The work presented in this thesis was conducted at the department of Medical Informatics of the Erasmus Medical Center, Rotterdam, the Netherlands

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# QT Variability and Other Electrocardiographic Predictors of Sudden Cardiac Death

Marten Enne van den Berg

**QT Variability and Other Electrocardiographic Predictors of Sudden Cardiac Death**

**QT variabiliteit en andere electrocardiografische voorspellers van plotse hartdood**

Thesis

to obtain the degree of Doctor from the

Erasmus University Rotterdam

by command of the

rector magnificus

Prof.dr. H.A.P. Pols

and in accordance with the decision of the Doctorate Board.

The public defense shall be held on

Wednesday October 4, 2017 at 15:30 hours

by

Marten Enne van den Berg

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**Chapter 1.2** Noordam R, Van den Berg ME, Niemeijer MN, Aarts N, Hofman A, Tiemeier H, Kors JA, Stricker BH, Eijgelsheim M, Visser LE Rijnbeek PR Antidepressants and heart-rate variability in older adults: a population-based study. *Psychol Med.* 2016 Apr;46(6):1239-47.

**Chapter 1.3** Noordam R, Van den Berg ME, Niemeijer MN, Aarts N, Leening MJ, Deckers JW, Hofman A, Rijnbeek PR, Eijgelsheim M, Kors JA, Stricker BH, Visser LE. Assessing Prolongation of the Heart Rate Corrected QT Interval in Users of Tricyclic Antidepressants: Advice to Use Fridericia Rather Than Bazett's Correction. *J Clin Psychopharmacol.* 2015 Jun;35(3):260-5.

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**Chapter 2.1** Niemeijer MN, Van den Berg ME, Eijgelsheim M, van Herpen G, Stricker BH, Kors JA, Rijnbeek PR. Short-term QT variability markers for the prediction of ventricular arrhythmias and sudden cardiac death: a systematic review. *Heart*. 2014 Dec;100(23):1831-6.

**Chapter 2.2** Rijnbeek PR, Van den Berg ME, van Herpen G, Ritsema van Eck HJ, Kors JA. Validation of automatic measurement of QT interval variability. Rijnbeek PR, van den Berg ME, van Herpen G, Ritsema van Eck HJ, Kors JA. *PLoS One*. 2017 Apr 12;12(4):e0175087.

**Chapter 2.3** Van den Berg ME, Kors JA, Niemeijer MN, Van Herpen G, Bots ML, Hillege H, Swenne CA, Eijgelsheim M, Stricker BH, Rijnbeek PR. Normal values of QT variability in 10-second electrocardiograms for all ages. *Submitted*

**Chapter 2.4** Van den Berg ME, Niemeijer MN, Deckers JW, Franco OH, Hofman A, Van Herpen G, Kors JA, Stricker BH, Eijgelsheim M, Rijnbeek PR. QT variability as risk factor for sudden cardiac death, cardiac mortality, and all-cause mortality. *Submitted*

**Chapter 2.5** Van den Berg ME, Niemeijer MN, Eijgelsheim M, Deckers JW, Hofman A, Nieboer D, Franco OH, Stricker BH, Kors JA, Rijnbeek PR. Additional value of electrocardiographic markers for predicting sudden cardiac death in the middle-aged and elderly. *In preparation*

**Chapter 2.6** Van den Berg ME, Niemeijer MN, Leening MJ, Deckers JW, Van Herpen G, Kors JA, Stricker BH, Eijgelsheim M, Rijnbeek PR. QT variability is associated with incident heart failure in a prospective population-based cohort study. *In preparation*

**Chapter 2.7** Van den Berg ME, Chaker L, Niemeijer MN, Kors JA, Eijgelsheim M, Rijnbeek PR, Peeters RP, Stricker BH. Does thyroid function affect QT variability? A population-based study. *In preparation*

**Chapter 3.1** Van den Berg ME, Stricker BH, Brusselle GG, Lahousse L. Chronic obstructive pulmonary disease and sudden cardiac death: A systematic review.*Trends Cardiovasc Med.* 2016 Oct;26(7):606-13.

**Chapter 3.2** Chaker L, Van den Berg ME, Niemeijer MN, Franco OH, Dehghan A, Hofman A, Rijnbeek PR, Deckers JW, Eijgelsheim M, Stricker BH, Peeters RP. Thyroid Function and Sudden Cardiac Death: A Prospective Population-Based Cohort Study. *Circulation.* 2016 Sep 6;134(10):713-22.

# General Introduction

Sudden cardiac death (SCD), the definition of which will be given later, comprises half of all cases of coronary heart disease (CHD) mortality,[1] which in turn account for half of all deaths globally.[2] Assessment of the risk of SCD in members of the general population is of great importance because about half of the cases of SCD occur in people without a previous history of cardiac disease, and rates of successful resuscitation remain low at about 8%).[3] The electrocardiogram (ECG) is already being used as a tool to predict SCD,[4] but it has not yet reached its full potential Further studying and combining currently used ECG markers and exploring the potential of new ones could aid in the prediction of SCD in the future.

*Historical background of sudden death*

In biblical times, sudden death was often viewed as divine punishment. In the Old Testament, this view is expressed by Elihu to Job in the book of Job. Speaking of the unrighteous, he says:

> "In a moment they die;
> at midnight the people are shaken and pass away,
> and the mighty are taken away by no human hand." (Job 34:20, NRSV)

In the New Testament we find the cautionary tale of Ananias and Sapphira (Acts 5:1-11) who die instantly as they are lying to the apostle Peter about money held back from the communal fund. However, where Elihu describes a natural phenomenon (sudden death at night) and gives it a supernatural explanation, the timing of the deaths in the story of Ananias and Sapphira seems to indicate a wholly supernatural or legendary phenomenon, outside the purview of medical science.

In classical mythology, the god Apollo's arrows strike swiftly and deadly from far. He kills the 7 sons and 7 daughters of Niobe in quick sequence for the sole reason that she had affronted his mother Latona (Ovid's Metamorphoses VI:148-312). Also, in the first book of Homer's Iliad, his arrows wreak havoc on the Greek warriors in retaliation for the slight they had inflicted on his priest Chryses. Contrary to this view of sudden death as a manifestation of divine wrath, there were not a few who hailed it as a divine grace, mostly extended by the same [marksman] Apollo, who therewith spared one a perhaps miserable natural deathbed.

The first person to describe sudden death not as divine intervention but as a natural phenomenon was Hippocrates of Cos (460BC - 370 BC). In *Aphorisms*, one of the books ascribed to him, he makes the following observation:

> "Those who are subject to frequent and severe fainting attacks without obvious cause die suddenly" (Aphorisms II, No. 41)

This observation not only describes sudden death, but also indicates that it might be predicted by signs, just like any other disease. According to some modern medical historians, it is not unlikely that

this observation describes congenital long-QT syndrome, currently one of the known causes of SCD.[5] In another observation in the same book, Hippocrates describes another warning sign for sudden death:

> "Those who are constitutionally very fat are more apt to die quickly than those who are thin" (Aphorisms II, No. 44)

This observation is correct in marking obesity as a risk factor for SCD, often by way of CHD.

*Modern view of SCD*

Based on the circumstances during death, two types of SCD are distinguished in the modern medical literature. The first is that of witnessed SCD. The most common definition of witnessed SCD, which is endorsed by the European Society of Cardiology, is given by Myerburg: "Natural death due to cardiac causes, heralded by abrupt loss of consciousness within one hour from onset of acute symptoms. Preexisting heart disease may have been known to be present, but the time and mode of death are unexpected."[6,7] The second type is that of unwitnessed SCD. A case is considered unwitnessed SCD if the person is found dead, be it in bed or on the street, having been known to be in a stable medical condition in the 24 hours before discovery, and if there is no evidence of a non-cardiac cause of death.[8] Although the distinction between unwitnessed and witnessed SCD rests on coincidence i.e. whether others happened to be present at the incident, the distinction is important in view of the difference in certainty and level of information between the circumstances of death.

Most cases of SCD are thought to be the result of an acute event or trigger acting in the context of an earlier disease substrate.[4] The acute triggers include acute ischemia, environmental stress, and acute hemodynamic stress.[4] The disease substrate can be any cardiac pathology that interferes with the ability of the heart to prevent fatal arrhythmias, such as ventricular tachycardia or ventricular fibrillation.[9] There are many different disease substrates that can lead to SCD, but the most common in both men and women is CHD.[10] Other common disease substrates are valvular heart disease, dilated cardiomyopathy, and hypertrophic cardiomyopathy.[4,10,11] A rarer but well-described group of disease substrates is that of the long-QT syndromes.[12,13] These are syndromes characterized by a long QT interval on the ECG, the occurrence of syncope, and a high risk of SCD. Long QT syndromes can be congenital or drug-induced. The congenital variants are caused by mutations in the cardiac myocyte ion channels that are responsible for the repolarization of the heart,[12] while the drug-induced variants are caused by drugs that block these ion channels.[13] Subsidiary risk factors for SCD that are contributory to the aforementioned disease substrates are smoking,[14,15] hypertension,[16] high serum LDL/HDL cholesterol ratio,[17] diabetes mellitus,[18] etc. The aforementioned long QT interval on the ECG is a risk factor in the general population, but not the

only ECG-based harbinger of serious cardiac trouble.[19] Many aspects of cardiac function and pathology associated with underlying causes of SCD can be detected by the ECG (e.g., CHD, cardiac hypertrophy), but accurate prediction of SCD has not yet been achieved.[20]

*The incidence and impact of SCD*

SCD is the outcome of various disease processes, the main one being CHD.[11] CHD is the number one cause of death globally,[2] and it is estimated that half of all cases of cardiac death occur suddenly and as the first, and last, sign of cardiac disease.[1] The scope of the problem can be illustrated with the fact that an estimated 210,000 cases of SCD occur in the United States annually.[21] Although the incidence of SCD seems to be decreasing somewhat in the Western world, there remains a large burden of mortality.[22] Resuscitation techniques have improved, but the results of resuscitation remain poor,[21] and the impact and costs of the post-cardiac arrest syndrome makes prevention and recognition of potential SCD cases in the general population a priority.[23] CHD is a much more frequent cause of SCD in men than in women. A study of survivors of cardiac arrest found that in men, CHD accounted for 80% of the cases, while in women, this is only 45%. The same study found that the second common underlying cause of SCD, dilated cardiomyopathy, accounts for 19% of the cases in women, but for only 10% in men. The less common disease substrates, such as valvular heart disease and long QT syndrome also differ in frequency between men and women.[11,24]

*The electrocardiogram*

It was the British physiologist Augustus Waller who coined the term “electrocardiogram” and who made the first ECG in 1887. However, with the technology available at the time, it took hours to obtain a somewhat decent recording.[25] In the 1890s, the Dutch scientist Einthoven devised the string galvanometer, which allowed for quicker and infinitely more accurate ECG recordings. Einthoven’s string galvanometer was a device based on a 3-micron thick silver-coated filament of quartz, suspended within the magnetic field of two poles of a large, water-cooled electromagnet. The filament was made by attaching the molten quartz to the tail of an arrow, which was then fired with a bow across the lab. As the arrow shot away, a thin filament was created.[26] The device weighed 270 kg, occupied two rooms, and required 5 people to operate.[27] Einthoven introduced the standard ECG leads I, II, and III, together forming an equilateral triangle, and coined the terms P, Q, R, S and T marking the successive waves, all of which are still in use today.[28] He received the Nobel Prize for his work on electrocardiography in 1924.[29]

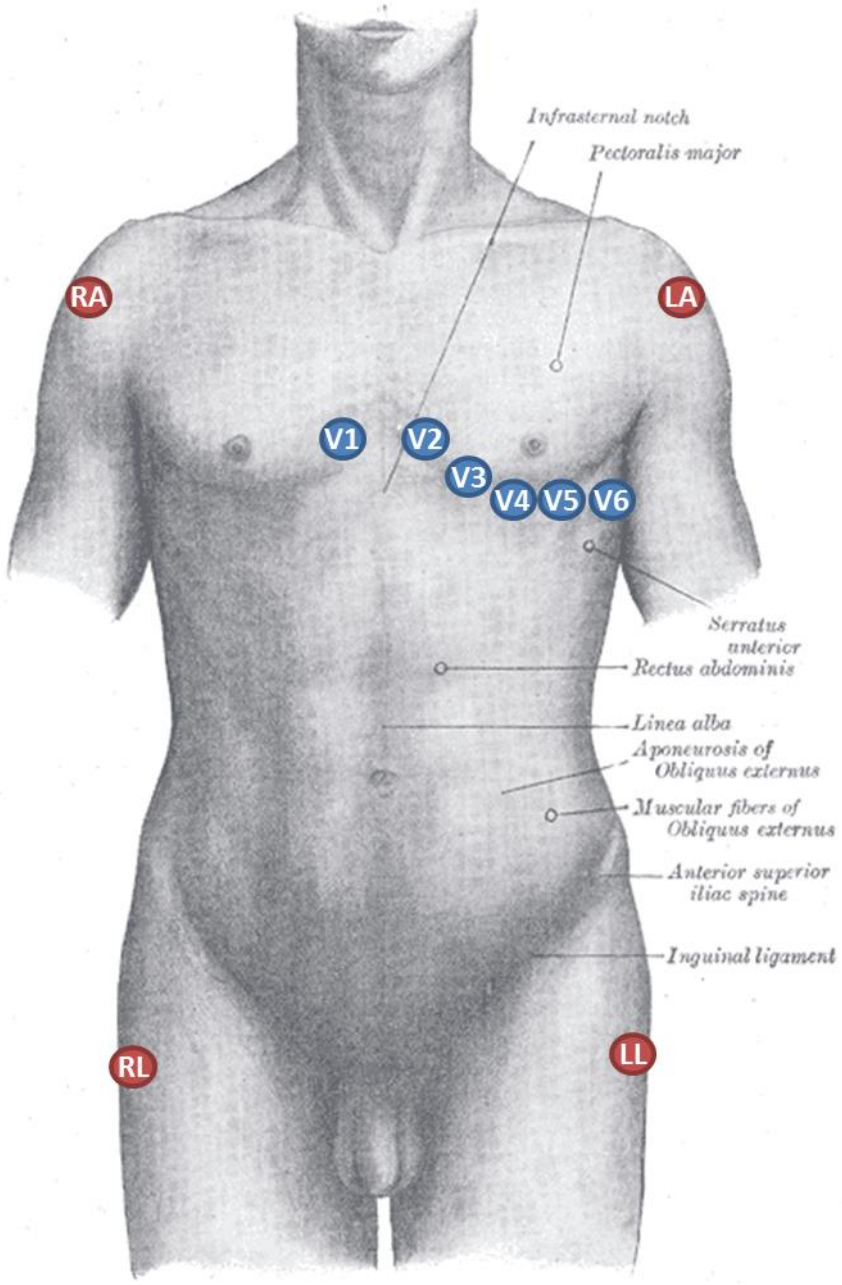
The standard ECG is recorded by ten electrodes that are placed on the body.[30] Figure 1 shows the placement of these electrodes. The voltages measured between opposing electrodes are

called leads. Bipolar leads are voltages measured between two electrodes. Unipolar leads are the voltages measured between an electrode and a common connection of two or three electrodes. The standard ECG consists of 12 leads: three bipolar limb leads (I, II and III), three unipolar augmented limb leads (aVR, aVL and aVF) and six unipolar precordial leads (V1 to V6).[30] The resulting ECG signal is characterized by a series of deflections from the baseline on the aforementioned leads. The general schematic shape of the ECG signal is shown in Figure 2. A positive deflection (going up) indicates that a current is flowing towards the electrode designated as positive, while a negative deflection indicates the opposite.[28] The P wave is the first wave, indicating the depolarization of the atria. The first negative deflection, if there is one, is referred to as the Q wave. This wave indicates the beginning of the depolarization of the ventricles. The Q wave, R wave, and S wave together form the QRS complex, and reflect the depolarization of the ventricles. The T wave represents the repolarization of the ventricles. The QT interval spans the beginning of the Q wave to the end of the T wave (see Figure 1), including both depolarization and repolarization of the ventricles. The last wave, the small U wave, also described by Einthoven, is more prominent in the case of bradycardia, or in cases of hypokalemia. Its origin has not yet been fully clarified.[31]

*Heart-rate variability and QT variability*

Heart-rate variability (HRV) denotes the variability of the duration of the intervals between heart beats (denoted by RR intervals in Figure 1).[32] The earliest studies about HRV date from the 1930s, and were mainly focused on psychiatric patients, an interest that remains to this day.[33,34] A paper from 1936 noted that psychotic patients had a lower HRV than healthy controls.[33] A publication from 1949 reported that women had a higher HRV than men, and that HRV decreased over age.[35]

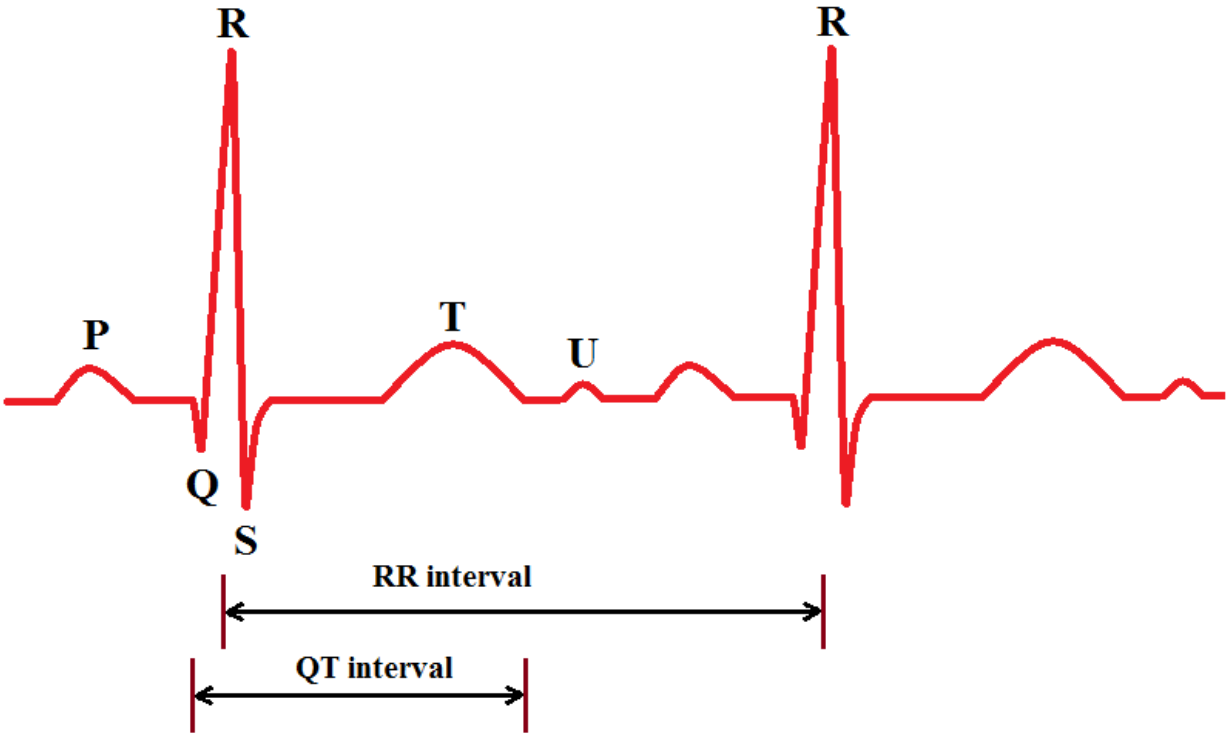
**Figure 1. Placement of the ECG electrodes**




*Based on FIG. 1219 from Anatomy of the Human Body. 1918. by Henry Gray (1825–1861).*

Abbreviations: LA: Left arm, LL: Left leg, RA: Right Arm, V1-V6: Precordial leads 1 to 6.

**Figure 2. The shape of the electrocardiographic recording of two beats**




It should be noted that these old publications have serious limitations: sample sizes were very small (less than 100 participants), in studies that used psychiatric patients, the criteria for diagnosis were not reported, and most important, statistical and computational methods were very crude all models were univariate. Today it is thought that HRV reflects the activity of the autonomic nervous system: high parasympathetic nervous activity is associated with an increased HRV, while high sympathetic nervous activity is associated with a decrease.[36-38] In a healthy heart, HRV and its concomitant parasympathetic activity are relatively high,[38] whereas a low HRV is associated with high adrenergic activity and an increased risk of all-cause mortality,[39-44] cardiac mortality,[41,45] and sudden cardiac death.[46,47]

While HRV in the ECG denotes the variability in RR-interval durations, QT variability denotes the variability in QT-interval durations. QT variability has been studied extensively in animal models (e.g., dogs[48] and rabbits[49]). One study in dogs with a chronic AV-block showed that QT prolongation alone could not predict ventricular arrhythmias, but increased QT variability could.[48] The first paper that studied QT variability in humans is by Berger *et al.* and was published in 1997.[50] This study showed that QT variability is higher in patients with dilated cardiomyopathy than in control subjects. Because dilated cardiomyopathy is associated with a high incidence of malignant ventricular

arrhythmias and sudden death, this study suggested that QT variability is a promising marker for sudden cardiac death,[50] which was an incentive for other studies.[51,52]

*Aim of this thesis*

Prediction and prevention of SCD remain difficult due to a number of factors: The ascertainment of cases is often equivocal, and information on patient characteristics shortly before the occurrence of SCD (e.g., heart rhythm) is not available most of the time.[22] The aetiology of SCD is multifactorial[53] and it is difficult to retrieve the underlying disease state and the triggers that led to the event.[54] About half of the cases of SCD occur in persons without a history of cardiac disease, which calls for tools to estimate their risk of SCD and possibly to prevent it.[55] The ECG is certainly one of these tools,[20] and the aim of this thesis is to explore and extend the utility of the ECG in the risk assessment of SCD.

*General outline of the thesis*

In the first part of the thesis, we closer explore old ECG markers that have been used to predict SCD: heart rate, heart-rate variability, and QT interval. In chapter 1.1, we determine age- and sex dependent normal values for heart-rate variability. In chapter 1.2, we address the issue of whether depression or antidepressants are responsible for the decrease in heart-rate variability in patients with depression. The topic of chapter 1.3 is the method to correct the QT interval for heart rate. We show that Bazett's heart-rate correction for QT duration may lead to overestimation or underestimation of the QTc. We end this part with chapter 1.4, a study of the genetic determinants of heart rate using the exome-chip data in a large meta-analysis involving more than thirty population-based cohorts.

In the second part we focus on QT variability, a relatively new marker for the risk of SCD. In chapter 2.1 we review the current status of the literature about short-term QT variability in humans, including the methods that are used to measure and quantify QT variability, and the topics that have been studied in relation to QT variability. In chapter 2.2 we introduce fiducial segment averaging (FSA), a new method to reliably determine the inflectional points in the ECG and accurately measure individual QT intervals. In the following chapters, we use the FSA algorithm to measure QT variability in standard 10-second ECGs in population-based studies. In chapter 2.3 we present normal limits for QT variability for both men and women, for all ages. In chapter 2.4 we study QT variability as a risk factor for total mortality, CHD mortality, and SCD. In chapter 2.5 we analyse QT variability as a risk factor for heart failure. This part of the thesis ends with chapter 2.6, where we study the association of QT variability and thyroid function.

The third part of this thesis addresses diseases associated with sudden cardiac death. In chapter 3.1, a literature review of the association between SCD and chronic obstructive pulmonary disease (COPD) is presented, and in chapter 3.2, we study the association of thyroid function with SCD. These two studies point to future research of ECG markers for diseases associated with SCD.

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# Part I

# Commonly Used ECG Markers for Sudden Cardiac Death

# Chapter 1.1

# Normal Values of Heart-Rate Corrected Heart-Rate Variability in 10-Second Electrocardiograms for All Ages

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**Abstract**

*Purpose* Heart-rate variability (HRV) measured on standard 10-second electrocardiograms (ECGs) has been associated with increased risk of cardiac and all-cause mortality, but age- and sex-dependent normal values have not been established. Since heart rate strongly affects HRV, its effect should be taken into account. We determined a comprehensive set of normal values of heart-rate corrected HRV derived from 10-second ECGs for both children and adults, covering both sexes.

*Methods* Five population studies in the Netherlands (Pediatric Normal ECG Study, Leiden University Einthoven Science Project, Prevention of Renal and Vascular End-stage Disease Study, Utrecht Health Project, Rotterdam Study) provided 10-second, 12-lead ECGs. ECGs were stored digitally and analyzed by well-validated analysis software. We included cardiologically healthy participants, 46% being women. Their ages ranged from 11 days to 91 years. After quality control, 14,004 ECGs were available. Heart-rate correction formulas were derived using an exponential model. Two time-domain HRV markers were analyzed: the corrected standard deviation of normal-to-normal RR intervals (SDNNc) and corrected root mean square of successive RR-interval differences (RMSSDc).

*Results* There was a considerable age effect. For both SDNNc and RMSSDc, the median and the lower limit of normal decreased steadily from birth until old age. The upper limit of normal decreased until the age of 60, but increased markedly after that age. Differences of the median were minimal between men and women.

*Conclusions* We report the first comprehensive set of normal values for heart-rate corrected 10-second HRV, which can be of value in clinical practice and in further research.

**Introduction**

Heart-rate variability (HRV) as measured on the electrocardiogram (ECG) is the variability of intervals between QRS complexes generated by sinus node depolarization in one continuous recording [1]. Many studies have indicated that reduced HRV is a strong, independent, and consistent risk factor for all-cause and cardiac mortality [2]. HRV guidelines recommend that measurements be based on 5-minute or 24-hour ECG recordings, but 10-second ECGs are more commonly made during routine medical care and are faster, cheaper, and more patient-friendly than longer ECG recordings. Furthermore, even though it is not possible to determine frequency-domain measurements on a 10-second signal, it is possible to obtain valid time-domain measurements, like the standard deviation of the normal-to-normal RR intervals (SDNN) and the root mean square of successive RR-interval differences (RMSSD) [1]. It has recently been demonstrated that time-domain HRV as measured in a given 10-second episode of a 4- to 5-minute pulse-wave recording was in substantial agreement (correlation coefficients varied between 0.76 and 0.93) with HRV derived from the full-length recording [3]. In fact, time-domain HRV markers as measured on 10-second ECGs have been associated with heart failure [4], cardiac mortality [5], and all-cause mortality [6] in population-based studies. In one of these studies [5], both high HRV and low HRV were associated with adverse outcomes. Thus, 10-second HRV seems a promising tool in both research and clinical practice.

HRV is known to have a strong, inverse relationship with heart rate [7-11]. It has been suggested by Monfredi et al. [9] that this relationship is exponential, and that HRV parameters should be exponentially corrected for heart rate. However, the above-mentioned studies either did not adjust for heart rate, or did a linear adjustment for heart rate. As heart-rate itself is a strong risk factor for cardiac morbidity and mortality [12,13], the results of the earlier studies might have been confounded.

Knowledge of normal values of heart-rate corrected HRV markers from 10-second ECGs would allow researchers to derive well-grounded thresholds for continuous variables and enable clinicians to establish diagnostic criteria. A number of studies have reported normal HRV values for 5-minute [14-16] and 24-hour [17] ECG recordings, but only one recent study reported normal values for 10-second ECGs [18], without investigating the age-dependency of HRV. Moreover, none of these studies applied heart-rate correction. Therefore, in this study we determine heart-rate corrected normal values for HRV as derived from 10-second ECGs across all age groups.

**Materials and Methods**

*Study populations*

In this study we combined data from five population studies conducted in the Netherlands. The 10-second 12-lead ECGs from these studies were digitally recorded and stored at sampling rates of at least 500 Hz, up to 1200 Hz in the pediatric group. All data were anonymized.

(1) Pediatric Normal ECG Study [19]. The population of this study consists of 1,912 children, their ages ranging from 11 days to 16 years. The children were recruited in the year 2000 at three child health centers, three primary schools, and one secondary school in the city of Rotterdam. The children's height and weight, measured before ECG recording, corresponded well with the Dutch growth standard. ECGs were recorded with a portable PC-based acquisition system (Cardio Control, Delft, Netherlands).

(2) The Leiden University Einthoven Science Project [20]. The population of this study contains 787 medical students of Leiden University. The ages of the participants range between 17 and 29 years, and all attested to be in good health. The ECGs were recorded from 2005 until 2007 with Megacart electrocardiographs (Siemens, Erlangen, Germany).

(3) The Prevention of Renal and Vascular End-stage Disease (PREVEND) Study [21]. This study, which started 1997, has as its goal to investigate the natural course of microalbuminuria and its relation to renal and cardiovascular disease in the general population. The PREVEND population consists of 8,592 participants aged 28-75 years, from the city of Groningen. Medical records, including medication use, were available for all participants. ECGs were recorded with CardioPerfect equipment (Welch Allyn Cardio Control, USA).

(4) The Utrecht Health Project [22]. This ongoing study started in 2000 in Leidsche Rijn, a newly developed residential area of Utrecht. All new inhabitants were invited by their general practitioner to participate. The population of this study consists of 6,542 participants. Written informed consent was obtained and an individual health profile was made by dedicated research nurses. Baseline assessment included physical examination, ECG, blood tests, and interview-assisted questionnaires. Pharmacy records were used to obtain medication use. ECGs were recorded with CardioPerfect equipment (Welch Allyn Cardio Control, USA).

(5) The Rotterdam Study [23]. This study, which started in 1990, investigates determinants of a number of age-related disorders in an elderly population, prominently among them cardiovascular disease. The Rotterdam Study population consists of 10,994 inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years or older. Participants were visited at home for an interview and were subsequently examined at the research center. Detailed information was collected on health status, medical history, and medication use. ECGs were recorded with an ACTA electrocardiograph (Esaote, Florence, Italy).

From these five populations, totaling 28,827 participants, we selected a subgroup of participants with no indication of cardiac disease. Reasons for exclusion were a history of myocardial infarction, heart failure, coronary bypass surgery, coronary angioplasty, or pacemaker implantation. Other exclusion criteria were hypertension and diabetes mellitus. Hypertension was defined as a systolic blood pressure ≥160 mmHg or a diastolic blood pressure ≥100 mmHg or use of antihypertensive medication, including use of beta-blockers. Diabetes mellitus was defined as a non-fasting serum glucose ≥11 mmol/l or use of glucose-lowering drugs. After applying these criteria, 15,248 individuals were available. We further removed ECGs with excessive noise, excessive baseline wander, premature ventricular beats, premature supraventricular beats, and second or third degree atrioventricular block. Outliers of HRV values were visually checked and discarded if related to poor signal quality. This resulted in 14,004 participants with a low-noise ECG containing only normal beats available for analysis.

*HRV measurement and correction*

RR intervals for all ECGs were automatically determined by the Modular ECG Analysis System (MEANS), an ECG computer program that has been evaluated extensively [24,25]. MEANS signals excessive noise, baseline wander, ventricular complexes, and supraventricular complexes. We calculated two time-domain HRV markers: SDNN and RMSSD. As proposed by Monfredi et al. [9], we corrected the HRV markers for heart rate (HR) with an exponential formula, taking 60 beats per minute as the reference:

$$HRVc = HRV\, e^{\beta\,(60 - HR)}$$

To determine the correction parameter β, we fitted an exponential model:

$$HRV = e^{\alpha + \beta\, HR}$$

After log-transformation, the parameters α and β were estimated using linear regression. To deal with possible confounding by age, regression was performed separately in predetermined age groups as specified in Table 1. Whereas the estimated parameters α considerably differed across age groups, the parameters β were largely similar. We computed an aggregate β as the weighted mean of the age-specific estimates, taking the inverse of the variance of the estimates as the weights [26]. This resulted in the following correction formulas:

$$SDNNc = SDNN\ e^{-0.02294\ (60 - HR)}$$

$$RMSSDc = RMSSD\ e^{-0.03147\ (60 - HR)}$$

*Estimation of normal values*

Centile curves were estimated using the Box-Cox *t* distribution in a semi-parametric model for location, scale and shape [27,28]. The Box-Cox *t* distribution allows for modeling of the distribution of the median, skewness, and kurtosis as functions of age. The *lms* function of the R-package *gamlss* was used for the creation of the centile curves. The 2$^{nd}$ percentile was taken as the lower limit of normal (LLN) and the 98$^{th}$ percentile as the upper limit of normal (ULN). Normal values for all age categories were estimated using the *predict.gamlss* function of the *gamlss* package. The normal values of all age categories in Tables 2 and 3 were estimated based on the modeled Box-Cox *t* distribution, taking the central age in the age group. For example, normal values for the category of 16-20 years were based on the values of participants aged 18 years.

This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center. Since all data were anonymized and retrospectively collected, informed consent of the subjects was not required according to Dutch legislation.

**Results**

Table 1 shows the age and sex distribution of the study population. Most age groups have more than 100 ECGs, only the age groups below six months or above 90 years consist of fewer ECGs.

The median, LLN and ULN of the heart-rate corrected HRV markers, stratified by sex, are shown in Table 2 (SDNNc) and Table 3 (RMSSDc) per age group, and in Figure 1 (SDNNc) and Figure 2 (RMSSDc) as continuous age-dependent curves. Other percentile values of SDNNc and RMSSDc are provided as Supplementary Material. SDNNc and RMSSDc display the same age-dependent pattern. The median and LLN of both markers steadily decrease from childhood to the years of middle and

older age. The ULN also decreases till the age of 50-60, after which both markers show a marked increase of their ULN, resulting in a greater range of normal values in the elderly. The difference between men and women appears to be small until 50 years of age, after which men attain higher ULN and lower LLN values than women.

**Table 1. Age and sex distribution of the study population**

| Age group | No. of boys/men | No. of girls/women | Total |
|---|---|---|---|
| Younger than 1 month | 11 | 8 | 19 |
| 1 to 3 months* | 27 | 23 | 50 |
| 3 to 6 months | 34 | 38 | 72 |
| 6 to 12 months | 69 | 54 | 123 |
| 1 to 3 years | 51 | 52 | 103 |
| 3 to 5 years | 60 | 62 | 122 |
| 5 to 8 years | 123 | 104 | 227 |
| 8 to 12 years | 115 | 164 | 279 |
| 12 to 16 years | 141 | 108 | 249 |
| 16 to 20 years | 156 | 385 | 541 |
| 20 to 30 years | 522 | 844 | 1,366 |
| 30 to 40 years | 1,947 | 1,406 | 3,353 |
| 40 to 50 years | 1,497 | 584 | 2,081 |
| 50 to 60 years | 1,275 | 951 | 2,226 |
| 60 to 70 years | 1,180 | 1,070 | 2,250 |
| 70 to 80 years | 338 | 439 | 777 |
| 80 to 90 years | 51 | 110 | 161 |
| 90 years and older | 1 | 4 | 5 |
| Total | 7,598 | 6,406 | 14,004 |

*The term “to” specifies the upper limit in the sense of “less than”.

**Table 2. Normal values for heart-rate corrected SDNN (in ms) per age group and for both sexes**

| | Median ($2^{nd}$ percentile; $98^{th}$ percentile) | |
|---|---|---|
| Age group | Boys/men | Girls/women |
| < 1 month | 99.9 (32.4; 267.6) | 109.5 (35.1; 281.4) |
| 1 to 3 months* | 99.7 (32.3; 267.2) | 109.1 (34.9; 280.8) |
| 3 to 6 months | 99.1 (32.1; 266.2) | 108.5 (34.7; 279.6) |
| 6 to 12 months | 98.3 (31.8; 264.8) | 107.4 (34.3; 277.7) |
| 1 to 3 years | 95.7 (30.6; 260.1) | 104.1 (33.0; 271.6) |
| 3 to 5 years | 91.5 (28.9; 252.6) | 99.0 (31.1; 262.1) |
| 5 to 8 years | 86.2 (26.8; 242.7) | 92.8 (28.8; 250.5) |
| 8 to 12 years | 78.7 (24.0; 227.7) | 84.4 (25.8; 234.5) |
| 12 to 16 years | 70.1 (21.0; 209.1) | 75.4 (22.6; 216.6) |
| 16 to 20 years | 62.1 (18.3; 190.6) | 67.3 (19.9; 199.5) |
| 20 to 30 years | 50.7 (14.8; 162.8) | 55.5 (16.4; 172.3) |
| 30 to 40 years | 39.8 (11.7; 135.1) | 42.6 (12.9; 138.0) |
| 40 to 50 years | 31.9 (9.5; 117.0) | 32.5 (10.2; 111.4) |
| 50 to 60 years | 25.2 (7.3; 105.2) | 25.1 (8.1; 94.2) |
| 60 to 70 years | 20.9 (5.8; 109.7) | 20.5 (6.8; 90.3) |
| 70 to 80 years | 18.0 (4.7; 136.1) | 18.0 (6.0; 105.7) |
| 80 to 90 years | 15.6 (3.9; 203.4) | 16.7 (5.6; 157.8) |

*The term “to” specifies the upper limit in the sense of “less than”.

**Table 3. Normal values for heart-rate corrected RMSSD (in ms) per age group for both sexes**

| | Median (2nd percentile; 98th percentile) | |
|---|---|---|
| Age group | Boys/men | Girls/women |
| < 1 month | 152.5 (51.6; 443.3) | 162.9 (56.9; 463.4) |
| 1 to 3 months* | 151.8 (51.4; 441.8) | 162.1 (56.5; 461.7) |
| 3 to 6 months | 150.4 (50.8; 438.8) | 160.6 (55.9; 458.3) |
| 6 to 12 months | 148.3 (49.9; 434.2) | 158.3 (55.0; 453.1) |
| 1 to 3 years | 141.5 (47.2; 419.3) | 151.0 (52.0; 436.3) |
| 3 to 5 years | 131.1 (43.1; 396.0) | 139.8 (47.5; 410.6) |
| 5 to 8 years | 118.8 (38.3; 367.5) | 126.8 (42.4; 380.2) |
| 8 to 12 years | 102.7 (32.3; 328.2) | 110.3 (36.0; 340.2) |
| 12 to 16 years | 86.2 (26.5; 284.8) | 93.8 (29.9; 298.8) |
| 16 to 20 years | 72.3 (22.0; 245.7) | 80.2 (25.1; 262.9) |
| 20 to 30 years | 55.1 (16.8; 193.1) | 62.9 (19.4; 213.8) |
| 30 to 40 years | 41.1 (13.0; 146.4) | 46.7 (14.8; 161.7) |
| 40 to 50 years | 32.3 (10.5; 119.4) | 35.0 (11.6; 124.9) |
| 50 to 60 years | 25.3 (8.1; 106.9) | 26.8 (9.3; 104.3) |
| 60 to 70 years | 21.4 (6.4; 122.2) | 22.2 (7.8; 105.6) |
| 70 to 80 years | 19.4 (5.4; 186.5) | 20.3 (7.1; 139.5) |
| 80 to 90 years | 17.8 (4.5; 307.9) | 19.6 (6.7; 209.9) |

*The term “to” specifies the upper limit in the sense of “less than”.

**Figure 1. Median, 2nd and 98th percentiles for heart-rate corrected SDNN in men and women**

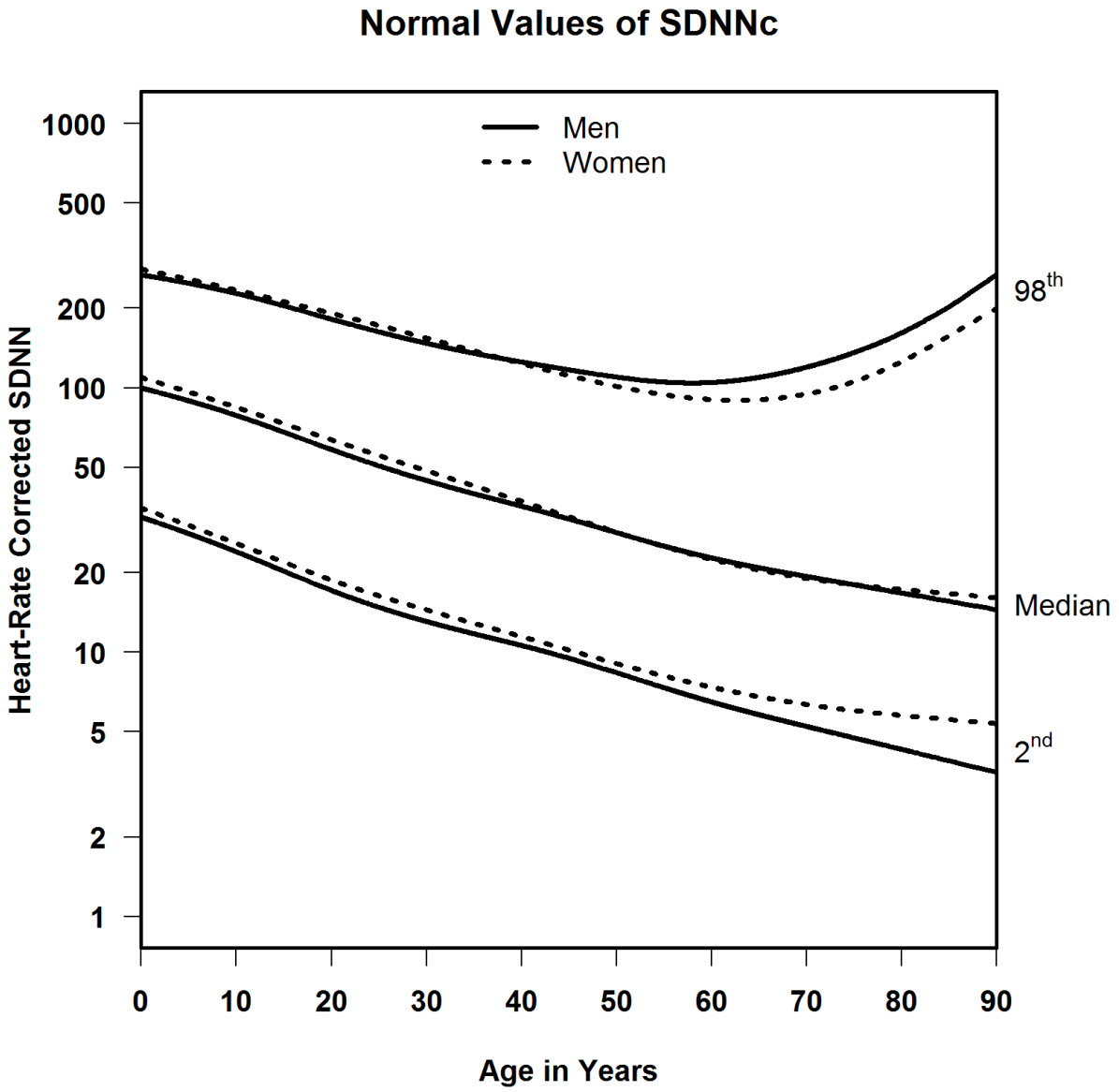


**Figure 2. Median, 2nd and 98th percentiles for heart-rate corrected RMSSD in men and women**

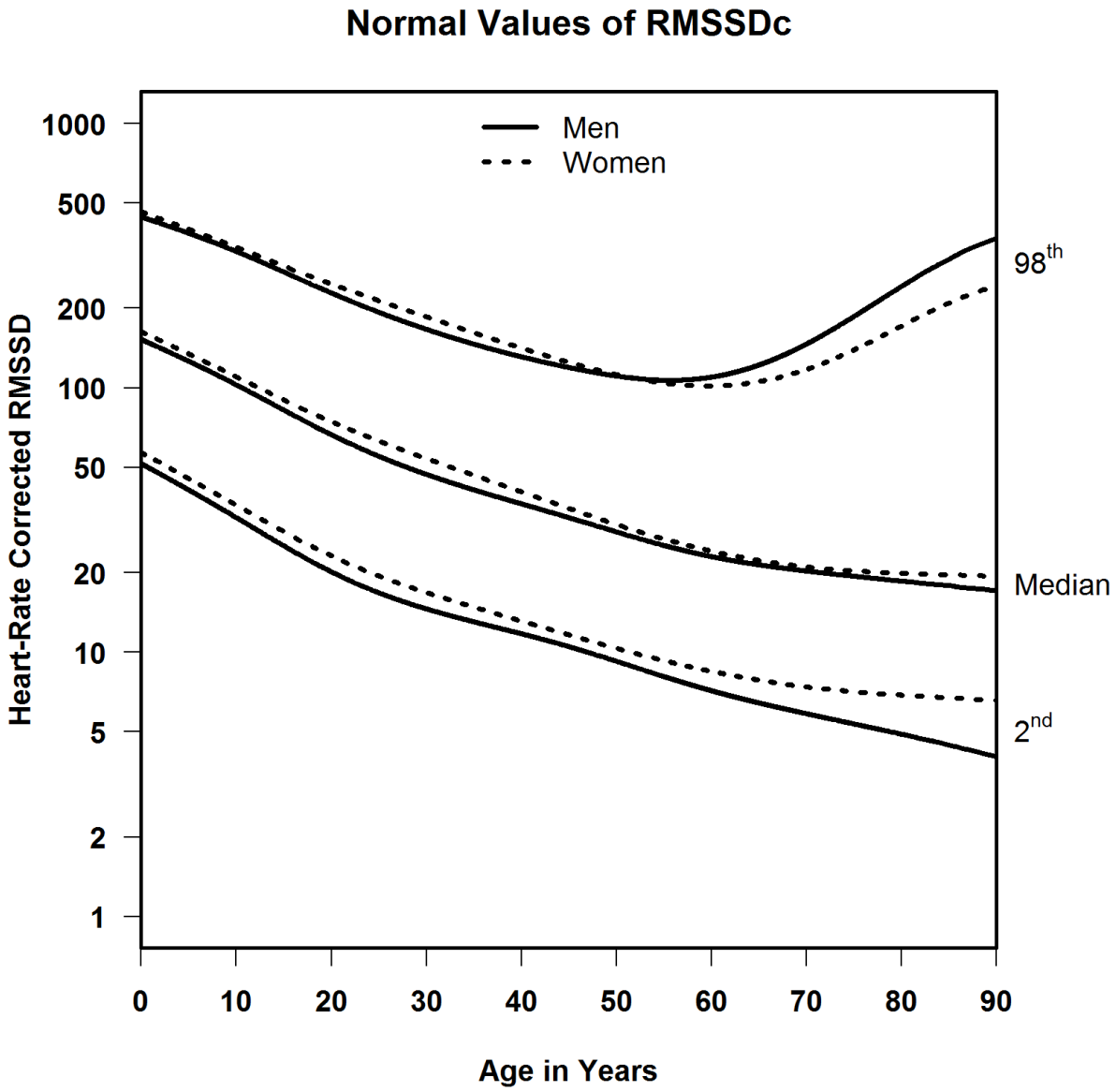
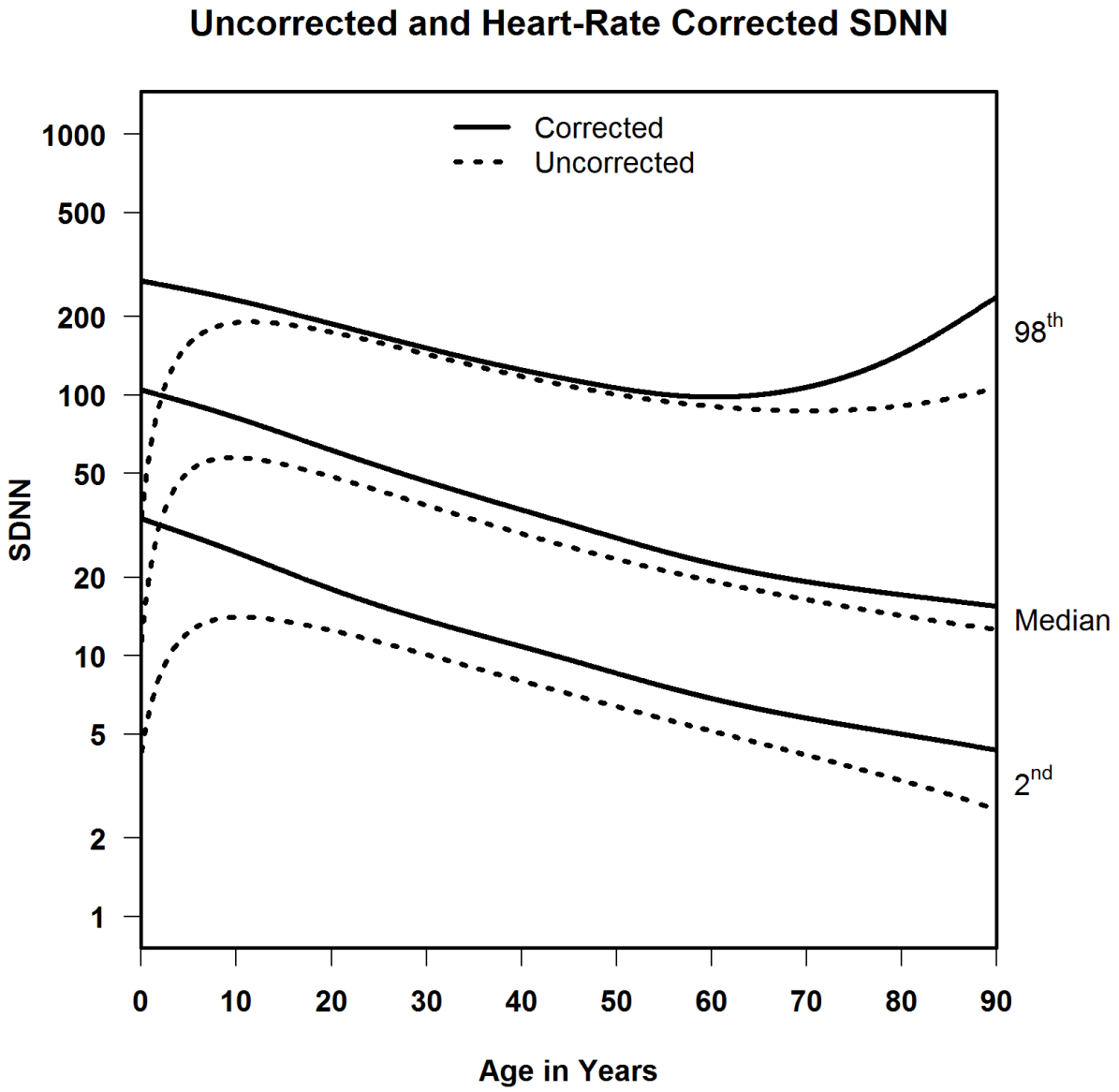


**Figure 3. Median, 2nd and 98th percentiles for heart-rate corrected SDNN and uncorrected SDNN**



**Discussion**

This is the first population-based study to provide heart-rate corrected normal values for SDNN and RMSSD as derived from 10-second ECGs across all ages and for both sexes. Our study shows that in both men and women, the LLN of heart-rate corrected SDNN and RMSSD decreases continuously from birth to old age, whereas the ULN decreases until the age of 50-60 and then starts to rise again.

Several studies calculated uncorrected normal values for HRV from 5-minute or 24-hour ECG signals [14-17]. Nunan et al. [14] published normal values for middle-aged and elderly people in a systematic review of 5-minute SDNN and RMSSD using 44 studies containing 21,438 participants. However, their data were not stratified by age. Seppala et al. [15] reported normal HRV values but only for children aged 6 to 8 years. Additionally, two studies looked at the effect of age. Kim et al. [16] found that 5-minute SDNN and RMSSD decreased between the age of 18 and 50 in both men and women. Umetani et al. [17] also found that 24-hour SDNN decreases in adults, as recorded in 260 healthy participants aged 10-99 years. The decrease of uncorrected 5-minute or 24-hour HRV in aging adults was similar to the decrease in uncorrected 10-second HRV found in our study, as illustrated in Figure 3. There is one other study that calculated normal values of uncorrected 10-second HRV, for middle-aged and elderly participants, but this study did not take age into account [18].

When HRV is not heart-rate corrected, we find a sharp increase between birth and adolescence, a pattern that was also observed by others [29,30]. However, this increase is connected to heart rate, which is known to be strongly age-dependent in the young [19]. After heart-rate correction, SDNNc and RMSSDc decrease continuously from birth to adolescence, and further into older ages. A continuous decrease in HRV indices was also observed in a recent study in children aged 6-13 years [11], after correcting the indices by different powers of the heart rate. These findings underline the fact that meaningful comparison of HRV measurements, and their possible association with adverse outcomes, can only be made if the relationship between HRV and heart rate is properly taken into account, especially in children. Our correction formulas for SDNN and RMSSD can be applied to deal with this issue

We made the observation, not previously reported in the literature, that after the age of 60 the ULN turns upwards, in men even more than in women, while the median and LLN continue their downward course. This finding implies a growing instability in sinus node activity in a part of the aging population. This is perhaps caused by incipient dysfunction of the cardiac excitation and conduction tissues.

Our study has a number of strengths. First, we are the first study to report HRV normal values that are corrected for heart rate. We have a total of 14,004 ECGs with wide age coverage,

from children of 11 days to 90-year old men and women. All ECGs from the five included study cohorts were analyzed automatically by a well-validated program, MEANS, which eliminates intra-observer bias. The use of the 10-second ECG may be seen as a strength and as a limitation. Admittedly, the 10-second ECG contains less information than longer recordings, and may sometimes contain only a few RR intervals for HRV calculation. On the other hand, it was demonstrated in pulse wave recordings that 10-second HRV appears a valid proxy for HRV as derived from longer ECG recordings[3]. Moreover, the 10-second ECG is in universal use, cheap, and easily obtained without extra annoyance for the patient. Its benefits then outweigh its inadequacies. A further limitation of our study is the low number of ECGs in the extremes of the age distribution. For this reason, the normal limits of the groups younger than six months and older than ninety years should be used with caution.

In conclusion, normal limits have been established for heart-rate corrected SDNN and RMSSD, derived from 10-second ECGs, using a consistent and automatic methodology for all ages and both sexes. Our coverage of the pediatric population allows age-specific comparisons of HRV of the pediatric ECGs, which change rapidly from birth to puberty, independent of the rapid change in heart rate in the same life period. Using these normal values, both researchers and clinicians have a tool to decide upon cut-off values of HRV, which improves the applicability of 10-second HRV in practice.

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**Supplementary Table 1. Percentiles of heart-rate corrected SDNN (in milliseconds) for men**

| Age group | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | 51.6 | 64.6 | 78.4 | 107.8 | 152.5 | 215.4 | 294.8 | 356.6 | 443.3 |
| 1 to 3 months* | 51.4 | 64.2 | 78.0 | 107.2 | 151.8 | 214.5 | 293.6 | 355.3 | 441.8 |
| 3 to 6 months | 50.8 | 63.5 | 77.2 | 106.2 | 150.4 | 212.7 | 291.3 | 352.6 | 438.8 |
| 6 to 12 months | 49.9 | 62.5 | 76.0 | 104.6 | 148.3 | 209.9 | 287.8 | 348.6 | 434.2 |
| 1 to 3 years | 47.2 | 59.2 | 72.1 | 99.5 | 141.5 | 201.0 | 276.5 | 335.7 | 419.3 |
| 3 to 5 years | 43.1 | 54.2 | 66.2 | 91.8 | 131.1 | 187.2 | 259.0 | 315.6 | 396.0 |
| 5 to 8 years | 38.3 | 48.4 | 59.3 | 82.7 | 118.8 | 170.8 | 237.8 | 291.1 | 367.5 |
| 8 to 12 years | 32.3 | 41.1 | 50.5 | 70.9 | 102.7 | 148.9 | 209.2 | 257.8 | 328.2 |
| 12 to 16 years | 26.5 | 33.9 | 41.8 | 59.1 | 86.2 | 125.9 | 178.6 | 221.6 | 284.8 |
| 16 to 20 years | 22.0 | 28.1 | 34.8 | 49.3 | 72.3 | 106.3 | 151.9 | 189.6 | 245.7 |
| 20 to 30 years | 16.8 | 21.5 | 26.6 | 37.6 | 55.1 | 81.3 | 117.1 | 147.2 | 193.1 |
| 30 to 40 years | 13.0 | 16.5 | 20.3 | 28.4 | 41.1 | 60.3 | 87.0 | 110.1 | 146.4 |
| 40 to 50 years | 10.5 | 13.3 | 16.2 | 22.5 | 32.3 | 47.2 | 68.6 | 87.9 | 119.4 |
| 50 to 60 years | 8.1 | 10.3 | 12.6 | 17.6 | 25.3 | 37.6 | 56.2 | 74.3 | 106.9 |
| 60 to 70 years | 6.4 | 8.3 | 10.3 | 14.6 | 21.4 | 32.8 | 52.3 | 74.2 | 122.2 |
| 70 to 80 years | 5.4 | 7.1 | 9.0 | 12.9 | 19.4 | 31.0 | 54.5 | 87.4 | 186.5 |
| 80 to 90 years | 4.5 | 6.2 | 7.9 | 11.7 | 17.8 | 29.7 | 58.3 | 107.8 | 307.9 |

*The term “to” specifies the upper limit in the sense of “less than”.

**Supplementary Table 2. Percentiles of heart-rate corrected SDNN (in milliseconds) for women**

| Age group | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | 35.1 | 45.0 | 55.6 | 77.5 | 109.5 | 151.4 | 200.1 | 235.3 | 281.4 |
| 1 to 3 months* | 34.9 | 44.9 | 55.4 | 77.3 | 109.1 | 151.0 | 199.6 | 234.8 | 280.8 |
| 3 to 6 months | 34.7 | 44.6 | 55.1 | 76.8 | 108.5 | 150.1 | 198.6 | 233.6 | 279.6 |
| 6 to 12 months | 34.3 | 44.1 | 54.5 | 76.0 | 107.4 | 148.9 | 197.0 | 231.9 | 277.7 |
| 1 to 3 years | 33.0 | 42.5 | 52.6 | 73.5 | 104.1 | 144.6 | 191.9 | 226.4 | 271.6 |
| 3 to 5 years | 31.1 | 40.1 | 49.7 | 69.6 | 99.0 | 138.1 | 184.0 | 217.6 | 262.1 |
| 5 to 8 years | 28.8 | 37.2 | 46.2 | 65.0 | 92.8 | 130.1 | 174.4 | 207.0 | 250.5 |
| 8 to 12 years | 25.8 | 33.4 | 41.5 | 58.7 | 84.4 | 119.3 | 161.2 | 192.4 | 234.5 |
| 12 to 16 years | 22.6 | 29.4 | 36.7 | 52.1 | 75.4 | 107.4 | 146.6 | 176.2 | 216.6 |
| 16 to 20 years | 19.9 | 25.9 | 32.4 | 46.2 | 67.3 | 96.6 | 133.0 | 160.9 | 199.5 |
| 20 to 30 years | 16.4 | 21.2 | 26.6 | 37.9 | 55.5 | 80.5 | 112.2 | 137.1 | 172.3 |
| 30 to 40 years | 12.9 | 16.6 | 20.5 | 29.1 | 42.6 | 62.0 | 87.5 | 108.1 | 138.0 |
| 40 to 50 years | 10.2 | 12.9 | 15.9 | 22.3 | 32.5 | 47.7 | 68.3 | 85.5 | 111.4 |
| 50 to 60 years | 8.1 | 10.2 | 12.4 | 17.2 | 25.1 | 37.3 | 54.7 | 70.0 | 94.2 |
| 60 to 70 years | 6.8 | 8.4 | 10.1 | 14.0 | 20.5 | 31.1 | 47.5 | 63.1 | 90.3 |
| 70 to 80 years | 6.0 | 7.4 | 8.8 | 12.2 | 18.0 | 28.3 | 46.1 | 65.6 | 105.7 |
| 80 to 90 years | 5.6 | 6.8 | 8.1 | 11.2 | 16.7 | 27.3 | 49.0 | 78.1 | 157.8 |

*The term “to” specifies the upper limit in the sense of “less than”.

**Supplementary Table 3. Percentiles of heart-rate corrected RMSSD (in milliseconds) for men**

| Age group | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | 51.6 | 64.6 | 78.4 | 107.8 | 152.5 | 215.4 | 294.8 | 356.6 | 443.3 |
| 1 to 3 months* | 51.4 | 64.2 | 78.0 | 107.2 | 151.8 | 214.5 | 293.6 | 355.3 | 441.8 |
| 3 to 6 months | 50.8 | 63.5 | 77.2 | 106.2 | 150.4 | 212.7 | 291.3 | 352.6 | 438.8 |
| 6 to 12 months | 49.9 | 62.5 | 76.0 | 104.6 | 148.3 | 209.9 | 287.8 | 348.6 | 434.2 |
| 1 to 3 years | 47.2 | 59.2 | 72.1 | 99.5 | 141.5 | 201.0 | 276.5 | 335.7 | 419.3 |
| 3 to 5 years | 43.1 | 54.2 | 66.2 | 91.8 | 131.1 | 187.2 | 259.0 | 315.6 | 396.0 |
| 5 to 8 years | 38.3 | 48.4 | 59.3 | 82.7 | 118.8 | 170.8 | 237.8 | 291.1 | 367.5 |
| 8 to 12 years | 32.3 | 41.1 | 50.5 | 70.9 | 102.7 | 148.9 | 209.2 | 257.8 | 328.2 |
| 12 to 16 years | 26.5 | 33.9 | 41.8 | 59.1 | 86.2 | 125.9 | 178.6 | 221.6 | 284.8 |
| 16 to 20 years | 22.0 | 28.1 | 34.8 | 49.3 | 72.3 | 106.3 | 151.9 | 189.6 | 245.7 |
| 20 to 30 years | 16.8 | 21.5 | 26.6 | 37.6 | 55.1 | 81.3 | 117.1 | 147.2 | 193.1 |
| 30 to 40 years | 13.0 | 16.5 | 20.3 | 28.4 | 41.1 | 60.3 | 87.0 | 110.1 | 146.4 |
| 40 to 50 years | 10.5 | 13.3 | 16.2 | 22.5 | 32.3 | 47.2 | 68.6 | 87.9 | 119.4 |
| 50 to 60 years | 8.1 | 10.3 | 12.6 | 17.6 | 25.3 | 37.6 | 56.2 | 74.3 | 106.9 |
| 60 to 70 years | 6.4 | 8.3 | 10.3 | 14.6 | 21.4 | 32.8 | 52.3 | 74.2 | 122.2 |
| 70 to 80 years | 5.4 | 7.1 | 9.0 | 12.9 | 19.4 | 31.0 | 54.5 | 87.4 | 186.5 |
| 80 to 90 years | 4.5 | 6.2 | 7.9 | 11.7 | 17.8 | 29.7 | 58.3 | 107.8 | 307.9 |

*The term “to” specifies the upper limit in the sense of “less than”.

**Supplementary Table 4. Percentiles of heart-rate corrected RMSSD (in milliseconds) for women**

| Age group | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | 35.1 | 45.0 | 55.6 | 77.5 | 109.5 | 151.4 | 200.1 | 235.3 | 281.4 |
| 1 to 3 months* | 34.9 | 44.9 | 55.4 | 77.3 | 109.1 | 151.0 | 199.6 | 234.8 | 280.8 |
| 3 to 6 months | 34.7 | 44.6 | 55.1 | 76.8 | 108.5 | 150.1 | 198.6 | 233.6 | 279.6 |
| 6 to 12 months | 34.3 | 44.1 | 54.5 | 76.0 | 107.4 | 148.9 | 197.0 | 231.9 | 277.7 |
| 1 to 3 years | 33.0 | 42.5 | 52.6 | 73.5 | 104.1 | 144.6 | 191.9 | 226.4 | 271.6 |
| 3 to 5 years | 31.1 | 40.1 | 49.7 | 69.6 | 99.0 | 138.1 | 184.0 | 217.6 | 262.1 |
| 5 to 8 years | 28.8 | 37.2 | 46.2 | 65.0 | 92.8 | 130.1 | 174.4 | 207.0 | 250.5 |
| 8 to 12 years | 25.8 | 33.4 | 41.5 | 58.7 | 84.4 | 119.3 | 161.2 | 192.4 | 234.5 |
| 12 to 16 years | 22.6 | 29.4 | 36.7 | 52.1 | 75.4 | 107.4 | 146.6 | 176.2 | 216.6 |
| 16 to 20 years | 19.9 | 25.9 | 32.4 | 46.2 | 67.3 | 96.6 | 133.0 | 160.9 | 199.5 |
| 20 to 30 years | 16.4 | 21.2 | 26.6 | 37.9 | 55.5 | 80.5 | 112.2 | 137.1 | 172.3 |
| 30 to 40 years | 12.9 | 16.6 | 20.5 | 29.1 | 42.6 | 62.0 | 87.5 | 108.1 | 138.0 |
| 40 to 50 years | 10.2 | 12.9 | 15.9 | 22.3 | 32.5 | 47.7 | 68.3 | 85.5 | 111.4 |
| 50 to 60 years | 8.1 | 10.2 | 12.4 | 17.2 | 25.1 | 37.3 | 54.7 | 70.0 | 94.2 |
| 60 to 70 years | 6.8 | 8.4 | 10.1 | 14.0 | 20.5 | 31.1 | 47.5 | 63.1 | 90.3 |
| 70 to 80 years | 6.0 | 7.4 | 8.8 | 12.2 | 18.0 | 28.3 | 46.1 | 65.6 | 105.7 |
| 80 to 90 years | 5.6 | 6.8 | 8.1 | 11.2 | 16.7 | 27.3 | 49.0 | 78.1 | 157.8 |

*The term “to” specifies the upper limit in the sense of “less than”.

# Chapter 1.2

# Antidepressants and Heart-Rate Variability in Older Adults: A Population-Based Study

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**Abstract**

*Background* Tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) may be associated with lower heart rate variability (HRV), a condition associated with increased mortality risk. We aimed to investigate the association between TCAs, SSRIs and HRV in a population-based study.

*Methods* In the prospective Rotterdam Study cohort, up to five ECGs per participant were recorded (1991 – 2012). Two HRV variables were studied: standard deviation of normal-to-normal RR intervals (SDNN) and root mean square of successive RR-interval differences (RMSSD). We compared the HRV on ECGs recorded during use of antidepressants with the HRV on ECGs recorded during non-use of any antidepressant. Additionally, we analyzed the change in HRV on consecutive ECGs. Those who started or stopped using antidepressants before the second ECG were compared with non-users on two ECGs.

*Results* We included 23,647 ECGs from 11,729 participants (59% women, mean age 64.6 years at baseline). Compared with ECGs recorded during non-use of antidepressants (22,971 ECGs), SDNN and RMSSD were lower in ECGs recorded during use of TCAs (296 ECGs) and SSRIs (380 ECGs). Participants who started using TCAs before the second ECG had a lower HRV, and those who stopped had a higher HRV than consistent non-users (p-value < 0.001) at the time of the second ECG recording. Starting or stopping SSRIs was not associated with HRV changes.

*Conclusion* TCAs were associated with a lower HRV in all analyses, indicating a real drug effect. For SSRIs the results are mixed, indicating a weaker association, possibly due to other factors.

**Introduction**

Heart-rate variability (HRV) refers to the beat-to-beat variability in heart rate. HRV is influenced by the parasympathetic and sympathetic autonomous nervous system. HRV is lowered when parasympathetic nerve activity is decreased or when sympathetic nerve activity is increased[1-3]. A relatively low HRV is associated with an increased risk of all-cause mortality[4-9], cardiac mortality[8,10], and sudden cardiac death [11].

Use of antidepressants has been associated with a lower HRV in a number of studies[12-18]. Two population-based studies on the relation between antidepressants and HRV[12,16] reported that use of tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) is associated with a lower HRV. In both studies, the effect of the TCAs on HRV was more pronounced than that of SSRIs[12,16]. This is possibly caused by the anticholinergic effects of TCAs[19]. A meta-analysis reported that depression itself – and not antidepressant use – is associated with a lower HRV[17], while the association between depression and HRV was found to be predominantly driven by the use of antidepressants in a different and larger study[16].

To date, only one population-based cohort study has addressed the association between TCAs, SSRIs and HRV[12]. However, this study neither investigated longitudinal effects of antidepressant use on HRV nor a possible dose-response relationship of antidepressants on HRV. These analyses can add to the evidence for a drug effect instead of an effect observed due to residual confounding. In addition, no studies to date have examined individual antidepressants with regard to their association with HRV, as only class effects were considered. Therefore, in order to address these issues, our objective was to investigate the association between TCA use, SSRI use, and HRV in a general middle-aged and elderly population.

**Methods**

*Study setting*

This study is part of the Rotterdam Study, a prospective population-based cohort study. The design and rationale of the Rotterdam Study have been described in more detail elsewhere[20,21]. In short, from 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district in Rotterdam, the Netherlands, were invited to participate in the initial cohort. A total of 7,983 individuals agreed to participate (response rate 78%). In 2000, the cohort was extended by including all inhabitants from the same district who became 55 years or older, or who had moved into the district after the start of the initial cohort. In this extension of the cohort, 3,011 individuals agreed to participate (response rate 67%). The cohort was additionally extended in 2006 by inviting inhabitants of the same district aged 45 years and older. In total, 3,932 individuals agreed to participate (response rate

65%). Follow-up examinations were conducted approximately every four to five years after baseline examination, with a maximum of five center visits per participant. The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sport of the Netherlands. Written informed consent was obtained from all study participants.

*Study population and selection of ECGs*

ECGs of participants of the Rotterdam Study recorded between January 1st, 1991 and December 31st, 2012 were included. ECGs recorded before January 1st, 1991 were excluded because pharmacy dispensing records were not available. We also excluded ECGs with less than five normal heartbeats and ECGs on which the following pathology was detected: left or right bundle branch block, second degree or third degree atrioventricular block, ventricular hypertrophy according to Sokolow-Lyon criteria, atrial fibrillation. ECGs recorded with a pacemaker rhythm or recorded during the use of monoamine-oxidase inhibitors (ATC code: N06AF/AG) and other antidepressants (ATC code: N06AX) were excluded, because of the low number of prescriptions and heterogeneous pharmacodynamics.

*Antidepressant exposure assessment*

At study entry, more than 95% of the participants had their drug prescriptions filled at one of the seven fully computerized regional pharmacies, which use one common computer network. Dispensing data was available on a day-to-day basis, which included the anatomical therapeutic chemical (ATC) code, the dispensing date, the total amount of tablets/capsules per dispensing, the prescribed daily number of tablets/capsules, and the product name of the drug. Dispensing episodes were calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. If the date of an ECG recording fell within a dispensing episode of TCAs (ATC code: N06AA) or SSRIs (ATC code: N06AB), the participant was considered as being exposed during that ECG recording. We allowed a carry-over period of 7 days to define current users. For individual antidepressants, the complete, 7-digit ATC code was used. The dosage was defined as the ratio between the prescribed daily dosage by the defined daily dosage (PDD/DDD ratio), as determined by the World Health Organization[22].

*Heart-rate variability*

A standard 10-second, 12-lead resting ECG was recorded with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florance, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were

processed by the standardized modular ECG analysis system (MEANS), which has been described previously and has been validated and applied extensively[23-27].

HRV was calculated based on RR intervals between normal heart beats: RR intervals were excluded if they immediately preceded or followed premature ventricular complexes or premature supraventricular complexes [28]. We selected two of the most commonly used HRV variables; the standard deviation of normal-to-normal RR intervals (SDNN) and the root mean square of successive RR-interval differences (RMSSD) [29,30].

*Depression score*

A Dutch version of the Center for Epidemiological Studies Depression (CES-D) scale was used to screen for depressive symptoms. The outcome of this questionnaire is a score ranging between 0 and 60. A higher score indicates more depressed feelings [31,32]. The CES-D questionnaire was taken at visits from 1993 onwards. Therefore, we adjusted for CES-D score in a subsample analysis, using only those persons and ECGs for which a CES-D score is available.

*Covariables*

The following covariables were considered: age, sex, smoking status, body-mass index (BMI), RR interval, hypertension, prevalent coronary heart disease, prevalent diabetes mellitus, heart failure, use of beta-blockers, use of verapamil and use of diltiazem. All covariables were determined at the date of ECG recording. Smoking status (current smoker or non-smoker) was determined by home interview. BMI was calculated by dividing weight by the squared height, with weight in kilograms and height in meters, which were both measured at the study center. Heart rate was taken into account in the form of the RR interval as recorded on the ECG (heart rate equals 60,000 / RR interval), because both HRV measures are also based on the RR interval. Blood pressure was measured twice in sitting position at the upper right arm. The average of these measurements was used. Hypertension was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, or the use of blood pressure lowering medication for the indication hypertension. Coronary heart disease was defined as a prevalent myocardial infarction or as a history of coronary artery bypass graft surgery (CABG) or percutaneous coronary intervention (PCI). Myocardial infarction was adjudicated based on a combination of symptoms, ECG measurements, and enzyme markers indicative for the presence of myocardial infarction [33]. Diabetes mellitus was defined by the use of blood-glucose lowering drugs, a non-fasting glucose level of more than 11.0 mmol/L, or a fasting glucose level of more than 6.9 mmol/L[34]. The diagnosis of heart failure was based on typical signs and symptoms confirmed by objective cardiac dysfunction[33]. Use

of heart-rate affecting drugs (beta-blockers, verapamil, and diltiazem) at the date of ECG recording was based on pharmacy dispensing records.

*Statistical analyses*

Baseline characteristics were assessed at the first eligible ECG recording for each participant. We log-transformed the HRV measures for all analyses, but results are presented back-transformed to the geometric scale. We used linear mixed models to take into account the within-person correlation between multiple visits. We fitted models with different covariance matrices and selected the model that had the lowest Akaike's information criterion (AIC). All statistical models were adjusted for age, sex, RR interval, and heart-rate affecting drugs (beta-blockers, verapamil, and diltiazem). In a second model, we included all other available covariables.

In the first set of analyses, we compared the HRV recorded during TCA use and SSRI use with the HRV recorded during non-use of antidepressants. We repeated these analyses for the individual antidepressants if more than 10 exposed ECGs were available. For paroxetine and amitriptyline, the most frequently prescribed drugs in the Rotterdam Study cohort[35], we additionally analyzed a possible dose-response relationship. In a subsample analysis, we adjusted all previously mentioned analyses for the CES-D score, in the subgroup for which CES-D scores were available. With this adjustment, we aimed to determine if the association between the antidepressants and HRV is mediated by depressive symptoms. In the second set of analyses, we compared the change in HRV of those who started or stopped using TCAs or SSRIs by the time of the second visits with the HRV of non-users on two consecutive visits. Because the HRV variables were log-transformed and because *log(a) – log(b) = log(a/b),* the difference between two log-transformed measurements is presented as the fold-change in HRV of the back-transformed measurement. We used IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, USA) for all analyses. Two-sided p-values below 0.05 were considered statistically significant.

**Results**

*Study characteristics*

In total, 27,833 ECGs were recorded. We excluded 3,004 ECGs (10.8%) because cardiac pathologies had been detected. An additional 1,072 ECGs (3.9%) were excluded because they had less than five normal heartbeats. 110 ECGs were excluded because they were recorded during use of antidepressants other than TCAs and SSRIs. In the final selection, 23,647 ECGs from 11,729 participants were used. Baseline characteristics of the final study population are shown in Table 1. In short, 59 percent of the total study population was woman, and the mean age was 64.6 years with a standard deviation (SD) of 9.5 years.

**Table 1. Baseline characteristics of the study population**

| Characteristic | Total study population<br>N = 11,729 |
|---|---|
| Number of ECGs | 23,647 |
| ECGs per participant, median (range) | 2 (1 – 5) |
| Time between ECG recordings in years, median (IQR) | 4.1 (2.4 – 5.0) |
| | |
| Age in years, mean (SD) | 64.6 (9.5) |
| Women, N (%) | 6,896 (58.8) |
| Body-mass index in kg/$m^2$, mean (SD) | 27.0 (4.1) |
| CES-D score, median (IQR) | 3 (1 – 8) |
| RR interval in milliseconds (SD) | 881 (141) |
| Heart rate in beats per minute, mean (SD) | 70 (11) |
| | |
| Beta-blocker use, N (%) | 1,470 (12.5) |
| Verapamil use, N (%) | 67 (0.6) |
| Diltiazem use, N (%) | 134 (1.1) |
| | |
| Current smoking, N (%) | 2,458 (21.0) |
| Coronary heart disease, N (%) | 677 (5.8) |
| Heart failure, N (%) | 179 (1.5) |
| Hypertension, N (%) | 6,060 (51.7) |
| Diabetes mellitus, N (%) | 845 (7.2) |

Abbreviations: CES-D, Center of Epidemiological Studies Depression Scale; ECG, electrocardiogram; IQR, interquartile range; SD standard deviation; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal RR intervals.

*Antidepressant use and HRV*

The lowest Akaike's information criterion was achieved with the 'first-order autoregressive covariance structure with heterogeneous variances'. This covariance structure was therefore used in all analyses. Table 2 presents the geometric estimated means of SDNN and RMSSD for ECGs made during non-use of antidepressants and during use of TCAs and use of SSRIs. Of all 23,647 included ECGs, 296 were recorded during TCA use and 380 ECGs were recorded during SSRI use.

Compared with ECGs recorded during non-use of antidepressants, ECGs recorded during TCA use had a significant (p-values < 0.05) lower SDNN (18.3 ms for non-use, and 15.6 ms for TCAs) and RMSSD (19.8 ms for non-use and 16.7 ms for TCAs). ECGs recorded during SSRI use also showed a significantly lower SDNN (18.3 ms for non-use and 15.4 ms for SSRI use) and a lower RMSSD (19.8 ms for non-use and 17.1 ms for SSRI use) than ECGs recorded during non-use. Of the individual antidepressants, ECGs recorded during use of clomipramine (27 ECGs), amitriptyline (185 ECGs), nortriptyline (13 ECGs), fluoxetine (35 ECGs) and paroxetine (237 ECGs) showed significantly lower HRV measures than ECGs recorded during non-use of antidepressants. A dose-response relationship was analyzed for amitriptyline and paroxetine, which were the most frequently prescribed antidepressants. A higher prescribed dosage of both amitriptyline (Figure 1A) and paroxetine (Figure 1B) was associated with a statistically significant trend toward a lower SDNN. Results were similar for RMSSD (results not shown).

Similar results were observed when adjusted for all considered covariables in this study (results not shown).

*Analyses for possible mediation by depressive symptoms*

A total of 14,693 ECGs from 9,194 participants were recorded during visit rounds when information of depressive symptoms was available. Of those ECGs, 180 were recorded during use of TCAs and 318 ECGSs were recorded during use of SSRIs. In this subgroup, the associations between TCAs, SSRIs and the HRV measures were similar as observed in the total cohort. Additional adjustment for CES-D score did not materially change these results (Supplementary Tables 1 and 2).

*Longitudinal analysis of antidepressant use and HRV*

Table 3 shows the ECGs made on two consecutive visits, and the estimated mean fold-change in SDNN and RMSSD between two visits. The results show that HRV is reduced when TCA use is started, and that HRV is increased when TCA use is stopped. For SSRIs the same pattern is observed, but with a smaller effect size and no statistically significant difference with consistent non-users.

**Table 2. Heart-rate variability recorded during non-use and use of antidepressants**

| | | | SDNN (ms) | RMSSD (ms) |
|---|---|---|---|---|
| | ECGs | Median PDD / DDD | $Mean_{adj}$ in ms (95% CI) | $Mean_{adj}$ in ms (95%CI) |
| Non-use of antidepressants (ref) | 22,971 | NA | 18.3 (17.0 – 19.7) | 19.8 (18.4 – 21.4) |
| TCA use | 296 | 0.33 | 15.6 (14.0 – 17.4) ‡ | 16.7 (15.0 – 18.7) ‡ |
| - Imipramine | 16 | 0.50 | 15.9 (11.3 – 22.5) | 19.3 (13.6 – 27.4) |
| - Clomipramine | 27 | 0.50 | 10.9 (8.3 – 14.4) ‡ | 11.4 (8.6 – 15.0) ‡ |
| - Amitriptyline | 185 | 0.33 | 16.3 (14.4 – 18.5) | 17.3 (15.2 – 19.7) * |
| - Nortriptyline | 13 | 0.67 | 10.3 (6.8 – 15.5) † | 11.3 (7.4 – 17.2) † |
| - Maprotiline | 48 | 0.50 | 15.5 (12.5 – 19.3) | 17.0 (13.6 – 21.1) |
| SSRI use | 380 | 1.00 | 15.4 (14.0 – 17.0) ‡ | 17.1 (15.4 – 19.0) ‡ |
| - Fluoxetine | 35 | 1.00 | 12.5 (9.8 – 15.8) † | 14.1 (11.1 – 18.0) † |
| - Citalopram | 28 | 1.00 | 16.8 (13.0 – 21.8) | 18.0 (13.9 – 23.3) |
| - Paroxetine | 237 | 1.00 | 15.2 (13.5 – 17.0) ‡ | 16.9 (15.0 – 19.0) † |
| - Sertraline | 37 | 1.00 | 15.8 (12.4 – 20.0) | 16.8 (13.2 – 21.5) |
| - Fluvoxamine | 39 | 1.00 | 17.6 (14.0 – 22.2) | 19.6 (15.5 – 24.7) |

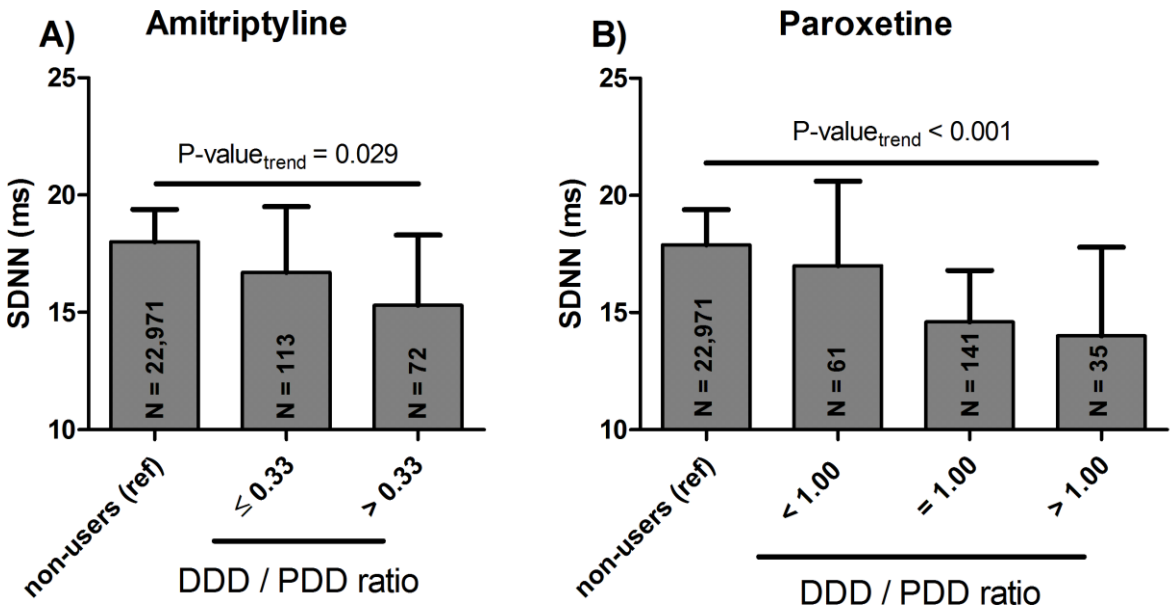
Abbreviations: ref, reference; CI, confidence interval; DDD, defined daily dosage; ECGs, number of electrocardiograms; $Mean_{adj}$ : geometric mean adjusted for covariables; ms: milliseconds; NA, not applicable; PDD, prescribed daily dosage; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal RR-interval; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant. Only individual antidepressants prescribed during more than 10 ECG recordings were analyzed. Analyses were adjusted for age, sex, RR interval, use of beta-blockers, verapamil and diltiazem. * p-value <0.05; † p-value < 0.01; ‡ p-value < 0.001 compared with non-use of antidepressants.

**Table 3. Antidepressant use and fold-change in heart-rate variability between two consecutive visits**

| | | | $Mean_{adj}$ fold-change (95%CI) | |
|---|---|---|---|---|
| Use on first visit | Use on second visit | ECGs | SDNN | RMSSD |
| None | None | 11,103 | 1.06 (0.96 – 1.18) | 1.08 (0.97 – 1.20) |
| None | TCA | 82 | 0.76 (0.62 – 0.94) † | 0.75 (0.61 – 0.93) † |
| None | SSRI | 94 | 0.90 (0.74 – 1.10) | 0.98 (0.80 – 1.20) |
| TCA | None | 65 | 1.49 (1.18 – 1.87) † | 1.64 (130 – 2.08) † |
| SSRI | None | 62 | 1.10 (0.87 – 1.39) | 1.10 (0.87 – 1.40) |

Abbreviations: CI: confidence interval; ECGs : number of electrocardiograms; $Mean_{adj}$ : geometric mean adjusted for covariables; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal RR intervals; SSRI; selective serotonin reuptake inhibitor; TCA: tricyclic antidepressant. Analyses were adjusted for age, sex, RR interval, use of beta-blockers, verapamil and diltiazem. † p-value < 0.001 compared with non-use of antidepressants during both ECGs.

**Figure 1. Association of amitriptyline and paroxetine dosages with heart-rate variability**




Abbreviations: DDD, defined daily dosage; ms, milliseconds; PDD, prescribed daily dosage; SDNN, standard deviation of normal-to-normal RR intervals. The bars represent the mean SDNN, the whiskers are the upper limit of the 95% confidence interval. P-value$_{trend}$ indicates the level of statistical significance across the strata. Analyses were adjusted for age, sex, RR interval, use of beta-blockers, verapamil and diltiazem.

**Discussion**

In this population-based study in older adults, use of TCAs and SSRIs was associated with a lower HRV. In addition, the individual antidepressants clomipramine, amitriptyline, nortriptyline, fluoxetine and paroxetine were significantly associated with a lower HRV and a dose-response relationship of amitriptyline and paroxetine with HRV was observed. In the longitudinal analysis, the start of TCA use, and not SSRI use, was associated with significant changes in HRV. Additional adjustment for CES-D score did not materially change the results.

To our knowledge, only one study to date has analyzed the effect of antidepressant use on HRV in the general older population[12]. This study reported a lower HRV in users of TCAs and SSRIs, which is in line with the results of our study. However, we additionally investigated the effect of individual antidepressants on HRV: all previous studies only considered antidepressant classes. We observed a lower HRV for all individual TCAs, but the difference with non-use of antidepressants was largest for clomipramine and nortriptyline. Imipramine and maprotiline showed much smaller differences in HRV compared with non-use, while the prescribed daily dosages were similar. For amitriptyline, the smaller effect on HRV can be explained by the fact that it was prescribed at a lower median daily dosage than the other TCAs. This is supported by the fact that we found a significant trend towards a lower HRV with higher amitriptyline dosages. For individual SSRIs, the results were difficult to interpret. Users of fluoxetine had a notably lower HRV than users of the other SSRIs while the median daily dosage of all individual SSRIs was the same. Although HRV was somewhat lower during the use of all individual SSRIs, the difference for these individual SSRIs was not statistically significant, which could be due to a low number of users. Therefore, we were not able to exclude the possibility that HRV is dependent on the use of only certain SSRIs. Nevertheless, we observed a significant dose-response relationship for paroxetine, which was not statistically significant in the overall analysis. For this reason, these analyses should be repeated in other studies. Additional adjustment for depressive score did not materially change the results. This finding contradicts the meta-analysis[17], but is in line with a different and larger cohort study[16]. That cohort study suggested that the association between depression and HRV was predominantly driven by the use of antidepressants.

The previously mentioned population-based study[12] had only one measurement per participant and could not assess longitudinal changes over time. One study did assess longitudinal changes in HRV with respect to starting and stopping of TCA treatment and SSRI treatment[18]. They found that starting TCA treatment or starting SSRI treatment was associated with a reduction in HRV. However, starting SSRI treatment was associated with less reduction in HRV than starting TCAs[18]. In our study, we observed no significant decrease in HRV after starting SSRIs, and no significant

increase after stopping SSRIs. The inconsistent results for SSRIs for the cross-sectional and longitudinal analyses might suggest that other, unexamined, factors than the drug itself lead to a lower HRV, but additional research is required to support this suggestion.

The relatively low HRV in users of TCAs might have clinical consequences for an individual patient, as a relatively low HRV has been associated with an increased risk of all-cause mortality[4-9], cardiac mortality[8,10], and sudden cardiac death[11]. TCAs have also been associated with an increased risk of cardiac mortality[36]. Whether this increased risk is due to a low HRV should be determined in subsequent studies.

This study has a number of strengths and limitations. Major strengths were the availability of detailed pharmacy-dispensing data and the availability of multiple ECG recordings for most participants. Also, we used MEANS, which calculates the RR interval and heart-rate variability systematically and automatically. This enhances precision of the measurements and prevents bias in ECG assessment[24,26]. Information bias for drug use was limited as pharmacy-dispensing data was collected prospectively and irrespective of disease state. The analysis using the calculated within-person changes of HRV between two consecutive visit rounds is less subjected to confounding than our cross-sectional analysis and the results are complementary. Finally, we added a variable for depressive symptoms which did not change the results. There are also some limitations to address. First, some of the individual antidepressants were dispensed in low numbers. Second, the median time interval between two ECG recordings was 4.1 years, which limits the interpretation of a change in HRV between visits, as other confounding factors may occur in the long time period. Finally, we used standard 10-second ECG recordings, while the major studies in the literature are based on 5-minute or longer ECGs. However, in two previous studies, HRV derived from 10-second ECGs was still predictive for cardiovascular disease and mortality[9,37], and the results of the first set of analyses in this paper are in line with that of previous studies[12,18]. Besides this, 10-second ECGs are cheaper and more patient friendly.

In conclusion, the results indicate that TCAs are associated with a lower HRV in a general middle-aged and elderly population, a condition associated with an increased risk of mortality. For SSRIs this is less clear, although we observed an association between SSRIs and HRV and a dose-response relationship of paroxetine with HRV.

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# Chapter 1.3

# Assessing Prolongation of the Heart-Rate Corrected QT Interval in Users of Tricyclic Antidepressants: Advice to use Fridericia Rather than Bazett's Correction

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**Abstract**

*Background* A prolonged heart-rate corrected QT interval (QTc) increases the risk of sudden cardiac death. Some methods of heart-rate correction (notably Bazett) overestimate QTc in people with high heart rates. Studies suggest that tricyclic antidepressants (TCAs) can prolong the QTc and increase heart rate. Therefore, we aimed to study whether TCA-induced QTc prolongation is a false-positive observation due to overestimation at high heart rates.

*Methods* For this, we included 12,734 participants from the prospective population-based Rotterdam Study, with a total of 27,068 electrocardiograms (ECGs) of which 331 during TCA use. Associations between use of TCAs, QTc, and heart rate were studied with linear repeated measurement analyses. QT was corrected for heart rate according to Bazett ($QTc_{Bazett}$), Fridericia ($QTc_{Fridericia}$) or a correction based on regression coefficients obtained from the Rotterdam Study data ($QTc_{Statistical}$).

*Results* On ECGs recorded during TCA use, $QTc_{Bazett}$ was 6.5 milliseconds (95% confidence interval: 4.0 – 9.0) longer and heart rate was 5.8 beats per minute (95% confidence interval: 4.7 – 6.9) faster than during nonuse. $QTc_{Fridericia}$ and $QTc_{Statistical}$ were not statistically significantly longer during TCA use than during non-use. Furthermore, $QTc_{Bazett}$ was similar for ECGs recorded during TCA use and nonuse after statistical adjustment for heart rate. According to our results, TCA use appears not to be associated with QTc prolongation.

*Conclusion* Therefore, the current advice of regulatory authorities to restrict use of these drugs and to do regular check-ups of the QTc may need to be revised. Other formulas, like Fridericia's, might be preferred.

**Introduction**

A prolonged heart-rate corrected QT interval (denoted hereafter as QTc) on the electrocardiogram (ECG) is associated with an increased risk of torsade de pointes and sudden cardiac death[1,2]. QTc prolongation can be hereditary, environmentally, or drug-induced[2]. Drug-induced QTc prolongation is a frequent reason for withdrawal of a drug from the market[3]. In recent years, a large number of cardiac and non-cardiac drugs have been associated with QTc prolongation[4].

The QT interval and heart rate are highly correlated. Therefore, several formulas have been developed to correct the QT interval for heart rate. Both in clinical practice and in research, the formula by Bazett is used most frequently[5-7]. However, this correction is suboptimal because the resulting QTc is not completely independent of heart rate[6,8]. More specifically, Bazett's QTc is overestimated in individuals with a high heart rate, and underestimated in individuals with a low heart rate[6,8]. Nevertheless, QTc corrected with the formula of Fridericia is also overestimated in individuals with higher heart rate, but to a lesser extent than with Bazett's[8]. Therefore, the American Food and Drug Administration recommends that studies assessing the QTc-prolonging effects of new drugs (thorough QT/QTc studies) should not use Bazett's formula alone[7] but also other correction formulas, such as Fridericia's[9] or correction formulas based on regression coefficients[10-12].

One of the drug groups thought to prolong the QTc are tricyclic antidepressants (TCAs)[13-16]. However, since TCAs also increase heart rate due to anticholinergic effects[17], it is possible that the increase in QTc can be explained by a higher heart rate. The objective of our study was to reassess the association between use of TCAs and QTc using different heart-rate correction methods in a population-based cohort.

**Methods**

*Study setting*

Our study was part of the Rotterdam Study, a prospective population-based cohort of middle-aged and elderly participants. The design and rationale of the Rotterdam Study is described in detail elsewhere[18,19]. In short, from 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district in Rotterdam, the Netherlands, were asked to participate. In total, 7983 individuals agreed to participate (response rate 78%). The original cohort was extended in 2000. In this first subcohort, all inhabitants who turned 55 years of age or moved into the district (matching the age criterion) were asked to participate, of whom 3011 individuals agreed (response rate 67%). A second extension of the cohort was initiated in 2006. In this subcohort, inhabitants from the same district aged 45 years and older were invited to participate. In total, 3932 inhabitants agreed to participate (response rate 65%). Follow-up examinations were conducted every 4 to 5 years, with a current maximum of 5 center visits per participant. The Rotterdam Study has been approved by the

medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all study participants.

*Study population*

We included all participants with at least one ECG recorded in the period that drug-dispensing data was available (January 1, 1991 – December 31, 2011). ECGs on which atrial fibrillation or pacemaker rhythm was determined were excluded from the study.

*Assessment of TCA use*

At study entry, more than 99% of the participants had their drug prescriptions filled at one of the seven regional pharmacies. Drug dispensing at these pharmacies was fully computerized. Dispensing data included the product name, the anatomical therapeutic chemical (ATC) code, dispensing date, total amount of tablets/capsules per prescription and dispensed daily number of tablets/capsules. Dispensing episodes were calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. If the date of a center visit during which the ECG was recorded fell within a dispensing episode of TCA use (ATC code: N06AA), the participant was considered as a current user.

*ECG measurements*

A standard 12-lead resting ECG was recorded with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florance, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS) program, which has been described previously and validated and applied extensively[20-24]. MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with use of template matching techniques[22]. The duration of the QT interval was measured by the MEANS program from the start of the QRS complex to the end of the T wave, which is described more extensively elsewhere[25]. The median RR and average QT interval was computed after exclusion of RR intervals that immediately preceded or followed premature ventricular complexes[25].

The following methods were used to correct the QT interval for heart rate: Bazett's formula ($QTc_{Bazett}$: QT / $\sqrt{RR}$)[5], Fridericia's formula ($QTc_{Fridericia}$: QT / $\sqrt[3]{RR}$ )[9], and one based on regression coefficients measured in the Rotterdam Study ($QTc_{Statistical}$), which was similarly done elsewhere[11]. A prolonged $QTc_{Bazett}$ was defined as a QTc >450 ms for men and >470 ms for women. The method of the regression-based model will be discussed in the statistical analyses paragraph. Heart rate in beats per minute (bpm) was calculated by dividing 60,000 by the RR interval in milliseconds (ms).

*Covariables*

The following covariables were considered additionally to age and sex: body mass index (BMI), use of drugs that affect the heart rate and/or QTc, heart failure, hypertension, myocardial infarction and diabetes mellitus. Weight and height were measured at the study center at the date of ECG examination. BMI was calculated with the following formula: weight (in kilograms) / height$^2$ (in meters). Use of drugs associated with an altered heart rate was determined on the date of ECG examination, and was based on the pharmacy dispensing records. These drugs were beta-blockers (ATC code: C07), verapamil (ATC code: C08DA01), diltiazem (ATC code: C08DB01), and digoxin (ATC code: C01AA05). Of the QTc-prolonging drugs, we only considered those having probable QTc-prolonging properties. Probable QTc-prolonging drugs are generally thought to increase the risk of torsade de pointes. This list contains drugs from various classes, including several antibiotics and antipsychotics[4]. TCAs on this list were not taken into account in this variable. The diagnosis of heart failure was based on typical signs and symptoms confirmed by objective evidence of cardiac dysfunction[26]. The presence of hypertension at the center visit was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, or use of blood-pressure lowering medication with the indication hypertension. Myocardial infarction was adjudicated based on a combination of symptoms, ECG measurements and enzyme markers[26]. The presence of diabetes mellitus was defined as use of glucose-lowering drugs, a non-fasting glucose level of more than 11.0 mmol/L, or a fasting glucose level of more than 6.9 mmol/L.

*Statistical analyses*

Characteristics of the study population were studied at baseline, which was defined as the first eligible ECG recording of a participant included in the study irrespective of the TCA-use status. As most participants had multiple ECG recordings, analyses were conducted with linear mixed models to correct for within-person correlations between visits. We tested covariance structures and selected the one with the lowest Akaike's information criterion. We based $QTc_{Statistical}$ on the regression coefficients of the independent variables heart rate and heart rate squared as computed in the Rotterdam Study. Heart rate squared was included in the regression model to take into account the nonlinear relationship between heart rate and QT interval. $QTc_{Statistical}$ was calculated with the following formula: QT – 3.984323*(60 – heart rate) + 0.014126*(3600 – heart rate squared).

We analyzed the data in two ways: cross-sectional and longitudinal. In the cross-sectional analysis all ECGs made during TCA use were compared with ECGs made during nonuse, with respect to $QTc_{Bazett}$, $QTc_{Fridericia}$, $QTc_{Statistical}$ and heart rate. After assessing the effects of TCAs as a class, we

analyzed the effect of individual drugs. We also stratified the analysis based on tertiles of heart rate. For the longitudinal analysis, we studied the within-person changes in $QTc_{Bazett}$, $QTc_{Fridericia}$, $QTc_{Statistical}$ and heart rate in between two consecutive visits (an ECG pair). The within-person changes were calculated by subtracting the first measurement from the second measurement. We classified the following exposure classes in an ECG pair: no TCA use at both visits (none-none), use of TCAs only at the second visit (none-TCA), use of TCAs only at the first visit (TCA-none), and use of TCAs at both consecutive visits (TCA-TCA). For the analyses, we compared the within-person changes between exposure classes using none-none as the reference. All analyses were adjusted for age and sex. Other covariables were included if they changed the effect size by a minimum of 10%.

In the sensitivity analysis we excluded all ECGs in which left bundle branch block, right bundle branch block, second and third degree atrioventricular block, left ventricular hypertrophy (according to Sokolow-Lyon criteria) or QRS duration > 120 milliseconds, were detected as well as ECGs recorded during use of beta-blocking agents, verapamil, diltiazem and digoxin. For every analysis, we used SPSS (version 21.0, IBM Corp., Somers, NY, USA). Two-sided p-values below 0.05 were considered statistically significant.

**Results**

*Baseline characteristics*

Baseline characteristics of the study population are presented in Table 1. We included 12,734 participants with a total of 27,048 ECGs. At baseline, the mean age of the study population was 65.1 years (standard deviation (SD) = 9.8), and 58.1% of the participants were women. Mean $QTc_{Bazett}$ was 430 ms (SD = 25), $QTc_{Fridericia}$ 421 ms (SD = 22), $QTc_{Statistical}$ 422 ms (SD = 21) and mean heart rate was 69 bpm (SD = 11).

*Association between TCAs, QTc and heart rate*

Of the tested covariance matrices, the first-order autoregressive covariance structure with homogenous variances had the lowest Akaike's information criterion, which we therefore used in the rest of the study for all analyses. The results of the cross-sectional analyses are presented in Table 2. Compared to ECGs made during nonuse, $QTc_{Bazett}$ was 6.5 ms (95% confidence interval (CI): 4.0 – 9.0 longer in ECGs made during TCA use. However, when heart rate was added to the statistical model, the difference was no longer statistically significant. Furthermore, neither $QTc_{Fridericia}$ nor $QTc_{Statistical}$ were significantly different when comparing ECGs made during TCA use with ECGs made during nonuse. These results remained similar when we added heart rate to the statistical model.

**Table 1. Baseline characteristics of the study population**

| Characteristics | Total population N=12,734 |
|---|---|
| Number of ECGs | 27,068 |
| ECGs per participant, median (range) | 2 (1 – 5) |
| Age in years | 65.1±9.8 |
| Females | 7,402 (58.1) |
| Body mass index in kg/$m^2$ | 26.9±4.2 |
| $QTc_{Bazett}$ in milliseconds | 430±25 |
| Prolonged $QTc_{Bazett}$ in men(>450 ms) | 714 (13.4) |
| Prolonged $QTc_{Bazett}$ in women (>470 ms) | 445 (6.0) |
| $QTc_{Fridericia}$ in milliseconds | 421±22 |
| $QTc_{Statistical}$ in milliseconds | 422±21 |
| QRS in milliseconds | 101±18 |
| QRS in milliseconds | 101±18 |
| Diabetes mellitus | 967 (7.6) |
| Hypertension | 6,761 (53.1) |
| History of myocardial infarction | 952 (7.4) |
| History of heart failure | 261 (2.0) |
| Left ventricular hypertrophy | 391 (3.1) |
| Left bundle branch block | 193 (1.5) |
| Right bundle branch block | 315 (2.5) |
| $2^{nd}$ or $3^{rd}$ degree atrioventricular block | 12 (0.1) |
| Beta blockers | 1,666 (13.1) |
| Verapamil | 83 (0.7) |
| Diltiazem | 156 (1.2) |
| Digoxin | 157 (1.2) |
| Probable QTc-prolonging drugs* | 271 (2.1) |

Abbreviations: ECG: electrocardiogram; $QTc_{Bazett}$: heart-rate corrected QT interval according to Bazett's formula; $QTc_{Fridericia}$: heart-rate corrected QT interval according to Fridericia's formula; $QTc_{Statistical}$: heart-rate corrected QT interval using linear regression coefficients observed in the Rotterdam Study. Presented data refer to the first eligible center visit with an ECG measurement of a participant. Data presented as mean±standard deviation or number of participants (percentage), or as indicated otherwise.

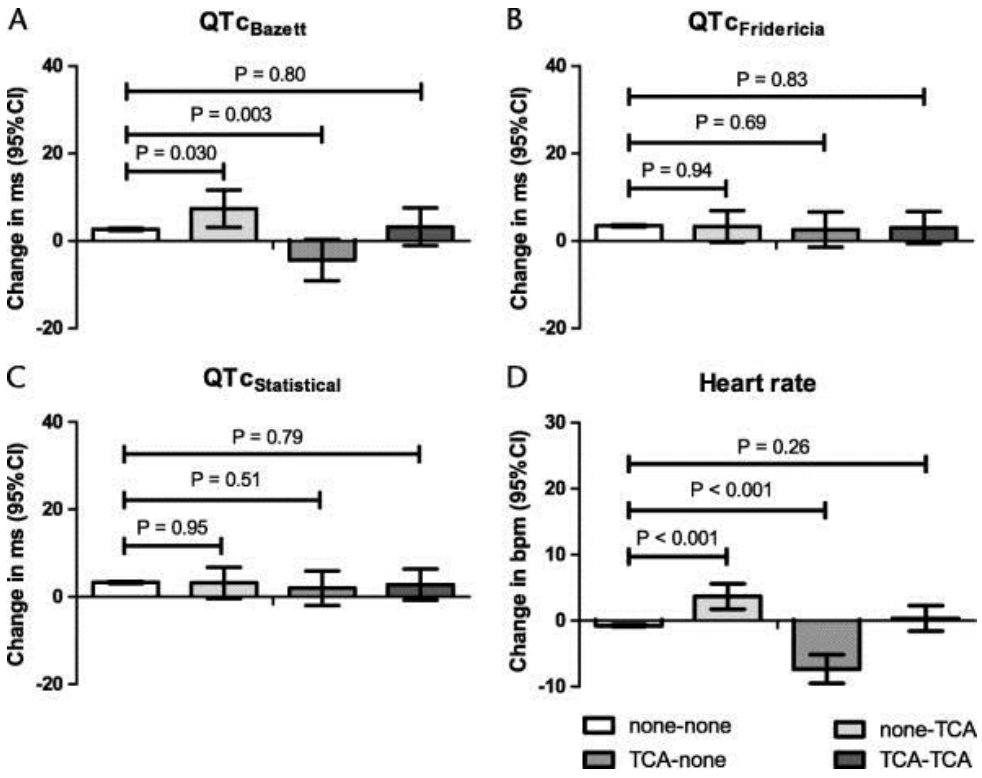
* Tricyclic antidepressants defined as probable QT prolonging drugs were excluded.

**Table 2. Cross-sectional association between use of tricyclic antidepressants and QT interval using different heart-rate correction methods and heart rate**

| | | $QTc_{Bazett}$ | | $QTc_{Fridericia}$ | | $QTc_{Statistical}$ | | Heart rate | |
|---|---|---|---|---|---|---|---|---|---|
| | N | β in ms | 95%CI | β in ms | 95%CI | β in ms | 95%CI | β in bpm | (95%CI) |
| No TCA use | 26,737 | ref | | ref | | ref | | Ref | |
| Any TCA | 331 | 6.5 | 4.0 – 9.0† | 0.6 | -1.5 – 2.8 | 0.9 | -1.2 – 3.0 | 5.8 | 4.7 – 6.9† |
| Amitriptyline | 200 | 5.5 | 2.3 – 8.8† | -0.5 | -3.3 – 2.3 | -0.2 | -3.0 – 2.5 | 5.8 | 4.4 – 7.3† |
| Maprotiline | 56 | 10.8 | 4.9 – 16.7† | 5.1 | 0.0 – 10.2* | 5.0 | 0.1 – 9.9* | 5.7 | 3.0 – 8.4† |
| Clomipramine | 31 | 3.6 | -4.0 – 11.1 | 0.5 | -0.6 – 7.0 | 0.6 | -5.7 – 6.9 | 3.0 | -0.3 – 6.4 |
| Imipramine | 17 | 11.3 | 0.7 – 21.9* | 0.5 | -8.7 – 9.7 | 0.7 | -8.1 – 9.6 | 10.9 | 6.0 – 15.7† |
| Nortriptyline | 16 | 13.0 | 1.8 – 24.2* | 1.9 | -8.1 – 11.9 | 2.4 | -7.3 – 12.1 | 11.5 | 6.7 – 16.3† |
| Doxepin | 7 | -7.8 | -24.3 – 22.8 | -3.4 | -17.6 – 10.8 | -3.2 | -16.7 – 10.4 | -5.0 | -12.8 – 2.7 |
| Dosulepin | 4 | -2.2 | -27.2 – 22.7 | -2.2 | -24.2 – 19.6 | -1.8 | -22.9 – 19.3 | -0.7 | -12.1 – 10.7 |

Abbreviations: bpm: beats per minute; CI: confidence interval; ECG: electrocardiogram; ms: milliseconds; N: number of ECGs; $QTc_{Bazett}$: heart-rate corrected QT interval according to Bazett's formula; $QTc_{Fridericia}$: heart-rate corrected QT interval according to Fridericia's formula; $QTc_{Statistical}$: heart-rate corrected QT interval using linear regression coefficients observed in the Rotterdam Study; TCA: tricyclic antidepressant. Data presented as the mean adjusted difference in milliseconds of the QTc or beats per minute of heart rate between TCA users and nonusers. Analyses are adjusted for age and sex. * P-value < 0.05; † P-value <0.001.

**Figure 1. Changes in intervals between repeated center visits**




None-none: no use of tricyclic antidepressants (TCAs) at both consecutive visits; None-TCA: used a TCA at the second of two consecutive visits; TCA-none: used a TCA at the first of two consecutive visits: TCA-TCA, used TCAs at both consecutive visits. **A)** Change in $QTc_{Bazett}$. **B)** Change in $QTc_{Fridericia}$. **C)** Change in $QTc_{Statistical}$. **D)** Change in heart rate. Data presented as the mean change in milliseconds with 95% confidence interval between consecutive center visits. Analyses were adjusted for age and sex.

When we stratified on tertiles based on heart rate, $QTc_{Bazett}$ was 4.4 ms (95%CI: 1.0 – 7.7) longer in ECGs made during use than in ECGs made during nonuse, but only in the highest tertile (heart rate > 73 bpm). In all tertiles, there was no association between TCA use during ECG recording and $QTc_{Fridericia}$ and $QTc_{Statistical}$. When stratified on individual TCAs, $QTc_{Bazett}$ was statistically longer than nonuse during use of the following TCAs: imipramine, amitryptiline, nortriptyline and maprotiline. However, only maprotiline use of showed a significantly longer $QTc_{Fridericia}$ or $QTc_{Statistical}$ than nonuse. Heart rate was 5.8 bpm (95%CI: 4.7 – 6.9) faster on ECGs recorded during TCA use than on ECGs recorded during nonuse. Of the individual TCAs, heart rate was significantly faster on ECGs recorded during use of imipramine, amitriptyline, nortriptyline and maprotiline.

Results did not materially change when ECGs were excluded that were made in participants with cardiac pathology and that were made during use of heart-rate affecting drugs.

*Longitudinal analyses: changes in QTc and heart rate*

A total of 13,885 ECG pairs were available with a median time interval between two consecutive visits of 4.3 years (interquartile range: 2.4 – 5.0). In the TCA exposure class none-TCA, $QTc_{Bazett}$ was 4.7 ms (95%CI: 0.5 – 9.0) higher at the second ECG (Figure 1A) than the none-none class. Conversely, in the TCA exposure class TCA-none, $QTc_{Bazett}$ was lower at the second ECG (-7.1 ms, 95%CI: -11.8 – 2.3) than at the first ECG. These associations were neither observed for $QTc_{Fridericia}$ (Figure 1B) nor for $QTc_{Statistical}$ (Figure 1C). In the same TCA exposure classes, heart rate was higher at the second ECG than at the first ECG when TCAs were used during the second ECG, and heart rate was lower at the second ECG than at the first ECG when TCAs were used during the first ECG.

Results did not materially change when we excluded ECGs made in participants with cardiac pathology and ECGs made during use of drugs affecting the heart rate.

**Discussion**

In the present study, use of TCAs was associated with a prolonged QTc according to Bazett's formula, but not with a significant increase in QTc when using Fridericia's formula or when QT was corrected for heart rate using regression coefficients obtained from the included ECGs of the present study. Furthermore, TCA use was not associated with prolongation of $QTc_{Bazett}$ when we additionally adjusted for heart rate. Of the individual TCAs, only use of maprotiline showed a significantly higher QTc with the correction methods other than Bazett's, although the effect size with these methods was lower than with Bazett. $QTc_{Bazett}$ was only higher in ECGs made during TCA use than in ECGs made during nonuse in the tertile with the highest heart rates. Results were similar in the

longitudinal analyses of QTc interval and heart rate. In addition, use of TCAs was associated with a faster heart rate of 5.8 bpm.

Because the association between TCAs and $QTc_{Bazett}$ was no longer significant when additionally adjusted for heart rate, the higher heart rate is likely the cause of the overestimation of the TCA-induced QTc prolongation. Therefore, the results of our study indicate that the previously described TCA-induced prolongation of $QTc_{Bazett}$ is possibly observed because of overestimation instead of a real drug effect. This is supported by our finding that TCA-induced prolongation of $QTc_{Bazett}$ was only observed in participants with the highest heart rates (i.e. upper tertile). Furthermore, of the individual TCAs, we observed the largest difference between QTcBazett and other correction methods for those TCAs with the largest effect on heart rate.

The association between TCA use and $QTc_{Bazett}$ prolongation has been consistently observed in cohort studies[13-15]. In addition, a number of case reports on TCA-induced QTc prolongation have been published[16]. Although these case reports did not support our findings directly, it should be noted that these case studies reported TCA-induced QTc prolongation in patients with severe cardiac pathologies or in patients with a TCA overdose[16]. As further support of our findings, no association was observed in population-based studies between use of TCAs and sudden cardiac death, which is one of the hard endpoints associated with QTc prolongation[27,28]. However, this does not imply that use of TCAs is without risk with respect to cardiovascular end points. A higher heart rate has previously been associated with an increased risk of cardiac conditions like heart failure and overall cardiovascular mortality[29-31]. As use of TCAs is associated with an increased risk of cardiovascular mortality[32], detrimental cardiovascular effects of TCAs can be caused by an increase in heart rate rather than a prolonged QT interval. TCAs have also been associated with an increased risk of torsade de pointes[16]. Because the correlation between QTc prolongation and torsade de pointes is modest at best, this may still be in line with our findings[33].

Bazett's formula is the most often used method to correct the QT interval for heart rate in both clinical practice and in research[6,7]. The two other studied correction formulas are less dependent on heart rate after correction, as point estimates did not materially differ when additionally adjusted for heart rate in the statistical model. This indicates that these formulas are less prone to overestimation of QTc in persons with high heart rates. To study the safety of TCAs with respect to QTc prolongation, these formulas might be more appropriate. The correction formula based on regression coefficients would be the best, as the coefficients were obtained from the same population as the one we used for the analyses, and therefore this formula would correct for heart rate optimally. However, such a model is specific for a study population, and thus the clinical use is limited[8]. Heart-rate correction with Fridericia's formula might be preferred in patients

taking heart-rate increasing drugs, as it is also easily calculated and results were similar with respect to the model based on regression coefficients.

A strength of our study is the analysis of longitudinal changes in QTc and heart rate. The cross-sectional analysis is more prone to be confounded by a contraindication, as participants with a longer QTc interval are less likely to be prescribed TCAs. Also, the cross-sectional analysis cannot infer causality between TCAs and QTc prolongation. Although the longitudinal analysis is still observational, it can provide additional arguments in favor of a causal relationship. As similar results were obtained with cross-sectional and longitudinal analyses, our findings are more likely due to the drug rather than due to other unmeasured factors. Another strength of our study is the MEANS algorithm to calculate QT interval and heart rate. This algorithm works systematically and automatically and has been evaluated extensively[21-23]. Finally, information bias was limited as pharmacy-dispensing data was recorded irrespective of disease state. Our study also has some limitations. In our study, only a limited number of participants were using TCAs. Second, the median time interval between two examination rounds is 4.3 years, which might be too lengthy for the calculation of changes in QTc intervals. Therefore, it is still possible that there is an acute QTc-prolonging effect of TCA use, which is visible with all heart-rate correction models. However, our results indicate that long-term treatment with TCAs is not accompanied by QTc prolongation. And last, being an observational study, our study is subjected to confounding. However, none of the considered covariables changed the effect sizes substantially.

In conclusion, the results of our study suggested that TCA-induced prolongation of $QTc_{Bazett}$ might be the result of overestimation that occurs in persons with high resting heart rates. TCA prescribing should nonetheless still be done with caution due to the associated increase in resting heart rate. When the QT interval was corrected for heart rate with Fridericia's formula or with a model based on regression coefficients, no increase in QTc was observed. Reported effects on the QTc interval should be reconsidered for heart-rate increasing drugs. For such drugs Fridericia's correction might be preferred. The advice of regulatory authorities to restrict use of these drugs and to do regular check-ups of the QTc, may need to be revised.

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# Chapter 1.4

# Discovery of Novel Heart Rate-Associated Loci Using the Exome Chip

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**Abstract**

*Background* Resting heart rate is a heritable trait, and an increase in heart rate is associated with increased mortality risk. GWAS analyses have found loci associated with resting heart rate, at the time of our study these loci explained 0.9% of the variation.

*Aim* To discover new genetic loci associated with heart rate from Exome Chip meta-analyses.

*Methods* Heart rate was measured from either elecrtrocardiograms or pulse recordings. We meta-analysed heart rate association results from 104,452 European-ancestry individuals from 30 cohorts, genotyped using the Exome Chip. Twenty-four variants were selected for follow-up in an independent dataset (UK Biobank, N = 134,251). Conditional and gene-based testing was undertaken, and variants were investigated with bioinformatics methods.

*Results* We discovered five novel heart rate loci, and one new independent low-frequency non-synonymous variant in an established heart rate locus (*KIAA1755*). Lead variants in four of the novel loci are non-synonymous variants in the genes *C10orf71, DALDR3, TESK2*, *SEC31B*. The variant at *SEC31B* is significantly associated with *SEC31B* expression in heart and tibial nerve tissue. Further candidate genes were detected from long range regulatory chromatin interactions in heart tissue (*SCD*, *SLF2, MAPK8*). We observed significant enrichment in DNase I hypersensitive sites in fetal heart and lung. Moreover, enrichment was seen for the first time in human neuronal progenitor cells (derived from embryonic stem cells) and fetal muscle samples by including our novel variants.

*Conclusion* Our findings advance the knowledge of the genetic architecture of heart rate, and indicate new candidate genes for follow-up functional studies.

**Introduction**

Increased resting heart rate (HR) is a known risk factor for cardiovascular morbidity and mortality [1-3], including stroke [4] and sudden cardiac death [5,6]. A HR increased by 20 beats per minute (bpm) is associated with 30-50% higher mortality that appears independent of confounding factors [7]. High HR increases myocardial oxygen consumption yet lessens oxygen delivery to myocardial tissue. It also increases arterial stiffness and risk of plaque rupture [8]. Although HR can be influenced by many non-genetic factors (e.g., exercise, smoking and cardiovascular drugs), the heritability of resting HR is estimated to be 26–32% from family studies [9,10], and 55–63% from twin studies [11].

Several meta-analyses of genome-wide association studies (GWAS) studies have been undertaken to detect genetic determinants of HR [12-16]. There were 21 HR loci previously reported at the time of our study by den Hoed *et al.* [12] in a GWAS analysis of 180,000 individuals, predominantly of European ancestry. The study implicated 20 candidate genes from follow-up functional studies in *Danio rerio* and *Drosophila melanogaster* models. Smaller GWAS analyses have also been performed in Icelandic and Norwegian populations [15], African Americans [13], and genetically isolated European populations [16]. The variants discovered by GWAS are common, and are mostly in introns or intergenic regions. Together the previous loci from GWAS at the time of our study only explain a small percentage (0.9% of the variability in heart rate [12,17].

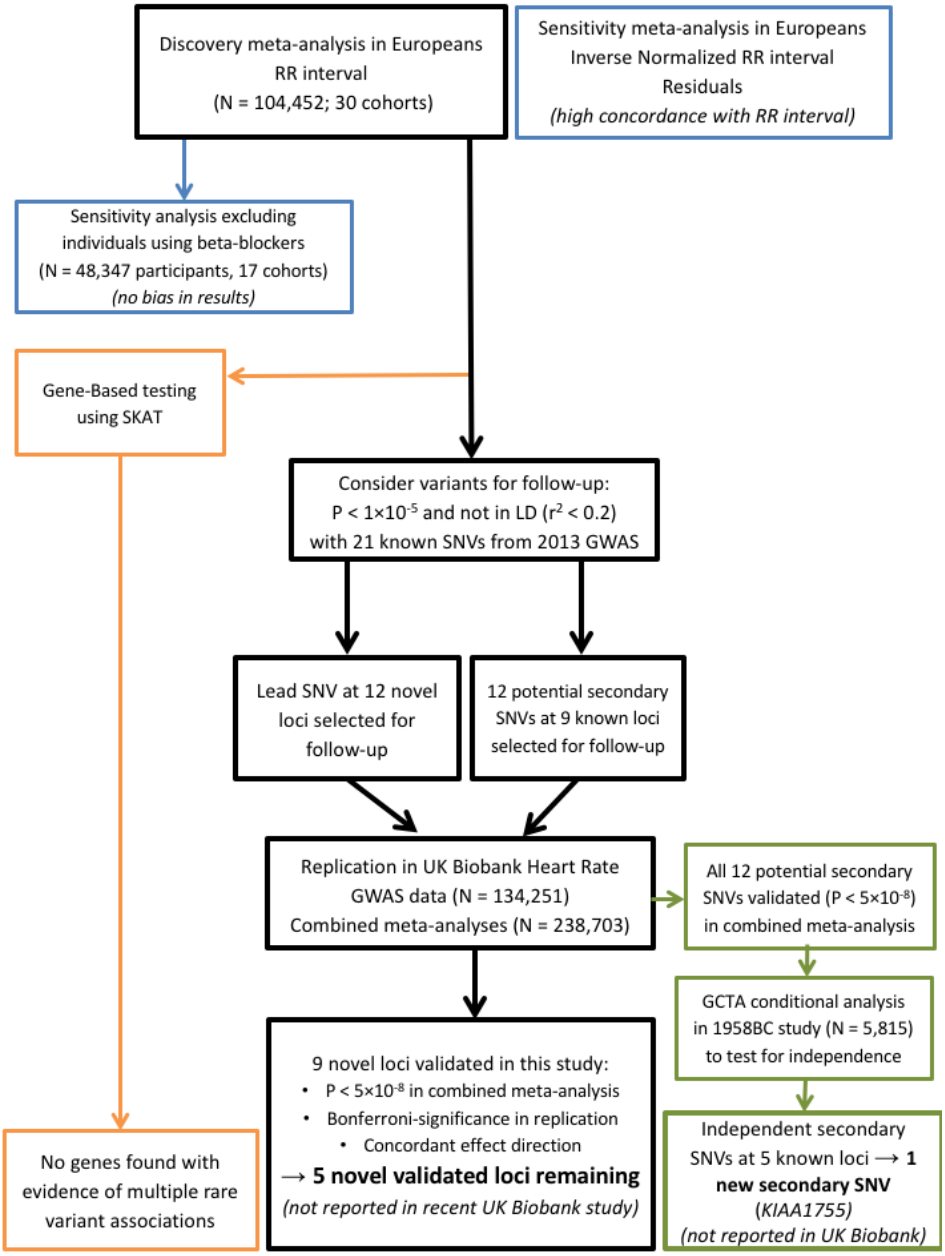
To increase our knowledge of genetic determinants influencing HR and discover novel loci, especially rare or low frequency coding variants with larger effects, we meta-analysed data from 104,452 individuals of European-ancestry using the Exome Chip, from cohorts that participated in the Cohorts for Heart & Aging Research in Genomic Epidemiology (CHARGE) EKG consortium. The Exome Chip permits a cost-efficient analysis of coding variants derived from sequencing of >12,000 individuals and includes many rare and low frequency variants . We performed a validation experiment using independent replication samples from UK Biobank data, and bioinformatics investigations to gain an understanding of the new HR loci.

**Results**

*Single nucleotide variant analysis in individuals of European-ancestry*

In the discovery phase, association results of 235,677 single nucleotide variants (SNVs) from 104,452 individuals were meta-analysed using a fixed-effects model (Supplementary Figure 1). Two analyses were performed. The first used RR intervals (RR in milliseconds= 60,000/HR, in beats per minute, according to the inverse relationship between HR and RR). The second used the inverse-normalized residuals of the linear regression RR interval adjusted for age + sex + BMI as covariates (denoted as RR-INVN). An overview of the study design is provided in Figure 1.

**Figure 1. Schematic flow diagram of the study design**




N: sample size; SKAT: SNV-set Kernel Association Test; *P*: *P*-value; LD: linkage disequilibrium; SNV: single nucleotide variant; GCTA: Genome-wide Complex Traits Analysis software; 1958BC: 1958 Birth Cohort. UKB = UK Biobank.

We observed a high correlation of effect sizes and *P*-values between the RR-interval and RR-INVN meta-analyses ($r^2$ = 0.99 and 0.98, respectively; Supplementary Figure 2). Furthermore, the RR interval was near-normally distributed, so inverse normalization was deemed unnecessary (Supplementary Figure 3).

Beta-blockers are clinically known to lower HR, therefore the phenotype measurements of beta-blocker users may be under-estimated, and hence the inclusion of beta-blocker users in our analysis may potentially bias our analysis results. We therefore performed a sensitivity analysis by also meta-analysing a subgroup of cohorts that provided beta-blocker data (N = 48,347; 17 cohorts). Results including or excluding beta-blocker users were highly correlated ($r^2$ of the betas = 0.97; $r^2$ of the *P* values = 0.74; Supplementary Figure 4), suggesting there is little or no bias from including beta-blocker users in the analysis. Therefore we report the meta-analysis results from the full dataset for the RR-interval, to maximize sample size and power.

*Replication and meta-analysis with the UK Biobank dataset*

To identify novel associated loci, we selected 12 variants with $P < 1 \times 10^{-5}$ that mapped outside the 21 HR loci reported in the previous GWAS [12] for follow-up in an independent dataset. Within each unknown locus, there were no potential secondary SNVs not in LD with the lead SNV ($r^2 < 0.2$) and meeting our look-up significance threshold $P < 1 \times 10^{-5}$). Hence only 12 new lead SNVs were carried forward. We also followed-up 12 potential secondary signals at nine of the 21 previously reported HR loci (further details on selection criteria are provided in the Methods).[12] None of the selected variants were in LD ($r^2 < 0.2$) with each other, or with the published SNVs. Thus a total of 24 variants were taken forward into replication. The UK Biobank dataset provided results for the selected genetic variants (N = 134,251 individuals).

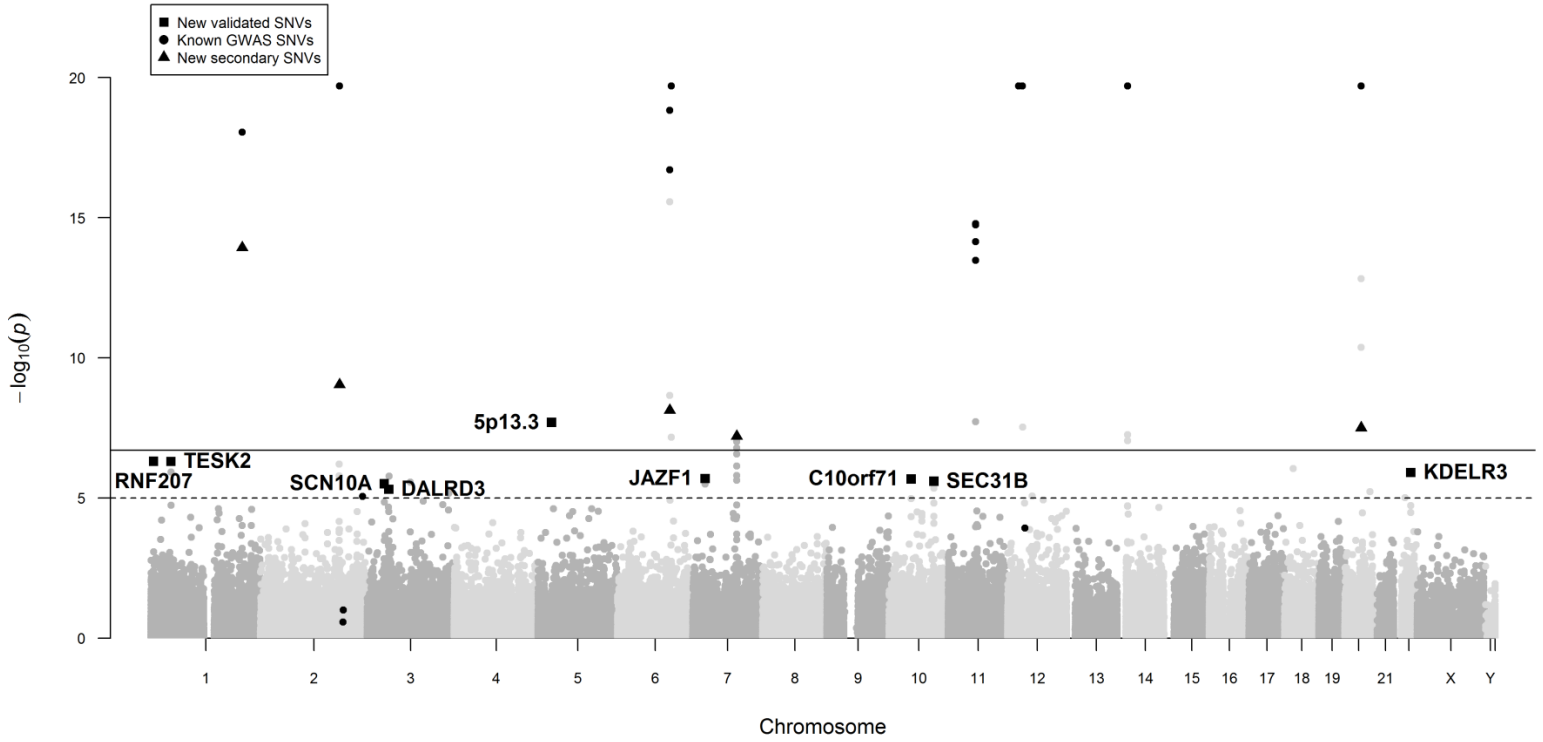
Nine of the 12 previously unknown variants were validated based on exome-wide significance ($P \leq 2.12 \times 10^{-7}$) in the combined meta-analysis of CHARGE and UK Biobank data, and on Bonferroni-adjusted significance ($P \leq 0.0042$ for 12 tests) in the replication dataset alone, with concordant directions of effects taking into account the inverse relationship between the RR-interval from the discovery data and HR from the replication data (Table 1; Figure 2). Indeed all nine SNV associations were genome-wide significant in the combined meta-analysis ($P < 5.0 \times 10^{-8}$). Four of our nine validated novel loci were reported in a UK Biobank study [17], that was published after completion of our study (Table 1B). Hence, we present results here for five unreported novel loci (Table 1A; Supplementary Figures 5 and 6).

**Table 1. Heart rate associated loci identified from Exome Chip analysis**

| SNV | Locus | Chr:Pos | EA | EAF | $N_{DISCOVERY}$ | BETA-RR (SE) | $P_{DISCOVERY}$ | BETA-HR (SE) | $P_{REPLICATION}$ | $P_{COMBINED}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **(A) Five unreported novel loci** | | | | | | | | | | |
| rs17853159* | *TESK2* | 1:45810865 | A | 0.07 | 104,452 | -6.03 (1.20) | $5.02\times10^{-7}$ | 0.31 (0.08) | $9.55\times10^{-5}$ | $4.09\times10^{-10}$ |
| rs3087866* | *DALRD3* | 3: 49054692 | T | 0.25 | 104,452 | 3.29 (0.72) | $4.92\times10^{-6}$ | -0.31 (0.05) | $7.06\times10^{-10}$ | $2.09\times10^{-14}$ |
| rs1635852 | *JAZF1* | 7: 28189411 | C | 0.50 | 104,452 | 2.96 (0.62) | $2.04\times10^{-6}$ | -0.15 (0.04) | $4.10\times10^{-4}$ | $6.97\times10^{-9}$ |
| rs10857472* | *C10orf71* | 10: 50534599 | A | 0.45 | 104,452 | -2.97 (0.63) | $2.11\times10^{-6}$ | 0.16 (0.04) | $1.49\times10^{-4}$ | $2.21\times10^{-9}$ |
| rs3793706*‡ | *SEC31B* | 10: 102269085 | A | 0.22 | 104,452 | 3.52 (0.75) | $2.54\times10^{-6}$ | -0.19 (0.05) | $2.06\times10^{-4}$ | $3.72\times10^{-9}$ |
| **(B) Four loci validated in our study and also recently published in the UK Biobank study** | | | | | | | | | | |
| rs709209* | *RNF207* | 1:6278414 | G | 0.35 | 104,452 | -3.30 (0.66) | $4.94\times10^{-7}$ | 0.27 (0.04) | $2.14\times10^{-9}$ | $5.44\times10^{-15}$ |
| rs6795970* | *SCN10A* | 3: 38766675 | A | 0.40 | 104,452 | 2.97 (0.64) | $3.10\times10^{-6}$ | -0.24 (0.04) | $1.81\times10^{-8}$ | $2.73\times10^{-13}$ |
| rs4282331 | *5p13.3* | 5: 30881510 | G | 0.42 | 104,452 | -3.56 (0.63) | $2.03\times10^{-8}$ | 0.26 (0.04) | $2.97\times10^{-9}$ | $3.34\times10^{-16}$ |
| rs12004* | *KDELR3* | 22: 38877461 | G | 0.30 | 104,452 | 3.30 (0.68) | $1.24\times10^{-6}$ | -0.31 (0.05) | $4.92\times10^{-11}$ | $4.04\times10^{-16}$ |

Abbreviations: SNV: single-nucleotide variant, Chr:Pos: Chromosome:Position based on HG build 19, EA: Effect allele, EAF: Effect allele frequency from the discovery data, BETA-RR: Beta effect estimate of RR Interval (milliseconds) taken from the ExomeRR discovery data, SE: Standard error of the effect estimate, N: sample size analyzed per variant (provided for genotyped discovery data only, as replication data was imputed so N=maximum N for all variants), BETA-HR: Beta effect for heart rate (in beats per minute) taken from the UK Biobank replication data, *P*: *P*-value from either the discovery meta-analysis, the replication data, or the combined meta-analysis of discovery and replication data. Locus name indicates the nearest gene to the HR-associated SNV. * indicates that the lead or a proxy SNV ($r^2$>0.8) is a non-synonymous SNV. ‡ indicates if the lead SNV is predicted to be damaging. A novel locus is a genomic region with no SNVs in LD ($r^2$ < 0.2) with HR-associated SNVs and (ii) mapping to more than 500 kb from either side of a previously reported HR-associated SNV. Note: due to the inverse relationship between RR-interval and HR that opposite beta directions do relate to concordant directions of effect between discovery and replication.

**Figure 2. Manhattan plot for the RR-interval discovery meta-analysis in European individuals**




The Manhattan plot displays the results from the discovery meta-analysis of RR-intervals from N = 104,452 individuals of European ancestry (from 30 cohorts). On the X axis, *P*-values are expressed as -$\log_{10}(P)$ are plotted according to physical genomic locations by chromosome. The Y-axis is truncated to -$\log_{10}(P)$ = 20 with any variants with $P < 1x10^{-20}$ displayed on the -$\log_{10}(P)$ = 20 line. The nine novel variants validated from the combined meta-analysis with UK Biobank data are shown as squares. Variants in linkage disequilibrium (LD; $r^2 > 0.8$) with published GWAS variants are shown as circles[12]. New secondary variants validated in our analysis are shown as triangles. Locus names of the novel loci correspond to the nearest annotated gene, with 5p13.3 denoting an intergenic variant. The dashed line indicates a *P*-value threshold of $1x10^{-5}$, corresponding to the lookup significance threshold and the continuous line indicates a *P*-value threshold of $2x10^{-7}$, corresponding to exome-wide significance.

Twelve of the 21 HR-associated SNVs from the previously reported GWAS [12] were covered on the Exome Chip, either directly or by a proxy SNV in high linkage disequilibrium (LD; $r^2 > 0.8$). Our discovery meta-analysis showed strong support for the previous findings, with 11 of the 12 SNVs validated at Bonferroni-adjusted significance ($P \leq 0.0042$ for 12 tests), of which 9 were validated at exome-wide significance ($P < 2 \times 10^{-7}$; Figure 2). Only rs4140885 at the *TFPI* locus was not supported in our data ($P = 0.10$; Supplementary Table 1).

*Independent secondary signals at known loci*

All 12 potential secondary signals at loci previously reported by Den Hoed *et al.*[12] were genome-wide significant in the combined meta-analysis (Supplementary Table 2) and are independent to the known SNPs according to LD ($r^2 < 0.2$). We performed a conditional analysis using GCTA to formally identify secondary signals of association. Five of the 12 validated potential secondary SNVs (within *CD46*, *CCDC141*, *SLC35F1*, *ACHE* and *KIAA1755* loci) were selected within the final GCTA model (Supplementary Table 3). At four of the previously reported HR regions the secondary signals that we identified were confirmed to be statistically independent signals of association: *CD46* (rs2745967), *CCDC141* (rs10497529), *SLC35F1* (rs12210810) and *KIAA1755* (rs41282820) in addition to the known SNV, as both the published SNV and the new secondary SNV were present in the final GCTA model of jointly independent associated variants. Hence we identified two distinct signals of association at each of these four known HR loci. However, the published SNV at the *ACHE* locus (rs13245899) is not covered on the Exome-chip, or by any proxies (Supplementary Table 1), so the GCTA analysis does not include the known variant. As we are not able to condition on the unavailable published SNV and formally test association jointly with the known SNV, we are unable to statistically confirm the total number of independent signals at the *ACHE* locus.

The secondary SNVs at *CCDC141, ACHE* and *KIAA1755* are non-synonymous variants. Furthermore, the SNVs at *CCDC141* and *KIAA1755* are low-frequency with minor allele frequencies (MAFs) of 3.6% and 1.7%, respectively. Secondary signals have also recently been observed at four of the five loci (*CD46*, *CCDC141*, *SLC35F1* and *ACHE*) in UK Biobank data [17], since completion of our meta-analysis. At *CD46*, our secondary SNV (rs2745967) is in high LD ($r^2 = 0.78$) with the secondary SNV (rs2745959) reported in UK Biobank, so likely to be the same signal. At *CCDC141* our secondary variant is exactly the same SNV as from UK Biobank (rs10497529). Similarly, at *SLC35F1*, our secondary SNV (rs12210810) is in very high LD ($r^2 = 0.98$), so is likely to be the same signal. Hence at these three known loci (*CD46*, *CCDC141*, *SLC35F1*), all existing data suggests there are two independent signals of association. At the *ACHE* locus, our secondary SNV (rs542137; ~38kb and $r^2 < 0.2$ from the published SNV) is not in LD ($r^2 < 0.2$) with the secondary SNV from UK Biobank

(rs140367586; ~659kb and $r^2 < 0.2$ from the published SNV). We are unable to clearly determine the number of distinct signals at the *ACHE* locus from our Exome-chip RR-interval discovery meta-analysis data, without the published SNV being covered on the Exome-chip. The low-frequency non-synonymous variant (rs41282820) at the known *KIAA1755* locus is a new, secondary variant, with strong evidence of independent association, it does not overlap with other published findings.

*Variance explained*

Twelve of the 21 previously reported HR-associated SNVs [12] covered on the Exome Chip explain 1.14% of RR interval variance ($P = 3.96 \times 10^{-10}$) within the 1958 Birth Cohort study (Methods). The added contribution of the lead SNVs at our five unreported novel loci, combined with the 12 previously reported SNVs, increases the variance explained to 1.28% overall ($P = 9.17 \times 10^{-11}$).

*Comparison of results between European and non-European populations*

To investigate our data from non-European samples (9,358 African Americans (AA), 1,411 Hispanic (HIS), and 754 Chinese-Americans (CH); Supplementary Table 4), we first extracted results for the 12 of the 21 previously reported HR-associated SNVs covered on the Exome Chip [12]. In contrast to previous results for Europeans, only two known HR-SNVs showed evidence of association ($P < 0.05$), at the *GJA1* and *MYH6* loci, in the AA population only. This is likely due to a lack of power from the smaller non-European sample sizes, considering the power was calculated to be only 48%, 11.7% and 8.5% for AA, HIS and CH, respectively. Concordance in the direction of effects compared to Europeans was only significant for AA, with 92%, 64% and 50% concordance, corresponding to *P*-values of $2.9\times10^{-3}$, 0.16 and 0.23 from binomial tests for AA, HIS and CH, respectively. The lack of support of previous findings from the under-powered non-European data led us to restrict our primary discovery meta-analysis to Europeans only.

We also performed a look-up of the nine validated SNV associations in the non-European samples. Due to the lack of power, and different allele frequencies compared to Europeans, none of the SNVs had results with $P < 0.05$ within any ancestry (Supplementary Figure 6), and there was little concordance in effect directions: 56% and $P = 0.246$ for AA; 33% and $P = 0.164$ for HIS and CH.

*Gene-based tests*

Gene-based testing was performed to identify genes which may have multiple rare variant associations. None of the gene-based test results were significant, after excluding the single most significant low-frequency variant from the tests (Supplementary Table 5).

*Look-up of UK Biobank heart rate SNVs*

Since completion of our meta-analysis of Exome Chip genotypes, a genome-wide scan for HR has been completed in UK Biobank [17]. This study published 46 new HR loci. Four of these novel loci were simultaneously discovered in our analyses (*RNF207*, *SCN10A*, *5p13.3*, *KDELR3:* Table 1B). Among the 42 remaining UK Biobank loci, only 5 of the lead SNVs were covered on the Exome Chip at $r^2 \geq 0.8$. Results from our exome RR European-ancestry meta-analyses show support for all five of these loci ($P < 0.01$; Bonferroni-adjusted significance for 5 tests; Supplementary Table 6).

*Heart rate loci and association with other traits*

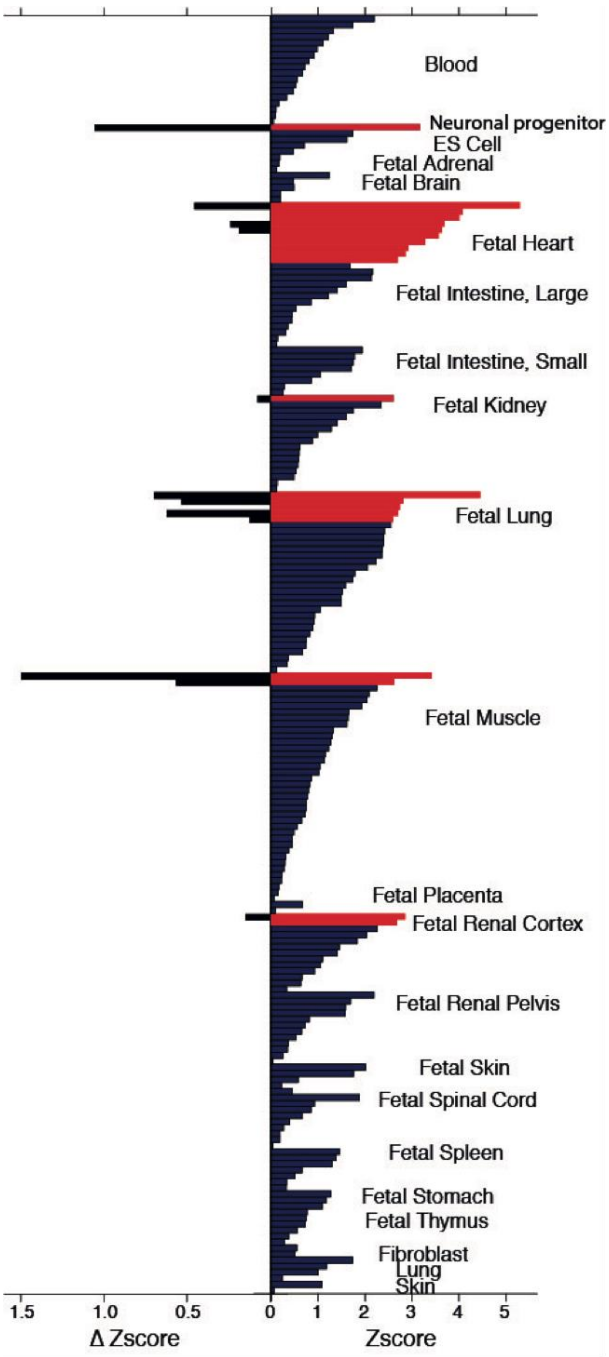
To provide insights into possible shared aetiologies or mechanisms of disease, we assessed association of our five unreported novel HR SNVs (and their proxies, $r^2 \geq 0.8$) with other traits. Genome-wide significant phenotype-genotype associations were observed for three novel loci (Supplementary Table 7). The SNV at the *DLRD3* locus was associated with age of menarche. The SNV at the *JAZF1* locus was highly pleiotropic, as shown by associations with several autoimmune disorders (systemic lupus erythematosus, Crohn's disease, and selective immunoglobulin A deficiency), height, type 2 diabetes, and *JAZF1* transcript levels in adipose tissue. The SNV at the *SEC31B* locus was associated with plasma palmitoleic acid levels and differential exon expression of *SEC31B*.

*Functional annotation of novel heart rate SNVs and candidate genes*

Four of the five unreported novel HR SNVs or their proxies ($r^2 > 0.8$) are non-synonymous SNVs in *TESK2*, *DALRD3*, *C10orf71,* and *SEC31B* (Table 1A). The non-synonymous SNV in *SEC31B* (rs2295774, c.1096T>G, p.Ser332Ala) is in a conserved region of the protein, and is predicted to be damaging using three different algorithms in ANNOVAR [19]. We also investigated if the novel HR-associated SNVs or their proxies ($r^2 > 0.8$) were associated with changes in expression levels of nearby genes (i.e., as expression quantitative trait loci, or eQTLs) in the GTEx dataset [20]. We observed a significant eQTL association at one novel HR locus (Supplementary Table 8). Specifically, the HR increasing allele of the non-synonymous SNV at *SEC31B* was associated with increased levels of *SEC31B* in tibial nerves ($P = 8.08 \times 10^{-33}$), lung ($P = 1.22 \times 10^{-23}$), atrial appendage tissue ($P = 4.56 \times 10^{-11}$), and the left ventricle ($P = 4.0 \times 10^{-9}$), tissues which may be regarded as physiologically relevant for HR.

We also observed HR loci to be significantly enriched for DNase I hypersensitive sites (DHSs; Figure 3). We evaluated regions containing the five unreported novel HR loci and five independent secondary variants at previously reported HR loci,[12] together with all 67 published HR-associated SNVs (21 loci reported from the original GWAS [12] plus 46 loci recently published from UK Biobank[17]).

**Figure 3. Enrichment of HR–SNVs in DNase I hypersensitive sites of 299 tissue samples.**




The right panel shows the enrichment of the combined known and novel (all) HR-SNVs in DNase I hypersensitivity sites of 212 Roadmap Epigenome tissue samples (those with positive Z-scores). Enrichment is expressed as a Z-score compared to the distribution of 1000 matched background SNV sets. Significant enrichments are shown in red (Z-score ≥ 2.58, FDR <1.5%), enrichments below this threshold are shown in blue. The left panel shows the enrichment difference ($\Delta$ Zscore= $Zscore_{all}$-$Zscore_{known}$) for those tissue samples in which we found significant enrichment using all SNPs and that further show a positive change using all SNVs compared to only known SNVs, with increased enrichment hence due to the novel loci identified.

Highest enrichment for DHSs in HR loci occurred within regions that are transcriptionally active in fetal heart tissue and fetal lung, as reported in the UK Biobank study. Moreover, for the first time we found significant enrichment for DHSs in human neuronal progenitor cells (derived from embryonic stem cells) and fetal muscle samples, with the inclusion of our novel loci.

*Pathway analyses*

We used Ingenuity pathway analyses to determine if there was any increased enrichment in HR associated pathways with the contribution of our five newly identified loci. We identified 16 significantly enriched pathways at $P < 1 \times 10^{-4}$. Most of these pathways are related to the cardiovascular system and involve, for example, supraventricular arrhythmias, dilated cardiomyopathy, and heart rate (Supplementary Table 9).

*Coding variants at heart rate loci*

The Exome Chip provides a unique opportunity to search for coding variants within known HR loci. Whereas GWAS analyses typically identify intron or intergenic variants, Exome Chip analysis may identify HR-associated coding variants, which would point to candidate causal genes. We considered all 67 published HR loci (21 previously reported GWAS loci,[12] plus 46 recently published loci from UK Biobank[17]) and extracted all SNVs in high LD with the lead variants ($r^2 \geq 0.8$), tagging the same association signal, restricted to variants covered on the Exome Chip. We further filtered variants to obtain SNVs that reached exome-wide significance for associations with RR interval in our primary discovery meta-analysis, to ensure that variants have a highly significant association with the trait. Coding SNVs were identified, using the CHARGE Exome Chip annotation file.

We only observed two such coding variants in two reported loci: *CCDC141* and *KIAA1755*. The published *CCDC141* coding variant was previously annotated as being non-synonymous [12], and is predicted to be damaging in our annotation (rs17362588; p.Arg935Trp). The coding SNV at *KIAA1755* is the best proxy ($r^2$~1) for the published non-synonymous SNV (rs6127471) covered on the Exome Chip (Supplementary Table 1). The original GWAS [12] had reported this signal as non-synonymous. Therefore, our Exome Chip analyses do not reveal any new evidence of likely causal coding variants at well-established HR loci.

*Regulatory variants at heart rate loci*

Our analyses of coding variants at all known HR loci indicated that the majority of HR-associated SNVs and the variants in high LD with them are non-coding. We thus investigated which variants could have a causal effect through regulatory chromatin interactions, such as promoter-enhancer

contacts. We considered all 67 published HR loci (21 previously reported GWAS loci [12], plus 46 recently published loci from UK Biobank [17]), and the five novel loci reported here. We found variants that potentially affect enhancer function using RegulomeDB [21] and found genes whose promoter regions form significant chromatin interaction with them from right ventricle Hi-C data [22]. We found 64 potential target genes in 49 HR loci (4 new loci, 18 loci from the GWAS study and 27 loci from the UK Biobank study; Supplementary Table 10). Including these long range interactors in the candidate causal genes list increased the significance of enrichment for many HR related terms, such as arrhythmia and cardiac fibrillation in our Ingenuity® Pathway Analysis (IPA®; Supplementary Table 11).

For newly identified loci, the *TESK2* promoter had a long-range interaction with the SNVs with highest regulatory potential in the locus, underlining it as a candidate. *LOC441204*, a gene of unknown function was found to interact with the *JAZF1* locus. At the *SEC31B* locus, there were interactions with two genes, *SCD* and *SLF2*. At the *C10orf71* locus, *MAPK8* showed the most significant interaction.

In the 21 loci from the previously published GWAS [12], we identified significant chromatin contacts for the regulatory SNVs of 18 loci. We found *CALCRL*, *TTN*, *HTR2B*, *PLD1* and *CHRM2* as strongest interactors at the *TFPI*, *CCDC141*, *B3GNT7*, *FNDC3B* and *CHRM2* loci, respectively, out of these only *CALCRL* is in LD ($r^2 > 0.8$) with the lead SNV. The previous study [12] functionally tested 31 candidate genes, they found 20 of them to have a HR phenotype in either *Drosophila melanogaster* or *Danio rerio* experiments. All five of the strongest interactor genes were amongst the 20 genes with a HR phenotype.

Finally, we found 41 potential causal genes that have not been implicated by previous GWASs. A few of these genes have a cardiac function, including *RAPGEF4* [18] and *PIM1* [23], while some are involved in neuronal development and function e.g. *PBX3*, *NRNX3*. These candidates open up new avenues that may aid our understanding of heart rate biology.

**Discussion**

Our meta-analysis of Exome Chip genotypes yielded five unreported novel HR loci, and one unreported independent new secondary signal, which was a low-frequency non-synonymous SNV at the previously reported *KIAA1755* locus. Our data strongly supported the association of SNVs at 11 of the 12 previously reported GWAS loci that were covered on the Exome Chip. All lead SNVs at all validated novel loci are common (MAF ≥ 5%) and have similar effect sizes, which are smaller than the effect sizes for the majority of previously reported SNVs (Supplementary Figure 7). Our study did not yield any rare SNV associations with HR, indicating that much larger sample sizes will be required in

future studies to have sufficient power to detect effects of any rare variants and assess their contributions to HR heritability.

The same observation of the need of larger sample sizes applies to the analysis of HR loci identified within Europeans in other ancestries, where the lack of significance and concordance in the results from non-European populations is most likely due to a lack of power, as well as differences in the allele frequencies and LD patterns between Europeans and non-Europeans. As the non-European samples were much smaller, we did not perform a comprehensive comparison across populations or a robust trans-ethnic meta-analysis.

Annotation of novel HR SNVs or their close proxies, eQTL analyses and long range chromatin interactions in heart tissue reveal new potential causal candidate HR genes (Supplementary Tables 10 and 12). At the *SEC31B* locus there is a predicted damaging non-synonymous variant in *SEC31B,* and SNVs at this locus are also significantly associated with *SEC31B* expression levels. Although its precise function is unknown, the *SEC31B* gene encodes SEC31 homolog B, a COPII coat complex component. SEC31B has been proposed to function in vesicle budding, and cargo export from the endoplasmic reticulum [24]. The gene is ubiquitously expressed at low levels, but there are higher levels of expression in the cerebellum. There are 13 transcripts, and thus several predicted SEC31B proteins. The major isoform is 129 kDa, but the HR-associated non-synonomous SNV maps to all *SEC31B* transcripts. There are no existing mouse models, and the predicted protein does not directly interact with other proteins or pathways currently recognized as being important to HR. Chromatin interactions in heart tissue indicate *SCD* and *SLF2* as two other candidate genes for consideration at this locus. *SCD* encodies a stearoyl-CoA desaturase, which has a role in myocardial dysfunction [25] and *SLF2* encodes the SMC5-SMC6 complex localization factor 2. *TESK2*, *C10orf71* and *DALRD3* can be considered as candidates for further analyses, based on the lead SNVs being non-synonymous variants in each gene. *TESK2* encodes a serine/threonine protein kinase with an N-terminal protein kinase domain that is structurally similar to the kinase domains of testis-specific protein kinase-1 and the LIM motif-containing protein kinases (LIMKs). *TESK2* is ubiquitously expressed, but its function is unknown [26]. There is also support for *TESK2* from the chromatin interaction analyses. *C10orf71* encodes an ORF of unknown function, that is highly expressed in heart and skeletal muscle. Chromatin interaction analyses indicate *MAPK8* as a second candidate gene at the *C10orf71* locus, *MAPK8* is involved in formation of the heart as well as heart rate regulation [27,28]. *DALRD3* encodes a protein with a DALR anticodon binding domain similar to that of class Ia aminoacyl tRNA synthetases [29].

The conditional analysis results provided one new, unreported association at a previously reported HR locus, *KIAA1755* (rs41282820; c.1528C>T or c.1528C>A; p. Arg510Ter, a loss of function

variant). *KIAA1755* is predicted to encode an uncharacterized protein, and is only characterized at the transcriptional level. The transcript is highly expressed in the brain and nerves, and it is also expressed in the heart.

Our analyses and the recently published UK Biobank analyses [17] discovered a second low-frequency non-synonymous SNV at *CCDC141* (rs10497529, c. 442C>T, P. Ala141Val). *CCDC141* (also known as *CAMDI*) encodes the coiled-coil domain containing 141 protein and interacts with DISC1 (disrupted in schizophrenia 1) and MYL2 (phosphorylatable myosin light chain). *CCDC141* is highly expressed in heart muscle [30]. Knockdown of CCDC141 in neurons leads to abnormal cortical neuronal migration, but there are otherwise limited functional studies of CCDC141 [30]. The *CCDC141* locus includes *TTN* (titin), which encodes a major structural protein in striated muscle. *TTN* mutations are associated with a range of hereditary myopathies [31]. Prior work [12] using RNA interference (RNAi) in *Drosophila melanogaster* have shown that knockdown of *TTN* leads to significant changes in resting HR and HR post tachypacing, supporting *TTN* is a causal candidate gene at this locus. The new data described here implicates *CCDC141* as a second candidate gene at this locus for functional follow-up.

Enrichment analysis of HR variants in DNase I hypersensitivity sites across nearly 300 tissue samples and cell lines indicated new candidate tissues, such as neuronal progenitors and fetal muscle as being functionally relevant. Our data suggest these tissues should be targeted for future functional studies.

Our long range regulatory chromatin interaction analyses provided additional support for some of the candidate genes have been experimentally tested previously [12] and shown to have a HR related phenotype (*CALCRL*, *TTN*, *HTR2B*, *PLD1* and *CHRM2*). By expanding the list of HR loci to include new and published, several new candidate genes are highlighted for functional studies in Supplementary Table 10.

The Exome Chip contains non-synonymous, splicing, and stop-coding variants that are thought to alter protein expression and function. Our analyses discovered four novel coding variants, indicating potential candidate causal genes at these loci. Our two-stage study design permitted the robust validation of all our novel loci findings, with a large replication sample size from UK Biobank (N = 134,251) to add together to our European discovery data (N = 104,452) for a large combined meta-analysis. However, due to the Exome Chip covering mainly coding regions, we were not able to compare results with all previous GWAS findings. In conclusion, our results taken together with recent studies [12] indicate HR-associated SNVs are mostly common (MAF > 5%) and have relatively small effect sizes. The maximum effect sizes reported thus far are ~0.70 bpm per allele and MAF of 1% for SNVs at *CCDC141* (rs17362588) and *GJA1* (rs1015451). An analysis of much larger sample sizes (1M and above) including rare and common SNVs, and samples across different ancestries may

provide further information on the contributions of both coding and non-coding variants, and the importance of rare coding variants in HR.

**Materials and Methods**

*Study populations, phenotypes and exclusions*

Thirty cohorts contributed data to the discovery meta-analysis in individuals of European ancestry. Details of all participating cohorts are provided in Supplementary Table 13, including phenotype, cohort ancestry, study design, and key references. The UK Biobank study, which was only recently published since the completion of our meta-analysis [17], provided results for replication analyses. Details of this study are also included in Supplementary Table 13.

All participating cohorts either measured RR intervals from the standard 12-lead electrocardiogram (ECG) or used HR measurements (in beats per minute) from peripheral pulse measurements (Supplementary Table 14), which were converted to the RR interval scale (in milliseconds) using the inverse relationship formula: RR (ms) = 60,000/HR (bpm). The discovery analysis was undertaken using the RR interval phenotype. The exclusion criteria included: extreme RR intervals (< 600 ms or > 1500 ms), atrial fibrillation on the ECG, a history of myocardial infarction or heart failure, use of non-dihydropyridine calcium-antagonists (Anatomic Therapeutic Chemical (ATC) code C08D), digoxin (ATC code C01AA5), second or third degree atrioventricular block, and a pacemaker signal on the ECG. Local ethics committees approved the contributing studies from the CHARGE consortium, and all individuals provided their consent in writing. The UK Biobank study has approval from the North West Multi-centre Research Ethics Committee (MREC) and has Research Tissue Bank (RTB) approval.

*Study level genotyping and quality control*

All discovery cohorts were genotyped using a human Exome Chip array (exact details of the chip for each study are provided in Supplementary Table 15). Quality Control (QC) was done according to CHARGE Exome QC guidelines, including joint variant calling with zCall [32]. At the study-level, the sample-level QC consisted of excluding samples of non-European ancestry (for European-ancestry cohorts), samples with call rates < 95%, samples with sex discordance, or related samples with an unexpected high identical by descent (IBD) estimate. It was recommended that principal components (PCs) be obtained using variants with MAF ≥ 1%. The variant QC consisted of exclusion of SNVs with call rate < 95%, with Hardy-Weinberg equilibrium values of $P < 1 \times 10^{-6}$, and of variants that were strongly associated with plate assignment.

*Study-level statistical analysis*

Each cohort performed two SNV association analyses using an additive model implemented with the R package *seqMeta* [33]. Analyses were stratified by ancestry. One SNV association analysis used an untransformed model with RR interval as the outcome, adjusted for age, sex, body-mass index (BMI) and cohort-specific adjustments. The other SNV association analysis was a model based on the rank-based inverse-normal transformed residuals (RR-INVN), with residuals taken from a linear regression RR interval adjusted for age, sex and BMI covariates. The RR-INVN analysis was performed to check for potential sensitivity to deviations from normality within the analysis of rare variants. Additional cohort-specific covariate adjustments were also applied, which included for example principal components (PCs) or family structure.

*Central QC and meta-analyses*

We performed additional QC checks centrally. For each study we checked the sample size and the total number of SNVs (monomorphic and polymorphic) and assessed the beta distribution. Within each cohort's results, all monomorphic SNVs were checked to have NA results. In order to detect potential strand-flip issues, the cohort coded effect allele frequencies (EAF) of each SNV were compared to the meta-analyzed EAF of a group of CHARGE cohorts (AGES, ARIC, CHS, FHS and WHI). Any discordant SNVs showing cohort-EAF ~ 0 in at least one study, but meta-EAF ~ 1, or vice versa, were excluded from the central meta-analysis. A set of approximately 11,000 SNVs that were known to have QC issues from central CHARGE QC were also excluded from the meta-analysis. Quantile-Quantile (QQ) plots were produced to inspect each cohort. After all QC steps were completed 235,677 SNVs remained. The results from all cohorts were then combined into a discovery meta-analysis using the *SeqMeta* R package.

*Sensitivity analyses*

A sensitivity analysis was performed on the use of beta-blockers (ATC code C07) due to the recognized effects of beta-blockers on HR. All cohorts with data on beta-blocker use were re-analyzed with exclusion of individuals using beta-blockers at the time of phenotype measurement. Results of this meta-analysis were compared with the results from the same subset of cohorts with beta-blocker users included.

*Selection of variants for replication*

All SNVs with $P < 1 \times 10^{-5}$ from the discovery meta-analysis in European individuals were considered for follow-up. As a QC step after meta-analysis, we excluded four SNVs with unrealistically high beta values, large standard errors, and results that were reported in less than four studies. We defined a

novel locus as a genomic region (i) with SNVs not in LD ($r^2 < 0.2$) with any well-established HR-associated SNVs from the previously reported GWAS [12] (Supplementary Table 1), and (ii) mapping to more than 500 kb from either side of a previously reported HR-associated SNV. At the time of our study, there were 21 loci reported from GWAS analyses with HR-associated SNVs [12]. A potential secondary signal within a previously reported locus was defined as being within a 1 Mb region centred around the published SNV, but not in LD ($r^2 < 0.2$) with the published SNV in that region. LocusZoom plots were produced for all selected SNVs. Only the lead SNV was carried forward, for each signal being followed up. Specifically, the most significantly associated SNV was selected for any SNVs in pairwise-LD ($r^2 > 0.2$). LD was calculated within UK Biobank genetic data, in order to calculate pairwise-LD for all 21 known SNVs (not only those covered on the Exome Chip).

*Replication analyses*

We used data from UK Biobank for replication of the selected SNVs (at the time of analysis genetic data was available for 150,000 individuals). The UK Biobank data were analysed with untransformed HR as the phenotype, with no exclusions for medication use. In UK Biobank resting heart rate was assessed by two methods: first, pulse rate using an automated reading during blood pressure measurement, and second, pulse rate during arterial stiffness measurement using the pulse wave form obtained of the finger with an infra red sensor. When multiple heart rate measurements were available during the first visit for an individual, these measurements were averaged. In 99.7% of participants at least one single measurement was available. Individuals were excluded with extreme (> 4SD) values (N=818). Further details are provided [17]. The results of our European exome discovery meta-analysis for RR were combined with the UK Biobank replication results for HR (N = 134,251), and a combined meta-analysis, using sample-size weighted fixed effects meta-analysis in METAL was performed [34]. All alleles were aligned between the discovery and replication data, and the inverse relationship between RR-interval and HR was taken into account, i.e. so that a negative beta direction from our discovery data for a decreased effect on RR-interval was made equivalent to a positive beta

A novel locus was declared if the lead SNV reached exome-wide significance in the combined meta-analysis of discovery and replication data ($P < 2.12 \times 10^{-7}$) and replicated with Bonferroni-adjusted significance ($P < 0.0042$ for 12 tests) in the replication data alone. In addition, the directions of effect between the discovery and replication data were required to be concordant, taking into account the inverse relationship between RR from our discovery data and HR from the replication data.

Potential secondary SNVs at known regions were declared as validated if there was an exome-wide significant association in the combined meta-analysis. Variants that validated were subsequently tested for independence from previously reported HR variants in a conditional analysis.

*Conditional analysis*

In order to determine whether the validated secondary signals at previously reported loci were independent of the published SNV, conditional analysis was performed within Genome-wide Complex Traits Analysis (GCTA) software [35] applying the –cojo method (consisting of conditional and joint analysis with stepwise model selection). The input data was the exome-wide summary statistics from the full discovery meta-analysis of RR interval in Europeans. The 1958 Birth Cohort Study (1958BC; N = 5,815) dataset was used as the reference for genotype data, because it represents one of the largest discovery studies (See Supplementary Table 13). LD was calculated between pairwise SNVs, but any SNVs further than 10 Mb apart were assumed to not be in LD. All autosomal chromosomes were analyzed, with MAF restricted to ≥ 0.01%, to allow for low frequency secondary SNVs, whilst taking into account the statistical power achievable. To allow for secondary associations a *P*-value cut-off of $1 \times 10^{-4}$ was used as the modelling selection threshold within the GCTA analysis. Results were then extracted for the nine previously reported regions, within which potential secondary signals had been validated from the combined meta-analysis. To be consistent with the look-up threshold for selecting SNVs to carry forward from discovery to replication, results were restricted to SNVs with a significance level of $P < 1 \times 10^{-5}$ from both the discovery meta-analysis and the joint association from GCTA.

*Gene-based testing*

Gene-based testing was conducted using the primary discovery data in Europeans. Analysis was performed using the SNV-set Kernel Association (SKAT) test within the *seqMeta* R Package. SKAT tests were performed according to two different MAF filters of 1% and of 5%, and three different levels of variant filtering, based on annotations within the CHARGE Exome SNP Info annotation file: (i) all variants, (ii) variants deemed predicted to be damaging and (iii) variants that were non-synonymous or leading to abnormal splicing. For gene-based tests we adjusted for multiple testing using the Bonferroni correction, according to the number of genes tested. The gene-wide significance level was calculated as $1.98 \times 10^{-6}$ for 25,241 tests (i.e. the number of genes on the Exome Chip). For any genes attaining significance, the gene-based tests were repeated with

exclusion of the most significantly associated lead variant, in order to confirm that the association was due to multiple rare variants.

*Non-European ancestry analyses*

Association results were also received for non-European samples. Analysis and QC were performed as described for the European data. A meta-analysis was performed centrally in seqMeta for AA ancestry, combining data from the five AA cohorts. Study-level results remained for HIS and CH ancestries (from only the MESA cohort), in order to consider the three non-European ancestries (AA, CH, and HIS) separately from stratified analyses. Due to the smaller sample sizes, power calculations were performed using the Genetic Power Calculator [36], based on the average percent trait variance explained per locus being 0.04%, according to the recently published results from 64 validated HR loci explaining ~2.5% of HR variance [17]. To assess the level of heterogeneity by ancestry in non-European data, we performed a look-up of SNVs at the 12 published HR loci covered on the Exome Chip, extracting results for these variants from each of the AA, CH and HIS results. We restricted our primary discovery analysis to Europeans only after finding a lack of significant validation and concordance between EUR and non-EUR data for previously reported HR variants. As a secondary analysis, we performed look-ups of all validated novel loci within the non-European data. The forest plots for all validated novel loci display non-European results, to serve as a comparison to results within Europeans. In addition to calculating the percentage of concordance of effect directions for each ancestry compared to Europeans, a Binomial sign test was also performed in R. This test was based on the number of SNVs with consistent effect directions, and it was done to determine whether the concordance was higher than expected by chance alone, using $P < 0.05$ to declare significant concordance.

*Variance explained*

The percentage variance explained for RR interval was calculated using data from all subjects in the 1958BC study. The SNV genotypes were extracted from the 1958BC Exome Chip data and considered in two different sets: the 12 previously reported SNVs covered on the chip including proxies ($r^2 \geq 0.8$; see Supplementary Table 1); and the lead SNVs from the five unreported novel loci (see Table 1A). First, RR interval was regressed in a linear model against the sex and BMI covariates (not age, as all 1958BC subjects are of same age). Then the trait residuals from this first model were used as the phenotype in a second linear regression model, with all SNVs in the given set analysed jointly as multiple predictors, and adjusted for the top ten PCs. The percentage trait variance explained by the set of SNPs was estimated from this second model, according to the adjusted $R^2$ value.

*Heart rate loci annotation*

For the purposes of annotation, all signals were expanded to include SNVs in LD. LD was calculated within the UK Biobank full genetic dataset using PLINK (v1.9). All variants with an $r^2 \geq 0.8$ within 500 kb downstream or upstream of the SNVs of interest were identified. These variants were annotated using ANNOVAR (vJun2015) [19]. ANNOVAR functionally annotates variants, provides their conservation score, identifies SNVs that may cause protein-coding changes and reports their damaging prediction scores. Various prediction scores are available in ANNOVAR, including SIFT, PolyPhen, and MutationTaster, among others.

We investigated the unreported novel SNVs and their proxies ($r^2 \geq 0.8$) across 44 tissues available in the Genotype-Tissue Expression database (GTEx) dataset [20] for expression quantitative trait loci (eQTL). We reviewed the results for SNV-eQTL associations across all tissues, focusing on the heart, nerve, lung, muscle, adrenal, and brain tissues which may be relevant tissues for HR based on known physiology of heart rate and our results from the enrichment analysis. Genes reported as eQTLs are based on study specific significance thresholds (*P* values $< 10^{-8}$) and $r^2 \geq 0.8$ between HR-SNV and top-eQTL SNV (the SNV most significantly associated with transcript).

*PhenoScanner*

PhenoScanner [37]was used to identify variants that are associated with other traits. All proxy SNVs in high LD ($r^2 \geq 0.8$) with the lead SNVs at our five unreported novel loci were investigated in the PhenoScanner 1000 Genomes reference dataset. Results were filtered to those reaching a genome-wide significance *P*-value $\leq 5 \times 10^{-8}$.

*Potential candidate genes at new heart rate loci*

Candidate genes at each locus were compiled using LD information, ANNOVAR-derived-annotation, and eQTL lookup results. A literature review was conducted for potential candidate genes at each new HR locus. Sources of information included: published articles, GeneCards, Online Mendelian Inheritance in Man®, the Human Protein Atlas, STRING, and UniProt. We searched for information on the corresponding mouse models via the International Mouse Phenotyping Consortium (IMPC) and the Jackson Laboratory online catalog. URLs for each of the sources is provided in the URL section below.

*Pathway analyses*

Pathway analyses were performed using QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City) software. In order to distinguish the pathway enrichment contribution of novel loci from known HR loci, two sets of analyses were conducted. The first analysis captured the total known signal to date, investigating all 67 loci currently published, which include the 21 loci from the previously reported GWAS [12] and the 46 loci recently published from UK Biobank [17] since the completion of our meta-analysis. The second analysis included our five unreported novel loci in addition to all the previously reported loci. In each case, the analysis included all genes annotated from the lead SNVs and their proxies ($r^2 \geq 0.8$). Results were filtered for pathway enrichment of *P*-values $\leq 10^{-4}$. We specifically report the pathways for which enrichment is increased with the inclusion of genes from our novel loci.

*Enrichment in DNaseI hypersensitive sites*

To identify the tissues in which HR associated SNVs are active, we used FORGE to look for enrichment of DNaseI hypersensitive sites (DHS) in 299 tissue samples from the Roadmap Epigenome Project [38]. FORGE calculates enrichment for overlap of HR variants with DHS by comparison with overlap of DHSs with 1000 matched background variant sets (matching distance to transcriptional start sites, GC content, and minor allele frequency).

We performed two different enrichment analyses. First, we did a 'known' analysis using all 67 currently published lead SNVs to date [21 previously reported from the original GWAS [12] and 46 new loci from the recently published UK Biobank study [17]]. Second, we did an 'all' analysis using the lead SNVs at our five unreported novel loci and the 5 independent secondary SNVs that we found at previously reported loci; together with the 67 known signals, denoted as the "all" analysis. We compared the enrichment results of the two analyses, in order to identify any new enrichment due to the inclusion of our novel loci. The enrichment is expressed as Z-score statistics. A Z-score of 2.58 was used as a threshold for statistical significance, which corresponds to FDR<1.5%. We calculated the $\text{Z-score}_{\text{all}}$-$\text{Z-score}_{\text{known}}$ ($\Delta$ Z-score) for those tissue samples that were found statistically significant in the "all" analysis in order to assess the effect of the ten new, additional SNVs from our study.

*Regulatory potential of SNVs*

We selected the HR associated SNVs and proxies in LD ($r^2 \geq 0.8$; calculated using the UK Biobank full genetic dataset) that were identified in this study, and from the previous GWAS (16) and UK Biobank studies (17) for annotation. To identify the potential regulatory variants, we retrieve the functional confidence score for SNVs from the RegulomeDb database [21]. RegulomeDb assigns a functional

confidence score to each SNV by overlapping them with functional genomic data mainly from ENCODE (e.g. DNase I hypersensitivity, DNase I footprinting, ChIP-seq), with eQTL data and with computational prediction (e.g. TF-binding sites and their disruption). We considered any SNP with at least one functional annotation to have regulatory potential (this corresponds to functional confidence scores: 1a-6).

*Long-range regulatory contacts*

Using significant long-range chromatin interactions as identified by Fit-Hi-C in right ventricle Hi-C data (40kb resolution) [22], we annotated the potential regulatory SNVs with potential target genes, whose promoter is in contact with the given SNV. Where the 40kb genomic region containing the SNV had more significant promoter interactions, we show the genes in order of most significant interaction to least significant. We took the genes with the most significant promoter interaction with the SNVs with regulatory potential per locus, and using IPA®, we assessed which pathways were affected, and specifically those that were enriched compared to using only genes in LD with HR-SNVs.

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**Abbreviations**

| | |
|---|---|
| AA | African American |
| CH | Chinese American |
| DHS | DNase I hypersensitive site |
| eQTL | expression quantitative trait loci |
| GTEx | genotype-tissue expression database |
| GWAS | genome-wide association study |
| HIS | Hispanic |
| HR | heart rate |
| IPA® | Ingenuity® Pathway Analysis |
| LD: | linkage disequilibrium |
| MAF | minor allele frequency |
| SNV | single nucleotide variant |

Supplementary tables and figures are available at HMG online: http://academic.oup.com/hmg/

# Part II

# QT Variability

# Chapter 2.1

# Short-Term QT Variability Markers for the Prediction of Ventricular Arrhythmias and Sudden Cardiac Death: A Systematic Review

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**Abstract**

*Objective:* Sudden cardiac death (SCD) is a major health burden and is primarily caused by ventricular arrhythmias. Currently, the most well-known marker for the risk of ventricular arrhythmias is QT/QTc prolongation. Animal studies indicate that QT variability might be a better indicator. Our objective was to give an overview of the literature on QT variability in humans.

*Methods:* We performed a free-text search in PubMed and Embase, from inception through February 2013.

*Results:* We identified nine QT variability markers in 109 studies reporting on QT variability markers, measured on the surface ECG. QT variability can be distinguished using two characteristics: heart-rate normalisation and whether QT interval is measured on consecutive beats. Most study populations were small (median 48 subjects, range 1-805) and different methods, time intervals, and leads for measurement were used. QT variability markers were determinants for the risk of ventricular arrhythmias, (sudden) cardiac death and total mortality. Few studies compared the predictive value of QT variability with that of QT/QTc prolongation. A study comparing all different QT variability markers is lacking.

*Conclusions:* QT variability markers are potential determinants of ventricular arrhythmias and cardiac mortality. However, it is unclear which marker and methodology is clinically most useful as well as reliable reference values. More studies on larger datasets are needed to find the most accurate marker for the prediction of arrhythmias and SCD to assess its value in addition to QT/QTc duration and its role in drug-induced arrhythmia and sudden death.

**Introduction**

Sudden cardiac death (SCD) is a common lethal manifestation of heart disease.[1,2] It is primarily caused by ventricular arrhythmias such as ventricular fibrillation (VF), ventricular tachycardia (VT), and torsade de pointes.[3,4] Risk factors include drug and alcohol use, history of heart failure or cardiac ischemia, and certain syndromes with electrocardiogram (ECG) abnormalities, e.g. long QT (LQTS) and Brugada syndrome.[5,6] Although specific groups with these risk factors have a high risk of SCD, 50% of all SCDs due to coronary heart disease occur as a first event of this condition or in patients thought to be at low risk.[7] Risk assessment has two main clinical applications: drug safety testing and risk stratification for SCD.

In clinical drug-safety studies, the applied method for predicting the arrhythmogenic potential is prolongation of the heart-rate corrected QT (QTc) interval on the ECG.[8,9] However, studies have shown that some QTc prolonging drugs do not increase the incidence of ventricular arrhythmias,[10,11] while some drugs that do carry an arrhythmogenic risk do not always show QTc prolongation.[12] Through animal studies an increased QT interval variability has been proposed as a less equivocal method for the prediction of arrhythmogenic effects.[13-23]

Another application of QT variability is risk stratification for ICD implantation. Berger *et al*. hypothesized that cardiac abnormalities lead to changes in ventricular repolarization.[24] However, these changes will not necessarily lead to arrhythmias, but it will lower the repolarisation reserve.[25,26] Repolarization reserve is the concept that the complexity of the repolarisation includes some redundancy.[26] QT variability is thought to be a measure of decreased repolarization reserve and therefore of the risk for arrhythmias.[25]

In high-risk patients, antiarrhythmic therapy is administered using implantable cardioverter-defibrillators (ICD).[27-29] Although ICD therapy reduces mortality due to SCD, it has a high cost-effectiveness ratio,[30-32] (inappropriate) shocks can lower quality of life[33] and are related to adverse outcomes.[34] Therefore improved risk stratification for ICD therapy is necessary.

The objective of this review is to provide an overview of the literature regarding short-term QT variability.

**Methods**

A PubMed and Embase search from inception until 4 February 2013 was performed. Because no MeSH-terms exist for QT variability we used the following free-text keywords: QTVI, QTV index, QT variability index, QT variability, QT interval variability, short term variability, beat-to-beat variability, STV, BVR, temporal variability, QT, repolarization, monophasic action potential duration or MAPD. We excluded papers with the term “Quantitative Tissue Velocity Imaging”, a term not related to our topic but also abbreviated QTVI. We limited the search to papers in English. In Supplement 1 the

exact search strategy is outlined. We hand-searched citation lists of all studies for publications not captured by our electronic search. Results were stored in an EndNote X5 (version 5.0.1, Thomson Reuters, USA) database.

Study selection was performed in two stages. Primary physiological or clinical studies measuring QT variability on the surface ECG were candidates for inclusion. First, publications were independently assessed on title and abstract by two reviewers (MNN, MEB). Second, the two reviewers independently assessed the full text manuscripts. When full text publications could not be retrieved online, we contacted the authors.

QT variability can be distinguished into short- and long-term. Currently, the most frequently studied QT variability markers have been measured on signal windows of 256 seconds[24] or 30 beats.[23] Long-term variability has been measured over periods of up to 24 hours.[35] We limited this review to short-term variability, and applied a maximum time window of 10 minutes or 600 beats. Disagreements between reviewers were resolved in consensus meetings. For each included publication all relevant information was extracted. This review was conducted according to the PRISMA guideline.[36]

**Results**

*Search results*

We retrieved 297 unique publications from the electronic search and included 109 publications after full-text review. These studies included 58 case-control studies, 23 cross-sectional studies, 15 clinical trials, 12 cohort studies and one case report. The selection process and reasons for exclusion are shown in Figure 1. In Supplement 3, a full overview of all included studies is shown. We could not retrieve the full text of nine publications: one review,[37] one conference paper,[38] one comment,[39] one clinical trial with nadolol and metoprolol,[40] two studies of post-coronary artery bypass graft patients[41,42] and case-control studies of patients with diabetes,[43] non-alcoholic liver cirrhosis,[44] and congestive heart failure.[45]

**Figure 1. Overview of selection process**

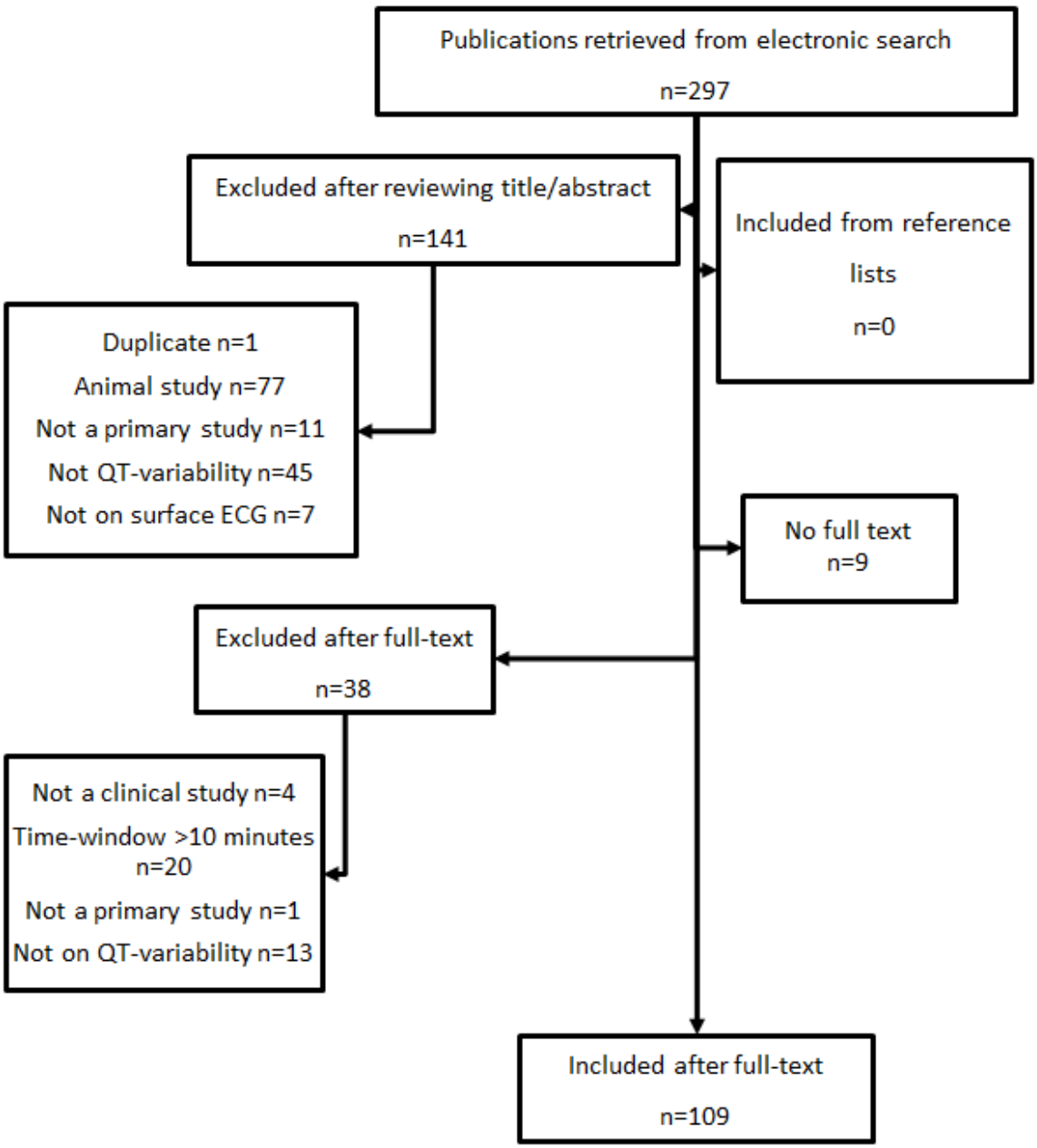


**Table 1. Overview of markers and studies included**

| | | Marker | Formula | N studies | N participants Total Median (IQR) Range |
|---|---|---|---|---|---|
| Not normalised for RR | Not on consecutive beats | QTvar | $\frac{1}{n-1}\sum_{i=1}^{n}(QT_i - QT_m)^2$ | 25 | 2262<br>59 (24-134)<br>11-396 |
| | | QTVN | $\frac{QT_{var}}{QT_m^2}$ | 31 | 4209<br>54 (29-106)<br>16-805 |
| | | SDqt | $\sqrt{QT_{var}}$ | 13 | 369<br>20 (10-48)<br>1-86 |
| | | MADqt | $\text{Median}(\lvert QT_i - \text{Median}(QT)\rvert)$ | 1 | 8 |
| | Consecutive beats | STVqt | $\sum_{i=1}^{n}\frac{\lvert QT_{i+1} - QT_i\rvert}{n\sqrt{2}}$ | 6 | 418<br>52 (27-128)<br>9-152 |
| | | RMSSDqt | $\sqrt{\frac{1}{n-1}\sum_{i=1}^{n-1}(QT_{i+1} - QT_i)^2}$ | 7 | 312<br>53 (20-68)<br>8-80 |
| Normalised for RR (variability) | Not on consecutive beats | QTVI | $\log_{10}\left[\frac{(QT_{var}/QT_m^2)}{(HR_{var}/HR_m^2)}\right]$ | 89 | 8526<br>49 (24-99)<br>6-805 |
| | | $\frac{MADqt}{MADrr}$ | $\frac{\text{Median}(\lvert QT_i - \text{Median}(QT)\rvert)}{\text{Median}(\lvert RR_i - \text{Median}(RR)\rvert)}$ | 1 | 34 |
| | Consecutive beats | QTRR | $\sum_{i=1}^{n}\frac{[(QT_{i+1} - QT_i)/(RR_i - RR_{i-1})}{n}$ | 2 | 123<br>61.5±9.262*<br>55-68 |

* mean±SD. The sum of studies exceeds 109 because several papers cover more than one marker.

QTvar: QT variance; QTm: mean QT interval; QTVN: QT variance normalised for mean QT interval; SDqt: standard deviation of QT intervals; MADqt: median absolute difference of QT intervals; STVqt: short-term variability of QT interval; RMSSDqt: root meand square of the successive QT interval differences; QTVI: QT variability index; HRvar: heart-rate variance; HRm: mean heart rate

*General findings*

Nine QT variability markers were identified: QT variance (QTvar), QT variance normalised for mean QT interval (QTVN), standard deviation of the QT intervals (SDqt), median absolute difference of QT intervals (MADqt), short-term variability of the QT interval (STVqt), root mean square of the successive QT interval differences (RMSSDqt), QT variability index (QTVI) MADqtc/MADrr and QTRR (Table 1). QT variability measures can be grouped based on the combination of two characteristics. The first is whether or not the variability is derived from measurements on consecutive beats. When measured on consecutive beats, the differences in QT interval between the first and second beat, the second and third beat, and so on are the basis for the variability measure (consecutive QT variability). Alternatively, beat order is not taken into account and QT intervals of all beats in a certain time window are used to calculate variability (non-consecutive QT variability). This distinction may have important consequences for the result. Figure 2 shows two different hypothetical patterns of four QT intervals. In the case of non-consecutive QT variability measures, the variability in pattern 1 and 2 is equal because the order is irrelevant. Consecutive QT variability measures will be larger in pattern 1 than in pattern 2.

The second characteristic to classify the different measures is whether QT variability is normalised for heart-rate (variability) (QTn). It is well-known that the QT interval duration is influenced by the duration of the RR interval, therefore in clinical practice it is common to apply a heart-rate correction method. Through the same mechanism, it could also be important whether or not QT variability is normalized for heart-rate (variability). However, heart-rate correction methods for QT interval have limitations, e.g. an overestimation of the QTc interval at higher heart rates when using Bazett's formula. Similar problems could arise with QT variability.

Thus, four groups can be distinguished: non-consecutive QT variability, consecutive QT variability, non-consecutive QTn variability, and consecutive QTn variability. Table 1 gives an overview of the nine QT variability markers, classified into these four groups, with their formulas, the number of studies per marker and the number of participants. Table 1 and Figure 3 illustrate that the study populations are generally small with a median size of 48 subjects (range 1-805 subjects).

A variety of ECG leads and time windows are used to calculate the QT variability. The majority used either lead II or V5. Time windows ranged from 10 seconds to 10 minutes. In a small number of studies measurements were performed manually, in most studies semi-automatic methods were applied, usually template stretching according to Berger.[24] In this method, a digitised ECG is displayed on a graphical interface. The operator defines the template QT interval by selecting the beginning of the QRS complex and the end of the T wave for one beat. The algorithm then

detects all R waves with automatic peak detection and subsequently determines the QT interval of all other beats by stretching or compressing each beat in time to best match the user-defined template.[24]

We will first describe available physiological and clinical information for all QT variability markers. After that we will summarise information from publications comparing different QT variability markers. Finally, we will discuss publications comparing QT variability markers and QT(c) duration.

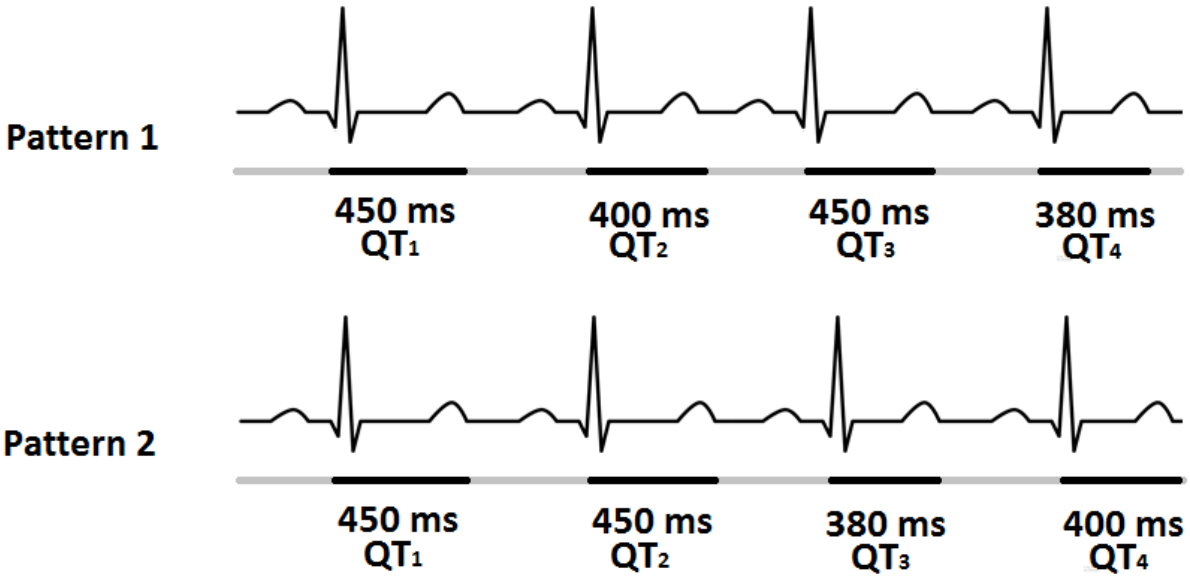
*Association between QT variability and physiological and clinical endpoints*

QT variability markers had a diurnal pattern being low at night and during sleep, increasing in the morning, peaking at noon.[46-49] Variability was lower in children than in adults and further increased with age[49-53] No conclusion could be drawn on gender differences.[51-57] Variability was higher in standing position compared to supine.[58-60] Passive head tilting,[61-63] head-down bed rest (simulating a long-duration space flight),[64] job strain and exercise showed inconsistent results, but most studies showed an increased QT variability during exercise.[54,56,65-69]

An increased variability was found in patients with cardiac abnormalities,[61-63,66,70-100] e.g. congenital LQTS,[78,95,96,101,102] drug-induced LQTS[77,98] and was associated with the occurrence of VF/VT.[70-76] Ten cohort studies (n=132-687) on patients with cardiac diseases showed that an increased variability was associated with the occurrence of VF/VT, (cardiovascular) mortality and SCD.[55,71,73,76,85,88,103-106] However, three case-control studies did not show an increased QT variability in patients with SCD.[72,107,108] Case-control studies further showed that QT variability was increased in patients with cardiomyopathies,[24,62,79,109-111] coronary artery disease,[80,84,86,112,113] and hypertension.[94,109,114]

When patients with structural heart disease received cardiac resynchronization therapy, QT variability did not change when electrical remodeling was reversed (reversal of electrical remodeling was defined as a decrease of the QRS duration with more than 10 ms.),[71] QT variability was not increased in patients with postural tachycardia syndrome[61] and in teenage patients who underwent an arterial switch operation in the neonatal period.[115] Three studies showed that sympathetic activity of the heart elevated QT variability.[61,114,116] Six studies showed an increased variability in patients who had a disturbed cardiac autonomous regulation.[87,117-121] This indicates that neuronal regulation of cardiac function influences repolarization patterns and stability represented in QT variability. Several other studies report on QT variability in conditions that have a weak association with SCD. Most of these studies found a higher QT variability in cases.[49,59,93,116-119,122-150]

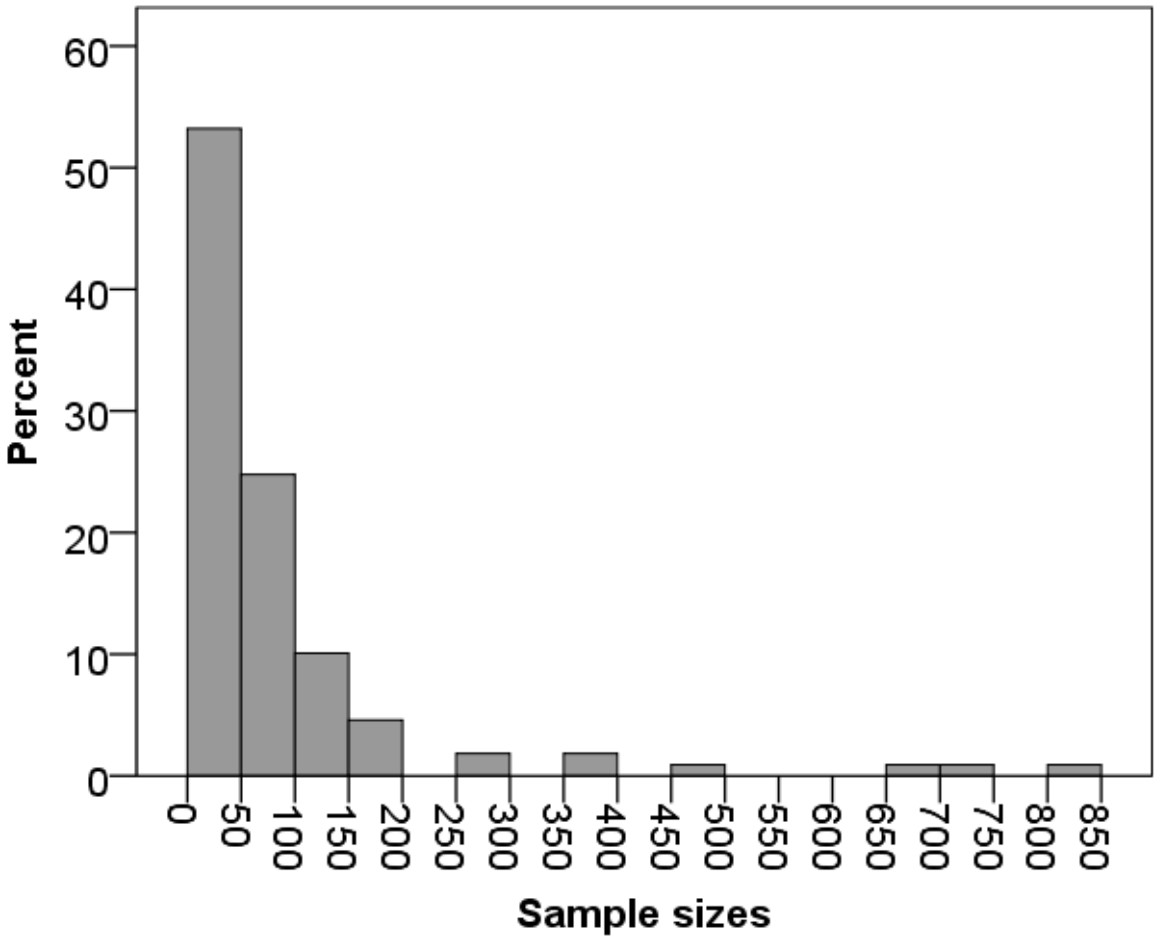
**Figure 2. Effect of QT interval patterns on QT variability measures**




| Type of QT variability | Pattern 1 ——— — ——— — | Pattern 2 ——— ——— — — |
|---|---|---|
| consecutieve QT variability: STVqt $\sum_{i=1}^{n} \frac{\lvert QT_{i+1} - QT_i \rvert}{n\sqrt{2}}$ | (\|400-450\| + \|450-400\| + \|380-450\|) / (4√2) **30.05** | (\|450-450\| + \|380-450\| + \|400-380\|) / (4√2) **15.91** |
| non-consecutive QT variability: SDqt $\sqrt{\frac{1}{n}\sum_{i=1}^{n}(QT_i - QT_m)^2}$ | sqrt(1/4 ((450-420)$^2$ + (400-420)$^2$ + (450-420)$^2$ + (380-420)$^2$)) **35.59** | sqrt(1/4 ((450-420)$^2$ + (450-420)$^2$ + (380-420)$^2$ + (380-420)$^2$)) **35.59** |

Two hypothetical ECG patterns consisting of four beats are shown. The duration of QT intervals are identical in pattern 1 and 2, but their order is different. In case of non-consecutive QT variability measures, e.g. the standard deviation, there is no difference between pattern 1 and 2 because the order of beats is irrelevant. Consecutive QT variability, e.g. short-term variability, will be larger in pattern 1 than in pattern 2.

**Figure 3. The distribution of population sizes across studies**




In Table 2 the effect of drugs on QT variability is shown.[53,63,68,77,89,110,116,127,128,135,151-158] Variability was decreased by β-blockers, but increased by sotalol, a drug that can cause ventricular arrhythmias. Drugs that stimulate the sympathetic nervous system as well as most QTc interval prolonging drugs increased QT variability. Amiodarone neither increases QT variability, nor does it increase the risk of ventricular arrhythmias, but it does prolong the QTc interval. This observation is of particular interest given the inconsistent relation between observed QTc prolongation and SCD risk by certain drugs.

**Table 2. Effects of several drugs on non-consecutive QTn variability**

| Drug | Effect | Drug | Effect |
|---|---|---|---|
| β-blocker | | Antidepressants | |
| Carvedilol[W63 | ↓ | Clonidine[W158 | ↑ |
| Metoprolol[W63 | ↓ | Pemoline[W157 | ↑ |
| Sotalol[W53,W 77,W110,W151 | ↑/= | Venlafaxine[W135 | ↑ |
| Antiarrhythmics | | Duloxetine[W135 | ↑ |
| Digoxin[W151 | ↑ | Nortriptyline[W128 | ↑ |
| Amiodarone[W110 | = | Yohimbine[W158 | ↑ |
| Ibutilide[W152 | ↑ | Fluoxetine[W157 | = |
| Atorvastatin[W154 | = | Fluvoxamine[W116 | = |
| β-sympaticomimetics | | Sertraline[W116 | = |
| Dobutamine[W89 | ↑ | Citalopram[W116,W135 | ↑/= |
| Epinephrine[W153 | ↑ | Paroxetine[W128,W135 | ↑/= |
| Isoproterenol[W127 | ↑ | Magnesium sulfate[W151 | ↓ |
| Sevoflurane[W155 | ↑ | Sildenafil[W156 | ↑ |

*Comparison between QT variability marker groups*

As described earlier, QT variability markers can be divided into four groups based on whether or not the measurement is performed on consecutive beats and whether or not it is normalized for heart-rate (variability). Because these marker groups are physiologically different, as mentioned in the general findings section, there could be differences in the association with the endpoints between marker groups. In 57 studies, the results for different QT variability markers are described, however no direct comparisons are made. Most studies (51) described non-consecutive QT and non-consecutive QTn variability together. In Table 3 all possible combinations of simultaneous analyses are shown. There are no studies that studied all four QT variability marker groups. In the majority of studies comparing two or three markers, the markers showed similar results, i.e. they showed a

significantly higher QT variability in persons with a higher risk for arrhythmias and SCD. None of the studies compared sensitivity and specificity or predictive power of different QT variability markers.

*Comparison between QT variability and QTc interval*

Seventy of the 109 included publications reported on the QT/QTc interval alongside QT variability. In 40 of these publications, the QT interval was corrected for heart-rate. Only two studies made a direct comparison. Hinterseer *et al*. compared the sensitivity and specificity of QTc interval and QT variability for diagnosing congenital LQTS among 40 patients and 40 controls, and found that sensitivity of QT variability was higher than for QTc interval (83% vs. 43%), but specificity was lower (68% vs. 97%).[96] When tested in 20 patients with drug-induced LQTS, the predictive power of QT variability was superior to that of QTc prolongation (area under the receiver operating characteristics curve 0.89 vs. 0.57).[98] Of the other 68 studies, 66 described an increased QT variability, while only 27 found an increased QT(c) interval. Unfortunately in these studies nothing was reported on the predictive power of QT variability compared to QTc prolongation.

**Table 3. Studies comparing different QT variability groups**

| 1st marker | 2nd marker | Difference | No difference | Total |
|---|---|---|---|---|
| Non-consecutive QT | Consecutive QT | 1 | 6 | 7 |
| Non-consecutive QT | Non-consecutive QTn | 19 | 32 | 51 |
| Non-consecutive QT | Consecutive QTn | - | 2 | 2 |
| Consecutive QT | Non-consecutive QTn | 2 | 1 | 3 |
| Consecutive QT | Consecutive QTn | - | 2 | 2 |
| Non-consecutive QTn | Consecutive QTn | - | - | 0 |

All possible combinations of comparison are shown in column 1 and 2. The third column shows in how many studies there is a difference in association of the markers with the outcome. The fourth column shows how many studies show no difference.

**Discussion**

*Key findings*

This is the first review that focuses on all QT variability markers. It shows that QT variability markers are potentially useful determinants of ventricular arrhythmias, (sudden) cardiac death and total mortality, both in the context of risk stratification and drug safety. Possibly, QT variability could be a useful addition to risk stratification for ventricular arrhythmias and SCD. However, many studies used small populations and a few studies did not show an association between QT variability markers and conditions assumed to enhance the risk of arrhythmias and SCD.[71,77,99,108,115] Moreover, a wide variety of markers and methods is used while studies that compare the predictive value of markers directly are unavailable.

*Overview of results and further research*

We identified nine different markers of QT variability. Non-consecutive QTn was the most prominent, covered in 83% of the included studies. Most studies were case-control studies with small sample sizes. Reference values and differences between sex, age groups and ethnicities have not been established.

The measurement methods lack uniformity. First, there is no consensus on which lead to use, although most studies use either lead II or V5. Two studies found significant differences in QTVI between the standard ECG leads.[57,159] All studies used a single lead to determine the markers. It is plausible that some method of combining leads will give better results, but this has not been investigated to date.

Second, there are different methods to measure the subsequent QT intervals. While a few studies measured manually, the majority used the semi-automatic algorithm as proposed by Berger *et al*.[24] Since beat-to beat changes in the QT interval are in the magnitude of a few millisecond, QT variability can considerably be affected by accuracy of measurement. For comparison of the results between studies, a standard method of measuring QT intervals should be chosen. A recent study by Baumert *et al.* showed that template matching algorithms, in particular a time shifting algorithm, performs better than conventional methods.[160] Third, the time window in which QT variability is determined varies between studies. As indicated by Magri *et al*., variation in time window can give different results for the same marker.[112] As of yet, no studies have been performed to assess the optimal time window. Fourth, the effect of heart-rate (variability) normalization on QT variability is unclear. One study specifically comparing QTVN with QTVI noted that an increase in QTVI could be due to a decrease in heart-rate variability or an increase in QT variability.[88] The other studies we

found however, did not consistently report the heart-rate variability and QT variability component of QTVI. Therefore we could not elucidate this further.

Studies that described different QT variability marker groups did not unequivocally show that one specific marker group performs better than the others. However, without direct comparison of the predictive value of these markers, we cannot, on the basis of the current literature, conclude that one marker or marker group is superior. In a paper by Oosterhoff *et al*. predictive value of consecutive and non-consecutive QT variability on intracardiac ECGs were compared. They hypothesized that consecutive QT variability accentuates sudden changes in QT interval, which are likely due to stochastic activity of ion channels and thus are arrhythmogenic. Conversely, non-consecutive QT variability should be more sensitive to slow changes in QT due to variation in autonomic tone or respiration, and reflects changes in the autonomic tone in the ventricles.[161] In Figure 2, pattern one corresponds to the sudden i.e. high-frequency changes, and pattern two corresponds to the slow, low-frequency changes. As non-consecutive variability is the same in both patterns, it cannot be said to be more sensitive to slow changes.
In two small studies direct comparison of the predictive and discriminative power showed that QT variability performs better than QTc prolongation. Although in other studies no direct comparisons of the predictive value was made, QT variability discriminated between cases with an increased risk for SCD and controls in more studies than QTc prolongation. Evidence for superiority of QT variability over QT duration is limited. However, it can be of high relevance, even if the predictive power in the general population is limited. It could lead to re-evaluating the place of the obliged assessment of QT duration prolonging effects in drug development. Also the withdrawal of several drugs could be disputed.

*Clinical relevance*

To date no study systematically investigated which marker and measuring method is optimal for risk prediction. For drug safety studies, study sample sizes were small and methodological heterogeneity was substantial. Therefore we cannot draw any firm conclusions. For risk stratification there is evidence that QT variability is a marker of cardiovascular mortality and SCD, including a number of cohort studies, indicating that QT variability might indeed be a useful risk indicator. However, further research is needed before this promising marker can be used in clinical practice.

*Limitations*

As our intention was to restrict ourselves to the literature on short-term QT variability we only included studies that measured over at most 10 minutes. Consequently, we excluded 20 publications

on long-term QT variability. The value of long-term variability is still unknown and remains a subject for further investigation. Furthermore, we only included studies performed in humans on surface ECGs since these studies have the highest clinical impact and are of interest for future epidemiologic research. Finally, due to substantial clinical heterogeneity we were not able to quantify our results through meta-analysis. This heterogeneity was caused by differences in signal processing, formulas used and clinical endpoints that were studied.

*Conclusion*

This review shows that QT variability might be a clinically useful determinant of ventricular arrhythmias and SCD. However, more comparative studies are needed to find the most accurate QT variability marker and the best method for measurement. We believe this overview of the current scientific status of QT variability markers aids clinicians and epidemiologist in future research on ECG derived markers for sudden cardiac death and arrhythmias.

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structural heart disease: a comparison with QT variability index. *Heart Rhythm.* 2011;**8**:1584-90.

**Supplement 1. Exact search strategy**

*Embase*

**#1** qt OR repolarization

**#2** 'monophasic action potential duration' OR mapd

**#3** 'short term variability' OR 'beat-to-beat variability' OR stv OR bvr OR 'temporal variability'

**#4** qtvi OR 'qtv index' OR 'qt variability index' OR 'qt variability' OR 'qt interval variability' NOT 'quantitative tissue velocity imaging'

**#5** (#1 AND #3) OR (#2 AND #3) OR #4

**#6** #5 AND [english]/lim AND ([article]/lim OR [article in press]/lim)

→283 publications

(((qt OR repolarization) AND ('short term variability' OR 'beat-to-beat variability' OR stv OR bvr OR 'temporal variability')) OR (('monophasic action potential duration' OR mapd) AND ('short term variability' OR 'beat-to-beat variability' OR stv OR bvr OR 'temporal variability')) OR (qtvi OR 'qtv index' OR 'qt variability index' OR 'qt variability' OR 'qt interval variability' NOT 'quantitative tissue velocity imaging')) AND [english]/lim AND ([article]/lim OR [article in press]/lim)

*Pubmed*

**#1** qt OR repolarization OR repolarisation

**#2** "monophasic action potential duration" OR mapd

**#3** "short term variability" OR "beat-to-beat variability" OR stv OR bvr OR "temporal variability"

**#4** qtvi OR "qtv index" OR "qt variability index" OR "qt variability" OR "qt interval variability" NOT "quantitative tissue velocity imaging"

**#5** (#1 AND #3) OR (#2 AND #3) OR #4 Filters activated: Journal Article, English

**#6** (#1 AND #3) OR (#2 AND #3) OR #4 Filters activated: Review, English

**#7** #5 NOT #6

→268 publications

((qt OR repolarization OR repolarisation) AND ("short term variability" OR "beat-to-beat variability" OR stv OR bvr OR "temporal variability")) OR (("monophasic action potential duration" OR mapd) AND ("short term variability" OR "beat-to-beat variability" OR stv OR bvr OR "temporal variability")) OR qtvi OR "qtv index" OR "qt variability index" OR "qt variability" OR "qt interval variability" NOT "quantitative tissue velocity imaging"

Filters activated: Journal Article [or review], English

**Supplement 2. Overview of included studies**

| | Number of participants | | | QT variability markers | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st Author and year of publication | Total | Cases | Topic | QTVI | QTvar | QTVN | $STV_{QT}$ | QTsd | $SDNN_{QT}$ | $RMSSD_{QT}$ | $MAD_{QT}$ |
| Alam 2009 | 11 | 11 | Obese subjects after surgery | ■ | | | | | | | |
| Arnol 2008 | 68 | 28 | Renal transplant recipients | | | | | | ■ | ■ | |
| Atiga 1998 | 105 | 81 | SCD, heart disease | ■ | ■ | | | | | | |
| Atiga 2000 | 62 | 36 | Hypertrophic CM | ■ | ■ | | | | | | |
| Bar 2007 | 50 | 25 | Paranoid schizophrenia | ■ | | ■ | | | | | |
| Bar 2007 | 51 | 18 | Acute alcohol withdrawal | ■ | | ■ | | | | | |
| Bar 2008 | 30 | 15 | Paranoid schizophrenia | ■ | | | | | | | |
| Bar 2010 | 108 | 36 | Relatives of schizophrenics | ■ | | | | | | | |
| Baumert 2008 | 12/5 | | Depression/panic disorder | ■ | ■ | | | | | | |
| Baumert 2008 | 20 | | OSAS | ■ | ■ | | | | | | |
| Baumert 2011 | 25 | 13 | Postural tachycardia syndrome | ■ | ■ | | | | | | |
| Baumert 2011 | 32 | 23 | Essential hypertension | ■ | | | | | | | |
| Berger 1997 | 147 | 83 | Dilated CM | ■ | ■ | | | | | | |
| Berger 2003 | 30 | 22 | Dilated CM | ■ | ■ | | | | | | |
| Boettger 2010 | 23 | | Sport students | ■ | | | | | | | |
| Boettger 2010 | 131 | | Age, sex differences | ■ | | | | | | | |
| Bonnet 2005 | 15 | | Young adult subjects | ■ | | | | | | | |
| Carney 2003 | 36 | 18 | Depression in MI patients | ■ | ■ | ■ | | | | | |
| Cheng 2009 | 21 | 13 | Heart failure | ■ | | | | | | | |
| Collins 2005 | 36 | | Job strain in middle-aged men | ■ | | | | | | | |
| Couderc 2009 | 34 | 17 | Drug-induced LQTS | | | | | | | | ■ |
| Couderc 2010 | 8 | 3/2 | Drug-induced/congenital LQTS | | | | | | | | ■ |
| Desai 2004 | 34 | 17 | Heart failure, effect of posture | ■ | | | | | | | |
| Dobson 2009 | 372 | | Diurnal changes, heart failure | ■ | | ■ | | | | | |
| Dobson 2011 | 268 | 55 | Mortality, heart failure | ■ | | | | | | | |
| Falkenberg 2013 | 23 | 8 | Arterial switch operation | ■ | | | | | | | |
| Frljak 2003 | 47 | 27 | Cardiac surgery | | | | | ■ | | | |
| Furakawa 2006 | 26 | 12 | B-blockers in MI patients | ■ | | ■ | | ■ | | | |
| Galeano 2003 | 86 | 69 | VF/VT in organic heart disease | ■ | | | | ■ | | | |
| Gao 2005 | 46 | 17 | Chronic renal failure | ■ | | | | | | | |
| Haigney 2004 | 463 | 104 | VF/VT in patients with ICD | ■ | | ■ | | | | | |
| Haigney 2006 | 29 | | Effect of cocaine | ■ | | ■ | | | | | |
| Haigney 2009 | 805 | 663 | Sexe, VF/VT, ischemic CM, ICD | ■ | | ■ | | | | | |
| Haigney 2009 | 70 | 47 | Coronary artery disease, ICD | ■ | | ■ | | | | | |

| Author & year | Total | Cases | Topic | QTVI | QTvar | QTVN | $STV_{QT}$ | QTsd | $SDNN_{QT}$ | $RMSSD_{QT}$ | $MAD_{QT}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hasan 2012 | 72 | | Healthy subjects | ■ | | | | | | | |
| Hinterseer 2008 | 40 | 20 | Drug-induced LQTS | | | | ■ | | | | |
| Hinterseer 2009 | 64 | 40 | Congenital LQTS | | | | ■ | | | | |
| Hinterseer 2010 | 120 | 60 | Heart failure, ICD | | | | ■ | | | | |
| Hiromoto 2005 | 59 | | Previous anterior MI | ■ | ■ | | | ■ | | | |
| Huang 2012 | 23 | | Effect of air pollution | ■ | | | | | | | |
| Ince 2001 | 15 | | Heart failure, effect of MgSO4 | ■ | | | | | | | |
| Jie 2010 | 33 | 16 | Drug-induced LQTS | | | | ■ | | | | |
| Jindal 2009 | 50 | 24 | 1st episode psychosis | ■ | | | | | | | |
| Johansson 2004 | 153 | 67 | Chronic renal failure | ■ | ■ | | | | | | |
| Kanemori 2008 | 23 | 13 | Brugada syndrome | | | | | ■ | | | |
| Koschke 2009 | 150 | 75 | Major depression | ■ | | | | | | | |
| Koschke 2010 | 40 | 20 | Anorexia nervosa | ■ | | ■ | | | | | |
| Kudaiberdieva 2003 | 54 | 27 | MI patients, VF/VT | ■ | | | | | | | |
| Kuriki 2011 | 50 | 25 | Kawasaki disease | ■ | ■ | | | | | | |
| Kusuki 2011 | 173 | | QT variability in healthy children | ■ | ■ | ■ | | | | | |
| La Fountaine 2010 | 114 | 83 | Spinal cord injury | ■ | | ■ | | | | | |
| La Fountaine 2011 | 6 | 3 | Concussion in athletes | ■ | | | | | | | |
| Lengyel 2011 | 152 | 76 | Professional soccer players | | | | ■ | | | | |
| Lewis 2006 | 17 | | Exercise | ■ | | ■ | | | | | |
| Lewis 2008 | 8 | | Progressive bicycle exercise | ■ | | | | ■ | | ■ | |
| Lewis 2012 | 38 | | QT variability in the eldery | ■ | | | | | | | |
| Magri 2007 | 60 | 30 | β-thalassemia major | ■ | ■ | ■ | | | | | |
| Magri 2012 | 50 | 20 | MI, β-blocker therapy | ■ | | | | | | | |
| Magri 2012 | 63 | 43 | Myotonic dystrophy type 1 | ■ | | | | | | | |
| Mezilis 1998 | 10 | | Hypertrophic CM | | | | | ■ | | | |
| Mine 2008 | 11 | | Atrial pacing, propranolol, atropin | | ■ | | | ■ | | | |
| Murabayashi 2002 | 68 | | Ischemic episodes during holter | ■ | | ■ | | | | | |
| Myredal 2005 | 121 | 20 | Renovascular hypertension | ■ | ■ | | | | | | |
| Myredal 2008 | 93 | 61 | Recovery after CABG | ■ | ■ | ■ | | | | | |
| Nishi 2011 | 9 | | Cardiac rehabilitation program | | | | ■ | | | | |
| Nussinovitch 2010 | 24 | 12 | Familial dysautonomia | ■ | | ■ | | | | | |
| Nussinovitch 2012 | 106 | 53 | Familial Mediterranean fever | ■ | | ■ | | | | | |
| Nussinovitch 2012 | 88 | 43 | Anorexia nervosa | ■ | | ■ | | | | | |
| Nussinovitch 2012 | 26 | 12 | Familial Mediterranean fever | ■ | | | | | | | |
| Piccirillo 2001 | 143 | 91 | Propranolol, sotalol, age | ■ | | | | | | | |
| Piccirillo 2002 | 82 | | Heart failure | ■ | ■ | | | | | | |
| Piccirillo 2002 | 20 | 10 | Heart failure | ■ | | | | | | | |
| Piccirillo 2002 | 83 | 44 | Hypertension, HCM, ICD | ■ | | | | | | | |
| Piccirillo 2006 | 190 | 44 | Heart failure | ■ | ■ | | | | | | |

| Author & year | Total | Cases | Topic | QTVI | QTvar | QTVN | $STV_{QT}$ | QTsd | $SDNN_{QT}$ | $RMSSD_{QT}$ | $MAD_{QT}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Piccirillo 2007 | 396 | 42 | Heart failure | ■ | ■ | ■ | | | | | |
| Piccirillo 2008 | 32 | 22 | CM | ■ | | | | | | | |
| Piccirillo 2008 | 32 | 22 | CADASIL patients | ■ | ■ | | | | | | |
| Pohl 2001 | 17 | 6 | Panic disorder | ■ | | ■ | | | | | |
| Pohl 2003 | 23 | | Effect of fluoxetine, pemoline | ■ | | | | | | | |
| Sachdev 2010 | 63 | 30 | ICU patients | ■ | | | | | | | |
| Sacre 2012 | 31 | 16 | Cardiac dysinnervation | ■ | | ■ | | | | | |
| Sakowski 2011 | 20 | | Head-down bed rest | ■ | | | | ■ | | ■ | |
| Satomi 2005 | 19 | 14 | Congenital LQTS | | | | | ■ | | | |
| Seethala 2011 | 10 | | Atrial pacing, dobutamine | ■ | | | | ■ | | | |
| Segerson 2008 | 687 | 12/3 | Patients 6-8 weeks post MI | ■ | | | | | | | |
| Singh 1997 | 49 | | Sleep, awakening | | | | | ■ | | | |
| Singh 1997 | 1 | | Cardiac arrest | | | | | ■ | | | |
| Solaimanzadeh 2008 | 28 | 14 | Familial dysautonomia | ■ | | | | | ■ | ■ | |
| Spears 2012 | 21 | 11 | SCD, epinephrine | ■ | | | | | | | |
| Sullivan 2004 | 16 | | Panic disorder | ■ | | | | | | | |
| Svernhage 1998 | 12 | 4 | Torsades de Pointes | | ■ | | | | | | |
| Tereschenko 2009 | 298 | | VF/VT, ICD | ■ | ■ | | | | | | |
| Tereschenko 2011 | 69 | 22 | CRT-D, VF/VT | ■ | | ■ | | | | | |
| Tereschenko 2012 | 714 | 533 | Heart failure | ■ | | ■ | | | | | |
| Vrtovec 2000* | 55 | 25 | Angina pectoris | | | | | | ■ | ■ | |
| Vrtovec 2001 | 53 | 30 | Hormone replacement therapy | | | | | | ■ | ■ | |
| Vrtovec 2005 | 80 | 40 | Heart failure, atorvastatin | | | | | | ■ | ■ | |
| Vrtovec 2008 | 110 | 29 | Heart failure | ■ | | | | | | | |
| Vrtovec 2009 | 142 | 55 | Dilated cardiomyopathy | ■ | | | | | | | |
| Yeragani 2000 | 49 | | Posture, breathing, age | ■ | ■ | ■ | | | | | |
| Yeragani 2000 | 19 | | Posture, breathing | ■ | ■ | ■ | | | | | |
| Yeragani 2000 | 16 | | Panic disorder | ■ | | ■ | | | | | |
| Yeragani 2000 | 30 | 16 | Panic disorder | ■ | | | | | | | |
| Yeragani 2000 | 88 | 36 | Panic disorder, depression | ■ | | ■ | | | | | |
| Yeragani 2001 | 22 | 6 | Overanxious disorder | ■ | ■ | ■ | | | | | |
| Yeragani 2002 | 54 | 22 | Panic disorder | ■ | | ■ | | | | | |
| Yeragani 2003 | 31 | 19 | Panic disorder | ■ | | | | | | | |
| Yeragani 2005 | 49 | | Diurnal changes in QT variability | ■ | | ■ | | | | | |
| Yeragani 2006 | 48 | 25 | Anxiety, panic disorder | ■ | | | | | | | |

# Chapter 2.2

# Validation of Automatic Measurement of QT Interval Variability

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**Abstract**

*Background* Increased variability of beat-to-beat QT-interval durations on the electrocardiogram (ECG) has been associated with increased risk for fatal and non-fatal cardiac events. However, techniques for the measurement of QT variability (QTV) have not been validated since a gold standard is not available. In this study, we propose a validation method and illustrate its use for the validation of two automatic QTV measurement techniques.

*Methods* Our method generates artificial standard 12-lead ECGs based on the averaged P-QRS-T complexes from a variety of existing ECG signals, with simulated intrinsic (QT interval) and extrinsic (noise, baseline wander, signal length) variations. We quantified QTV by a commonly used measure, short-term QT variability (STV). Using 28,800 simulated ECGs, we assessed the performance of a conventional QTV measurement algorithm, resembling a manual QTV measurement approach, and a more advanced algorithm based on fiducial segment averaging (FSA).

*Results* The results for the conventional algorithm show considerable median absolute differences between the simulated and estimated STV. For the highest noise level, median differences were 4-6 ms in the absence of QTV. Increasing signal length generally yields more accurate STV estimates, but the difference in performance between 30 or 60 beats is small. The FSA algorithm proved to be very accurate, with most median absolute differences less than 0.5 ms, even for the highest levels of disturbance.

*Conclusion* In conclusion, artificially constructed ECGs with a variety of disturbances allow validation of QTV measurement procedures. The FSA algorithm provides highly accurate STV estimates under varying signal conditions, and performs much better than traditional beat-by-beat analysis. The fully automatic operation of the FSA algorithm enables STV measurement in large sets of ECGs.

**Introduction**

The duration of the QT interval in the electrocardiogram (ECG) may vary between individual beats, reflecting beat-to-beat changes in ventricular depolarization and repolarization[1]. A recent position paper about QT-interval variability (QTV) extensively reviewed the measurement, physiological basis, and clinical value of QTV.[2] Increased QT-interval variability (QTV) has been associated with increased risk for arrhythmias and cardiovascular events in general.[2,3]

The measurement of QTV is a challenging task because the QT-interval variations are usually subtle, in the order of milliseconds, and noise or baseline wander may further complicate the determination of the end of the T wave, which in itself is ill-defined. QT intervals have been measured manually, which is time-consuming and cumbersome. Alternatively, several (semi-)automatic techniques have been proposed[2], but little is known about their measurement accuracy. Validation of manual or automatic measurement techniques, preferably under different operating conditions, is needed. However, validation is equivocal because no reference standard is available.

This issue was in part addressed by Baumert et al.,[4] who constructed artificial ECGs by concatenating a single, noise-free ECG beat, and then added various forms of simulated disturbances (noise, baseline wander, amplitude modulation). The simulated ECGs were then used for testing the performance of three QTV measurement algorithms. These authors did not simulate beat-to-beat QT-interval variations, and thus could only validate the performance of the algorithms in the absence of QTV. Moreover, all simulated ECGs were based on just one ECG beat from a single lead.

Here we present a validation method that generates artificial standard 12-lead ECGs based on the averaged P-QRS-T complexes from a variety of existing ECG signals, with simulated intrinsic (QT interval) and extrinsic (noise, baseline wander, signal length) variations. Using the simulated ECGs, we assessed the performance of two fully-automatic QTV measurement algorithms, viz. a conventional QTV measurement algorithm, resembling a manual QTV measurement approach, and a more advanced algorithm based on the fiducial segment averaging technique.[5]

**Methods**

Our validation approach consists of the following steps. First, low-noise artificial ECGs of different durations are constructed from a collection of 12-lead ECGs, and initial QT intervals of the individual beats in each artificial ECG are set. Various amounts of intrinsic variability (QTV) and extrinsic variations (noise and baseline wander) are simulated and added to the artificial ECGs. Second, the artificial ECGs are processed by a QTV measurement program and the computed QTV is compared with the simulated QTV to assess program performance. These steps are discussed in more detail below.

*Construction of artificial ECGs*

For a given standard 12-lead ECG, we constructed an artificial ECG by computing an averaged P-QRS-T complex for each lead and concatenating this single complex at the same heart rate as in the original ECG. Since the complexes of the artificial ECG are per lead exactly identical, there is no QTV. To determine the averaged complex, we had recourse to the Modular ECG Analysis System (MEANS). This program for automatic ECG measurement and diagnosis has been evaluated extensively, both by its developers and by others.[6-8] For each lead, MEANS performs baseline correction, removes mains interference, and determines an averaged complex from the dominant beats after having excluded ectopic beats. This results in a low-noise representative complex without baseline wander. MEANS determines global fiducial points in the averaged beats of all 12 leads, resulting in a common P onset, P end, QRS onset, QRS end, and T end over all leads. The fiducial points determined by MEANS are transferred to each beat in the artificial ECG, and serve as the reference points for subsequent evaluation of the QTV measurement algorithms.

*Simulation of intrinsic and extrinsic variations*

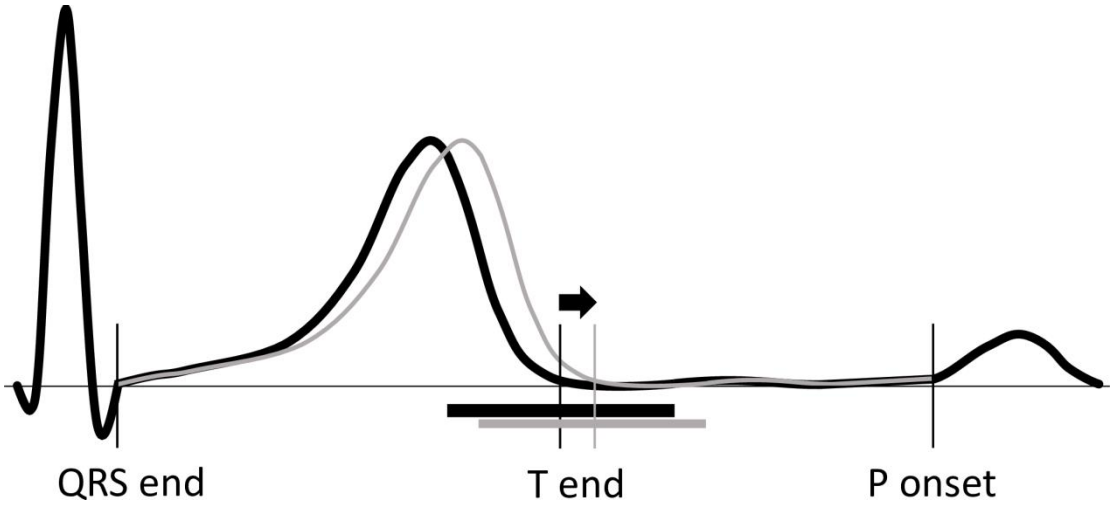
Assuming that QTV is mainly determined by ventricular repolarization, we simulated QT interval changes by stretching or compressing the ST-T wave of complexes, effectively shifting the end of the T wave. We did not change the onset of the QRS complex. The end of the T wave as determined by MEANS was taken as starting point. Simulated changes in the end of the T wave always consisted of an integer number of sample points (sampling interval 2 ms). A symmetric window of 90 sample points around T end was shifted in time foreward or backward without deformation, bringing about a compression or extension of the signal segments before and after the window (see Figure 1). The samples in the T wave before this window were shifted proportionally in time, interpolated, and resampled at the original sampling frequency (500 Hz). Similarly, the samples after the window till the start of the next P wave were shifted, interpolated, and resampled. For a given complex, the shift in T end was the same across all leads.

We quantified QTV by a commonly used measure, short-term QT variability (STV), which is defined as the mean absolute difference between successive QT intervals [9]:

$$STV = \sum_{i=1}^{N} \frac{|QT_{i+1} - QT_i|}{N\sqrt{2}}$$

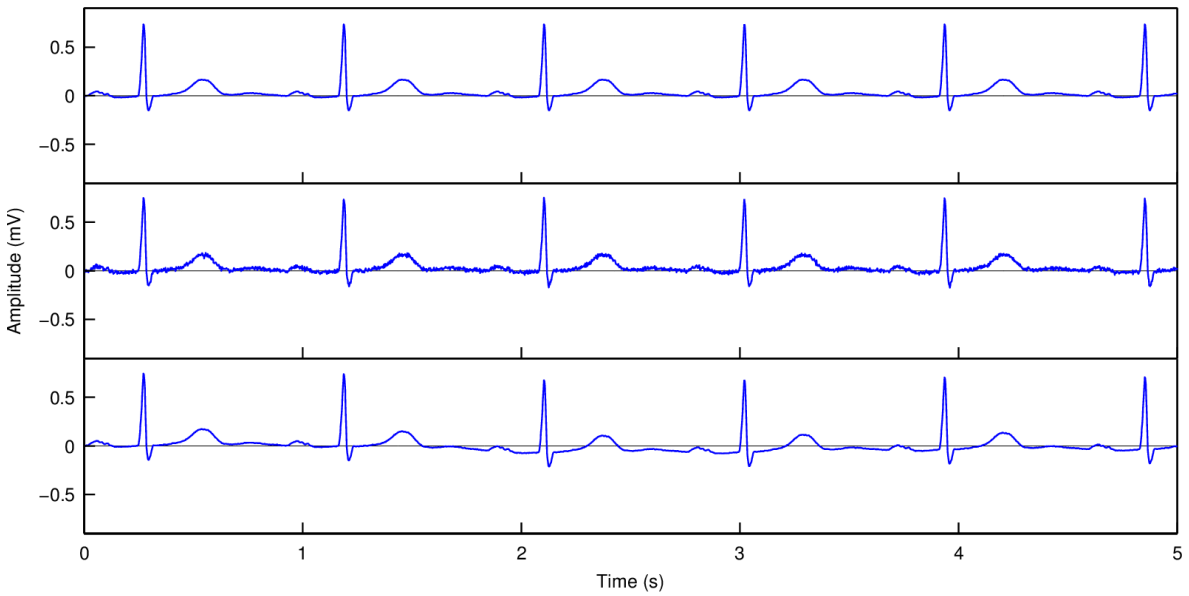
To simulate a particular STV value for a signal consisting of N+1 beats, we generated a sequence of N absolute QT-interval differences (i.e., $|QT_{i+1} - QT_i|$) by drawing from a uniform distribution centered around the required STV value, with a minimum of 0 and a maximum of twice the required STV.

**Figure 1. Example of a simulated QT-interval change**




The black line indicates the original ECG signal with the vertical line denoting the end of the T wave as determined by the MEANS program. The grey line indicates the signal with a shifted end of the T wave. The horizontal bars below the signals mark symmetric windows of 180 ms around the end of the T wave in which the signal is not deformed. The signal segment from QRS end till the start of the window is extended, whereas the signal segment from the end of the window till the onset of the next P wave is compressed.

**Figure 2. Example of simulated extrinsic disturbances**




Top panel: artificial ECG signal constructed by concatenating the averaged P-QRS-T complex of the original ECG. Middle panel: artificial signal with added noise (SNR 20). Bottom panel: artificial signal with added residual baseline wander (standard deviation of slope 30 µV/s).

If the absolute difference between the STV of the sequence and the required value was greater than 0.1 ms, the sequence was rejected and a new sequence was generated. This was repeated until the difference was $\leq$ 0.1 ms. The QT durations of the individual beats were then derived from the generated QT differences, taking for the first beat the original QT interval as determined by MEANS. To avoid an ever-increasing QT interval, each (absolute) difference was added to or subtracted from the preceding QT interval so that the cumulative sum of the (signed) differences was minimized. Two types of extrinsic variation were simulated, muscle noise and baseline wander (see Figure 2). To simulate muscle noise, we generated white noise. For each lead, this noise was added after scaling of the noise amplitude to a prespecified signal-to-noise ratio (SNR). Baseline wander was simulated by piecewise linear baseline shifts, where each piece started at the onset of a QRS complex and ended at the onset of the next QRS complex. The slope of each piece of baseline shift was randomly selected from a normal distribution with a prespecified standard deviation and zero mean. Since the simulated baseline wander might easily be removed by an automatic correction method, we chose to simulate small baseline shifts that were considered to constitute the residual baseline wander that remained after a (hypothetical) baseline correction algorithm was applied. Since small simulated pieces of baseline wander may add up to a large baseline shift if successive pieces have slopes with the same sign, we applied the following rule: if the simulated baseline amplitude at the end of a particular complex was positive, the slope of the next piece of baseline was taken negative, and vice versa, if the baseline amplitude was negative, the slope of the next piece was taken positive.

*QT variability measurement*

We assessed the performance of two fully automatic QTV measurement algorithms: a conventional method based on the processing and measurement of individual ECG beats, and fiducial segment averaging, which exploits the correlation between signal segments across beats.

*Conventional computerized measurement*

The MEANS program described above also has the option to measure each individual beat in a recording separately. We used this option to determine beat-to-beat QT interval estimates for the artificial ECGs. The baseline correction of MEANS was turned off to assess the effect of residual baseline wander on QTV measurement. The processing of individual beats by MEANS reflects a manual measurement process in which QT intervals are also measured separately.

*Fiducial segment averaging*

Fiducial segment averaging (FSA) uses beat-to-beat coherence of relatively small segments within the P-QRS-T complex to improve the accuracy of fiducial point estimates. A semi-automatic version of the measurement process using FSA was first described by Ritsema van Eck.[5] In this study, we have implemented a fully automatic version (Figure 3).

**Figure 3. Pseudocode of the FSA algorithm**

```
Input: Standard 12-lead ECG.
Result: Locations of the fiducial points in the individual ECG beats.

L = locations loc_i of the initial fiducial points in the ECG beats;
S = signal segments segm_i around loc_i in the root-mean-square ECG signal;
do
  N = |L|;                          // number of beats
  for i = 1 to N do                 // for each beat
    Construct averaged segment avg_segm from all segments except segm_i;
    Determine shift_i of segm_i at which correlation between segm_i and avg_segm is maximum;
    loc_i = loc_i + shift_i;        // update location
  end
  removedSegment = False;
  for i = 1 to N do                 // check for outliers
    Construct averaged segment avg_segm from all segments except segm_i;
    if averaged absolute amplitude differences between segm_i and avg_segm > threshold
      Remove segm_i from S and loc_i from L;
      removedSegment = True;
    end
  end
while any shift_i > 0 or removedSegment          // perform new round of shifting if true
```

First, MEANS determines the initial fiducial points (onset of QRS complex and end of T wave) and constructs a detection function consisting of the root-mean-square ECG signal.[10] Second, the fiducial point in each individual beat is shifted until maximum correlation is achieved between a 120-ms signal segment of the detection function around this fiducial point and the average of the segments around the fiducial points of all complexes. The amount of shifting is retained and constitutes the individual beat variation in the fiducial point estimate. Based on the new fiducial point estimates another round of shifting is carried out. This process is repeated until the correlations cannot be further improved. Finally, the QT interval for each beat is calculated taking into account the final shifts.

To safeguard against signal segments with excessive noise or baseline wander, the FSA algorithm applies an additional test after each round of shifting. If the averaged absolute amplitudes of the difference between the ST-T wave of an individual beat and the averaged ST-T wave of the remaining beats is larger than a preset value, the beat is discarded and the iteration process is repeated for the remaining beats. It should be noted that a rejected beat may reduce the number of QT-interval differences in the STV computation by more than one because only differences between QT intervals of consecutive beats are taken into account. Since we did not intend to simulate excessive noise or baseline wander, the number of rejected beats was expected to be negligible.

Validation experiments

To validate the two measurement algorithms, we used the first 200 ECGs from the Common Standards for Electrocardiography (CSE) diagnostic ECG library.[8] The CSE library consists of 1,220 fully anonymized ECGs that have previously been used in various studies to assess and compare the performance of computerized ECG programs. The leads of these ECGs were recorded simultaneously at a sampling rate of 500 Hz during 10 seconds. The diagnostic classification of individual ECGs has not been released, but the database is known to contain 382 normal ECGs while the rest have various abnormalities.[8]

Each of the 200 ECGs was processed by MEANS to construct averaged beats, which were used to generate artificial noise-free ECGs without QTV consisting of 10, 30, and 60 beats, as described above. For each of these ECGs, new ECGs with simulated STV values of 2, 4, 6, 8, and 10 ms were generated. For each of the resulting ECGs, further ECGs were generated by adding different amounts of noise (SNR 40, 30, or 20), residual baseline wander (standard deviation of the distribution of slopes 10, 20, or 30 μV/s), or a combination (SNR 30 and 20 μV/s baseline wander), for a total of 28,800 ECGs.

## Results

### Conventional computerized measurement

Table 1 shows the median and 95th percentile (p95) of the absolute differences between the simulated STV and the STV estimated by the conventional, beat-by-beat measurement of MEANS. For disturbance-free ECGs, the median absolute differences are in the order of 15% of the simulated STV, while p95 values are about twice as high. For low and medium noise levels (SNR 40 or 30), similar results are observed for simulated STV values of 4 ms or larger. Interestingly, the median and p95 values of the absolute differences in the absence of STV are higher than those for a simulated STV of 2 ms. This may be explained by the fact that if the simulated STV is 0, any QT-interval mismeasurement will yield an estimated STV > 0, whereas if the simulated STV is larger than 0 and QT mismeasurements are made, the estimated STV can be lower or higher, or even the same, as the simulated STV. For the highest noise level (SNR 20), performance deteriorates greatly, with median differences of 4-6 ms in the absence of STV and p95 values varying between 10 and 20 ms. Measurements are much more robust for ECGs with residual baseline wander. The absolute differences are comparable to those of slightly noisy ECGs (SNR 40). The amount of residual baseline wander hardly affects the estimates. The combination of medium noise and residual baseline (SNR 30 + slope 20 µV/s) shows similar performance as medium noise alone. An increase in number of beats generally results in more accurate STV estimates, but the difference in performance between 30 or 60 beats is small in most cases.

### *FSA measurement*

Table 2 shows the median and p95 of the absolute differences between simulated and estimated STV for the FSA algorithm. For ECGs without artifacts, FSA perfectly estimates the different simulated STV values, i.e., all differences between simulated and estimated STV are zero. For ECGs with low or medium noise, most of the differences are very small (p95 well below 1 ms). For higher noise levels (SNR 20), the median absolute differences are still very small (about 1 ms for STV = 0 and less than 0.5 ms for STV > 0), while p95 values are in the range of 1-2 ms. A similar pattern with very low differences is observed for various amounts of residual baseline wander. The combination of medium noise and baseline residual gives slightly worse results than those of either artifact separately, but almost all median values remain below 0.5 ms, and most p95 values below 1 ms.

**Table 1. Median (95th percentile) of the absolute differences between simulated STV and STV as measured by the MEANS algorithm for different signal-to-noise ratios (SNR), residual baseline wander, and number of beats.**

| | | Simulated STV (ms) | | | | | |
|---|---|---|---|---|---|---|---|
| Artifact | No. of beats | 0 | 2 | 4 | 6 | 8 | 10 |
| None | 10 | 0.00 (0.00) | 0.31 (0.79) | 0.63 (1.34) | 1.06 (2.12) | 1.26 (2.99) | 1.65 (3.50) |
| | 30 | 0.00 (0.00) | 0.27 (0.49) | 0.59 (1.02) | 0.83 (1.43) | 1.17 (2.23) | 1.46 (2.57) |
| | 60 | 0.00 (0.00) | 0.26 (0.40) | 0.55 (0.84) | 0.83 (1.34) | 1.09 (1.78) | 1.40 (2.30) |
| SNR 40 | 10 | 1.02 (2.59) | 0.31 (1.34) | 0.67 (1.65) | 1.02 (2.44) | 1.34 (2.79) | 1.69 (4.32) |
| | 30 | 1.15 (2.44) | 0.22 (1.05) | 0.44 (1.24) | 0.80 (1.77) | 1.18 (2.46) | 1.39 (2.97) |
| | 60 | 1.20 (2.18) | 0.18 (1.14) | 0.42 (0.91) | 0.71 (1.58) | 1.05 (1.73) | 1.31 (2.40) |
| SNR 30 | 10 | 1.49 (3.22) | 0.47 (2.12) | 0.79 (2.44) | 1.02 (3.02) | 1.22 (3.42) | 1.53 (3.77) |
| | 30 | 1.63 (3.91) | 0.37 (2.45) | 0.46 (1.27) | 0.68 (1.91) | 1.12 (2.35) | 1.46 (3.16) |
| | 60 | 1.64 (4.25) | 0.43 (2.25) | 0.32 (1.73) | 0.66 (1.47) | 0.95 (2.04) | 1.27 (2.56) |
| SNR 20 | 10 | 4.48 (19.05) | 2.36 (16.42) | 1.81 (14.89) | 1.57 (18.38) | 1.89 (10.21) | 2.12 (10.69) |
| | 30 | 5.73 (18.08) | 3.71 (18.28) | 2.07 (14.24) | 1.54 (11.29) | 1.22 (14.78) | 1.45 (12.19) |
| | 60 | 5.82 (17.49) | 3.74 (17.31) | 2.40 (14.81) | 1.53 (15.02) | 1.10 (13.96) | 1.13 (11.69) |
| Baseline 10 µV/s | 10 | 0.94 (2.63) | 0.39 (1.26) | 0.71 (1.49) | 1.02 (2.24) | 1.41 (2.99) | 1.65 (3.77) |
| | 30 | 1.05 (2.24) | 0.22 (1.13) | 0.46 (1.21) | 0.78 (1.71) | 1.09 (2.01) | 1.46 (3.06) |
| | 60 | 1.03 (2.08) | 0.20 (0.89) | 0.47 (1.03) | 0.77 (1.53) | 1.06 (2.04) | 1.40 (2.40) |
| Baseline 20 µV/s | 10 | 1.02 (2.71) | 0.39 (1.41) | 0.79 (1.73) | 1.02 (2.47) | 1.37 (3.22) | 1.57 (3.89) |
| | 30 | 1.02 (2.24) | 0.24 (1.27) | 0.44 (1.15) | 0.84 (1.55) | 1.11 (2.21) | 1.51 (2.91) |
| | 60 | 1.07 (2.26) | 0.22 (1.10) | 0.46 (1.05) | 0.74 (1.53) | 1.08 (2.10) | 1.32 (2.62) |
| Baseline 30 µV/s | 10 | 1.02 (2.44) | 0.39 (1.61) | 0.79 (1.96) | 1.10 (2.40) | 1.41 (3.10) | 1.73 (4.01) |
| | 30 | 1.07 (2.46) | 0.24 (1.68) | 0.51 (1.29) | 0.72 (1.84) | 1.12 (2.45) | 1.39 (2.84) |
| | 60 | 1.10 (2.54) | 0.19 (1.14) | 0.41 (0.96) | 0.77 (1.50) | 1.04 (2.16) | 1.35 (2.84) |
| SNR 30 + | 10 | 1.49 (3.18) | 0.47 (2.20) | 0.71 (2.12) | 1.02 (2.87) | 1.57 (3.50) | 1.73 (3.89) |
| baseline 20 µV/s | 30 | 1.65 (4.17) | 0.43 (2.12) | 0.45 (1.66) | 0.73 (1.99) | 1.04 (2.33) | 1.38 (2.96) |
| | 60 | 1.75 (4.45) | 0.36 (2.97) | 0.32 (1.97) | 0.71 (1.38) | 0.97 (1.89) | 1.33 (2.56) |

**Table 2. Median (95th percentile) of the absolute differences between the simulated STV and STV as measured by the FSA algorithm for different signal-to-noise ratios (SNR), residual baseline wander, and number of beats.**

| | | Simulated STV (ms) | | | | | |
|---|---|---|---|---|---|---|---|
| Artifact | No. of beats | 0 | 2 | 4 | 6 | 8 | 10 |
| None | 10 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | 30 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | 60 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| SNR 40 | 10 | 0.00 (0.31) | 0.00 (0.24) | 0.00 (0.16) | 0.00 (0.31) | 0.00 (0.31) | 0.00 (0.31) |
| | 30 | 0.00 (0.24) | 0.00 (0.10) | 0.00 (0.10) | 0.00 (0.10) | 0.00 (0.10) | 0.00 (0.15) |
| | 60 | 0.00 (0.18) | 0.00 (0.05) | 0.00 (0.05) | 0.00 (0.05) | 0.00 (0.05) | 0.00 (0.05) |
| SNR 30 | 10 | 0.47 (1.34) | 0.16 (0.79) | 0.31 (0.71) | 0.16 (0.63) | 0.16 (0.94) | 0.16 (0.79) |
| | 30 | 0.20 (0.93) | 0.10 (0.39) | 0.10 (0.39) | 0.10 (0.39) | 0.10 (0.39) | 0.10 (0.29) |
| | 60 | 0.22 (0.79) | 0.05 (0.31) | 0.05 (0.24) | 0.05 (0.24) | 0.05 (0.22) | 0.05 (0.29) |
| SNR 20 | 10 | 1.26 (2.75) | 0.31 (1.81) | 0.47 (1.57) | 0.47 (1.41) | 0.47 (1.96) | 0.47 (1.57) |
| | 30 | 0.88 (2.32) | 0.24 (1.02) | 0.20 (1.02) | 0.20 (0.95) | 0.20 (1.07) | 0.22 (1.19) |
| | 60 | 0.77 (2.21) | 0.19 (0.95) | 0.14 (0.90) | 0.14 (0.77) | 0.17 (1.21) | 0.16 (1.19) |
| Baseline 10 µV/s | 10 | 0.16 (0.94) | 0.00 (0.47) | 0.00 (0.63) | 0.00 (0.63) | 0.00 (0.63) | 0.00 (0.47) |
| | 30 | 0.20 (1.02) | 0.05 (0.34) | 0.05 (0.29) | 0.05 (0.29) | 0.05 (0.34) | 0.05 (0.29) |
| | 60 | 0.19 (0.90) | 0.05 (0.34) | 0.05 (0.22) | 0.02 (0.24) | 0.05 (0.26) | 0.05 (0.24) |
| Baseline 20 µV/s | 10 | 0.63 (2.12) | 0.16 (1.02) | 0.16 (0.94) | 0.16 (0.94) | 0.16 (0.79) | 0.16 (0.94) |
| | 30 | 0.68 (1.80) | 0.15 (0.76) | 0.10 (0.44) | 0.10 (0.49) | 0.10 (0.51) | 0.10 (0.59) |
| | 60 | 0.67 (1.95) | 0.12 (0.68) | 0.10 (0.44) | 0.10 (0.46) | 0.10 (0.38) | 0.07 (0.40) |
| Baseline 30 µV/s | 10 | 0.94 (2.91) | 0.31 (1.49) | 0.31 (1.49) | 0.31 (1.41) | 0.31 (1.26) | 0.31 (1.41) |
| | 30 | 0.98 (2.37) | 0.24 (1.24) | 0.19 (0.95) | 0.15 (0.88) | 0.19 (0.68) | 0.15 (0.78) |
| | 60 | 0.96 (2.34) | 0.26 (1.09) | 0.13 (0.85) | 0.11 (0.79) | 0.10 (0.54) | 0.12 (0.50) |
| SNR 30 + | 10 | 0.79 (1.73) | 0.31 (1.10) | 0.31 (0.94) | 0.31 (0.94) | 0.31 (1.10) | 0.31 (1.10) |
| baseline 20 µV/s | 30 | 0.59 (1.41) | 0.19 (0.59) | 0.15 (0.49) | 0.10 (0.44) | 0.15 (0.49) | 0.15 (0.59) |
| | 60 | 0.50 (1.41) | 0.10 (0.52) | 0.10 (0.34) | 0.07 (0.35) | 0.10 (0.37) | 0.10 (0.38) |

The number of ECGs in which the FSA algorithm rejected beats for further analysis was very low: one ECG for the highest level of simulated noise (SNR 20), and two ECGs for the largest slope of residual baseline wander (30 µV/s).

**Discussion**

We have validated the performance of two QTV measurement tools under different operating conditions by constructing artificial ECGs with different amounts of simulated STV and disturbances. Our results indicate that the FSA algorithm produces highly accurate STV estimates. A traditional beat-by-beat measurement algorithm performed less well, especially for higher levels of noise or residual baseline wander.

We are not the first to use simulated data as a means to validate the performance of QTV measurement algorithms [4,11]. Baumert et al. [4] concatenated a noise-free beat of one ECG lead and added different forms of artifacts to validate several (semi-)automatic measurement techniques. The same data were also used in a later study, in which the authors evaluated an alternative measurement approach [11]. Beat-to-beat QT-interval variations were not simulated, and thus the performance of the algorithms was only validated in the absence of QTV. Moreover, all simulated ECGs were constructed from just one ECG beat from a single lead. We used a set of 200 different artificial 12-lead ECGs, and also simulated different amounts of STV. Contrary to the previous studies, this allowed us to validate the performance of measurement algorithms for non-zero STV values, in a morphologically diverse set of ECGs.

The same approach that we applied to validate automatic algorithms, could, in principle, also be used to validate a manual measurement procedure. We did not attempt to do this since the effort of measuring individual QT intervals in thousands of ECGs was considered prohibitive. However, the MEANS algorithm, like the manual method, also measures on a beat-by-beat basis. Our results clearly indicate that this beat-by-beat measurement is inferior to an approach that exploits the correlation between individual beats, as is done in FSA. In particular for larger noise levels, the errors in the MEANS estimates become unacceptably large. This suggests that STV estimates obtained with a beat-by-beat measurement procedure, automatic or manual, must be interpreted cautiously.

Previous studies that used STV have measured QT intervals in 30 or 60 consecutive beats [9,12], but the effect of varying recording durations on the accuracy of STV estimates has not been investigated. Our results indicate that accuracy generally improves with increasing signal length. This effect is more pronounced for FSA than for MEANS, likely because FSA employs an averaged signal segment that will become less noisy with increasing signal length, whereas MEANS does not use averaging when measuring individual beats. We also found that FSA already performs very well for signal

durations of 10 s. This finding increases the practical utility of STV as the far majority of ECGs that are recorded in clinical practice or epidemiological studies are standard 10-s ECGs. The ability to process large sets of ECGs also allows to quantify circadian effects and establish normal values of QTV, as recommended in a recent QTV position paper [2].

In this study we have focused on the validation of STV measurement. The same approach can be used to validate the measurement of other QTV parameters, such as the standard deviation of QT-interval durations. QTV parameters that normalize for heart rate variability, like the QTV index [1], would require additional modeling of variations in RR-interval duration. The approach could also be applied to validate measurement algorithms of other types of variability, such as T-wave alternans, after appropriate modelling.

Our study has several limitations. First, our simulation of QTV by shifting the tail of individual T waves, preserving their shape, is straightforward but may not fully reflect reality. Unfortunately, little is known about the underlying mechanisms that affect QTV and the shape of the T wave. Once such knowledge becomes available, a more elaborate simulation is imaginable. Second, for practical reasons we only tested the effect of a limited set of artifacts, i.e., noise and residual baseline wander, but simulation of other types of artifacts can be envisaged. For example, simulated respiratory modulation of T-wave amplitudes has previously been shown to affect QTV estimates based on single-lead measurement [4]. Although we expect our algorithms to be less sensitive for respiratory movements because we combine information from all ECG leads, this may be investigated in future research.

In conclusion, artificially constructed ECGs with a variety of disturbances allow validation of QTV measurement procedures. The FSA algorithm provides accurate STV estimates under varying signal conditions, and performs significantly better than traditional beat-by-beat analysis. The fully automatic operation of the FSA algorithm enables STV measurement in large sets of ECGs.

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# Chapter 2.3

# Normal Values of QT Variability in 10-Second Electrocardiograms for All Ages

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**Abstract**

*Aims* QT variability is a promising electrocardiographic marker. It has been studied as a possible screening tool for patients with coronary artery disease and left ventricular hypertrophy, and it is a known risk factor for sudden cardiac death. Because comprehensive normal values for QT variability are lacking, we aim to establish these in standard 10-second electrocardiograms (ECGs) covering both sexes and all ages.

*Methods* Ten-second, 12-lead ECGs were provided by five Dutch population studies (Pediatric Normal ECG Study, Leiden University Einthoven Science Project, Prevention of Renal and Vascular End-stage Disease Study, Utrecht Health Project, Rotterdam Study). ECGs were recorded digitally and processed by well-validated analysis software. We selected cardiologically healthy participants, 46% being women. Ages ranged from 11 days to 91 years. After quality control, 13,882 ECGs were available. We assessed three markers: standard deviation of QT intervals (SDqt), short-term QT variability (STVqt), and QT variability index (QTVI).

*Results* For SDqt and STVqt, the median and lower limit of normal remained stable with age. The upper limit of normal declined until around age 45, and increased strongly in the elderly. For QTVI, median and normal limits decreased until age 20, and steadily went up afterwards except for the lower limit of normal, which decreased after age 65. Generally, women had higher SDqt, STVqt, and QTVI than men, notably so after age 30.

*Conclusion* We report the first comprehensive set of normal values for QT variability based on 10-second ECGs. These normal values should be valuable for research and clinical practice.

**Introduction**

QT variability is a measure of the spontaneous fluctuations in the duration of the QT interval on the electrocardiogram (ECG). These fluctuations are thought to be the result of local variations in cardiac repolarization, and to reflect cardiac electrical instability.[1] For this reason, QT variability has been studied in relation to ventricular arrhythmias[2] and sudden cardiac death (SCD).[3] Moreover, QT variability was found to be an effective screening tool for coronary artery disease and left ventricular hypertrophy, which are important underlying causes of SCD.[4] The effects of cardiac drugs on QT variability have also been studied: β-blockers decrease QT variability (except sotalol), while digoxin and β-sympathomimetic drugs increase QT variability.[5] The most commonly used QT variability markers are the standard deviation of QT intervals (SDqt), short-term QT variability (STVqt), and the QT variability index (QTVI).[1,5]

QT variability has been measured on ECG signals as short as 10 seconds, and as long as 24 hours.[1,5] Because the standard 10-second ECG is cheap, non-burdening, and in ubiquitous use, it would be valuable to have comprehensive normal values for QT variability based on 10-second ECGs. A recent position paper on QT variability presented a meta-analysis of SDqt and QTVI of healthy participants from previous studies.[1] However, this meta-analysis was of limited size (1,954 adults for QTVI, and 1,190 adults for SDqt) and did not stratify on age or sex. Also, differences between the individual studies in ECG processing and in duration of ECG recording were not addressed.[1] The various studies referred to in this meta-analysis reported normal limits for QT variability, but these studies often did not use standard 10-second ECGs, but analyzed much longer ECG signals.[6-10]

In the present study we provide comprehensive normal values for QT variability, applying the commonly used QT variability markers SDqt, STVqt, and QTVI to a large set of standard 10-second, 12-lead ECGs of cardiologically healthy persons, covering both sexes and all ages.

**Methods**

*Study population*

We combined data from five population studies conducted in the Netherlands. The 10-second, 12-lead ECGs from these studies were digitally recorded and stored at sampling rates of at least 500 Hz, up to 1200 Hz in the pediatric group. All data were anonymized. The following studies were included:

(1) Pediatric Normal ECG Study:[11] The population of this study consists of 1,912 children, their ages ranging from 11 days to 16 years. The children were recruited in the year 2000 at three children's health centers, three primary schools, and one secondary school in the city of Rotterdam. The children's height and weight, measured before ECG recording, corresponded well with the Dutch

growth standard.[11] ECGs were recorded with a portable PC-based acquisition system (Cardio Control, Delft, The Netherlands).

(2) Leiden University Einthoven Science Project:[12] The population of this study contains 787 medical students of Leiden University. The ages of the participants ranged between 17 and 29 years, and all attested to be in good health. The ECGs were recorded from 2005 until 2007 with Megacart electrocardiographs (Siemens, Erlangen, Germany).

(3) Prevention of Renal and Vascular End-stage Disease (PREVEND) Study:[13] This study, which started 1997, aims to investigate the natural course of microalbuminuria and its relation to renal and cardiovascular disease in the general population. The PREVEND population consists of 8,592 participants aged 28-75 years, from the city of Groningen. Medical records, including medication use, were available for all participants. ECGs were recorded with CardioPerfect equipment (Welch Allyn Cardio Control, USA).

(4) Utrecht Health Project:[14] This ongoing study started in 2000 in Leidsche Rijn, a newly developed residential area of Utrecht. All new inhabitants were invited by their general practitioner to participate. The population of this study consists of 6,542 participants. Written informed consent was obtained and an individual health profile was made by dedicated research nurses. Baseline assessment included physical examination, ECG, blood tests, and interview-assisted questionnaires. Pharmacy records were used to obtain medication use. ECGs were recorded with CardioPerfect equipment (Welch Allyn Cardio Control, USA).

(5) Rotterdam Study:[15] This study, which started in 1990, investigates determinants of a number of age-related disorders in an elderly population, prominently among them cardiovascular disease. The first two cohorts of the Rotterdam Study population consist of 10,994 inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years or older. Participants were visited at home for an interview and were subsequently examined at the research center. Detailed information was collected on health status, medical history, and medication use. ECGs were recorded with an ACTA electrocardiograph (Esaote, Florence, Italy).

From these five populations, totaling 28,827 participants, we selected a subgroup with no indication of cardiac disease. Reasons for exclusion were a history of myocardial infarction, heart failure, coronary bypass surgery, coronary angioplasty, or pacemaker implantation. Other exclusion criteria were hypertension and diabetes mellitus. Hypertension was defined as a systolic blood pressure ≥160 mmHg or a diastolic blood pressure ≥100 mmHg or use of antihypertensive medication, including use of beta-blockers. Diabetes mellitus was defined as a non-fasting serum glucose ≥11 mmol/l or use of glucose-lowering drugs. After applying these criteria, 15,248 individuals remained available.

*ECG quality control*

We removed ECGs with excessive noise, excessive baseline wander, ventricular arrhythmias, and second or third degree atrioventricular block. This resulted in 13,882 ECGs available for analysis. In 11 ECGs all measured QT intervals were identical, producing a SDqt and STVqt of zero. In those cases, the log-transformation contained in the QTVI formula produced values of minus infinite. These values were set to the lowest non-infinite QTVI value of –3.60.

*QT-variability measurement*

All QT intervals on the ECGs were measured automatically by the Modular ECG Analysis System (MEANS) using fiducial segment averaging (FSA),[16] a technique that exploits the correlation between signal segments from individual beats. First, MEANS determines the locations of the individual QRS complexes and initial fiducial points (i.e., the onset of the QRS complex and the end of T wave). Baseline wander is corrected using restricted splines, and a detection function consisting of the root-mean-square ECG signal is computed. Second, the fiducial point in each individual beat is shifted in an iterative procedure until maximum correlation is achieved between a small signal segment of the detection function around this fiducial point and the average of the segments around the fiducial points of the remaining complexes. The amount of shifting is retained and constitutes the individual beat variation in the fiducial point estimate. Finally, the QT interval for each beat is calculated taking into account the shifts of the initial QRS onset and T end. MEANS automatically detects ectopic beats, such as premature (supra)ventricular complexes, and excludes them from further processing. Also, the beats immediately before and after an ectopic beat are not used. To safeguard against signal segments with excessive noise or baseline wander, an additional test is applied after each FSA iteration. If the averaged absolute amplitudes of the difference between the ST-T wave of an individual beat and the averaged ST-T wave of the remaining beats is larger than a preset value, the beat is discarded and the iteration process is repeated for the remaining beats.

We calculated the QT-variability markers SDqt, STVqt, and QTVI.[5] SDqt is defined as the standard deviation of the QT intervals on a 10-second ECG. STVqt is defined as the average of the absolute differences between the QT intervals of subsequent beats: STVqt = $\sum_{i=1}^{n} \frac{|QT_{i+1} - QT_i|}{n\sqrt{2}}$, where *n* is the number of differences. QTVI normalizes the variance of the QT intervals (QTv) for mean QT (QTm) squared, and the variance of the heart rate (HRv), for mean heart rate (HRm) squared, and is defined as QTVI = $log_{10}\left[\left(\frac{QT_v}{QT_m^2}\right)/\left(\frac{HR_v}{HR_m^2}\right)\right]$.

*Estimation of normal values*

We used the Box-Cox *t* distribution in a semi-parametric model for location, scale, and shape to estimate centile curves.[17] The Box-Cox *t* distribution allows for modeling of the distribution of the median, skewness, and kurtosis as functions of age. The lower limit of normal (LLN) was defined as the 2nd percentile and the upper limit of normal (ULN) was defined as the 98th percentile. The Box-Cox *t* distribution was implemented with the *lms* function of the R-package *gamlss*, and the normal values for all age categories were estimated using the *predict.gamlss* function of the *gamlss* package. The normal values of a given age category were estimated for the central age in that category. For example, normal values for the category of 30-40 years are based on the estimated values for participants aged 35 years.

This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center. Since all data were anonymized and retrospectively collected, informed consent of the subjects was not required according to Dutch legislation.

**Results**

Table 1 shows the number of available ECGs grouped by sex and age category. Most age groups had more than 100 ECGs, only the age groups below six months or above 90 years contained fewer ECGs. Table 2 gives the age-dependent median, ULN, and LLN of SDqt, stratified by sex. Table 3 shows these statistics for STVqt and table 4 for QTVI. Additional percentile values for the three markers are given in Supplementary tables 1, 2, and 3. Figures 1, 2, and 3 show continuous age-dependent curves of the median and the normal limits of SDqt, STVqt and QTVI, respectively.

The median and LLN of SDqt remain relatively constant over age, but the ULN changes considerably: it decreases from birth to approximately 45 years, after which age it starts to increase again, the rate of ascent being higher than that of descent. The age-dependent pattern of STVqt is similar to that of SDqt: the median and LLN are fairly constant, while the ULN decreases from birth to age 45, and increases strongly afterwards. The normal values of QTVI show a decrease until about 20 years, after which they start to rise.

There were some notable sex differences. For SDqt, boys have a higher ULN than girls until the age of 15, but after that age, women have a higher ULN than men. The sex differences in the median or LLN are minimal. For STVqt, the point at which women have a higher ULN than men lies at around 20 years, while the sex differences in the median and LLN are again minimal. QTVI follows a similar pattern (men higher values at younger ages, women have higher values later in life), but less pronounced than SDqt and STVqt.

**Table 1. The study population stratified on sex and age groups**

| Age group | Boys/Men | Girls/Women | Total |
|---|---|---|---|
| Younger than 1 month | 9 | 8 | 17 |
| 1 to 3 months† | 26 | 22 | 48 |
| 3 to 6 months | 33 | 37 | 70 |
| 6 to 12 months | 69 | 53 | 122 |
| 1 to 3 years | 50 | 51 | 101 |
| 3 to 5 years | 60 | 61 | 121 |
| 5 to 8 years | 123 | 104 | 227 |
| 8 to 12 years | 115 | 163 | 278 |
| 12 to 16 years | 139 | 100 | 239 |
| 16 to 20 years | 155 | 384 | 539 |
| 20 to 30 years | 522 | 844 | 1,366 |
| 30 to 40 years | 1,942 | 1,402 | 3,344 |
| 40 to 50 years | 1,492 | 583 | 2,075 |
| 50 to 60 years | 1,270 | 934 | 2,204 |
| 60 to 70 years | 1,173 | 1,038 | 2,211 |
| 70 to 80 years | 329 | 427 | 756 |
| 80 to 90 years | 49 | 110 | 159 |
| 90 years and older | 1 | 4 | 5 |
| Total | 7,557 | 6,325 | 13,882 |

†The term “to” specifies the upper limit in the sense of “less than”.

**Table 2. Normal values for SDqt in milliseconds per age group and sex**

| | Median (2nd Percentile, 98th Percentile) | |
|---|---|---|
| **Age Category** | **Boys/Men** | **Girls/Women** |
| < 1 month | 3.15 (1.13; 10.77) | 3.13 (1.12; 8.70) |
| 1 to 3 months† | 3.15 (1.13; 10.76) | 3.13 (1.12; 8.70) |
| 3 to 6 months | 3.15 (1.13; 10.74) | 3.13 (1.13; 8.71) |
| 6 to 12 months | 3.16 (1.13; 10.71) | 3.13 (1.13; 8.73) |
| 1 to 3 years | 3.16 (1.14; 10.62) | 3.14 (1.13; 8.78) |
| 3 to 5 years | 3.16 (1.15; 10.47) | 3.15 (1.12; 8.87) |
| 5 to 8 years | 3.16 (1.15; 10.28) | 3.15 (1.11; 9.01) |
| 8 to 12 years | 3.12 (1.14; 9.98) | 3.13 (1.08; 9.25) |
| 12 to 16 years | 3.02 (1.10; 9.55) | 3.08 (1.01; 9.60) |
| 16 to 20 years | 2.88 (1.05; 9.06) | 2.99 (0.95; 9.85) |
| 20 to 30 years | 2.63 (0.95; 8.32) | 2.90 (0.96; 9.43) |
| 30 to 40 years | 2.34 (0.83; 7.59) | 2.79 (1.00; 8.94) |
| 40 to 50 years | 2.14 (0.76; 7.19) | 2.63 (0.97; 9.16) |
| 50 to 60 years | 2.11 (0.73; 7.84) | 2.54 (0.94; 10.22) |
| 60 to 70 years | 2.24 (0.77; 8.95) | 2.61 (0.98; 12.44) |
| 70 to 80 years | 2.48 (0.90; 10.17) | 2.81 (1.05; 16.25) |
| 80 to 90 years | 2.78 (1.02; 12.47) | 3.11 (1.16; 22.67) |

†The term “to” specifies the upper limit in the sense of “less than”.

**Table 3. Normal values for STVqt in milliseconds per age group and sex**

| | Median (2nd Percentile, 98th Percentile) | |
|---|---|---|
| **Age Category** | **Boys/Men** | **Girls/Women** |
| < 1 month | 2.22 (0.46; 7.57) | 2.13 (0.61; 7.16) |
| 1 to 3 months† | 2.22 (0.46; 7.58) | 2.14 (0.61; 7.16) |
| 3 to 6 months | 2.22 (0.46; 7.59) | 2.14 (0.61; 7.17) |
| 6 to 12 months | 2.23 (0.46; 7.61) | 2.14 (0.61; 7.19) |
| 1 to 3 years | 2.23 (0.47; 7.69) | 2.15 (0.60; 7.23) |
| 3 to 5 years | 2.25 (0.47; 7.79) | 2.17 (0.60; 7.30) |
| 5 to 8 years | 2.25 (0.48; 7.90) | 2.17 (0.60; 7.36) |
| 8 to 12 years | 2.23 (0.48; 7.97) | 2.16 (0.58; 7.38) |
| 12 to 16 years | 2.15 (0.47; 7.84) | 2.12 (0.55; 7.28) |
| 16 to 20 years | 2.03 (0.45; 7.51) | 2.06 (0.53; 7.11) |
| 20 to 30 years | 1.82 (0.43; 6.85) | 2.01 (0.50; 7.04) |
| 30 to 40 years | 1.58 (0.39; 6.07) | 1.98 (0.49; 7.18) |
| 40 to 50 years | 1.42 (0.36; 5.56) | 1.87 (0.47; 7.33) |
| 50 to 60 years | 1.42 (0.35; 5.92) | 1.83 (0.46; 8.05) |
| 60 to 70 years | 1.58 (0.38; 7.02) | 1.92 (0.49; 9.51) |
| 70 to 80 years | 1.83 (0.43; 8.51) | 2.13 (0.56; 11.55) |
| 80 to 90 years | 2.13 (0.51; 10.45) | 2.44 (0.67; 14.27) |

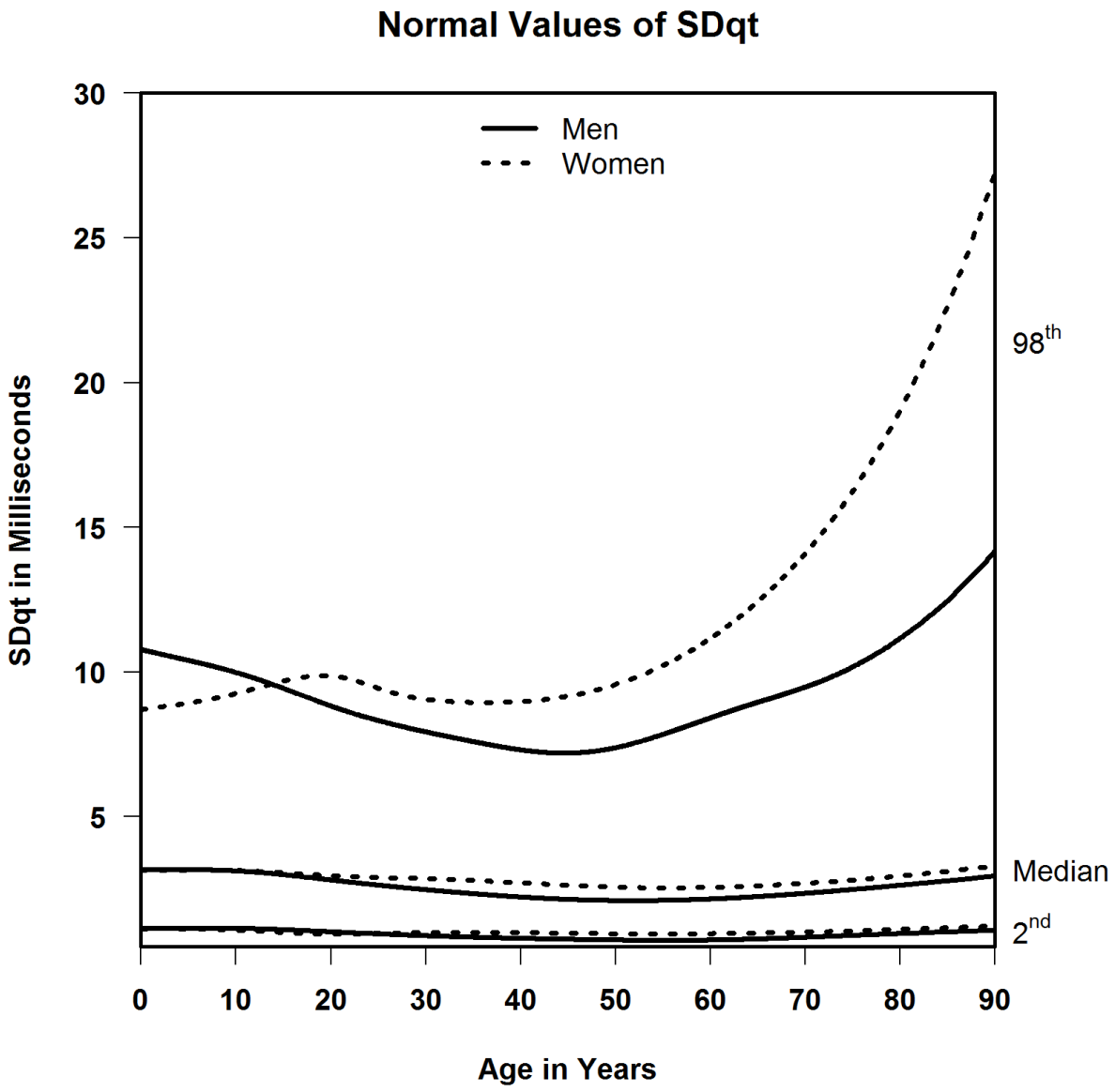
†The term “to” specifies the upper limit in the sense of “less than”.

**Table 4. Normal values for QTVI per age group and sex**

| | Median (2nd Percentile , 98th Percentile) | |
|---|---|---|
| **Age Category** | **Boys/Men** | **Girls/Women** |
| 1 month | -1.48 (-2.36; 0.17) | -1.48 (-2.42; -0.13) |
| 1 to 3 months* | -1.48 (-2.36; 0.16) | -1.48 (-2.42; -0.14) |
| 3 to 6 months | -1.49 (-2.37; 0.15) | -1.49 (-2.43; -0.14) |
| 6 to 12 months | -1.50 (-2.38; 0.13) | -1.50 (-2.44; -0.15) |
| 1 to 3 years | -1.52 (-2.40; 0.08) | -1.53 (-2.47; -0.19) |
| 3 to 5 years | -1.57 (-2.44; 0.01) | -1.58 (-2.51; -0.24) |
| 5 to 8 years | -1.62 (-2.49; -0.08) | -1.63 (-2.57; -0.29) |
| 8 to 12 years | -1.67 (-2.55; -0.17) | -1.69 (-2.64; -0.35) |
| 12 to 16 years | -1.72 (-2.62; -0.24) | -1.74 (-2.71; -0.38) |
| 16 to 20 years | -1.74 (-2.67; -0.28) | -1.76 (-2.75; -0.39) |
| 20 to 30 years | -1.75 (-2.73; -0.26) | -1.73 (-2.75; -0.35) |
| 30 to 40 years | -1.67 (-2.77; -0.16) | -1.58 (-2.67; -0.16) |
| 40 to 50 years | -1.55 (-2.71; -0.08) | -1.39 (-2.55; 0.08) |
| 50 to 60 years | -1.36 (-2.61; 0.12) | -1.18 (-2.41; 0.38) |
| 60 to 70 years | -1.14 (-2.53; 0.39) | -0.96 (-2.29; 0.69) |
| 70 to 80 years | -0.93 (-2.46; 0.69) | -0.78 (-2.23; 0.97) |
| 80 to 90 years | -0.68 (-2.41; 1.05) | -0.63 (-2.23; 1.25) |

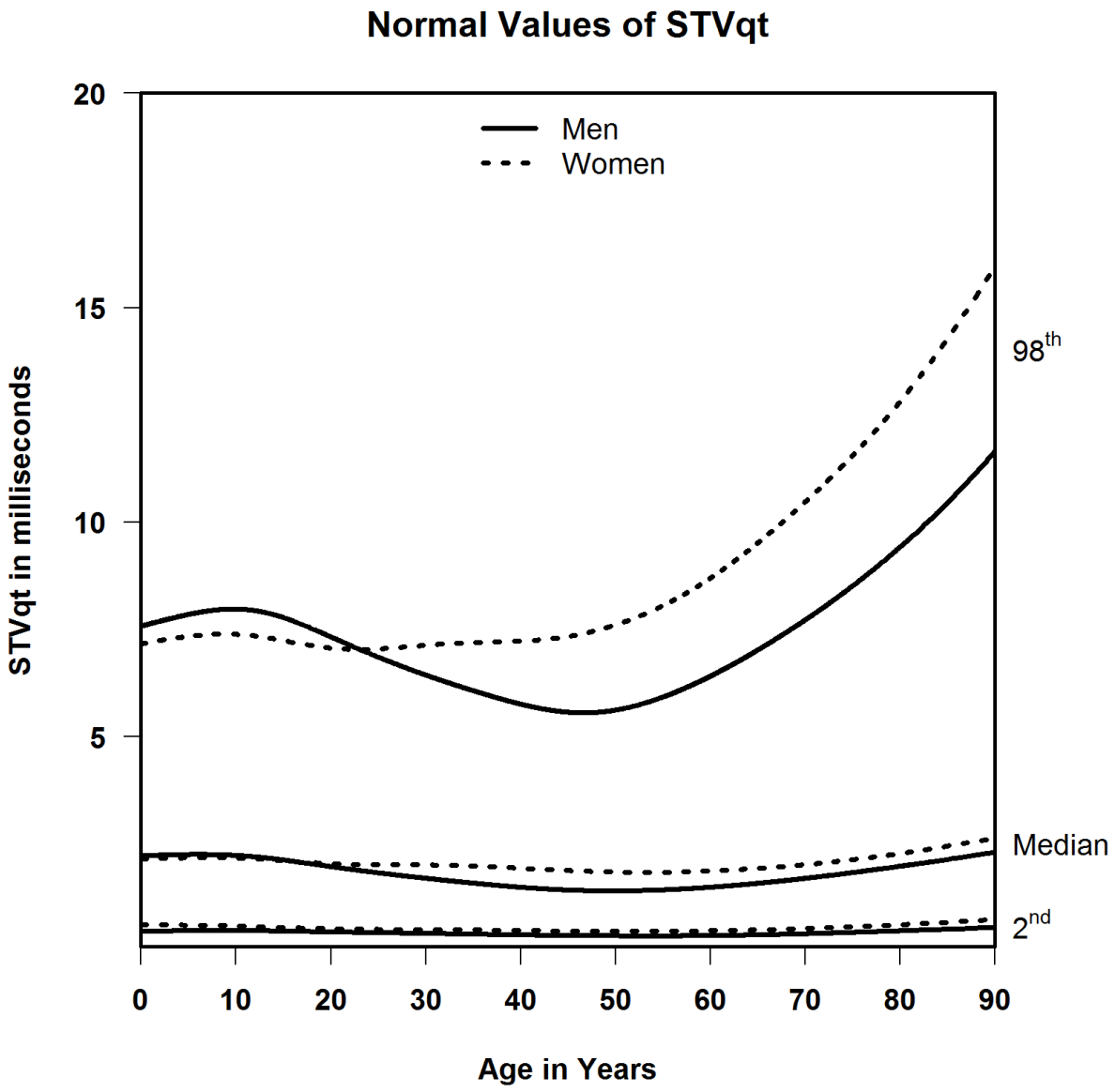
†The term “to” specifies the upper limit in the sense of “less than”.

**Figure 1. Median, 2nd and 98th percentiles for SDqt for all ages in men and women**



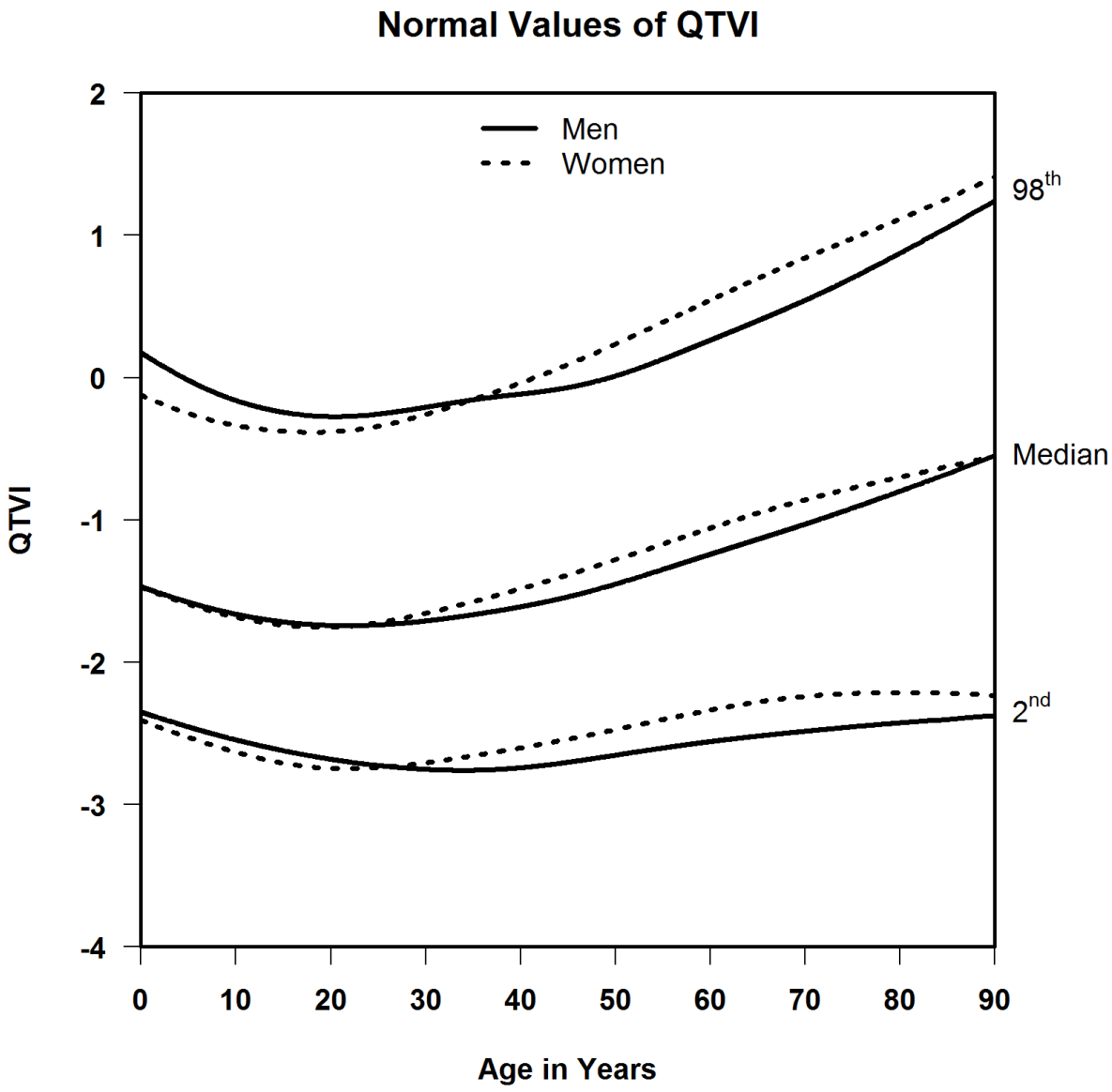

Abbreviation: SDqt: Standard deviation of QT intervals

**Figure 2. Median, 2nd and 98th percentiles for STVqt for all ages in men and women**




Abbreviation: STVqt: Short-term variability of the QT interval

**Figure 3. Median, 2nd and 98th percentiles for QTVI for all ages in men and women**




Abbreviations: QTVI: QT variability index

**Discussion**

This is the first paper to report comprehensive normal values of QT variability based on 10-second, standard 12-lead ECGs. We report the median, LLN, and ULN of SDqt, STVqt and QTVI across all ages and stratified on sex.

A recent paper by Baumert *et al.* presents a meta-analysis of normal values from 45 studies involving 1,954 adults with QTVI measurements and from 23 studies involving 1,190 adults with SDqt measurements.[1] Without taking age or ECG-recording duration into account, this meta-analysis found for men a mean QTVI of –1.6 and a mean SDqt of 3.3 milliseconds. Some of the larger component studies of this meta-analysis did not use standard 10-second ECGs, but analyzed much longer ECG signals. In children, there is a study by Kusuki *et al.* that reported normal values of children without organic heart disease aged 0 to 7 years, using ECG recordings containing 120 beats.[9] This paper concluded that QTVI decreased from birth to age 7, which is in accordance with our findings. Unfortunately this study did not report percentiles. A study by Yeragani *et al.* using 10-minute recordings found no significant difference in QTVI between 15 children aged 6-14 years and 34 normal adults aged 20-55 years.[6] Assuming the 10-minute recordings have the same age-dependent pattern as 10-second recordings, this may be explained by the children being on the descending limb of the QTVI curve, while the adults are at the same height on the ascending limb. There are three studies that track normal values for QTVI over a longer age range, between 20 and 90 years.[7,8,10] These studies, with 40,[10] 131,[8] and 143[7] healthy participants respectively, showed that QTVI increases over this age range, which is similar to our findings. These three studies used 10-minute, 15-minute and 30-minute recordings, respectively. SDqt was analyzed by Kraus *et al.*, showing that SDqt is higher in women and that SDqt increases with age;[18] both findings are consistent with the results of this study. Kraus *et al.* used 24-hour Holter recordings, and calculated hourly mean values for SDqt. To the extent comparisons are possible, the current findings are in general agreement with these previous studies that used longer recordings. The age and sex dependent normal values of QT variability reported here may serve to establish cut-off values for various clinical and pharmacological applications. For example, a drug associated with risk of ventricular arrhythmias might be withheld if the QT variability is above a certain limit.

Our study has a number of strengths. With 13,882 ECGs it is by far the largest study reporting normal values for QT variability. We include both men and women, and have a wide age coverage, from 11 days to 90 years. Although the ECGs in this study were retrieved from five different population studies, they were all analyzed consistently and automatically with the well-validated MEANS program and FSA technique.[16] This approach eliminates intra-observer bias, and uses all 12 ECG leads, which limits dependency of QT variability on the T-wave amplitude.

Our study also has some limitations. First, we had a relatively low number of ECGs in the extremes of the age distribution. For this reason, the normal limits of the groups younger than six months and older than ninety years should be used with caution. Second, the use of 10-second recordings prohibits measurement of QT variability over longer time intervals and detection of, e.g., respiratory modulation and diurnal changes. However, respiratory modulation was studied by Emori *et al.*, and this study concluded that respiratory modulation does not affect QT variability in healthy participants.[19] As for diurnal changes, a study by Yeragani *et al.* has shown that QT variability differs between the waking and sleeping hours, but it has also shown that QT variability is fairly constant within the waking or sleeping period.[20] Therefore, if all ECGs are recorded in awake participants, this should not materially affect the results. Finally, 10-second ECGs may sometimes contain only a few QT intervals for QT-variability calculation, and may render QT-variability estimates less reliable than those from longer recordings.

In conclusion, normal limits have been established for SDqt, STVqt, and QTVI derived from 10-second ECGs, using a consistent and automatic methodology for all ages and both sexes. We found strong age effects for SDqt and STVqt, as the ULN increases in the elderly. Using these normal values, both researchers and clinicians have a tool to decide upon cut-off values of QT variability, which improves the applicability of 10-second QT variability in practice.

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**Supplementary Table 1. Percentiles of SDqt in milliseconds by age category of men and women**

| | Percentiles for Men | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Age | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
| < 1 month | 1.13 | 1.37 | 1.64 | 2.21 | 3.15 | 4.58 | 6.57 | 8.24 | 10.77 |
| 1 to 3 months† | 1.13 | 1.37 | 1.64 | 2.22 | 3.15 | 4.58 | 6.56 | 8.23 | 10.76 |
| 3 to 6 months | 1.13 | 1.37 | 1.64 | 2.22 | 3.15 | 4.58 | 6.56 | 8.22 | 10.74 |
| 6 to 12 months | 1.13 | 1.38 | 1.64 | 2.22 | 3.16 | 4.58 | 6.55 | 8.21 | 10.71 |
| 1 to 3 years | 1.14 | 1.38 | 1.65 | 2.23 | 3.16 | 4.58 | 6.52 | 8.16 | 10.62 |
| 3 to 5 years | 1.15 | 1.39 | 1.66 | 2.24 | 3.16 | 4.56 | 6.48 | 8.07 | 10.47 |
| 5 to 8 years | 1.15 | 1.40 | 1.66 | 2.24 | 3.16 | 4.54 | 6.40 | 7.96 | 10.28 |
| 8 to 12 years | 1.14 | 1.39 | 1.65 | 2.22 | 3.12 | 4.46 | 6.26 | 7.75 | 9.98 |
| 12 to 16 years | 1.10 | 1.35 | 1.61 | 2.16 | 3.02 | 4.30 | 6.01 | 7.42 | 9.55 |
| 16 to 20 years | 1.05 | 1.29 | 1.54 | 2.07 | 2.88 | 4.08 | 5.69 | 7.03 | 9.06 |
| 20 to 30 years | 0.95 | 1.17 | 1.41 | 1.89 | 2.63 | 3.70 | 5.15 | 6.38 | 8.32 |
| 30 to 40 years | 0.83 | 1.05 | 1.26 | 1.70 | 2.34 | 3.25 | 4.53 | 5.68 | 7.59 |
| 40 to 50 years | 0.76 | 0.97 | 1.17 | 1.57 | 2.14 | 2.96 | 4.13 | 5.24 | 7.19 |
| 50 to 60 years | 0.73 | 0.93 | 1.14 | 1.54 | 2.11 | 2.94 | 4.19 | 5.44 | 7.84 |
| 60 to 70 years | 0.77 | 0.98 | 1.19 | 1.61 | 2.24 | 3.18 | 4.63 | 6.10 | 8.95 |
| 70 to 80 years | 0.90 | 1.10 | 1.32 | 1.77 | 2.48 | 3.60 | 5.32 | 7.02 | 10.17 |
| 80 to 90 years | 1.02 | 1.23 | 1.46 | 1.96 | 2.78 | 4.14 | 6.30 | 8.46 | 12.47 |

| | Percentiles for Women | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Age | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
| < 1 month | 1.12 | 1.38 | 1.66 | 2.24 | 3.13 | 4.37 | 5.91 | 7.08 | 8.70 |
| 1 to 3 months | 1.12 | 1.38 | 1.66 | 2.24 | 3.13 | 4.37 | 5.91 | 7.08 | 8.70 |
| 3 to 6 months | 1.13 | 1.38 | 1.66 | 2.24 | 3.13 | 4.37 | 5.91 | 7.09 | 8.71 |
| 6 to 12 months | 1.13 | 1.38 | 1.66 | 2.24 | 3.13 | 4.38 | 5.92 | 7.10 | 8.73 |
| 1 to 3 years | 1.13 | 1.39 | 1.66 | 2.25 | 3.14 | 4.39 | 5.94 | 7.13 | 8.78 |
| 3 to 5 years | 1.12 | 1.39 | 1.66 | 2.25 | 3.15 | 4.41 | 5.98 | 7.19 | 8.87 |
| 5 to 8 years | 1.11 | 1.38 | 1.66 | 2.25 | 3.15 | 4.42 | 6.01 | 7.26 | 9.01 |
| 8 to 12 years | 1.08 | 1.35 | 1.64 | 2.23 | 3.13 | 4.41 | 6.04 | 7.35 | 9.25 |
| 12 to 16 years | 1.01 | 1.29 | 1.58 | 2.18 | 3.08 | 4.35 | 6.04 | 7.45 | 9.60 |
| 16 to 20 years | 0.95 | 1.23 | 1.52 | 2.11 | 2.99 | 4.26 | 5.98 | 7.47 | 9.85 |
| 20 to 30 years | 0.96 | 1.22 | 1.49 | 2.05 | 2.90 | 4.13 | 5.79 | 7.22 | 9.43 |
| 30 to 40 years | 1.00 | 1.22 | 1.46 | 1.98 | 2.79 | 3.99 | 5.61 | 6.95 | 8.94 |
| 40 to 50 years | 0.97 | 1.17 | 1.38 | 1.86 | 2.63 | 3.81 | 5.49 | 6.93 | 9.16 |
| 50 to 60 years | 0.94 | 1.13 | 1.33 | 1.78 | 2.54 | 3.77 | 5.63 | 7.34 | 10.22 |
| 60 to 70 years | 0.98 | 1.16 | 1.36 | 1.82 | 2.61 | 3.96 | 6.17 | 8.38 | 12.44 |
| 70 to 80 years | 1.05 | 1.24 | 1.45 | 1.94 | 2.81 | 4.36 | 7.10 | 10.10 | 16.25 |
| 80 to 90 years | 1.16 | 1.36 | 1.59 | 2.13 | 3.11 | 4.96 | 8.49 | 12.73 | 22.67 |

†The term “to” specifies the upper limit in the sense of “less than”

**Supplementary Table2. Percentiles of STVqt in milliseconds by age category of men and women**

**Percentiles for Men**

| Age | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | 0.46 | 0.67 | 0.91 | 1.43 | 2.22 | 3.34 | 4.78 | 5.92 | 7.57 |
| 1 to 3 months† | 0.46 | 0.67 | 0.91 | 1.43 | 2.22 | 3.34 | 4.78 | 5.93 | 7.58 |
| 3 to 6 months | 0.46 | 0.67 | 0.91 | 1.43 | 2.22 | 3.35 | 4.79 | 5.93 | 7.59 |
| 6 to 12 months | 0.46 | 0.68 | 0.92 | 1.43 | 2.23 | 3.35 | 4.80 | 5.95 | 7.61 |
| 1 to 3 years | 0.47 | 0.68 | 0.92 | 1.44 | 2.23 | 3.37 | 4.82 | 5.99 | 7.69 |
| 3 to 5 years | 0.47 | 0.69 | 0.93 | 1.45 | 2.25 | 3.39 | 4.86 | 6.05 | 7.79 |
| 5 to 8 years | 0.48 | 0.69 | 0.93 | 1.45 | 2.25 | 3.40 | 4.89 | 6.11 | 7.90 |
| 8 to 12 years | 0.48 | 0.69 | 0.93 | 1.44 | 2.23 | 3.37 | 4.87 | 6.11 | 7.97 |
| 12 to 16 years | 0.47 | 0.67 | 0.90 | 1.39 | 2.15 | 3.25 | 4.73 | 5.96 | 7.84 |
| 16 to 20 years | 0.45 | 0.65 | 0.86 | 1.32 | 2.03 | 3.07 | 4.48 | 5.68 | 7.51 |
| 20 to 30 years | 0.43 | 0.60 | 0.79 | 1.19 | 1.82 | 2.74 | 4.01 | 5.11 | 6.85 |
| 30 to 40 years | 0.39 | 0.55 | 0.71 | 1.06 | 1.58 | 2.36 | 3.45 | 4.44 | 6.07 |
| 40 to 50 years | 0.36 | 0.51 | 0.66 | 0.96 | 1.42 | 2.09 | 3.07 | 3.98 | 5.56 |
| 50 to 60 years | 0.35 | 0.50 | 0.65 | 0.96 | 1.42 | 2.11 | 3.12 | 4.11 | 5.92 |
| 60 to 70 years | 0.38 | 0.54 | 0.71 | 1.05 | 1.58 | 2.38 | 3.59 | 4.79 | 7.02 |
| 70 to 80 years | 0.43 | 0.61 | 0.80 | 1.20 | 1.83 | 2.81 | 4.33 | 5.81 | 8.51 |
| 80 to 90 years | 0.51 | 0.70 | 0.91 | 1.38 | 2.13 | 3.36 | 5.26 | 7.11 | 10.45 |

**Percentiles for Women**

| Age | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | 0.61 | 0.79 | 0.99 | 1.44 | 2.13 | 3.16 | 4.50 | 5.59 | 7.16 |
| 1 to 3 months | 0.61 | 0.79 | 0.99 | 1.44 | 2.14 | 3.16 | 4.50 | 5.59 | 7.16 |
| 3 to 6 months | 0.61 | 0.79 | 0.99 | 1.44 | 2.14 | 3.16 | 4.51 | 5.60 | 7.17 |
| 6 to 12 months | 0.61 | 0.79 | 1.00 | 1.44 | 2.14 | 3.17 | 4.52 | 5.61 | 7.19 |
| 1 to 3 years | 0.60 | 0.79 | 1.00 | 1.45 | 2.15 | 3.18 | 4.54 | 5.64 | 7.23 |
| 3 to 5 years | 0.60 | 0.79 | 1.00 | 1.45 | 2.17 | 3.21 | 4.58 | 5.69 | 7.30 |
| 5 to 8 years | 0.60 | 0.79 | 1.00 | 1.45 | 2.17 | 3.23 | 4.61 | 5.73 | 7.36 |
| 8 to 12 years | 0.58 | 0.77 | 0.98 | 1.44 | 2.16 | 3.22 | 4.60 | 5.73 | 7.38 |
| 12 to 16 years | 0.55 | 0.74 | 0.95 | 1.41 | 2.12 | 3.16 | 4.53 | 5.64 | 7.28 |
| 16 to 20 years | 0.53 | 0.71 | 0.92 | 1.36 | 2.06 | 3.07 | 4.41 | 5.51 | 7.11 |
| 20 to 30 years | 0.50 | 0.68 | 0.89 | 1.32 | 2.01 | 3.02 | 4.35 | 5.44 | 7.04 |
| 30 to 40 years | 0.49 | 0.67 | 0.86 | 1.29 | 1.98 | 3.00 | 4.36 | 5.50 | 7.18 |
| 40 to 50 years | 0.47 | 0.63 | 0.82 | 1.22 | 1.87 | 2.87 | 4.26 | 5.46 | 7.33 |
| 50 to 60 years | 0.46 | 0.62 | 0.80 | 1.19 | 1.83 | 2.85 | 4.35 | 5.73 | 8.05 |
| 60 to 70 years | 0.49 | 0.66 | 0.85 | 1.26 | 1.92 | 3.01 | 4.72 | 6.41 | 9.51 |
| 70 to 80 years | 0.56 | 0.75 | 0.95 | 1.39 | 2.13 | 3.34 | 5.35 | 7.44 | 11.55 |
| 80 to 90 years | 0.67 | 0.88 | 1.11 | 1.61 | 2.44 | 3.86 | 6.27 | 8.88 | 14.27 |

†The term “to” specifies the upper limit in the sense of “less than”

**Supplementary Table3. Percentiles of QTVI by age category of men and women**

**Percentiles for Men**

| Age | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | -2.36 | -2.22 | -2.08 | -1.82 | -1.48 | -1.05 | -0.59 | -0.26 | 0.17 |
| 1 to 3 months† | -2.36 | -2.22 | -2.09 | -1.83 | -1.48 | -1.06 | -0.59 | -0.26 | 0.16 |
| 3 to 6 months | -2.37 | -2.23 | -2.09 | -1.83 | -1.49 | -1.06 | -0.60 | -0.27 | 0.15 |
| 6 to 12 months | -2.38 | -2.24 | -2.10 | -1.84 | -1.50 | -1.07 | -0.61 | -0.28 | 0.13 |
| 1 to 3 years | -2.40 | -2.26 | -2.13 | -1.87 | -1.52 | -1.11 | -0.65 | -0.33 | 0.08 |
| 3 to 5 years | -2.44 | -2.30 | -2.17 | -1.91 | -1.57 | -1.15 | -0.71 | -0.39 | 0.01 |
| 5 to 8 years | -2.49 | -2.35 | -2.21 | -1.95 | -1.62 | -1.21 | -0.77 | -0.46 | -0.08 |
| 8 to 12 years | -2.55 | -2.41 | -2.27 | -2.01 | -1.67 | -1.27 | -0.84 | -0.55 | -0.17 |
| 12 to 16 years | -2.62 | -2.47 | -2.32 | -2.06 | -1.72 | -1.32 | -0.90 | -0.61 | -0.24 |
| 16 to 20 years | -2.67 | -2.51 | -2.36 | -2.09 | -1.74 | -1.35 | -0.93 | -0.64 | -0.28 |
| 20 to 30 years | -2.73 | -2.56 | -2.39 | -2.10 | -1.75 | -1.34 | -0.92 | -0.63 | -0.26 |
| 30 to 40 years | -2.77 | -2.56 | -2.37 | -2.05 | -1.67 | -1.26 | -0.84 | -0.54 | -0.16 |
| 40 to 50 years | -2.71 | -2.48 | -2.27 | -1.93 | -1.55 | -1.14 | -0.72 | -0.44 | -0.08 |
| 50 to 60 years | -2.61 | -2.35 | -2.13 | -1.76 | -1.36 | -0.93 | -0.51 | -0.23 | 0.12 |
| 60 to 70 years | -2.53 | -2.24 | -1.99 | -1.59 | -1.14 | -0.69 | -0.25 | 0.04 | 0.39 |
| 70 to 80 years | -2.46 | -2.14 | -1.86 | -1.41 | -0.93 | -0.43 | 0.03 | 0.33 | 0.69 |
| 80 to 90 years | -2.41 | -2.04 | -1.72 | -1.22 | -0.68 | -0.15 | 0.35 | 0.67 | 1.05 |

**Percentiles for Women**

| Age | 2nd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 98th |
|---|---|---|---|---|---|---|---|---|---|
| < 1 month | -2.42 | -2.25 | -2.09 | -1.82 | -1.48 | -1.10 | -0.72 | -0.46 | -0.13 |
| 1 to 3 months | -2.42 | -2.25 | -2.10 | -1.82 | -1.48 | -1.11 | -0.72 | -0.46 | -0.14 |
| 3 to 6 months | -2.43 | -2.26 | -2.10 | -1.83 | -1.49 | -1.11 | -0.73 | -0.47 | -0.14 |
| 6 to 12 months | -2.44 | -2.27 | -2.11 | -1.84 | -1.50 | -1.12 | -0.74 | -0.48 | -0.15 |
| 1 to 3 years | -2.47 | -2.30 | -2.14 | -1.87 | -1.53 | -1.16 | -0.77 | -0.51 | -0.19 |
| 3 to 5 years | -2.51 | -2.34 | -2.19 | -1.91 | -1.58 | -1.21 | -0.82 | -0.56 | -0.24 |
| 5 to 8 years | -2.57 | -2.40 | -2.24 | -1.96 | -1.63 | -1.26 | -0.88 | -0.62 | -0.29 |
| 8 to 12 years | -2.64 | -2.46 | -2.30 | -2.02 | -1.69 | -1.32 | -0.94 | -0.68 | -0.35 |
| 12 to 16 years | -2.71 | -2.52 | -2.36 | -2.07 | -1.74 | -1.37 | -0.99 | -0.72 | -0.38 |
| 16 to 20 years | -2.75 | -2.56 | -2.39 | -2.10 | -1.76 | -1.39 | -1.00 | -0.74 | -0.39 |
| 20 to 30 years | -2.75 | -2.56 | -2.38 | -2.08 | -1.73 | -1.34 | -0.95 | -0.68 | -0.35 |
| 30 to 40 years | -2.67 | -2.47 | -2.28 | -1.96 | -1.58 | -1.17 | -0.76 | -0.49 | -0.16 |
| 40 to 50 years | -2.55 | -2.34 | -2.14 | -1.80 | -1.39 | -0.95 | -0.52 | -0.25 | 0.08 |
| 50 to 60 years | -2.41 | -2.19 | -1.98 | -1.61 | -1.18 | -0.71 | -0.25 | 0.04 | 0.38 |
| 60 to 70 years | -2.29 | -2.04 | -1.82 | -1.42 | -0.96 | -0.46 | 0.02 | 0.32 | 0.69 |
| 70 to 80 years | -2.23 | -1.96 | -1.71 | -1.28 | -0.78 | -0.26 | 0.25 | 0.58 | 0.97 |
| 80 to 90 years | -2.23 | -1.92 | -1.64 | -1.17 | -0.63 | -0.07 | 0.48 | 0.83 | 1.25 |

†The term “to” specifies the upper limit in the sense of “less than”

# Chapter 2.4

# QT Variability as Risk Factor for Sudden Cardiac Death, Cardiac Mortality, and All-Cause Mortality

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**Abstract**

*Aims* Increased QT variability (QTV), the variation of QT-interval durations on the electrocardiogram (ECG), has been proposed as a risk factor for cardiac morbidity and mortality in general, and for ventricular arrhythmias and sudden cardiac death (SCD) in particular. However, QTV has not yet been assessed as a risk factor in the general population. We therefore aimed to assess QTV as a risk factor for total mortality, coronary heart disease (CHD) mortality, and SCD in a population-based cohort.

*Methods and Results* The Rotterdam Study is a cohort of middle-aged and elderly people. We included 9,024 participants, with 22,610 ECGs in total. 3,847 participants died after a median follow-up of 10.5 years. Total mortality, CHD mortality, SCD, and witnessed SCD were the endpoints in Cox' regressions. The standard deviation of the QT intervals (SDqt), short-term variability (STVqt), root-mean-square of successive QT-interval differences (RMSSDqt), and QT variability index (QTVI) were measured automatically from 10-second ECGs. QTVI and $QTVI^2$ were significant risk factors for total mortality (hazard ratios (HRs) 1.06 and 1.01, respectively). SDqt, STVqt, and RMSSDqt were significant risk factors for total mortality (HRs 1.12, 1.12, and 1.11, respectively), CHD mortality (HRs 1.22, 1.23, and 1.21), and in women, for witnessed SCD (HRs 1.44, 1.63, and 1.50).

*Conclusions* The association of QTV markers with total mortality and CHD mortality was confirmed in a population-based cohort. An association with witnessed SCD was established only in women. Future research may elucidate the underlying sex-specific pathophysiology of these findings.

## Introduction

Despite improvements in prevention and treatment, cardiovascular diseases still impose a major burden of morbidity and mortality in Western Europe.[1] About half of the deaths associated with heart disease are sudden cardiac deaths (SCDs).[2] SCD has a multifactorial etiology: various underlying pathologies may cause electric instability, which in turn can lead to ventricular arrhythmias and ensuing SCD.[3] The underlying pathologies have different prevalences in men and women, but coronary heart disease (CHD) and dilated cardiomyopathy are most common in both.[4] Still, SCD can occur in a structurally normal heart and can also be drug-induced.[4] As SCD is often the first expression of heart disease, prediction of SCD in the general population remains a challenging problem.[5]

The heart-rate corrected QT (QTc) interval is the best known electrocardiographic (ECG) marker of ventricular arrhythmias and SCD.[5] Yet, QTc prolongation has limited predictive value for SCD.[6] QT variability (QTV), the beat-to-beat variation of QT-interval duration, has been advocated as a more specific risk factor for ventricular arrhythmias.[7] It is hypothesized that regional heterogeneity of cardiac repolarization leads to beat-to-beat changes in the QT interval,[7] and thus, QTV is thought to be a marker of electric instability.[3] QTV has come forward as a promising marker of SCD: an increase of a few milliseconds in QTV was predictive of ventricular arrhythmias in animal models of cardiac pathology,[8] QTV had a higher sensitivity and specificity for drug-induced arrhythmias than QTc prolongation in a case-control study in humans,[6] and QTV was associated with total mortality,[9] cardiovascular mortality,[9] and SCD[10] in cohort studies. These cohort studies, however, were small (at most 805 participants) and exclusively contained heart-failure patients or patients that had had a myocardial infarction (MI), while improvement of SCD prediction is most needed in the general population.[5] Several QTV markers have been proposed, but the predictive value of a comprehensive set of QTV markers has not yet been assessed and compared.[11] Finally, studies have not specifically addressed older adults, in whom the incidence of cardiovascular disease and SCD is highest.[1] Therefore, we aimed to establish the association of four different QTV markers with total mortality, CHD mortality, and SCD in a community-dwelling population of older adults.

## Methods

### *Setting*

Our study is part of the prospective population-based Rotterdam Study. The design and rationale of the Rotterdam Study have been described in more detail elsewhere.[12] In short, from 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district in Rotterdam, the Netherlands, were invited to participate in the initial cohort, and 7,983 individuals agreed to participate (response

rate 78%). In 2000, the cohort was extended by inviting all inhabitants from the same district who had turned 55 or who had moved into the district after the start of the initial cohort. For this extension, 3,011 individuals agreed to participate (response rate 67%). Of the 10,994 participants we included 8,990 in our study. Figure 1 shows a flowchart of the selection of the study population. Follow-up examinations were conducted every four to five years. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". We included only participants who provided written informed consent to participate in the study, including access to information from their general practitioner for follow-up monitoring.

*Electrocardiography*

Standard 12-lead ECGs were recorded at rest with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS), which has been validated and applied extensively.[13] We excluded ECGs with atrial fibrillation, atrial flutter, pacemaker rhythm, or left bundle branch block. We calculated the short-term variability of QT intervals (STVqt), the root mean square of successive QT-interval differences (RMSSDqt), the standard deviation of QT intervals (SDqt), and the QT variability index (QTVI).[11] Supplementary Table 1 shows their formulas.

To determine QTV, QT intervals were measured automatically using the fiducial segment averaging (FSA) technique.[14] Briefly, MEANS determines the locations of the individual QRS complexes in the ECG and their initial fiducial points (global onset of QRS complex and end of T wave across all leads), and computes a detection function consisting of the root-mean-square ECG signal. MEANS automatically detects and excludes ectopic beats from further processing together with the beats immediately before and after the ectopic beat. Second, the fiducial point of each individual beat is shifted in an iterative procedure until maximum correlation is achieved between a small signal segment of the detection function around this fiducial point (the "fiducial segment") and the average of the fiducial segments of the remaining complexes. The amount of shifting is retained and constitutes the individual beat variation in the fiducial point estimate. Finally, the QT interval for each beat is calculated while taking into account the shifts of the initial QRS onset and T end. To safeguard against signal segments with excessive noise or baseline wander, an additional test is applied after each FSA iteration. If the averaged absolute amplitudes of the difference between the ST-T wave of an individual beat and the averaged ST-T wave of the remaining beats is larger than a preset value, the beat is discarded and the iteration process is repeated for the remaining beats.

The following ECG-derived markers were analyzed as covariables: heart rate, heart-rate variability (HRV), average QT interval, and Sokolow-Lyon index. HRV was expressed as the standard deviation of normal-to-normal RR intervals (SDNN).[15] Based on an earlier study that showed a quadratic relationship between SDNN and both total and cardiovascular mortality,[16] SDNN squared was also added to the model. The Sokolow-Lyon index was taken as the sum of the voltages of the S in lead V1 and the R in lead V5 or V6, whichever was larger.

*Covariables*

We analyzed age, sex, smoking, body-mass index (BMI), hypertension, history of CHD, history of diabetes mellitus, and history of heart failure. All covariables were determined at the date of each ECG recording. Based on a home interview, participants were classified into never, former, and current smokers. BMI was defined as weight in kilograms divided by height squared in meters. Blood pressure was measured twice in the sitting position on the right upper arm. The average of two measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg, or a diastolic blood pressure ≥90mmHg, or the use of blood-pressure-lowering medication with the indication hypertension. CHD was defined as a history of a MI or a percutaneous or surgical coronary revascularization procedure.[17] The diagnosis of heart failure was established in accordance with the guidelines of the European Society of Cardiology[18] and was based on typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction.[17] Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L or a non-fasting serum glucose level ≥11.1 mmol/L, or use of glucose-lowering medication, or a previous diagnosis of diabetes mellitus.

*Outcome definitions*

Total mortality was death due to any cause. The definition of CHD mortality in the Rotterdam Study has been described previously.[17] In short, all available information for each candidate case of fatal CHD was reviewed by research physicians to ascertain the cardiac cause of death. CHD mortality as used in this study includes mortality due to definite fatal MI, definite fatal CHD and possible fatal CHD.[17]

SCD was defined in accordance with the Myerburg definition, which is endorsed by the European society of Cardiology,[19] as “a natural death due to cardiac causes, heralded by abrupt loss of consciousness within one hour from onset of acute symptoms. Pre-existing heart disease may have been known to be present, but the time and mode of death are unexpected.” Cases of unwitnessed death were labeled as SCD if the person had been seen in a stable medical condition 24 hours preceding death and if there was no evidence of a non-cardiac cause of death. In addition, in

case of sparse information, cases were labelled as SCD when the attending physician recorded death as sudden or unexpected.[1] Identification and validation of SCD cases was based on a two-step process. Initially, when a participant died, research assistants gathered the relevant portions from the medical records. A study physician then coded the death according to the International Classification of Disease, 10th revision (ICD-10). A medical specialist pertinent to the field validated the study physician's coding. The decision of the medical specialist was decisive. In the second step, the medical files of participants whose deaths were coded according to the following ICD-10 codes were assessed: I05-I09 (Chronic rheumatic heart diseases), I20-I25 (Ischemic heart diseases), I30-I52 (Other forms of heart disease), I60-I69 (Cerebrovascular diseases), and R96-R99 (Ill-defined and unknown causes of mortality). Classification as SCD was done independently by two research physicians and subsequently by an experienced cardiologist. For the outcome "witnessed SCD", we subtracted SCD cases that were unwitnessed, or for which it was unclear if death was witnessed. Follow-up was complete for 96% of all mortality events until January 1st, 2011.

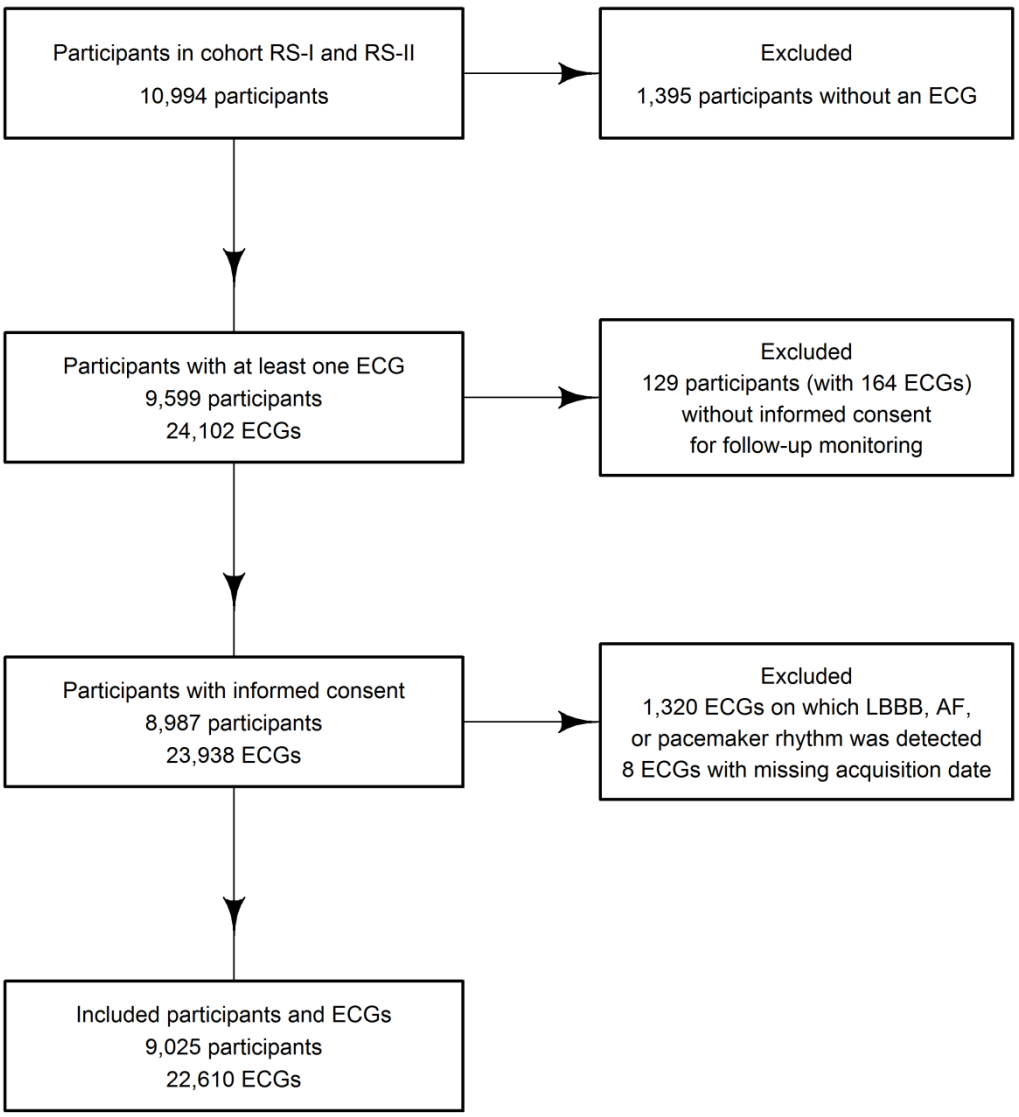
*Statistics*

All variables were used as time-dependent variables in a Cox' proportional hazard model, updated at each center visit. ECG variables were updated when a new ECG had been made. QTV variables that were not normally distributed were log-transformed before analysis. We plotted the Schoenfeld residuals to assess the proportional hazards assumption. In case of missing values, last observations were carried forward if a previous center visit was available. Remaining missing values were imputed using 10-times multiple imputation.

For every QTV marker we reported both crude hazard ratios (HRs) and HRs adjusted for the covariables age, sex, QT interval, heart rate, and the additional confounders smoking, body-mass index (BMI), hypertension, history of CHD, history of diabetes mellitus, and history of heart failure. The additional confounders were only included if they changed the beta estimate of the QTV marker by more than 10%. For CHD mortality, history of CHD was excluded because it is highly associated with the event. For analyses with QTVI we did not consider HRV because this variable is part of the QTVI formula. If a quadratic term for a QTV marker was significant, this was added to all analyses. In case of a significant interaction between age or sex and the QTV marker, results were presented stratified by age group or sex. The correlation between QTV markers was assessed on baseline ECGs with the Pearson correlation coefficient.

For all analyses, we used R (R Foundation for Statistical Computing, Vienna, Austria), specifically the packages *survival*, *rms* and *Hmisc.* Results were presented as HRs for each QTV marker with 95% confidence intervals (95%CIs). Two-sided p-values <0.05 were considered significant.

**Figure 1. Flowchart of the generation of the study population.**




Abbreviations: ECG: electrocardiogram; LBBB: Left bundle branch block; AF: atrial fibrillation; RS: Rotterdam Study cohort; QTV: QT variability; n: number

**Results**

Baseline characteristics of the study population are shown in Table 1. In short, 58.3% were women, and the mean age at baseline was 68 years. Median follow-up duration was 10.5 years. During follow-up, 3,847 persons died, of whom 774 due to CHD and 401 due to SCD. There was considerable overlap of SCD and CHD: 366 SCD cases were also classified as CHD (91%). Of the 401 SCD cases, 215 cases were witnessed SCD. No covariable had more than 5% missing values.

Table 2 presents the association of all QTV markers with all four outcomes. SDqt, RMSSDqt, and STVqt were not normally distributed, and therefore log-transformed. ln(SDqt), ln(STVqt), and ln(RMSSDqt) were significantly associated with total mortality. QTVI had a significant quadratic term for total mortality. All four markers were significantly associated with total mortality. ln(SDqt), ln(STVqt), and ln(RMSSDqt) were significantly associated with CHD mortality. None of the QTV markers were significantly associated with SCD. These were all multivariable-adjusted results. We stratified the analyses for witnessed SCD on sex because the interaction term of ln(STVqt) and ln(RMSSDqt) with sex was significant. Table 3 shows that ln(STVqt) and ln(RMSSDqt) were significantly associated with a higher risk of witnessed SCD in women, but not in men.

The Pearson correlation coefficients of STVqt, SDqt, RMSSDqt ranged between 0.82 and 0.97, showing a high correlation, but each of these had a weaker correlation with QTVI, as the Pearson Correlation Coefficients of STVqt, SDqt and RMSSDqt with QTVI ranged between 0.46 and 0.48.

**Table 1. Baseline characteristics**

| | |
|---|---|
| Participants, N | 9,024 |
| Women, N (%) | 5,265 (58.3%) |
| Number of ECGs, N | 22,610 |
| Follow-up time in years, median (IQR) | 10.5 (7.7-17.3) |
| Age in years, mean (SD) | 68.0 (8.7) |
| BMI in kg/$m^2$, mean (SD) | 26.6 (3.9) |
| Smoking Never, N (%) | 2,968 (33.8%) |
| Former, N (%) | 3,825 (43.5%) |
| Current, N (%) | 1,993 (22.7%) |
| Hypertension, N (%) | 5,256 (59.1%) |
| Coronary heart disease, N (%) | 653 (7.5%) |
| Diabetes mellitus, N (%) | 949 (10.9%) |
| Heart failure, N (%) | 190 (2.1%) |
| Heart rate in beats per minute, mean (SD) | 70.1 (11.8) |
| QT interval in milliseconds, mean (SD) | 402 (30) |
| QTc interval (Bazett) in milliseconds, mean (SD) | 431 (25) |
| ln(SDNN), mean (SD) | 2.9 (0.8) |
| Sokolow-Lyon index, mean (SD) | 2,304 (748) |
| ln(SDqt), mean (SD) | 1.5 (0.6) |
| QTVI, mean (SD) | -0.9 (1.0) |
| ln(STVqt), mean (SD) | 1.3 (0.6) |
| ln(RMSSDqt), mean (SD) | 1.7 (0.6) |

Abbreviations: BMI: body-mass index; ECG: electrocardiogram; IQR: interquartile range; QTVI: QT variability index; RMSSDqt: Root mean square of successive differences in QT intervals; SD: standard deviation; SDNN: Standard deviation of normal-to-normal RR intervals; SDqt: Standard deviation of QT intervals; STVqt: Short-term QT variability.

**Discussion**

We analyzed the association of QTV as measured on 10-second ECGs with four endpoints in a large population of older adults. With adjustment for relevant covariables, an elevated QTV was significantly associated with an increased risk of total mortality and CHD mortality. There was also an association of QTV with witnessed SCD, but only in women.

We found that QTV markers were associated with total mortality in a community-dwelling older population, confirming previous studies[11] conducted in cohorts of patients with cardiac diseases. The associations of QTV markers with CHD mortality were significant, with higher point estimates than those of total mortality, confirming a study that found an association of QTV markers with cardiovascular death and SCD in a cohort of heart failure patients.[9]

The association of QTV with SCD was not significant, even though this association was reported in cardiac patients in previous cohort studies.[10] Nevertheless, in women we found such an association for witnessed SCD, and with a higher point estimate of the HR than that for total mortality or CHD mortality. We venture the following explanation for the observed difference between witnessed and unwitnessed SCD: because information of symptoms and of the timeframe previous to death is lacking, unwitnessed deaths due to a non-arrhythmic (non-cardiac) cause could more readily be classified as SCD than witnessed deaths.[19] Thus, the unwitnessed cases dilute the pool of deaths of pure arrhythmic origin and weaken the association between QTV and SCD. The difference between men and women may be explained by different underlying pathologies.[4] According to our study, QTV is not significantly associated with CHD mortality, implying that QTV is not or weakly associated with SCD when caused by underlying CHD. A study of cardiac arrest survivors found that in men, 80% of the cardiac arrests are related to CHD, while in women only 45% of the cardiac arrests are related to CHD.[4] Therefore, it is possible that most cases of witnessed SCD in men are due to CHD and thus only weakly associated with QTV. However, a study in patients with ischemic cardiomyopathy found that QTV is a risk factor for ventricular arrhythmias in men only.[20] The opposite results of that study might be explained by the widely different participant selection. Our study indicates that further research on the difference in SCD risk prediction between men and women is warranted.

Two observations can be made with regard to the different types of QTV markers. First, consecutively measured QTV (e.g. RMSSDqt) and non-consecutively measured QTV (e.g. SDqt) were highly correlated and showed similar results in the analyses, even though some studies have previously found a difference between SDqt and RMSSDqt.[21] The absence of this difference in our study might derive from the use of 10-second ECGs. Second, both a decreased and an increased QTVI was associated with a higher risk of total mortality (the significant term $QTVI^2$), while for the

other three QTV markers, only an increase was associated with a higher risk. As QTVI is defined by a formula with QTV in the numerator and HRV in the denominator (Table 1), the association between QTVI and total mortality can be mediated through both QTV and HRV. As both a low and a high HRV have been associated with an increased risk for total mortality,[16] a low QTVI may be associated with total mortality by way of a high HRV or a low QTV and vice versa. A separate assessment of HRV and QTV would yield more specific information than a combined marker.

Our study has several strengths. First, it is part of a population-based study of community-dwelling middle-aged and elderly participants. Clinical and ECG data were available for many relevant cardiovascular risk factors and for multiple center visits, with <5% missing values. Successive ECGs were available for many participants. Second, all QTV markers were automatically calculated by MEANS in combination with FSA, which enhanced precision and prevented bias in ECG assessment, whereas most previous studies used semi-automatic or manual methods.[11] There are, however, also some limitations. First, the QTV markers are based on 10-second routine ECGs, whereas most other studies of QTV used longer ECG recordings.[11] However, one study reported good agreement between STVqt estimates from 10-second ECGs and 3-minute ECGs (Pearson correlation coefficient 0.81). [22] Second, even though we adjusted for the most relevant clinical risk factors, we cannot rule out residual confounding. Third, we did not have information on the underlying causes of SCD (e.g., by autopsy), and the arrhythmia before death was unknown. However, incidence rates of SCD in our study are comparable to those of other studies, indicating sufficient validation quality.[1,3] Fourth, there are conflicting studies about the confounding effect of respiration on QTV[23,24] , but we had no data on respiration. Fifth, we could not adjust the sex-stratified model for witnessed SCD due to the small number of events. The inclusion of too many covariables would lead to overfitting. Additionally, multiple testing might be an issue due to the multiple QTV markers and the multiple outcomes. However, the effect of multiple testing is likely to be limited, as the QTV markers are highly correlated, and the HR estimates of the QTV markers are highly similar, with the exception of QTVI.

In conclusion, we found an association of QTV markers with total mortality and CHD mortality, and a stronger association with witnessed SCD, but only in women. Further study of the underlying pathophysiology of QTV and its gender-specific components is necessary.

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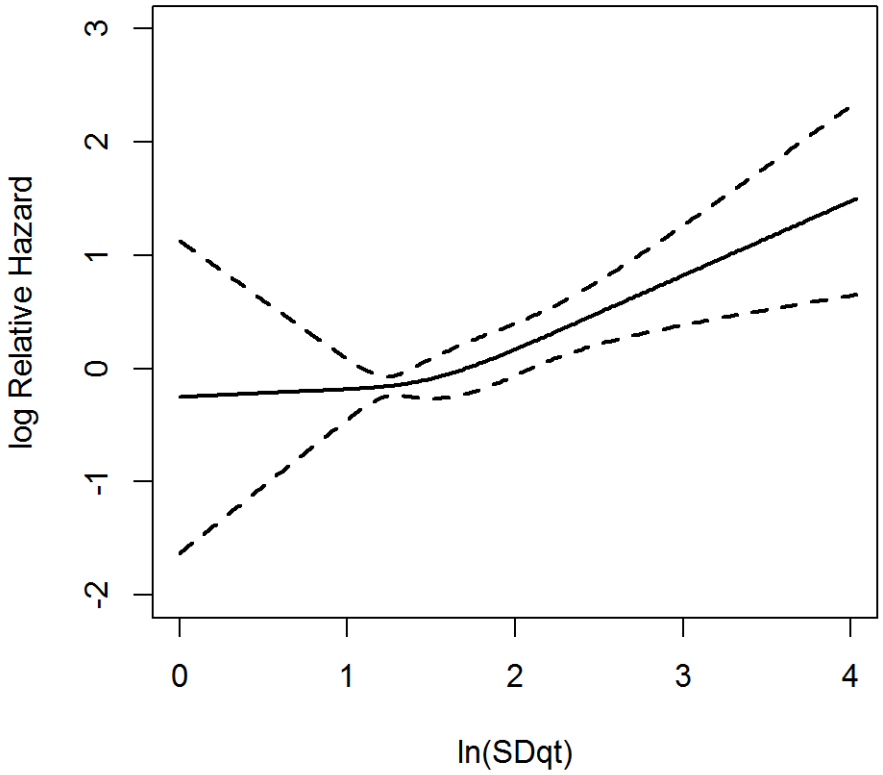
**Supplementary table 1. QT-interval variability measures**

| Variable | Based on consecutive differences | Heart-rate Normalization | Formula |
|---|---|---|---|
| SDqt | No | No | $\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(QT_i - QT_m)^2}$ |
| QTVI | No | Yes | $log_{10}\left[\frac{\left(\frac{QT_v}{QT_m^2}\right)}{\left(\frac{HR_v}{HR_m^2}\right)}\right]$ |
| STVqt | Yes | No | $\sum_{i=1}^{n}\frac{\lvert QT_{i+1} - QT_i\rvert}{n\sqrt{2}}$ |
| RMSSDqt | Yes | No | $\sqrt{\frac{1}{n}\sum_{i=1}^{n}(QT_{i+1} - QT_i)^2}$ |

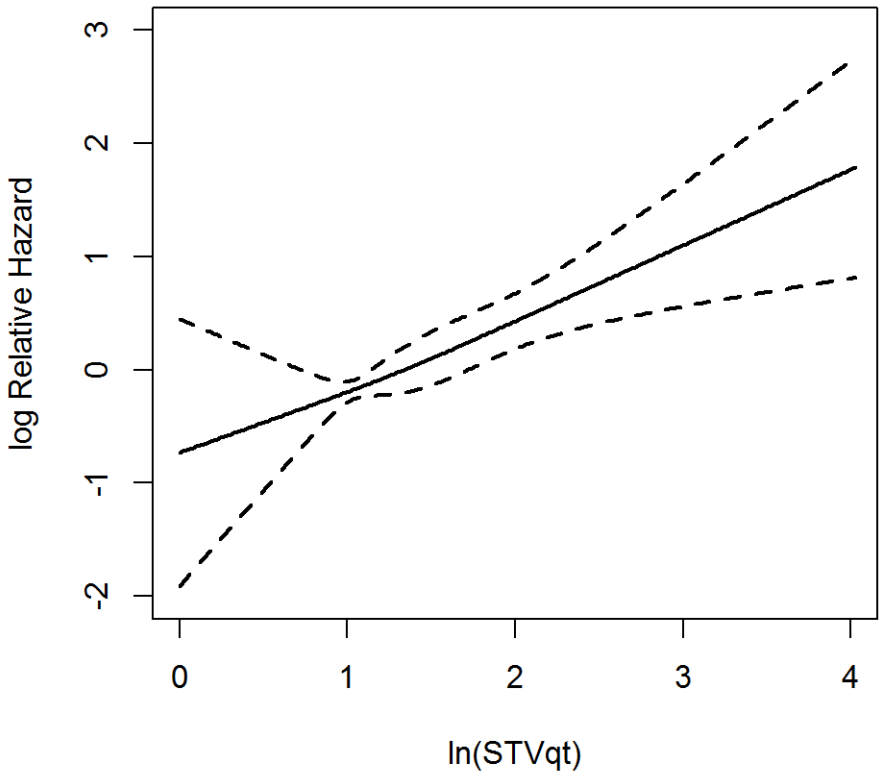
Abbreviations: $HR_m$: mean heart rate; $HR_v$: variance of heart rate; n: number of beats used for calculation; $QT_i$: QT interval of beat i; $QT_m$: mean QT interval; $QT_v$: variance of QT interval; QTVI: QT variability index; RMSSDqt: root mean square of successive differences in QT intervals; SDqt: standard deviation of QT intervals; STVqt: short-term QT variability.

**Supplementary figure 1. Log relative hazards of SDqt for witnessed SCD in women**



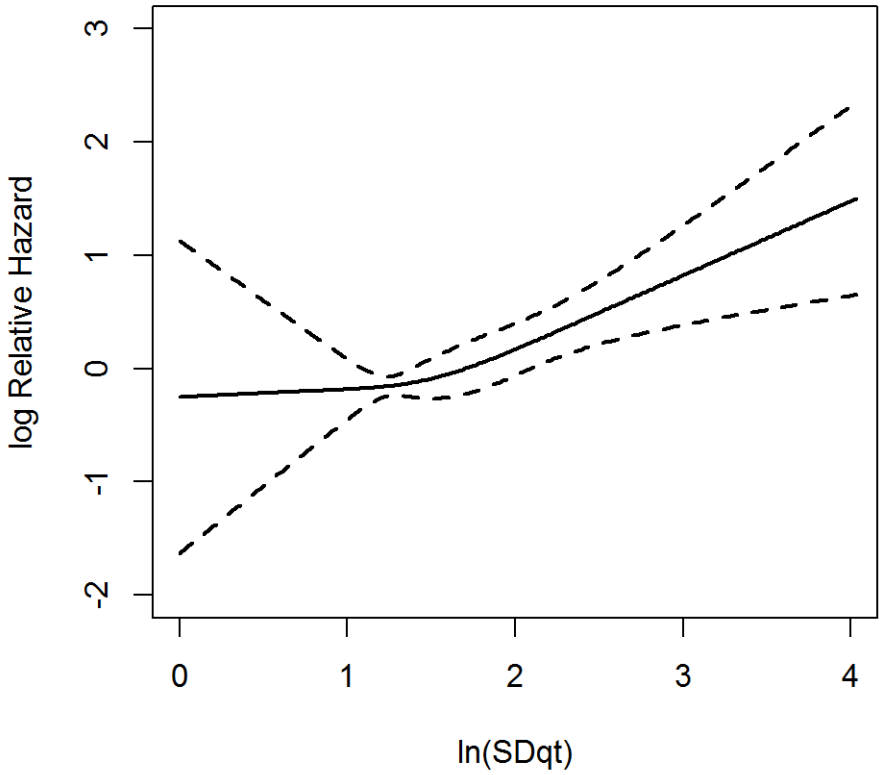

Log relative hazards were estimated with 3-knot restricted cubic splines. The solid line represents the estimate, the dotted lines represent the limits of the 95% confidence interval. Abbreviations: SCD: sudden cardiac death, SDqt: standard deviation of QT intervals

**Supplementary figure 2. Log relative hazards of STVqt for witnessed SCD in women**




Log relative hazards were estimated with 3-knot restricted cubic splines. The solid line represents the estimate, the dotted lines represent the limits of the 95% confidence interval. Abbreviations: SCD: sudden cardiac death, STVqt: short-term variablity of QT intervals

**Supplementary figure 3. Log relative hazards of RMSSDqt for witnessed SCD in women**




Log relative hazards were estimated with 3-knot restricted cubic splines. The solid line represents the estimate, the dotted lines represent the limits of the 95% confidence interval. Abbreviations: SCD: sudden cardiac death, RMSSDqt: root mean square of successive differences between QT intervals.

# Chapter 2.5

# Additional Value of Electrocardiographic Markers for Predicting Sudden Cardiac Death in The Middle-Aged and Elderly

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**Abstract**

*Background* Various electrocardiographic (ECG) markers have been proposed as risk factors for sudden cardiac death (SCD). Because these markers were usually studied individually, we aimed to evaluate the additional value of a comprehensive set of ECG markers for predicting SCD.

*Methods and Results* We used data from the Rotterdam Study, a cohort of community-dwelling participants. SCD within ten years was the endpoint in a proportional subdistribution hazards model. We assessed a model combining ECG markers and clinical risk factors over a basic model with only clinical risk factors. Variables for these models were chosen by backwards selection. When creating the combined model, the variables of the basic model were fixed. There were 13 candidate clinical risk factors and 24 candidate ECG markers. The discrimination of the basic and the combined model was evaluated with a bootstrapped Harrell's C-statistic. The study cohort consisted of 5,558 participants, of whom 149 died of SCD. The basic model consisted of age, sex, total/HDL cholesterol ratio, smoking, diabetes mellitus, and use of agents acting on the renin-angiotensin system. The combined model additionally contains heart rate, Sokolow-Lyon index, spatial J amplitude, T amplitude in lead aVR, right bundle branch block, and heart-rate corrected SDNN. The Model with the additional ECG variables had a significantly higher C-statistic than the basic model.

*Conclusion* This study suggests that the prediction of SCD in the general population can be improved by combining clinical risk factors with ECG markers, as adding the ECG markers significantly improved the discrimination of the model.

**Introduction**

Sudden cardiac death (SCD) is a major health burden. Incidence rates in the general population range from 50 to 100 cases per 100,000 person-years in developed countries.[1] The underlying causes of SCD include coronary heart disease (CHD),[2] cardiomyopathies,[3] and ion-channelopathies.[4] SCD is often the first presentation of heart disease, which makes the occurrence of SCD difficult to predict in the general population.[5]

The opportunities for SCD risk prediction in the general population have been described by Wellens *et al.*[6] In their paper, it was recommended that integrated risk models be investigated by combining parameters that characterize the underlying causes of SCD. As a matter of course, electrocardiographic (ECG) markers can be useful for this, as they represent different aspects of cardiac function and pathology. Many ECG markers have been associated with increased risk for cardiac mortality or SCD in population-based studies. Examples are the heart-rate corrected QT (QTc) interval,[7] the Sokolow-Lyon index, and T axes.[8] However, these population-based studies have analyzed only one ECG marker or a limited number of combinations of markers.[6] The most comprehensive study evaluated five basic ECG risk factors (atrial fibrillation, Cornell voltage, corrected QT interval, QRS duration, and heart rate) for an SCD prediction model.[9]

In this study, we evaluated a large number of ECG markers, in order to discover which markers are associated with SCD, independently of clinical risk factors. By creating a combination of ECG markers, we aimed to assess the true additional worth of the ECG in addition to clinical risk factors.

**Methods**

*Setting and study population*

This study was performed as part of the Rotterdam Study, a prospective population-based cohort study. Details regarding design, objectives, and methods of the Rotterdam Study have been described previously.[10] In short, the Rotterdam Study started in 1990 with an initial cohort of 7,983 persons (response rate 78%) aged 55 years or older living in the Ommoord district of the city of Rotterdam in the Netherlands. In 2000, the cohort was extended with 3,011 participants (response rate 67%) who had become 55 years of age or who moved into the study district. Follow-up examinations were conducted approximately every four to five years. These examinations consisted of a home interview and an extensive set of tests at a research center located in the study district. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the “Wet Bevolkingsonderzoek: ERGO” (Population Study Act: Rotterdam Study). We included only

participants who provided written informed consent to participate in the study and who provided written informed consent to obtain information from their treating physicians. By linking the general practitioners' and municipality records to the study database, participants were continuously monitored for major morbidity and mortality during follow-up. Other exclusion criteria were a missing ECG, an unreadable ECG (e.g., due to excessive noise), or an ECG showing a pacemaker rhythm or atrial fibrillation.

*Clinical risk factors*

Candidate clinical risk factors were age, sex, body-mass index (BMI), total cholesterol/high-density lipoprotein cholesterol (TC/HDL) ratio, smoking, systolic blood pressure, diastolic blood pressure, prevalent heart failure, and prevalent diabetes mellitus. We additionally considered use of four groups of cardiovascular drugs as candidate covariables: diuretics (Anatomical Therapeutic Chemical (ATC) code C03), beta-blocking agents (ATC C07), agents acting on the renin-angiotensin system (ATC C09), and lipid-lowering drugs (ATC C10). TC and HDL levels were acquired by an automated enzymatic procedure (Boehringer Mannheim System). Smoking status was assessed during a home interview, and participants were classified into never, past, and current smoker categories. Systolic blood pressure and diastolic blood pressure were measured in sitting position at the right upper arm. For each visit, the average of two consecutive blood pressure measurements was used. Heart failure diagnosis was established in accordance with the guidelines of the European Society of Cardiology[11] and included typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction.[12] Diabetes mellitus was defined as a fasting serum glucose level ≥ 7.0 mmol/L, a non-fasting serum glucose level ≥11.1 mmol/L, or use of blood-glucose lowering medication. Prevalent CHD was defined as a previous myocardial infarction or a coronary revascularization procedure.[12] More than 99% of the participants had their drug prescriptions filled at one of the seven fully computerized regional pharmacies, which use one common computer network. Dispensing data was available on a day-to-day basis, and included the ATC code. Participants were considered exposed to a drug group if there was overlap of a dispensing episode of this drug group and a window of 90 days before the date of ECG recording.

*ECG markers*

A standard 12-lead resting ECG was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECG and VCG measurements were obtained with the Modular ECG Analysis System (MEANS).[13] The MEANS program uses template-matching techniques to determine common wave onsets and offsets for all 12 leads together on one

representative averaged beat. The X, Y, and Z leads of the VCG were synthesized from the 12-lead ECGs in good approximation.[14]

Supplementary Table 1 in gives an overview of the 24 candidate ECG and VCG markers with their formulas and definitions. These ECG and VCG markers were selected because they have been associated in previous studies with cardiac death or SCD. Fridericia's formula was used to calculate the QTc interval since this formula is more accurate than Bazett's formula in correcting the QT interval for heart rate.[15] Heart-rate variability (HRV) markers standard deviation of normal-to-normal RR-intervals (SDNN) and root-mean-square of successive RR-interval differences (RMSSD) were corrected for heart rate with an exponential formula, denoted with SDNNc and RMSSDc.[16] The measurement of QT variability markers is given in the supplementary methods.

*Sudden cardiac death*

SCD was defined in accordance with the Myerburg definition, endorsed by the European Society of Cardiology[17] as "a natural death due to cardiac causes, heralded by abrupt loss of consciousness within one hour from onset of acute symptoms; pre-existing heart disease may have been known to be present, but the time and mode of death are unexpected". We included cases of unwitnessed death if the person was seen in a stable medical condition in the twenty-four hours preceding death and if there was no evidence of a non-cardiac cause of death. In the case of sparse information, cases were labelled as SCD when treating physicians labelled their deaths as sudden or unexpected.[1] Identification and validation of SCD cases was based on medical files, which were reviewed independently by two research physicians and subsequently by an experienced cardiologist.

*Statistics*

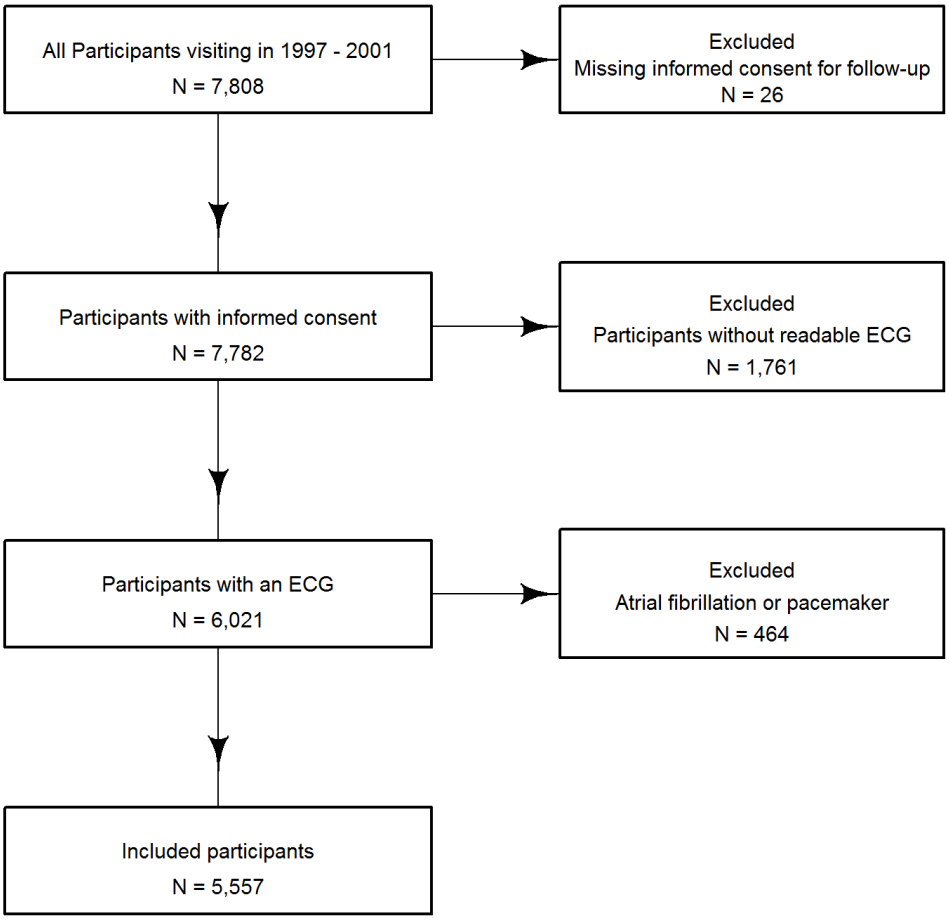
The primary outcome was SCD within ten years. Total mortality was defined as death from any cause within ten years. Follow-up was calculated from date of the ECG recording until date of death, end of study period, after ten years follow-up, or loss to follow-up (n = 223, 4%). All visits occurred between 1997 and 2001, corresponding with the third visit of the initial cohort (which originally started in 1990) and the first visit of the additional cohort.

We evaluated the risk factors and ECG markers for SCD in proportional subdistribution hazards models according to Fine and Gray.[18] The variable selection procedure was as follows: all candidate variables were included in the model and the variable with the highest p-value was determined. If this p-value was larger than 0.2,[19] the variable was removed and a new model was built. This elimination procedure was repeated until all p-values were below 0.2. Two models were created. First, a basic model was built from 13 candidate clinical risk factors. Second, the risk factors

selected in the basic model were kept fixed, and 24 candidate ECG and VCG variables were added as input for the elimination procedure. In a separate analysis, we bootstrapped Harrell's C-statistic 100 times for both the basic model and the combined model in order to obtain confidence intervals. In order to assess the influence of prevalent CHD, one of the most important underlying causes of SCD,[20] we performed a sensitivity analysis including prevalent CHD at baseline as a candidate covariable for the basic model and the combined model.

We used multiple imputation, specific methods are given in the supplementary methods.

**Figure 1. Flowchart of included and excluded participants of the Rotterdam Study**




Abbreviations: ECG: electrocardiogram; N: number of ECGs

**Results**

*Study population*

Figure 1 shows the flowchart of the study population, consisting of 5,558 participants. Baseline characteristics are shown in Table 1. In short, the mean age was 69.2 years with a standard deviation of 8.0 years, and 43.1% of the participants were men. During median follow-up of 10 years, 1,395 participants died, of whom 149 due to SCD.

*Variable selection*

The selected variables associated with increased risk of SCD are shown in Table 2. The basic model consisted of six clinical risk factors: age, sex, TC/HDL ratio, smoking, diabetes mellitus, and use of agents acting on the renin-angiotensin system (ATC 09). The combined model contained seven additional ECG markers: heart rate, Sokolow-Lyon index, spatial J amplitude, T amplitude in lead aVR, (T aVR) right bundle branch block (RBBB), and SDNNc.

*Characteristics of the basic model and the combined model*

Table 2 shows the variables selected for the basic model and the combined model, with subdistribution hazard ratios (SHRs) and 95% confidence intervals (CIs). Based on 100 bootstraps, the C-statistic of the basic model was 0.75, while the C-statistic of the combined model was 0.78. The bootstrapped difference in C-statistic between these models was statistically significant: 0.03, 95%CI 0.02-0.06.

*Sensitivity analyses*

Additional inclusion of prevalent CHD as a candidate covariable resulted in the same basic model (with the addition of prevalent CHD). The combined model with CHD additionally included heart rate, Sokolow-Lyon index, T aVR, right RBBB and SDNNc. Spatial J amplitude was not selected, but - otherwise - the combined model with CHD was comparable to the model from the main analysis. The bootstrapped C-statistic was 0.75 for the basic model with prevalent CHD, and 0.79 for the combined model with prevalent CHD. The bootstrapped difference between these models was 0.03, 95%CI 0.02-0.06.

**Table 1. Baseline characteristics of the study population**

| | |
|---|---|
| Total study population | 5557 |
| Women, N(%) | 3,162 (56.9%) |
| Death within ten years, N(%) | 1397 (25.1%) |
| SCD within ten years, N(%) | 149 (2.7%) |
| Follow-up (years), Median ($1^{st}$; $3^{rd}$ quartile) | 10.0 (9.0; 10.0) |
| age (years), Mean ± SD | 69.2 ± 7.9 |
| Body mass index ($kg/m^2$) , Mean ± SD | 27.0 ± 3.9 |
| TC/HDL ratio, Mean ± SD | 4.5 ± 1.3 |
| Systolic blood pressure (mmHg) , Mean ± SD | 144 ± 21 |
| Diastolic blood pressure (mmHg) , Mean ± SD | 77 ± 11 |
| Smoking never, N(%) | 1,765 (32%) |
| past, N(%) | 2,702 (48.9%) |
| current, N(%) | 1,053 (19.1%) |
| Use of diuretics (C03†), N(%) | 604 (10.9%) |
| Use of beta-blocking agents (C07†), N(%) | 890 (16.0%) |
| Use of renin-angiotensin agents (C09†), N(%) | 634 (11.4%) |
| Use of lipid-lowering drugs (C10†), N(%) | 662 (11.9%) |
| Coronary heart disease, N(%) | 449 (8.2%) |
| Heart failure, N(%) | 137 (2.5%) |
| Diabetes mellitus, N(%) | 628 (11.7%) |
| Heart rate (bpm), Mean ± SD | 69 ± 11 |
| QTc interval (ms), Mean ± SD | 423 ± 21 |
| QRS duration in (ms), Mean ± SD | 99 ± 17 |
| JT interval in (ms), Mean ± SD | 308 ± 28 |
| Cornell index (µV), Mean ± SD | 1,456 ± 578 |

| | |
|---|---|
| 12-lead sum (µV), Mean ± SD | 14,228 ± 3,113 |
| Sokolow-Lyon index (µV), Mean ± SD | 2,254 ± 695 |
| QRS-T angle (°), Median ($1^{st}$ ; $3^{rd}$ quartile) | 58 (40; 82) |
| Frontal T-axis deviation (°), Median ($1^{st}$ ; $3^{rd}$ quartile) | 12 (6; 21) |
| Horizontal T-axis deviation (°), Median ($1^{st}$ ; $3^{rd}$ quartile) | 15 (7; 27) |
| Spatial J amplitude (µV), Median ($1^{st}$ ; $3^{rd}$ quartile) | 39 (26; 55) |
| Max. spatial T amplitude (µV), Median ($1^{st}$ ; $3^{rd}$ quartile) | 354 (267; 449) |
| T-loop width (°), Median ($1^{st}$ ; $3^{rd}$ quartile) | 23 (15; 35) |
| T amplitude in aVR (µV), Mean ± SD | -193 ± 96 |
| STVqt, Median ($1^{st}$ ; $3^{rd}$ quartile) | 1.98 (1.26; 3.34) |
| QTVI, Mean ± SD | -0.94 ± 0.84 |
| SDNNc, Median ($1^{st}$ ; $3^{rd}$ quartile) | 18.7 (12.3; 29.5) |
| RMSSDc, Median ($1^{st}$ ; $3^{rd}$ quartile) | 20.5 (14.2; 31.4) |
| ST depression, N(%) | 374 (6.7%) |
| T wave inversion, N(%) | 381 (6.9%) |
| Early repolarization, N(%) | 16 (0.3%) |
| Right bundle branch block, N(%) | 155 (2.8%) |
| Left bundle branch block, N(%) | 92 (1.7%) |
| One or more PVCs on the ECG, N(%) | 325 (5.8%) |

Abbreviations: bpm: beats per minute; mmHg: millimeter of mercury; ms: millisecond; µV: microvolt; N : number of participants; PVCs: premature ventricular contractions; Q: quartile; QTc: heart-rate corrected QT interval; QTVI: QT variability index; RMSSDc: heart-rate corrected root mean square of successive RR interval differences. SD: standard deviation; SCD: sudden cardiac death; SDNNc: heart-rate corrected standard deviation of normal-to-normal RR intervals; STVqt: short-term variability of the QT interval; TC/HDL ratio: total cholesterol / high-density lipoprotein cholesterol ratio.
†According to the Anatomical Therapeutic Chemical (ATC) codes.

**Table 2. Subdistribution hazard ratios for SCD with 95% confidence intervals**

| Variable (units) | basic model SHR (95%CI) | combined model SHR (95%CI) |
|---|---|---|
| Age (years) | **1.08 (1.06-1.10)¶** | **1.06 (1.04-1.09)¶** |
| Male sex | **2.00 (1.37-2.94)¶** | **1.71 (1.17-2.51)‡** |
| TC/HDL ratio | 1.10 (0.98-1.23) | 1.10 (0.98-1.23) |
| Smoking: never | 1 (reference) | 1 (reference) |
| past | 1.13 (0.76-1.69) | 1.16 (0.78-1.74) |
| current | **2.08 (1.26-3.44)§** | **1.99 (1.19-3.32)‡** |
| Diabetes mellitus | **2.02 (1.38-2.97)¶** | **1.88 (1.28-2.77)§** |
| Use of RAS blockers (ATC C09) | **1.61 (1.06-2.45)†** | 1.27 (0.81-1.98) |
| Heart rate (10bpm) | | 1.14 (0.99-1.32) |
| Sokolow-Lyon index (100µV) | | 1.02 (1.00-1.05) |
| Spatial J Amplitude (10µV) | | 1.03 (0.98-1.09) |
| T aVR (10µV) | | **1.03 (1.01-1.05)‡** |
| Right bundle branch block | | **2.92 (1.52-5.61)‡** |
| SDNNc (10 milliseconds) | | **1.06 (1.02-1.10)§** |

Subdistribution hazard ratios were calculated according to Fine and Gray. Abbreviations: ATC: Anatomical Therapeutic Chemical code; bpm: beats per minute; CI: confidence interval; HR: hazard ratio; µV: millivolt; PVCs: premature ventricular complexes; RAS blockers: renin-angiotensin system blockers; SCD: sudden cardiac death; SHR: subdistribution hazard ratio; TC/HDL ratio: total cholesterol/high-density lipoprotein cholesterol ratio.

†p-value <0.05; ‡p < 0.01; §p< 0.005; ¶p <0.001

**Discussion**

In the present study, we considered a large number of ECG markers previously associated with cardiac mortality or SCD. We created a basic model containing age, sex, total/HDL cholesterol ratio, smoking, diabetes mellitus, and use of agents acting on the renin-angiotensin system and a combined model with the additional ECG markers heart rate, Sokolow-Lyon index, spatial J amplitude, T amplitude in lead aVR, right bundle branch block, and heart-rate corrected SDNN. The Model with the additional ECG markers had a significantly higher bootstrapped C-statistic than the basic model. The majority of previous studies analyzed ECG markers individually, although a number

of previous studies have assessed risk models with a limited number (<6) of ECG or VCG markers combined, while adjusting for clinical risk factors.[8,9,21,22]

Some ECG parameters that were selected or eliminated merit additional explanation. First, a previous publication from the Rotterdam Study analyzed the association of uncorrected HRV with cardiac mortality,[23] and reported that both high and low HRV were associated with increased cardiac mortality risk. In this analysis, we found that only an increase in SDNNc was associated with SCD. It is possible that the previously reported U-shaped association of HRV with SCD was caused by residual confounding by heart rate, which has a strong inverse association with HRV.[24] Second, a previous study reported that isolated RBBB is a risk factor for cardiovascular morbidity and mortality.[25] The selection of RBBB in our study in the combined model confirmed this finding. Third, early repolarization has been shown to be a promising risk factor, [26] but was not selected for the prediction model, and in only 26% of the bootstraps. This is probably due to the fact that early repolarization was much rarer in the current cohort of elderly persons than in a cohort of younger people.[27] Fourth, the QTc interval is a known risk factor for SCD in the Rotterdam Study and other study populations,[7,26] and is important in drug-safety research,[28] but was eliminated during variable selection. It is possible that the predictive value of QTc prolongation for SCD was limited in this study because the most common underlying cause of SCD in the general population is CHD,[20] whereas QTc prolongation is associated with drug-induced arrhythmias like torsade de pointes, which are relatively uncommon in the general population.[5] Fifth, ace-inhibitors were selected for the basic model, while ace-inhibitors have been associated with a reduction in SCD.[29] We think that the association of ace-inhibitors with SCD is probably based on confounding by indication. Sixth, QTVI and STVqt, markers of QT variability,[30] were not selected for the final model, although found to be promising in the literature. A previous study showed that QT variability is a risk factor only in women, and it is possible that using both men and women in this cohort lead to the elimination of QTVI and STVqt.[31]

The sensitivity analysis showed results similar to the main analysis. Additionally including prevalent CHD in the list of candidate variables resulted in a model with the same variables as the main analysis, except for the inclusion of prevalent CHD and the elimination of spatial J amplitude. The C statistic of the combined model with CHD was 0.79, versus 0.78 without CHD. More importantly, the ECG markers significantly improved the models' performance in both the main analysis and the sensitivity analysis.

This study has a number of strengths. The Rotterdam Study is a cohort of community-dwelling elderly representative of the general older population.[12] ECG markers were based on standard 12-lead ECGs, which are commonly used in clinical practice. Inter- and intra-observer

variability was reduced by calculating all ECG markers systematically and automatically with MEANS, a program that has been evaluated extensively.[32] We are the first to use competing risk analysis for a combination of ECG markers and clinical risk factors. The SHRs from the competing risk model take into account that most people die of other causes than SCD.[18]

A limitation of this study was the low number of SCD cases. Therefore, we could not create a prediction model for SCD for men and women separately. Furthermore, both witnessed and unwitnessed SCD cases were included in the definition of SCD and the information on symptoms prior to death and the timing of death was incomplete in unwitnessed cases. This could have increased the risk of including deaths due to other causes,[17] resulting in a more heterogeneous SCD phenotype. However, since it is likely that this heterogeneity would underestimate the observed effects of the included parameters, its contribution would be minor / small it is consequently conservative. Another limitation of this study is that a family history of SCD is often considered to represent a risk factor for SCD,[33,34] but information on such data was unavailable to us. Finally, our prediction model is based on an elderly population. As the risk factors for SCD and the underlying causes of SCD are probably age-dependent, this prediction model is probably not valid for a younger population. This is illustrated by the fact that early repolarization was rare in this population, while it has been shown to be an important risk factor in a younger population.[27] We were not able to do external validation and calibration of our model.

In conclusion, we report here that a combination of seven ECG markers improves the discrimination of a model with basic cardiovascular risk factors. Our findings strongly suggest that future research on novel ECG markers should focus on the additional value of combinations of ECG markers, rather than studying ECG markers individually.

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**Supplementary Methods**

*QT variability*

QT variability was assessed with two variables: short-term variability of the QT interval (STVqt) and the QT variability index (QTVI).[1] In order to measure STVqt and QTVI, all QT intervals on an ECG were measured automatically using fiducial segment averaging (FSA):[2] First, MEANS determines the locations of the individual QRS complexes on the ECG and initial fiducial points (onset of QRS complex and end of T wave), and computes a detection function consisting of the root-mean-square ECG signal. Second, the fiducial point in each individual beat is shifted in an iterative procedure until maximum correlation is achieved between a small signal segment of the detection function around this fiducial point and the average of the segments around the fiducial points of the remaining complexes. The amount of shifting is retained and constitutes the individual beat variation in the fiducial point estimate. Finally, the QT interval for each beat is calculated taking into account the shifts of the initial QRS onset and T end. MEANS automatically detects ectopic beats, and excludes them from further processing. To safeguard against signal segments with excessive noise or baseline wander, an additional test is applied after each FSA iteration. If the averaged absolute amplitudes of the difference between the ST-T wave of an individual beat and the averaged ST-T wave of the remaining beats is larger than a preset value, the beat is discarded and the iteration process is repeated for the remaining beats. QT intervals of premature ventricular complexes and of beats immediately before and after these beats were not used.

*Imputation*

Before imputation, we set values more extreme than the 1$^{st}$ or 99$^{th}$ percentile of the continuous variables to the value of the 1$^{st}$ or 99$^{th}$ percentile. Missing values were imputed with ten-times multiple imputation. If reported in de literature, non-linearity was assessed by adding a quadratic term to an age-and sex adjusted model. If this term was significant, it was added in all models. SDNNc and RMSSDc had 12% missing values, the other covariables had less than 10% missing values. The proportional hazards assumption was assessed by evaluating the Schoenfeld residuals over follow-up time. We used R (R Foundation for Statistical Computing, Vienna, Austria) for all analyses.

**References for supplementary methods**

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**Supplementary Table 1. Candidate electrocardiographic variables**

| Name | Definition | Unit | Reference for association with cardiac mortality or sudden cardiac death |
|---|---|---|---|
| QTc interval[1] | Heart rate-corrected QT interval. Fridericia's correction formula was used: $QTc = \frac{QT(milliseconds)}{\sqrt[3]{RR(seconds)}}$ | milliseconds | Straus SM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MC, Stricker BH, Witteman JC. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. J Am Coll Cardiol Jan 17 2006;47:362-367. |
| Heart rate[2] | 60,000/RR interval in milliseconds | beats per minute | Chang HY, Yin WH, Lo LW, et al. The utilization of twelve-lead electrocardiography for predicting sudden cardiac death after heart transplantation. Int J Cardiol 2013;168:2665-2672. |
| QRS duration[3] | Interval from start to end of QRS complex | milliseconds | Aro AL, Huikuri HV. Electrocardiographic predictors of sudden cardiac death from a large Finnish general population cohort. J Electrocardiol 2013;46:434-438. |
| JT interval[2] | Interval from J point (end of QRS complex) to end of T wave | milliseconds | Chang HY, Yin WH, Lo LW, et al. The utilization of twelve-lead electrocardiography for predicting sudden cardiac death after heart transplantation. Int J Cardiol 2013;168:2665-2672. |
| Sokolow-Lyon index[3] | S in V1 + R in V5 or V6 (whichever is larger) | microvolts | Aro AL, Huikuri HV. Electrocardiographic predictors of sudden cardiac death from a large Finnish general population cohort. J Electrocardiol 2013;46:434-438. |
| 12-lead sum[4] | Sum of top-top deflections in all 12 ECG leads | microvolts | Ostman-Smith I, Wisten A, Nylander E, Bratt EL, Granelli A, Oulhaj A, Ljungstrom E. Electrocardiographic amplitudes: a new risk factor for sudden death in hypertrophic cardiomyopathy. Eur Heart J Feb 2010;31:439-449. |

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|---|---|---|---|
| Cornell index[5] | S in V3 + R in aVL | microvolts | Schroder K, Wegscheider K, Wenger NK, Vettorazzi E, Schroder R. Resting electrocardiogram predicts mortality in postmenopausal women with coronary heart disease or with risk factors for coronary heart disease. Eur J Prev Cardiol Jun 29 2012;21:749-757. |
| Spatial QRS-T angle[6, 7] | Spatial angle between the QRS axis and the T axis | degrees | Kardys I, Kors JA, van der Meer IM, Hofman A, van der Kuip DA, Witteman JC. Spatial QRS-T angle predicts cardiac death in a general population. Eur Heart J Jul 2003;24:1357-1364. |
| Frontal T-axis deviation[8, 9] | Absolute difference between 45 degrees and the T-wave axis in the frontal plane | degrees | Kors JA, de Bruyne MC, Hoes AW, van Herpen G, Hofman A, van Bemmel JH, Grobbee DE. T axis as an indicator of risk of cardiac events in elderly people. Lancet Aug 22 1998;352:601-605. |
| Horizontal T-axis deviation[8, 9] | Absolute difference between 45 degrees and the T-wave axis in the horizontal plane | degrees | Kors JA, de Bruyne MC, Hoes AW, van Herpen G, Hofman A, van Bemmel JH, Grobbee DE. T axis as an indicator of risk of cardiac events in elderly people. Lancet Aug 22 1998;352:601-605. |
| Spatial J amplitude[9] | Spatial amplitude at the J point | microvolts | Kors JA, Rijnbeek P, Van Herpen G, Keulers PP, Hofman A, Witteman JC. Spatial repolarization parameters for predicting cardiac death in the elderly. J Electrocardiol 2004;37 Suppl:198-200. |
| Max. spatial T amplitude[9] | Maximum spatial amplitude of the T wave | microvolts | Kors JA, Rijnbeek P, Van Herpen G, Keulers PP, Hofman A, Witteman JC. Spatial repolarization parameters for predicting cardiac death in the elderly. J Electrocardiol 2004;37 Suppl:198-200. |
| T-loop width[9] | Spatial angle between the axes of the first and the second part of the T loop | degrees | Kors JA, Rijnbeek P, Van Herpen G, Keulers PP, Hofman A, Witteman JC. Spatial repolarization parameters for predicting cardiac death in the elderly. J Electrocardiol 2004;37 Suppl:198-200. |

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|---|---|---|---|
| T amplitude in aVR[10] | Amplitude of the T wave in lead aVR | microvolts | Rautaharju PM, Zhang ZM, Warren J, Gregg RE, Haisty WK, Kucharska-Newton AM, Rosamond WD, Soliman EZ. Electrocardiographic predictors of coronary heart disease and sudden cardiac deaths in men and women free from cardiovascular disease in the Atherosclerosis Risk in communities study. J Am Heart Assoc 2013;2. |
| Short-term QT variability (STVqt)[11] | $\sum_{i=1}^{n} \frac{\lvert QT_{i+1} - QT_i \rvert}{n2}$ | milliseconds | Niemeijer MN, van den Berg ME, Eijgelsheim M, van Herpen G, Stricker BH, Kors JA, Rijnbeek PR. Short-term QT variability markers for the prediction of ventricular arrhythmias and sudden cardiac death: a systematic review. Heart Dec 2014;100:1831-1836. |
| QT variability index (QTVI)[11] | $\log_{10}\left[\frac{(\mathrm{QT_v}/\mathrm{QT_m^2})}{(\mathrm{HR_v}/\mathrm{HR_m^2})}\right]$ | - | Niemeijer MN, van den Berg ME, Eijgelsheim M, van Herpen G, Stricker BH, Kors JA, Rijnbeek PR. Short-term QT variability markers for the prediction of ventricular arrhythmias and sudden cardiac death: a systematic review. Heart Dec 2014;100:1831-1836. |
| SDNNc[12] | Standard deviation of normal-to-normal RR intervals, adjusted for heart rate | milliseconds | de Bruyne MC, Kors JA, Hoes AW, Klootwijk P, Dekker JM, Hofman A, van Bemmel JH, Grobbee DE. Both decreased and increased heart rate variability on the standard 10-second electrocardiogram predict cardiac mortality in the elderly: the Rotterdam Study. Am J Epidemiol Dec 15 1999;150:1282-1288. |
| RMSSDc[12] | Root of the mean squared differences between consecutive RR intervals, adjusted for heart rate | milliseconds | de Bruyne MC, Kors JA, Hoes AW, Klootwijk P, Dekker JM, Hofman A, van Bemmel JH, Grobbee DE. Both decreased and increased heart rate variability on the standard 10-second electrocardiogram predict cardiac mortality in the elderly: the Rotterdam Study. Am J Epidemiol Dec 15 1999;150:1282-1288. |

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|---|---|---|---|
| ST depression[13] | Minnesota code 4.1 or 4.2* | - | Laukkanen JA, Makikallio TH, Rauramaa R, Kurl S. Asymptomatic ST-segment depression during exercise testing and the risk of sudden cardiac death in middle-aged men: a population-based follow-up study. Eur Heart J Mar 2009;30:558-565. |
| T-wave inversion[15] | Minnesota code 5.1 or 5.2* | - | Laukkanen JA, Di Angelantonio E, Khan H, Kurl S, Ronkainen K, Rautaharju P. T-wave inversion, QRS duration, and QRS/T angle as electrocardiographic predictors of the risk for sudden cardiac death. Am J Cardiol Apr 1 2014;113:1178-1183. |
| Early repolarization[3] | J amplitude > 100 microvolts in at least 1 of the lateral (II, III, aVF) or inferior (I, aVL, V4-V6) leads | - | Aro AL, Huikuri HV. Electrocardiographic predictors of sudden cardiac death from a large Finnish general population cohort. J Electrocardiol 2013;46:434-438. |
| RBBB[3] | Right bundle branch block | - | Aro AL, Huikuri HV. Electrocardiographic predictors of sudden cardiac death from a large Finnish general population cohort. J Electrocardiol 2013;46:434-438. |
| LBBB[3] | Left bundle branch block | - | Aro AL, Huikuri HV. Electrocardiographic predictors of sudden cardiac death from a large Finnish general population cohort. J Electrocardiol 2013;46:434-438. |
| PVCs[16] | Presence of one or more premature ventricular complexes | - | Cheriyath P, He F, Peters I, Li X, Alagona P, Jr., Wu C, Pu M, Cascio WE, Liao D. Relation of atrial and/or ventricular premature complexes on a two-minute rhythm strip to the risk of sudden cardiac death (the Atherosclerosis Risk in Communities [ARIC] study). Am J Cardiol Jan 15 2011;107:151-155. |

# Chapter 2.6

# QT Variability is Associated with Incident Heart Failure in a Prospective Population-Based Cohort Study

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**Abstract**

*Background* QT variability reflects beat-to-beat changes of QT-interval duration on the electrocardiogram (ECG). QT variability is proposed as a risk factor for mortality in patients with heart failure (HF), but no study has assessed QT variability as a risk factor for HF itself. We aimed to study QT variability as a risk factor for HF.

*Methods* We included 8,091 participants of the Rotterdam Study, a community-dwelling cohort of middle-aged and elderly persons, from whom 17,281 standard ECGs were recorded. We analyzed the short-term variability of the QT interval (STVqt) and the QT variability index (QTVI). Hazard ratios (HRs) and 95% confidence intervals (CIs) of the association between QT variability and HF were estimated with time-dependent Cox' proportional-hazard models. Adjustment included ECG markers and known clinical risk factors for HF. We assessed non-linearity with restricted cubic splines and tested for an interaction with sex.

*Results* A total of 787 participants developed HF. In a multivariable-adjusted model, a one standard deviation increase of STVqt was significantly associated with a higher risk of HF (HR 1.19, 95%CI 1.11-1.26). QTVI was not significantly associated with HF in a multivariable-adjusted model. Tests for non-linearity were not significant, and there was no interaction with sex.

**Conclusion:** STVqt from standard 10-second ECGs was associated with an increased risk of HF. STVqt might be indicative of the myocardial remodeling and electrical instability. QTVI was not associated with HF, possibly because it is a marker containing both QT variability and heart rate variability.

## Introduction

Heart failure (HF) is a common and disabling disease. In developed countries, between 6 and 10% of people older than 65 suffer from HF, and 60-70% of patients die within five years after diagnosis.[1,2] Early diagnosis of these risk factors is important, as prevention can delay the onset of HF and mortality due to HF.[3] The electrocardiogram (ECG) is currently a diagnostic tool for patients suspected of having HF,[4] capable of detecting several underlying causes of HF.[4] One of the main pathophysiological processes in the development of HF is ventricular remodeling.[5] There is currently no established ECG marker for ventricular remodeling, but variability of QT-interval duration has been suggested as a marker for this process.[6]

It is hypothesized that QT variability is a marker of increased repolarization instability in cardiomyopathies and of cardiac tissue remodeling.[7] Previous studies have shown that for HF patients or people with mild to moderate left ventricular dysfunction, an increased QT variability is a risk factor for all-cause mortality,[8,9] cardiovascular mortality[8,10], ventricular fibrillation, ventricular tachycardia[6,11], and sudden cardiac death.[9,12] Two case-control studies found that QT variability was significantly higher in patients with heart failure than in healthy controls,[10,13] while one case-control study reported that QT variability was only significantly increased in HF during ventricular pacing.[14] However, all those studies were relatively small (the smallest had 29, the largest 714 participants, of which 533 HF cases), all used a cross-sectional design and no study to date has assessed whether QT variability is prospectively associated with HF in the general population.

Therefore, we analyzed the association of two commonly-used QT variability markers with HF in a prospective population-based cohort. We used the short-term variability of QT intervals (STVqt) and the QT variability index (QTVI).[15]

## Methods

*Research setting*

This study is based on the prospective population-based Rotterdam Study. The design and rationale of the Rotterdam Study have been described in more detail elsewhere.[16,17] In short, from 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district in Rotterdam, the Netherlands, were invited to participate in the initial cohort. A total of 7,983 individuals agreed to participate (response rate 78%). In 2000, the cohort was extended by including all inhabitants from the same district who had become 55 years or had moved into the district after the start of the initial cohort. In total, 3,011 individuals agreed to participate (response rate 67%). The cohort was additionally extended in 2006 by inviting inhabitants of the same district aged 45 years and older. In total, 3,932 individuals agreed to participate (response rate 65%). Follow-up examinations were

conducted every four to five years, with a current maximum of five center visits per participant. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO" (Population Studies Act: Rotterdam Study).

*Study population and selection of ECGs*

We included all participants who provided informed consent for follow-up monitoring, and with at least one ECG available. We excluded ECGs on which we detected atrial fibrillation, atrial flutter, pacemaker rhythm, left bundle branch block, right bundle branch block and third degree atrioventricular block, because these interfere with accurate measurement of QT variability. We also excluded ECGs with excessive noise, excessive baseline wandering, or with premature ventricular or supraventricular complexes.

*Electrocardiography*

Standard 10-second, 12-lead ECGs were recorded at rest with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florance, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS) program, which has been described previously and has been validated and applied extensively.[18-22] QT intervals were measured automatically using fiducial segment averaging (FSA).[23] This algorithm is explained more extensively in the supplementary methods. Briefly, MEANS determines the initial fiducial points (onset of QRS complex and end of T wave) and a detection function consisting of the root-mean-square ECG signal,[24] is computed. Second, the fiducial point in each beat is shifted until maximum correlation is achieved between a small segment of the detection function around this fiducial point and the average around the fiducial points of the other complexes. The amount of shifting is retained and the QT interval for each beat is calculated taking into account the shifts of the initial QRS onset and T end.

*QT variability markers*

We assessed two commonly used QT variability markers: the short-term variability of QT intervals (STVqt)[25] and the QT variability index (QTVI).[7] The formulas of these markers are shown in Table 1. There are two differences between STVqt and QTVI. First, STVqt is a marker of beat-to-beat differences, while QTVI is based on the variance of QT. Second, QTVI is normalized for average QT, heart rate, and heart-rate variably, while STVqt is not. On ten ECGs all QT intervals had equal duration, which would result in an infinite value of QTVI. QTVI for these ECGs was therefore set to

the lowest QTVI value that was observed in the other ECGs (QTVI = -3.33). Because of the skewed distribution of STVqt, we log-transformed this variable in all analyses. In order to deal with those cases in which STVqt was zero, we added 1 to STVqt before transformation, ln(STVqt + 1).

*Potential covariables*

The following ECG variables were considered as covariable: heart rate, (average) QT interval, and Sokolow-Lyon index. For STVqt we additionally considered heart-rate variability and heart-rate variability squared, because of a previous study that showed that both a high and low heart-rate variability is a risk factor for total mortality and cardiovascular mortality.[26,27] Heart-rate variability was expressed as the standard deviation of normal-to-normal RR intervals (SDNN). Heart rate variability was not included in the models for QTVI, because heart-rate variability is already included in the denominator of QTVI.

We considered the following non-ECG covariables, which reflect common HF risk factors: age, sex, smoking status, body-mass index (BMI), systolic blood pressure, diastolic blood pressure, history of coronary heart disease, and history of diabetes mellitus.[28] The covariables for each participant were determined at the date of the ECG recording. Smoking status was assessed during a home interview: participants were classified as never, former, or current smokers. Weight and height were measured at the study center. Blood pressure was measured twice in a sitting position at the right upper arm. The average of the two measurements was used. A history of coronary heart disease was defined as myocardial infarction or a percutaneous or surgical coronary revascularization procedure.[29] Diabetes mellitus was defined as a fasting serum glucose level greater than or equal to 7.0 mmol/L or, if fasting glucose was not available, a non-fasting serum glucose level greater than or equal to 11.1 mmol/L, use of glucose-lowering medication, or a previous diagnosis of diabetes mellitus.[30]

*Outcome definition*

Methods on event adjudication of incident HF for the Rotterdam Study have been described previously.[2,29] Adjudication of HF cases is described extensively in the supplementary methods. Briefly, we used automated linkage with files from general practitioners to allow for continuous monitoring, and the diagnosis of HF was based on criteria from the European Society of Cardiology.[4] Follow-up for heart failure ended at the first occurrence of heart failure, death, or end of the study period.

**Table 1. Formulas of QT interval variability markers**

| Variable | Full name | Formula |
|---|---|---|
| QTVI | QT variability index | $\log_{10}\left[\frac{(QT_v/QT_m^2)}{(HR_v/HR_m^2)}\right]$ |
| STVqt | Short-term QT variability | $\sum_{i=1}^{n}\frac{\lvert QT_{i+1}-QT_i\rvert}{n\sqrt{2}}$ |

Abbreviations: $HR_m$: mean heart rate; $HR_{var}$: variance of heart rate; n: number of beats used for calculation; $QT_i$: QT interval of beat i; $QT_m$: mean QT interval; $QT_{var}$: variance of QT interval

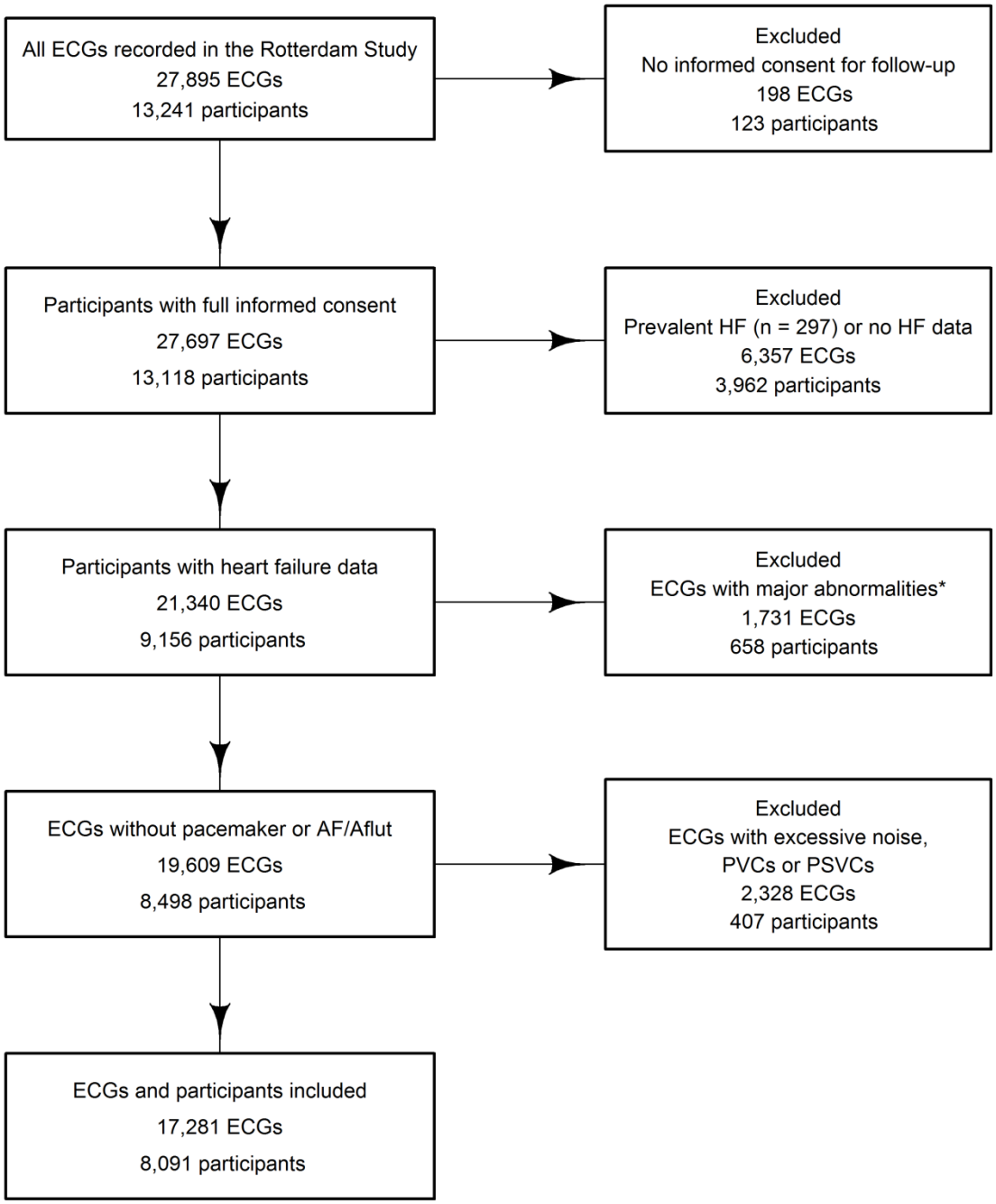
*Statistical analyses*

The association of QT variability with HF was assessed using Cox' proportional hazards models. All determinants were used as time-dependent covariables. The proportional hazards assumption was tested by assessing the slope of the time-dependent beta, and this was not significant.

In the main analyses, we used STVqt and QTVI as continuous variables. We calculated a crude hazard ratio, and then two models with multivariable adjustment. In Model 1 we adjusted for age, sex, and study cohort. In Model 2, additional covariables were added if they changed the beta estimate of either STVqt or QTVI by more than 10%. We considered the possibility of a non-linear association by adding a quadratic term ($STVqt^2$ or $QTVI^2$). We additionally used QTVI and STVqt divided into quartiles to illustrate the association of QT variability with HF. The quartile limits are specified in Supplementary Table 1. Missing values were imputed using 10-times multiple imputation. All covariables had less than 6% missing values.

The analysis were performed with R Statistical Software version 3.0.1 (Foundation for Statistical Computing, Vienna, Austria).[31] The time-dependent Cox' proportional hazard regressions were done with the R package *survival*.[32] Non-linearity was assessed with the R package *rms*.[33] For multiple imputation and subsequent pooling we used the function *aregImpute* and *fit.mult.impute* from the *Hmisc* package.[34] Results were shown as hazard ratios (HRs) for each QT-variability marker with 95% confidence intervals (CIs). Two-sided p-values less than 0.05 were considered statistically significant.

**Figure 1. Flowchart of in-and exclusion criteria resulting in the study population**




*Major abnormalities include atrial fibrillation, atrial flutter, third degree atrioventricular block, left bundle branch block and right bundle branch block.

Abbreviations: ECG: Electrocardiogram; HF: heart failure; AF: atrial fibrillation; PVC: premature ventricular complex; PSVC: premature supraventricular complex.

**Results**

*Population characteristics*

The flowchart of the population selection is shown in Figure 1. There were 8,091 participants with 17,281 ECGs available after applying the inclusion and exclusion criteria. Baseline characteristics of the study population are shown in Table 2. The study population had 59% women, and the mean age was 67.7 years. The median follow-up duration was 8.8 years. During follow-up, 787 participants were diagnosed with HF.

*QT variability and HF*

Table 3 shows that STVqt as a continuous variable was a significant risk factor for HF in the crude model, in Model 1 with adjustment for age, sex and Rotterdam study cohort, and in Model 2 with additional adjustment for other ECG parameters, coronary heart disease, systolic blood pressure and diabetes mellitus. The risk of HF increased per standard deviation of ln(STVqt + 1) with a HR of 1.19 (95%CI 1.11 - 1.26), p-value = $1.07 \times 10^{-7}$. In the categorical analysis, which was also fully adjusted, higher quartiles of STVqt had higher risk estimates for HF, but only the fourth quartile carried a significantly increased risk, with a HR of 1.52 (1.23 – 1.88), p-value = $1.01 \times 10^{-4}$. Figure 2 illustrates the analysis with quartiles, Supplementary Table 1 shows the HRs and 95% CIs.

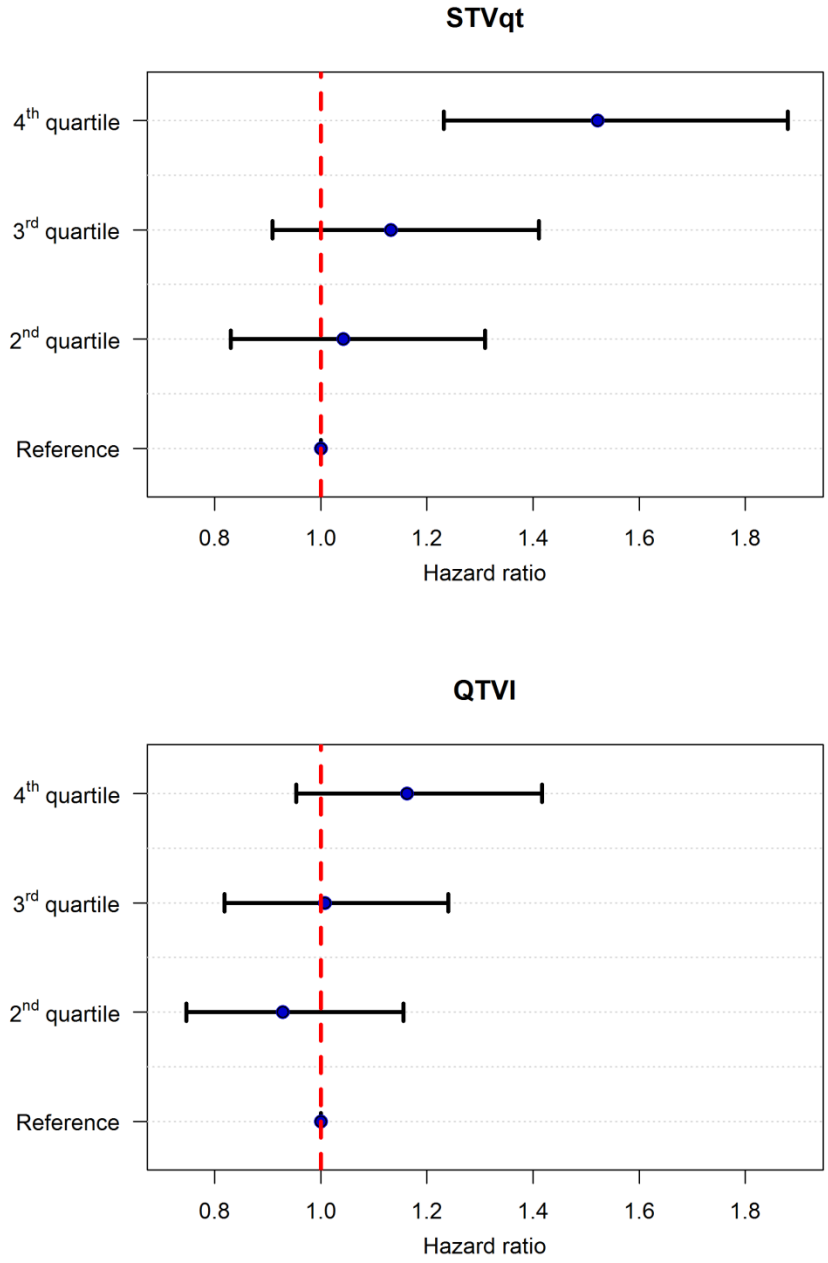
QTVI as a continuous variable was a significant risk factor in the crude model and in Model 1, but was not significantly associated with HF in the fully adjusted model. In the categorical analysis, none of the quartiles carried a significantly increased risk of HF, shown in Figure 2. Quadratic terms of STVqt and QTVI were not significant, and there was no significant interaction of STVqt or QTVI with sex.

**Table 2. Baseline characteristics of the study population**

| | |
|---|---|
| Participants, N | 8,091 |
| Women, N (%) | 4,798 (59%) |
| Follow-up time in years, median (IQR) | 8.7 (6.0; 15.1) |
| Age in years, mean (SD) | 67.8 (8.3) |
| BMI in kg/m$^2$, mean (SD) | 26.7 (3.9) |
| Smoking | |
| Never, N (%) | 2,668 (34%) |
| Former, N (%) | 3,472 (44%) |
| Current, N (%) | 1,780 (22%) |
| Hypertension, N (%) | 4,818 (60%) |
| Systolic blood pressure, mean (SD) | 140.3 (21.7) |
| Diastolic blood pressure, mean (SD) | 75.7 (11.5) |
| Coronary heart disease, N (%) | 506 (6%) |
| Diabetes mellitus, N (%) | 795 (10%) |
| Number of available ECGs, N | 17,281 |
| Participants with one/two/three/four ECGs, N | 2,724/2,761/1,389/1,217 |
| Participants with one/two/three/four ECGs, % | 34%/34%/17%/15% |
| Heart rate in bpm, mean (SD) | 69.7 (11.6) |
| QT interval in ms, mean (SD) | 401 (29) |
| QTc interval in ms, mean (SD) | 429 (23) |
| Sokolow-Lyon index in mV, mean (SD) | 2,325 (723) |
| SDNN, median (IQR) | 15.5 (9.8; 25.3) |
| STVqt, median (IQR) | 2.0 (1.3; 3.3) |
| QTVI, median (IQR) | -0.9 (-1.4; -0.3) |

Abbreviations: BMI: body-mass index; bpm: beats per minute; ECG: electrocardiogram; IQR: interquartile range; ms: milliseconds; N: number of participants; QTc: heart-rate corrected QT interval (using Bazett's formula); QTVI: QT variability index; SD: standard deviation; SDNN: standard deviation of normal-to-normal RR intervals; STVqt: short-term QT variability.

**Figure 2. Estimates and 95% confidence intervals for of STVqt and QTVI in quartiles for risk of heart failure**




The blue dot represents the estimate, the whiskers represent the upper and lower limit of the 95% confidence interval. The estimates are adjusted for age, sex, cohort, heart rate, heart-rate variability (only for STVqt), Sokolow-Lyon index, QT interval, coronary heart disease and diabetes mellitus. Abbreviations: STVqt: short-term variability of QT intervals; QTVI: QT variability index.

**Table 3. Association between QT variability and incident heart failure**

| | Crude | Model 1 | Model 2 |
|---|---|---|---|
| ln(STVqt + 1) per SD | **1.34 (1.27 - 1.42)** | **1.21 (1.14 - 1.29)** | **1.19 (1.11 - 1.26)** |
| QTVI per SD* | **1.23 (1.15 - 1.32)** | **1.10 (1.03 - 1.18)** | 1.07 (1.00 - 1.14) |

All results are shown as Hazard ratios (95% Confidence intervals). Model 1 is adjusted for age, sex and cohort. Model 2 is adjusted for age, sex, cohort, heart rate, heart-rate variability, Sokolow-Lyon index, QT interval, coronary heart disease and diabetes mellitus. *Not adjusted for heart-rate variability. Abbreviations: QTVI: QT variability index; SD: standard deviation; STVqt: short-term variability of QT intervals. Bold indicates p-value below 0.05

**Discussion**

We report that a higher STVqt is associated with an increased risk of HF, but QTVI is not associated with HF after adjustment for other ECG parameters and well-known HF risk factors.

Our study is the first study to analyze the association of QT variability with HF in a large population-based setting. Previous studies focused on QT variability as a risk factor for mortality or sudden death within groups of patients with HF or mild-to-moderate left ventricular systolic dysfunction.[6,8-12] Three previous case-control studies compared HF patients with healthy controls. The first study looked into the standard deviation of QT intervals (SDqt),[14] a marker similar to STVqt, and found a significant difference between 20 HF patients and 9 healthy controls, but only during ventricular pacing (80 and 100 beats per minute). During pacing, SDqt was approximately 5 milliseconds (ms) in the healthy control group, but 11 ms in the HF group. In the second study, the average QTVI was lower (-0.16) in a group of healthy controls (n = 146) than in a group of patients with chronic heart failure (-1.40, n = 44), a statistically significant difference.[13] The third study found that QTVI as measured in 3-minute recordings was significantly higher in patients with HF compared with in healthy controls: -1.56, 95% CI -2.61 to -0.42 versus -2.23, 95% CI -3.39 to -1.05.[10] The results of these three papers are in line with our study. The estimates of risk of HF were higher in the third and fourth quartile of STVqt and QTVI, and STVqt was a significant risk factor for HF, even after adjustment for clinical covariables. The results for QTVI were significant without adjustment for clinical covariables, confirming the three aforementioned case-control studies, which also did not adjust or matched their results.[10,13,14]

Based on the differences in the formulas of STVqt and QTVI, a number of explanations are possible for the fact that we found significant results for STVqt, but not for QTVI. First, STVqt measures beat-to-beat changes and takes the order of the beats into account while QTVI uses the

variance of QT intervals, which is not affected by beat order. A second possible explanation is that QTVI is a composite marker: the numerator of the QTVI formula contains QT variability, while the denominator contains heart-rate variability. This means that QTVI is increased in two situations: when QT variability is high, and/or when heart-rate variability is low.[10] A previous study in the Rotterdam Study population found that both high and low heart-rate variability are associated with cardiac mortality.[26] Participants with a high heart-rate variability and consequently a high risk of heart disease could, as a result of the QTVI formula, have a high risk of HF with a low QTVI. This would dilute the effect of those participants who have a higher risk of HF and an increased QTVI due to a higher QT variability. This effect does not exist for STVqt, because we adjusted for heart-rate variability by using heart-rate variability in the model.

HF is usually caused by abnormalities in cardiac structure and function resulting from myocardial infarction, hypertension, or cardiomyopathies.[1,4] QT variability has previously been associated with both hypertrophic[35,36] and dilated[7,13,37] cardiomyopathies. As this study shows that STVqt is a risk factor for heart failure, future studies could focus on the interplay between STVqt, markers of cardiac pathology, and risk of HF.

This study has a number of strengths. First, it is a population-based study of community-dwelling middle-aged and elderly persons and data was collected prospectively. The diagnosis of HF was based on criteria from the European Society of Cardiology.[4] QT variability was calculated automatically, thereby preventing observer bias in ECG assessment. There are also some limitations that need to be considered. First, most previous studies used longer ECG recordings than the standard 10-second ECGs.[15] While this could be a limitation, a previous study reported that QTVI as measured on 10-second ECGs compared to QTVI measured on 3-minute ECGs had a high correlation, with a Pearson correlation coefficient of 0.81. Furthermore, our results do show that QT variability measured on 10-second ECGs is associated with HF. Second, data on echocardiographic parameters at the time of HF diagnosis were not systematically collected, therefore we could not discern between HF with reduced ejection fraction or HF with preserved ejection fraction.[38] Third, we did not assess the effect of atrial fibrillation on the relation between QT variability and HF, as individuals with atrial fibrillation at the time of ECG measurement were excluded.

In conclusion, we show an association of STVqt, but not of QTVI, with a higher risk of HF in the general middle-aged and elderly population. Our results show that STVqt measured on 10-second ECGs is a promising marker for HF.

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**Supplementary Methods**

*Fiducial segment averaging (FSA)*

The main goal of FSA is to measure the QT interval of each beat separately, while still using the information from the other beats, in order to reduce the effect of noise and to increase accuracy. FSA works as follows: first, MEANS determines the locations of the individual QRS complexes on the ECG and initial fiducial points (onset of QRS complex and end of T wave) and a detection function consisting of the root-mean-square ECG signal,[1] is computed. Second, the fiducial point in each individual beat is shifted in an iterative procedure until maximum correlation is achieved between a small signal segment of the detection function around this fiducial point and the average of the segments around the fiducial points of the remaining complexes. The amount of shifting is retained and constitutes the individual beat variation in the fiducial point estimate. Finally, the QT interval for each beat is calculated taking into account the shifts of the initial QRS onset and T end. To safeguard against signal segments with excessive noise or baseline wander, an additional test is applied after each FSA iteration. If the averaged absolute amplitudes of the difference between the ST-T wave of an individual beat and the averaged ST-T wave of the remaining beats is larger than a preset value, the beat is discarded and the iteration process is repeated for the remaining beats.

*Adjudication of HF cases*

Automated linkage with files from general practitioners allowed for continuous monitoring of participants of the Rotterdam Study for the occurrence of HF during follow-up. The date of incident HF was defined as the day of the first occurrence of symptoms suggestive of HF, obtained from the medical records, or if information on onset of symptoms was missing, the day of receipt of a first prescription for a loop diuretic or an ACE-inhibitor in someone with confirmed HF, whichever came first. The diagnosis of HF was classified as definite, probable, possible, or unlikely.[2,3] For our analyses, we used only definite and probable cases. Definite HF was defined as the presence of at least one of the typical signs or symptoms of HF, such as breathlessness at rest, breathlessness during exertion, ankle edema, and pulmonary crepitations combined with confirmation by objective evidence of cardiac dysfunction by chest X-ray or echocardiography. This definition is in accordance with the criteria of the European Society of Cardiology.[4] To be classed as definite HF, the diagnosis of a medical specialist was necessary. HF was classified as probable when at least two typical symptoms suggestive of HF was present, and at least 1 of the following criteria: a history of cardiovascular disease (e.g. myocardial infarction or hypertension), response to treatment for HF, or objective evidence of cardiac dysfunction, while symptoms could not be attributed to another underlying disease, such as chronic obstructive pulmonary disease.

**References of the supplementary methods**


**1.** Lux RL, Sower CT, Allen N, Etheridge SP, Tristani-Firouzi M, Saarel EV. The application of Root Mean Square Electrocardiography (RMS ECG) for the detection of acquired and congenital long QT syndrome. *PLoS ONE.* 2014;9(1):e85689.

**2.** Bleumink GS, Knetsch AM, Sturkenboom MC, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. *Eur Heart J.* Sep 2004;25(18):1614-1619.

**3.** Leening MJ, Kavousi M, Heeringa J, et al. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. *Eur J Epidemiol* Mar 2012;27(3):173-185.

**4.** Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail.* Aug 2016;18(8):891-975.

**Supplementary Table 1. QT variability in quartiles and its association with heart failure**

| **STVqt** | **Quartile limits** | **HR (95%CI)** |
|---|---|---|
| **1st quartile 1** | ≤ 1.27 | 1.00 (ref) |
| **2nd quartile** | > 1.27 and ≤ 1.98 | 1.04 (0.83 – 1.31) |
| **3rd quartile** | > 1.98 and ≤ 3.21 | 1.13 (0.91 – 1.41) |
| **4th quartile** | > 3.21 | 1.52 (1.23 – 1.88) |
| **QTVI** | **Quartile limits** | **HR (95%CI)** |
| **1st quartile 1 (ref)** | ≤ -1.39 | 1.00 (ref) |
| **2nd quartile** | > -1.39 and ≤ -0.89 | 0.93 (0.75 – 1.16) |
| **3rd quartile** | > -0.89 and ≤ -0.37 | 1.01 (0.82 – 1.24) |
| **4th quartile** | > -0.37 | 1.16 (0.95 – 1.42) |

The estimates and confidence intervals were adjusted for age, sex, cohort, heart rate, heart-rate variability (only for STVqt), Sokolow-Lyon index, QT interval, coronary heart disease and diabetes mellitus. Abbreviations: CI: confidence interval; HR: hazard ratio; STVqt: short-term variability of QT interval; QTVI: QT variability index

# Chapter 2.7

# Does Thyroid Function Affect QT Variability? A Population-Based Study

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**Abstract**

*Background* Short-term variability of QT intervals (STVqt) is a promising electrocardiographic marker for ventricular arrhythmias and sudden cardiac death (SCD). Both hypothyroidism and hyperthyroidism have been linked to ventricular arrhythmias; while higher thyroid function is associated with SCD. Our objective was to study the association between thyroid function and STVqt.

*Methods and Results* The association of thyroid function and STVqt was analyzed in a cross-sectional study using linear regression models. Thyroid function was expressed by thyroid-stimulating hormone (TSH) and free thyroxine (FT4), and by thyroid status (i.e. hypothyroidism, subclinical hypothyroidism, euthyroidism, subclinical hyperthyroidism, and hyperthyroidism). Nonlinearity was tested by adding polynomial terms of FT4. Analyses were stratified on age and sex. In sensitivity analyses we excluded users of thyroid medication, participants with anti-TPO antibodies above 35kU/mL and non-euthyroid participants.
The setting was the Rotterdam Study, a cohort of community-dwelling participants. The study population comprised 8,708 participants, 56.9% were women. The mean age was 64.5 years. FT4 had a significant quadratic association with STVqt; both low and high levels of FT4 associated with a higher STVqt. The association was significantly stronger in men than in women (p interaction FT4 0.014; FT42 0.006). After excluding users of thyroid medication, TSH was significantly associated with a higher STVqt. STVqt was not significantly different across thyroid categories (p-trend 0.41).

*Conclusions* Both high and low levels of FT4 are associated with a higher STVqt, which is stronger in men. Further studies can elucidate if the association of FT4 and SCD is mediated via STVqt.

**Introduction**

The short-term variability of QT intervals (STVqt) is a marker of QT variability.[1] QT variability reflects the beat-to-beat changes in QT interval duration on the electrocardiogram (ECG).[1-3] It has been hypothesized that a high QT variability is indicative of repolarization instability. For example, QT variability has been investigated in studies that focused on cardiomyopathies or drug use as the substrate leading to repolarization instability.[2,4] Moreover, an increase in QT variability has been associated with an increased risk of ventricular arrhythmias [5] and sudden cardiac death (SCD).[6]

Thyroid hormones (triiodothyronine ($T_3$) and its precursor, thyroxine ($T_4$)), could also affect repolarization stability. Thyroid hormones can influence repolarization indirectly via the heart rate,[7] or directly via potassium, sodium and calcium channels,[8] or by changing the expression of Ca2+ ATPase and its inhibitor Phospholamban in the sarcoplasmic reticulum.[9] The possible effect of thyroid hormones on repolarization instability is reflected by the fact that higher thyroid hormone levels are associated with an increased risk of atrial fibrillation, even in euthyroid subjects,[10] and that high free thyroxine ($FT_4$), even within the normal range, is associated with SCD.[3] On the other side of the thyroid spectrum, lack of thyroid hormone (i.e. hypothyroidism) is related to bradycardia and prolongation of the QT interval. [11] Case reports have suggested that both hyperthyroidism[12-14] and hypothyroidism[11,15-17] are associated with ventricular arrhythmias, albeit probably through different mechanisms.

Although both high thyroid function[3] and high QT variability[6] have been associated with SCD, the association of thyroid function with QT variability, and the marker STVqt, has not yet been studied. Analysis of this association would elucidate the relation of thyroid function with repolarization instability, and could provide pathophysiological insights into why higher $FT_4$ levels are associated with an increased risk of SCD. We therefore assessed the association of thyroid function with STVqt as measured on standard ECGs in a community dwelling middle-aged and elderly cohort.

**Methods**

*Research Setting*

The study is embedded in the prospective population-based Rotterdam Study. Design and rationale of the Rotterdam Study have been described in more detail elsewhere.[18,19] In short, 10,215 inhabitants aged 55 years and older from the well-defined Ommoord district in the city of Rotterdam were invited from 1990 to 1993 to participate in the initial cohort. Of the invitees, 7,983 persons agreed to participate (response rate 78%). In 2000, the cohort was extended by inviting 4,472 inhabitants of the same district who had turned 55 or who had moved into the district after the start of the initial cohort. In total, 3,011 individuals agreed to participate (response rate 67%). A second

extension of the cohort was created in 2006 by inviting 6,057 persons living in the Ommoord district aged 45 years or over, of whom 3,932 subjects were included in the study (response rate 65%). Thus, by the end of 2008, 14,926 persons were included in the Rotterdam Study. Follow-up examinations were conducted every four to five years. This study used thyroid measurements from the third examination of the first cohort and the first examination of the second and third cohorts, 11,740 participants in total. Thyroid measurements taken in the first visit of the first cohort were not used because these were measured with a different assay. No thyroid measurements were taken during the second visit of the first cohort.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". We only included participants who provided written informed consent to participate in the study, to give access to information from their attending physicians and for follow-up monitoring.

*Assessment of Thyroid Function*

Thyroid function assessment included measurement of thyroid-stimulating hormone (TSH), $FT_4$, and thyroid peroxidase antibodies (TPOAb) in serum samples stored at -80°C (electrochemiluminescence immunoassay for thyroxine, thyrotropine and thyroid peroxidase antibodies, "ECLIA", Roche, Basel, Switzerland). The cut-off values of the normal range of TSH were set to 0.4-4.0 mIU/L, while the reference range for $FT_4$ was 11-25 pmol/L, in accordance with previous studies and in line with Dutch national guidelines.[3,10,20] TPOAb levels greater than 35 kU/mL were regarded as positive, as recommended by the assay manufacturer. Euthyroidism was defined as a TSH value within the reference range. Hypothyroidism was defined by TSH > 4.0 and $FT_4$ <11, whilst subclinical hypothyroidism was defined as TSH > 4.0 and $FT_4$ within the reference range. Hyperthyroidism was defined by TSH < 0.4 and $FT_4$ > 25, while subclinical hyperthyroidism was defined as TSH < 0.4 and $FT_4$ within the reference range.

*Electrocardiography*

ECGs were recorded during the same center visit as the thyroid function measurements. Standard 12-lead 10-second ECGs were recorded during rest with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florence, Italy). We used a sampling frequency of 500 Hz and the ECGs were stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS), which has been described previously and has been validated and applied extensively.[21,22] Exclusion criteria for ECGs

were atrial fibrillation, atrial flutter, or pacemaker rhythm. We used short-term variability of QT intervals (STVqt)[1] as marker for QT variability. STVqt is defined as

$$\mathrm{STVqt} = \sum_{i=1}^{n} \frac{|QT_{i+1} - QT_i|}{n\sqrt{2}}$$

where $QT_i$ is the QT interval of beat i, and n is the total number of beats in the ECG recording.

QT intervals were measured automatically using fiducial segment averaging (FSA).[23] First, MEANS determines the locations of the individual QRS complexes on the ECG and initial fiducial points (onset of QRS complex and end of T wave) and a detection function consisting of the root-mean-square ECG signal,[24] is computed. Second, the fiducial point in each individual beat is shifted in an iterative procedure until maximum correlation is achieved between a small signal segment of the detection function around this fiducial point and the average of the segments around the fiducial points of the remaining complexes. The amount of shifting is retained and constitutes the individual beat variation in the fiducial point estimate. Finally, the QT interval for each beat is calculated taking into account the shifts of the initial QRS onset and T end. MEANS automatically detects ectopic beats, and excludes them from further processing. To safeguard against signal segments with excessive noise or baseline wander, an additional test is applied after each FSA iteration. If the averaged absolute amplitudes of the difference between the ST-T wave of an individual beat and the averaged ST-T wave of the remaining beats is larger than a preset value, the beat is discarded and the iteration process is repeated for the remaining beats.

*Covariables*

The following covariables were derived from the ECG: heart rate, heart-rate variability, (average) QT interval, and Sokolow-Lyon index. Heart-rate variability was expressed as the standard deviation of normal-to-normal RR intervals (SDNN, in milliseconds)[25] and log-transformed in the analyses to approximate a normal distribution. The Sokolow-Lyon index was defined as the sum of the voltages (in millivolt) of the S wave in lead V1 and the R wave in lead V5 or V6, whichever was larger. The following clinical covariables were used: age, sex, smoking, BMI, hypertension, coronary heart disease (CHD), and diabetes mellitus. Smoking was based on a home interview. Participants were classified into never, former, and current smokers. BMI was defined as weight/height$^2$, with weight in kg and height in meters. Blood pressure was measured twice in the sitting position on the right upper arm. The average of two measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg, or a diastolic blood pressure ≥90 mmHg, or the use of blood-pressure-lowering medication with the indication hypertension. CHD was defined as a history of a myocardial infarction (MI) or a surgical or percutaneous coronary revascularization procedure.[26] Diabetes

mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L or a non-fasting serum glucose level ≥11.1 mmol/L (if fasting serum glucose was not present), or use of glucose-lowering medication, or a previous diagnosis of diabetes mellitus.

*Statistics*

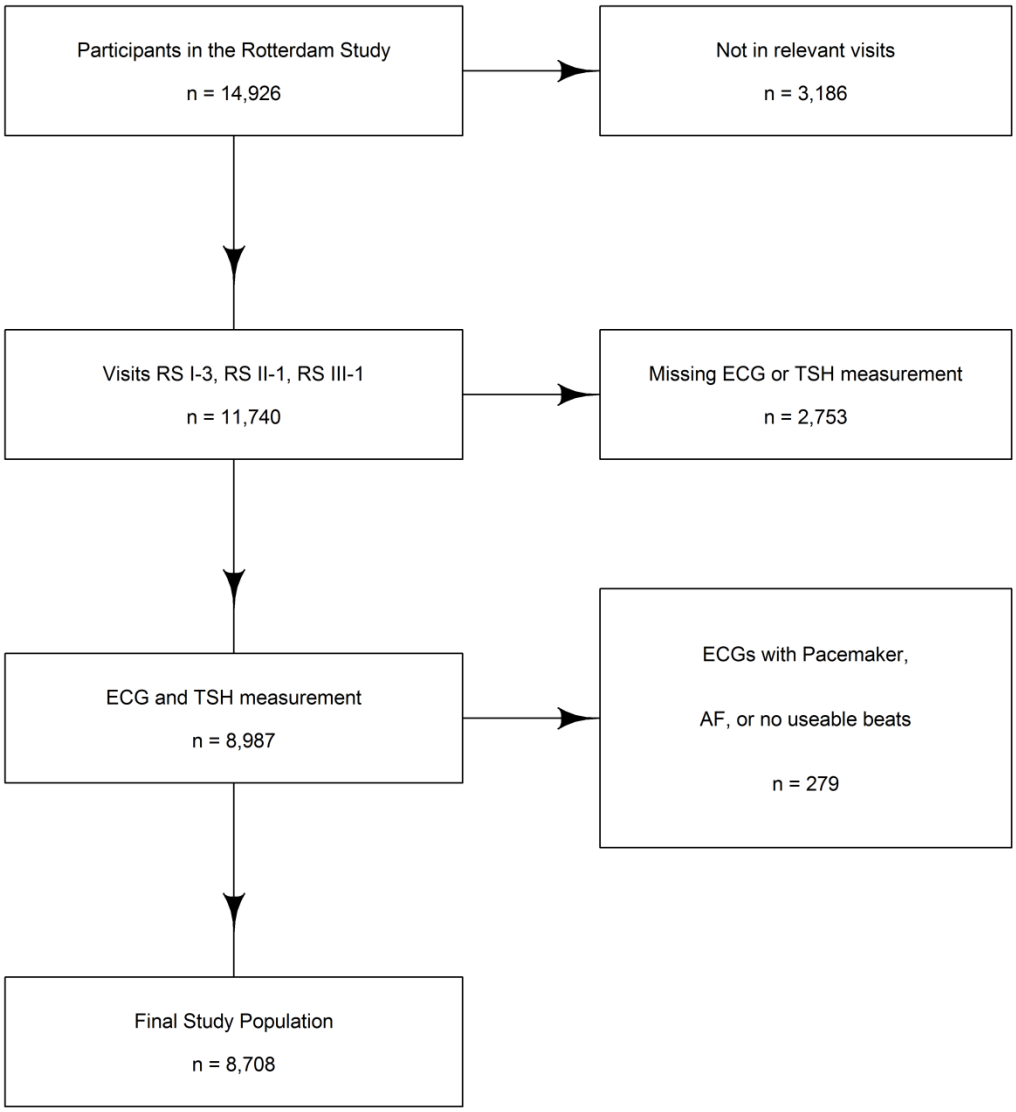
STVqt and TSH were log-transformed to normalize their distributions for all continuous analyses. Zero values for STVqt (n = 14) were set to one fourth of the lowest nonzero value to allow log-transformation. We implemented linear regression models to study the association of $FT_4$ with STVqt, using the Wald-test for confidence intervals (CIs) and p-values. The possibility of a nonlinear association between log-transformed TSH or $FT_4$ and STVqt was tested by successively adding polynomial terms of $FT_4$: $FT_4^2$, $FT_4^3$ and $FT_4^4$. The value of adding these terms was assessed with the likelihood ratio test.

All participants with a TSH measurement and an STVqt measurement were included in the main analysis (six missing values for $FT_4$ were imputed). Three sensitivity analyses were performed. First, to assess the possible influence of drug use, we excluded all users of thyroid function altering preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B), iodine therapy (ATC H03C), corticosteroids for systemic use (ATC H02A and H02B), or amiodarone. Second, we limited the analyses to euthyroid participants (as defined above). Third, to rule out a potential effect of auto-immunity on STVqt, we excluded participants with TPOAb >35 kU/l. Furthermore, we performed pre-specified analyses stratified by sex and stratified by age (older or younger than 65 years). For all analyses, we created a basic model adjusted for age and sex and a multivariable model with adjustment for age, sex, cohort, averaged QT interval, heart rate, and log-transformed heart-rate variability, BMI, hypertension, smoking status, serum total cholesterol, prevalent diabetes mellitus, and prevalent CHD. If the association of STVqt with $FT_4$ or with TSH was significant, we plotted the estimated regression line with 95% confidence intervals of $FT_4$ or TSH versus values of STVqt. The association of thyroid function with STVqt was additionally assessed using thyroid status, a categorical variable with the categories hypothyroid, subclinical hypothyroid, euthyroid, subclinical hyperthyroid and hyperthyroid. This categorical variable was analyzed in a linear regression model, using the same covariables and sensitivity analyses as in the analyses with TSH and $FT_4$.

After selecting those participants with both a TSH measurement and an ECG, remaining missing values in the other covariables were imputed with five-times imputation. There were no variables with more than 5% missing values. For all analyses we used SPSS (IBM SPSS Statistics for

Windows, version 21.0. Armonk, NY). The regression line and 95% confidence intervals were calculated in R.

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




Abbreviations: RS, Rotterdam Study; RS I-3, Third visit of the first cohort; RS II-1, First visit of the second cohort; RS III-1, First visit of the third cohort; ECG, electrocardiogram; TSH, Thyroid-stimulating hormone; AF, Atrial fibrillation

***Results***

*Population Characteristics*

Figure 1 shows the flowchart of the study population. There were 8,987 participants with a TSH measurement and an ECG. We excluded 279 participants because their ECG showed a pacemaker signal (n = 8), atrial fibrillation (n = 138), atrial flutter (n = 13), or because their ECG had no usable consecutive QT intervals (n = 120). Of the remaining 8,708 participants, 4,955 were women (56.9%) and the average age was 64.5 years, with a standard deviation of 9.7 years. Further characteristics of the study population are shown in Table 1.

*Association of TSH and $FT_4$ with STVqt*

Table 2 shows the association of TSH and $FT_4$ with STVqt in the multivariable models. Both $FT_4$ and $FT_4^2$ were significantly associated with STVqt, indicating a U-shaped relationship of $FT_4$ with STVqt. TSH was not significantly associated with STVqt, except in the sensitivity analysis excluding users of thyroid-affecting medication. Otherwise, the sensitivity analyses did not reveal relevant changes in the association between thyroid function and STVqt. Results of the basic model were very similar to the multivariate model, e.g. main analysis $FT_4$ -0.086 (95%CI-0.124; -0.047), $FT_4^2$ 0.003 (95%CI 0.001; 0.004) and thus not shown for all analyses.

Table 3 shows the analyses stratified on age and sex. There was a significant interaction of sex with $FT_4$ and $FT_4^2$ (p-values 0.014 and 0.006, respectively). The association of $FT_4$ with STVqt was significantly stronger in men than in women $FT_4$ : -0.143 (95% CI -0.202; -0.085) in men versus -0.048 (95% CI -0.096; 0.001) in women, $FT_4^2$ : 0.004 (95% CI 0.003; 0.006) in men versus 0.001, 95% CI 0; 0.001 in women. In the stratification on age, there was no significant interaction with $FT_4$ or $FT_4^2$ when comparing those younger than 65 with those older than 65.

Figure 2 shows the regression lines of $FT_4$ and back-transformed STVqt, based on the model including all men. The figure shows that STVqt is higher with an increased or a decreased $FT_4$. Figure 3 shows the same plot but then for all women, revealing a weaker U-shaped association between $FT_4$ and STVqt. Supplementary Figure 1 and 2 show the same regression lines for men and women with euthyroid status, showing a similar pattern.

**Table 1. Baseline characteristics of included participants**

| Characteristic | | Result |
|---|---|---|
| Participants, N | | 8,708 |
| Women, N (%) | | 4,955 (56.9%) |
| Age (years), mean (SD) | | 64.5 (9.7) |
| Diabetes mellitus | | 1,604 (18.4%) |
| Smoking, N (%) | never | 2,704 (31.1%) |
| | former | 4,247 (48.8) |
| | current | 1,711 (19.6%) |
| Hypertension, N (%) | | 5,306 (60.9%) |
| Prevalent CHD, N (%) | | 575 (6.6%) |
| BMI ($kg/m^2$), mean (SD) | | 27.3 (4.2) |
| Total cholesterol (mmol/L), mean (SD) | | 5.7 (1.0) |
| TSH (mU/l), median (IQR) | | 1.9 (1.3; 2.8) |
| $FT_4$ (pmol/l), mean (SD) | | 15.7 (2.3) |
| TPOAb > 35 (kU/mL), N (%) | | 1,147 (13.2%) |
| Hypothyroidism, N (%) | | 67 (0.8%) |
| Subclinical hypothyroidism, N (%) | | 815 (9.4%) |
| Euthyroidism, N (%) | | 7,591 (87.2%) |
| Subclinical hyperthyroidism, N (%) | | 215 (2.5%) |
| Hyperthyroidism, N (%) | | 20 (0.2%) |
| Heart rate (bpm), mean (SD) | | 68.9 (10.9) |
| QT interval (ms) / QTc bazett, mean (SD) | | 405 (30) / 431 (24) |
| SDNN (ms), median (IQR) | | 16.4 (10.4; 27.0) |
| STVqt (ms), median (IQR) | | 1.8 (1.2; 3.0) |

Abbreviations: BMI, body-mass index; CHD, coronary heart disease; $FT_4$, thyroxine; IQR, interquartile range; QTc, heart-rate corrected QT interval; SD, standard deviation; SDNN, standard deviation of normal-to-normal RR intervals; STVqt, short-term variability of the QT interval; TPOAb, thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone

**Table 2. Association of thyroid function with log-transformed STVqt**

| Population | Determinant | Beta (95% CI) |
|---|---|---|
| All participants | log-transformed TSH | 0.016 (-0.004; 0.036) |
| n = 8,708 | $FT_4$ | **-0.079 (-0.117; -0.041)** |
| | $FT_4^2$ | **0.002 (0.001; 0.003)** |
| No thyroid | log-transformed TSH | **0.023 (0.001; 0.045)** |
| medication | $FT_4$ | **-0.088 (-0.138; -0.039)** |
| n = 8,181 | $FT_4^2$ | **0.003 (0.001; 0.004)** |
| Euthyroid status | log-transformed TSH | 0.013 (-0.023; 0.048) |
| n = 7,591 | $FT_4$ | **-0.168 (-0.244; -0.092)** |
| | $FT_4^2$ | **0.005 (0.003; 0.007)** |
| TPOAb ≤ 35 | log-transformed TSH | 0.009 (-0.014; 0.033) |
| n = 7,552 | $FT_4$ | **-0.076 (-0.122; -0.031)** |
| | $FT_4^2$ | **0.002 (0.001; 0.004)** |

Notes: TSH and $FT_4$ were assessed in separate linear regression models. Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. *No thyroid medication* excludes users of thyroid function altering preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B) , iodine therapy (ATC H03C), corticosteroids for systemic use, (ATC H02A and H02B) and Amiodarone. Bold indicates P-value below 0.05.

Abbreviations: $FT_4$, Thyroxine; STVqt, short-term variability of the QT interval; TPOAb, thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone.

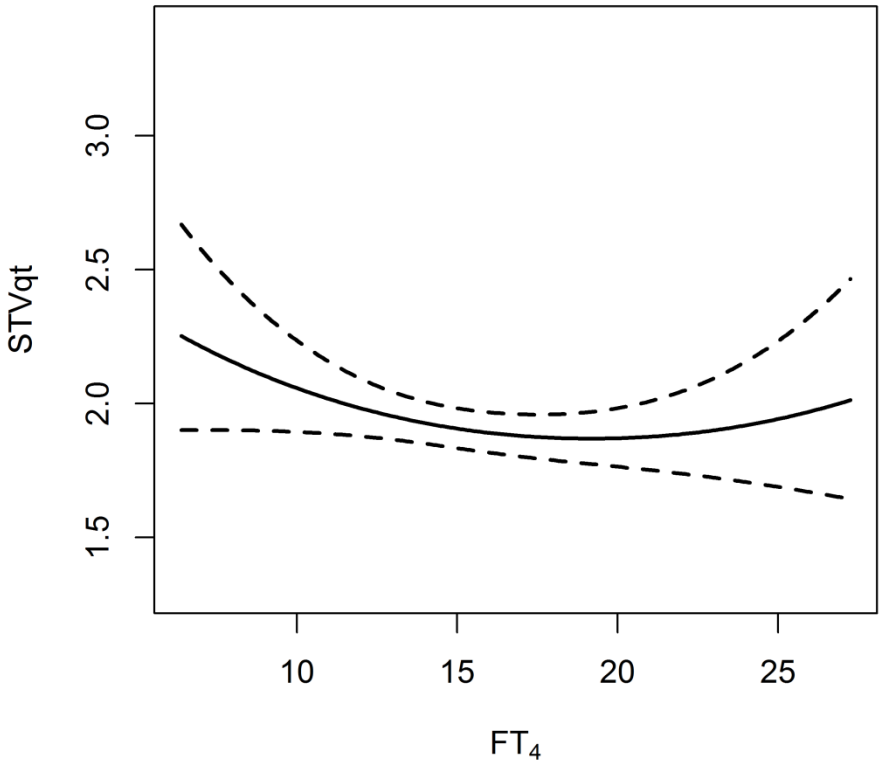
**Table 3. Association of thyroid function with log-transformed STVqt stratified on age and sex**

| | **Men; n = 3,753** | **Women; n = 4,955** |
|---|---|---|
| | **Beta (95% CI)** | **Beta (95% CI)** |
| log-transformed TSH | -0.001 (-0.036; 0.034) | 0.021 (-0.004; 0.046) |
| (log-transformed TSH)$^2$ | **0.022 (0.007; 0.038)** | |
| $FT_4$ | **-0.136 (-0.195; -0.078)** | -0.044 (-0.094; 0.006) |
| $FT_4^2$ | **0.004 (0.002; 0.006)** | 0.001 (0.000; 0.003) |
| | Age ≤ 65; n = 5,018 | Age > 65; n = 3,690 |
| | Beta (95% CI) | Beta (95% CI) |
| log-transformed TSH | 0.004 (-0.023; 0.030) | 0.028 (-0.003; 0.058) |
| FT4 | -0.036 (-0.099; 0.026) | **-0.102 (-1.53; -0.052)** |
| $FT_4^2$ | 0.001 (-0.001; 0.003) | **0.003 (0.001; 0.004)** |

Notes: TSH and $FT_4$ were assessed in separate linear regression models. Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. Bold indicates P-value below 0.05

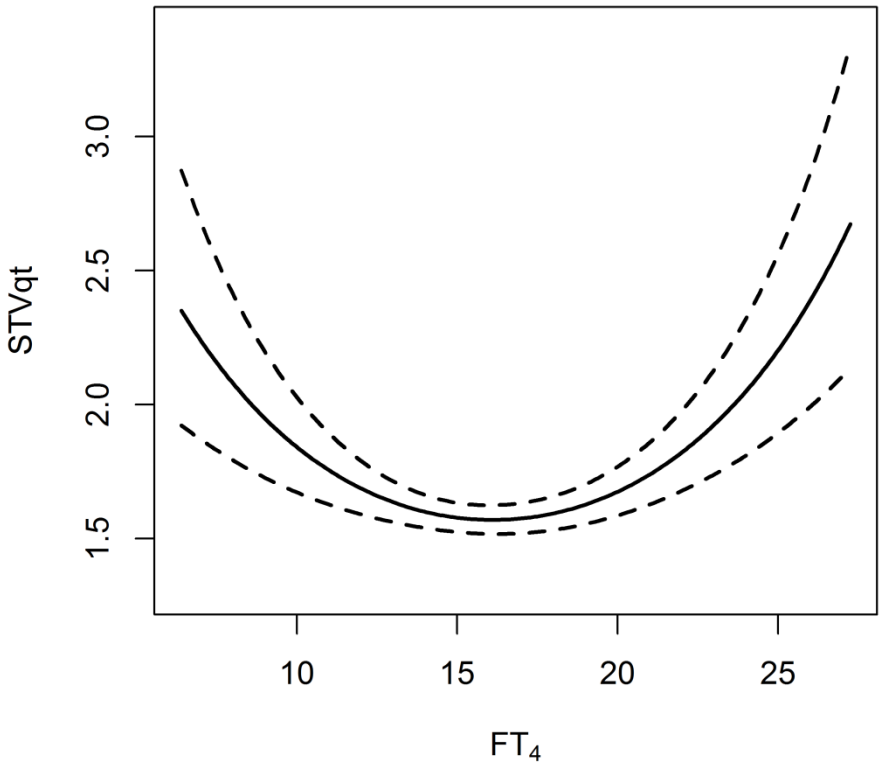
Abbreviations: $FT_4$, thyroxine; STVqt, short-term variability of the QT interval; TSH, thyroid-stimulating hormone.

**Figure 2. Estimated regression line of STVqt by FT4 status with 95% confidence intervals in men**




STVqt was used as a log-transformed variable in the analysis and back-transformed for presentation. The prediction was based on all included men (n = 3,753). The analysis was adjusted for cohort, age, sex, QT interval, heart rate, heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. The continuous line indicates the estimate, the dotted lines indicate the limits of the 95% confidence interval.

**Figure 3. Estimated regression line of STVqt by FT4 status with 95% confidence intervals in women**




STVqt was used as a log-transformed variable in the analysis and back-transformed for presentation. The prediction was based on all included women (n = 4,955). The analysis was adjusted for cohort, age, sex, QT interval, heart rate, heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. The continuous line indicates the estimate, the dotted lines indicate the limits of the 95% confidence interval.

*Categorical Analysis of the Association between Thyroid Status and STVqt*

The categorical analyses are shown in Supplementary Tables 1 and 2. Supplementary Table 1 first shows the main analysis, where subclinical hyperthyroid participants had the lowest beta for STVqt. However, differences with the other categories were not statistically significant, and the p-for trend was 0.41. In the sensitivity analysis excluding subjects that used thyroid function altering drugs, the hyperthyroid participants had the lowest beta for STVqt (Supplementary Table 1). When excluding participants with TPOAbs > 35, the hypothyroid participants had the lowest beta for STVqt. It should be noted that there were very low numbers (n = 8 and n = 10, respectively) in those categories. Supplementary Table 2 shows the categorical analysis stratified on sex. In the stratified analysis, no results were significant, except that hyperthyroid women had a significantly higher STVqt than euthyroid women.

**Discussion**

Our study shows that there is a U-shaped association between $FT_4$ and STVqt. This association is significantly stronger in men than in women. TSH was only significantly associated with STVqt when excluding users of thyroid-affecting drugs. The analysis of overt thyroid dysfunction with STVqt showed no significant results, probably due to small numbers in several categories.

This is the first study that analyzes the association between thyroid function and QT variability.[4] Studies of heart-rate variability (HRV) found that HRV was decreased in both subclinical hyperthyroidism[27] and overt hypothyroidism,[28] suggesting a U-shaped association of thyroid function with heart-rate variability. Our study demonstrates a similar relationship of $FT_4$ with STVqt. We did not find a significant association of TSH with STVqt in the main analysis. Similarly, in previous studies in the elderly, $FT_4$, but not TSH, was associated with SCD [3] or atrial fibrillation[10]. This might be due to changes in the set point of the hypothalamic-pituitary-thyroid axis, with an altered TSH secretion in reaction to negative feedback of thyroid hormone due to ageing.[20] The fact that the association of TSH with STVqt is significant when excluding thyroid-affecting drugs could indicate a different association between TSH and STVqt in users of thyroid-affecting drugs, which interferes with the association in the main analysis.

QT variability is thought to be increased when channels of the inward rectifying current ($I_{kr}$) in cardiac myocytes are blocked. Blockade of $I_{kr}$ delays repolarization and increases the QT interval. [1] $I_{kr}$ potassium channels can be blocked by the use of certain drugs,[4] or in congenital long-QT syndromes,[29] which are also associated with an increase in QT variability. It has been shown in animal models that an increase in $FT_3$ leads to a quick decrease in the $I_{kr}$ current, prolonging repolarization.[30] it is possible that the $I_{kr}$ potassium channels are the pathway by which higher levels

of thyroid hormone lead to an increase in STVqt. The association of thyroid function and STVqt may also be indirect: case reports suggest that both hyperthyroidism[31] and hypothyroidism[32] can lead to dilated cardiomyopathy, and it is known that STVqt and other QT variability markers are increased in dilated cardiomyopathy.[2,33] However, we could not address dilated cardiomyopathy in our study, as we did not have data of this diagnosis, and future studies should address this issue. Other possible pathways, e.g. common genetic factors, have also not yet been investigated.

Previous studies have reported that an increased QT variability is associated with ventricular arrhythmias[5,34] and sudden cardiac death.[35-38] Our study indicates that both lower and higher levels of FT4 lead to an increase in STVqt, which suggests that both hyperthyroidism and hypothyroidism could lead to ventricular arrhythmias. This is supported by a number of case reports of ventricular arrhythmias and sudden cardiac death in both hyperthyroidism[12-14] and hypothyroidism.[11,15-17] In a previous study we reported an association of higher $FT_4$ with an increased risk of SCD.[3] Also, there seemed to be a stronger positive association of $FT_4$ with SCD in men than in women, but this was not statistically significant. This suggests that STVqt could be involved in underlying mechanisms of the association between $FT_4$ and SCD. In our previous study of thyroid function and SCD,[3] there was no association between a low $FT_4$ and SCD, and it is possible that when a low $FT_4$ leads to a higher STVqt, other morbidity and mortality than SCD occurs. Also, the previously mentioned association of $FT_3$ and the $I_{kr}$ potassium channels[30] cannot explain why hypothyroidism is associated with an increased STVqt. Thus, further studies are needed to elucidate the role of STVqt in mortality associated with thyroid disease.

We found that the (U-shaped) association of $FT_4$ with STVqt was stronger in men than in women, with significant interaction terms between sex and $FT_4$, $FT_4^2$. A previous study in the Rotterdam Study population reported that an increased $FT_4$ was associated with a higher risk of (average) QTc prolongation, but only in men.[39] Moreover, animal studies in dogs have shown that testosterone increases expression of potassium channels in cardiac myocytes and the QT interval on the ECG.[40] These studies suggest that repolarization of the heart affected both by testosterone and by thyroid hormone, perhaps leading to interaction between the effects of the two hormones.

Our study has a number of strengths and limitations. Strengths of our study include the use of a large population-based cohort of community-dwelling middle-aged and elderly participants. Furthermore, ECGs and thyroid measurements were performed prospectively without knowledge of the study question, and the ECGs were processed automatically, which reduced observer bias.

A limitation of our study is that most previous studies used longer ECG recordings (usually 3 to 5 minutes) than the standard 10-second ECGs used in this study,[4] and the relationship between STVqt measured on short ECGs and STVqt measured on longer ECGs has not yet been studied.

However, despite this limitation, we were able to find a significant association between $FT_4$ and STVqt. Additionally, the results that are obtained in this middle-aged and elderly population might not be representative of younger populations. We could not evaluate temporal relationships between $FT_4$ and STVqt due to the cross-sectional design of our study.

In conclusion, we found that $FT_4$ has a U-shaped association with STVqt, as measured on 10-second ECGs. Since STVqt is a marker of repolarization instability, our study suggests that both high $FT_4$ and low $FT_4$ levels lead to repolarization instability. Further studies should elucidate the role of STVqt in mortality associated with thyroid disease, and possible sex differences in the relationship between thyroid function, STVqt and sudden cardiac death.

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**Supplementary table 1. Effect of thyroid status on log-transformed STVqt**

| | All participants | | No thyroid medication | | TPOAb ≤35 | |
|---|---|---|---|---|---|---|
| **Thyroid status** | **n** | **β (95% CI)** | **n** | **β (95% CI)** | **n** | **β (95% CI)** |
| Hypothyroid | 67 | 0.08 (-0.09; 0.27) | 59 | 0.13 (-0.06 - 0.33) | 10 | -0.02 (-0.48; 0.45) |
| Sub. Hypothyroid | 815 | 0.05 (0.00; 0.10) | 712 | 0.06 (0.00; 0.12) | 502 | 0.08 (0.01; 0.15) |
| Euthyroid | 7,591 | Reference | 7,238 | Reference | 6,844 | Reference |
| Sub. Hyperthyroid | 215 | -0.02 (-0.13; 0.08) | 164 | 0.00; (-0.11; 0.12) | 179 | 0.00 (-0.11; 0.11) |
| Hyperthyroid | 20 | 0.06 (-0.27; 0.39) | 8 | -0.05 (-0.56; 0.47) | 17 | 0.11 (-0.25; 0.47) |

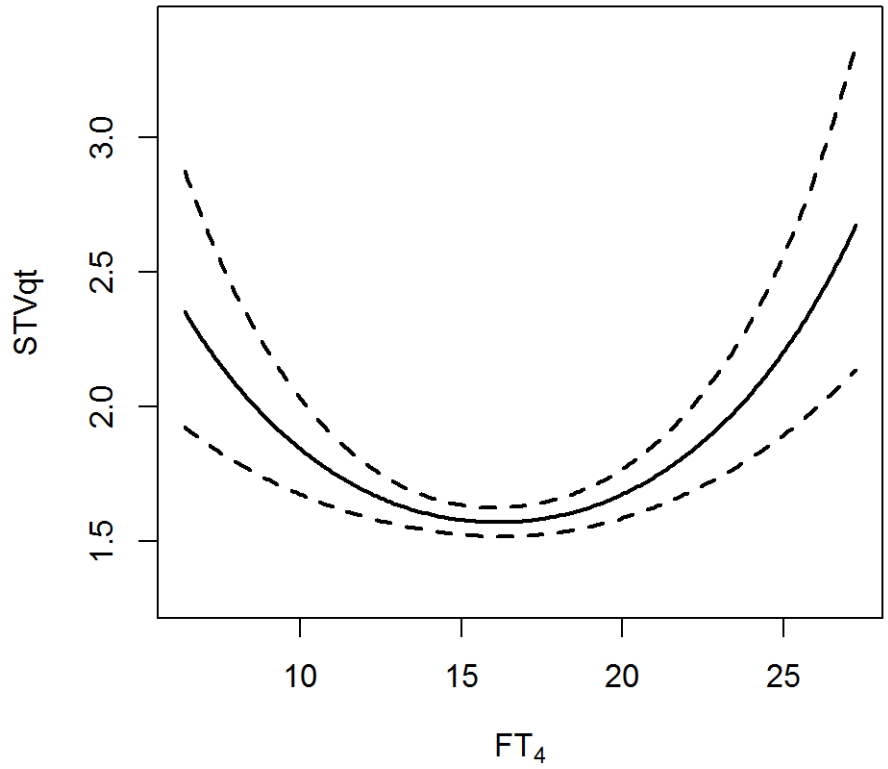
Notes: β Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. *No thyroid medication* excludes users of thyroid preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B) , iodine therapy (ATC H03C), corticosteroids for systemic use, (ATC H02A and H02B) and Amiodarone. Abbreviations: STVqt, short-term variability of the QT interval; Sub, Subclinical; BMI, Body-mass index

**Supplementary table 2. Effect of thyroid status on log-transformed STVqt stratified on sex**

| | Men | | Women | |
|---|---|---|---|---|
| **Thyroid status** | **n** | **β (95% CI)** | **n** | **β (95% CI)** |
| Hypothyroid | 17 | 0.21 (-0.13; 0.55) | 50 | 0.04 (0.01; 0.13) |
| Sub. Hypothyroid | 240 | 0.03 (-0.06; 0.13) | 575 | 0.05 (-0.17; 0.27) |
| Euthyroid | 3,427 | Reference | 4,164 | Reference |
| Sub. Hyperthyroid | 66 | 0.10 (-0.08; 0.27) | 149 | -0.08 (-0.20; 0.05) |
| Hyperthyroid | 3 | 0.41 (-0.40; 1.21) | 17 | 0.00 (-0.37; 0.38) |

Notes: β Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. *No thyroid medication* excludes users of thyroid preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B) , iodine therapy (ATC H03C), corticosteroids for systemic use, (ATC H02A and H02B) and Amiodarone. Abbreviations: STVqt, short-term variability of the QT interval; Sub, Subclinical

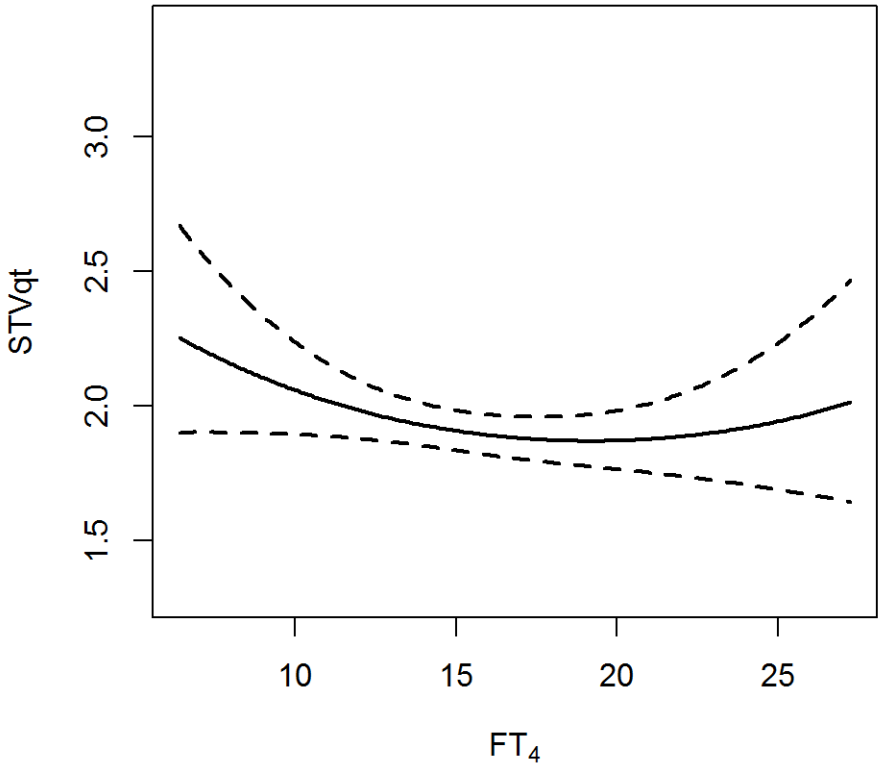
**Supplementary figure 1. Estimated regression line with 95% confidence intervals of STVqt by $FT_4$ status in euthyroid men**




Notes: STVqt was used as a log-transformed variable in the analysis and back-transformed for presentation. The prediction was based on men with euthyroid status (n = 3,427). The analysis was adjusted for cohort, age, sex, QT interval, heart rate, heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. The continuous line indicates the estimate, the dotted lines indicate the limits of the 95% confidence interval.

Abbreviations: STVqt, short-term variability of the QT interval; BMI, Body-mass index

**Supplementary figure 2. Estimated regression line of STVqt by $FT_4$ status with 95% confidence intervals in euthyroid women**




Notes: STVqt was used as a log-transformed variable in the analysis and back-transformed for presentation. The prediction was based on women with euthyroid status (n = 4,164). The analysis was adjusted for cohort, age, sex, QT interval, heart rate, heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. The continuous line indicates the estimate, the dotted lines indicate the limits of the 95% confidence interval.

Abbreviations: STVqt, short-term variability of the QT interval; BMI, Body-mass index

# Part III

# Risk Factors for Sudden Cardiac Death Not Based on the Electrocardiogram

# Chapter 3.1

# Chronic Obstructive Pulmonary Disease and Sudden Cardiac Death: a Systematic Review

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**Abstract**

*Background* Both chronic obstructive pulmonary disease (COPD) and sudden cardiac death (SCD) are major health burdens. A number of studies have addressed their interrelationship, but currently no systematic review has been published. Our objective is to give an overview of the literature of the association between COPD and SCD.

*Methods* A search on PubMed with both MeSH headings and free-text key words was performed. We selected all original articles of studies in humans that assessed COPD on the on hand and SCD, electrocardiographic markers for SCD, ventricular arrhythmias or asystole on the other.

*Results* The electronic search yielded 251 papers, from which 27 full publications were selected after careful evaluation of the full-text papers. In these studies, COPD was associated with a prolonged and with a shortened QT interval. In patients with a myocardial infarction (MI), COPD was associated with an increased risk of ventricular arrhythmias and decreased survival. COPD was a risk factor for SCD both in cardiovascular patient groups and in community based studies, independent from cardiovascular risk profile. Studies of the potential impact of respiratory treatment on the occurrence of SCD showed conflicting results.

*Conclusion* Cumulating evidence associates COPD with an increased risk of SCD. Asystole and pulseless electric activity could be more common than VT/VF in deaths associated with COPD. Underlying mechanisms explaining this association require further investigation.

**Introduction**

Cardiovascular disease is the leading cause of death and imposes a large global burden of morbidity and mortality.[1-4] About half of all cardiovascular deaths are sudden cardiac deaths (SCDs). SCDs occur suddenly and unexpectedly, often as a first sign of cardiac disease.[5,6] SCD remains a major cause of death, even though the incidence seems to be decreasing in Western Europe.[2,7] The majority of SCDs is thought to result from ventricular arrhythmias.[8] However, in recent years the proportion of SCDs resulting from pulseless electrical activity (PEA) and asystole is increasing,[9] possibly due to advances in treatment of coronary heart disease (CHD) and increased use of implantable cardioverter-defibrillators (ICDs).[10] SCD can be the result of various of underlying causes, such as CHD,[11] cardiomyopathies[12] or use of QT-prolonging drugs.[13] However, many possible etiological pathways have not yet been established.[6]

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death globally.[4] Progressive airflow limitation is the hallmark of COPD. Progressive airflow limitation is associated with a local chronic inflammatory response in the airways and lungs,[14] but in a subgroup of patients with COPD biomarkers of systemic inflammation (e.g. fibrinogen, C-reactive protein) are also increased.[15-17] COPD and cardiovascular disease are notably linked. For example, ECG abnormalities such as bundle branch blocks and axis deviations are common in COPD patients.[18] COPD patients also have a higher rate of cardiovascular morbidity and mortality than the general population.[19] Moreover, half of the deaths of COPD patients is attributable to cardiovascular disease.[19] The association of COPD with cardiovascular disease in general suggests that there could also be an association between COPD and SCD. Indeed, COPD can cause respiratory arrest, which can lead to PEA and asystole, and ultimately SCD. Accurate prediction of SCD in the general population is still a challenge because most cases of SCD occur in people with seemingly low cardiovascular risk.[6] Thus, risk assessment of SCD might be improved by studying the link between COPD and SCD. Studying the association of COPD with SCD includes assessing possible common risk factors (e.g. ageing or smoking) and the effect of respiratory drugs on SCD risk. To date, there has not yet been a systematic review of the literature that has studied the association of COPD with SCD. The objective of this paper is to provide an overview of the published literature on this association.

**Methods**

We searched PubMed from inception until December 10, 2015 using a combination of MeSH terms and free-text keywords. For COPD we used the MeSH term "Pulmonary Disease, Chronic Obstructive ". The free-text keywords were all terms derived from COPD and pulmonary emphysema, including the terms “obstructive pulmonary disease” and “COPD”. For SCD we used the MeSH terms "Death,

Sudden, Cardiac", "Heart Arrest", "Tachycardia, Ventricular", "Ventricular Fibrillation", "Ventricular Flutter", and "torsades de pointes". Free-text keywords for SCD were all derived from these MeSH terms. In order to retrieve unindexed papers we searched without additional filters for the publication dates after 2014. After that, we filtered our search results on papers written in English and on human participants aged older than 18 years. The online supplement contains the complete search strategy, including all MeSH terms and free-text keywords. Citation lists of the publications found by the electronic search were hand-searched for additional relevant publications.

Papers were deemed relevant for this review if they contained original research, included COPD patients or lung function measurements and the outcome SCD, ventricular arrhythmias, or electrocardiographic (ECG) markers for SCD. We extracted relevant papers from the list of the initial search in a two-step process. In the first step, two reviewers (MvdB, LL) independently screened the abstracts of the list of papers. Disagreements in abstract selection were resolved with consensus meetings. In the second step, the full-text articles were retrieved and all relevant information was extracted and tabulated. Based on this, a final selection was made.

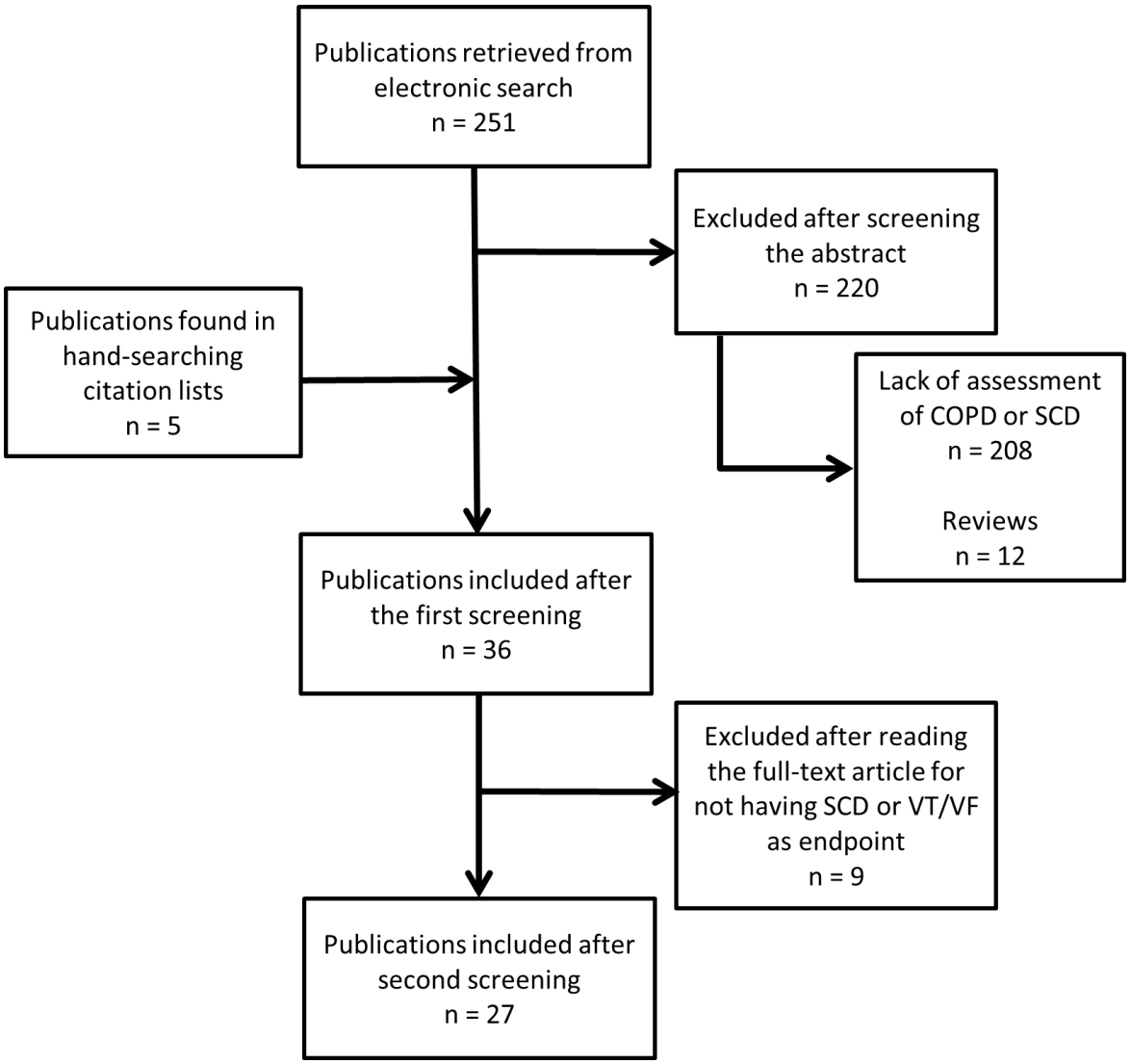
**Results**

*General findings*

The electronic search on PubMed yielded 251 publications. A further 5 publications were found by hand- searching citation lists. After screening the abstracts, we included 27 publications for reviewing. The selection process and its results are shown in more detail in Figure 1. The selected papers included 26 observational studies (10 cohort studies, 9 cross-sectional studies and 7 case-control studies) and 1 randomized controlled clinical trial (RCT). A list with summaries of all included papers is provided in supplementary Table 1.

The results section consists of two parts. Part one addresses the association of COPD with SCD, ventricular arrhythmias and ECG markers for SCD. First, we review COPD and electrocardiographic markers associated with SCD. Then, we review publications addressing COPD, ventricular arrhythmias, and cardiac arrest; and provide an overview of studies addressing COPD and SCD in patient cohorts and community-based studies. In part two, we discuss potential sex differences in the association between COPD and SCD. Finally, we focus on possible mechanisms underlying the association of SCD and COPD, including smoking, hypertension, respiratory drugs and the role of COPD exacerbations.

**Figure 1. An overview of the electronic search and selection process**




Abbreviations: COPD: chronic obstructive pulmonary disease, SCD: sudden cardiac death, VT/VF: ventricular tachycardia/ventricular fibrillation.

**Part one: association of COPD with SCD, ventricular arrhythmias and ECG markers for SCD.**

*COPD and electrocardiographic markers of SCD*

The heart-rate corrected QT (QTc) interval is a known risk factor for SCD,[20] and it was the most commonly used ECG marker in the studies we found. In the study by Zulli *et al*,[21] QTc prolongation was associated with an increased mortality risk in a cohort of 246 COPD patients.[21] The association of COPD with the QTc interval was addressed in fours studies, with mixed results: Tukek *et al.* (total n = 73)[22] and Sievi *et al.*(n = 164)[23] found that QTc was significantly higher in COPD cases compared to controls,[22,23] but the study by Lahousse *et al,*[24] which compared 1615 COPD patients with all other participants (n = 11,856) at baseline, and Zupanic *et al.*(n = 62)[25] did not find a significant difference. However, Lahousse et *al.* only compared the median QTc of COPD patients to participants without COPD at baseline[24] and the study by Zupanic *et al.* was possibly underpowered.[25] A fifth study by Yildiz *et al*,[26] measured the maximum QT interval without heart-rate correction on 24-hour Holter recordings. This study found that the uncorrected maximum 24-hour QT interval was significantly higher in COPD patients.[26]

An abnormally short QT interval (≤ 300 milliseconds) has also been associated with SCD.[27] A large cross-sectional population-based study found that although QT shortening was rare (incidence of 2.7 per 100,000 persons), it was significantly associated with COPD with an odds ratio(OR) of 2.4 (95% confidence interval (CI) 1.3 - 3.5).[28] This study included 6,547,785 ECGs of which 660,534 were recorded in COPD patients.[28]

In addition to the QTc interval, heart-rate variability has also been associated SCD.[29] Heart-rate variability was assessed next to the QTc interval by Tukek *et al*,[22] Yildiz *et al*,[26] and Zupanic *et al.*[25] These studies found that heart-rate variability was significantly decreased in COPD patients.[20, 21, 23] A reduced heart-rate variability is indicative of an increased sympathetic tone and a reduced vagal tone, which might be pathways from COPD to an increased SCD risk.[30]

QT dispersion (QTD) is the difference between the longest and shortest QT interval in any lead for a given set of ECG leads.[31] QTD has been challenged on the ground that it is only a derivative attribute of T-loop morphology and not indicative of repolarization instability, and therefore an unsound marker for the prediction of ventricular arrhythmias.[31,32] Nevertheless, four studies addressed this marker:[21-23,26] In the study by Sievi et al,[23] QTD was not significantly increased in COPD patients compared with controls matched for age, cardiovascular risk and medication use, but it was increased in COPD patients in Tukek *et al.*[22] and Yildiz et al.[22,26] Finally, the cohort study of Zulli *et al,*[21] an increased QTD was associated with higher mortality risk.[21]

*COPD, ventricular arrhythmias and cardiac arrest*

Between 30% and 70% of SCD cases result from ventricular tachycardia and ventricular fibrillation (VT/VF).[9,10] For this reason, the association of COPD with ventricular arrhythmias can be relevant for the association of COPD with SCD. Two studies addressed the underlying rhythm of COPD patients with a cardiac or a respiratory arrest. Frist, a study[33] in 70 patients with chronic airway obstruction admitted for acute respiratory failure noted that 15 patients (21.4%) developed cardiac arrest (which included VF and asystole), and 4 patients (5.7%) developed VT.[33] In a cross-sectional study (n = 5,415)[34] of people who were resuscitated for out-of-hospital cardiac arrests (OHCAs), 20% of OHCAs were judged to be caused by COPD based on case history. The initial rhythm of resuscitation differed between the causes of the OCHAs: For the OHCAs with a cardiac cause, the initial rhythm was VT/VF in 48% of the cases, and PEA or asystole in 50% of the cases. For the OHCAs caused by COPD, the initial rhythm at was VT/VF in 8% of the cases, and PEA or asystole in 88% of the cases.[34] These studies indicate that the occurrence of VT/VF in COPD patients is relatively low and PEA might be more frequently associated with SCD in COPD.[33,34] Furthermore, there was no significant increase of sustained VTs on a 24-hour ECG in stable COPD patients after adjustment for cardiovascular risk factors.[35] In patients with a non-ST-segment elevation myocardial infarction (non-STEMI), the association of COPD with VT/VF seems to be stronger: In 26,416 patients with a non-STEMI, a history of COPD was associated with a significantly increased risk of VT (HR 1.9, 95% CI 1.1 - 3.1) and VF (HR 2.5, 95% CI 1.6 - 4.1).[36] In addition to this, one study found that after an OHCA with VT/VF, 30-day survival was significantly lower in COPD patients.[37]

*COPD and SCD in cohorts of patient populations*

Almost all studies defined SCD first as a witnessed unexpected death within one hour of onset of abrupt change in symptoms and second as an unwitnessed death within 24 hour of the last observation as medically stable. Supplementary Table 1 shows the definitions of SCD for all included studies.

A study in 5,992 COPD patients reported that 7.7% of all deaths were SCDs,[38] while a study of non-traumatic prehospital SCDs (defined as death within one hour post-ER arrival, n = 905) found that COPD was relatively common in those SCD cases (11% had COPD).[39] However, neither study had a control group (non-COPD patients and non-SCD deaths, respectively).

Studies in patient groups with cardiac diseases found that COPD as comorbidity is a risk factor for SCD. These clinic-based cohorts included patients who survived percutaneous coronary interventions (PCIs, n = 6,846)[40] and coronary artery bypass graft (CABG, n = 2,910)[40] and patients with atrial fibrillation (AF, n = 334) who underwent atrioventricular-node ablation.[41] However, COPD

was not a significant risk factor for SCD in a study in elderly patients (n = 3,726, mean age 81 years) who underwent transcatheter aortic valve replacement (TAVR).[42]

Patients with an ICD are a special patient group for the study of ventricular arrhythmias and SCD. COPD was a risk factor for appropriate ICD shocks, which indicate the occurrence of ventricular arrhythmias in a cohort study of patients with an ICD (n = 628).[43] A second cohort study (n = 202) found that COPD was also associated with ICD shocks, but this study did not differentiate appropriate from inappropriate ICD shocks (due to non-ventricular arrhythmias).[44]

*COPD and SCD in community-based studies*

Three community-based studies investigated the association of SCD or SCA with COPD. First, SCA risk was increased in COPD patients (OR 1.4, 95% CI 1.2 - 1.6), in a case-control study that compared SCA patients with VT/VF (n = 1,310) to controls matched for age, sex and index date (n = 5,793). This study adjusted for cardiovascular risk factors, which were estimated by the use of cardiovascular drugs.[45] Second, COPD was a risk factor for SCD in a population-based cohort of middle-aged and elderly participants (HR 1.4, 95% CI 1.0 - 1.5). This study additionally created competing risk models, which showed that COPD is a risk factor for SCD beyond the generalized increased risk of death in COPD patients.[24] Third, a higher frequency of respiratory diseases (international classification of diseases 10 - group J which includes COPD) was observed in SCA patients. However, this was statistically not significant (p=0.184). In this study, a large cross-sectional study of emergency medical service interventions, the medical records on comorbid conditions of patients with out-of-hospital sudden cardiac arrest (SCA, n = 245) were examined retrospectively and compared with patients that underwent interventions for other reasons (n = 26,398).[46] The fact that the higher frequency of respiratory diseases was not significant could be explained by the lack of specific COPD phenotyping or by the underdiagnosis of COPD in the medical records of emergency operations.

**Part two: sex differences and mechanisms of the association of SCD and COPD**

*Sex differences in the association of COPD with SCD*

Three studies analyzed sex differences as possible effect modifiers of the association between COPD and VT/VF or SCD. First, men were more prone to VT than women in a cross-sectional study of ambulatory ECGs of 69 COPD patients.[47] In contrast, there was no sex difference in the risk of SCA between participants with COPD or asthma and participants without COPD or asthma in a study of SCA cases (n = 1,568) [48] Third, SCD occurred more frequently in men with COPD in the population-based study of Lahousse *et al.* with 13,471 community-dwelling participants.[24] However, this study found that frequent COPD exacerbations carried a slightly higher risk for women (HR 3.5, 95%CI 1.8 -

6.8) than for men (HR 3.1, 95%CI 1.7 - 5.5).[24] Since the risk increased further when restricted to post-menopausal women, a role of stress-induced cardiomyopathy during COPD exacerbations was suggested.[24]

*Smoking*

The association of COPD with SCD was adjusted for smoking in two cohort studies. The first cohort study (total n = 7,441) found that low $FEV_1$ and FVC were associated with SCD both in smokers and non-smokers,[15] and the study by Lahousse *et al.* reported that the association between COPD and SCD was independent of smoking behavior.[24] Moreover, smoking was not a significant predictor for ICD shocks in a study in a cohort of patients with ICDs.[44] These studies suggest that the association of COPD and SCD is independent of smoking.

*Hypertension and timing of SCA/SCD*

Two studies assessed factors associated with both COPD and cardiac endpoints (myocardial infarction (MI) or OHCA). First, hypertension: acute MI patients admitted to the ICU with hypertension more often had COPD than normotensive MI patients, but they presented less often with VF.[49] Second, the time of incidence: nighttime admissions for OHCAs more often had COPD as comorbidity, and lower 1-year survival.[49] Furthermore, in the study by Lahousse *et al,*[24] SCDs in COPD patients occurred more often at night than SCDs in non-COPD patients (53% vs. 36%).[24] The increased number of ventricular ectopic episodes during sleep could be the result of reduced ventilation and a blunted response to hypercapnia.[50,51]

*Respiratory drugs*

The study by Warnier *et al.*[45] reported that use of inhaled short-acting beta-agonists (SABAs) and use of inhaled muscarinic antagonists (the study did not specify if these were long-acting or short-acting anticholinergics) were associated with sudden cardiac arrest (SCA) due to VT/VF. The OR for SABAs was 3.9 (95% CI 1.7 - 8.8) and for anticholinergics 2.7 (95% CI 1.5 - 4.8), after adjustment for concomitant cardiovascular disease. Use of inhaled corticosteroids was not associated with SCA in this study.[45] However, increased drug use during exacerbations or increased drug use due to more severe COPD might be plausible confounders for the increased risk of SCA in users of SABAs and anticholinergics.[52] In contrast, two studies found no effect of respiratory drugs on the risk of SCD in COPD. First, use of the long-acting anticholinergic tiotropium did not increase the risk of VT/VF in a meta-analysis combining thirty clinical trials and including 19,545 COPD patients.[53] However, because there were only five fatal VT/VF cases this meta-analysis was underpowered for this

endpoint.[53] Clinical trials of COPD patients possibly have a low incidence of VT/VF due to the selection of relatively stable patients without significant cardiovascular comorbidities.[54] Second, use of sympathomimetic respiratory drugs did not significantly interact with COPD on the risk of SCD in the study by Lahousse *et al*, but this study did not take duration of drug use or dosage into account.[24]

*COPD exacerbations*

In the study by Lahousse *et al,*[24] long-term COPD patients with frequent exacerbations had a higher risk of SCD (HR 3.2, 95% CI 2.1 – 5.0) than long-term COPD patients without frequent exacerbations (HR 1.52, 95% CI 1.1 – 2.2). Exacerbations only increased the risk of SCD in those participants with elevated systemic inflammation at cohort entry, indicating that the association of COPD exacerbations with SCD is at least partially mediated by systemic inflammation. Thus, severity and duration of COPD are of importance for the risk of SCD.[24]

**Discussion**

The majority of the studies supports the hypothesis that COPD is associated with SCD. COPD was a risk factor for SCD independent of cardiovascular risk profile, both in cohorts of cardiovascular patients and in community based studies. Evidence for the association of COPD with VT/VF is much weaker, and the evidence for the potential impact of respiratory treatment on the onset of SCD is inconclusive. COPD was also associated with reduced heart-rate variability, while the studies for QTc prolongation were inconclusive.

There are several theoretical explanations for an association between COPD and SCD, which are illustrated in Figure 2. We will review each of these mechanisms and note what has been studied, and identify gaps needing further research. First, COPD might cause pathophysiological changes which directly increase the risk of SCD. Examples of previously studied pathological changes in COPD are changes in cardiac repolarization, represented by QT or QTc interval abnormalities,[22-26,28] and increased sympathetic tone or reduced vagal tone indicated by a reduced heart-rate variability.[22,25,26] Other studies have also found that COPD is associated with baroreceptor sensitivity abnormalities[55], heart-rate variability abnormalities,[56] and a direct increase in muscle sympathetic nerve activity.[57] Further research might focus on the interaction of COPD and autonomic neuropathy for the risk of SCD. The evidence for the association of COPD with QTc interval prolongation was inconclusive, and QT interval variability, a promising ECG marker of ventricular arrhythmias and SCD,[58] has not yet been studied in COPD. Therefore, QT variability might be an avenue for future research. Besides ECG markers, the effect of systemic inflammation in COPD on the risk of SCD has

been assessed.[24] However, there are also a number of pathophysiological changes associated with COPD that have not yet been studied in relation to SCD risk, including hypoxia and hypoxemia,[14] cardiac ischemia,[8,19] heart failure,[59] and possibly pulmonary hyperinflation and pulmonary hypertension.[24]

Second, there may be common risk factors acting as confounders. The association of COPD with SCD was independent of smoking, which was addressed in two studies.[15,24] The role of common genetic risk factors need to be elucidated, since these studies will provide more insights into common pathogenic pathways that lead to increased SCD risk in COPD (e.g. systemic inflammation or tissue remodeling). No study to date has assessed common genetic risk factors between COPD and SCD.

Third, use of respiratory drugs could be associated with SCD. The studies included in this review give contradictory results and no study assessed the dose-response relationship of respiratory drugs and SCD.[24,45,53] The TIOSPIR[60] and SUMMIT[61] randomized controlled trials were not included in our review because they have not (or not yet) reported on the endpoint SCD. Because the association of respiratory drugs with SCD risk is probably dose-dependent, future studies should address the dose-effect. In addition to inhaled COPD drugs, none of the included studies have addressed the oral macrolide antibiotics, which are often prescribed for COPD exacerbations. These antibiotics are known QT-prolonging drugs, and suspected of increasing the risk of ventricular arrhythmias and SCD.[20,62]

Fourth, the diagnosis of COPD could change the prescription pattern of cardiovascular drugs. On the one hand, contra-indications for drugs like beta-blockers might lead to inadequate cardiovascular prevention, which might increase the risk of SCD. On the other hand, the medical attention which is sought for COPD may also lead to improved cardiovascular prognosis by active prevention of cardiovascular risks (such as smoking and hypertension). Warnier *et al.*[45] studied the prevalence of use of cardiovascular drugs in SCD cases and controls, but did not assess the interaction with COPD. Therefore, a time-dependent and dose-dependent assessment of individual cardiovascular drugs in COPD patients and non-COPD patients, and the associated SCD risk, is needed.

Fifth, the association of COPD with SCD could be caused by misclassification of acute respiratory deaths as sudden cardiac deaths especially when non-witnessed. However, the ratio of witnessed to non-witnessed SCD was not different for participants with COPD compared to participants without COPD in the largest study of the association between COPD and SCD.[24]

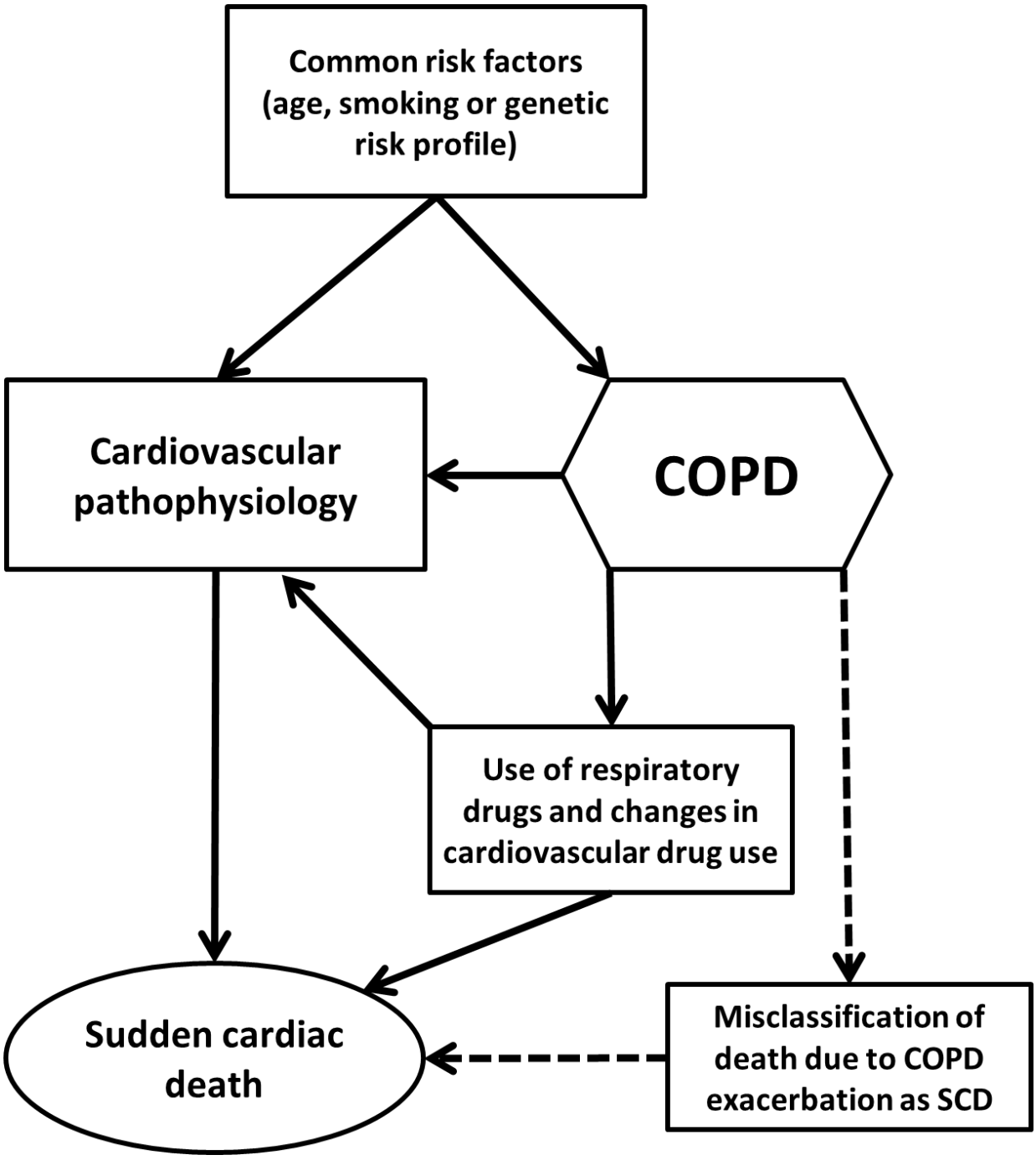
Lastly, the occurrence of a higher rate of exacerbations has also been reported to increase the risk of SCD in COPD patients.[24] Among explanatory mechanisms recently reviewed,[52] this

association can be caused by a direct increase in systemic inflammation associated with frequent exacerbations,[63] or indirectly by use (and abuse) of SABAs and LABAs. Excessive use of SABAs and LABAs may lead to hypomagnesemia,[64] which has also been associated with an increased risk of SCD.[65]

A strength of the studies addressing SCD is the uniform definition of SCD as a witnessed unexpected death within one hour of onset of abrupt change in symptoms or an unwitnessed death within 24 hour of the last observation as medically stable. However, none of the studies with the endpoint SCD reported the preceding cardiac arrhythmia. Therefore, we were unable to distinguish between SCD caused by VT/VF and SCDs resulting from PEA and asystole. One study indicates that PEA and asystole are more common than VT/VF in COPD patients with cardiac arrest.[34] As these different direct causes of SCD are possibly the results of different pathologies and associated with different risk factors, this is a limitation of the studies included in this review.

In conclusion, accumulating evidence associates COPD with an increased risk of SCD. Asystole and PEA could be more common than VT/VF in COPD-associated deaths. Underlying mechanisms explaining this association require further investigation. Promising avenues of further research include studies of QT variability and hypoxia, genetic studies and pharmaco-epidemiological studies.

**Figure 2: Possible mechanisms of the association between COPD and SCD**




*Note: Besides potential misclassification, COPD exacerbations are known to amplify both cardiovascular pathophysiology and the use of respiratory drugs.*

Abbreviations: COPD: chronic obstructive pulmonary disease, SCD: sudden cardiac death

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## Supplementary table 1. Overview of all included studies

| First author year | Study type | Population | Cases | Controls | Exposure or Determinants | Endpoint | follow-up time | Conclusion |
|---|---|---|---|---|---|---|---|---|
| Abrignani[1] 2005 | Case-control | Consecutive patients with AMI admitted to the ICU (n=4,994) | Hypertensive patients with first infarction (n=915) Age: 68.8±11.4 years | Gender- and Age-matched normotensives (n=915) | COPD VF From hospital records | | | Cases more often COPD (13.1 vs.7.5%, p<0.001), less VF (2.2 vs. 3.7%, p = 0.036) |
| Al Khatib[2] 2002 | Cross-sectional | Pooled datasets from 4 trials of patients with non–ST-segment elevation MI (n=26,416) | | | History of COPD | In-hospital VT or VF (n=552) | | COPD OR for VT 2.5 (1.6–4.1) COPD OR for VF 1.9 (1.1–3.1) |
| Blom[3] 2013 | Cohort | Patients with non-traumatic OHCA with ECG-documented VT/VF (n=1,172) | Patients with OPD (n=178) OPD: at least 2 prescriptions of any meds with ATC R03 in the year before OHCA. | Non-OPD patients (n=993) | | I. 30-day survival II. Survival to hospital discharge | 30 days | I. OR 0.7 (0.5-0.97) II. OR 0.6 (0.4-0.9) For OPD |
| Celli[4] 2010 | Clinical trial | COPD Patients: at least 40 years of age, ≥10 pack-years, airflow limitation $FEV_1$ ≤70% of FVC | Users of tiotropium (n=10,846) | Placebo users (n=8,699) | | VT/VF: IR users: 0.24 IR placebo: 0.16 Cardiac arrest: IR users: 0.26 IR placebo 0.16 | 4 years | Users of tiotropium VT/VF: RR 0.67 (0.38-1.19) Cardiac arrest: RR 0.68 (0.39-1.16) |
| Chugh[5] 2009 | Cross-sectional | SCA: a sudden unexpected pulseless condition <1h of symptom onset or <24h of having been seen alive and symptom-free. (n=1,568) | Men with adult SCA (n=805) | Women with adult SCA (n=453) | History of COPD or asthma | | | No sex-related difference COPD / asthma in SCA (P=0.10) |

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| Dougherty[6] 2009 | Cohort | SCA survivors or those with malignant ventricular arrhythmias who received an ICD for the first time | No ICD shock within 12 months (n = 151) | ICD shock within 12 months (n = 51) | COPD collected from self-report and/or from medical records review at the time of study entry | | 12 months | COPD, congestive HF or VT at ICD implant were significant predictors of an ICD shock in the first year |
| Engdahl[7] 2003 | Cross-sectional | All patients suffering from OHCA with resuscitation in Goteborg in years 1981-2000. | Cardiac arrests from a non-cardiac aetiology (n=1,360) of which 20% by COPD (n=275) | Cardiac arrests from a cardiac aetiology (n=4,055) | | Initial rhythm VT/VF: Cases: 8% COPD cases: 8% Controls: 48% | | Lower occurrence of ventricular fibrillation in cases ($p<0.001$). |
| Hudson[8] 1973 | Cross-sectional | Patients with chronic airway obstruction (n=70) from 147 consecutive admissions for acute respiratory failure. | | | | Cardiac arrest (n=15) VF (n=4) | | |
| Iribarren[9] 2014 | Cross-sectional | 12-lead ECGs recorded in 1.7 million participants (n = 6,547,785) | | | ECGs in patients with COPD ascertained by combining inpatient and outpatient diagnostic and procedure codes (n = 660,534) | Confirmed ECGs with $QT_{creg}$ (corrected by regression) ≤300, n=45 (from1,086 machine-read short QTs) | | COPD: OR of short QT 2.4 (1.3–4.5) Short QT known risk factor of SCD |
| Karlsson[10] 2014 | Cross-sectional | The nationwide Danish Cardiac Arrest Registry (2001-2010); OHCA patients (n=18,929) | | | Daytime (07.00–14.59h) Evening (15.00–22.59h) Nighttime (23.00–06.59h) | COPD discharge diagnosis codes up to 10 years before OHCA (Danish National Registry) | | For OHCA during nighttime significantly higher percentage COPD ($p<0.001$) |
| Konecny[11] 2014 | Case-control | Consecutive adults who underwent clinically indicated pulmonary function testing and 24h Holter (n=7,441) | Patients with COPD based in electronical medical records (n=3,121) | Patients without COPD based in electronical medical records (n=4,320) | | Sustained VT | | COPD patients ↑ sustained VT ($p=0.012$), multivariate model ns. |

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| Kurl[12] 2015 | Cohort | Participants with lung function test at baseline. (n=1,250) | $FEV_1$ FVC $FEV_1$ to FVC-ratio in quintiles | SCD: Death <1h of onset of abrupt change in symptoms or <24h of last observed medically stable, without non-cardiac cause. (n = 95) | Mean 20 years Range 0.3-25.7 | Low $FEV_1$ risk factor for SCD Low FVC risk factor for SCD $FEV_1$/FVC ratio n.s. |
| Lahousse[13] 2015 | Cohort | Participants aged ≥45 (n=13,471) living in Ommoord area of Rotterdam | COPD diagnosis ($FEV_1$/FVC < 70% from medical records, n=1,615) | SCD: Death <1h of onset of abrupt change in symptoms or <24h of last observed medically stable, without non-cardiac cause. (n=551) | Median 8.8 years IQR 10 years | COPD ↑ risk of SCD HR, 1.34 (1.06-1.70) Frequent exacerbations ↑↑ risk of SCD |
| McGarvey[14] 2012 | Cohort | Study participants with a clinical diagnosis of COPD (n=5,992) | | Unexpected deaths in stable patients <1h. (SCD, 4.4%) or >1h and <24h of last observed medically stable (sudden death, 3.4%) | 4 years | SD occurs relatively commonly in COPD patients:7.7% of fatal cases in patients. Seems independent of disease severity |
| Naksuk[15] 2013 | Cohort | Consecutive patients who underwent ICD implantation. (n=628) | COPD diagnosis ($FEV_1$/FVC < 70%) from medical records (n=1615) | Death (n=152) Appropriate ICD shock (n=138) | Median 4.1 years IQR 2.2-5.7 | COPD ↑ risk Death (p=0.02) and appropriate ICD shock.(p<0.001) |
| Nishiyama[16] 2010 | Cohort | Consecutive patients who survived first elective PCI (n=6,846) and CABG (n=2,910) (total n=9,756) | COPD | SCD: Death <1h of onset of abrupt change in symptoms or <24h of last observed medically stable, without HF, MI, other. (n=140) | Mean 3.5 years SD 1.3 years | COPD HR 2.04 (1.48-2.70, p< 0.001) for SCD |

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| Ozcan[17] 2002 | Cohort | Consecutive patients with AF who underwent AV node ablation. (n=334) | Participants without SCD (n=327) | SCD: death <1h of onset of symptoms or <1h from last seen to when the body was found. (n=9) | COPD from medical records | | Mean 36 months SD 26 months | COPD for SCD RR 14.23 (1.69-119.71, p=0.01) |
| Shih[18] 1988 | Cross-sectional | Ambulatory 24-hour ECGs from stable COPD patients (n=69) | | | | VT (n=22) | | Men and patients with edema are more prone to repetitive ventricular arrhythmia. |
| Sievi[19] 2014 | Case-control | | COPD patients objectively confirmed according to GOLD-guidelines (n=91) | Controls matched for age, CV risk and meds (n=32), and healthy subjects (n=41) | QTc interval from the ECG<br>QT dispersion >60ms | | | QTc was significantly longer in cases vs. matched controls and healthy subjects<br>QT dispersion was not significantly different in cases vs. matched controls. |
| Tukek[20] 2003 | Case-control | | COPD patients (n=41) | Healthy controls (n=32) | SDNN, SDANN , QTc, QTc dispersion from 24h ECG recordings | | | SDNN, SDANN ↓ in cases<br>Max. QTc and QT dispersion↑ in cases |
| Uchmanowicz[21] 2015 | Cross-sectional | Emergency medical service interventions (n=26,219) | Emergency medical service interventions with ICD-10 code I46 (OHSCA, n=245) | Emergency medical service interventions without ICD-10 code I46 (n=26,398) | ICD-10 group J (COPD & bronchial asthma) | | | Chi-square group J and OHSCA p-value 0.184 |
| Urena[22] 2015 | Cohort | Patients who underwent transcatheter aortic valve replacement (n=3,726) | | | COPD | SCD: death within 1h of symptom onset if witnessed or within the previous 24h if unwitnessed. (n=57) | Mean 22 months SD 18 months | COPD ns. HR 1.34(0.77–2.35, p-value 0.305) for SCD |

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| Wang[23] 2007 | Cross-sectional | NPSD due to heart disease or rapid organ deterioration outside hospital or <1h post-ER arrival ( n = 605) | | | COPD (11%) | | | COPD common in NPSD |
| Warnier[24] 2013 | Case-control | | SCA patients with VT/VF age > 40 years (n=1,310) | Controls matched by age, sex and index date (n=5,793) | OPD users of β-blockers, Short-acting β-agonists (SABA), and anticholinergics (ACs) | | | SCA risk 40%↑ in OPD. SCA risk ↑↑ in OPD patients with high CV risk-profile, users of SABA, and AC |
| Yildiz[25] 2002 | Case-control | | COPD patients (n=30) | Age- and sex matched controls (n=31) | 24h ambulatory ECG: SDNN, SDANN, QT interval, QT dispersion | | | In cases SDNN, SDANN↓, QTmax, QTmin , QT dispersion↑ All $p < 0.05$ |
| Zulli[26] 2006 | Cohort | COPD patients without significant comorbidities, and mild to moderate functional impairment (n=246) | | | QTcmax > 440 ms QTc max (continuous variable) QT dispersion, QTc dispersion IC < 80% predicted $PaO_2$ < 60 mmHg | Death (n = 81) | Mean 59.7 range 5-114 months | QTcmax, QT dispersion, IC and $Pa0_2$ associated with mortality (NB: paper uses RR instead of HR for Cox' model.) |
| Zupanic[27] 2014 | I. Case-control II. Pseudo-randomized control study | | I. Patients with COPD (n=31) II. COPD patients who underwent a 4-week rehabilitation program (n=18) | I. Healthy controls (n=31) II. COPD patients who did not undergo 4-week rehabilitation program(n=13) | I. 20-min ECGs SDNN, RMSSD, QTc II. 20-min ECGs SDNN, RMSSD, QTc | | II. 4 weeks | I. SDNN, RMSSD↓in cases. QTc ns. II. SDNN ↑ after program. |

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**Abbreviations of supplementary table 1**

AMI: acute myocardial infarction, AF: atrial fibrillation, ATC code: anatomical therapeutic chemical code, AV-node: atrioventricular node, CABG: coronary artery bypass graft, COPD: chronic obstructive pulmonary disease, CV: cardiovascular, ECG: electrocardiogram, ER: emergency room, $FEV_1$: forced expiratory volume in one second, FVC: forced vital capacity, GOLD: global initiative for chronic obstructive lung disease, h: hour, IC: inspiratory capacity, ICD: implantable cardioverter-defibrillator, ICD-10: international classification of diseases and related health problems 10th revision, ICU: intensive care unit, IQR: interquartile range, IR: incidence ratio, NPSD: non-traumatic prehospital sudden death, ns: not significant, OHCA: out-of-hospital cardiac arrest, OHSCA: out-of-hospital sudden cardiac arrest, OPD: obstructive pulmonary disease, OR: odds ratio, PaO2: partial oxygen pressure in arterial blood, PCI: percutaneous coronary intervention, QTc: heart-rate corrected QT interval, RMSSD: root mean square of successive RR-interval differences, RR: risk ratio, SCA: sudden cardiac arrest, SCD: sudden cardiac death, SD: standard deviation, SDANN: standard deviation of the average of the normal-to-normal RR intervals over 5 minutes, SDNN: standard deviation of normal-to-normal RR intervals, VF: ventricular fibrillation, VT: ventricular tachycardia

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# Chapter 3.2

# Thyroid Function and Sudden Cardiac Death: A Prospective Population-Based Cohort Study

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**Abstract**

*Background* The association between thyroid function and cardiovascular disease is well-established, but no study to date has assessed whether it is a risk factor for sudden cardiac death (SCD). Therefore we studied the association of thyroid function with SCD in a prospective population-based cohort.

*Methods* Participants from the Rotterdam Study ≥45 years with thyroid-stimulating hormone (TSH) or free thyroxine (FT4) measurements and clinical follow-up were eligible. We assessed the association of TSH and FT4 with the risk of SCD using an age- and sex-adjusted Cox-proportional hazards model, in all participants and also after restricting to euthyroid subjects (defined by TSH 0.4-4.0 mIU/L). Additional adjustment included cardiovascular risk factors, notably hypertension, serum cholesterol and smoking. We stratified by age and sex and performed sensitivity analyses by excluding subjects with abnormal FT4 values (0.85-1.95 ng/dL) and including only witnessed SCDs as outcome. Absolute risks were calculated in a competing risk model by taking death by other causes into account.

*Results* We included 10,318 participants with 261 incident SCDs (median follow-up of 9.1 years). Higher levels of FT4 were associated with an increased SCD risk, even in the normal range of thyroid function (hazard ratio 2.28 per 1ng/dL FT4, 95% Confidence Interval, 1.31-3.97). Stratification by age or sex and sensitivity analyses did not change the risk estimates substantially. The absolute 10-year risk of SCD increased in euthyroid participants from 1% to 4% with increasing FT4 levels.

*Conclusions* Higher FT4 levels are associated with an increased risk of SCD, even in euthyroid subjects.

**Introduction**

Thyroid hormone is critical for the development and function of nearly all organs and tissues, with the cardiovascular system being one of the major targets. Thyroid hormone is known to increase heart rate, increase cardiac contractility, alter systolic and diastolic function and decrease systematic vascular resistance [1]. Thyroid dysfunction, even in the subclinical range, is associated with an increased incidence of cardiovascular risk factors and disease [2-4]. Both overt and subclinical hypothyroidism are associated with hypertension, dyslipidemia and coronary heart disease (CHD)[4,5], whereas excess of thyroid hormone, subclinical and overt hyperthyroidism, increases the risk of atrial fibrillation (AF), CHD and heart failure (HF) [2,6]. However, little is known about the association between thyroid (dys)function and the risk of sudden cardiac death (SCD).

SCD is defined as unexpected natural death from a cardiac cause within a short time period, generally <1 hour from the onset of symptoms, in a person without any prior condition that would appear fatal [7]. SCD accounts for over 50% of cardiovascular deaths and 15% to 20% of total mortality [8]. As much as 75 percent of SCDs have been attributed to CHD (known or unknown) and the risk factors for CHD and SCD are therefore very similar and include older age, male sex, hypertension, HF, smoking and dyslipidemia [7,9,10]. Additional risk factors include non-ischemic cardiac disease (e.g. congenital heart disease) and non-cardiac disorders (e.g. drug-induced) [10]. However, the predictability of SCD in the general population remains poor, as almost half of the SCD cases are the first presentation of cardiac disease [11]. It is therefore of crucial importance to identify additional risk factors other than well-established cardiovascular risk factors. We hypothesized that thyroid function is associated with an increased risk of SCD and set to determine this association and possible subgroups at risk in a population-based cohort study.

**Methods**

*Setting*

All analyses were performed in the Rotterdam Study (RS), a prospective population-based cohort study that investigates determinants and occurrence of cardiovascular, neurological, ophthalmologic, psychiatric, and endocrine diseases in the middle-aged and elderly population. The aims and design of the Rotterdam study have been described in detail elsewhere [12]. We included participants from three independent cohorts within the Rotterdam Study. The RS Cohort 1 (RSI) includes participants aged 55 years and older and baseline data were collected during 1990-1993. RS Cohort II (RSII) includes participants aged 55 years and older and baseline data were collected from 2000-2001. For the RS Cohort 3 (RSIII), all residents of Ommoord aged 45 years and over who had not been invited before, were asked to participate and baseline data were collected from 2006 to

2008. The Medical Ethics Committee of the Erasmus University approved the study protocols and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All included participants provided a written informed consent in accordance with the Declaration of Helsinki to participate in the study and to obtain information from their family physicians.

*Study Population*

Participants were eligible for inclusion if they had thyroid-stimulating hormone (TSH) or free thyroxine (FT4) measurements made at baseline visit of the study cohorts RSI (RSI-1), RSII (RSII-1) and RSIII (RSIII-1). Since a number of participants from RSI did not have thyroid measurements at the first visit, they were included in the analyses using data from their third visit 3 (RSI-3) and were follow-up since the date of their laboratory measurement. A total of 10,318 participants from the three cohorts were included in our analyses. All study participants were followed up from the day of baseline laboratory testing to date of SCD, to death from other causes, or to December 12th, 2010, whichever came first.

*Assessment of thyroid function*

For RSI-1, serum TSH (TSH Lumitest; Henning, Berlin, Germany) and F T4 levels (FT4; Vitros, ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Amersham, UK) were determined in a random subset of the baseline serum samples (n=1855). For RSI-3, RSII-1 and RSIII-1, thyroid function assessment was also performed in baseline serum samples for TSH and FT4 (The electrochemiluminescence immunoassay for thyroxine and thyrotropine, "ECLIA", Roche). The two tests' TSH reference ranges did not differ substantially and had a good Spearman correlation co-efficient (0.96 for TSH, $p < 0.0001$and 0.81 for FT4, $p < 0.0001$). We determined the cut-off values for normal range TSH as 0.4-4.0 mIU/L according to national guidelines and our previous studies.[13] The reference range for FT4 was 0.85-1.95 ng/dL (=11-25 pmol/L) [14]. Euthyroidism was defined as a TSH value within the reference range. Hypothyroidism was defined by TSH mIU/L > 4.0 and FT4 <0.85 ng/dL whilst with subclinical hypothyroidism FT4 was still within the reference range. Hyperthyroidism was defined by TSH mIU/L < 0.4 and FT4 > 1.95 ng/dL whilst with subclinical hyperthyroidism FT4 was still within the reference range.

*Sudden cardiac death definition and case ascertainment*

Information on SCD was obtained from medical records and death certificates. Deaths were considered from study entry through December 12$^{th}$ 2010. SCD was defined in accordance with the

Myerburg definition[15], which is endorsed by the European Society of Cardiology[16], as "a natural death due to cardiac causes, heralded by abrupt loss of consciousness within one hour from onset of acute symptoms. Pre-existing heart disease may have been known to be present, but the time and mode of death are unexpected. We included cases of unwitnessed death if the person was seen in a stable medical condition 24 hours preceding death and if there was no evidence of a non-cardiac cause of death. We considered both witnessed and unwitnessed SCD cases for the main outcome, and conducted a sensitivity analysis excluding participants with unwitnessed SCD. In addition, in case of sparse information, cases were labelled as SCD when treating physicians labelled death as sudden or unexpected [17]. Case validation and definition of SCD has been described earlier in previous publications from the Rotterdam Study [17,18]. In short, SCD cases were adjudicated by two independent reviewers. SCD cases were coded as "possible" if there was insufficient information in the medical file regarding the period prior to and until death to code an SCD as "certain". We conducted a sensitivity analysis excluding SCD cases coded as possible. If there was disagreement, this was resolved by consensus meetings. All cases were ultimately reviewed by a senior cardiologist.

*Baseline and other measurements*

Blood pressure was measured twice using a random-zero sphygmomanometer and averaged. Hypertension was defined as having a systolic blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg or using antihypertensives at baseline (diuretics, anti-adrenergic agents, β blockers, calcium channel blockers and RAAS inhibitors). Smoking was categorized in current or non-current smokers. QT-interval was measured on a resting ECG. Heart-rate variability was automatically determined by MEANS. We assessed the standard deviation of the normal-to-normal RR intervals (SDNN)[19]: a time-domain HRV marker, based on all the RR intervals on the 10-second ECGs. For the analyses with heart-rate variability, we removed ECGs with excessive noise, excessive baseline wander, premature ventricular beats, and premature supraventricular beats. Outliers of heart-rate variability values were visually checked and discarded if related to poor signal quality. Pulse rate was measured twice with a pulse oximeter and averaged. Serum total cholesterol was measured using standard laboratory techniques. Diabetes was defined by an impaired fasting glucose ≥ 7 mmol/L, non-fasting glucose use ≥ 11.1 mmol/L or use of glucose lowering medication. Body-mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Ascertainment of AF in the Rotterdam Study has been described in detail previously [20]. In short, it was ascertained using three methods 1) ECG's at baseline and during follow-up, ascertained by a cardiologist, 2) medical information obtained from the General Practitioners, after ascertainment of

ECG and 3) national registry of hospital discharge diagnosis. Cases of incident HF were obtained by continuous monitoring of participants during follow-up through automated linkage with files from general practitioners [12,21]. All available data on HF, such as hospital discharge letters and notes from general practitioners, were copied from the medical records. HF was adjudicated in accordance with in accordance with the guidelines of the European Society of Cardiology [22] and included typical signs and symptoms of heart failure confirmed by objective evidence of cardiac dysfunction.

*Statistical analysis*

We assessed the association of TSH or FT4 at baseline, in separate models, with the risk of SCD both in the full and the euthyroid range of thyroid function (defined by TSH 0.4-4.0 mIU/L), using Cox-proportional hazards model. We also evaluated the risk of SCD according to thyroid state with euthyroidism as reference. All primary analyses were sex and age-adjusted. Multivariable models additionally adjusted for cohort, pulse rate, hypertension, serum cholesterol, diabetes mellitus, BMI, smoking and QT-interval, after applying multiple imputation for missing data of these covariates (missingness < 3% for all covariates). Variables in the multivariable models represent the most common confounding or mediating factors of the association between thyroid function and SCD. Absolute 10 year risk-probabilities were estimated, given the covariates used in the primary Cox-proportional hazards model, according to the Fine and Gray model [23]. This model takes the competing risks of death due to all other (non-SCD) causes into account. We also derived the subdistribution HRs (SHR) for included variables from this model.

Pre-defined stratification by sex, age categories and cohort were performed. The cut-off of the age categories was 65 years, which is close to the median age of our population. Sensitivity analyses included 1) restricting to the analyses euthyroid individuals with witnessed SCDs as outcome 2) excluding possible SCD events 3) restricting the analyses to euthyroid subjects with normal FT4 values and excluding individuals using thyroid medication defined as hormone replacement therapy or anti-thyroid drugs (e.g. Methimazole), 4) additionally censoring participants at thyroid medication use during follow-up 5) excluding abnormal FT4 values and thyroid function altering medication (defined as thyroid medication, amiodarone or corticosteroids) and 6) additionally excluding participants with CHD and HF at baseline 7) adjusting the analyses additionally for HRV. We explored the possible role of incident AF or HF in the association between thyroid function and SCD by censoring the analyses at time of AF or HF. Follow-up data on HF incidence was available in 6893 participants. Furthermore, we calculated the hazard ratios for total mortality, total cardiovascular mortality as well as non-SCD cardiovascular mortality for comparison. The proportional hazards assumption was assessed with plots and test of Schoenfeld residuals. The proportional hazards

assumption was met for all analyses. We also performed a goodness-of-fit test for the Fine and Gray model for the absolute risk estimation, using the Zou Laird Fine test, and this revealed no linear, quadratic or log time varying effects of ft4 in the full or euthyroid range ($p > 0.35$ for all analyses). Non-linearity was tested using fractional polynomials and adding quadratic terms to the model. The best fit was determined to be linear. TSH was log transformed for all continuous analyses to approximate normality while FT4 levels were normally distributed. Fractional polynomials were performed using STATA (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). Schoenfeld plot, Schoenfeld test and competing risk calculations were performed in R (survival and cmprsk packages R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2). All other statistical analyses were performed using SPSS version 21 (SPSS IBM, New York, U.S.A). Results of this study are reported according to the STROBE statement guidelines [24].

**Results**

*Baseline characteristics*

We included 10,318 participants with a maximum follow-up of 21.2 years and a median of 9.2 years (interquartile range 3.8-11.1). A total of 261 SCD events occurred during follow-up (incidence rate = 3.1 per 1000 person-years) and completeness of follow-up was 97.9% [25]. When restricting to euthyroid subjects, there were 231 cases. Of the total number of participants, 10,314 had TSH measurements and 10,225 had FT4 measurements. Baseline characteristics are shown in Table 1.

*Thyroid function and SCD*

Higher levels of FT4 were associated with an increased risk of SCD with a hazard ratio (HR) of 1.87 per 1 ng/dL increase of FT4 (95% Confidence Interval [CI], 1.09-2.86) (Table 2). The log relative hazard between FT4 and SCD as well as the distribution of FT4 in the population are plotted in Figure 1. When restricting to euthyroid subjects, there was an increased risk of SCD events with a HR of 2.26 (95% CI , 1.30-3.94). In line with an increased risk for higher FT4 levels, higher TSH levels were associated with a concomitant decreased risk but failed to reach statistical significance (HR 0.92, 95% CI, 0.80-1.04, per one unit increase of natural log transformed TSH). The highest compared to the lowest tertile of FT4 had a higher risk of SCD in the full range (HR 1.35, 95% CI, 1.01-1.83, p for trend 0.022) (Table 3). Sensitivity analyses did not alter risk estimates substantially (Table 4). When restricting to euthyroid subjects with normal FT4 values, the absolute 10 year risks increased from 1% to almost 4% with increasing FT4 values (Figure 2).

There seemed to be a differential risk of SCD in different age groups (Supplemental Table 1).

However, the number of cases in the younger age category (≤ 65 years) was small and the p for interaction (p >0.30) insignificant. Stratification analyses for age, sex and cohort did not show differential risks (p for interaction for all analyses > 0.40) (Supplemental Table 1). Participants with subclinical or overt hypo- or hyperthyroidism did not have a higher risk of SCD compared to euthyroid participants (Supplemental Table 2), but the number of events per category was small. Censoring the analyses at time of incident AF or HF did not alter risk estimates meaningfully (Supplemental Table 3 and 4), neither did censoring the analyses for both AF and HF (data not shown). The SHRs obtained from the competing risk model were slightly more attenuated for all variables compared to the HRs obtained from the conventional Cox-proportional hazard model, but remained qualitatively similar for the FT4 analyses (Supplemental Table 5). As compared to the SCD analyses the risk estimates were slightly higher for non-SCD cardiovascular mortality and slightly lower for total mortality (Supplemental Table 6).

**Table 1. Baseline characteristics of included participants**

| Variable | Mean (SD)* |
|---|---|
| Number of individuals in the study | 10,318 |
| Age, in years | 64.7 (9.5) |
| Age range, years | 46-106 |
| Women N (%) | 5886 (57.0) |
| Diabetes mellitus N ( %) | 1042 (10.1) |
| BMI | 27.2 (4.2) |
| Cholesterol | 5.89 (1.40) |
| Smoking current N (%) | 2372 (23.0) |
| Hypertension N (%) | 6142 (59.5) |
| Pulse rate | 71 (11) |
| Median TSH (IQR) mIU/L | 1.85 (1.23-2.72) |
| FT4 ng/dL | 1.23 (0.20) |
| Thyroid hormone replacement therapy use N (%) | 298 (2.9) |

*unless specified otherwise

Abbreviations: BMI Body-Mass Index, IQR interquartile range, FT4 free thyroxine, SCD Sudden Cardiac Death, SD standard deviation, TSH Thyroid-Stimulating Hormone, N number, TSH is missing in 4 participants, FT4 is missing in 93 participants

**Table 2. Association between thyroid function and the risk of SCD**

| Thyroid Function Measurements | SCD Events | Total Participants | HR (95% CI) Model 1 | HR (95% CI) Model 2 | HR (95% CI) Model 3 |
|---|---|---|---|---|---|
| Full range of measurement | | | | | |
| TSH mIU/L | 261 | 10,314 | 0.91 (0.80-1.04) | 0.91 (0.80-1.03) | 0.92 (0.80-1.04) |
| FT4 ng/dL | 249 | 10,225 | 1.87 (1.18-2.96) | 1.76 (1.10-2.86) | 1.77 (1.09-2.86) |
| | | | | | |
| Euthyroid participants* | | | | | |
| TSH mIU/L | 231 | 8953 | 0.81 (0.63-1.04) | 0.80 (0.62-1.03) | 0.80 (0.62-1.04) |
| FT4 ng/dL | 222 | 8881 | 2.54 (1.48-4.40) | 2.24 (1.31-4.40) | 2.26 (1.30-3.94) |

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes, body-mass index and smoking. Model 3: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes, body-mass index, smoking and QT-interval. *Euthyroidism is defined by TSH 0.4-4.0 mIU/L.

TSH was log transformed for the continuous analyses, results for TSH are per one unit increase of the natural logarithm of TSH and results for FT4 per 1 ng/dL.

Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

**Table 3. Associations of tertiles of TSH and FT4 with SCD**

| | SCD Events | Total Participants | HR (95% CI) Model 1 | HR (95% CI) Model 2 | HR (95% CI) Model 3 |
|---|---|---|---|---|---|
| Full range | | | | | |
| TSH tertiles | | | | | |
| 0.01-1.43 | 113 | 3436 | REFERENCE | REFERENCE | REFERENCE |
| 1.44-2.36 | 81 | 3442 | 0.88 (0.66-1.17) | 0.87 (0.65-1.15) | 0.86 (0.65-1.15) |
| 2.37-80.64 | 67 | 3436 | 0.78 (0.57-1.05) | 0.79 (0.58-1.07) | 0.78 (0.58-1.06) |
| *P for trend* | | | *0.17* | *0.18* | *0.18* |
| FT4 tertiles | | | | | |
| 0.12-1.14 | 72 | 3420 | REFERENCE | REFERENCE | REFERENCE |
| 1.14-1.29 | 71 | 3404 | 1.03 (0.74-1.43) | 1.03 (0.74-1.44) | 1.03 (0.74-1.44) |
| 1.29-4.73 | 106 | 3401 | 1.39 (1.03-1.87) | 1.35 (1.01-1.83) | 1.35 (1.01-1.83) |
| *P for trend* | | | *0.008* | *0.026* | *0.022* |

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking. Model 3: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes, body-mass index, smoking and QT-interval.

*Euthyroidism is defined by TSH 0.4-4.0 mIU/L. FT4 is in ng/dL.

Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

**Table 4: Sensitivity analyses for association between thyroid function and risk of SCD in euthyroid subjects***

| Sensitivity analysis | SCD Events | Total participants | HR (95% CI) Model 1 | HR (95% CI) Model 2 |
|---|---|---|---|---|
| Restricting to witnessed SCD's | | | | |
| TSH mIU/L | 133 | 8953 | 0.74 (0.53-1.04) | 0.74 (0.53-1.03) |
| FT4 ng/dL | 126 | 8881 | 3.36 (1.69-6.66) | 3.39 (1.68-6.81) |
| Excluding possible SCD's | | | | |
| TSH mIU/L | 195 | 8953 | 0.85 (0.65-1.21) | 0.84 (0.64-1.11) |
| FT4 ng/dL | 186 | 8881 | 2.20 (1.17-4.15) | 2.02 (1.07-3.82) |
| Excluding abnormal FT4 values† & thyroid medication at baseline ‡ | | | | |
| TSH mIU/L | 216 | 8642 | 0.83 (0.64-1.08) | 0.83 (0.64-1.08) |
| FT4 ng/dL | 216 | 8642 | 3.00 (1.44-6.18) | 2.79 (1.34-5.71) |
| Excluding abnormal FT4 values† & thyroid medication at baseline and censoring participants with thyroid medication use at during follow-up‡ | | | | |
| TSH mIU/L | 216 | 8642 | 0.83 (0.64-1.08) | 0.83 (0.64-1.08) |
| FT4 ng/dL | 216 | 8642 | 2.99 (1.45-6.17) | 2.80 (1.34-5.82) |
| Excluding abnormal FT4 values† and thyroid function altering medication at baseline§ | | | | |
| TSH mIU/L | 206 | 8519 | 0.81 (0.62-1.06) | 0.81 (0.62-1.06) |
| FT4 ng/dL | 206 | 8519 | 2.42 (1.14-5.16) | 2.25 (1.05-4.82) |
| Excluding abnormal FT4 values†, thyroid function altering medication§ & prevalent HF and CHD | | | | |
| TSH mIU/L | 145 | 7746 | 0.80 (0.58-1.09) | 0.80 (0.58-1.10) |
| FT4 ng/dL | 145 | 7746 | 2.48 (1.00-6.11) | 2.22 (0.95-5.52) |

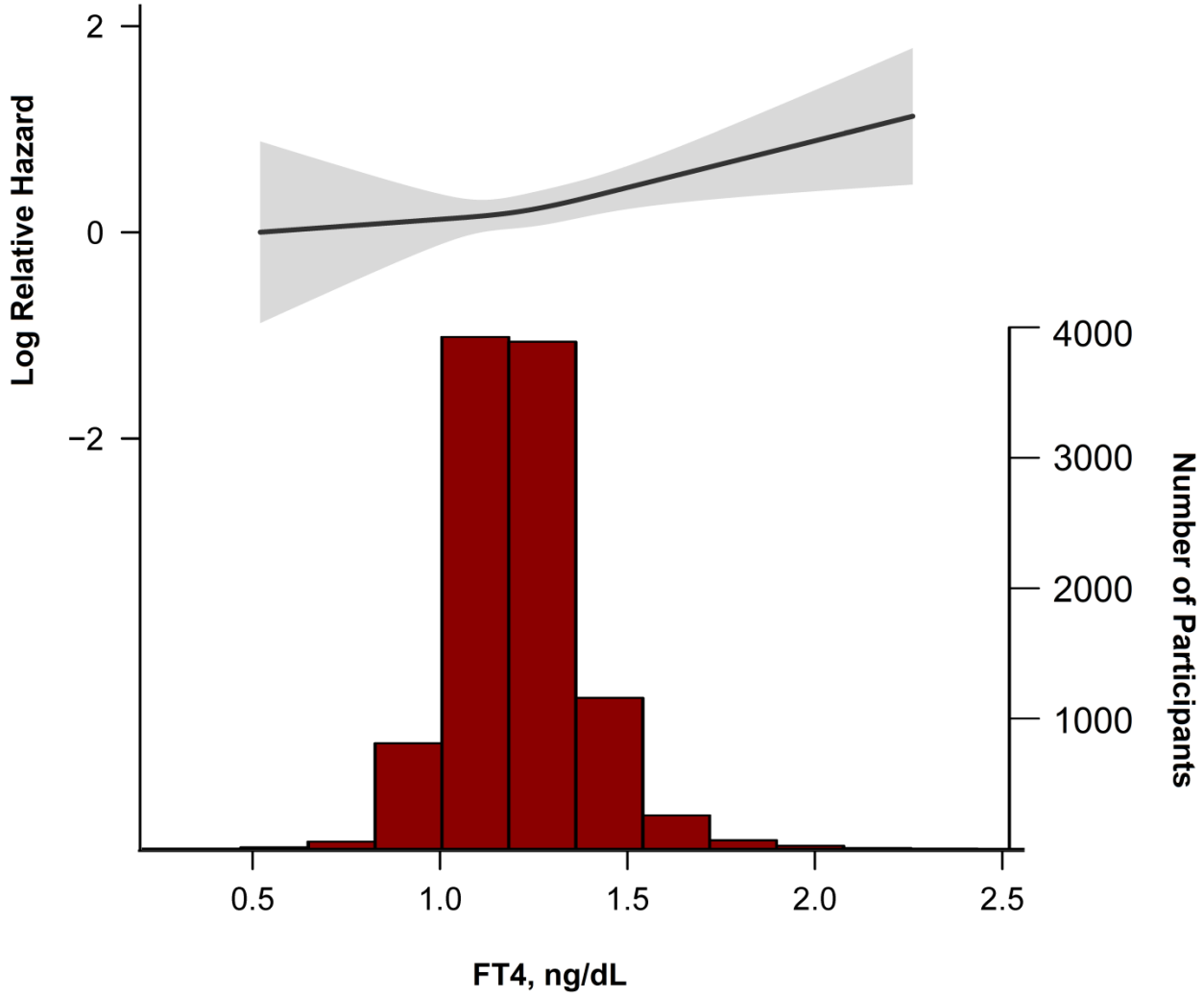
| | | | | |
|---|---|---|---|---|
| Additionally adjusting for HRV | | | | |
| TSH mIU/L | 135 | 7146 | 0.91 (0.65-1.28) | 0.91 (0.65-1.28) |
| FT4 ng/dL | 133 | 7131 | 3.38 (1.41-8.14) | 3.04 (1.27-7.03) |

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking.* Euthyroidism is defined by TSH 0.4-4.0 mIU/L. †Normal range of FT4 0.85-1.95 ng/dL. ‡Thyroid medication is defined as use of thyroid hormone replacement therapy or any anti-thyroid drug. §Thyroid function altering medication is defined as use of thyroid medication, amiodarone or corticosteroids.

TSH was log transformed for the continuous analyses, results are per one unit increase of the natural logarithm of TSH. Results for FT4 are per 1 unit increase.

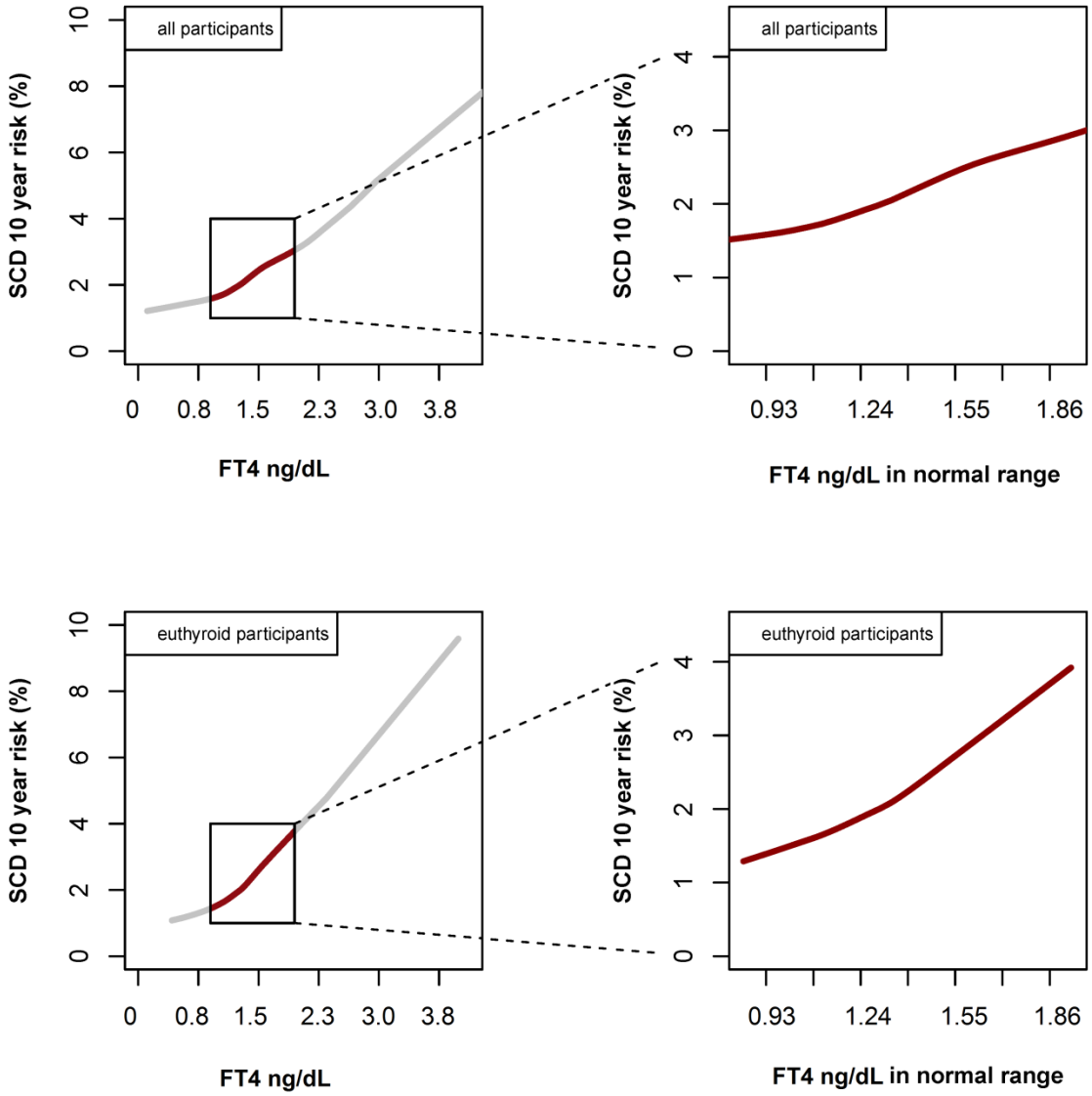
Abbreviations: CHD coronary heart disease, CI confidence interval, FT4 free thyroxine, HF heart failure, HR hazard ratio, HRV heart rate variability, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

**Figure 1. Log relative hazard plotted against absolute FT4 ng/dL values with historgram of FT4 distribution in the population**




Estimates for the relative hazard were derived using 3 restricted cubic splines with a p for non-linearity = 0.49

**Figure 2. Absolute 10-years risks of SCD by FT4 values**




Absolute 10-years risks of SCD were calculated taking competing risk of death by other causes into account, and are plotted against FT4 values in all participants in the two upper figures and in euthyroid participants in the lower two. The normal range of FT4 is highlighted in the two right figures.

FT4 free thyroxine, SCD Sudden Cardiac Death.

**Discussion**

In the current study, higher FT4 levels were associated with an increased relative and absolute risk of SCD, even in the normal range of thyroid function. The relative risk estimates were similar in the analyses with only witnessed SCDs as outcome or when excluding prevalent cases of cardiac diseases and those using thyroid-function altering medication.

This is the first study addressing the association between thyroid function and SCD in the general population. Two earlier studies that evaluated the relation between thyroid function and SCD were conducted in specific patient populations (HF patients and diabetic hemodialysis patients). These studies did not investigate the association of FT4 with SCD and had conflicting results [26,27]. The SCD-HeFT study, conducted in HF patients, found no difference in SCD risk between TSH categories [27]. The 4D study, investigating 1000 diabetic hemodialysis patients, reported a higher risk of SCD in subjects with subclinical hyperthyroidism [26]. In the current study, additional adjustment for diabetes mellitus at baseline and exclusion of participants with prevalent HF did not alter risk estimates substantially. In addition, stratification by age and sex showed no differential risk. This suggests that the association of higher FT4 levels with an increased risk of SCD is not driven by a certain subgroup.

Thyroid hormone is an important overall regulator of the cardiovascular system [1]. The pathways through which thyroid hormone can interfere with the cardiovascular system are both direct as well as indirect (e.g. cardiovascular risk factors). Thyroid hormone directly influences the adrenergic system where it has a stimulatory effect on beta-adrenergic signaling leading to positive chronotopic, dromotropic and inotropic effects [28]. This hyperdynamic state, which causes many of the symptoms in hyperthyroid patients, could be one of the mechanisms explaining the relation between thyroid hormone and SCD. Furthermore, thyroid hormones have been shown to lead to QT-interval prolongation [29,30], which in turn is related to cardiovascular disease in general [31] and SCD in particular [18]. Another pathway could be through various cardiovascular risk factors related to thyroid dysfunction, and thus leading to ischemic heart disease, in turn a large contributor in SCD. Subclinical thyroid dysfunction has also been related to CHD and HF In large collaborative individual participant efforts[2-4]. In the current study we did not find evidence for either hypotheses as including pulse rate, reflecting a chronotropic effect of adrenergic system stimulation, and various cardiovascular risk factors, including QT-interval duration and HRV, did not change risk estimates in the multivariable analyses. The exact mechanism for the association between thyroid hormone and SCD therefore still needs to be determined. Alternative pathways could be via the effects of thyroid hormone on the activity and availability of several cation transporters such as cardiac Na-K-ATPase [32,33] or via the transcription and translation of several cardiac genes (e.g. α- and β-myosin heavy chain genes) [34].

We find an effect of FT4 on the risk of SCD, while the association with TSH is less evident. These findings seem to be in line with previous literature investigating the risk of thyroid function on several clinical endpoints [14,35-37]. Thyroid hormone levels are regulated by the hypothalamus-pituitary-thyroid axis, which has a unique set point for each individual [38]. There is a wide variety of factors that can modulate this set point, including illness and ageing. A change in set point over time might be an explanation why the association with FT4 is stronger than for TSH. We do not have repeated measures of thyroid function and can therefore not investigate if changes in TSH over time could explain the apparent discrepant findings between FT4 and TSH.

We find roughly similar estimates for SCD, (non-SCD) CVD mortality, and total mortality. Similar associations for these outcomes are not surprising, since the association between thyroid dysfunction and CVD mortality (mainly consisting of CHD mortality) has been described in several previous studies and is well established [4,39]. Guidelines recommend treatment of subclinical thyroid disease from a certain TSH value cut-off mainly based on CHD outcomes from large collaborative studies. Cardiovascular diseases are the leading cause of burden of disease and mortality in elderly worldwide[40]. In high-income country, cancer mortality is the second leading cause. Although the underlying mechanisms are probably different to CVD mortality, the association of high thyroid dysfunction and cancer has been previously described and could therefore also contribute to the association found with total mortality. The associations of thyroid function with CVD mortality and of thyroid function and SCD seem to both be independent of cardiovascular risk factors. However, bigger sample size and more detailed data are needed to determine whether these association share the same or have distinct pathways.

SCD develops within a short timeframe and there is limited time to start intervention and cardiopulmonary resuscitation. Therefore, identification of modifiable risk factors is crucial in the setting of identification of certain populations or subgroups at risk, as well as screening and prevention. In the current study we were not able to demonstrate differential risks by age and sex, but the association between thyroid function and SCD was more pronounced within the normal range of thyroid function. This can likely be explained by the fact that participants with a thyroid function outside the reference range have a higher probability of being treated, which will alter their risk. In contrast, people with FT4 levels in the normal range have no indication for treatment and are usually stable with very little intra-individual variation, reflecting the individual set point. When we exclude those using levothyroxine or anti-thyroid drugs at baseline or during follow-up, risk estimates only slightly shift towards the risk in euthyroid participants. However in our study, no conclusions on the benefits or risks of thyroid medication can be drawn. Ideally, this should be

investigated in a randomized controlled trial.

*Strengths and limitations*

Major strengths of our study are the number of participants and covariates included in the analyses. All data were collected irrespective of the current hypothesis. Furthermore, we were able to phenotype SCD in detail and make a distinction between witnessed and unwitnessed types. The setting of a population-based cohort and the long follow-up allowed for estimation of long-term absolute risks. Limitations include possible residual confounding and the availability of one baseline measurement of thyroid function, not allowing for assessment of temporal changes of TSH and FT4. Furthermore, there were a limited number of participants with FT4 values outside the reference range (190 participants). The vast majority of participants in the study were of Caucasian descent and therefore our results might not be generalizable to other populations.

*Conclusions*

In summary, we describe an increased risk of SCD with increasing FT4 levels, even when restricting to euthyroid participants.

**Clinical Perspective**

*1) What is new?*

Thyroid dysfunction, even in the subclinical range, is a known risk factor for coronary heart disease, but the association with SCD is unknown. In this study, we evaluated the association of thyroid function measurements and SCD in over 10,000 participants of a population-based study. In participants with a normal thyroid function (i.e. euthyroid participants), we show that higher free thyroxine levels are associated with a 2.5-fold increased risk of SCD, independent of cardiovascular risk factors. The absolute risk to die from SCD in the upcoming 10 years increased from 1% to 4% with higher free thyroxine levels.

*2) What are the clinical implications?*

The predictability of sudden cardiac death (SCD) in the general population is poor and almost half of the SCD cases are the first presentation of cardiac disease. Therefore, identification of additional risk factors for SCD is important. Free thyroxine could be an additive marker in risk prediction and screening in the general population. Further research is needed to assess the possible additional benefit of the use of FT4 levels in risk stratification for prevention of SCD.

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**Supplemental table 1. Stratified associations for thyroid function and SCD**

| Stratification variables | **Thyroid Function Measurement** | **SCD events /participants** | **HR (95% CI), Model 1** | **HR (95% CI), Model 2** |
|---|---|---|---|---|
| Age categories | TSH mIU/L | | | |
| **≤ 65 years** | | 68/5871 | 1.08 (0.79-1.48) | 1.09 (0.93-1.28) |
| **> 65 years** | | 193/4443 | 0.88 (0.77-1.01) | 0.87 (0.77-1.00) |
| | *P for interaction* | | *0.31* | *0.31* |
| | FT4 ng/dL | | | |
| **≤ 65 years** | | 66/5855 | 0.80 (0.23-2.86) | 0.75 (0.21-2.69) |
| **> 65 years** | | 183/4370 | 2.28 (1.37-3.74) | 2.09 (1.26-3.49) |
| | *P for interaction* | | *0.74* | *0.60* |
| Sex | TSH mIU/L | | | |
| **Men** | | 139 / 4432 | 0.92 (0.75-1.13) | 0.91 (0.74-1.13) |
| **Women** | | 122 / 5882 | 0.91 (0.78-1.07) | 0.91 (0.78-1.08) |
| | *P for interaction* | | *0.93* | *0.93* |
| | FT4 ng/dL | | | |
| **Men** | | 132/4397 | 2.48 (1.05-5.84) | 2.30 (0.96-5.46) |
| **Women** | | 117/5828 | 1.64 (0.90-2.96) | 1.50 (0.81-2.82) |
| | *P for interaction* | | *0.43* | *0.42* |
| Cohort | TSH mIU/L | | | |
| **I** | | 210/4453 | 0.94 (0.81-1.08) | 0.92 (0.80-1.07) |
| **II** | | 42/2477 | 0.89 (0.64-1.23) | 0.93 (0.79-1.09) |
| **III** | | 9/3451 | 0.69 (0.37-1.26) | 0.74 (0.54-1.01) |
| | *P for interaction* | | *0.51* | *0.58* |
| | FT4 ng/dL | | | |
| **I** | | 198/4335 | 1.84 (1.13-3.00) | 1.83 (1.08-3.10) |
| **II** | | 42/2466 | 2.30 (0.49-10.93) | 2.43 (0.70-8.47) |
| **III** | | 9/3424 | 2.71 (0.08-97.26) | 3.02 (0.53-17.06) |
| | *P for interaction* | | *0.87* | *0.72* |

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking. TSH was log transformed for the continuous analyses, results are per one unit increase of the natural logarithm of TSH. Results for FT4 are per 1 ng/dL increase. Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

**Supplemental table 2: Thyroid function and SCD risk**

| Thyroid Function Measurement | SCD events | Participants | HR (95% CI), Model 1 | HR (95% CI), Model 2 |
|---|---|---|---|---|
| Hypothyroidism | 4 | 80 | 1.59 (0.59-4.29) | 1.53 (0.57-4.16) |
| Subclinical hypothyroidism | 14 | 945 | 0.64 (0.37-1.10) | 0.65 (0.38-1.12) |
| Euthyroidism | 231 | 9006 | REFERENCE | REFERENCE |
| Subclinical hyperthyroidism | 11 | 312 | 1.02 (0.56-1.88) | 1.05 (0.57-1.93) |
| Hyperthyroidism | 1 | 32 | 1.40 (0.20-10.00) | 1.18 (0.16-8.48) |

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking.

Abbreviations: CI confidence interval, HR hazard ratio, SCD sudden cardiac death

**Supplemental Table 3: Association between thyroid function and the risk of SCD when censoring for incident atrial fibrillation**

| Thyroid function measurements | SCD Events | Total participants | HR (95% CI), Model 1 | HR (95% CI), Model 2 | HR (95% CI), Model 3 |
|---|---|---|---|---|---|
| Full range of measurement | | | | | |
| TSH mIU/L | 238 | 10,314 | 0.94 (0.82-1.08) | 0.94 (0.82-1.08) | 0.93 (0.81-1.07) |
| FT4 ng/dL | 228 | 10,225 | 1.91 (1.17-3.11) | 1.85 (1.11-3.09) | 1.85 (1.10-3.09) |
| Euthyroid participants* | | | | | |
| TSH mIU/L | 211 | 8953 | 0.87 (0.67-1.14) | 0.87 (0.66-1.13) | 0.86 (0.66-1.13) |
| FT4 ng/dL | 203 | 8881 | 2.91 (1.56-5.44) | 2.61 (1.40-4.88) | 2.60 (1.39-4.87) |

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking. Model 3: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes, body-mass index, smoking and QT-interval. *Euthyroidism is defined by TSH 0.4-4.0 mIU/L.

TSH was log transformed for the continuous analyses, results are per one unit increase of the natural logarithm of TSH. Results for FT4 are per 1 ng/dL increase.

Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

**Supplemental Table 4: Sensitivity analysis of the association between thyroid function and the risk of SCD in those with follow-up data on incident heart failure available**

| Thyroid function measurements | SCD Events | Total participants | HR (95% CI), Model 1 | HR (95% CI), Model 2 | HR (95% CI), Model 3 |
|---|---|---|---|---|---|
| **All participants with incident heart failure data** | | | | | |
| Full range of measurement | | | | | |
| TSH mIU/L | 252 | 6892 | 0.92 (0.81-1.05) | 0.92 (0.81-1.05) | 0.92 (0.80-1.05) |
| FT4 ng/dL | 240 | 6801 | 1.87 (1.17-2.98) | 1.77 (1.09-2.87) | 1.77 (1.08-2.88) |
| Euthyroid participants* | | | | | |
| TSH mIU/L | 222 | 5898 | 0.83 (0.64-1.07) | 0.83 (0.64-1.07) | 0.83 (0.64-1.07) |
| FT4 ng/dL | 213 | 5827 | 2.57 (1.49-4.54) | 2.29 (1.31-4.01) | 2.29 (1.31-4.02) |
| **Censoring at time of incident heart failure** | | | | | |
| Full range of measurement | | | | | |
| TSH mIU/L | 210 | 6892 | 0.93 (0.81-1.07) | 0.93 (0.80-1.07) | 0.93 (0.80-1.07) |
| FT4 ng/dL | 202 | 6801 | 1.80 (1.07-3.02) | 1.69 (0.98-2.91) | 1.69 (0.98-2.91) |
| Euthyroid participants* | | | | | |
| TSH mIU/L | 183 | 5898 | 0.81 (0.61-1.07) | 0.81 (0.61-1.07) | 0.81 (0.61-1.07) |
| FT4 ng/dL | 177 | 5827 | 2.48 (1.34-4.59) | 2.23 (1.20-4.15) | 2.23 (1.19-4.16) |

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking. Model 3: adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index, smoking and QT-interval. *Euthyroidism is defined by TSH 0.4-4.0 mIU/L. TSH was log transformed for the continuous analyses, results are per one unit increase of the natural logarithm of TSH. Results for FT4 are per 1 unit increase.

Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

**Supplemental Table 5: Methodological sensitivity analysis for the association of thyroid function, age and sex with the risk of SCD**

| Model variables | SCD Events | Total participants | HR (95% CI), Model 1 | SHR (95% CI), Model 1 |
|---|---|---|---|---|
| Full range of measurement of thyroid function | | | | |
| FT4 ng/dL | 249 | 10,225 | 1.87 (1.18-2.96) | 1.65 (1.02-2.69) |
| Age per year | 249 | 10,225 | 1.09 (1.08-1.09) | 1.05 (1.03-1.06) |
| Sex (female vs male) | 249 | 10,225 | 0.51 (0.46, 0.57) | 0.60 (0.47,0.77) |
| | | | | |
| Euthyroid participants* | | | | |
| FT4 ng/dL | 222 | 8881 | 2.54 (1.48-4.40) | 2.10 (1.20-3.68) |
| Age per year | 222 | 8881 | 1.09 (1.08-1.10) | 1.05 (0.03-1.07) |
| Sex (female vs male) | 222 | 8881 | 0.51 (0.46, 0.57) | 0.59 (0.45-0.77) |

Model 1: including, FT4, age and sex. *Euthyroidism is defined by TSH 0.4-4.0 mIU/L. Results for FT4 are per 1 ng/dL. HR are obtained from a Cox-proportional Hazards Model, SHR are obtained from the estimates of a competing risk model taking death by other causes into account.

Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, SHR subdistribution hazard ratio, TSH thyroid-stimulating hormone.

**Supplemental Table 6: Comparison analysis for the association of thyroid function, with the risk of SCD, CVD mortality non-SCD CVD mortality and total mortality**

| | HR (95% CI), CVD deaths | HR (95% CI), CVD non-SCDs | HR (95% CI), Total mortality |
|---|---|---|---|
| Full range of measurement of thyroid function | | | |
| FT4 ng/dL | 1.88 (1.69-2.08) | 1.93 (1.71-2.18) | 1.67 (1.57-1.78) |
| Euthyroid participants* | | | |
| FT4 ng/dL | 2.47 (2.19-2.79) | 2.58 (2.24-2.97) | 2.08 (1.92-2.25) |

These analyses are adjusted for sex, age, cohort, pulse rate, hypertension, cholesterol, diabetes, body-mass index, smoking and QT-interval. *Euthyroidism is defined by TSH 0.4-4.0 mIU/L. Results for FT4 are per 1 ng/dL. From the total of 10.381 participants, 611 had a non-SCD CVD death and 872 total CVD deaths. There were 2541 deaths in total.

Abbreviations: CVD cardiovascular disease, CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death,

# General Discussion

*Overview*

The overarching goal of this thesis was to contribute to the knowledge of electrocardiographic (ECG) markers for the risk assessment of sudden cardiac death (SCD). ECG markers are commonly used for research and in the clinic, but their capability to predict SCD is still less than perfect.[1] To improve the situation, new ECG markers have been (and are being) devised, e.g. QT variability, but these have to be studied intensively before they can be put to practical use by clinicians. Necessary studies include analyses of the associations of a marker with cardiac morbidity and mortality, and studies of a marker's dependence on and correlation with other markers, most importantly heart rate. Studies of normal limits of a marker can be used to set optimal cut-off values for risk stratification.

*Definition of SCD*

One of the hurdles in the study of SCD is its definition. The most commonly used definition stipulates that SCD is a natural unexpected death due to cardiac causes that occurs within one hour of the onset of acute symptoms.[2] This definition has obvious limitations: the interval of one hour is arbitrary and not grounded in any study linking the time frame to underlying pathology. Also, it does not go into the various causes and is not concerned with a differentiation between arrhythmias directly leading to SCD, like ventricular tachycardia and ventricular fibrillation (VT/VF), pulseless electric activity (PEA), and asystole.[3] In most studies the culprit rhythm of the vast majority of SCD cases is unknown, so it is evident that the definition of SCD is based on practicality rather than on pathology and physiology. A practical adaptation to the definition of SCD is the inclusion by many studies of unwitnessed cases.[4] In the definition for the unwitnessed cases, death has occurred in a person observed to be in a stable medical condition for at least 24 hours before discovery, and in whom a non-cardiac cause has not been found. Again, the time frame is grounded in common sense and educated opinion, but has no evidential support. It could be argued that there is more misclassification of unwitnessed cases due to the inherent lack of information about symptoms before death. However, whether a case of sudden death is witnessed or not is essentially a matter of chance, and by excluding all unwitnessed cases the incidence of SCD is probably underestimated. Future studies could significantly improve upon this by recording the rhythm before death: large-scale unobtrusive ECG monitoring in high-risk groups could yield the necessary empirical data. This material could also be used to empirically ground the time frame used in the definition of SCD.

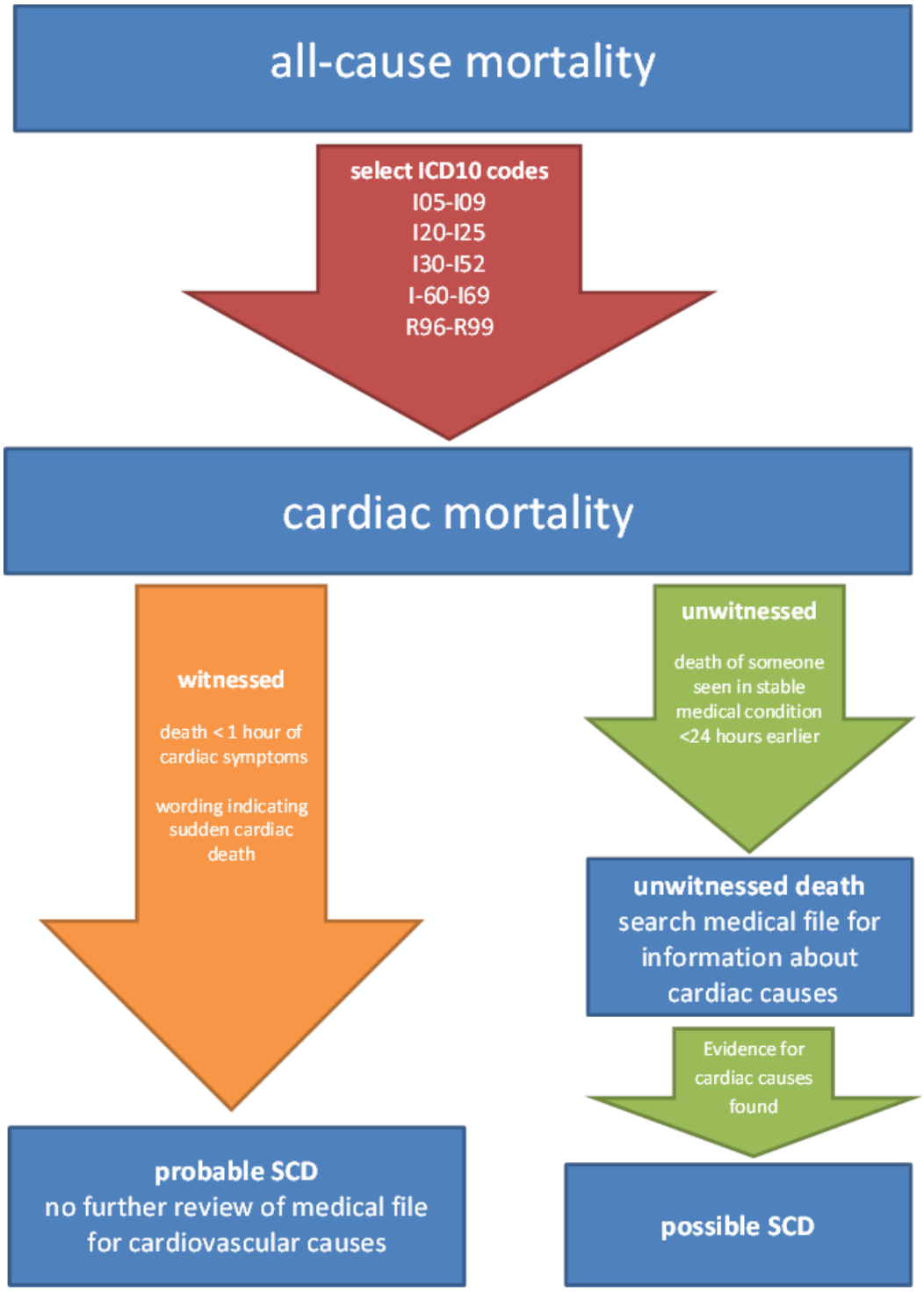
*Validation of SCD*

SCD cases can be ascertained either by retrieving and scrutinizing medical records immediately after death, or by using death certificates. Unfortunately, death certificates often lack information

concerning the circumstances of death, and it was discovered that the use of death certificates leads to an overestimation of the SCD incidence.[4] More importantly, SCD cases ascertained by death certificates correlated poorly with physician diagnoses.[4] Most papers referred in this thesis used data from the Rotterdam study using a two-step prospective method of SCD ascertainment and validation. This process is described in detail in Figure 1. The initial ICD10 [International statistical classification of diseases and related health problems, 10th revision] coding was performed by a study physician and validated by a medical specialist pertinent to the field. For validation of SCD, the medical files of deaths associated with relevant ICD10 codes were selected for a second review by two physicians working independently. The relevant ICD10 codes were mainly cardiac causes of death, but also included deaths due to strokes. We checked the strokes to make sure that none of them were misclassified cardiac deaths. Suspected SCD cases were reviewed and discussed in consensus meetings with an experienced cardiologist. Using this methodology, we found an incidence of SCD in the Rotterdam Study that was comparable to the incidence found in other studies (42 per 1000 person-years).[5]

Although the use of witnessed SCD cases only is too restrictive, suppletion with unwitnessed cases is likely to introduce misclassification due to the relative dearth of information about the circumstances at death. For that reason, it is advisable to use only the witnessed SCDs in sensitivity analyses.

**Figure 1: Flowchart of ascertainment of SCD cases**




*Abbreviations: SCD: Sudden cardiac death, ICD10: International statistical classification of diseases and related health problems, 10th revision*

*Re-evaluating the heart-rate correction of established ECG markers*

Bazett's formula for heart-rate correction of the QT interval overestimates the QTc at higher heart rates,[6,7] It is nevertheless still the most used QT correction formula in practice. Chapter 1.3 highlights one of the problems that can occur when using a biased heart-rate correction: when we studied the effects of tricyclic antidepressants on the QT interval, we found that QTc Bazett was spuriously increased due to the concomitant increase in heart rate caused by these drugs. When we used QTc according to Fridericia, no such increase appeared. Heart-rate variability (HRV) is another ECG marker known to have a strong association with heart rate.[8,9] One study showed this association to be exponential, which would imply that the usual adjustment for heart rate by adding heart rate as a covariable in a linear regression model (as, for example, done by De Bruyne *et al.* in the Rotterdam study population[10]) is misleading. When we determined normal values for HRV for all ages, we at first noticed a very low value in infancy. This is counterintuitive because low HRV is usually associated with increased cardiovascular risk.[11-13] Thereupon we estimated the exponential correction parameter separately for predetermined age groups, and then computed an inverse-variance-weighted average. After correction for heart rate in this manner, HRV proved to continuously decrease from birth to old age, as was expected. One might ask whether the results of previous work on HRV in the Rotterdam study and in other population-based studies would not come out differently when repeated with the new heart-rate corrected markers. Future work could focus on the interplay of age, heart rate, and HRV with cardiovascular morbidity and mortality. Our observations show that even established markers like QT and HRV need critical revision.

*QT variability: a promising new marker for SCD*

The main marker studied in this thesis is QT variability. QT variability is a measure of the beat-to-beat changes in the duration of the QT interval. This temporal variability is thought to be a measure of instability in the repolarisation of the cardiac ventricles, and therewith of the risk of arrhythmias leading, in the worst case, to SCD.[14] A number of encouraging animal studies indicated (I) that an increased QT variability had a higher sensitivity and specificity for the prediction of torsade de pointes [a specific ventricular arrhythmia] than QT interval prolongation,[15] and (II) that QT variability was consistently increased in animals treated with drugs of a known arrhythmogenic potential.[16] The first step in our study of QT variability was to review all relevant investigations in humans (chapter 2.1). We found 109 publications that covered QT variability. The majority of these studies found associations between QT variability and cardiac morbidity, cardiac mortality and SCD. Also, a higher QT variability was found to accompany the use of potentially arrhythmogenic QT prolonging drugs. It should be noted that the median sample size of the 109 studies in humans was 48 participants, and

that the largest study consisted of a mere 805 participants. Moreover, most studies were conducted in specific patient populations and no study was carried out in a community-dwelling population. Therefore, although QT variability is a promising feature, there is still room for improvement with studies in population-based cohorts using a consistent methodology for all ECGs.

*Fiducial segment averaging*

The sample sizes reported in chapter 2.1 were small because the researchers probably did not have a fast automatic method to measure all QT intervals on an ECG recording. The most commonly-used method was first proposed by Berger *et al.*[14] This method was based on template matching, and had to be primed by human interaction for each ECG. To be able to study QT variability in larger numbers, we employed an automated fiducial segment averaging (FSA) algorithm as shown in chapter 2.2. FSA uses the coherence of subsequent beats in small segments around so-called fiducial points in the ECG signal, in our case the beginning of the Q wave and the end of the T wave, in order to determine the location of these points more accurately with less sensitivity to noise. Our FSA algorithm is an automated version of the semi-automatic measurement process devised by Ritsema van Eck.[17]

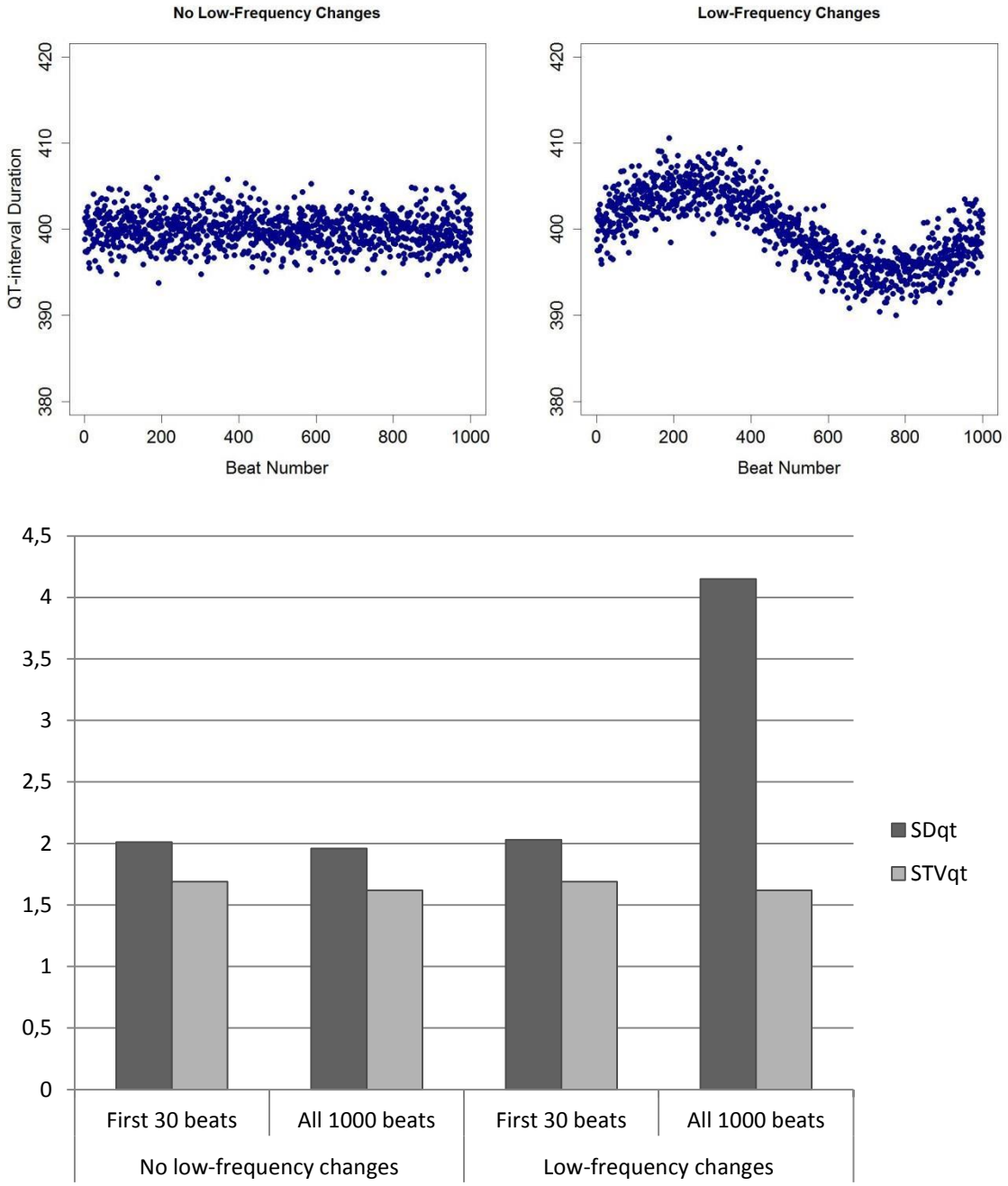
*Consecutive vs. non-consecutive QT variability*

Using the FSA algorithm, we were able to determine all nine different formulas for QT variability found in our literature review quickly and easily on a large number of standard 10-second ECGs. For reasons of practicality we focused on the most commonly used makers and did not analyse all nine, noting that several markers were statistically equivalent in theory (like SDqt = $\sqrt{\mathrm{QTvar}}$) or highly correlated in practice. However, one fundamental difference between QT variability formulas was of special interest: on the one hand formulas based on the beat-to-beat variability (called consecutive QT variability, e.g. STVqt) and on the other hand formulas based on variability compared to the mean (non-consecutive QT variability, e.g. SDqt). It is not apparent which of these formulas result in markers that better reveal repolarisation instability and are better predictors of ventricular arrhythmias. One study proposed that only consecutive QT variability would be arrhythmogenic and that non-consecutive QT variability would be the result of low-frequency changes caused by respiration and fluctuations of autonomic tone.[18]

At variance with this idea, we found in chapter 2.4 that SDqt and STVqt are strongly correlated (Pearson correlation coefficient of 0.96), and that the results of SDqt and STVqt are similar in almost all analyses performed in this thesis.

A possible explanation of the high correlation between SDqt and STVqt in our measurements lies in the use of 10-second ECGs. For longer ECG signals, low-frequency variability would increase SDqt but not STVqt. This is illustrated in figure 2, where we simulated 1000 subsequent QT intervals with and without (exaggerated) long-term low-frequency changes. Introducing low-frequency changes did not materially increase either SDqt or STVqt when calculated over the first 30 beats, but when calculated over all 1000 beats, SDqt almost doubled while STVqt stayed the same. Incidentally, Feeny *et al.*[19] found a high concordance (Lin's rho 0.79) of STVqt based on a three-minute recording and STVqt based on a 10-second recording, which is in line with this simulation. These results show that STVqt does not increase in response to low-freqency changes, no matter the recording length and that SDqt only increases in response to low-freqency changes when the ECG recording is long enough to show them.

**Figure 2. Comparing consecutive and non-consecutive QT variability on long and short simulations.**




*Top left figure: simulated QT intervals based on a normal distribution with mean 400 and standard deviation of 2. Top right figure: the same simulated QT intervals from the top figure but with added low-frequency changes with amplitude of 5 milliseconds and a period of 1000 beats. Bottom figure: simulated values of SDqt and STVqt for the different scenarios.*

*QT variability: normal limits and association with SCD*

As recommended in chapter 2.1, we conducted population-based studies of QT variability. An important but often overlooked aspect of any marker resides in its normal limits. Usually, a new biomarker is only evaluated by its distribution in a population (and log-transformed if necessary to create a normal distribution). But ideally, we think, the study of normal limits should be performed in population-based cohorts, covering all ages and both sexes with sufficient numbers in each category, using the same methodology for all participants. This was what we attempted for HRV in chapter 1.1 and for QT variability in chapter 2.3. Previous studies of normal values often retrospectively collected and meta-analyzed data from a large number of previous studies. Nunan *et al.*[20] did this for heart rate variability and Baumert *et al.*[21] for QT variability. Unfortunately, these meta-analyses too often yield limited information - despite the large collective sample size - and suffer from methodological variation between the component studies. The normal values in these meta-analyses are often derived from healthy controls from case-control studies, who tend to be too young and fit to be representative of the general normal population. In any case, these retrospective normal-limit studies do not report continuous change of the studied marker with age. The main additional worth of our normal-value studies of QT variability and HRV lies in showing the changes over all life stages for both sexes. Future research of new markers could incorporate more large-scale normal value studies, and address other fundamental factors of influence on ECG markers like ethnicity.

*Creating a prediction model for SCD*

In chapter 2.4 we analyzed the association of QT variability with SCD in the general population, and found that QT variability was a significant risk factor for witnessed SCD, but only in women. In addition we adjusted for the ECG variables heart-rate, HRV and QT interval, but we did not address how QT variability performs compared to other ECG variables. As Wellens *et al.*[1] show, there is a long list of ECG variables that have been found to have prognostic value, but no study has really compared and combined them. We therefore set out in chapter 2.5 to find the additional value of ECG markers to a prediction model for SCD based on clinical risk factors. A prediction model ideally specifies which variables are important and which must be left out. This signifies that one must choose the right candidate ECG markers before creating a model at all.[22] A previous study that created a prediction model for SCD considered six candidate ECG markers, viz. atrial fibrillation, Cornell voltage, QTc interval, QRS interval, heart rate and left bundle branch block, of which only the QTc interval was selected. This paper did not assess the value of the ECG in addition to other risk factors.[23] However, in our opinion six candidate ECG markers is not nearly enough: a large number of

ECG markers have been described in the literature (for example, Kors et al. cover 18 repolarization markers derived from the ECG in one paper alone![24]) and one marker can be expressed in multiple ways, e.g. the QT interval has many derivative markers, each with a different heart-rate correction.[6] Hence, we started our modeling with 24 candidate ECG markers that had been significantly associated with cardiac death or SCD in previous studies. Starting with 24 candidate markers we found that a model with six ECG markers (heart rate, Sokolow-Lyon index, spatial J amplitude, amplitude of the T wave in lead aVR, right bundle branch block, and SDNN adjusted for heart rate) and six clinical risk factors (age, sex, total cholesterol/HDL cholesterol ratio, smoking, diabetes mellitus and use of renin-angiotensin system blockers) performed better in the prediction of SCD than a model with clinical risk factors alone. This shows that the ECG has its own contribution to make in SCD risk prediction. We were hampered however, by the small number of SCD cases (n = 149) and the lack of a validation cohort. Some variables were unexpectedly eliminated. To our surprise, QT variability, the main topic of chapter 2.4, and the QTc interval, a commonly used and well-studied predictor of SCD,[25] were not selected in the SCD prediction model of chapter 2.5. This suggests that both QTc and QT variability are weak predictors of SCD. A possible explanation lies in the heterogeneous nature of SCD. As Albert *et al.* show in survivors of cardiac arrest, there are a number of different causes of SCD in the general population,[26] and we surmise that QTc and QT variability are associated with only one or a small subset of these causes. For the marker QTc the specific cause would be ventricular arrhythmias like torsade de pointes, associated with use of QTc prolonging drugs[27] or congenital long-QT syndromes.[28] Thus, these conditions are only responsible for a small number of cases of SCD in the general population, although QTc is an important predictor in users of QTc prolonging drugs and in people with congenital long QT-syndromes.[29] We conjecture that an increased QT variability is associated with a high risk of SCD mainly in patients with cardiac hypertrophy and associated heart failure. To test this hypothesis in the general population, we analyzed the association of QT variability with heart failure, the result of which was that QT variability has a stronger association with heart failure than with SCD, the estimate of the multivariate adjusted hazard ratio of STVqt being 1.06 for SCD and 1.36 for heart failure. Although the association of QT variability with witnessed SCD was only significant in women, the association of QT variability with heart failure came out similar in both sexes.

The investigation of the different underlying causes and their determinants is a challenge for the future of SCD prevention. The inherently capricious nature of SCD makes it difficult to investigate, but some advancements have been made with the help of molecular autopsies.[30] A molecular autopsy is the analysis of genes encoding for cardiac ion channels in suddenly deceased persons with no other discernable cause found during regular autopsy.[31] This can also be done by

proxy by analyzing the genes of first- or second degree relatives of the patient.[32] Another opportunity would be unobtrusive ECG monitoring of at-risk groups. Current clinical use of QT variability is limited, but based on the previously mentioned clinical studies and the normal limits of QT variability determined in chapter 2.3, clinicians could use QT variability as an additional measure of SCD risk stratification in the general population. Future studies could address the specific pathways leading to SCD. Examples are studies of the association of drugs with QT variability and risk of SCD and studies of SCD risk among heart failure patients or among patients with cardiac hypertrophy.

Future aims in SCD research should be the combination of SCD cohorts in order to gain a larger combined sample size and testing of the model in other databases and other populations. Ideally the ascertainment of SCD cases should be done by identical criteria. In the field of genetic studies, this has already been accomplished: in the CHARGE consortium, a large genome-wide association study of genetic determinants of SCD, data from 10 cohorts were combined, creating a sample size of 4,496 SCD cases and over 25,0000 controls (manuscript in preparation in the CHARGE consortium). A similar set-up for the study of novel ECG markers would vastly improve the study of ECG markers and SCD. It would also allow for the creation of a general prediction model for SCD with more power.

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# Summary

Coronary heart disease is the number one cause of death globally.[1] Half of all cases of coronary heart disease occur suddenly and unexpectedly, and half of those cases occur in people without a previous history of cardiac disease. This is why prediction of sudden cardiac death (SCD) in the general population is of great importance. The electrocardiogram (ECG) is already used as a tool to predict SCD,[2] but it has not yet reached its full potential. The prediction of SCD could be improved by further study of ECG markers. This is the aim of this thesis: to explore and expand the use of the ECG in the prediction of SCD, in particular with respect to QT variability and heart-rate variability.

In the first section, we began with a closer examination of ECG markers already commonly used in clinical practice. The first ECG marker that we explored was heart-rate variability. Low heart-rate variability is a known risk factor for cardiac disease and SCD.[3,4] We explored two issues with this marker. The first issue is the lack of comprehensive age- and sex dependent normal limits. We addressed this by determining age-dependent and sex-specific normal values for heart-rate variability in chapter 1.1. In this study, we applied a novel exponential heart-rate correction formula to remove the effect of heart rate on heart-rate variability. In chapter 1.2, we addressed an issue with heart-rate variability in psychiatry. It is known that patients with anxiety and depression have an increased risk of acute myocardial infarction,[5,6] but it is unknown if the treatment with antidepressants might be partially responsible for this. Because heart-rate variability is associated with cardiovascular disease, [3,4] it is of interest to know what the interrelation is between depression, antidepressants and heart-rate variability. There are two opposing papers in the literature. One of these reported that depression lowers heart-rate variability [7] and the other one that antidepressants lowers heart-rate variability.[8,9] The main limitations of both studies were the cross-sectional study designs and the lack of reporting on dose-response relationships. Our study in chapter 1.2 was longitudinal and did assess dose-response relationships. We found that tricyclic antidepressants (TCAs) lower heart-rate variability, independently from heart-rate and depressive symptoms, while the evidence for selective serotonin reuptake inhibitors (SSRIs) was weaker, although there was a dose-response relationship of the SSRI paroxetine with a lower heart-rate variability. The next commonly used ECG marker that was examined more closely was the QT interval. A prolonged QT interval is a well-known risk factor for SCD.[10] Many formulas have been proposed to correct the QT interval for its dependency on heart rate. Two commonly used formulas were proposed by Bazett[11] and Fridericia,[12] already in the 1920s. The importance of heart-rate correction is notable in those cases where heart rate can be a confounder, in our case when testing drugs that influence the heart rate. Inaccurate correction for heart rate might underestimate or overestimate the heart-rate corrected QT interval (QTc), and thus give false-positive results. This is illustrated in chapter 1.3: TCAs are known to increase the heart rate,[13] and also are suspected of increasing the QTc,[14]

potentially increasing the risk of SCD. However, we found that the increased QTc associated with use of TCAs was based on overestimation of the QTc when using Bazett's formula. When we used Fridericia's formula, there was no QTc prolongation. We concluded that it is preferable to use that formula in future studies.

The second section of this thesis focused on the ECG marker QT variability. QT variability denotes the variability of QT-interval durations of individual beats on the ECG. It is thought to be indicative of repolarization instability, and thus to be predictive of ventricular arrhythmias and SCD.[15] In chapter 2.1 we show a review of all relevant papers that had previously studied QT variability. The review revealed that short-term QT variability had already been studied in over 100 papers, using nine different formulas for QT variability. However, we also noted a number of shortcomings in these papers: the studies were conducted either in selected healthy volunteers or in specific patient populations - not in the general population, where prediction of SCD is needed most. A second concern was the limited population size of these studies. It is possible that the population sizes were limited by the lack of an accurate and automatic method to measure all QT intervals on an ECG. To address this problem, we introduced in chapter 2.2 an automatic implementation of the fiducial segment averaging (FSA) algorithm devised by Ritsema van Eck.[16] The FSA algorithm is a relatively novel approach to accurately and automatically measure individual QT intervals on the ECG. What we did in chapter 2.2 was to propose a new method of validation of QT interval measurements. We simulated QT interval variability together with noise, baseline wander and signal-length variations in a signal based on the averaged P-QRS-T complexes of existing ECG recordings. In this set we compared the performance of a conventional QT variability measurement algorithm with that of FSA, and showed that FSA is more accurate than the conventional method.

We explored QT variability further in the next chapters. In chapter 2.3, we reported comprehensive age-and sex dependent normal limits of QT variability as measured on the 10-second ECG. We were the first to do this on such a large (>14,000 ECGs) dataset of 10-second ECGs.[17] In chapter 2.4 we studied the association of four QT-variability markers (QTVI, SDqt, STVqt, and RMSSDqt) with total mortality, coronary heart disease (CHD) mortality, SCD, and witnessed SCD. We found that these markers were associated with total mortality, CHD mortality, and with witnessed SCD, but the latter only in women and not in men. We noted that SDqt, STVqt, and RMSSDqt were highly correlated, which we thought is due to the shortness of the ECG signal. QTVI had a weaker correlation with the other three QT-variability markers due to its correction for QT interval, heart rate, and heart-rate variability. QTVI performed worse (i.e., had a weaker and non-significant association with clinical endpoints) than the three uncorrected markers. In chapter 2.5, we addressed a limitation of many studies of ECG markers pointed out by Wellens et al,[18] namely that

markers are often studied in isolation, adjusted for only a limited set of other ECG markers or clinical risk factors. Therefore, we created a prediction model for SCD to assess the additional value of 24 ECG markers over a model based on only clinical risk factors. After a backwards stepwise elimination procedure, six clinical risk factors were selected: age, sex, total/HDL cholesterol ratio, smoking, diabetes mellitus, and use of agents acting on the renin-angiotensin system. A second backwards elimination procedure with the 24 ECG markers as input and keeping the six clinical risk factors fixed, resulted in a combined model with six clinical risk factors and six additional ECG markers. The selected ECG markers in question were heart rate, Sokolow-Lyon index, spatial J amplitude, T amplitude in lead aVR, right bundle branch block, and heart-are corrected SDNN. We found that these ECG markers significantly improved the discrimination of the model for predicting SCD. We also noted the limitations of creating a prediction model with limited data. In the future, this might be addressed by combining cohorts that study SCD on an international scale. In the last two chapters of the second section, we explored how QT variability might be related to other diseases that are hypothesized to be intermediate causes of SCD. In chapter 2.6 we reported that QT variability is associated with heart failure and that this association is stronger than the association of QT variability with SCD or witnessed SCD. We speculated that this might be caused by the multifactorial nature of the causes of SCD. It could be argued that only a fraction of the underlying causes of SCD is characterized by an increased QT variability. In chapter 2.7, we analyzed whether thyroid function influences QT variability. Previous studies have shown that both a high and a low thyroid function are associated with an increased risk of ventricular arrhythmias and that a high thyroid function is a risk factor for SCD. In this chapter, we report that both a high and a low free thyroxine (FT4) is associated with an increase in STVqt, an association which was stronger in men than in women.

In the final section we investigated the association of two medical conditions with SCD. In chapter 3.1, we showed in a review of the literature that chronic obstructive pulmonary disease (COPD) is a risk factor for SCD. This chapter could prompt future research in the interplay between COPD, SCD, and ECG markers. In chapter 3.2, we explored the association between thyroid function and SCD. This chapter can be linked to chapter 2.7, where we studied the influence of thyroid function on QT variability, and to chapter 2.4, where we studied the association of QT variability and SCD. Taken together, these chapters show that an increased thyroid function can lead to an increased QT variability and an increased risk of SCD. Thus, one of the ways in which QT variability is associated with SCD is via the pathway of thyroid function.

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# Nederlandse Samenvatting

Wereldwijd zijn coronaire hartziekten de nummer één doodsoorzaak.[1] De helft van alle coronaire hartziekten treden plotseling en onverwachts op, en de helft van de plotse gevallen treden op bij mensen die geen voorgeschiedenis van hartziekten hebben. Daarom is het van groot belang is om plotse hartdood te kunnen voorspellen in de algemene bevolking. Het elektrocardiogram (ECG) wordt reeds gebruikt als instrument om plotse hartdood te voorspellen,[2] maar er is meer uit het ECG te halen dan nu gedaan wordt. Door de verschillende maten gebaseerd op het ECG nader te bestuderen, zou het voorspellen van plotse hartdood verbeterd kunnen worden. Hier ligt het doel van dit proefschrift: het verkennen en verbreden van het gebruik van het ECG bij het voorspellen van plotse hartdood, en meer specifiek met de ECG maten QT variabiliteit en hartslagvariabiliteit.

In het eerste deel vingen we aan met een nadere inspectie van ECG maten die al algemeen gebruikt worden in de klinische praktijk. De eerste ECG maat die we verkenden was hartslagvariabiliteit. Een lage hartslagvariabiliteit is een risicofactor voor hartziekten en plotse hartdood.[3,4] Twee aspecten van deze maat hebben we nader bekeken. Het eerste aspect is het feit dat er geen uitgebreide leeftijds- en geslachtsafhankelijke normale grenzen bekend zijn van hartslagvariabiliteit. Dit hebben we aangepakt door de leeftijds- en geslachtsafhankelijke normale grenzen te bepalen in hoofdstuk 1.1. In deze studie pasten we een nieuwe exponentiële hartslagcorrectieformule toe om het effect van hartslag op hartslagvariabiliteit te verwijderen. In hoofdstuk 1.2 verkenden we een tweede aspect van hartslagvariabiliteit. Het is namelijk bekend dat patiënten met angststoornissen en depressie een verhoogde kans op een myocardinfarct hebben,[5,6] maar het is niet bekend of dit mede veroorzaakt wordt door de behandeling met antidepressiva. Omdat hartslagvariabiliteit gerelateerd is aan hart- en vaatziekten,[3,4] is het interessant te zien wat de samenhang is tussen depressie, antidepressivagebruik en hartslagvariabiliteit. Er zijn twee tegengestelde publicaties hierover in de vakliteratuur. De eerste publicatie liet zien dat depressie hartslagvariabiliteit verlaagt,[7] en de andere publicatie dat antidepressiva verantwoordelijk zijn voor de verlaging van hartslagvariabiliteit.[8,9] De voornaamste beperkingen van deze beide studies zijn hun cross-sectionele opzet en het ontbreken van informatie over dosis-respons effecten. Onze studie in hoofdstuk 1.2 had een longitudinale opzet en gaf wel informatie over dosis-respons effecten. Wij lieten zien dat tricyclische antidepressiva (TCA's) hartslagvariabiliteit verlaagden, onafhankelijk van hartslagfrequentie en symptomen van depressie. Het bewijs voor serotonine heropnameremmers (SSRI's) was zwakker, maar er was wel een significant dosis-responseffect voor een lagere hartslagvariabiliteit bij hogere SSRI doses. De volgende veelgebruikte ECG maat die we nader onderzochten was het QT interval. Een verlengd QT interval is een bekend risicofactor plotse hartdood.[10] Het QT interval is afhankelijk van de hartslagfrequentie, en een groot aantal formules is voorgesteld om het QT interval voor de hartslagfrequentie te corrigeren. In de jaren '20 van de

vorige eeuw zijn de twee meest gebruikte formules bedacht door Bazett[11] en Fridericia.[12] Het belang van correctie voor hartslagfrequentie komt naar voren in die gevallen waar hartslagfrequentie een confounder kan zijn, in ons geval bij het testen van medicijnen die de hartslag beïnvloeden. Onnauwkeurige correctie voor hartslagfrequentie kan leiden tot een onder- of overschatting van het voor hartslagfrequentie gecorrigeerde QT interval (QTc), wat weer kan leiden tot vals positieve resultaten. Hoofdstuk 1.3 is een illustratie hiervan: het is bekend dat TCA's de hartslagfrequentie verhogen,[13] maar ze worden ook ervan verdacht de QTc te verlengen,[14] en dus in potentie het risico op plotse hartdood te verhogen. Desalniettemin vonden we dat de toename van het QTc gerelateerd aan het gebruik van TCA's gebaseerd was op een overschatting van het QTc bij het gebruik van de formule van Bazett. Toen we Fridericia's formule gebruikten, konden we geen QTc verlening vaststellen. Wij concludeerden dat het de voorkeur heeft om Fridericia's formule te gebruiken.

Het tweede deel van dit proefschrift is gericht op de ECG maat QT variabiliteit. QT variabiliteit geeft de variabiliteit van alle QT intervallen in een bepaalde tijdsspanne weer. Men denkt dat een verhoogde QT variabiliteit een indicatie is van instabiliteit van de repolarisatie, en dat het daarom voorspellend kan zijn voor ventriculaire aritmieën en plotse hartdood.[15] In hoofdstuk 2.1 laten we een overzicht en beoordeling zien van alle relevante vakliteratuur gericht op het bestuderen van QT variabiliteit. Deze review onthulde dat QT variabiliteit op ECG signalen van korte duur al onderzocht is in meer dan 100 publicaties, gebruikmakend van meer dan negen verschillende formules. Een aantal beperkingen van deze publicaties viel op: de studies waren uitgevoerd in een selectie van gezonde vrijwilligers of in specifieke patiëntgroepen, maar niet in de algehele bevolking, waar het voorspellen van plotse hartdood het meest van nut kan zijn. Een tweede probleem was de beperkte omvang van de studiepopulaties. Het is mogelijk dat de omvang van de studies beperkt was door het ontbreken van een nauwkeurige automatische methode om alle QT intervallen op een ECG te meten. Om dit probleem te verhelpen introduceerden wij in hoofdstuk 2.2 een automatische implementatie van het fiducial segment averaging (FSA) algoritme bedacht door Ritsema van Eck.[16] Het FSA algoritme is een relatief nieuwe benadering om QT intervallen op een ECG automatisch en accuraat te meten. Wij stelden ook in hoofdstuk 2.2 een nieuwe methode voor om metingen van QT intervallen te valideren. We simuleerden eerst QT variabiliteit samen met ruis, grondlijndwalen en variaties in signaallengte op een signaal gebaseerd op gemiddelde P-QRS-T complexen van bestaande ECG opnames. In deze dataset vergeleken we de prestaties van FSA met de conventionele methode om QT variabiliteit te meten, en toonden aan dat FSA veel nauwkeuriger is dan de conventionele methode.

In de volgende hoofdstukken gingen we verder met het onderzoeken van QT variabiliteit. In hoofdstuk 2.3 rapporteerden we uitgebreide leeftijds- en geslachtsafhankelijke normale grenzen van QT variabiliteit zoals gemeten op standaard ECG's van 10 seconden. Wij waren de eerste om dit te doen op zo'n grote schaal (±14,000 ECG's).[17] In hoofdstuk 2.4 bestudeerden we de relatie tussen vier maten van QT variabiliteit (QTVI, SDqt, STVqt, and RMSSDqt) met algehele mortaliteit, mortaliteit door coronair lijden (ook wel coronary heart disease mortality - CHD mortaliteit genoemd), plotse hartdood en geobserveerde plotse hartdood. We ontdekten dat deze maten geassocieerd waren met algehele mortaliteit, CHD mortaliteit en met geobserveerde plotse hartdood, maar in het laatste geval alleen bij vrouwen, en niet bij mannen. Het viel op dat SDqt, STVqt, en RMSSDqt onderling sterk gecorreleerd waren, wat mogelijk komt door de korte duur van het ECG signaal. QTVI was minder sterk gecorreleerd met de andere QT variabiliteitsmaten vanwege de correctie voor het gemiddelde QT interval, de gemiddelde hartfrequentie, en de hartslagvariabiliteit. QTVI had een zwakkere en statistisch niet significante associatie met de klinische eindpunten vergeleken met de drie ongecorrigeerde maten. In hoofdstuk 2.5 gaven we aandacht aan een zwak punt van veel voorgaande studies, opgemerkt door Wellens et al,[18] namelijk dat ECG maten vaak apart bestudeerd worden, slechts geadjusteerd voor een beperkt aantal andere ECG maten of klinische risicofactoren. Wij hebben een predictiemodel gemaakt voor plotse hartdood om de aanvullende waarde van 24 ECG maten te beoordelen boven een model gebaseerd op slechts klinische risicofactoren. Na stapsgewijze eliminatie werden zes klinische risicofactoren geselecteerd: leeftijd, geslacht, totaal/HDL cholesterol ratio, roken, diabetes mellitus en gebruik van medicatie die ingrijpen op het renine-angiotensine systeem. Een tweede stapsgewijze eliminatie, met behoud van de eerder geselecteerde zes risicofactor maar met de 24 ECG maten als extra input, resulteerde in een gecombineerd model met zes klinische risicofactoren en zes aanvullende ECG maten. De geselecteerde ECG maten in kwestie waren hartslagfrequentie, Sokolow-Lyon index, ruimtelijke J amplitude, T amplitude in afleiding aVR, rechter bundeltakblok en SDNN gecorrigeerd voor hartslagfrequentie. We ontdekten dat deze ECG maten samen de discriminatie van het model significant verbeterden, maar plaatsten ook enkele kanttekeningen bij het maken van een voorspellend model met beperkte data. Dit kan in de toekomst opgelost worden door op internationaal niveau cohortstudies naar plotse hartdood te combineren.

In de laatste twee hoofdstukken van het tweede deel onderzochten we of QT variabiliteit gerelateerd is aan andere ziektes die mogelijk een directe oorzaak voor plotse hartdood kunnen zijn. In hoofdstuk 2.6 meldden we dat QT variabiliteit geassocieerd is met hartfalen, en ook dat deze associatie sterker is met hartfalen dan met plotse hartdood (al dan niet geobserveerd). We speculeerden dat dit veroorzaakt wordt door de multifactoriële aard van plotse hartdood. Het is

immers mogelijk dat slechts een deel van de onderliggende oorzaken van plotse hartdood gekarakteriseerd worden door een toegenomen QT variabiliteit. We analyseerden in hoofdstuk 2.7 of de schildklierfunctie geassocieerd is met een verhoging van de QT variabiliteit. Voorgaande studies hebben aangetoond dat zowel een hoge als een lage schildklierfunctie geassocieerd zijn met een verhoogd risico op ventriculaire arithmieën en ook dat een verhoogde schildklierfunctie geassocieerd is met plotse hartdood. In dit hoofdstuk stellen wij dat zowel een hoog als een laag vrij thyroxine (FT4) gerelateerd is aan een verhoogde STVqt. Deze associatie was sterker in mannen dan in vrouwen.

In het laatste deel van het proefschrift onderzochten we de associatie van twee aandoeningen met plotse hartdood. In hoofdstuk 3.1 laten we een overzicht en beoordeling zien van de literatuur over chronisch obstructieve longziekten (COPD) als risicofactor voor plotse hartdood. Dit hoofdstuk is mogelijk een aanleiding voor verder onderzoek naar het samenspel van COPD, plotse hartdood en ECG maten. In hoofdstuk 3.2 verkenden we de associatie tussen schildklierfunctie en plotse hartdood. Dit hoofdstuk kan gerelateerd worden zowel aan hoofdstuk 2.7, waar we de invloed van de schildklier op QT variabiliteit onderzochten als aan hoofdstuk 2.4, waar de associatie van QT variabiliteit en plotse hartdood analyseerden. Deze hoofdstukken samen laten zien dat een toegenomen schildklierfunctie kan leiden tot een verhoogde QT variabiliteit en een verhoogd risico op plotse hartdood. Dit leidt tot de conclusie dat de schildklierfunctie één van de manieren zou kunnen zijn waarop QT variabiliteit geassocieerd is met plotse hartdood.

**Referenties**


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# Portfolio

| | |
|---|---|
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| Research School: | NIHES |
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**PhD Training** **Year**

*General Courses*

- Master of Science in Clinical Epidemiology, Netherlands Institute of Health Sciences (NIHES), Rotterdam, the Netherlands 2012-2014

*Specific Courses*

- SNP Course IX: SNPs and Human Diseases, Molecular Medicine Graduate School (MOLMED), Rotterdam, the Netherlands 2012
- English Biomedical Writing and Communication 2014
- Research Integrity, Erasmus MC, Rotterdam, the Netherlands 2014
- Arrhythmia Research Methodology COEUR PhD Course, Rotterdam, the Netherlands 2014

*Seminars and Workshops*

- Research Seminars, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands 2012-2016
- Presentation workshops, Department of Medical Informatics, Erasmus MC, Rotterdam, The Netherlands 2014

*National and International Conferences*

- Meeting on iMaging And eLectrical Technologies (MALT ) Noordwijk, the Netherlands 2014: “Electrocardiographic determinants of sudden cardiac death” [presentation] 2014
- ESC, London, UK 2015: “Combining cardiovascular and electrocardiographic risk factors improves the prediction of sudden cardiac death in the middle-aged and general elderly population.” [poster] 2015
- ICPE, Boston, USA 2015: The effect of antiarrhythmics on QT variability in a population-based cohort of middle-aged and elderly persons.[poster] 2015

*Teaching Activities: Supervision of Practicals*

- Pharmaco-epidemiology and Drug safety (NIHES) 2014-2016
- Principles of Research in medicine (NIHES) 2014-2016
- Systematic Reviews (2nd Year Medicine Students), Erasmus MC 2016

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Marten van den Berg was born on November 1st, 1986 in Leeuwarden, the Netherlands. He graduated from the Piter Jelles gymnasium in Leeuwarden in 2005. He started medical school at the University of Groningen in the same year. Here, he received his bachelor of science degree in 2008 and his master of science degree in 2012. Ensuing his medical studies, he started working on this thesis under the supervision of prof.dr. Bruno Stricker. While working on this thesis he completed a master of clinical epidemiology in 2014. He currently continues his scientific work with prof. dr. Bruno Stricker.