184
rs10820614	G	C	-0,185
rs41748	T	G	-0,193
rs12713404	G	T	-0,199
rs748802	G	A	-0,202
rs2358740	G	T	-0,208
rs1050288	C	T	-0,213
rs13165531	A	T	-0,221
rs11563648	G	C	-0,231
rs10841486	T	C	-0,238
rs58437978	T	C	-0,24
rs12579753	C	T	-0,246
rs1468333; (proxy of rs35284930 - R2=0,85)	T	C	-0,255
rs145358377	G	GA	-0,259
rs10739663	A	G	-0,266
rs11081761	G	A	-0,267
rs1260326	T	C	-0,275
rs11083258	A	C	-0,276
rs12721051	C	G	-0,287
rs7194801	T	C	-0,291
rs35284930	GA	G	-0,293

Variant	Non-coded Allele	Coded Allele	Beta-coefficient
rs2076028	G	A	-0,295
rs2152735	G	A	-0,306
rs41312411	C	G	-0,32
rs180239	G	C	-0,326
rs13002735	A	C	-0,331
rs138186803	AT	A	-0,333
rs907683	G	T	-0,334
rs6845865	T	C	-0,342
rs564190295	G	GCCGCCGC-CCCC	-0,355
rs4868243	G	A	-0,361
rs2283274	G	C	-0,405
rs17201923	A	G	-0,41
rs7612445	G	T	-0,428
rs79121763	C	T	-0,471
rs17180489	G	C	-0,49
rs75190942	C	A	-0,496
rs7173389	A	T	-0,539
rs6123471	T	C	-0,595
rs56233017	G	A	-0,666
rs4963772	G	A	-0,714
rs61735998	G	T	-0,834

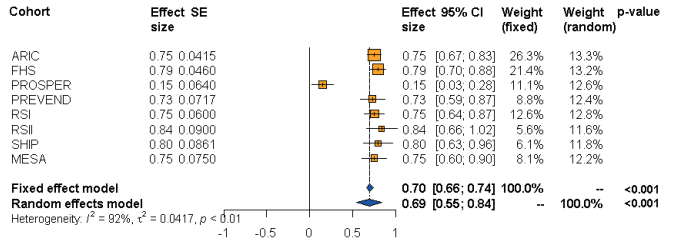
Figure legends

Figure 1. Association of resting heart rate polygenic risk score and resting heart rate in the AFGen consortium. The results of a regression analyses of the Heart rate PRS and resting Heart rate is shown. Figure 1A shows the results of the regression performed in the strata with instrumental variable-free resting heart rate below 65 bpm ($p<0.0001$), figure 1B shows the results of the regression performed in the strata with instrumental variable-free resting heart rate between 65 and 75 bpm ($p<0.0001$) and figure 1C shows the results of the regression performed in the strata with instrumental variable-free resting heart rate of and above 75 bpm ($p<0.0001$). I^2 reflects heterogeneity between studies, higher values reflect greater heterogeneity. Abbreviations: ARIC = Atherosclerosis Risk in Communities study, bpm = beats per minute, FHS = Framingham Heart Study, I^2 = heterogeneity, MESA= Multi-Ethnic Study of Atherosclerosis, PREVEND = Prevention of Renal and Vascular End-stage Disease study, PROSPER = PROspective Study of Pravastatin in the Elderly at Risk study, PRS = polygenic risk score, RS = Rotterdam Study, se = standard error of the effect size, SHIP = Study of Health in Pomerania, t^2 = between study variance.

Figure 2. Association of resting heart rate polygenic risk score and incident AF in the AFGen consortium. The results of a regression analyses of the Heart rate PRS and incident AF is shown. Figure 2A shows the results of the regression performed in the strata with instrumental variable-free resting heart rate below 65 bpm of the strata ($p=0.027$), figure 2B shows the results of the regression performed in the strata with instrumental variable-free resting heart rate between 65 and 75 bpm ($p=0.017$) and figure 2C shows the results of the regression performed in the strata with instrumental variable-free resting heart rate of and above 75 bpm ($p=0.29$). I^2 reflects heterogeneity between studies, higher values reflect greater heterogeneity. Abbreviations: ARIC = Atherosclerosis Risk in Communities study, bpm = beats per minute, FHS = Framingham Heart Study, I^2 = heterogeneity, MESA = Multi-Ethnic Study of Atherosclerosis, PREVEND = Prevention of Renal and Vascular End-stage Disease study, PROSPER = PROspective Study of Pravastatin in the Elderly at Risk study, PRS = polygenic risk score, RS = Rotterdam Study, se = standard error of the effect size, SHIP = Study of Health in Pomerania, t^2 = between study variance.

Figure 3. Association of resting heart rate polygenic risk score and incident AF adjusted for resting heart rate in the AFGen consortium. The results of a regression analyses of the Heart rate PRS and incident AF adjusted for heart rate is shown. The Heart rate PRS should not be associated with incident AF if adjusted for heart rate; this would be evidence for pleiotropic effects of the Heart rate PRS. Figure 3A shows the results of the regression performed in the strata with instrumental variable-free resting heart rate below 65 bpm of the strata ($p=0.052$), figure 3B shows the results of the regression performed in the strata with instrumental variable-free resting heart rate between 65 and 75 bpm ($p=0.111$), and figure 3C shows the results of the regression performed in the strata with instrumental variable-free resting heart rate of and above 75 bpm ($p=0.778$). I^2 reflects heterogeneity between studies, higher values reflect greater heterogeneity. Abbreviations: ARIC = Atherosclerosis Risk in Communities study, bpm = beats per minute, FHS = Framingham Heart Study, I^2 = heterogeneity, MESA = Multi-Ethnic Study of Atherosclerosis, PREVEND = Prevention of Renal and Vascular End-stage Disease study, PROSPER = PROspective Study of Pravastatin in the Elderly at Risk study, PRS = polygenic risk score, RS = Rotterdam Study, se = standard error of the effect size, SHIP= Study of Health in Pomerania, t^2 = between study variance.

▼ **Figure 1.** Association of resting heart rate polygenic risk score and resting heart rate in the AFGen consortium.



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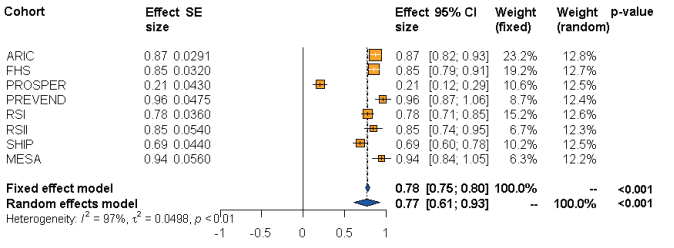

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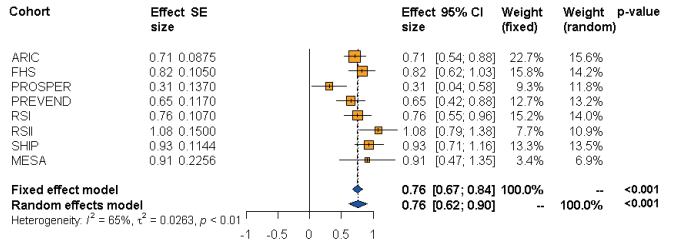

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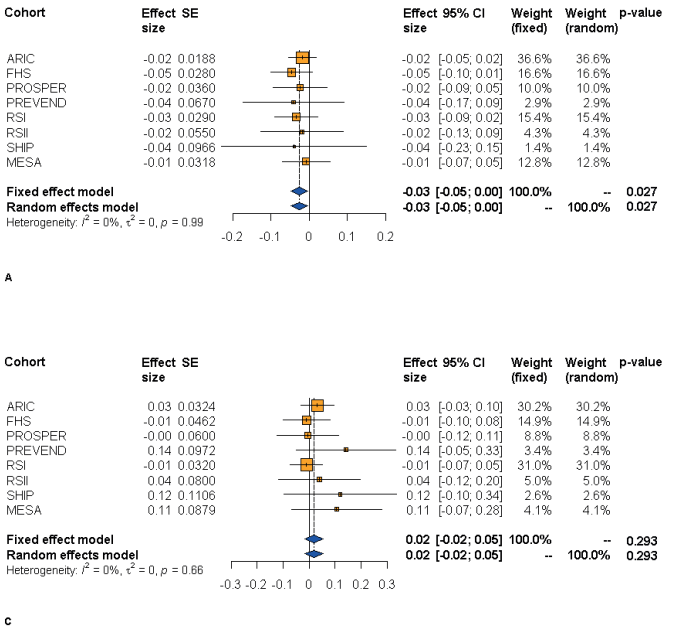
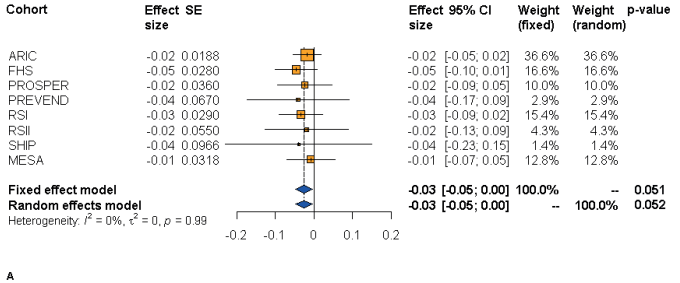
▼**Figure 2.** Association of resting heart rate polygenic risk score and incident AF in the AFGen consortium.



▼**Figure 3.** Association of resting heart rate polygenic risk score and incident AF adjusted for resting heart rate in the AFGen consortium.



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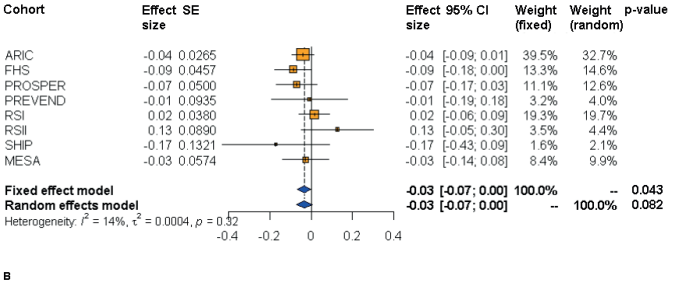

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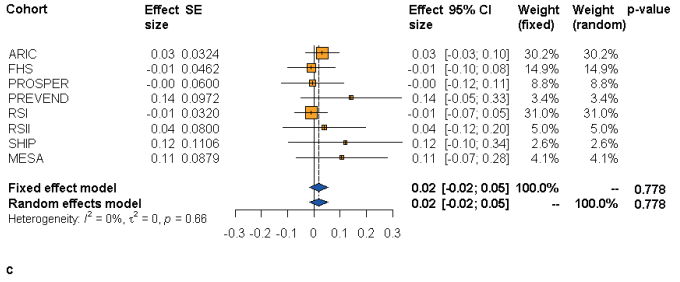


C

SUPPLEMENTARY NOTES

Description of included cohorts

The following cohorts were described previously: The Framingham Heart Study (FHS)[1] and the Prevention of Renal and Vascular End-stage Disease (PREVEND) study.[2]

The Atherosclerosis Risk in Communities study. Atherosclerosis Risk in Communities (ARIC) is a prospective population-based study of subjects in the United States (73% of European descent) aged 45 to 64 years at enrollment, recruited from four US communities (suburbs of Minneapolis, Minnesota; Washington County, Maryland; Jackson, Mississippi; and Forsyth County, North Carolina) between 1987-1989 to investigate the epidemiology of cardiovascular disease. Participants underwent electrocardiograms at baseline and at each follow-up exam (3 exams; 1 exam every 3 years). Incident atrial fibrillation was classified as the first occurrence of atrial fibrillation through 2005 as identified from electrocardiograms at study visits, hospital discharge codes or death certificates (ICD-9 code 427.31 or 427.32, or ICD-10 code I48). The sensitivity and positive predictive value of hospital discharge codes for the diagnosis of incident atrial fibrillation, as determined after review of hospital discharge summaries in a sample of ARIC participants, was close to 90%. Only subjects of self-reported European ancestry were included in this analysis; thus, subjects recruited from Jackson, MS, and a small group of subjects from Forsyth County, NC, were not included.

The Multi-Ethnic Study of Atherosclerosis. The Multi-Ethnic Study of Atherosclerosis (MESA) is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. The cohort is a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. Approximately 38 percent of the recruited participants are white, 28 percent African-American, 22 percent Hispanic, and 12 percent Asian (predominantly of Chinese descent). Participants were recruited during 2000-2002 from 6 field centers across the U.S. (at Wake Forest University; Columbia University; Johns Hopkins University; the University of Minnesota; Northwestern University; and the University of California – Los Angeles). All underwent anthropomorphic measurement and

extensive evaluation by questionnaires at baseline. Six exams have been completed since 2000. Participants are contacted every 9 to 12 months throughout the study to assess clinical morbidity and mortality. Further information can be found at: http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000209.v13.p3 and http://www.mesa-nhlbi.org.

The PROspective Study of Pravastatin in the Elderly at Risk. All data come from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). A detailed description of the study has been published elsewhere. PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5,804 subjects were randomly assigned to pravastatin or placebo. A large number of prospective tests were performed including Biobank tests and cognitive function measurements. A whole genome wide screening has been performed in the sequential PHASE project. Of 5,763 subjects DNA was available for genotyping. Genotyping was performed with the Illumina 660K beadchip, after QC (call rate <95%) 5,244 subjects and 557,192 SNPs were left for analysis. These SNPs were imputed to 2.5 million SNPs based on the HAPMAP built 36 with MACH imputation software. The study was approved by the institutional ethics review boards of centers of Cork University (Ireland), Glasgow University (Scotland) and Leiden University Medical Center (the Netherlands) and all participants gave written informed consent.

Rotterdam Study. The Rotterdam Study was designed as a prospective cohort study (RS I), initially comprising 7983 persons of 55 years or older, living in the well-defined Ommoord district in the city of Rotterdam in The Netherlands (78% of 10,215 invitees). From January 1990 onwards participants were recruited for the Rotterdam Study. In 2000, 3011 participants (out of 4472 invitees) who had become 55 years of age or moved into the study district since the start of the study were added to the cohort (RS II). In 2006, a further extension of the cohort was initiated in which 3932 subjects were included, aged 45–54 years, out of 6057 invited, living in the Ommoord district (RS III). By the end of 2008, the Rotterdam

Study therefore comprised 14,926 subjects aged 45 years or over. The overall response figure for all three cycles at baseline was 72.0% (14, 926 out of 20, 744). The participants were all extensively examined at study entry (i.e. baseline) and subsequent follow-up visits that take place every 3 to 6 years. They were interviewed at home (2 hours) and then underwent an extensive set of examinations (a total of 5 hours) in a specially built research facility in the centre of the district. These examinations focused on possible causes of invalidating diseases in the elderly in a clinically state-of-the-art manner, as far as the circumstances allowed. The emphasis was put on imaging (of heart, blood vessels, eyes, skeleton and later brain) and on collecting biospecimens that enabled further in-depth molecular and genetic analyses. The participants in the Rotterdam Study are followed for a variety of diseases that are frequent in the elderly, which include but are not exclusive to coronary heart disease, heart failure and stroke, Parkinson disease, Alzheimer disease and other dementias, depression and anxiety disorders, macular degeneration and glaucoma, COPD, emphysema, liver diseases, diabetes mellitus, osteoporosis, dermatological diseases and cancer. There is a complete coverage of filled prescriptions and there are repeated interviews of medicines use. The Rotterdam Study 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).

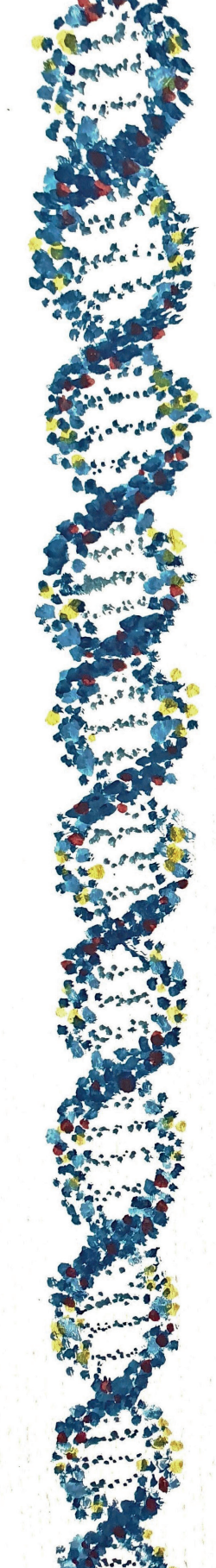
The Study of Health in Pomerania. The Study of Health in Pomerania (SHIP) is a prospective longitudinal population-based cohort study in Western Pomerania assessing the prevalence and incidence of common diseases and their risk factors. Participants aged 20 to 79 with German citizenship and principal residency in the study area were recruited from a random sample of residents living in the three local cities, 12 towns as well as 17 randomly selected smaller towns. Individuals were randomly selected stratified by age and sex in proportion to population size of the city, town or small towns, respectively. A total of 4,308 participants were recruited between 1997 and 2001 in the SHIP cohort. Individuals were invited to the SHIP study centre for a computer-assisted personal interviews and extensive physical examinations.

References

1. Ellinor PT, Lunetta KL, Albert CM, et al. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat Genet*. 2012;44(6):670-675.
2. Christophersen IE, Rienstra M, Roselli C, et al. Large-scale analyses of common and rare variants identify 12 new loci associated with atrial fibrillation. *Nat Genet*. 2017;49(6):946-952.

PART II

Genetics and determinants of atrial fibrillation after initial diagnosis



Clinical, biomarker and genetic predictors of specific types of atrial fibrillation in a community-based cohort: data of the PREVEND study

CHAPTER 4

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Abstract

Aims. Atrial fibrillation (AF) may present variously in time, and AF may progress from self-terminating to non-self-terminating AF, and is associated with impaired prognosis. However, predictors of AF types are largely unexplored. We investigate the clinical, biomarker, and genetic predictors of development of specific types of AF in a community-based cohort.

Methods. We included 8042 individuals (319 with incident AF) of the PREVEND study. Types of AF were compared, and multivariate multinomial regression analysis determined associations with specific types of AF.

Results. Mean age was 48.5 ± 12.4 years and 50% were men. The types of incident AF were ascertained based on electrocardiograms; 103(32%) were classified as AF without 2-year recurrence, 158(50%) as self-terminating AF, and 58(18%) as non-self-terminating AF. With multivariate multinomial logistic regression analysis, advancing age ($P< 0.001$ for all three types) was associated with all AF types, male sex was associated with AF without 2-year recurrence and self-terminating AF ($P= 0.031$ and $P= 0.008$, respectively). Increasing body mass index and MR-proANP were associated with both self-terminating ($P= 0.009$ and $P< 0.001$) and non-self-terminating AF ($P= 0.003$ and $P< 0.001$). The only predictor associated with solely self-terminating AF is prescribed anti-hypertensive treatment ($P= 0.019$). The following predictors were associated with non-self-terminating AF; lower heart rate ($P= 0.018$), lipid-lowering treatment prescribed ($P= 0.009$), and eGFR <60 mL/min/1.73 m^2 ($P= 0.006$). Three known AF-genetic variants (rs6666258, rs6817105, and rs10821415) were associated with self-terminating AF.

Conclusions. We found clinical, biomarker and genetic predictors of specific types of incident AF in a community-based cohort. The genetic background seems to play a more important role than modifiable risk factors in self-terminating AF.

Introduction

Nowadays, atrial fibrillation (AF) is one of the cardiovascular epidemics in Europe and the USA, and increases risk of stroke, heart failure, and death.[1,2] As a consequence, AF has extensive impact on public health. The toll of AF is expected to increase in the years to come.[3]

After a first episode of AF, rates of AF recurrences are extremely high, >90%.[4] Atrial fibrillation may have various presentations; AF may manifest as self-terminating episodes of AF, or more sustained forms of AF. Clinical risk factors of incident AF are well known, and include advancing age, male sex, hypertension, obesity, diabetes, heart failure, and valvular disease.[5,6] Data regarding risk factors for specific AF types are sparse.[7] Recent data suggest that more sustained forms of AF are at higher risk of vascular events, heart failure, and death.[4,8] Rates of AF progression vary between 5 and 15% per year depending on the population studied.[9–11] Recent studies identified risk factors for AF progression including advancing age, hypertension, heart failure, stroke, and chronic obstructive pulmonary disease.[8,11] Still, a large part of the risk of AF progression to non-self-terminating AF is unexplained.[6,12]Recently, 10 genetic variants have been discovered associated with AF;[13] however, no data are available regarding the association of these genetic variants with specific AF types.

We now investigate the clinical, biomarker, and genetic predictors of specific AF types, in a well-characterized community-based cohort, the Dutch Prevention of Renal and Vascular End-stage Disease (PREVEND) study.

Methods

Population. This study was performed using data from individuals participating in the PREVEND study, founded in 1997 in Groningen, The Netherlands. A detailed description of this study has been previously described.[14] In total, 8592 individuals were included and followed at 3-year intervals. AF assessment has been described in detail previously.[14]In brief, all electrocardiograms (ECGs) made at PREVEND screenings visits, hospital visits, or hospital admissions were screened. For present analysis, we excluded 248 individuals without any ECG. Of the 8344 individuals, 621 were diagnosed with AF. We excluded 79 individuals with prevalent AF. Of the 542 individuals with incident AF, we excluded those with <2 follow-up ECGs in the first 2 years after initial AF (n = 137). Additionally, we excluded those with <90 days

between first and last available ECG (*n* = 82), and those with insufficient ECG quality to determine the rhythm (*n* = 4), leaving 319 individuals with incident AF for analysis (**Supplementary Figure 1**). The PREVEND study was approved by the institutional medical Ethics Committee and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Atrial fibrillation definitions. Incident AF was assessed if either atrial flutter or AF was present on a 12-lead ECG at one of the three follow-up visits, or at an outpatient visit or hospital admission in the two hospitals in the city of Groningen (University Medical Center Groningen and Martini Hospital).[14] Based on all subsequent ECGs made in the first 2 years after initial AF detection, individuals were classified. If >1 ECG was performed on the same day, the ECG with AF was counted. Atrial fibrillation was classified as (i) AF without 2-year recurrence when AF was present on the initial ECG, but no AF was seen on all subsequent ECGs during 2-years after initial AF, (ii) self-terminating AF when AF was present on the initial ECG and on follow-up ECGs, but AF was seen on fewer than 90% of all follow-up ECGs, and (iii) non-self-terminating AF when AF was present on the initial ECG and on >90% of all follow-up ECGs.

Covariate definitions. Systolic and diastolic blood pressures were calculated as the mean of the last two measurements of the two visits, using an automatic Dinamap XL Model 9300 series device. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure <90 mmHg, or self-reported use of anti-hypertensive drugs. Anti-hypertensive drugs were defined as angiotensin converting enzyme inhibitors, angiotensin receptor blockers, diuretics, or calcium antagonists as a marker of hypertension.[14] Body mass index (BMI) was calculated as the ratio of weight to height squared (kg/m^2), and obesity was defined as a BMI > 30 kg/m^2. Diabetes was defined as a fasting plasma glucose ≥7.0 mmol/L (126 mg/dL), or a non-fasting plasma glucose ≥11.1 mmol/L, or use of anti-diabetic drugs. Hypercholesterolaemia was defined as total serum cholesterol >6.5 mmol/L (251 mg/dL) or a serum cholesterol >5.0 mmol/L (193 mg/dL) if a history of myocardial infarction was present or use of lipid-lowering drugs. Smoking was defined as nicotine use in the last 5 years. Previous myocardial infarction or stroke was defined as participant-reported hospitalization for at least 3 days as a result of this condition. Heart failure was ascertained by an expert panel as described in detail before.[15]

Laboratory testing. Fasting blood samples were obtained during the morning, and 24-h urine collections were obtained. The details on the laboratory measurements have been published previously.[16,17] Urinary albumin excretion was measured in the first morning void. The glomerular filtration rate was calculated using the simplified modification of diet formula.[18]

Genetic variants. Genotyping was performed using the Illumina CytoSNP12 v2 chip as previously described.[19] The single nucleotide polymorphisms (SNPs) from each of the 10 AF susceptibility loci identified by prior genome wide association studies[13] were selected for association testing. When the AF-related SNP was not directly genotyped on the Illumina CytoSNP12 v2 chip, imputed data was used (additional information in *Table S1*). Genotype data were only available for a subset of the included individuals (3419 individuals [42.5%]).

Statistical analysis. To adjust for the overselecting of individuals with microalbuminuria at study start, we added urine-albumin excretion as covariate in all regression analysis. Characteristics of the AF without 2-year recurrence, self-terminating, non-self-terminating AF, and no AF groups were presented as mean ± standard deviation or median (interquartile range) for continuous variables and counts with percentages for categorical variables. Comparisons between the specific AF types and the no AF group were evaluated using the *t*-test or the analysis of variance or the Wilcoxon rank test or Kruskal test, depending on normality of the data, for continuous data. For categorical data, the Fisher exact test (in case of binomial proportions) was used predominantly, and in the case of >2 response categories, the χ^2 test was used. We examined associations between AF-related SNPs and AF types using multinomial logistic regression analysis. We performed multivariate multinomial logistic regression analysis to assess the clinical, biomarker, and genetic predictors of specific types of AF (AF without 2-year recurrence, self-terminating, and non-self-terminating AF). In multinomial logistic regression, the different AF types are compared with the no AF group as reference. Covariates (except the genetic variants) with $P < 0.05$ in a urine-albumin excretion adjusted model were stepwise incorporated in a multivariable-adjusted model. The final multivariable model included all covariates with $P < 0.05$. Finally, interactions in the multivariate model were investigated. All analysis were performed using R package (version 3.0.3), and a *P*-value of <0.05 was considered statistically significant.

Results

Individuals' characteristics. We included 8042 individuals (319 with incident AF) in our analysis. The mean age was 48.5 ± 12.4 years and 49.5% were men. In **Table 1**, the clinical risk factors, cardiovascular diseases, and biomarkers at study start are depicted according to the types of incident AF. Of all included incident AF cases, 103 (32%) were classified as AF without 2-year recurrence, 158 (50%) as self-terminating AF, 58 (18%) as non-self-terminating AF (**Figure 1**). The median number of ECGs per individual was 15 (interquartile range 9–27). Age was significantly higher in each specific AF type group when compared with the no AF group. Sex differences were observed in all three AF type groups compared with no AF (49% men); 66% of AF without 2-year recurrence ($P < 0.001$), 63% of self-terminating AF ($P < 0.001$) and 74% of non-self-terminating AF ($P < 0.001$) individuals were men. Body mass index, systolic and diastolic blood pressure were significantly higher in each specific AF type group when compared with the no AF group. Hypertension, previous myocardial infarction, and diabetes were more common in each specific AF type group when compared with the no AF group. Heart rate was higher in the self-terminating and non-self-terminating AF group when compared with the no AF group. All measured biomarkers were significantly higher in each specific AF type group when compared with the no AF group.

▼**Figure 1.** Specific types of atrial fibrillation.

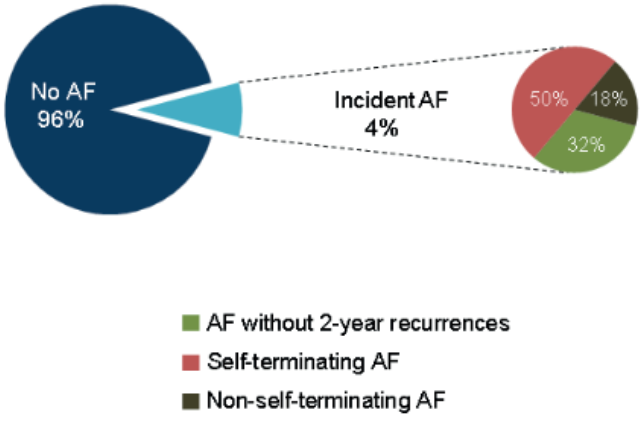

Table 1. Clinical and biomarker profile, according to type of incident AF.

	No. of AF (*n* = 7723)	**AF without 2-year recurrence (*n* = 103)**	***P*-value**	**Self-terminating AF (*n* = 158)**	***P*-value**	**Non-self-terminating AF (*n* = 58)**	***P*-value**
Clinical profile							
Age (years)	48 ± 12	59 ± 10	<0.001	59 ± 9	<0.001	62 ± 9	<0.001
Male sex	3770 (49%)	68 (66%)	<0.001	100 (63%)	<0.001	43 (74%)	<0.001
Caucasian	7322 (95%)	97 (94%)	0.657	155 (98%)	0.067	56 (97%)	0.769
BMI (kg/m^2)	26.0 ± 4.2	27.3 ± 3.6	<0.001	28.2 ± 4.6	<0.001	28.0 ± 3.7	<0.001
Obesity	1154 (15%)	26 (25%)	0.008	45 (29%)	<0.001	10 (17%)	0.585
systolic blood pressure (mmHg)	128 ± 20	138 ± 22	<0.001	144 ± 23	<0.001	148 ± 21	<0.001
Diastolic blood pressure (mmHg)	74 ± 10	78 ± 9	<0.001	79 ± 9	<0.001	79 ± 10	<0.001
Heart rate(bpm)	69 ± 10	68 ± 11	0.278	68 ± 11	0.045	66 ± 11	0.018
Anti-hypertensive treatment prescribed	912 (14%)	33 (35%)	<0.001	51 (36%)	<0.001	25 (53%)	<0.001
Hypertension	1944 (26%)	41 (41%)	0.001	91 (59%)	<0.001	38 (67%)	<0.001
Previous myocardial infarction	184 (2%)	11 (11%)	<0.001	17 (11%)	<0.001	10 (18%)	<0.001
Heart failure	12 (0.2%)	0 (0%)	1.000	1 (0.6%)	0.232	3 (5.2%)	<0.001
Glucose lowering treatment prescribed	97 (2%)	4 (4%)	0.058	6 (4%)	0.024	2 (4%)	0.165
Diabetes mellitus	259 (3%)	10 (10%)	0.003	13 (9%)	0.003	8 (14%)	<0.001
Previous stroke	48 (0.6%)	3 (3%)	0.029	4 (2.6%)	0.020	1 (1.8%)	0.307
Smoking	3451 (45%)	47 (46%)	0.842	69 (44%)	0.871	22 (38%)	0.354
Lipid-lowering treatment prescribed	272 (4%)	10 (11%)	0.007	19 (14%)	<0.001	8 (17%)	<0.001
Biomarker profile							
Glucose (mmol/L)	4.7 (4.3–5.1)	4.9 (4.5–5.3)	<0.001	5.0 (4.6–5.6)	<0.001	4.9 (4.5–5.7)	0.008
eGFR (mL/min/1.73 m^2)	80.5 (71.7–89.8)	75.7 (65.8–88.0)	0.006	75.2 (70.0–86.2)	0.002	75.8 (66.6–82.9)	0.020
eGFR ≤ 60 mL/min/1.73 m^2	414 (5%)	11 (11%)	0.028	12 (8%)	0.210	5 (9%)	0.236

	No. of AF (*n* = 7723)	**AF without 2-year recurrence (*n* = 103)**	***P*-value**	**Self-terminating AF (*n* = 158)**	***P*-value**	**Non-self-terminating AF (*n* = 58)**	***P*-value**
Urinary albumin concentration (mg/L)	11.8 (6.9–7.5)	13.5 (10.2–25.2)	0.009	15.8 (10.9–25.8)	<0.001	16.3 (10.1–54.7)	0.002
Creatinine (µmol/L)	82.0 (73.0–91.0)	86 (75–99)	0.005	85 (75–97)	0.009	89 (80–96)	0.001
Cystatine C (mg/L)	0.77 (0.68-0.87)	0.84 (0.73–0.95)	<0.001	0.87 (0.75–0.98)	<0.001	0.91 (0.82–1.02)	<0.001
NT-proBNP (ng/L)	35.1 (15.9–68.0)	74.1 (36.1–122.1)	<0.001	68.6 (30.7–153.4)	<0.001	123.2 (63.6–270.1)	<0.001
MR-proANP (ng/L)	46.7 (34.0–63.5)	64.0 (48.4–90.5)	<0.001	64.9 (46.2–95.2)	<0.001	83.3 (55.8–120.1)	<0.001
Highly sensitive-C-reactive protein (mg/L)	1.23 (0.54–2.85)	1.64 (0.83–3.83)	0.006	2.13 (0.86–4.04)	<0.001	2.11 (1.23–5.42)	<0.001

Data are expressed as mean ± SD, median (interquartile range), or numbers (%). Each AF group is compared with the no AF group. Abbreviations: AF = atrial fibrillation, BMI = body mass index, eGFR = estimated glomular filtration rate, MR-proANP = Mid-regional prohormone of the atrial natriuretic peptide, NT-proBNP = N-terminal prohormone of the brain natriuretic peptide.

Common genetic variants. With multinomial logistic regression analysis, and the no AF group as reference, rs6666258, on chromosome 1q21, in the *KCNN3/PMVK* locus [relative risk ratio (RRR) 1.58, 95% confidence interval (CI) 1.12–2.23, *P* = 0.009), rs6817105, on chromosome 4q25 near the *PITX2* locus (RRR 1.74, 95% CI 1.12–2.68, *P* = 0.013), and rs10821415, on chromosome 9q22, in the *C9orf3* locus (RRR 1.49, 95% CI 1.07–2.08, *P* = 0.019) were associated with self-terminating AF, and not with the other AF types (**Table 2**).

Table 2. Distribution of common AF-related genetic variants associated with type of incident AF.

			AF type					
Genetic variants			**AF without 2-year recurrence (*n* = 103)**		**Self-terminating AF (*n* = 158)**		**Non-self-terminating AF (*n* = 58)**	
AF SNP	Chromosome	Closest gene	RRR (95% CI)	***P*-value**	RRR (95% CI)	***P*-value**	RRR (95% CI)	***P*-value**
rs6666258	1q21	*KCNN3-PMVK*	0.94 (0.57–1.54)	0.795	1.58 (1.12–2.23)	0.009	1.11 (0.63–1.95)	0.715
rs3903239	1q24	*PRRX1*	1.04 (0.66–1.65)	0.860	1.33 (0.95–1.85)	0.094	1.27 (0.75–2.16)	0.369
rs6817105	4q25	*PITX2*	1.73 (0.96–3.13)	0.068	1.74 (1.12–2.68)	0.013	1.27 (0.59–2.70)	0.539
rs2040862	5q31	*WNT8A*	1.12 (0.62–2.01)	0.703	1.07 (0.70–1.66)	0.747	0.97 (0.48–1.96)	0.931
rs3807989	7q31	*CAV1*	0.90 (0.58–1.41)	0.656	1.08 (0.77–1.50)	0.666	0.72 (0.43–1.21)	0.218
rs10821415	9q22	*C9orf3*	1.14 (0.72–1.79)	0.571	1.49 (1.07–2.08)	0.019	0.93 (0.55–1.59)	0.798
rs10824026	10q22	*SYNPO2L*	1.32 (0.68–2.56)	0.406	1.04 (0.67–1.62)	0.862	1.22 (0.58–2.55)	0.604
rs1152591	14q23	*SYNE2*	1.01 (0.64–1.59)	0.956	1.09 (0.78–1.51)	0.626	0.72 (0.43–1.23)	0.229
rs7164883	15q24	*HCN4*	1.33 (0.76–2.33)	0.313	0.83 (0.51–1.35)	0.455	0.53 (0.21–1.33)	0.175
rs2106261	16q22	*ZFHX3*	1.00 (0.57–1.74)	0.987	1.30 (0.89–1.90)	0.177	1.72 (0.98–3.01)	0.060

In multinomial logistic regression, the AF groups are compared with the no AF group (n = 7723), which act as a reference. Adjusted for urinary albumin concentration (mg/L). Abbreviations: AF = atrial fibrillation, CI = confidence interval, RRR = relative risk ratio, SNP = single nucleotide polymorphism.

Predictors of specific atrial fibrillation types. With multivariate multinomial logistic regression analysis, advancing age ($P < 0.001$ for all AF types, **Table 3**) was associated with AF without 2-year recurrence, self-terminating, and non-self-terminating AF. Male sex was associated with AF without 2-year recurrence and self-terminating AF ($P = 0.031$ and $P = 0.008$). Increasing BMI and higher concentrations of mid-regional prohormone atrial natriuretic peptide (MR-proANP) were associated with both self-terminating ($P = 0.009$ and $P < 0.001$, respectively) and non-self-terminating AF ($P = 0.003$ and $P < 0.001$, respectively). Prescribed anti-hypertensive treatment ($P = 0.016$) was only associated with self-terminating AF. The following covariates were associated with non-self-terminating AF; lower heart rate ($P= 0.018$), lipid-lowering treatment prescribed ($P = 0.012$) and eGFR < 60 mL/min/1.73 m^2 ($P = 0.007$) (**Supplementary Figure 2**).

Table 3. Multivariate multinomial logistic regression comparing type of AF to no AF.

Covariate	AF type					
	AF without 2-year recurrence (*n* = 103)		Self-terminating AF (*n* = 158)		Non-self-terminating AF (*n* = 58)	
	RRR (95% CI)	*P*-value	RRR (95% CI)	*P*-value	RRR (95% CI)	*P*-value
Age (per 10 years)	1.70 (1.37–2.13)	<0.001	2.14 (1.48–3.07)	<0.001	1.84 (1.51–2.24)	<0.001
Male sex	1.66 (1.05–2.62)	0.031	2.82 (1.32–6.02)	0.008	1.47 (0.99–2.18)	0.053
Anti-hypertensive treatment prescribed	1.50 (0.88–2.56)	0.135	2.52 (1.19–5.33)	0.016	1.33 (0.85–2.08)	0.213
BMI (per 5 kg/m^2)	1.25 (0.94–1.66)	0.126	1.77 (1.15–2.71)	0.009	1.41 (1.12–1.78)	0.003
Heart rate (per 5bpm)	0.96 (0.86–1.07)	0.464	0.86 (0.73–1.01)	0.074	0.89 (0.80–0.98)	0.018
Lipid-lowering treatment prescribed	1.54 (0.76–3.14)	0.234	2.25 (0.96–5.26)	0.063	2.04 (1.17–3.56)	0.012
MR-proANP (per 50 ng/L)	1.33 (1.00–1.77)	0.051	1.78 (1.31–2.43)	<0.001	1.48 (1.19–1.84)	<0.001
eGFR ≤ 60 mL/min/1.73 m^2	0.92 (0.43–1.96)	0.828	0.62 (0.20–1.97)	0.416	0.33 (0.15–0.74)	0.007

In multinomial logistic regression, the AF groups are compared with the no AF group (n = 7723), which act as a reference. Adjusted for urinary albumine concentration.
AF, atrial fibrillation; BMI, body mass index; CI, confidence interval; eGFR, estimated glomular filtration rate; MR-proANP, Mid-regional prohormone of the atrial natriuretic peptide; RRR, relative risk ratio.

Discussion

In our contemporary community-based cohort, we determined clinical, biomarker, and genetic predictors of specific types of incident AF; AF without 2-year recurrence, self-terminating, and non-self-terminating AF.

Types of atrial fibrillation. A first episode of AF is always followed by a recurrence of AF, however the timing of recurrence maybe highly variable.[4] Different types of AF are described.[6,12] The most widely used classification system for temporal patterns of AF is the 3-P classification; paroxysmal, persistent, and permanent AF.[1] When AF terminates spontaneously it is called paroxysmal AF, when AF continues beyond 7 days, it is called persistent AF, when cardioversions of longstanding persistent AF are deemed unnecessary or have failed, it is called permanent AF. The designation of paroxysmal and persistent AF is not changed when the arrhythmia is terminated by pharmacological or electrical cardioversion.[1] However, above classification system is not ideal for several reasons. First, the categories of this classification are not mutually exclusive, and may differ within the same individual. Secondly, in daily clinical practice and in both hospital- and population-based studies, most often there is no continuous rhythm monitoring available and asymptomatic AF may be overlooked. Thirdly, the preferences of the individual having AF and the treating physician may influence the applied therapy and thereby the type of AF. This has led to the use of various classification systems in different studies.[4,9] In present study, we tried to use an intuitive classification system based on the availability of ECGs, with the AF without 2-year recurrence as AF once detected, and not found on subsequent ECGs within 2 years after AF detection, self-limiting AF as AF present on fewer than 90% of all available follow-up ECGs, and non-self-terminating AF as AF present on >90% of all follow-up ECGs. A third of the incident AF individuals had AF without 2-year recurrence, half of the individuals presented with self-terminating AF, and a minority of 18% had non-self-terminating AF as first presentation.

Clinical and biomarker predictors of specific atrial fibrillation types. Atrial fibrillation may progress from self-terminating to non-self-terminating forms, and relates to more cardiovascular morbidity and mortality,[11,20] whereby the rates of progression vary between 5 and 15% per year,[9–11]In hospital-based cohorts, a wide range of clinical predictors was found related to AF progression; advancing age, larger atrial size, heart failure valvular disease, hypertension, higher body mass

index, chronic obstructive pulmonary disease, and prior stroke.[10,11,20] However, it remains difficult to define the individual risk of non-self-terminating AF and AF progression. In PREVEND, we studied the clinical predictors of the individuals with different types of AF, and largely similar groups. Only distinct differences in age, male sex, anti-hypertensive treatment prescribed, BMI, heart rate, lipid-lowering treatment prescribed, MR-ANP, and eGFR ≤ 60 mL/min/1.73 m^2 were found as predictors of specific AF types.

In a recent analysis, comparing paroxysmal and non-paroxysmal AF in the community-based Women's Health Study differences were found in higher age and body mass index, but not in hypertension between both types of AF.[7]In an analysis of the aspirin-treated AF patients, included in hospital-based AF Clopidogrel Trial with Irbesartan for prevention of Vascular Events-Aspirin and Apixaban vs. acetylsalicylic acid to prevent stroke in AF patients who have failed or are unsuitable for vitamin K antagonist treatment trials, the clinical profile according to AF type was presented; and multiple differences were present. Patients with permanent AF were older, more men, and a greater cardiovascular disease burden.[8] Importantly, patients in those studies had AF at inclusion, whereas we studied the predictors of those at risk for a specific type of incident AF. Furthermore, the applied definitions were different, the cohort origin (hospital based vs. community based), and the selection of participants (AF patients vs. healthy population).

Genetic variants and specific atrial fibrillation types. We found a different distribution of risk alleles of three common AF-associated genetic variants for each AF type; all three associated with self-terminating AF, and not with AF without 2-year recurrence and non-self-terminating AF. Although it is not completely understood how these genetic variants increase the risk of (a specific type of) AF, the observed differences may support the idea that individuals may be susceptible to AF and even specific type of AF. The first genetic variant rs6666258 at chromosome 1q21 lies within a gene called *KCNN3* that encodes for a voltage-independent calcium-activated potassium channel.[21] In human and mouse cardiac repolarization models, KCNN3 channels are of importance during the late phase of cardiac action potential. In atrial myocytes of *KCNN3* knockout mice it has been observed that the action potential duration was prolonged, the number of early depolarizations was increased, and pacing-induced atrial arrhythmias were common.[22] The SNPs from each of the 10 AF susceptibility loci were identified by prior genome wide

association studies.[13] The second genetic variant rs6817105 at chromosome 4q25 lies near a gene called *PITX2* that encodes for the paired-like homeodomain transcription factor 2.[21]*PITX2c*$^{-/-}$predisposes mice to atrial arrhythmia.[23] Similarly, in human atrial tissue, *PITX2* expression levels were found ~2 times higher in the left atrium compared with the right atrium or the ventricles. *PITX2c* heterozygote mice had shorter atrial action potential durations compared with the wild type and were susceptible to AF induced by pacing, whereas no differences in cardiac morphology, including interstitial fibrosis and function, were observed.[24] The third genetic variant rs10821415 at chromosome 9q22 is located in an open reading frame C9orf3, also known as AP-O, encoding aminopeptidase O, which is expressed in the heart, and involved in cleavage of angiotensin subtypes.[25] No reports regarding its pathophysiological role in AF are available. The reported differences in genotypes found in those with self-terminating AF are intriguing, and suggest that there may be differences in pathophysiological pathways underlying the AF types. One may speculate that the genetic background is of relative more importance in those at risk for self-terminating AF, where the cardiovascular risk factors and disease are of relative more importance in those at risk for non-self-terminating AF. However, further studies are warranted to uncover the genetic contribution of specific AF types.

Strengths and limitations. Strengths of our analysis are the well-characterized cohort, the prospective design, long-term follow-up, and rigorous ascertainment of AF. The study also had potential limitations largely because of the observational study design. First, our AF ascertainment strategy may have been insensitive to asymptomatic paroxysms of AF, so asymptomatic AF may have been overlooked. Secondly, the number of ECGs per individual was highly variable especially in those with the minimum number of three ECGs in 2 years. In total, 61 (19%) individuals with incident AF had <5 ECGs; therefore, misclassification may have occurred. However, the total number of ECGs made in PREVEND participants was over 40,000. Thirdly, we were not informed about the treatment of AF, which may have impact the classification of AF. Information on rate- or rhythm control treatment was not available. Also, it is plausible that we may have been underpowered to study small size effects between the AF types, since numbers of individuals in each AF type were modest. Therefore, joint analyses in genetic consortia are necessary to increase statistical power, and extent present findings. Fourthly, since the majority

of individuals included were of European ancestry, results cannot be extended to other ethnicities. Finally, by design, our cohort was enriched for microalbuminuria, and although we adjusted for microalbuminuria in all regression analysis, we cannot exclude the possibility that it has impacted our results.

Conclusions

We found clinical, biomarker, and genetic predictors of specific types of incident AF in a community-based cohort. The genetic background seems to play a more important role than modifiable risk factors in self-terminating AF.

Funding

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Conflict of interest: none declared.

SUPPLEMENTARY DATA

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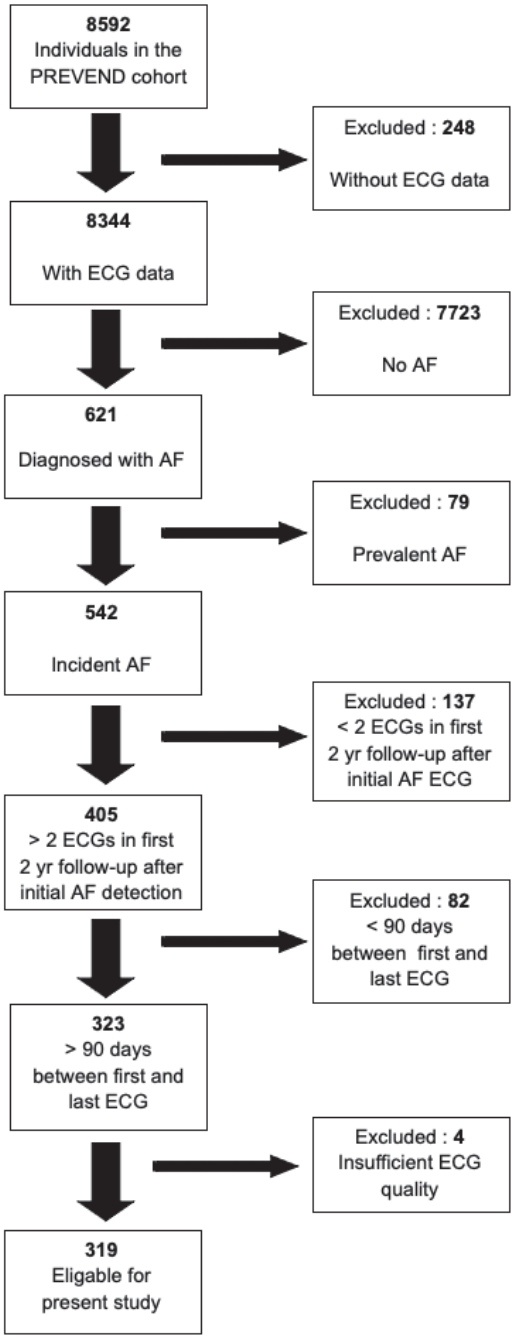
Supplementary Figure 1 Flow chart of selection process of eligible individuals for present analyses.

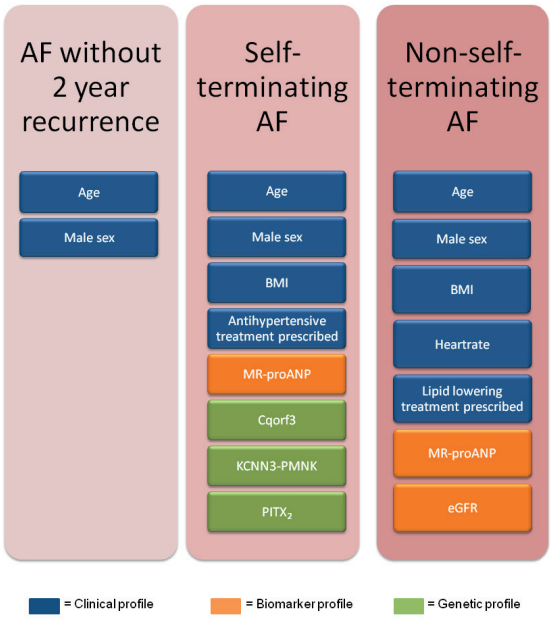
Supplementary Figure 2 Overall view of similarities and differences in predictors of AF without 2-year recurrence, self-terminating and non-self-terminating AF. The illustrated predictors were explained in more detail in table 2 and table 3.

Supplementary Table 1 Additional information regarding top common genetic variants found in the genome wide association study and on the Illumina HumanCytoSNP-12 chip.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Table 1. Additional information regarding top common genetic variants found in the genome wide association study and on the Illumina HumanCytoSNP-12 chip.

AF SNP	Imputation quality metric	Chromosome	Closest gene	SNP location relative to closest gene	Major allele	Minor allele	Minor allele frequency
rs6666258	0.952	1q21	*KCNN3-PMVK*	Intronic	G	C	0.32
rs3903239	0.994	1q24	*PRRX1*	Upstream	A	G	0.43
rs6817105	0.999	4q25	*PITX2*	Upstream	T	C	0.11
rs2040862	0.961	5q31	*WNT8A*	Intronic	C	T	0.18
rs3807989	1.000	7q31	*CAV1*	Intronic	G	A	0.44
rs10821415	0.998	9q22	*C9orf3*	Intronic	C	A	0.43
rs10824026	0.996	10q22	*SYNPO2L*	Upstream	A	G	0.16
rs1152591	0.986	14q23	*SYNE2*	Intronic	A	G	0.49
rs7164883	0.996	15q24	*HCN4*	Intronic	A	G	0.15
rs2106261	1.000	16q22	*ZFHX3*	Intronic	C	T	0.19

SNP = single nucleotide polymorphism

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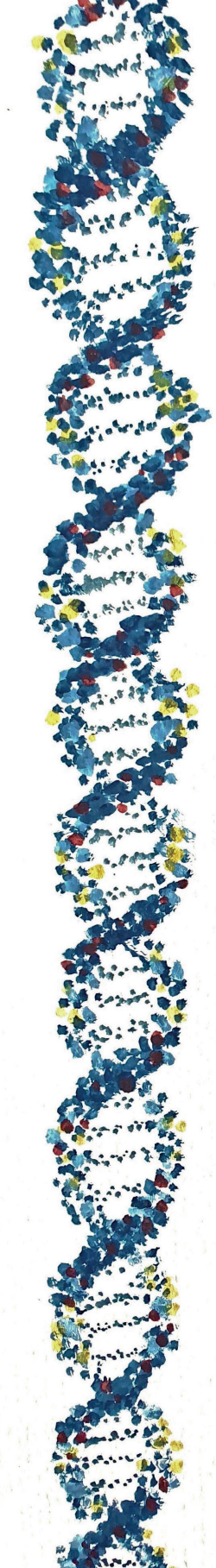

Genetically-determined body mass index and the risk of atrial fibrillation progression in men and women

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CHAPTER 5

Abstract

Aims. Limited causal evidence is available on the relationship between body mass index (BMI) and atrial fibrillation (AF) progression. Sex differences have been noted and may be relevant for AF progression. We investigated the association between the BMI Genetic Risk Score (GRS) and AF progression in men and women of the Groningen Genetic Atrial Fibrillation (GGAF) cohort.

Methods and results. The GGAF cohort (n=2207) is a composite of 5 prospective cohorts with individuals of European ancestry. AF patients with genetic information, with at least 12 months follow-up and AF progression data were included. AF progression was defined as progression from paroxysmal to persistent/permanent AF, or persistent to permanent AF. A BMI GRS was constructed of genetic variants associated with BMI. Univariate and multivariate Cox proportional hazard regression analyses were performed in the total population and in men and women, separately. During a median follow-up of 34 [interquartile range 19-48] months 630 AF patients (mean age 62±11, 36% women, BMI of 28±5) were analyzed, and men and women developed similar AF progression rates (respectively 6.5% versus 6.1%). The BMI GRS was not associated with AF progression either as a continuous variable or in tertiles in the overall population. However, AF progression was associated with the tertile of the highest BMI GRS in women (n=225), also after multivariable adjustments of clinical risk factors (Hazard ratio 2.611 (95% confidence interval 1.151–5.924) p=0.022).

Conclusions. Genetically-determined BMI is only associated with women at risk of AF progression. The results may be supporting evidence for a causal link between observed BMI and AF progression in women. We emphasize the need for further investigation of genetically-determined BMI and observed BMI to optimize AF management in women with increased risk for AF progression.

Introduction

Obesity and atrial fibrillation (AF) are both emerging global epidemics and causing a growing economic burden.[1] AF is associated with an increased risk of cardiovascular diseases like heart failure, stroke, dementia and death.[1] When AF progresses from paroxysmal / self-limiting to more sustained / non-self-limiting forms of AF, more cardiovascular events occur.[2] In an attempt to unravel the development of AF progression, several risk factors were identified. Higher observed body mass index (BMI) has been associated with an increased risk of AF. Previously, the association between observed BMI and the risk of incident AF differed between men and women.[3,4] Albeit contradictory findings, it is possible that sex is also a modifying factor in the relation between increased observed BMI and development or AF progression.

An increased observed BMI is one of the risk factors of AF progression.[5] One of the explanations is that an increased observed BMI causes atrial remodeling such as atrial stretch, and diastolic impairment.[6] These effects can be direct consequences of an increased observed BMI or indirectly via e.g. inflammation, sympathetic activation or associated comorbidities, such as hypertension and obstructive sleep apnea, that can induce atrial stretch.[6] However, evidence that an increased observed BMI causes AF progression is still limited.

Genetic variants associated with observed BMI could help to further elucidate its role in AF progression. If an association between genetic variants of BMI and AF progression exists, it may support the notion of a causal link. Therefore, we investigate the association between genetically-determined BMI and AF progression in men and women with short-lasting AF in the Groningen Genetic Atrial fibrillation (GGAF) cohort.

Methods

Study population. Three hospital-based, prospective, AF registries (AF-RISK study, Biomarker-AF study and Young-AF study) recruited in the University Medical Center Groningen and two age- and sex-matched reference cohorts (Prevention of Renal and Vascular End-stage Disease (PREVEND) study and the Glycometabolic Intervention as adjunct to primary Percutaneous coronary intervention in ST elevation myocardial infarction (GIPS) III) are enclosed in the GGAF cohort (n=2207).

The study protocols of the above-mentioned studies are in line with the Declaration of Helsinki, were approved by the local institutional review board (Medisch Ethische Toetsingscommissie Universitair Medisch Centrum Groningen), and written informed consent was received of all included individuals. A detailed description of participating cohorts was previously published.[7-9] In our study we selected AF patients of European descent with prospectively registered AF progression, successful genotyping and a follow-up of at least 12 months and with a maximum of 60 months in the AF-RISK study, Biomarker-AF study and Young-AF study. Individuals without AF at baseline, permanent AF or missing data at baseline were excluded (**Figure 1**).

Genotyping, quality control and imputation. The GGAF cohort was genotyped as part of the Broad AF Study (Broad AF) at the Broad Institute.[9] The Infinium PsychArray-24 v1.2 Bead Chip was used and GenomeStudio v1.6.2.2 and Birdseed v1.33 were used to call common variants (≥ 0.5% minor allele frequency (MAF)). In brief, pre-imputation quality control filtering of samples and variants was conducted. Samples were filtered based on completeness (>97% sample call rate), heterozygosity (>± 0.2), genetic ancestry outliers and related first- and second-degree AF patients (Identity By Descent < 0.24) to avoid bias. Variants were excluded when clear deviations from the Hardy-Weinberg proportions (HWE) (P-value in references > 1 x 10 -6) existed, high degree of missingness (<0.98) or low minor allele frequency (<0.005). Imputation was performed with HRC reference v1.1 panel on the Michigan Imputation Server v1.0.1 using Minimac. Further detailed information is described previously.[9] Only AF patients with sufficient quality control and imputation were included in current analyses.

BMI Genetic Risk Score. A Genetic Risk Score was constructed of 941 genetic variants associated with BMI available from previous meta-analysis of Yengo and colleagues,[10] and calculated for every AF patient by summing the dosage of each BMI risk allele weighted by the natural logarithm of the relative risk for each genetic variant. Thus, the effect size of every single genetic variant associated with BMI was summed into a Genetic Risk Score value for each AF patient, conditional of the relative risk for each genetic variant. The higher the BMI Genetic Risk Score in the AF patient, the higher the effect size of genetic variants associated with BMI present in the genotyped data of the AF patient. The Genetic Risk Score was constructed using the open source whole genome association data analyses toolset PLINK.[11]

Definitions. The AF diagnosis was based on the documentation of a 12-lead electrocardiogram (ECG), and was confirmed by a cardiologist. The AF type at baseline was determined by the information documented in the medical records. In line with the 2016 European Society of Cardiology AF guidelines the following types are distinguished: paroxysmal AF (≤ 7 consecutive days of AF), persistent AF (> 7 consecutive days of non-self-terminating AF) and permanent AF (conversion to sinus rhythm failed and cannot be restored and/or is no longer pursued by the treating cardiologist).[12] Heart failure was defined as the presence of New York Heart Association functional Class II or III, previous hospitalization for heart failure or left ventricular ejection fraction (LVEF) ≤ 45%. Hypertension was determined by a systolic blood pressure > 140 mmHg, diastolic blood pressure > 90mmHg, or by use of antihypertensive drugs. By dividing the weight to height squared (kg/m^2) observed BMI was calculated. Overweight was defined as observed BMI ≥ 25 kg/m^2, and obesity was defined as observed BMI ≥ 30 kg/m^2. Diabetes was defined as type I or type II diabetes with use of anti-diabetic drugs. Peripheral artery disease was defined by a clinical diagnosis of a vascular specialist or observed with Doppler ultrasonography. The clinical diagnosis of stroke or transient ischemic attack (TIA) and chronic obstructive pulmonary disease (COPD), and myocardial infarction were obtained from the medical records.

Follow-up data of AF progression. AF progression was defined as paroxysmal AF that developed into persistent or permanent AF, or persistent AF that developed into permanent AF. The time to AF progression was the time to development of persistent AF or electrical cardioversion or permanent AF. Follow-up visits were planned 3 monthly during the first year, thereafter yearly. If AF patients visited the treating cardiologist in the meantime, information from the medical records was collected and change of AF type was noted. Treatment after inclusion in the registry was not specified in the study protocols and led to discretion of the treating physician. The follow-up period started at the inclusion date (first inclusion November 2009) and was continued until the most sustained type of AF progression, the last contact date (last patient December 2017), or until death, with a maximum follow-up duration of 60 months.

Statistical analyses. The description of the statistics of the study population and separate analyses were presented as mean (standard deviation (SD)) or median [interquartile range] for continuous variables, depending on the normality of data.

Categorical data were presented as percentages and numbers. Chi-squared test was performed for categorical data and a t-test for continuous data. Cox proportional hazard regression analyses were used to find determinants of AF progression. In case of no progression, time until death or last contact date was used as time-to-event. Principal components of the genotype matrix of GGAF cohort were calculated to correct for population stratification. Results are given as hazard ratios (HR) with 95% confidence interval (CI). Analyses were performed for men and women separately. Univariate Cox proportional hazard regression was used to find determinants of AF progression. The multivariable Cox proportional hazard regression was adjusted for age, sex and 2 principal components determined by a scree plot (**Supplementary Figure 1**), AF type at baseline, time of follow-up, hypertension, age > 75 years, TIA or stroke, COPD, heart failure, diabetes, myocardial infarction, and peripheral vascular disease.[13,14] Only variables with $p<0.05$ were considered statistically significant. Schoenfeld residuals were evaluated to test the proportional hazard assumption. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA), R (version 3.1.6; R Foundation for Statistical Computing, Vienna, Austria) and PLINK.[11]

Results

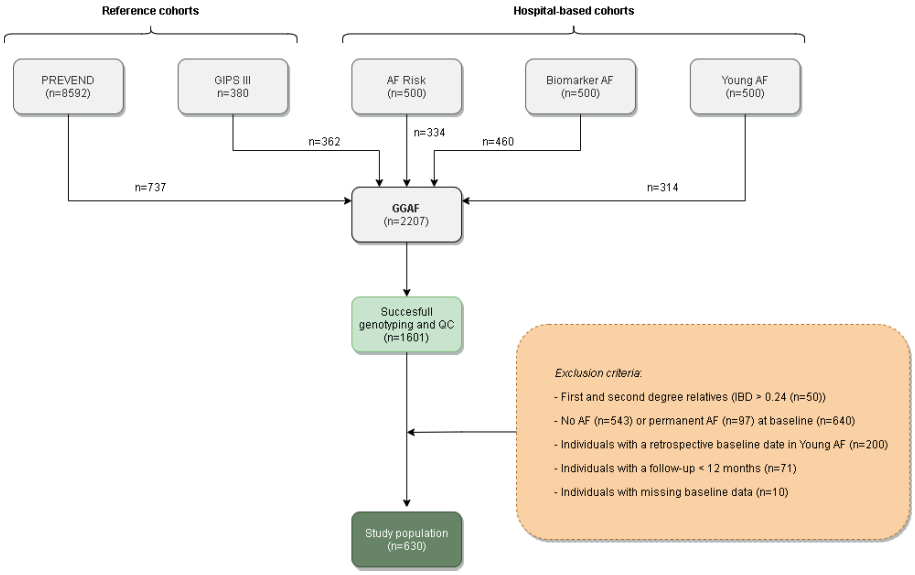
Patient characteristics. Of the 2207 individuals included in the GGAF cohort, we included 630 AF patients with short-lasting paroxysmal or persistent AF in present analysis (**Figure 1**). At baseline the mean age was 62±11 years, 225 (36%) were women and 496 (79%) AF patients were diagnosed with paroxysmal AF and 134 (21%) AF patients were diagnosed with persistent AF. AF patients had a observed BMI of 28 ± 5, and 180 (29%) AF patients were obese. Two-hundred-twenty-five (36%) women with a mean age of 63 ± 11 years, and 405 (64%) men with mean age of 61 ± 11 years were included. Obesity was not significantly more prevalent in women compared to men (respectively 26% versus 33%, $p = 0.060$). However, men were more often overweight than women (respectively 73% versus 64%, $p = 0.035$) (**Table 1, Figure 2**).

Table 1. Characteristics of the AF patients in the GGAF cohort.

	Study population (n=634)	**Women n=225 (35.7%)**	**Men n=405 (64.3%)**
Genetic Risk Score			
BMI Genetic Risk Score	1.766 ± 0.525	1.749 ± 0.528	1.777 ± 0.524
Clinical characteristics			
Age (years)	62 ± 11	63 ± 11	61 ± 11
BMI (kg/m²)	28 ± 5	28 ± 5	28 ± 4
Obesity	180 (28.6)	75 (33.3)	105 (25.9)
Overweight	440 (69.5)	144 (64.0)	296 (72.6)
Hypertension	333 (52.9)	127 (56.4)	206 (50.9)
Age > 75 years	63 (10.0)	25 (11.0)	38 (9.0)
TIA or stroke	63 (10.0)	24 (10.7)	39 (9.6)
COPD	49 (7.8)	14 (6.2)	35 (8.6)
Heart failure	101 (16.0)	30 (13.3)	71 (17.5)
Diabetes Mellitus	68 (10.8)	26 (11.6)	42 (10.4)
Myocardial infarction	65 (10.3)	19 (8.4)	46 (11.4)
Peripheral Artery disease	38 (6.0)	12 (5.3)	26 (6.4)

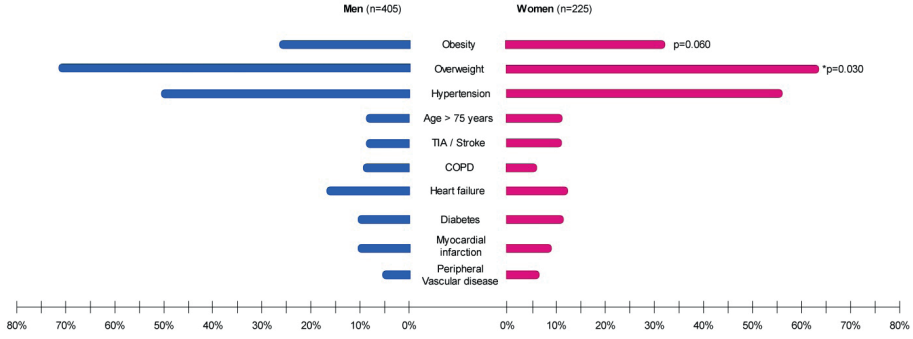
Values are mean ± SD or numbers (percentages). Abbreviations: AF = Atrial Fibrillation, BMI = Body Mass Index, COPD = Chronic Obstructive Pulmonary Disease, SD = standard deviation, TIA = Transient Ischemic Attack.

▼**Figure 1.** Flowchart of the selected study population.



Abbreviations: AF = Atrial fibrillation, AF Risk = AF Risk study, Biomarker AF = Biomarker AF study, GGAF = Groningen Genetic Atrial Fibrillation study, GIPS III = Glycometabolic Intervention as adjunct to primary Percutaneous coronary intervention in ST elevation myocardial infarction III study, IBD = Identity By Descent, PREVEND = Prevention of Renal and Vascular End-stage Disease study, QC = Quality control, Young AF= Young AF study.

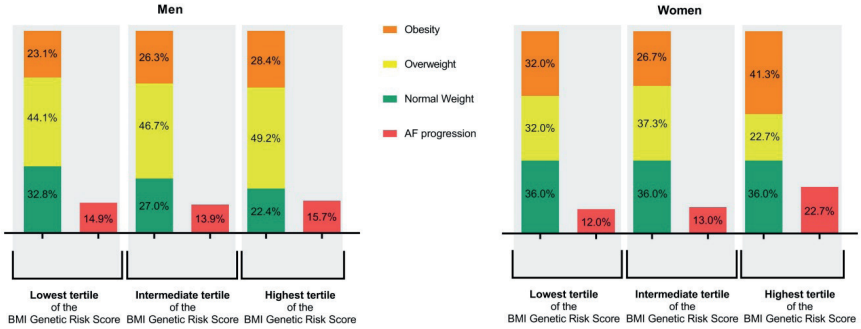
▼**Figure 2.** Sex-differences in clinical characteristics.



Results of Chi-squared test p < 0.1 are given. The asterisk (*) indicates statistical significance (p < 0.05). Abbreviations: COPD = Chronic Obstructive Pulmonary Disease, TIA = Transient Ischemic Attack.

BMI Genetic Risk Score. Women with the highest BMI Genetic Risk Score were not significantly more obese than women who did not have the highest BMI Genetic Risk Score (respectively 41% versus 29%, p = 0.099). Likewise, men with the highest BMI Genetic Risk Score were not significantly more obese than men who did not have the highest BMI Genetic Risk Score (respectively 28% versus 25%, p= 0.506) (**Figure 3, Supplementary Table 1, Supplementary Table 2**).

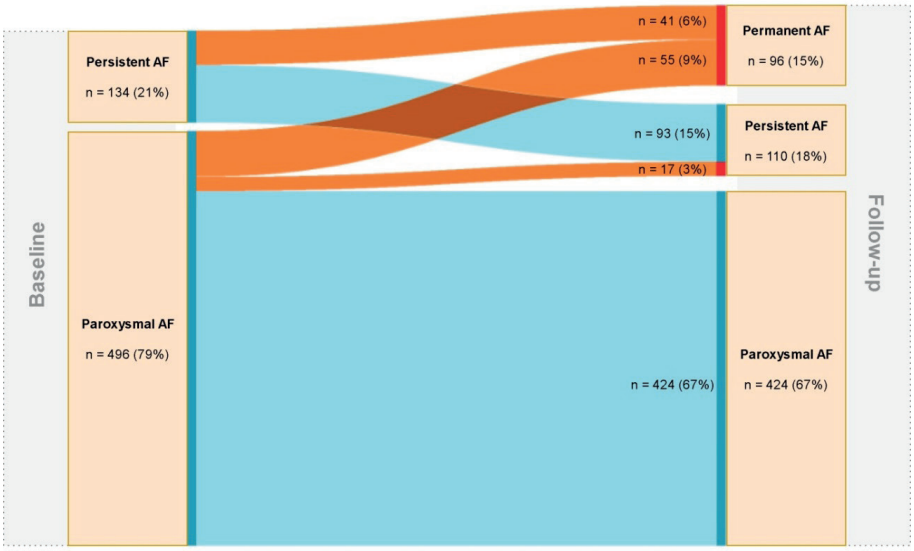
▼**Figure 3.** Characteristics of weight and AF progression in BMI Genetic Risk Score tertiles of both men and women.



In this figure the distribution of obesity, overweight and normal weight and AF progression in the BMI Genetic Risk tertiles are displayed in percentages. Abbreviations: AF = Atrial Fibrillation, BMI = Body Mass Index.

Follow up. During a median follow-up period of 34 [Interquartile range 19 – 48] months 113 (18%) AF patient developed AF progression. The development of AF to either persistent AF or permanent AF resulted in a total AF progression rate of 6.4% per patient year, and men and women had no significant different yearly AF progression rates (respectively 6.5% versus 6.1%) (**Figure 4**).

▼**Figure 4.** Overview of AF progression in the GGAF cohort.



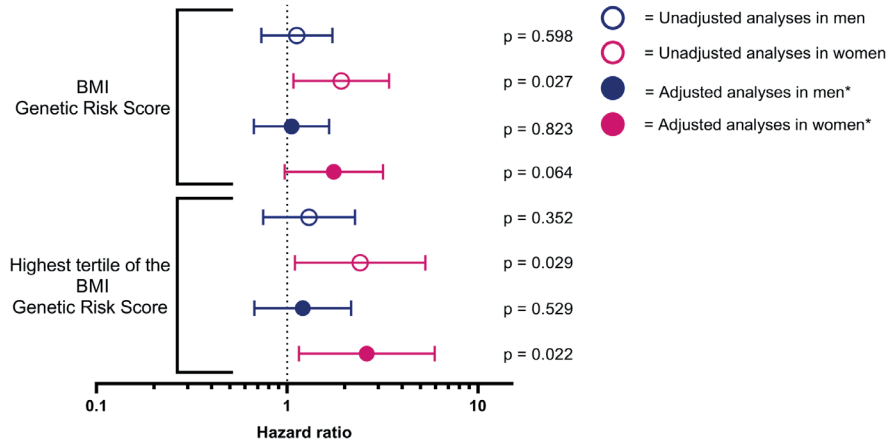
On the left side of figure 2 AF type and numbers (%) at baseline are shown. On the right side of figure 2 AF type and numbers (%) at follow-up are shown. The orange lines represent the transition to AF progression, the blue lines represent no progression. Abbreviations: AF = Atrial Fibrillation.

BMI Genetic Risk Score and AF progression. In univariate Cox proportional hazard regression analyses the BMI Genetic Risk Score as a continuous variable was not associated with AF progression (Hazard ratio (HR) 1.342 (95% confidence interval (CI) 0.953 - 1.891); p = 0.092). Subsequently, the BMI Genetic Risk Score was divided into tertiles, quartiles and quintiles to observe the trend and reliability of the data when age- and sex adjustments were performed. However, p-values were not larger than p = 0.1, even when correcting for age and sex (**Supplementary Table 3**).

BMI Genetic Risk Score and AF progression in men and women separately. Univariate Cox proportional hazard regression analyses of the tertile with the highest BMI Genetic Risk Score were significantly associated with AF progression in women (respectively HR 2.409 (95% CI 1.097 – 5.293) p = 0.029). In contrast, separate univariate Cox proportional hazard regression analyses of the tertile with the highest BMI Genetic Risk Score were not significantly associated with AF progression in men (HR 1.301 (95% CI 0.748 – 2.266) p = 0.352). In multivariate analyses the significant association between the tertile with highest BMI Genetic

Risk Score and AF progression remained statistically significant in women, when adjusted for principal components, age, AF type at baseline, follow-up duration, observed BMI, hypertension, age > 75 years, TIA or stroke, COPD, heart failure, and peripheral vascular disease (HR 2.611 (95% CI 1.151 – 5.924) p = 0.022) (**Figure 5**). Addition of antiarrhythmic medication, statins and pulmonary vein isolation in the multivariate analyses did not change our findings (**Supplementary Table 4, Supplementary Table 5**).

▼**Figure 5.** The association between the BMI Genetic Risk Score and AF progression in men and women.



Analyses are adjusted for principal components, age at inclusion, AF type at baseline, follow-up duration, BMI, hypertension, age > 75 years, transient ischemic attack or stroke, chronic obstructive pulmonary disease, heart failure, and peripheral vascular disease. Abbreviations: BMI = Body Mass Index.

Discussion

The main findings of our analysis were: 1) Men and women with short-lasting AF had similar AF progression rates in the GGAF cohort, 2) Genetically-determined BMI was associated with AF progression in women and not in men, 3) Our results may be supporting evidence that, in women, observed BMI may cause AF progression.

Genetically-determined BMI and observed BMI in the GGAF population. Observed BMI ≥ 30 kg/m^2 increases the risk by approximately 50% for developing AF.[15] On average, the AF patients of the GGAF cohort have an increased observed BMI, which is typical for individuals with AF.[2,13] The observed BMI distribution

in our study is similar to the observed BMI of European AF patients. In the European heart Survey several clinical types of AF were investigated in 5333 AF patients from 35 European countries.[16] The mean observed BMI of the included individuals with paroxysmal AF and persistent AF of European Heart Survey is comparable to our cohort (respectively mean (SD) observed BMI of 28±9 versus 28±5). Moreover, our cohort is similar to the AF population of the hospital-based Standard versus Atrial Fibrillation specific managemenT study (SAFETY), where more women presented obese and men were more overweight.[17] However, genetic predisposition to a high BMI may not be reflected in the observed BMI. More than half the individuals in the highest tertiles of the BMI Genetic Risk Score have an observed BMI ≥ 30 kg/m^2 (**Figure 3**). Still, genetically determined BMI may influence the development of AF progression.

Genetically-determined BMI and AF progression. In a recent systematic review with similar AF progression definitions, 47 studies with over 27 000 AF patients were included and the AF progression rate ranged from 0.8 to 35.6% per patient year. They showed a pooled AF progression rate of 8.2% per patient years in European countries.[13] AF progression in the GGAF cohort was comparable with 6.4% per patient years. The slightly lower AF progression rate in the GGAF cohort is potentially due to the mean age below 65 years. However, the total AF population did not show an association between genetically-determined BMI and AF progression. Absence of an association may be caused by lack of power, but may also be explained by potential differences in underlying mechanisms how obesity causes incident AF versus AF progression. It is possible that genetically-determined BMI is indeed causal to incident AF, but more a mediating factor regarding the progression to non-self-limiting forms of AF.

Genetically-determined BMI in women. In our cohort separate analyses for men and women showed that women with the highest BMI Genetic Risk were at increased risk of AF progression. Previously, the Woman's Health Study showed that an increased observed BMI may be of importance in women with AF progression. Women who developed AF progression were more obese compared to women who maintained paroxysmal AF two years after AF diagnosis.[18] In addition, obese women had more left atrial dilatation. In the FAT associated CardiOvasculaR dysfunction (FATCOR) study clinical and echocardiographic data from 581 individuals without cardiovascular disease and observed BMI > 27 kg/m^2 were analyzed.[19] Women had

a higher prevalence of left atrial dilatation than men, which suggests that there may be sex-specific differences in atrial remodeling and underlying comorbidities that eventually could results in AF development or progression.[20] However, the role of obesity in the development of AF progression in women remains largely uninvestigated.

Clinical perspective. Unveiling the impact of genetically-determined BMI and sex on AF progression may partly explain the existence of inter-individual differences in risk of AF progression. Our results using genetically-determined BMI as proxy for observed BMI / obesity, suggest that observed BMI may be of more importance in women than men regarding risk of AF progression. In the current European guidelines weight loss, combined with risk factor management is recommended.[12] Weight control may minimize their risk of AF progression more in women than in men. However, longitudinal observations of body mass index were not available in our cohort and clinical trials are warranted.

Strengths and limitations. Strengths of our study are the use of both genetic and phenotypic information to unravel the potential causal role of observed BMI to AF progression. AF patients of the GGAF cohort are deeply phenotyped, and AF ascertainment and AF progression types were defined according to the European guidelines.[12] Compared to prior studies of genetically-determined BMI and incident AF, the BMI Genetic Risk Score in our study has even more predictive power.[10] The increased amount of discovered associated genetic variants improves determination of genetic predisposition. Unfortunately, our sample size was not sufficient to perform Mendelian randomization analyses. However, we hope our results will encourage researchers in the field of AF to collect large scale data of genetics and AF progression and enable Mendelian randomization analyses in the future. Albeit that more information is needed to explain the effects of genetics on AF progression, clinicians and researchers may consider the findings of our study a pilot for research in the field of genetics and AF progression. Additionally, several other limitations need to be considered. Firstly, we did not perform continuous rhythm monitoring, and relied on intermittent ECGs to diagnose AF progression, this may have led to misclassification. Asymptomatic AF episodes may have been missed and concealed the time of AF progression. Secondly, one component of obesity is genetics, another component of obesity is lifestyle. We stress that important lifestyle risk factors of AF progression, such as physical activity,

fitness and alcohol intake were not investigated in our cohort. Adjustments for all environmental confounders of observed BMI could not possibly be made in our analyses. However, despite the fact that the used genetic variants are estimated to explain only 6% of observed BMI variance,[10] the association between genetically-determined BMI and AF progression maintained in women. Thirdly, heritability and genetic effects of BMI genetic variants could potentially be stronger in women, like genetic variants associated with waist-hip-ratio.[21] A stronger genetic effect of BMI genetic variants in women could have caused the significant association between BMI Genetic Risk Score and AF progression in women. Fourthly, by all means, the results should be interpreted with caution since a relatively small sample size was used, and the men-women ratio in this study (2:1) limits sex-specific analyses. Additionally, albeit that the use of antiarrhythmic medication, statins or pulmonary vein isolation did not change our findings fundamentally, no data of change in medication during follow-up was available. Furthermore other confounding factors may play a role. Further investigation of BMI genetic risk, AF progression and confounding factors should be conducted. Moreover, we would like to stress that it is important to replicate our results in independent cohorts across race-ethnicity to ensure the reliability of our findings. Finally, our results are not generalizable to all populations, only to individuals with short-lasting AF from European ancestry.

Conclusions

In the GGAF cohort men and women with short-lasting AF had similar AF progression rates. However, genetically-determined BMI was only associated with women at risk of AF progression, independent from clinical risk factors, including observed BMI. The association between genetically-determined BMI and AF progression was not present in men. Our study may provide supporting evidence that observed BMI may be causally linked to AF progression in women. However, the results should be interpreted with caution. The causality of observed BMI to AF progression and optimization of AF management should be investigated in other deep phenotyped AF populations and clinical trials considering genetically-determined BMI and intervention of the observed BMI.

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SUPPLEMENTARY DATA

Supporting information

Figure 1. Scree plot of 10 principal components in the GGAF cohort.

Table 1. Baseline characteristics of women divided into tertiles of the BMI Genetic Risk Score.

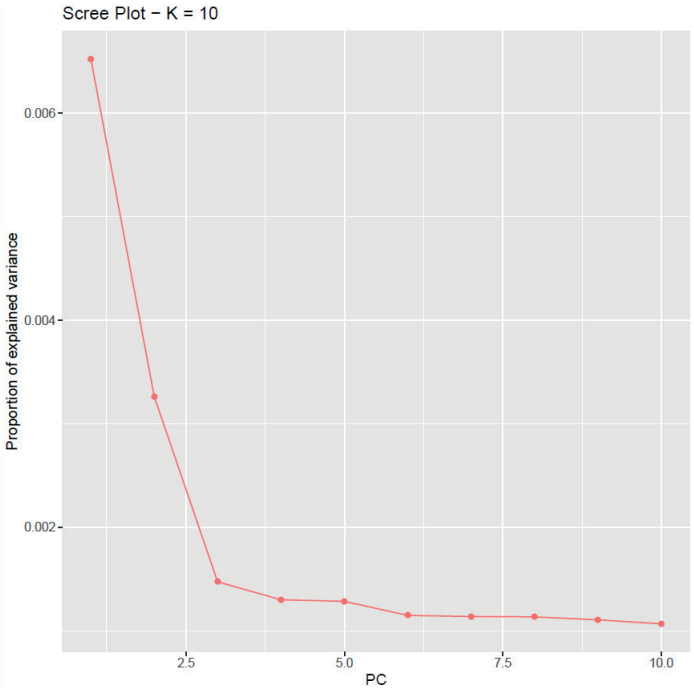
Table 2. Baseline characteristics of men divided into tertiles of the BMI Genetic Risk Score.

Table 3. Cox regression analyses with BMI Genetic Risk Score and AF progression.

Table 4. Medication at baseline.

Table 5. Multivariate regression in women.

▼**Supplementary Figure 1.** Scree plot of 10 principal components in the GGAF cohort.



Ten principal components in the GGAF cohort are shown in the scree plot. The point where the slope of the scree plot is leveling off indicates the number of the eigenvalue factors that should be retained. Abbreviations: PC = Principal components.

Supplementary Table 1. Baseline characteristics of women divided into tertiles of the BMI Genetic Risk Score.

	Total study population (n=225)	Lowest tertile of BMI GRS (n=75)	Intermediate tertile of BMI GRS (n=75)	Highest tertile of BMI GRS
Genetic Risk Score				
BMI Genetic Risk Score	1.749 ± 0.528	1.189 ± 0.280	1.721 ± 0.147	2.337 ± 0.274
Clinical characteristics				
Age (years)	63.4 ± 11.4	63.3 ± 12.0	63.5 ± 11.5	63.4 ± 10.6
BMI (kg/m^2)	28 ± 5	28 ± 6	27 ± 5	28 ± 5
Obesity	75 (33.3)	24 (32.0)	20 (26.7)	31 (41.3)
Overweight	144 (64.0)	48 (64.0)	48 (64.0)	48 (64.0)
Hypertension	127 (56.4)	46 (61.3)	41 (54.7)	40 (53.3)
TIA or stroke	24 (10.7)	10 (13.3)	5 (6.7)	9 (12.0)
COPD	14 (6.2)	4 (5.3)	3 (4.0)	7 (9.3)
Heart failure	30 (13.3)	9 (12.0)	4 (5.3)	17 (22.7)
Diabetes	26 (11.6)	8 (10.7)	8 (10.7)	10 (13.3)
Myocardial infarction	19 (8.4)	9 (12.0)	3,9 (5.3)	6 (8.0)
Peripheral artery disease	12 (5.3)	5 (6.7)	2 (2.7)	5 (6.7)

Values are mean (SD), numbers (percentages, median for categorical data and (interquartile range) for continuous variables. Abbreviations: AF = Atrial Fibrillation, BMI = Body Mass Index, COPD = Chronic Obstructive Pulmonary Disease, GRS= Genetic Risk Score, SD = standard deviation, TIA = Transient Ischemic Attack.

Supplementary Table 2. Baseline characteristics of men divided into tertiles of the BMI Genetic Risk Score.

	Total study population (n=405)	Lowest tertile of BMI GRS (n=134)	Intermediate tertile of BMI GRS (n=137)	Highest tertile of BMI GRS
Genetic Risk Score				
BMI Genetic Risk Score	1.777 ± 0.524	1.210 ± 0.275	1.774 ± 0.134	2.347 ± 0.297
Clinical characteristics				
Age (years)	60.7 ± 11.2	60.1 ± 12.8	61.3 ± 10.1	60.7 ± 10.6
BMI (kg/m^2)	28 ± 4	27 ± 5	28 ± 4	28 ± 5
Obesity	105 (25.9)	31 (23.1)	36 (26.3)	38 (28.4)
Overweight	294 (72.6)	90 (67.2)	100 (73.0)	104 (77.6)
Hypertension	206 (50.9)	62 (46.3)	67 (48.9)	77 (57.5)
TIA or stroke	39 (9.6)	13 (9.7)	9 (6.6)	17 (12.7)
COPD	35 (8.6)	12 (9.0)	10 (7.3)	13 (9.7)
Heart failure	71 (17.5)	26 (19.4)	22 (16.1)	23 (17.2)
Diabetes	42 (10.4)	10 (7.5)	14 (10.2)	18 (13.4)
Myocardial infarction	46 (11.4)	18 (13.4)	16 (11.7)	12 (9.0)
Peripheral artery disease	26 (6.4)	15 (11.2)	5 (3.7)	6 (4.5)

Values are mean (SD) or numbers (percentages) median for categorical data and (interquartile range) for continuous variables. Abbreviations: AF = Atrial Fibrillation, BMI = Body Mass Index, COPD = Chronic Obstructive Pulmonary Disease , GRS= Genetic Risk Score, SD = standard deviation, TIA = Transient Ischemic Attack.

Supplementary Table 3. Cox regression analyses with BMI Genetic Risk Score and AF progression.

Characteristics	Hazard ratio	95% Confidence Interval	P-value
BMI Genetic Risk Score	1.354	(0.959 – 1.912)	0.085
Tertile 1 of the BMI Genetic Risk Score	Reference	Reference	Reference
Tertile 2 of the BMI Genetic Risk Score	1.097	(0.6741 – 1.785)	0.710
Tertile 3 of the BMI Genetic Risk Score	1.555	(0.9897 – 2.443)	0.056
Quartile 1 of the BMI Genetic Risk Score	Reference	Reference	Reference
Quartile 2 of the BMI Genetic Risk Score	1.451	(0.827 – 2.544)	0.194
Quartile 3 of the BMI Genetic Risk Score	1.372	(0.779 – 2.415)	0.274
Quartile 4 of the BMI Genetic Risk Score	1.693	(0.986 – 2.908)	0.057
Quintile 1 of the BMI Genetic Risk Score	Reference	Reference	Reference
Quintile 2 of the BMI Genetic Risk Score	1.538	(0.817 – 2.896)	0.183
Quintile 3 of the BMI Genetic Risk Score	1.511	(0.798 – 2.861)	0.205
Quintile 4 of the BMI Genetic Risk Score	1.395	(0.733 – 2.657)	0.311
Quintile 5 of the BMI Genetic Risk Score	1.749	(0.946 – 3.233)	0.075
Genetic Risk Score adjusted for age and sex			
BMI Genetic Risk Score	1.309	(0.925 – 1.851)	0.129
Tertile 1 of the BMI Genetic Risk Score	Reference	Reference	Reference
Tertile 2 of the BMI Genetic Risk Score	1.072	(0.658 – 1.746)	0.780
Tertile 3 of the BMI Genetic Risk Score	1.523	(0.968 – 2.396)	0.069
Quartile 1 of the BMI Genetic Risk Score	Reference	Reference	Reference
Quartile 2 of the BMI Genetic Risk Score	1.380	(0.786 – 2.423)	0.263
Quartile 3 of the BMI Genetic Risk Score	1.281	(0.726 – 2.261)	0.392
Quartile 4 of the BMI Genetic Risk Score	1.654	(0.962 – 2.844)	0.067
Quintile 1 of the BMI Genetic Risk Score	Reference	Reference	Reference
Quintile 2 of the BMI Genetic Risk Score	1.461	(0.776 – 2.751)	0.241
Quintile 3 of the BMI Genetic Risk Score	1.412	(0.745 – 2.674)	0.291
Quintile 4 of the BMI Genetic Risk Score	1.357	(0.712 – 2.588)	0.354
Quintile 5 of the BMI Genetic Risk Score	1.654	(0.916 – 3.131)	0.093

Data of Cox regression of the tertiles, quartiles and quintiles of the BMI Genetic Risk Score and AF progression are shown, where the ascending numbers are coherent with increasing BMI Genetic Risk Score. Abbreviations: AF = Atrial Fibrillation, BMI = Body Mass Index.

Supplementary Table 4. Medication at baseline.

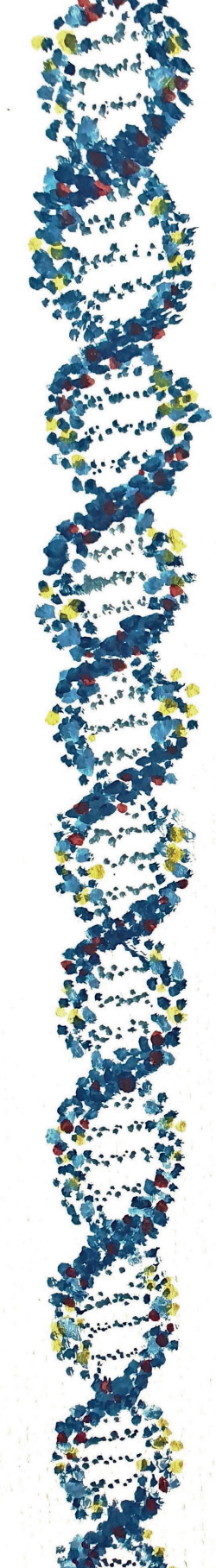
	All n=630	Men n=405	Women n=225	p-value
Use of class I antiarrhythmic medication	85 (13.5)	59 (14.6)	26 (11.6)	0.348
Use of beta blockers	388 (62.0)	238 (58.9)	150 (66.7)	0.067
Use of class III antiarrhythmic medication	98 (15.6)	66 (16.3)	32 (14.2)	0.566
Use of calcium antagonists	159 (25.2)	102 (25.2)	57 (25.4)	1.000
Use of statins	241 (38.3)	162 (40.0)	79 (35.1)	0.261
Pulmonary vein ablation	65 (10.4)	49 (12.2)	16 (7.21)	0.070

Values are numbers(percentages). Class I antiarrhythmic medication includes flecainide, propafenone, disopyramide, ritmoforine. Class III antiarrhythmic medication includes sotalol (dosage more than 160mg per day) and amiodaron. Calcium antagonist includes verapamil and diltiazem use. Pulmonary vein ablation at baseline is defined as a pulmonary vein ablation maximal 365 days before and maximal 14 days after the baseline visit.

Supplementary Table 5. Multivariate regression in women.

Adjustments	Significant variables	Hazard ratio (95% confidence interval)	p-value
Model 1	AF type at baseline (persistent AF)	2.78 (1.29 – 6.01)	0.009
	Highest BMI genetic risk score tertile	2.59 (1.13 – 5.96)	0.025
	Age at inclusion	1.06 (1.01 – 1.12)	0.027
Model 1 + antiarrhythmic medication	Highest BMI genetic risk score tertile	2.71 (1.13 – 6.50)	0.025
	AF type at baseline (persistent AF)	2.31 (1.05 – 5.06)	0.037
Model 1 + antiarrhythmic medication + statins	Highest BMI genetic risk score tertile	2.71 (1.13 – 6.50)	0.026
	AF type at baseline (persistent AF)	2.32 (1.06 – 5.09)	0.036
Model 1 + antiarrhythmic medication + statins + PVI	Highest BMI genetic risk score tertile	2.61 (1.07 - 6.37)	0.036
	AF type at baseline (persistent AF)	2.44 (1.09 – 5.49)	0.031
	BMI	1.07 (1.00 – 1.13)	0.037

In model 1 adjustments are made for principal components, age at inclusion, AF type at baseline, follow-up duration, BMI, hypertension, age > 75 years, transient ischemic attack or stroke, chronic obstructive pulmonary disease, heart failure, peripheral vascular disease. Antiarrhythmic medication is defined as class I antiarrhythmic medication, beta blockers, class III antiarrhythmic medication, calcium antagonists. Pulmonary vein isolation was performed maximal 365 days before or 14 days after the baseline visit. Abbreviations: AF = atrial fibrillation, BMI = body mass index. Abbreviations: AF = atrial fibrillation, BMI = body mass index, PVI = pulmonary vein isolation.



Atrial fibrillation and left atrial size and function: A Mendelian randomization study

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** These authors contributed equally*

Scientific reports 2021

CHAPTER 6

Abstract

Background. Atrial fibrillation (AF) patients have enlarged left atria (LA), but prior studies suggested enlarged atria as both cause and consequence of AF.

Aim. To study the causal association between AF and LA size and function.

Methods. In the UK Biobank, all individuals with contoured cardiovascular magnetic resonance data were selected. LA maximal volume (LA max), LA minimal volume (LA min), LA stroke volume and LA ejection fraction were measured and indexed to body surface area (BSA). Two-sample Mendelian randomization analyses were performed using 84 of the known genetic variants associated with AF to assess the association with all LA size and function in individuals without prevalent AF.

Results. A total of 4,274 individuals (mean age 62.0±7.5 years, 53.2% women) were included. Mendelian randomization analyses estimate a causal effect between AF and BSA-indexed LA max, LA min, and LA ejection fraction, but not between AF and LA stroke volume. Leave-one-out analyses showed that the causal associations were attenuated after exclusion of rs67249485, located near *PITX2 gene*.

Conclusion. Our results suggest that AF causally increases LA size through the risk allele of rs67249483 near the *PITX2* gene, and decreases LA ejection fraction.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide, and many patients with AF develop an enlarged left atrium (LA).[1,2] LA enlargement is associated with poorer prognosis of AF ablation outcomes and AF recurrences,[3] but may also increase the risk of stroke, adverse cardiovascular outcomes and death.[4,5]

LA enlargement is hypothesized to be a result of atrial remodeling, a persistent change in atrial structure or function.[6,7] However, pressure and/or volume overload commonly seen in conditions as hypertension, structural heart disease, mitral valve disease and heart failure may also induce change in atrial structure or function. As a consequence of atrial remodeling, it might trigger AF episodes, and then a vicious circle starts where AF episodes might trigger further atrial remodeling.[8,9] The degree of atrial remodeling can be assessed through measurement of LA volume with cardiovascular magnetic resonance (CMR) imaging.[10]

Co-existence of risk factors of AF and LA size and function make it difficult to determine causality. Uncertainty exists if atrial remodeling is the cause or the consequence of AF. The hypothesis of a causal link between AF and LA volume may be tested by applying a Mendelian randomization approach (MR). Since genetic variants of AF are randomly assigned at birth, a "naturally" randomized controlled trial can be performed, assuming that (1) genetic variants are reliably associated with AF, (2) genetic variants are independent of confounding factors and (3) genetic variants are only associated with LA volume through AF.[11] In present study, we investigate the causal hypothesis between AF and LA dimensions using a MR approach in individuals with CMR assessment of their LA size and function participating in the UK Biobank.

Methods

Study population.

The UK Biobank is a large, population-based cohort that included 503,325 individuals via general practitioners of the UK National Health Service (NHS) between 2006 and 2010. Informed consent was obtained from all included individuals and the North West Multi-centre Research Ethics Committee approved of the study.[20] Hospital

episode statistics were available up to 31-03-2017 for English participants, 29-02-2016 for Walsh participants and 31-10-2016 for Scottish participants. Individuals with contoured CMR data, as previously performed by Petersen *et al.*, were included in the current study[21]. Individuals were excluded in case of missing information on body surface area or any covariates (please see below), failure of genetic quality control (including heterozygosity, high missingness and a discrepancy between reported and inferred gender), familial relatedness, or a medical history of mitral valve disease, heart failure, valvular surgery, pulmonary hypertension or prevalent AF at the time of CMR. Definitions of prevalent incident and incident disease are presented in **Supplementary Table 1** and a flowchart depicting the study sample selection is shown in **Supplementary Figure 1**.

Left atrial dimensions.

CMR protocol and image analyses of left atrial dimensions have been described previously.[10] In brief, all CMR examinations in UK Biobank were performed on a clinical wide bore 1.5 Tesla scanner (MAGNETOM Aera, Sygno Platform VD13A, Siemens Healthcare, Erlangen, Germany) in Cheadle, United Kingdom. The LA dimensions were manually analyzed by two core laboratories based in London and Oxford and the returned volumes were used in the current study.[22] In each CMR examination, endocardial LA contours were manually traced at end-systole (maximal LA area) and end-diastole (minimal LA area) in the HLA (4-chamber) view and VLA (2-chamber) view. The biplane method was applied to calculate maximal and minimal areas. Maximal LA volume (LA max volume) is defined as the end of left ventricular systole. Minimal LA volume (LA min volume) is defined as the end of left ventricular diastole. LA stroke volume and LA ejection fraction were calculated as followed: LA stroke volume = *(LA max - LA min)* and LA ejection fraction= *100* × *(LA max - LA min)/(LA max)*.

LA volumes (LA max and LA min and LA stroke volume) were indexed to body surface area (BSA) to account for body size as well as gender differences.[12]

Genotype and imputed data.

The Wellcome Trust Centre for Human Genetics performed genotyping and quality control before imputation in the individuals of UK Biobank, and imputed to HRC v1.1 panel. The quality control of samples and variants, and imputation was previously described in detail[23].

Genetic variants: AF.

In this study, 111 genetic variants associated with AF (*P*-value < $5x10^{-8}$) from the prior GWAS of Nielsen *et al.* were selected as genetic instruments in current analyses.[24] The effect sizes of the genetic variants associated with AF within the independent cohorts of the Broad AF Study, BBJ, EGCUT, PHB, SiGN and the Vanderbilt AF Registry published by Roselli *et al.* were used (number of cases = 32,957, number of controls = 83,546)[14]. We opted for this approach to obtain one of the largest sets of robust AF genetic instruments, while also being able to use effect sizes that were independent of the UK Biobank to limit overlap of the exposure and outcome cohorts. One genetic variant (rs17005647) was a priori removed as we were unable to precisely calculate the beta with the provided odds ratio of 1.0.

Genetic variants: LA size and function.

Genetic variant-outcome associations were obtained for all genetic variants included in the current study. Effect sizes were obtained by performing linear regression analyses. Regression analyses were corrected for age, sex, 30 principal components and genotyping array.

Mendelian randomization analysis.

The genetic variants were tested for weak instrument bias (F-statistic) and reversed causation (MR-Steiger). F-statistics were calculated per genetic variant using the following formula: $F = R^2(n-2)/(1-R^2)$. Here, n is the sample size of the exposure and R^2 is the amount of variance of the exposure explained by the genetic variant.[25] An F-statistic <10 was considered to indicate weak-instrument bias and these genetic variants were removed from further analyses. Reversed causation was assessed through MR-Steiger filtering and genetic variants with a significantly higher (P < 0.05) R^2 for the outcome than for the exposure were removed.[26] The R^2 for AF (on the liability scale)[27] and linear outcomes[28] were calculated based on the summary statics provided in **Online Table 1** using previously established formulae.

MR estimates were generated using inverse-variance weighted random effects meta-analysis. The Rucker framework was applied to assess heterogeneity and thus potential pleiotropy within the MR effect estimates.[29] Balanced horizontal pleiotropy was assessed by calculating Cochran's Q (P < 0.05) and I^2 index (> 25%) as indicators of heterogeneity within the IVW model.[30] Potential unbalanced pleiotropy

was assessed by performing MR-Egger regression as the MR-Egger allows for a non-zero intercept.[31] The Rucker framework than assesses the difference between heterogeneity within the IVW effect estimate (Cochran's Q) and heterogeneity within the MR-Egger regression (Rucker's Q), called Q-Q'. A significant Q-Q' ($P < 0.05$), in combination with a significant non-zero intercept of the MR-Egger regression ($P < 0.05$), was considered to indicate unbalanced horizontal pleiotropy. Under this scenario, we report the MR-Egger effect estimates as it provides a causal estimate if the general InSIDE (Instrument Strength Independent of Direct Effect) assumption holds.[32] Weak instrument bias within the MR-Egger regression was assessed by I^2_{GX}. An I^2_{GX} of >95% was considered low risk of weak instrument bias within the MR-Egger estimates.[33]

Additional sensitivity analyses were performed; the Mendelian randomization-Pleiotropy Residual Sum and Outlier (MR-PRESSO),[34] MR-Lasso,[35] leave-one-out analyses,[36,37] weighted median,[38] weighted mode[39] and MR-Mix.[40] These all have their own strength and weaknesses and jointly provide information on the possibility of a true causal relationship. Outlier robust methods include MR-PRESSO (excludes outliers), leave-one-out analyses (excludes genetic variants one by one and reperforms IVW and MR-Egger analyses) and MR-Lasso (downweights outliers). Weighted median (majority valid), weighted mode and MR-MIX (plurality valid) generally have the potential to estimate true causal effects when larger proportions of genetic variants violate MR assumptions (generally at the cost of power).

Causal effect estimates are reported in β values, since LA volumes and fractions are continuous variables. Statistical significance is considered at a Bonferonni corrected α=0.05/7 for the IVW-RE (under the scenario of balanced horizontal pleiotropy) or MR-Egger estimate (under the scenario of unbalanced horizontal pleiotropy). Continuous variables are displayed as mean ± standard deviation when normally distributed and as median and interquartile ranges when skewed. Categorical variables are displayed as percentages. Regression analyses to obtain genetic variant-outcome associations were performed using statistical software STATA 15 (StataCorp LP). MR analyses were performed using R (version 3.6.3), the TwoSampleMR package 0.5.3,[41] MR-PRESSO (version 1.0),[34] MR-Lasso[35] and MR-mix.[40]

Results

In the current study, 4,274 individuals from the general population were included (mean age 62.0 ± 7.5, 53.2 % women). The mean body mass index (BMI) was 26.6 (SD 4.4) kg/m^2, the prevalence of hypertension and diabetes mellitus type II were 32.1% and 3.5%. Body surface area (BSA) indexed maximum LA volume (LA max), minimum LA volume (LA min) and LA stroke volume were 35.9 ± 10 ml/m^2, 14.1 ml/m^2 (Interquartile range (IQR) 10.9-17.9), and 21.1 ± 5.6 ml/m^2, respectively. LA ejection fraction was on average 59.4 ± 8.3%. A total of 36 individuals (0.8%) developed AF during the median follow-up of 2.0 years (IQR 1.8-2.4). Additional information on the cohort is provided in **Table 1**. A total of 24 genetic variants were removed from MR analyses to reduce risk of weak instrument bias (F-statistic <10) and 2 genetic variants were excluded during data harmonization. A total of 84 genetic variants were taken forward for further analyses. The total amount of genetic variants varies per outcome due to MR-Steiger filtering. Data supporting the genetic variants selection (F-statistics, data harmonization, Steiger filtering) and single genetic variant-estimates for all outcomes can be found in **Online Table 1**.

Table 1: Baseline characteristics

	Sample
No.	4,274
Age, y	62.0 ± 7.5
Sex, female, %	53.2
BMI, kg/m^2	26.6 ± 4.4
Diabetes mellitus type 2, %	3.5
Hypertension, %	32.1
Prevalent atrial fibrillation, %	0
Incident atrial fibrillation, %	0.8
LA max (ml)	67.0 ± 20.0
LA max, indexed (ml/m^2)	35.9 ± 10.0
LA min (ml)	26.0 (19.9 - 33.6)
LA min, indexed (ml/m^2)	14.1 (10.9 – 17.9)
LA SV (ml)	39.2 ± 11.1
LA SV, indexed (ml/m^2)	21.1 ± 5.6
LA EF (%)	59.4 ± 8.3
BSA (m^2)	1.9 ± 0.2

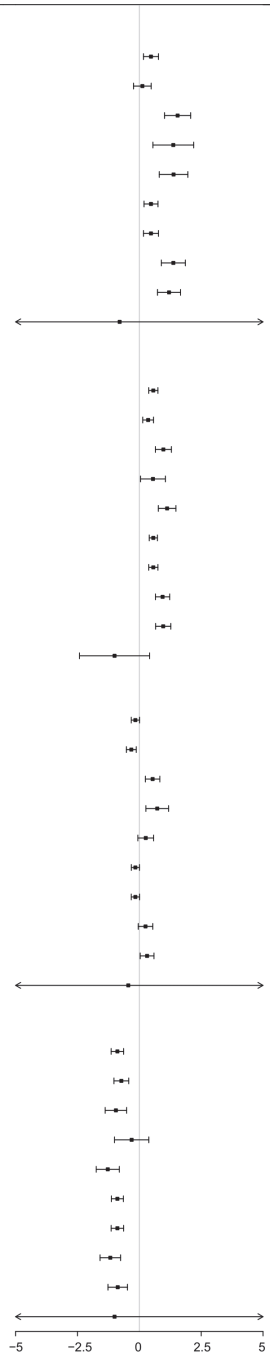
Results of the MR analyses between AF and indexed LA volumes and ejection fraction are shown in **Figure 1** and **Online Table 2**. Additional information on the association with the unadjusted LA volumes can be found in **Online Table 2**. Sensitivity analyses were performed to test whether the assumptions of the MR analyses were fulfilled (**Online Table 3**). MR-Steiger directionality test indicated that the 84 genetic variants known to be associated with AF explained ~7% of AF variance. The genetic variants explained more of AF variance than indexed LA max volume (1.7%), indexed LA min volume (1.7%), indexed LA stroke volume (1.8%) and LA ejection fraction (2.0%) (**Online Table 3**).

Using the Rücker framework, we found evidence for unbalanced horizontal pleiotropy in the MR estimates of indexed LA max and indexed LA stroke volume, indicated by significant Q-Q' and MR-Egger intercepts ($P < 0.05$) (**Online Table 3**). We therefore took forward the MR-Egger model as primary MR-method for indexed LA max and indexed LA stroke volume, whereas we adopted the more liberal inverse variance weighted random effects (IVW-RE) model for indexed LA min and LA ejection fraction. Using these models, we found evidence for a causal effect of incident AF on indexed LA max ($\beta = 1.56$, SE = 0.53, $P = 4.0 \times 10^{-3}$), indexed LA min ($\beta = 0.57$, SE = 0.19, $P = 2.0 \times 10^{-3}$) and LA ejection fraction ($\beta = -0.89$, SE = 0.25, $P = 4.1 \times 10^{-4}$) (**Figure 1**). Weak-instrument bias was indicated within the regression of AF on indexed LA max ($I^2_{GX} = 0.94$). We did not find evidence for a causal association between AF and indexed LA stroke volume ($\beta = 0.54$, SE = 0.29, $P = 6.98 \times 10^{-2}$). Scatter- and forest plots of the MR between AF and all LA dimensions are provided in **Supplementary Figures 2-8**.

►**Figure 1.** Summary MR estimates of the causal association between AF and LA size and function.

Figure 1 *displays the MR estimates on the association between AF and body surface area indexed left atrial maximal volume (LA max), minimal volume (LA min), stroke volume and ejection fraction. Inverse-variance-weighted (random effects) model, MR-Egger, MR pleiotropy residual sum and outlier (MR-PRESSO), weighted median, weighted mode-based estimator and MR-Mix are shown. Outlier-corrected MR-PRESSO estimates are not included, since no genetic variants were removed in the MR-PRESSO analyses. On the X-axis, the beta coefficient and its upper and lower bound standard error are shown. We considered a stringent two-sided Bonferonni corrected P<0.05/7 statistically significant for the main analyses (IVW under the scenario of balanced horizontal pleiotropy or MR-Egger estimate under the scenario of unbalanced horizontal pleiotropy). A P-value threshold of P<0.05 was adopted for the other MR-estimates. SE denotes standard error.*

Outcome and method	Nsnp	Beta (se)	Pvalue
LA max			
IVW RE	84	0.47 (0.30)	0.12
IVW RE, excluding rs67249485	83	0.12 (0.36)	0.73
MR Egger	84	1.55 (0.53)	4.4e−03
MR Egger, excluding rs67249485	83	1.37 (0.82)	0.1
Wald estimate, rs67249485	1	1.38 (0.58)	1.7e−02
MR−PRESSO	84	0.47 (0.28)	0.1
MR−Lasso	84	0.47 (0.30)	0.12
Weighted median	84	1.38 (0.49)	4.7e−03
Weighted mode	84	1.20 (0.47)	1.2e−02
MR−Mix	84	−0.80 (22.92)	0.97
LA min			
IVW RE	83	0.57 (0.19)	2.3e−03
IVW RE, excluding rs67249485	82	0.36 (0.22)	0.1
MR Egger	83	0.97 (0.32)	3.4e−03
MR Egger, excluding rs67249485	82	0.55 (0.50)	0.27
Wald estimate, rs67249485	1	1.12 (0.36)	1.6e−03
MR−PRESSO	83	0.57 (0.17)	9.6e−04
MR−Lasso	83	0.57 (0.19)	2.3e−03
Weighted median	83	0.94 (0.29)	1.1e−03
Weighted mode	83	0.97 (0.31)	2.3e−03
MR−Mix	83	−1.00 (1.42)	0.48
LA SV			
IVW RE	84	−0.16 (0.17)	0.35
IVW RE, excluding rs67249485	83	−0.32 (0.20)	0.10
MR Egger	84	0.54 (0.29)	0.07
MR Egger, excluding rs67249485	83	0.72 (0.46)	0.12
Wald estimate, rs67249485	1	0.26 (0.32)	0.41
MR−PRESSO	84	−0.16 (0.16)	0.34
MR−Lasso	84	−0.16 (0.17)	0.35
Weighted median	84	0.25 (0.29)	0.39
Weighted mode	84	0.31 (0.28)	0.26
MR−Mix	84	−0.44 (11.97)	0.97
LA EF			
IVW RE	82	−0.89 (0.25)	4.4e−04
IVW RE, excluding rs67249485	81	−0.73 (0.30)	1.5e−02
MR Egger	82	−0.95 (0.43)	3.2e−02
MR Egger, excluding rs67249485	81	−0.31 (0.69)	0.66
Wald estimate, rs67249485	1	−1.27 (0.47)	6.5e−03
MR−PRESSO	82	−0.89 (0.24)	4.7e−04
MR−Lasso	82	−0.89 (0.25)	4.4e−04
Weighted median	82	−1.17 (0.42)	4.8e−03
Weighted mode	82	−0.87 (0.39)	2.9e−02
MR−Mix	82	−1.00 (9.49)	0.92



Several sensitivity analyses were performed to test whether valid conclusions on causal inference could be made under different assumptions of possible underlying pleiotropy or instrumental invalidity. We investigated whether the results were consistent under the scenario where a relativity large portion of the genetic instruments is invalid using the weighted median approach. Using this approach, we found additional evidence for a significant causal estimate between AF and indexed LA max (β = 1.36, SE = 0.47, P = 3.83×10^{-3}), indexed LA min (β = 0.89, SE = 0.30, P = 2.8×10^{-3}) and LA EF (β = -1.17, SE = 0.42, P = 5.84×10^{-3}). MR-Lasso analyses suggested the associations between AF indexed LA min (β = 0.57, SE = 0.19, P = 1.98×10^{-3}) and LA ejection fraction (β = -0.89, SE = 0.25, P = 4.09×10^{-4}) and to be robust to the scenario in which a small proportion of the genetic variants are outliers. However, the association between AF and indexed LA max (β = 0.48, SE = 0.30, P = 1.13×10^{-1}) was attenuated (**Figure 1**).

We examined which genetic variant(s) drove the attenuation of the association between AF and LA dimensions by performing leave-one-out analyses. Results of the leave-one-out analyses using an IVW and MR-Egger approach are provided in **Online Table 4** and can be visually inspected in **Supplementary Figures 9-15**. We observed that the MR-Egger estimate was attenuated for indexed LA max after exclusion of rs67249485 (β = 1.41, SE = 0.82, P = 9.05×10^{-2}), a genetic variant located on the long arm of chromosome 4 in the proximity of the *PITX2* gene. However, the Wald estimate of rs67249485 did show a significant association for indexed LA max (β = 1.38, SE = 0.58, P = 1.65×10^{-2}). The results are shown in **Figure 1**. The leave-one-out analyses also showed an attenuation of IWR-RE estimates after exclusion of rs67249485 for indexed LA min (β = 0.36, SE = 0.22, P = 1.00×10^{-1}), and LA EF (β = -0.73, SE = 0.30, P = 1.52×10^{-2}). The Wald statistics for the association between rs67249485 and indexed LA min (β = 1.13, SE = 0.36, P = 1.44×10^{-3}), and LA ejection fraction (β = -1.29, SE = 0.47, P = 5.94×10^{-3}) were significant (**Figure 1**).

We performed several quality controls to gain insights in the statistical validity of rs67249485 driving the association between AF and LA dimensions and functions. Histograms of LA dimension distributions per AF increasing T allele showed absence of outliers which could drive current MR estimates (**Supplementary figure 16**). The genetic variant rs67249485 explained more variance for AF (MR-Steiger R^2 = 1.58%) than for any LA size or function, which ranged up to a maximum explained variance of 0.23% for LA min. This indicates that the Wald estimates assessed the true causal direction (**Online Table 1**).

The MR analyses for the non-indexed LA volumes are provided in **Online Table 1-4.** The results were consistent to the results on the indexed LA volumes. The MR analyses for LA min (indexed and non-indexed) were repeated using genetic variant-outcome effect estimates obtained from their log-transformed equivalents to account for right skewness. Results were comparable to the primary analyses (**Online Table 1-4**).

Discussion

Our study provides evidence to support the hypothesis that AF causally increases indexed LA max, LA min and decreases LA ejection fraction. We pinpoint that rs67249485, near the *PITX2* gene, is the driver of the association between AF and indexed LA max and LA min. The same variant contributed strongly to the association between AF and LA ejection fraction. However, we did not find evidence for a causal association between AF and LA stroke volume.

Our primary analysis indicates that AF causally increases indexed LA maximum and minimum volumes. However, both associations were attenuated after the exclusion of rs67249485 in the leave-one-out analyses. The Wald estimate of rs67249485 indicates that AF causally increases indexed LA max and LA min. Our results suggest evidence for rs67249485 to be the main driver of the association between AF and indexed LA volumes. A causal association between AF and LA stroke volume was not established. One potential explanation is that AF increases indexed LA max and indexed LA min in a similar degree, nullifying the effect on LA stroke volume. Another potential explanation is that a larger passive conduit function of the LA could compensate for a decreased pump function at larger maximal LA volume through the Frank-Starling law.[12,13] This would result in similar LA stroke volume and lower LA ejection fraction.[12,13] In fact, we do find that AF is associated with decreased LA ejection fraction. Again, the genetic variant rs67249485 also contributed strongly to the association between AF and LA ejection fraction. However, we still find a causal estimate between AF and LA ejection fraction after exclusion of this variant, which may suggest that other genetic variants may also contribute strongly in the association between AF and LA ejection fraction.

Our analyses show that rs67249485, located in an intergenic region near the PITX2 gene, is the driver of the association between AF and LA size and function.[14] The validity of this finding is statistically supported by several sensitivity

analyses which indicate the large effect of this genetic variants is very unlikely caused by measurement error or uneven population distribution and is in the correct causal direction. The biological role of *PITX2* in AF development has been extensively studied and many potential mechanisms have been suggested, including deviations in LA myocyte automaticity, impaired response to oxidative stress, inflammation and a role in the embryonic development of the heart.[15–18] The *PITX2* gene does not only increase the risk of AF development, but has been suggested as a determinant in the success of pulmonary vein ablation in preventing AF recurrence as well.[19] Our results provide evidence for another possible biological consequence of *PITX2,* as we show that LA volumes increase and LA ejection fraction decreases through the AF increasing T allele of rs67249485. However, further experimental validation is needed to investigate details of the mechanisms underlying the association of rs67249485, *PITX2*, AF and LA size and function.

Our study has several strengths. The strengths include the use state-of-art genetic and CMR data. The MR design is less susceptible to confounding and strongly contribute to previous work in the field. We excluded individuals with known prevalent AF and the MR was designed to study the effect of increased AF risk on LA dimensions before onset of the disease. Extensive sensitivity analyses were performed to further reduce the risk of pleiotropy and reversed causation and support our hypothesis.

Some limitations should be noted as well. First, we did not include all previously established genetic variants associated with AF as the UK Biobank was used as discovery cohort in the most recent GWAS of AF. We therefore took forward the largest set of genetic variants using effect sizes obtained without the UK Biobank to limit overlap of the exposure and outcome cohorts. We did not have data on LA volume at the onset of atrial contraction and were therefore unable to differentiate the effect of AF on the LA conduit and pump function separately. Pleiotropy cannot be ruled out completely despite rigorous sensitivity analyses. We were unable to perform a bidirectional MR to further entangle the cause and consequence in the association between AF and LA enlargement. Unfortunately, insufficient GWAS data of LA size and function have been performed so far and the current cohort is too small to identify robustly associated genetic variants. Lastly, the outcome cohort included individuals of mixed ancestry and this could introduce confounding by hidden population structure. However, we believe this to be highly unlikely given the stringent adjustments for genetic ancestry.

In conclusion, we provide evidence that AF causally increases indexed LA maximum and minimum volumes and decreases LA ejection fraction using the unique combination of CMR data and genetic information in UK Biobank. The genetic variant rs67249485, near the *PITX2* gene, drives the association between AF and indexed LA max and LA min. The genetic variant rs67249485 also contributed strongly to the causal estimate between AF and LA ejection fraction, but exclusion of this variant did not nullify the association. The association between AF and LA ejection fraction was robust to multiple sensitivity analyses and indicate genetic susceptibility to AF causally decreases LA ejection fraction. No causal association was established between AF and LA stroke volume.

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Competing interest declaration

None to declare

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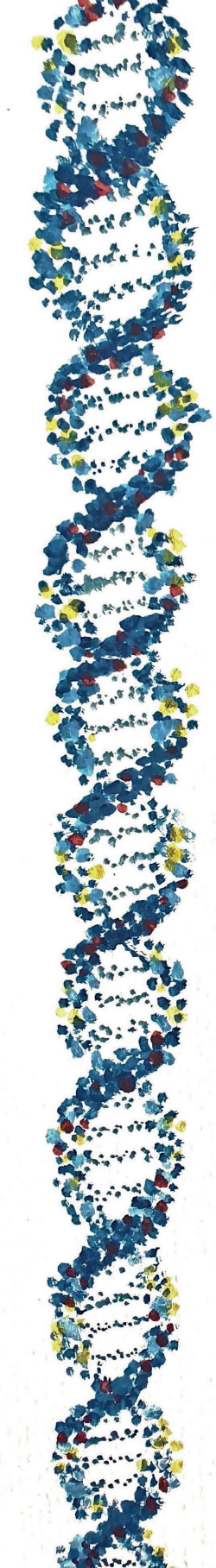
Content available at: ***https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8055882/bin/41598_2021_87859_MOESM1_ESM.xlsx***



Discussion and future perspectives

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Role of genetics in atrial fibrillation management

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CHAPTER 7

This thesis converges genetic and clinical information and demonstrates that genotypes associated with atrial fibrillation (AF) can help to understand (causal) interactions between risk factors and AF. In **part I** we addressed the role of genetics and the initiation of AF. We investigated potential (genetic) measures for risk of developing incident AF. In **part II** we demonstrated associations between genetics and determinants of AF after initial diagnosis, which entails profiling different AF types, AF progression and resulting dimensions of the heart. The results provide food for thought for clinical implementation of genetics in AF management.

Genetics and the initiation of AF

The scope and pace of AF genetics has been incredibly rapid and we currently have a solid understanding of the role of genetics in AF – at least in individuals of European descent. The lifespan in the current European society is expected to increase and efforts are made to improve healthy ageing.[1] Incident AF is associated with advancing age.[2,3] However, in some individuals their chronological age may not align with their biological age. Previous studies have shown that life stress, various metabolic traits and lifestyle habits as smoking accelerate biological ageing.[4,5] In **chapter 2** we investigated if the age-associated risk of AF is measurable through biological markers of ageing. Biological markers of ageing could provide evidence for the causal mechanisms underlying the association between chronological ageing and AF. The length of telomeres at the end of chromosomes are believed to be biological markers of ageing. In **chapter 2** we showed that telomere length of circulatory leukocytes is not independently associated with incident AF. Studies in other large population-based cohorts, as the Framingham Heart cohort and the Cardiovascular Health Study, were in line with our results.[6–8] Chronological ageing and other risk factors of AF may contribute more than telomere length to the increased risk of AF. The age-associated risk of AF may be expressed in other (yet unknown) genetic biological markers, (or) in cardiac tissue. Future studies are needed to confirm this hypothesis.

Optimal risk assessment is ideally based on causal associations. However, the exact contribution of risk factors to AF is hard to identify, since some risk factors may influence the existence or severity of other risk factors and AF (confounding factors). During the past decade, information on the genetic contribution to AF increased tremendously and this created the opportunity to study (causal) interactions

between risk factors and incident AF via Mendelian randomization analyses. In **chapter 3** we studied whether resting heart rate was causal to the development of AF in the AFGen consortium (a worldwide international collaboration to increase genetic data of AF). The genetic variants of resting heart rate were used as a polygenic risk score to infer causal associations. Our observational data of resting heart rate suggested a non-linear U-shaped association, similar to prior studies.[9,10] Both low and high resting heart rates were associated with increased risk of AF. Since Mendelian randomization can only assess linear associations, analyses were performed in three strata to reveal a causal non-linear association. Our data suggests that low resting heart rate may cause incident AF, below 65 beats per minute. Contradictory to our expectation no causal association was established above 65 beats per minute and a non-linear causal association could not be confirmed. While in literature associations were established between high resting heart rate and incident AF in observational data.[9,10] For example, a meta-analysis with 18,630 cases of the 431,432 included individuals showed a significant J-shaped association.[10] The observed association may have been a reflection of other cardiovascular conditions in the observational analyses, such as hypertension, diabetes and overweight. However, resting heart rate is easily measured during physical examination, which could make resting heart rate an excellent preventive measure in risk assessment of AF. Our results may support future studies that investigate low resting heart rate as a risk indicator or preventive measure of incident AF.

Genetics and determinants of AF after the initial diagnosis

The various AF types presenting in AF patients complicate the understanding of mechanisms underlying AF and the implementation of personalized AF management. In **chapter 4** we investigated the clinical, biomarker and genetic profiles of AF patients with different types of AF. AF episodes were defined as AF detected once, without any recurrence; or persistent AF, terminated by means of medication or a procedure. Additionally, AF episodes were defined to be chronic, when no medication or procedure could terminate the AF episode. Only in the self-terminating profile, genetic variants were associated with AF. Our results point out that genetic variants may play a more important role in certain AF patients. Current AF management guidelines emphasize the importance of implementing personalized treatment in clinical practice.[11] The identification of AF types and their concomitant genetic profile could improve personalization of AF management.

A powerful method for assessing the genetic profile to AF types or AF progression is to calculate a polygenic risk score that combines the genetic risk variants present in an individual patient. In **chapter 5** we constructed a polygenic risk score of body mass index (BMI) to investigate AF progression. BMI is one of the risk factors of AF progression with extensive genetic information available.[12–14] Sustained weight loss has shown to be beneficial for AF patients. Weight intervention showed a reduction of AF burden and symptoms.[11,15–21] Whether an increased BMI is causing AF progression is difficult to investigate since several AF-related conditions, such as hypertension, diabetes and heart failure, but also structural and hemodynamic changes are influenced by BMI.[22] Our results showed that an increased genetically determined BMI is associated with AF progression in women. The investigated genetic variants that determine BMI and are associated with AF progression support the notion of causality. Hypothetically, this may be due to sex-related heritability pathways of BMI genetic variants, as is seen with genetic variants associated with waist-hip-ratio.[23] Waist-hip-ratio heritability and genetic variants effects are stronger in women. One could hypothesize that our results suggest that genetic predisposition to a high BMI may potentially be more harmful in women, and weight management is important in this group. In the recent years it became more apparent that symptom presentation, burden of AF and (invasive) treatment attempts differ between men and women.[24–26] However, sex differences in AF genetics and pathophysiological mechanisms are yet to be explored. Whether changes in the observed BMI would influence the risk of AF progression in women with genetically determined high BMI, could not be confirmed in our data. Future studies should clarify whether weight loss in individuals with high genetic risk could lower the risk of AF progression.

In **chapter 6** we constructed a polygenic risk score of AF to show causality between AF, left atrial size enlargement and left atrial dysfunction. In the European guidelines, AF progression is described as the increase in frequency and duration of AF episodes.[11] Pressure and/or volume overload may be increasingly present when AF progresses and could change atrial structure or function. Persistent change in atrial structure and function is considered to be the result of atrial remodeling.[27] Atrial remodeling could trigger AF episodes. However, AF episodes could also trigger atrial remodeling and a vicious cycle may continue.[28,29] The association seems bidirectional and it is difficult to distinguish whether AF was cause or consequence of atrial changes.

Our study showed a genetic basis for causality between AF and left atrial size and function. Via Mendelian randomization analyses and through leave-one-out analyses we showed that the association between AF and left atrial size seems to be driven by a genetic variant located at the *PITX2* gene. This same variant was involved in the association between AF and decreased ejection fraction. The *PITX2* gene is highly associated with incident AF in previous studies.[30–32] Although the exact biological pathway of the genetic variant at the *PITX2* gene is unknown, this gene is suggested to play a role in left atrial myocyte automaticity, the response to oxidative stress, inflammation and embryonic development of the heart.[33–36] One may suggest that fewer AF episodes could cause less changes in left atrial size and function. Hypothetically, optimal rhythm control that could inhibit *PITX2,* may possibly prevent or minimalize changes in left atrial size and function, associated comorbidities and mortality.[37–39] However, data on genetically driven successful rate and/or rhythm control is scarce.

Future perspectives

During this thesis both the scale and the scope of the worldwide collaboration on AF genetics increased. One of the facilitators was the international Atrial Fibrillation Genetics (AFGen) Consortium. Through international collaboration, current AF GWAS analyses have increased their statistical power, which has enabled to uncover more of the missing heritability of AF.

AF heritability

In 2017, Weng et al. estimated that genetic variation contributes only with 22% to the development of AF.[40] Other contributions may be environmental, lifestyle or clinical determinants. At the time of that paper, only 25 AF loci were known and accounted for only a quarter of the 22% estimated genetic variation that contributes to AF. The fraction of the heritability of AF explained by common genetic variants has increased from 25% to 42% based on the results of the largest GWAS thus far.[31] As evident from the estimates of explained heritability, there is still a substantial proportion of "missing heritability" (**Figure 1**).

▼**Figure 1.** Heritability of AF.

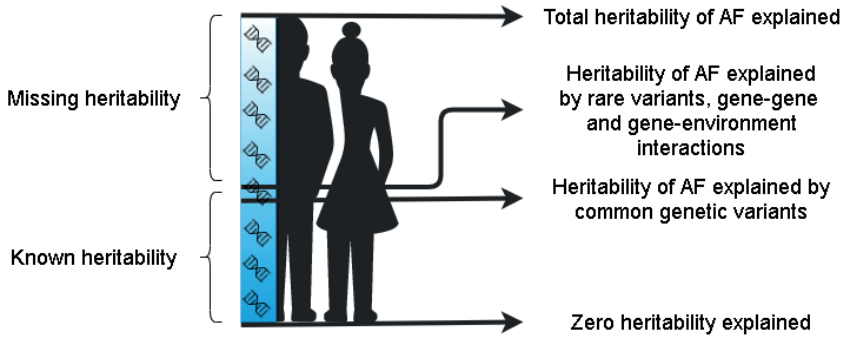

Figure 1 *illustrates that heritability of AF is partly explained by known genetic data and partly unknown, the missing heritability.*

Where the missing heritability could be found is debated. Common genetic variants identified through GWAS are mostly located in intergenic or intronic regulatory regions. They may participate in disease development through regulatory mechanisms, thereby affecting the expression of multiple genes located in proximity and possibly, further away. For example, the co-regulatory network involving *TBX5* and *PITX2* influencing atrial development.[31,41] Gene–gene interactions can play a significant role in regulating cellular behavior and biological events. Two variants with small individual effects may have large interaction effects. Lin et al. showed that two genetic variants, rs7164883 at the *HCN4* locus and rs4980345 at the *SLC28A1* locus, were highly significantly associated with AF in an European ancestry discovery cohort from 15 studies. Moreover, eight additional gene–gene interactions were marginally significant, albeit unreplicated thus far.[42]

Gene–environment interactions may also contribute to the missing heritability. Some cross-trait associations have been found between genetic variants related to AF risk factors, such as height, body mass index (BMI), hypertension.[31] These pleiotropic effects may also contribute to missing heritability. Moreover, unidentified common and rare genetic variants, including copy number variation, may partially explain the missing heritability of AF.[43] The fast-developing high-throughput sequencing technology may play an important role in uncovering missing heritability for AF. Finally, larger GWAS analyses in both European and non-European ancestry groups may reveal other loci associated with AF.

Nevertheless, the magnitude of recent GWAS analyses in individuals from European descent is close to reaching its saturation point. As a consequence, including more individuals in larger GWAS analyses will not lead to identification of more common genetic variants for AF.[44] Large GWAS made for other common complex diseases, such as major depressive disorder or schizophrenia, have already demonstrated this phenomenon. In 2018, Nishino et al. predicted the number of cases needed to detect significant genetic variants for these two psychiatric diseases.[45] The numbers of cases needed to detect 1, 10, and 100 genetic variants by GWAS were calculated to be 7,000, 18,000, and 51,000 cases for schizophrenia, and 34,000, 61,000, and 118,000 cases for major depressive disorder. The authors pointed out that the number of genetic variants associated to major depressive disorder would rapidly increase, when 50,000 cases or more were included. In comparison, the largest AF GWAS thus far, included a similar number of cases. However, the power of a GWAS depends not only on the sample size, but equally important are the genetic coverage (genotyping chip and imputation panel density), the frequency of the genetic variants, the ancestry group, and the effect size.[44,46–48] Nelson et al. have estimated the power of GWAS across a range of genotyping chips, ancestry groups, minor allele frequencies, and effect sizes, suggesting that we are close to having identified the most relevant common variants for AF in European ancestry groups. Thus, the pursuit of new common variants for AF will likely be more successful if we focus on non-European ancestry groups and on low-frequency variants with large effect size.[44]

A current challenge of GWAS is that the pace of genetic discovery has exceeded our capability to identify the causative genes. At many GWAS loci, there can be multiple potential candidate genes and deciphering which ones are causally related to AF is difficult. Genetic data has been combined with approaches such as expression quantitative trait loci (eQTL) mapping to discover the causative gene. In an eQTL analysis, a genetic variant is linked to expression of a gene in a relevant tissue, such as the left atrium or pulmonary veins.[49,50] Such an approach will identify a likely causative gene in ~25-30% of AF loci. A complimentary approach is to perform epigenetic analyses that focus on defining the three-dimensional architecture of the genome. Many AF genetic variants are in noncoding regions of the genome and likely regulate the expression of nearby genes. Epigenetic methods such as HiC, STARR-seq among others, can help to identify these three-dimensional interactions and lead to the identification of another subset of causative AF genes.[51,52]

In contrast to GWAS, high throughput exome and whole genome sequencing allow the identification of low-frequency genetic variants that can be associated with large effects on AF risk and may reveal missing heritability. Therefore, future sequencing efforts may focus on considerably larger sample sizes and on populations of non-European ancestry to enhance the diversity and generalizability of these findings. Ultimately, given the sheer number of AF related loci, it will be essential to develop high throughput methods for identifying and characterizing these genes. Large-scale overexpression or gene knockout in stem cell derived cardiomyocytes, followed by an assessment of the resulting electrical and structural effects, may be one approach to consider for gene prioritization.

Genetics and AF progression

Genetic data of AF progression is currently difficult to obtain. Many cohorts have different definitions of AF progression and this complicates merging data of different cohorts into large datasets for genetic analyses. This thesis contributed to more available genetic information and insight into AF progression. However, genetic data and continuous monitoring of rhythm would permit ultimate understanding of AF progression patterns. Recently, the first interim analyses were published of a multicenter Dutch initiative, the Reappraisal of AF: Interaction Between HyperCoagulability, Electrical Remodelling, and Vascular Destabilisation in the Progression of AF (RACE V) registry, that uses loop recorders to gain more insight into the progression patterns of AF (ClinicalTrials.gov Identifier: NCT03124576).[53] Results showed that within the current definition of paroxysmal AF, temporal patterns of AF are not one entity. Individuals with more comorbidities (worse renal function, higher calcium score, thicker intima media thickness) seemed to have a higher burden of AF. RACE V is unique in collecting genetic data in combination with detailed AF progression data. When RACE V is completed, interesting details on (causal) interactions and genetic predisposition for different AF temporal patterns may be published. Future results of large studies with similar continuous data from smartphones, smart watches[54] or implantable loop recorders in combination with genetic information may change current views of AF progression.

Genetics and AF management

Currently, the contribution of genetics to AF management is explored. Genetic risk prediction of AF and concomitant diseases is already possible with polygenic

risk scores. Polygenic risk scores could be used to identify individuals at high risk for AF and AF-associated diseases as, heart failure or stroke, to indicate who may benefit from screening for AF or to help guiding who may benefit from preventive therapies.[55] We envision that in the future it is highly likely that individuals at risk of developing AF, or diagnosed with AF, will be genotyped for common genetic variants, an approach that would enable the calculation of genetic risk for a range of complex diseases. Genetic risk scores may support screening of AF and, by matching genotype of patients to the best fitting treatment, may increase success rates of AF treatment.

Despite considerable advances in our understanding of genetics, clinical risk factors and AF management; progress has been quite limited on the development of new antiarrhythmic drugs to treat AF. While the causes of limited new antiarrhythmic drug development are multifactorial, it is sobering that the traditional modalities of antiarrhythmic drug development seldom lead to market approval in Europe. Many novel genetic loci for AF are not directly "drugable" but require years in the wet-lab for their functional effects to be mapped out. Identifying statistical associations between genetic variants and AF without knowing neither (I) which specific parts of the genetic loci carry the causal effects, nor (II) the underlying biological mechanisms carried by the causal variants, do limit our success in establishing new therapeutic targets. Interestingly, during this same time period, it has become increasingly clear that therapeutic targets search, using genetics in humans, seems to have a greater chance of ultimately making it to market.[44]

We need to identify the causal entities in the AF loci identified and disentangle their biological mechanisms in order to improve the development of new drugs and to understand how to improve AF interventions. One interesting application of this approach arose from the recent manuscript by Nielsen and colleagues. Out of 151 candidate genes for AF, they suggested 475 potential AF treatment targets, including 78 potential targets that are drugs that may control or trigger AF or other arrhythmias.[56] We anticipate that using the genetically driven therapeutic targets will be a promising avenue for future drug development.[57]

Genetics could support existing therapeutic strategies of AF, like rate- or rhythm control.[11] The goal of a rate control strategy is to lower heart rate in the presence of permanent AF in order to improve AF symptoms. Although the optimal heart rate target of AF patients is unclear, the RACE (Race control efficacy in Permanent

Atrial fibrillation) II randomized control trial showed that a lenient heart rate control was non-inferior to a more strict rate control strategy.[58,59] In rhythm control anti-arrhythmic drugs or (invasive) techniques as electrical cardioversion, AF ablation is used to return to and maintain sinus rhythm. Furthermore, targeted therapy of underlying conditions, in addition to rhythm control therapy have shown to improve maintenance of sinus rhythm even more than rhythm control only.[60] In the future, genetic variants may guide the choice of a rate- or rhythm control strategy by individually identifying those with higher chances of therapy success or higher risk of presenting side-effects.

Recently, the impact of genetics on rate control was investigated. In a recent study, the effectiveness for maintenance of sinus rhythm was compared between two rate control medications, bucindolol and metoprolol.[61] Bucindolol has greater benefit in heart failure patients with the beta1-adrenergic receptor (*ARDB1)* Arg389Arg genotype. Patients with heart failure with reduced ejection fraction, symptomatic AF and *ARDB1* Arg389Arg genotype were selected (*n*=267). Recurrences of AF were not reduced in the pharmacogenetically-guided bucindolol group. However, in the subgroup of patients with first diagnoses of heart failure less than 12 years prior to the diagnosis of AF, bucindolol reduced AF recurrences. Further research is needed to confirm this observation.

Some current antiarrhythmic drugs are already targeting processes influenced by AF loci. For example, the *SCN5A* gene associated with the sodium channel and the target for flecainide and propafenone.[62,63] *KCNH2* is associated with the alpha subunit of a potassium channel complex and is a target for sotalol and dofetilide.[31] One of the largest studies about genetics and pharmacologic rhythm control therapy in AF was reported by Parvez et al. in the Vanderbilt AF registry, including 478 Caucasian individuals with documented AF (32% women, age 63 ± 14 years), who used at least one antiarrhythmic drug.[64] During a 12-month follow-up period, successful rhythm control (> 75% reduction in symptomatic AF burden, defined as frequency, duration and severity of symptoms, in a patient who remained on the same antiarrhythmic drug for a minimum of 6 months) was established in 399 (84%) individuals. A genetic variant at chromosome 4q25 independently predicted successful rhythm control. As described previously, the gene closest to the 4q25 locus is *PITX2*, and loss of the cardiac isoform pitx2c can lead to failure to suppress a default pathway for the sinus node development of the

pulmonary myocardium or the cardiomyocytes in the left atrium.[64] More studies at the intersection with genetic data and detailed information of AF treatment are needed before genetically tailored treatment will be part of the guidelines. The predictive ability of the three most strongly associated AF susceptibility loci in relation to AF recurrence after successful electrical cardioversion has been evaluated in a cohort of 184 individuals in the Vanderbilt AF registry. One hundred sixty-two individuals underwent successful electrical cardioversion, of which 108 individuals had AF recurrence. A genetic variant at chromosome 4q25 (rs2200733) was significantly associated with AF recurrence after electrical cardioversion.[65] The outcome of electrical cardioversion of AF may be genetically predisposed; however, more and well-powered studies are needed to establish true associations. Other threads of exploration include genetic variants modulating outcomes of AF ablation. The use of genetic variants to predict AF catheter ablation outcome may be a promising clinical tool for the selection of patients for this procedure in the future. In 2013, Shoemaker et al. investigated 311 individuals in the Vanderbilt AF registry, of which 238 underwent only one ablation and 73 underwent two or more.[66] Of the 378 catheter-based AF ablations, 200 (53%) AF/atrial flutter recurrences were observed. The AF risk allele, rs2200733, at the 4q25 locus predicted shorter recurrence-free time after AF ablation. Park *et al.* found that the genetic variant rs2106216 at the *ZFHX3* locus on chromosome 16q22 was independently associated with a good response after AF ablation, but contradictory, no association was found with the *PITX* locus in the Korean Yonsei AF ablation cohort.[67] Increased polygenic risk for AF was strongly associated with AF recurrence in another Korean population from the Seoul National University Hospital and Korea University Guro Hospital.[68] During 23 months of follow-up in 746 individuals (74% men, mean age = 59 ± 11 years), 168 (22.5%) had AF recurrence after AF catheter ablation. Individuals carrying 7 to 10 risk alleles of the 5 AF risk variants (rs1448818, rs2200733, rs6843082, rs6838973, and rs2106261) had a 2.66-fold increased risk of recurrence compared to the lowest risk group, where individuals carried zero to three risk alleles. Larger, well-phenotyped populations are necessary to discover new genetic variants associated with outcomes of AF ablation, e.g., a large-scale AF ablation GWAS may expose more common genetic variants related to AF recurrence after ablation. Recently, a polygenic risk score that was associated with incident AF was used to evaluate recurrences of AF after AF ablation therapy in 3259 AF patients.[69] However, the incident AF polygenic risk score was not associated with AF recurrence after ablation.[70]

The time will come when sufficient information about genetics and AF management is readily available in clinical practice and can be used to implement genetically driven strategies in AF management guidelines. This may be an important step to further personalize AF management. Integrated AF management is essential to achieve personalized medicine.[11] The patient should be central in the decision making of AF management. In general, treatment options should be discussed and agreed with the patient and involved (multidisciplinary) healthcare professionals. One could imagine informing the patient about his/her own genetic profile and potential genetic risk for some diseases, like AF, may have a positive impact on treatment adherence.[70] However knowledge about genetic risk can also negatively impact the life of an individual and can have mental, societal and even financial (insurance) consequences. In addition, most current genetic studies focus on Caucasian populations. Other ethnicities and their characteristic genotype may have been underrepresented in current literature. Personalized medicine may not be readily available for members of underrepresented populations. Future studies should make an effort to investigate more ancestry groups to ensure availability of genetically driven personalized care for individuals of all ancestries at risk or diagnosed with AF.

Conclusion

The complex biology of AF and AF heritability has been increasingly elucidated in the era of AF genetics research. This thesis shows that genetic risk variants may support causal entities of clinical determinants of AF and reveal different genetic profiles of individuals with AF. Nevertheless, our knowledge of the biologic mechanisms underlying the genetic associations is still limited. The translation of genetic information into clinical practice is difficult and far from ready to be implemented. Overall, better understanding of the genetics of AF will hopefully provide better tools to prevent, detect, and treat AF, thus reducing the impact of the disease both at an individual level and in a public healthcare perspective (**Figure 2, Table 1**). In the past decade international collaborations contributed to GWAS, high-throughput sequencing, eQTL and epigenetic analyses that provided a new foundation for drug development, but also highlights the limitations of our understanding of the molecular mechanisms of AF. Small steps towards translation of genetic information into clinical implementation have been carefully taken. AF genetics could eventually be used to develop new therapeutics, when the

biological meaning of genetics variants is clarified. Implementation of AF genetics in the daily clinical routine is therefore still some steps away, but the paradigm of AF management is shifting (**Figure 3**). In the next decade it is critical to pivot from genetic discovery to translation into new molecular targets, treatment outcomes and personalized therapies. Thus, in the long run personalized care of AF can be improved and benefit the wellbeing of individuals at risk or diagnosed with AF.

▼**Figure 2.** Translation of genetics into clinical practice.

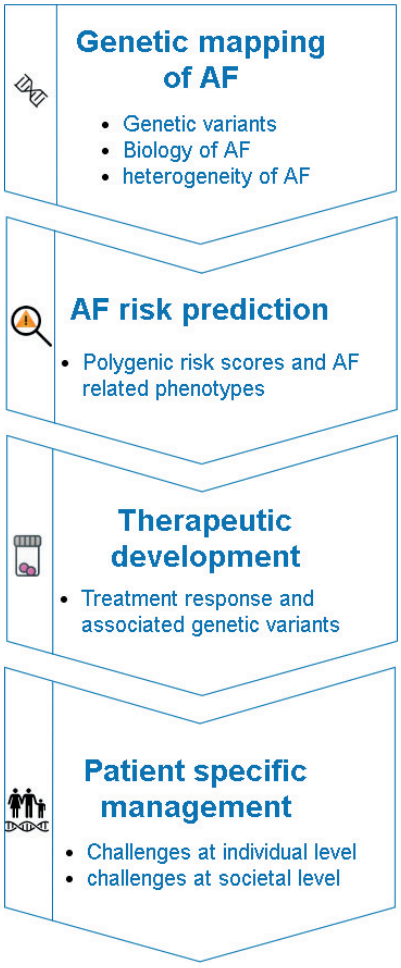

Figure 2 *The missing heritability of AF can be revealed by focusing on mapping the polygenic structure of AF, improving risk prediction, therapeutic development, and patient-specific management. Details of suggested studies are described in Table 1. Future perspectives—translating AF genetics into clinical practice. Abbreviations: AF = atrial fibrillation.*

Table 1. Future perspectives—translating AF genetics into clinical practice.

Direction	Knowledge gaps	Suggested studies
Genetic mapping of AF	*Total number of genetic variants*	• GWAS: Larger, non-European ancestry groups, more specific AF phenotypes
		• Whole-exome and -genome sequencing
	Biology of AF	• Expand eQTL studies
		• Network analyses
		• In vivo and in vitro experiments
		• Gene–gene interaction
		• Epigenetics
		• Transcriptomics
	Heterogeneity of AF	• Proteomics
		• Metabolomics
		• Discover new AF clinical risk factors
		• Validate AF risk models
		• Genetic classification of AF phenotypes
		• Investigate gene–environment interaction
		• PheWAS
		• Mendelian randomization studies of AF risk factors
AF prediction	*Polygenic risk scores*	• Improve genetic risk scores for AF, AF subtypes, and AF-related phenotypes
Therapeutic development	*Treatment response*	• Improve genetic risk scores for AF treatment
		• Increase cohorts with both genetics and treatment response data
		• Investigate the underlying biological mechanisms of genetic variants to identify new drug targets
Patient specific management	*Individual concerns*	• Focus on individualized risk assessment • How to relate to AF genetics and the ethical concept and resulting patient behavior
	Societal concerns	• Cost-effective consequences

Table 1 is published in 'Atrial fibrillation genetics update: towards clinical implementation' in Frontiers Cardiovascular Medicine 2019.

▼**Figure 3.** The shifting paradigm of AF management.

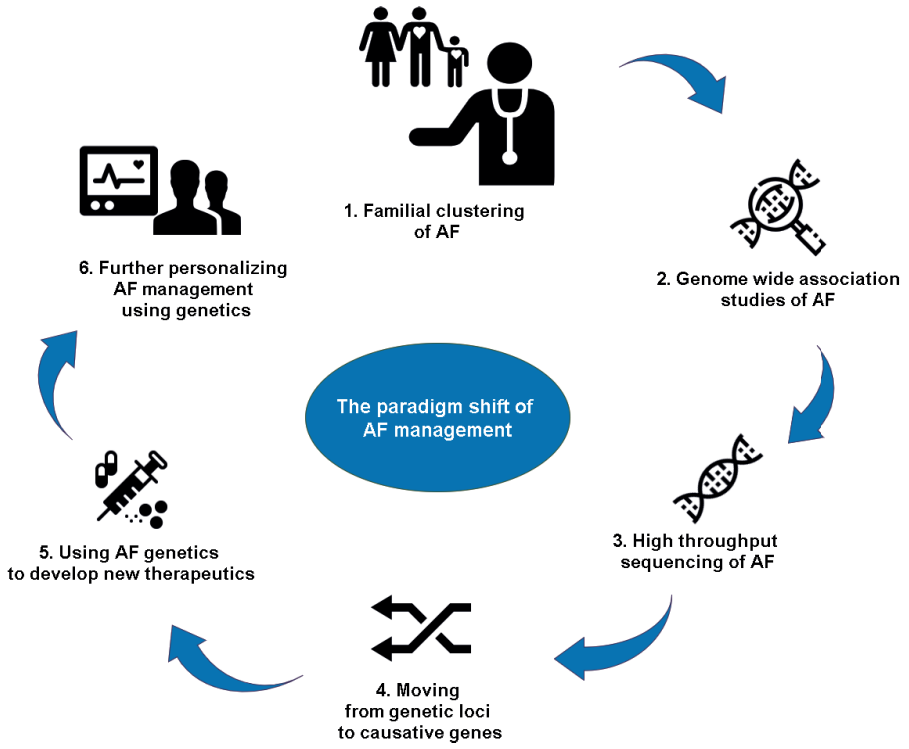

Figure 3 *illustrates the paradigm shift of AF management towards genetic implementation. Information of familial clustering of AF have led genome wide association studies of AF populations and high throughput secuencing of AF. Efforts are made to move from genetic loci to causative genes which will stimulate the development of new therapeutics for AF. Finally, genetically based treatment will further personalize and improve current AF management.*

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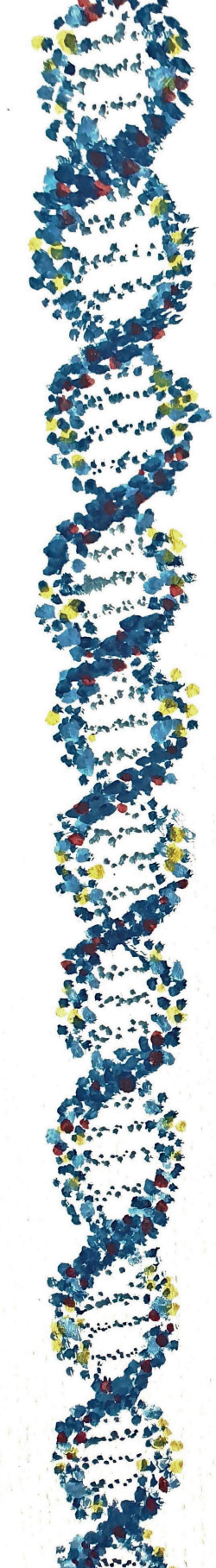

Appendices

Dutch summary
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Biography

Dutch summary | Nederlandse samenvatting

Boezemfibrilleren. Boezemfibrilleren is een veel voorkomende hartritmestoornis en komt steeds vaker voor.[1,2] Ongeveer 1 op de 3 mensen boven de 55 jaar ontwikkelt boezemfibrilleren.[3] Tijdens boezemfibrilleren veroorzaken chaotische impulsen in de boezems van het hart onregelmatige geleiding van impulsen naar de kamers van het hart.[4,5] Op het elektrische cardiogram (ECG) kan boezemfibrilleren worden vastgelegd en worden herkend aan een afwezige P golf en een onregelmatig hartritme. Volgens de Europese richtlijnen zijn er drie vormen van boezemfibrilleren te onderscheiden: paroxysmaal boezemfibrilleren (een episode korter bestaand dan 7 dagen en spontaan converterend naar een regelmatig ritme -- sinusritme), persisterend boezemfibrilleren (een episode langer bestaand dan 7 dagen en te converteren naar sinusritme door middel van behandeling) en permanent boezemfibrilleren (een episode langer bestaande dan 7 dagen en geaccepteerd door de behandelaar en enkel de hartslag frequentie wordt behandeld). De episodes van boezemfibrilleren kunnen over de tijd toenemen van paroxysmaal naar persisterend naar permanent boezemfibrilleren.[6] Klinische risico factoren van boezemfibrilleren zijn onder andere leeftijd, mannelijk geslacht, hoge bloeddruk, diabetes en een hoge body mass index (BMI).[1,7] Zo verdubbelt een hoge bloeddruk het risico op boezemfibrilleren.[8] Daarnaast geven bestaande hart- en vaatziekten zoals hartfalen, een doorgemaakt myocardinfarct of een CVA/ (ofwel cerebrovasculair accident (CVA) of transient ischemic accident (TIA)) een hoger risico op het ontwikkelen van boezemfibrilleren.[8] Patiënten met boezemfibrilleren kunnen ernstige beperkingen in het dagelijks leven ondervinden door bijvoorbeeld bijkomende klachten van kortademigheid, duizeligheid en hartkloppingen.[9–12] Echter, niet alle patiënten ervaren symptomen van boezemfibrilleren en het boezemfibrilleren kan dan onopgemerkt en onbehandeld blijven. Dit kan gevaarlijk zijn, aangezien patiënten met boezemfibrilleren een verhoogd risico hebben op hartfalen, dementie, een CVA of TIA en overlijden.[1,6] Het is daarom belangrijk te kunnen bepalen wie risico loopt om boezemfibrilleren te ontwikkelen. Boezemfibrilleren kan dan sneller worden ontdekt, behandeld en de risico's op geassocieerde ziekten worden verkleind.

Boezemfibrilleren en genetica

Ondanks dat veel klinische factoren het ontwikkelen en de progressie van boezemfibrilleren kunnen verklaren, blijft het ontstaan en de progressie van boezemfibrilleren bij een deel van de patiënten onverklaarbaar. In de afgelopen

jaren is er bewijs gevonden voor een genetische component die boezemfibrilleren veroorzaakt. De geschatte genetische bijdrage aan het ontstaan van boezemfibrilleren is ongeveer 22%.[13]

De genetische component van boezemfibrilleren werd eerst onderzocht in families waar boezemfibrilleren veel voorkwam. In 2004 onderzocht de Framingham Heart Study boezemfibrilleren in ouders en hun nageslacht. Zo bleek dat als één ouder met boezemfibrilleren was gediagnosticeerd het nageslacht een hoger risico had op het ontwikkelen van boezemfibrilleren.[14] Kort daarna lieten steeds meer analyses zien dat familiaire clustering van boezemfibrilleren veroorzaakt zou kunnen worden door genetische overdracht.[15–17] Eerder werden er genetische analyses gedaan met bijvoorbeeld "candidate genes". De genetische loci werden onderzocht op basis van hun waarschijnlijke biologische betrokkenheid met boezemfibrilleren. Echter waren de onderzoeksgroepen vaak te klein om daadwerkelijk een associatie aan te tonen. De genetische analyse technieken ontwikkelden zich en grootendeels door internationale samenwerking via het Atrial Fibrillation Genetics (AFGen) consortium groeide wereldwijd de database van veel voorkomende genetische varianten. In 2007 werd de eerste genome-wide association studies (GWAS) voor boezemfibrilleren verricht met 550 patiënten en 4476 controle personen.[18] Eén van de eerste genetische loci die met GWAS gevonden werd was *PITX2*, op het chromosoom 4q25. Pitxc2 is een transcriptiefactor die in de embryonale ontwikkeling van de atria, sinusknoop en links-rechts asymmetrie van het hart een rol speelt.[18,19] De populaties waarin GWAS werden uitgevoerd konden snel worden uitgebreid in aantal. Als gevolg hiervan groeide het aantal genetische varianten geassocieerd met boezemfibrilleren in de afgelopen decennia uit tot meer dan 100. Met de genetische varianten kunnen genetische risico scores worden gemaakt die het genetische risicoprofiel op boezemfibrilleren in de populatie zichtbaar kunnen maken.[20] Tevens kunnen genetische varianten meer inzicht geven in oorzakelijke verbanden tussen boezemfibrilleren en klinische risicofactoren. Mendelian randomisatie is een genetische analyse techniek die vergelijkbaar is met een gerandomiseerd onderzoek waarmee oorzakelijke verbanden kunnen worden onderzocht.[21,22] De uitkomst van de groep met genetische risico varianten wordt vergeleken met een groep zonder genetische risicovarianten. Zo worden genetische determinanten van een klinische risicofactor van boezemfibrilleren onderzocht op hun relatie met het ontstaan van boezemfibrilleren en kan bewijs worden geleverd voor een oorzakelijk verband tussen de onderzochte risicofactor van boezemfibrilleren en het ontstaan van boezemfibrilleren.

Doel van dit proefschrift. In dit proefschrift is met behulp van onder andere de internationale samenwerking binnen het AFGen consortium de bijdrage van genetische determinanten van boezemfibrilleren in combinatie met de huidige klinische informatie onderzocht. In deel I van het proefschrift worden voornamelijk de genetische en klinische determinanten van het ontwikkelen van boezemfibrilleren onderzocht. In deel II worden de genetische en klinische determinanten na de diagnose van boezemfibrilleren onderzocht.

Genetische determinanten van boezemfibrilleren. Het proefschrift begint met een uitgebreid overzicht van genetisch onderzoek over boezemfibrilleren (**hoofdstuk 1**). In **hoofdstuk 2** wordt ingezoomd op een genetische marker van leeftijd, de telomeerlengte. Telomeren zijn structuren die op het einde van chromosomen DNA degradatie tegen gaan. Echter, met elke celdeling verkorten de telomeren. Telomeer verkorting kan uiteindelijk leiden tot verminderde stabiliteit van chromosomen en celdood tot gevolg hebben.[23] In **hoofdstuk 2** wordt duidelijk dat, in tegenstelling tot de chronologische leeftijd, de biologische leeftijd in de vorm van telomeerlengte niet geassocieerd is met boezemfibrilleren. Chronologische leeftijd en andere risicofactoren zijn waarschijnlijk sterkere voorspellers van boezemfibrilleren. Het leeftijdsgeassocieerde risico op boezemfibrilleren wordt mogelijk in andere (nog onbekende) genetische markers in cardiaal weefsel geuit. Verder onderzoek is nodig om deze hypothese te bevestigen.[24,25]

Een lage, maar ook een hoge rust hartslag zijn in de literatuur geassocieerd met boezemfibrilleren.[26–31] Om te onderzoeken of de snelheid van de rust hartslag boezemfibrilleren veroorzaakt werd er in **hoofdstuk 3** Mendelian randomisatie toegepast. Genetische determinanten van de rust hartslag werden in een Mendelian randomisatie analyse gebruikt als afgeleide van de rust hartslag. De resultaten lieten zien dat een rust hartslag onder de 65 per minuut boezemfibrilleren zou kunnen veroorzaken. Echter, tegen onze verwachtingen in, was een hogere rust hartslag dan 65 slagen per minuut niet geassocieerd met het veroorzaken van boezemfibrilleren. Mogelijk zijn andere cardiovasculaire risicofactoren die geassocieerd zijn met een hoge rust hartslag medeverantwoordelijk voor het ontstaan van boezemfibrilleren. De resultaten van onze studie kunnen als basis gebruikt worden voor studies die in de toekomst een lage rust hartslag willen onderzoeken als risico indicator voor boezemfibrilleren.

Als patiënten boezemfibrilleren ontwikkelen zijn er verschillende vormen te onderscheiden.[6] In **hoofdstuk 4** hebben we de verschillende vormen van

boezemfibrilleren onderzocht. We ontdekten dat bepaalde genetische variaties alleen voorkwamen in patiënten met paroxysmaal boezemfibrilleren.[32] Onze resultaten geven aan dat genetische varianten een belangrijke rol kunnen spelen voor sommige patiënten. De huidige Europese richtlijnen benadrukken het belang van gepersonaliseerde zorg en behandeling. Het identificeren van de vormen van boezemfibrilleren en het bijbehorende genetische profiel kan het personaliseren van de zorg van boezemfibrilleren verbeteren.

Eén van de manieren om het genetische profiel van boezemfibrilleren zichtbaar te maken, is het construeren van een genetische risicoscore. In **hoofdstuk 5** hebben we met een genetische risicoscore van BMI de progressie van boezemfibrilleren onderzocht. BMI is één van de risicofactoren van progressie van boezemfibrilleren.[33] Gewichtsverlies kan de belasting van boezemfibrilleren en de symptomen van boezemfibrilleren ten goede komen.[34,35] Onze resultaten lieten zien dat genetische determinanten van BMI in vrouwen een verhoogd risico op progressie van boezemfibrilleren gaven.[36] Genetische determinanten van BMI kunnen mogelijk boezemfibrilleren veroorzaken. Echter, verder onderzoek is nodig om daadwerkelijk oorzakelijke verbanden te leggen. Ook zal er meer onderzoek moeten worden gedaan naar het effect van verandering in BMI en het genetische risico op het ontwikkelen van progressie van boezemfibrilleren.

In **hoofdstuk 6** onderzochten we genetische aanwijzingen voor een oorzakelijke verband tussen boezemfibrilleren en linker boezem grootte en functie. Druk en/of volume vergroting van het linker boezem is vaak aanwezig bij individuen met progressie van boezemfibrilleren en kan verandering in de structuur en functie van de linker boezem veroorzaken.[4,5,37] Verandering in structuur en functie van de boezem wordt ook wel remodellering genoemd. Remodellering kan episodes van boezemfibrilleren triggeren en episodes van boezemfibrilleren kunnen het proces van remodellering onderhouden – een vicieuze cirkel.[4,38] Onze resultaten laten via Mendelian randomization analyses zien dat er genetische aanwijzingen zijn voor een oorzakelijk verband tussen boezemfibrilleren en linker boezem grootte. In de "leave-one-out" analyse werd onderzocht of de associatie nog steeds bestaat als één genetische variant van boezemfibrilleren niet wordt meegenomen in de analyse. Een genetische variant op het *PITX2* gen lijkt een grote rol te spelen in de associatie tussen boezemfibrilleren en linker boezem grootte.[39] Het is vooralsnog onbekend of optimale behandeling, met als gevolg minder episodes van boezemfibrilleren, *PITX2* expressie kan verminderen en daarmee structurele

en functionele verandering van de linker boezem kan voorkomen of minimaliseren. Data over genetisch gedreven behandelingen zijn namelijk schaars.

Conclusies. In de afgelopen decennia is de kennis over genetica en boezemfibrilleren wereldwijd toegenomen. Internationale samenwerking via het AFGen consortium heeft geleid tot één van de grootste bijdrages aan GWAS analyses van boezemfibrilleren. Door internationale samenwerking binnen dit consortium konden in de afgelopen jaren de power van GWAS analyses toenemen en de voor alsnog missende informatie over genetica en boezemfibrilleren worden aangevuld. Zo kon het aantal genetische varianten geassocieerd met boezemfibrilleren worden uitgebreid.

Dit proefschrift laat zien dat verschillende genetische profielen van patiënten met boezemfibrilleren kunnen worden onderscheidden en genetische varianten oorzakelijke verbanden tussen klinische determinanten van boezemfibrilleren kunnen onthullen.

De vertaling van genetische informatie naar de klinische praktijk is nog beperkt, aangezien de onderliggende biologische mechanismen nog grotendeels onbekend zijn. Beter begrip van genetica in boezemfibrilleren zal hopelijk betere preventieve maatregelen, detectie en behandeling voor boezemfibrilleren opleveren en dus de impact van de ziekte op het individu en de samenleving verkleinen. In de komende decennia is het belangrijk om ontdekkingen op genetisch gebied verder te kunnen gaan vertalen naar nieuwe moleculaire aanknopingspunten, behandelresultaten en gepersonaliseerde therapieën. Immers, als de screening en behandeling voor boezemfibrilleren verder gepersonaliseerd kan worden, komt dat in de toekomst het welzijn van personen die risico lopen op het ontwikkelen van boezemfibrilleren ten goede.

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Biography

Joylene Elisabeth Siland was born in Gorinchem, the Netherlands, on the 24th of May 1993. She attended secondary school at Gymnasium Camphusianum in Gorinchem. After graduation, Joylene started studying medicine at the University of Groningen in 2011.

During her bachelor's degree she participated in Junior Scientific Masterclass activities, including a pilot project at the department of cardiology supervised by prof. dr. M. Rienstra and prof. dr. I.C. van Gelder. She gained interest in cardiovascular research and in 2015 she performed her master's research project at the department of cardiology at the University Medical Center Groningen. In 2016 she received a personal MD-PhD grant which allowed her to pursue a PhD degree while simultaneously completing a master's degree in medicine. Under the guidance of prof. dr. M. Rienstra, prof. dr. P. van der Harst and prof. dr. I. C. van Gelder she became involved in genetic research of atrial fibrillation. In collaboration with the international AFGen consortium DNA of atrial fibrillation patients from Groningen were analyzed and their data contributed to the discovery of new genetic loci and insights. This data was fundamental to her thesis "Genetic determinants of atrial fibrillation. Converging genetic and clinical information".

During her studies she was a competitive rower at the Groningen rowing society Aegir for four years, participated in several student committees, including as chairwoman of the charity committee, and joined the local gospel choir.

Joylene enjoyed her clinical rotations in the University Medical Center of Groningen, Medical Spectrum Twente in Enschede and Stanger Hospital in Kwa-Dukuza, South Africa. She developed a special interest in cardiothoracic surgery. In 2020 she graduated from medical school and started working as a physician at the department of cardiothoracic surgery department at the University Medical Center Utrecht. In September 2021 she will continue her career in cardiothoracic surgery at the University Medical Center Groningen.