RISK FACTORS FOR SUDDEN CARDIAC DEATH

EPIDEMIOLOGIC STUDIES IN AN AGEING POPULATION

Maartje Nieske den Besten - Niemeijer

The work presented in this thesis was conducted at the Department of Epidemiology of the Erasmus Medical Center, Rotterdam, the Netherlands.

The contribution of all of the participants and staff of the Rotterdam Study, as well as the general practitioners and pharmacists of the Ommoord district for help with data collection and validation, is gratefully acknowledged.

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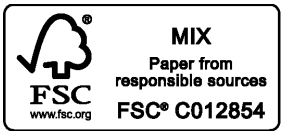

For reasons of consistency within this thesis, some terms have been standardized throughout the text. As a consequence the text may differ in this respect from the articles that have been published.

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Risk Factors for Sudden Cardiac Death

Epidemiologic studies in an ageing population

Risicofactoren voor acute hartdood

Epidemiologische studies in een ouder wordende populatie

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Content

Part I General introduction

Chapter 1.1	Introduction	11
Chapter 1.2	Aim, setting, and outline of this thesis	19

Part II Epidemiology of sudden cardiac death

Chapter 2.1	Declining incidence of sudden cardiac death from 1990-2010	27

Part III Electrocardiographic risk factors

Chapter 3.1	QT variability markers for the prediction of ventricular arrhythmias and sudden cardiac death	45
Chapter 3.2	Consistency of heart-rate corrected QT interval prolongation and risk of sudden cardiac death	75
Chapter 3.3	PR interval, P-wave indices, and sudden cardiac death	95

Part IV Clinical risk factors

Chapter 4.1	Subclinical cardiac dysfunction and sudden cardiac death	113
Chapter 4.2	Chronic obstructive pulmonary disease and sudden cardiac death	131
Chapter 4.3	Serum magnesium and the risk of death from coronary heart disease and sudden cardiac death	151

Part V Pharmacological risk factors

Chapter 5.1	Concomitant prescriptions of QT-prolonging drugs between 1996 and 2014	175
Chapter 5.2	Drugs and ventricular repolarization	193

Part VI Pharmacogenetic risk factors

Chapter 6.1	Genetics of drug-induced QT interval prolongation	211
Chapter 6.2	*ABCB1* gene variants, digoxin, and risk of sudden cardiac death	235
Chapter 6.3	*CACNA1C* variant rs2238018, calcium channel blockers, and heart rate	255

Part VII General discussion & Summary

Chapter 7.1	Discussion	273
Chapter 7.2	Summary	303
Chapter 7.3	Samenvatting	309

Part VIII Appendices

	Dankwoord	318
	PhD portfolio	321
	List of publications	323
	About the author	328

Seek the Lord and live!

Amos 5:6a

Part I

General introduction

Chapter 1.1

Introduction

Maartje N Niemeijer

Sudden cardiac death

Cardiovascular disease is the leading cause of death globally, giving rise to around 17 million deaths annually worldwide[1,2]. The World Health Organization has estimated that this number will increase to 23.3 million by 2030[1]. A substantial part of cardiovascular mortality consists of sudden cardiac death (SCD), with an estimated four to five million cases per year worldwide[3]. The currently used definition of SCD originates from 1997: "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"[4]. This definition has been endorsed by the European Society of Cardiology since 2001[5]. The definition applies to witnessed deaths. Unwitnessed deaths, e.g. when someone is found dead in bed, are usually considered as SCD if persons were unexpectedly found dead, while they were in a stable medical condition 24 hours before they were found, and without evidence of a non-cardiac cause[5-7]. SCD is mainly caused by ventricular arrhythmias, such as ventricular fibrillation, ventricular tachycardia and torsade de pointes[8-10], other causes include pulseless electric activity and asystole[11]. SCD is an important research topic as it poses a major burden on health care and is a substantial psychological burden for relatives of those struck by SCD because of the unexpected nature of death[12]. Preventive options, such as implantable cardioverter defibrillators and antiarrhythmic drugs are available, but not completely successful[13,14], partly because risk stratification for SCD is not optimal[11,12]. Risk stratification is hampered by the fact that in almost half of the SCD cases, no history of heart disease was present[12] and a clinically useful prediction model is not available[11,12]. Risk factors known to date include electrocardiogram (ECG) abnormalities, clinical, pharmacological, and genetic risk factors[15,16].

Electrocardiographic risk factors

The ECG measures the electrical activity of the heart using electrodes on the chest and limbs, which allows physicians to diagnose conduction disorders and cardiac arrhythmias. An ECG is a relatively easy, cheap, and non-invasive diagnostic test. In Figure 1, an ECG is shown with different waves and intervals that are most frequently measured in clinical practice. The different waves and intervals represent different parts of the conduction cycle. The P wave represents the depolarization of the atria. The QRS complex represents the ventricular depolarization, with at the same time the last part of the atrial repolarization. The JT interval represents the ventricular repolarization. Currently, the most well-known ECG-derived risk marker for SCD is the heart-rate corrected QT (QTc) interval[15-18]. Since the duration is highly dependent on the RR interval duration, it is common to apply a heart-rate correction method[19], e.g. as proposed by Bazett[20] or Fridericia[21]. A prolonged QTc interval is a contraindication for use

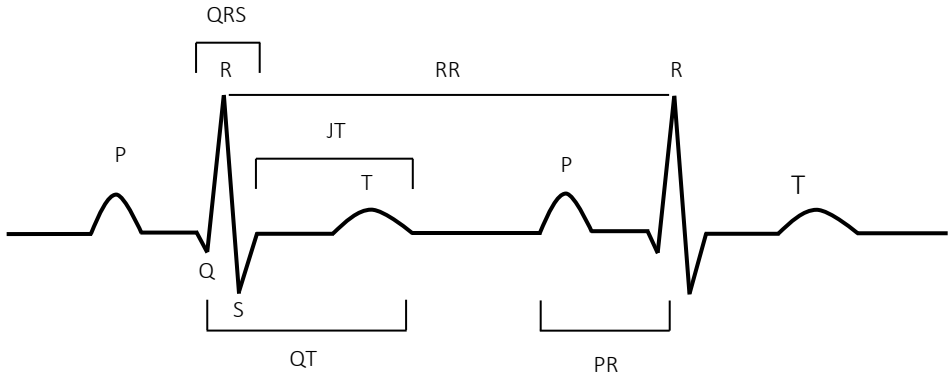

Figure 1. The electrocardiogram with several waves and intervals

of drugs with arrhythmogenic properties[17,18]. Although the QTc interval is the most well-known ECG-derived risk factor, it also has its limitations. Studies have shown that some drugs prolong the QTc interval but have never been associated with an increased risk of arrhythmias and SCD[22-24], while some drugs that do carry an arrhythmogenic risk do not cause QTc prolongation[23,25].

Besides the QTc interval, other ECG-derived markers have been associated with an increased risk of SCD. First, a delayed ventricular repolarization, i.e. a prolonged JT interval, is thought to be a cause of arrhythmias and SCD[19]. There is overlap between de QTc and JT interval measurement regarding ventricular repolarization, however the QTc interval also includes the QRS complex reflecting ventricular depolarization and therefore needs to be interpreted differently. Second, a shorter RR interval, i.e. a higher heart rate, has been associated with an increased risk of SCD, but also with total and cardiovascular mortality[26-29]. Third, a prolonged PR interval, also called a first-degree atrioventricular block, has been described as a risk factor for different adverse outcomes, such as atrial fibrillation[30], total mortality[31,32], and SCD[33]. However, results have not been consistent[32]. A recent study suggested that a higher risk is not dependent on the duration of the PR interval, but on features of the P wave itself, such as the duration of the P wave[34].

Clinical risk factors

Several clinical risk factors for arrhythmias and SCD have been identified. Heart diseases, such as myocardial infarction, heart failure, and atrial fibrillation are recognized as risk factors[12,15], but also non-cardiac diseases increase the risk of arrhythmias. These include psychiatric diseases, such as schizophrenia and depression[15], endocrinologic disorders, such as disorders of the thyroid gland and diabetes mellitus, and electrolyte disturbances[15]. Other factors such as a higher age, male sex, and smoking also increase the risk of arrhythmias[3,7,15].

Pharmacological risk factors

Several drugs have arrhythmogenic properties, which is mostly determined on the basis of their QTc-prolonging properties. These specific drugs are listed in, for example, the University of Arizona, Center for Education and Research on Therapeutics, list of QTc-prolonging drugs[35]. This list includes antiarrhythmic drugs, non-antiarrhythmic cardiovascular drugs, and non-cardiovascular drugs[36]. In the first group, the QTc-prolonging effect is related to the mechanism of action, contrary to the other two groups[37]. Drugs are classified as being known, possible, or conditional QTc-prolonging drugs. For the known category, a positive temporal relationship was demonstrated between drug administration and occurrence of the event. For the possible category less evidence is available. Conditional QTc-prolonging drugs have been proposed to cause risk, but evidence is inadequate or contradictory[38]. This classification only indicates the level of evidence available, and does not indicate the degree of QTc prolongation caused by a drug. The Food and Drug Administration considers an increase of the QTc interval of less than 5 milliseconds as not clinically relevant, an increase of 5-20 milliseconds as possibly clinically relevant, and an increase of more than 20 milliseconds as definitely clinically relevant with regard to the development of arrhythmias[17]. QTc-prolonging drugs are contraindicated in persons with an increased risk of QT prolongation[17], but it is unclear whether this is justified for all three drug categories. Regulatory agencies recommend avoiding concomitant use of QTc-prolonging drugs[17,18]. However, evidence to support this advice is lacking.

Genetic risk factors

Through genome-wide association studies and candidate-gene studies, several genes have been identified that influence the length of the QT interval and the risk of arrhythmias and SCD[12,39]. For example, variants in the *NOS1AP* gene influence the duration of the QTc interval[40] and the risk of arrhythmias and SCD[41]. The *CACNA1C* gene is associated with the RR interval[42] and mutations within this gene have been described in cases of arrhythmias[43]. Besides a direct effect on cardiac conduction and the risk of arrhythmias and SCD, there are also genes that have been associated with the bioavailability of certain drugs in the human body. One of these is the *ABCB1* gene, which encodes P-glycoprotein, a protein that influences, for example, digoxin serum concentrations[44]. Interaction between genetic variants and drug use might clarify why of a group of people using the same drug, only some encounter adverse effects such as arrhythmias or SCD. Therefore, with pharmacogenetic interaction studies (drug x gene), factors that are important for individualized risk stratification and safe drug use can be detected.

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Chapter 1.2

Aim, setting, and outline of this thesis

Maartje N Niemeijer

Aim

Despite the many known risk factors, as described in chapter 1.1, recent articles concluded that risk stratification for sudden cardiac death (SCD) is not yet optimal and should be improved further[1,2]. In this thesis, one aim was to study the current incidence of SCD in order to determine the magnitude of the problem. Another aim was to study risk factors for SCD and their clinical value. This was divided into two items: (i) identify new risk factors for SCD in the general population, and (ii) study characteristics of known risk factors, such as the consistency of a prolonged heart-rate corrected QT (QTc) interval over time, the association between concomitant use of multiple QTc-prolonging drugs and the QTc interval, and the interacting effect of digoxin and *ABCB1* gene variants on the risk of SCD. By studying these characteristics, we aim to improve the validity and usefulness of these markers in clinical practice.

Setting

Most of the studies in this thesis were performed as part of the Rotterdam Study, a population-based cohort in middle-aged and elderly persons[3,4]. This study started in 1990 in Ommoord, a district in Rotterdam. Overall, 14,926 persons aged 45 years and older were included in the study. These participants visit the research center approximately every four to five years for several tests. Besides that, the participants are continuously monitored for major morbidity and mortality through access to the medical files. Over the course of the study more than 5,500 participants died and 583 of these were attributed to SCD.

One study was performed within the framework of the Integrated Primary Care Information (IPCI) project. This is an integrated database of a selected group of more than 600 general practitioners' (GP) databases in the Netherlands, in which information on approximately 1.7 million patients is collected from 1996 onwards. As GPs in the Netherlands have a pivotal role in healthcare, the medical records within the GP databases contain all relevant information, including medical findings and diagnoses from secondary care.

Two studies in this thesis provided an overview of the currently available literature on two distinct topics.

Outline

In **part 2**, we describe the epidemiology of SCD in the general middle-aged and elderly population of the Rotterdam Study from 1990 to 2010. In **part 3**, we describe electrocardiographic risk factors, with in **chapter 3.1** a systematic overview of the literature regarding relatively novel ECG-derived risk factors: QT variability markers and their role in the

prediction of ventricular arrhythmias and SCD. In **chapter 3.2**, we describe the consistency of a prolonged QTc interval on two consecutive ECGs and how this consistency relates to the risk of SCD. To date, nothing is known about the consistency of QTc prolongation, and therefore it is unclear if and for how long the result of one QTc measurement is valid for decision-making with regard to the risk of SCD. In **chapter 3.3**, we study the association of PR interval duration and P-wave indices with the occurrence of SCD. In **part 4**, we study clinical risk factors, namely subclinical cardiac dysfunction (**chapter 4.1**), chronic obstructive pulmonary disease (**chapter 4.2**), and magnesium levels (**chapter 4.3**). In **Part 5**, we focus on concomitant use of QTc-prolonging drugs, a pharmacological risk factor. We study patterns of concomitant use of QTc-prolonging drugs within the IPCI database (**chapter 5.1**) and we describe the association between concomitant use and length of the QTc and JT interval (**chapter 5.2**). **Part 6** focuses on pharmacogenetic risk factors. In **chapter 6.1**, we provide an overview of the current knowledge on the genetic background of drug-induced QT prolongation. In **chapter 6.2**, we study the interaction between the *ABCB1* gene and digoxin on the risk of SCD. **Chapter 6.3** is about the interaction between the *CACNA1C* gene and calcium channel blockers on heart rate. In **part 7**, a summary and general discussion of the findings in this thesis can be found.

Contributions

All chapters, except chapter 4.3 were drafted by Maartje N Niemeijer. The data for all chapters except chapter 4.2 (Lies L Lahousse) and chapter 4.3 (Brenda C Kieboom) were analyzed by Maartje N Niemeijer. Marten E van den Berg contributed equally to chapter 3.1, and Lies L Lahousse contributed equally to chapter 4.2. Peter R Rijnbeek, Mark Eijgelsheim and Bruno H Stricker jointly supervised the chapters. All other co-authors critically revised the chapters or were active in data collection.

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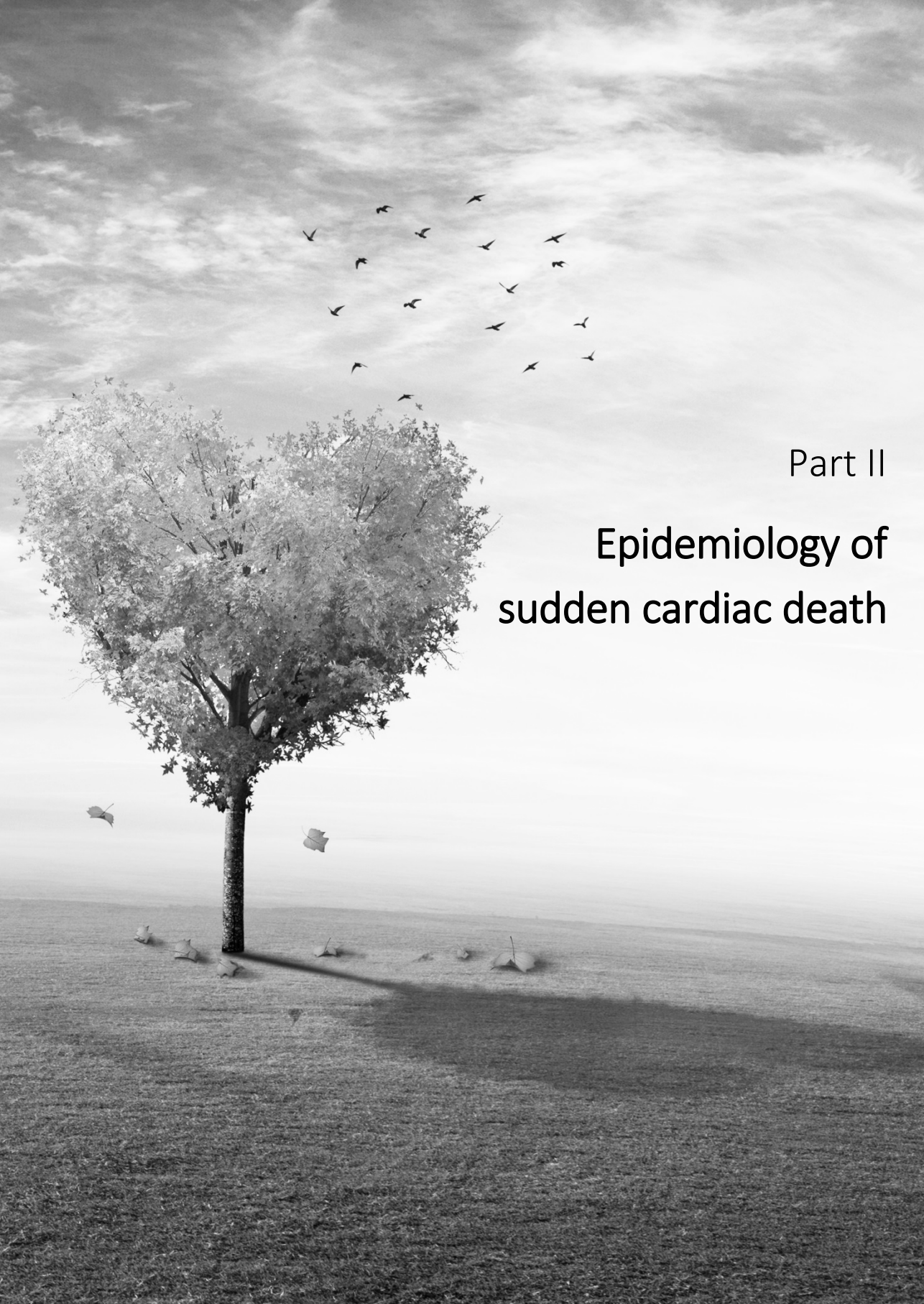

Part II

Epidemiology of sudden cardiac death

Chapter 2.1

Declining incidence of sudden cardiac death from 1990-2010

Maartje N Niemeijer, Marten E van den Berg, Maarten J G Leening, Albert Hofman, Oscar H Franco, Jaap W Deckers, Jan Heeringa, Peter R Rijnbeek, Bruno H Stricker, Mark Eijgelsheim

Abstract

Background Although sudden cardiac death (SCD) is relatively common, contemporary data on its incidence are lacking. Our objective was to investigate the current incidence of SCD and its trend over the last two decades in a general middle-aged and elderly population.

Methods This study was performed within the Rotterdam Study, a prospective population-based cohort study of persons aged 45 years and older. Age-standardized incidence rates of SCD were calculated. To study trends in incidence we compared two subcohorts within the total study population, one followed from 1990-2000 and the other from 2001-2010.

Results From 1990-2010 5,512 of 14,628 participants had died of whom 583 (4%) were classified as SCD. The overall incidence was 4.2 per 1000 person-years (py). Incidence was higher in men (5.2 per 1000py) than in women (3.6 per 1000py); age-adjusted HR 1.84 (95%CI 1.56;2.17) and risk of SCD increased with age (HR 1.10 per year; 95%CI 1.09;1.11). The incidence rate from 1990-2000 was 4.7 per 1000py vs. 2.1 per 1000py from 2001-2010 (age and sex adjusted HR of SCD 0.60; 95%CI 0.44;0.80). To check for cohort effects, we also analyzed the incidence of total mortality, and found an age and sex adjusted HR of total mortality of 0.82 (95%CI 0.75;0.90) for the second compared to the first subcohort, which was significantly higher than the decline in SCD incidence.

Conclusion We found an incidence of SCD of 4.2 per 1000py. The incidence decreased from 1990-2010, a period during which diagnosis and treatment of heart disease greatly improved.

Introduction

Cardiovascular disease is the leading cause of death globally, resulting in approximately 17 million deaths annually worldwide[1]. The World Health Organization has estimated that this number will increase to 23.3 million by 2030[1]. This increase predominantly will be seen in low and middle-income countries, as cardiovascular mortality in high-income countries has decreased over the previous decades because of better preventive measures and treatment[2]. Sudden cardiac death (SCD), defined as a sudden and unexpected death due to a cardiac cause[3], constitutes a substantial part of cardiovascular mortality, with an estimated 4 to 5 million cases per year worldwide[4]. Given the globally increasing cardiovascular mortality, the incidence of SCD is also expected to rise. However, because cardiovascular mortality in high-income countries is decreasing, it is assumed that the incidence of SCD in these countries will decrease. Nevertheless, as far as we know, recent incidence studies in the general population are lacking and studies that were performed some years ago do not describe trends in incidence[3-6]. Studies carried out between 1991 and 2000 reported incidence rates of 0.9-1.0 per 1000 person-years (py)[6,7] and up to 8.5 per 1000py in persons aged above 80 years[6]. A review, published in 2008, reported an incidence rate between 0.4-0.9 per 1000py in the general population older than 18 years from 1980 until 2000[4]. More recent incidence studies have been performed, but in specific high-risk populations such as athletes[8,9]. In these subjects aged 12 to 35 years, an incidence of 1.2 per 1000py was found.

The objective of this study was to determine the current incidence rate of SCD, and the differences in incidence with regard to age, sex, weekday, and season, and to investigate the incidence trend over the past two decades in a general middle-aged and elderly population.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study, which started in 1990 in the Ommoord district, in the city of Rotterdam, the Netherlands. Details regarding design, objectives, and methods of the Rotterdam Study have been described elsewhere[10,11]. In short, all inhabitants of the Ommoord district aged 55 years and older were invited to participate (n=10,215). No other inclusion or exclusion criteria were applied. At baseline, from 1990 through 1993, 7,983 participants (response rate 78%) were included (RS-I). In 2000, an additional 3,011 participants from among 4,472 invitees (response rate 67%) were enrolled (RS-II). This extension consisted of all persons living in the study district who had become 55 years of age or had moved into the study district. A second similar extension of the cohort was initiated in 2006, in which 3,932 participants of 6,057 invitees (response rate 65%) aged 45 years and

older were included (RS-III).

Follow-up examinations are conducted periodically, approximately every 4-5 years. Examinations consist of a home interview and an extensive set of tests at a research facility in the study district. In addition, participants are continuously monitored for major morbidity and mortality through access to general practitioners and municipality records. In the case of a participant's death, research assistants gather the relevant portions from the medical records at the offices of the general practitioners and nursing home physicians. All deaths are coded according to the International Classification of Disease, 10th revision (ICD-10), by a study physician and subsequently validated by a medical specialist in the relevant field, whose judgment is decisive. A level of certainty is adjudicated to each cause of death: definite, probable, or possible. More detailed information on the methods of data collection and definitions of cardiac outcomes have been previously reported[12]. The Rotterdam Study was 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. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Study population and validation of SCD cases

For all participants who provided informed consent for follow-up data collection, vital status and cause of death were ascertained until January 1st 2011. The current SCD case validation is an expansion on the data presented in a previous publication from the Rotterdam Study[13]. Of all the ICD-10 codes adjudicated, a pre-defined selection was made for further review: I05-I09 (chronic rheumatic heart disease, all certainty scores), I20-I25 (ischemic heart disease, all certainty scores), I30-I52 (other forms of heart disease, all certainty scores), I60-I69 with only possible certainty (possible cerebrovascular disease) and R96-R99 (sudden death and ill-defined causes of death, all certainty scores). Full medical records for this selection of participants were retrieved. For every participant, records were evaluated independently by two of the three research physicians (MNN, MEB, or MJGL) to assess whether SCD should be adjudicated. This was done without knowledge of the current research question.

Witnessed deaths were classified as SCD according to Myerburg's definition endorsed by the European Society of Cardiology: "a 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"[3,14]. Unwitnessed deaths, e.g. someone found dead in bed, cannot be classified using this definition. In many studies, such events are classified as SCD, even though the time between onset of symptoms and death often is impossible to ascertain [14]. In accordance with previous work, we applied an additional criterion; unwitnessed death was coded as SCD if death was unexpected in a person found dead, the person was in a stable medical condition 24 hours before he or she

was found dead, and no evidence of a noncardiac cause was found[6,14,15]. In addition, in case of sparse information, cases were labeled as SCD when treating physicians labeled the death as sudden or unexpected. If autopsy data were available and results indicated a noncardiac cause, that case was identified as a non-SCD. Any disagreement was resolved by consensus meetings. A senior cardiologist (JWD) reviewed all cases adjudicated as SCD and cases for which consensus could not be met. The cardiologist's judgment was considered decisive.

Cardiovascular risk factors

Body mass index (weight divided by height squared), blood pressure, and cholesterol levels are measured during visits to the research center. Hypertension is defined as systolic blood pressure above 140 mmHg, diastolic blood pressure above 90 mmHg or use of blood pressure lowering medication for the indication hypertension. Data on smoking and medication use are collected through structured interviews. Prevalent myocardial infarction is assessed using the medical records[12]. Heart failure diagnosis was adjudicated in accordance with the guidelines of the European Society of Cardiology[16] and included typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction[12]. Diabetes mellitus was defined as fasting glucose level above 6.9 mmol/l, non-fasting glucose level above 11.0 mmol/l, the use of blood-glucose lowering medication, previous diagnosis of diabetes mellitus, or positive response on the interview. Atrial fibrillation is ascertained by electrocardiogram measurements as well as medical records. Coronary revascularization was defined as either a percutaneous or surgical coronary revascularization procedure.

Statistical analysis

For the overall incidence all three subcohorts were used. First, the incidence rates of SCD were calculated by dividing the total number of SCD cases by the total number of person-years of follow-up observed. Age-standardized incidence rates and sex-specific incidence rates were calculated. Age-standardization was done in 5-year age categories. Person-years were calculated for each participant per age-category. Weights for age-standardization were calculated according to the total number of participants in a certain age category divided by the total number of participants. The 95% confidence intervals (CI) were calculated using a Poisson distribution. Cox proportional hazards models were used to analyze the association between age, sex, weekday and seasonal trends, and the occurrence of SCD. These models were adjusted for sex and age where applicable and results are presented as hazard ratio (HR) with 95%CI. Subsequently, to investigate incidence trends over time, SCD incidence from two subcohorts within the Rotterdam Study, RS-I and RS-II, were compared. From both subcohorts, persons aged older than 95 years were excluded because there were too few participants and cases in this age category. RS-III was not used for this analysis because age for inclusion was lower and follow-up time was shorter. Follow-up time was censored at date of death, date of reaching 95

years of age, date of reaching 10 years of follow-up or at January 1st 2001 for RS-I and January 1st 2011 for RS-II, whichever came first. RS-I was analyzed from 1990-2000, RS-II from 2001-2010 to ensure reasonably comparable age ranges and similar follow-up time. A Cox model adjusted for age and sex was used to determine the difference between RS-I and RS-II with regard to the occurrence of SCD. To check for a possible cohort effect with regard to mortality, a Cox model was also used to determine the difference between the two subcohorts with regard to all-cause mortality and the difference between the two HRs was determined using a Z-test. Baseline differences between the two subcohorts were analyzed using a chi-square test for dichotomous variables. A two-sided p-value below 0.05 was considered statistically significant. Analyses were performed using IBM SPSS Statistics Version 21.0 (IBM Corp., Somer, NY, USA).

Results

General characteristics

General characteristics of the population are presented in Table 1. The mean age of the total population at baseline was 65.8±10.4 years and 41% were men. The 14,628 participants contributed 137,944 person-years of follow-up (median 9.3 years; interquartile range 11.4). A total of 5,512 participants died during follow-up. The cause of death could not be established in 223 (4%), and in 583 subjects the cause of death was adjudicated as SCD. The process of identifying SCD cases is summarized in Figure 1.

Table 1. Baseline characteristics of the total Rotterdam Study (RS) and the two subcohorts

	Total RS (RS-I, RS-II and RS-III)	RS-I (1990-1993)	RS-II (2000-2001)
n	14,628	7,758	2,985
Cases	583	518	53
Men	6,027 (41%)	3,061 (40%)	1,310 (44%)
Age (years)	65.8±10.4	70.3±9.6	65.2±8.4
Body mass index (kg/m^2)	26.4 [5.0]	26.0 [4.6]	26.2 [5.7]
Blood pressure (mmHg) Systolic	138±22	139±22	143±22
Diastolic	77±12	74±12	79±11
Hypertension	4,283 (29%)	1,778 (23%)	662 (22%)
Current smoker	3,483 (23%)	1,705 (22%)	688 (23%)
Total cholesterol (mmol/l)*	6.2±1.6	6.6±1.2	5.8±1.0
Statin use	1,057 (7%)	144 (2%)	358 (12%)
Myocardial infarction	750 (5%)	504 (7%)	142 (5%)
Heart failure	320 (2%)	257 (3%)	40 (1%)
Diabetes mellitus	1,480 (10%)	839 (11%)	321 (11%)
Atrial fibrillation	553 (4%)	373 (5%)	81 (3%)
Coronary revascularization	431 (3%)	210 (3%)	108 (4%)
Resuscitation attempt	74 (13%)	59 (11%)	12 (23%)

* RS-I non-fasting, RS-II & RS-III fasting

Data presented as number (percentage), median [interquartile range] or mean±standard deviation

Table 2. Circumstances of sudden cardiac death events in the Rotterdam Study

Circumstances	n (%)
Home	319 (55%)
Residential care home	77 (13%)
Nursing home	55 (9%)
Ambulance	6 (1%)
Hospital	58 (10%)
During admission	*45*
Emergency room	*12*
Outpatient clinic	*1*
During physical activity	16 (3%)
Sport	*9*
House holding activities	*6*
Sexual intercourse	*1*
Other	46 (8%)
On vacation	*14*
On the street	*13*
Visiting friends/relatives	*6*
Restaurant	*3*
Car	*3*
Work	*2*
Airplane/helicopter	*2*
Airport	*1*
Pedicure	*1*
Singing club	*1*
Not specified	6 (1%)

Some people could be placed in more than one category, we chose the most prominent place or activity as category

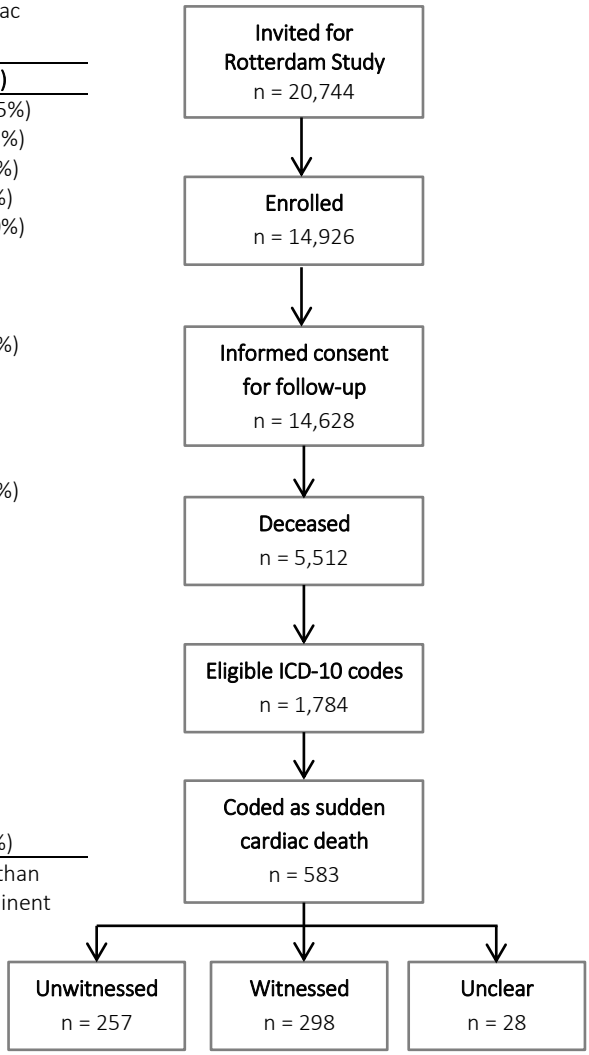

Figure 1. Flowchart of study population selection and sudden cardiac death case adjudication

Of the SCD cases, 257 (44%) were unwitnessed; in 28 cases (5%) it was unclear whether death was witnessed because of sparse information. Circumstances of SCD are summarized in Table 2. The majority of SCDs occurred at home (55%, n=319), in a residential care home (13%, n=77), or in a nursing home (9%, n=55). Of these subjects, almost 20% (n=87) died in their sleep; 58 persons died in hospital, most during admission or in the emergency room, and 1 participant died while visiting an outpatient clinic. Sixteen persons died during physical activity. In 74 cases (13%), an attempt to resuscitate was reported.

Incidence of SCD

The overall age-standardized incidence of SCD in this population of people aged 45 years and older was 4.2 per 1000py. The incidence of SCD was higher in men (5.2 per 1000py) than in women (3.6 per 1000py; age-adjusted HR 1.84; 95%CI 1.56;2.17). With increasing age, the risk of SCD increased (sex-adjusted HR per year 1.10; 95%CI 1.09;1.11). Specific incidence rates for age and gender categories are given in Table 3. SCD incidence showed a seasonal variation (Figure 2), which indicates a decrease in the summer (HR 0.75; 95%CI 0.59;0.94) compared to the winter. There were no significant differences in incidence of SCD on different days of the week or between weekdays and weekend days for the total population.

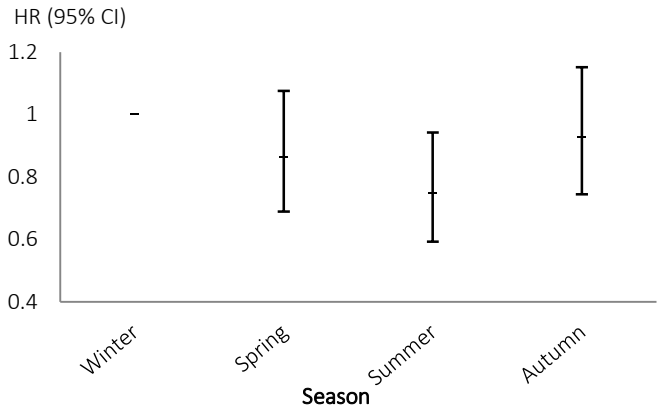

Figure 2. Hazard ratio (HR) and 95% confidence intervals for sudden cardiac death by season adjusted for age and sex, with winter as reference category

Trend analysis

For trend analysis, the first subcohort consisted of 7,678 participants with 262 SCD cases; the second subcohort consisted of 2,973 participants with 53 SCD cases. The numbers of participants and SCD cases are different from the total RS-I and RS-II population given in Table 1 because participants aged older than 95 years at baseline were excluded for this analysis. Also, for RS-I, follow-up was censored at January 1st 2001. Follow-up of vital status was complete for both subcohorts. Information on cause of death was available for all participants of RS-I and for 99.3% of the participants of RS-II. For RS-I, at baseline the prevalence of coronary revascularization procedures was 3% versus 4% in RS-II ($p<0.001$). Statin uptake at baseline increased significantly from RS-I (2%) to RS-II (12%; $p<0.001$). Prevalence of myocardial infarction, heart failure, and atrial fibrillation all were significantly higher in RS-I compared to RS-II ($p<0.001$). In RS-II, more resuscitation attempts were reported ($p<0.02$). In RS-I, from 1990-2000, the age-standardized incidence rate was 4.7 per 1000py (95%CI 4.1;5.3). In RS-II, from

Table 3. Age and gender-specific incidence rates of sudden cardiac death based on the Rotterdam Study (1990-2010)

Age category	Total population		Men		Women	
	Cases/py	IR (95%CI)	Cases/py	IR (95%CI)	Cases/py	IR (95%CI)
45-54	3/4,398	0.7 (0.1;2.0)	1/1,887	0.5 (0.0;3.0)	2/2,512	0.8 (0.1;2.9)
55-59	8/8,948	0.9 (0.4;1.8)	6/3,871	1.6 (0.6;3.4)	2/5,077	0.4 (0.1;1.4)
60-64	28/20,885	1.3 (0.9;1.9)	20/8,968	2.2 (1.4;3.5)	8/11,917	0.7 (0.3;1.3)
65-69	38/24,508	1.6 (1.1;2.1)	25/10,618	2.4 (1.5;3.5)	13/13,890	0.9 (0.5;1.6)
70-74	76/25,031	3.0 (2.4;3.8)	45/10,716	4.2 (3.1;5.6)	31/14,315	2.2 (1.5;3.1)
75-79	105/22,326	4.7 (3.9;5.7)	67/8,902	7.5 (5.8;9.6)	38/13,424	2.8 (2.0;3.9)
80-84	139/16,736	8.3 (7.0;9.8)	57/5,754	9.9 (7.5;12.8)	82/10,982	7.5 (5.9;9.3)
85-89	109/9,943	11.0 (9.0;13.2)	40/2,630	15.2 (10.9;20.7)	69/7,312	9.4 (7.3;11.9)
>90	77/5,169	14.9 (11.8;18.6)	18/994	18.1 (10.7;28.6)	59/4,176	14.1 (10.8;18.2)
Overall	583/137,944	4.2 (3.9;4.6)	279/54,340	5.1 (4.6;5.8)	304/83,605	3.6 (3.2;4.1)

Due to rounding, numbers can differ

Abbreviations: CI: confidence interval; IR: incidence rate per 1000 person-years; py: person-years

2001-2010, the age-standardized incidence rate was 2.1 per 1000py (95%CI 1.5;2.7), incidence rate ratio 0.42 (95% CI 0.31;0.57). In the Cox regression the sex and age-adjusted HR was 0.60 (95%CI 0.44;0.81). Further adjustment for known cardiovascular risk factors did not provide significantly different HRs. For total mortality, the age and sex adjusted HR was 0.82 (95%CI 0.75;0.90), which could indicate a cohort effect on mortality. However, the decline in SCD incidence was significantly larger ($p<0.01$) indicating that the decline of SCD is independent of total mortality. Incidence rates for RS-I and RS-II for the different age categories are shown in Figure 3. For men, the age-adjusted incidence decreased from 6.4 per 1000py from 1990-2000 to 3.0 per 1000py from 2001-2010 (age-adjusted HR 0.61; 95%CI 0.41;0.90). For women, the incidence decreased from 3.8 per 1000py in RS-I to 1.4 per 1000py in RS-II (age-adjusted HR 0.55; 95%CI 0.34;0.90). The difference in decrease between men and women was not statistically significant (p=0.74).

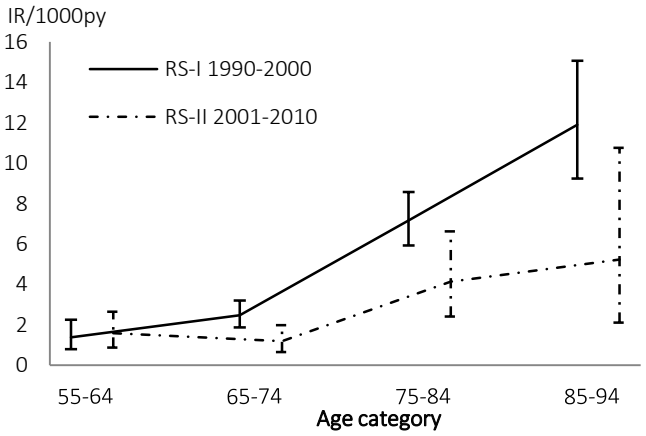

Figure 3. Incidence rates (IR) of sudden cardiac death per 1000 person-years (py) with their 95% confidence intervals by calendar year separate for the RS-I (1990-2000) and RS-II (2001-2010) subcohorts

Discussion

Key findings

In this study, we assessed the incidence rate of SCD in a general middle-aged and elderly population. In our study, the age-standardised incidence rate was 4.2 per 1000py, and the incidence was higher in men than in women. Incidence has declined over the past two decades, from 4.7 per 1000py from 1990-2000, to 2.1 per 1000py from 2001-2010.

Overview of results and interpretation

Most studies on the incidence of SCD showed an incidence rate of aprroximately 1 per 1000py in the total adult population[6,7,17]. However, in men, aged 60-69 years, an incidence up to 8 per 1000py has been reported[6,18]. Our population consisted of persons aged 45 years and older. Our results are in line with these earlier findings reported in the literature.

Because the incidence of cardiovascular mortality in high-income countries has decreased over the last decades[2], it seems reasonable to assume that the incidence of SCD will follow the same trend. In our study, we showed that this expected decrease in incidence indeed occurred in the Netherlands. We postulate that the decrease is potentially due to better diagnosis and treatment of heart diseases[19,20] and most importantly primary prevention of SCD[21] and cardiovascular disease in general through improved lifestyle and risk factor management[22,23]. This hypothesis is supported by the larger percentage of participants with a history of coronary revascularization procedures in the second subcohort as well as the much higher uptake of statins. However, when adjusting the Cox model for these factors, we could not demonstrate a less significant decline. Furthermore, a better recording of death could play a role; however, we had access to the full medical records, and there was no selection or change in reporting of death events except for changes in reporting by physicians, which would influence every study done on this topic. We also analyzed the incidence trend for total mortality to check for cohort effects, and found that total mortality also declined from 1990-2010; however, the decline in SCD incidence was significantly larger. This indicates that there is a real decline of SCD incidence other than cohort effects with regard to total mortality. This cohort effect could be the result of better health care and an increased life expectancy.

The incidence in men was higher than in women. Although this finding is consistent with other studies[6,17,24], the observed difference in our study was smaller. In earlier studies, men had a threefold higher risk of SCD compared to women[24] whereas in our study this was a 1.48-fold higher risk.

In our population, we saw a seasonal variation with a reduction in the summer and a peak during the winter. This is in line with findings from earlier studies[6,25,26]. The higher incidence in the winter could result from cold-related physical stress, but also from a change in biological rhythms linked to day and night[26]. Unlike other studies that showed a peak in incidence on Mondays and Tuesdays[26,27], indicating an effect of work-related stress, we could not demonstrate this finding in our population. However, in the Rotterdam Study the mean age is above 65 years of age, the age of formal retirement in the Netherlands. We only had 38 cases in participants up to 65 years of age. Therefore, in our study, the effect of work-related stress might not be well represented.

Limitations

Some limitations of our study merit attention. First is the possibility of misclassification of SCD cases. We had information on almost all deaths in our study (96%), including circumstances in the period before and around death, and the time between onset of symptoms and death. This information allowed us to determine whether participants met the criteria for the SCD definition. However, some potential false-positive misclassification may have occurred by inclusion of unwitnessed deaths. In our population of SCD cases, 44% of deaths were unwitnessed, which is comparable to previously reported numbers[6,15,24].

Another potential source of misclassification in population-based studies in community-dwelling elderly is that few participants die while being monitored by electrocardiogram. Therefore, we had to use a definition that relies on symptoms and time from onset of symptoms until death. By using this definition potential heterogeneity in our case set was introduced. For instance, intracerebral hemorrhage, ruptured aortic aneurysms, or massive pulmonary embolisms could lead to abrupt loss of consciousness and near-instant death. Unfortunately, we do not have consistent data on whether an autopsy was conducted and on the outcome of the autopsy if one was done. Nevertheless, we applied a universal SCD definition and this allowed us to compare our study to other studies. Next, within the Rotterdam Study no systematic data is collected on successful cardiac resuscitations, cardioverter defibrillator implants, and appropriate defibrillator shocks. Such data could have been helpful in explaining the decrease in SCD incidence over time. Last, we used two different subcohorts to study the incidence trend. Participation rates were lower in RS-II than in RS-I. It is a well-recognised phenomena that non-participants are less healthy than participants in population research[28,29]. In theory, the estimates of decline in the incidence of SCD therefore may be overestimated; however, this would also hold true for the total mortality. The decline in SCD incidence is significantly larger than the decline for total mortality, indicating that the declining SCD incidence cannot be fully attributed to lower participation rates and inclusion of a healthier population.

Clinical implications and future research

Even though our study showed a decreasing incidence of SCD in a study population from a high-income country, the number of SCDs is expected to increase on a global scale because cardiovascular morbidity and mortality are increasing worldwide[1]. SCD and other cardiac arrhythmias are a major burden for health care and a substantial psychological burden for relatives of those struck by SCD. Therefore, SCD remains an important global health issue. To prevent SCD, risk stratification is crucial to identify persons at high risk and offer tailored individualized preventive measures. However, accurate risk assessment remains challenging, and potentially additional risk indicators should be identified that would be of use in risk stratification and targeted prevention.

Conclusion

SCD incidence is declining in the western population but remains a major health problem. Therefore, awareness and prevention should remain important issues in clinical care as well as at the public health level. Additional preventive care and further improvements in cardiovascular disease management and better risk assessment most likely will lead to a further reduction in the health burden of SCD in the future.

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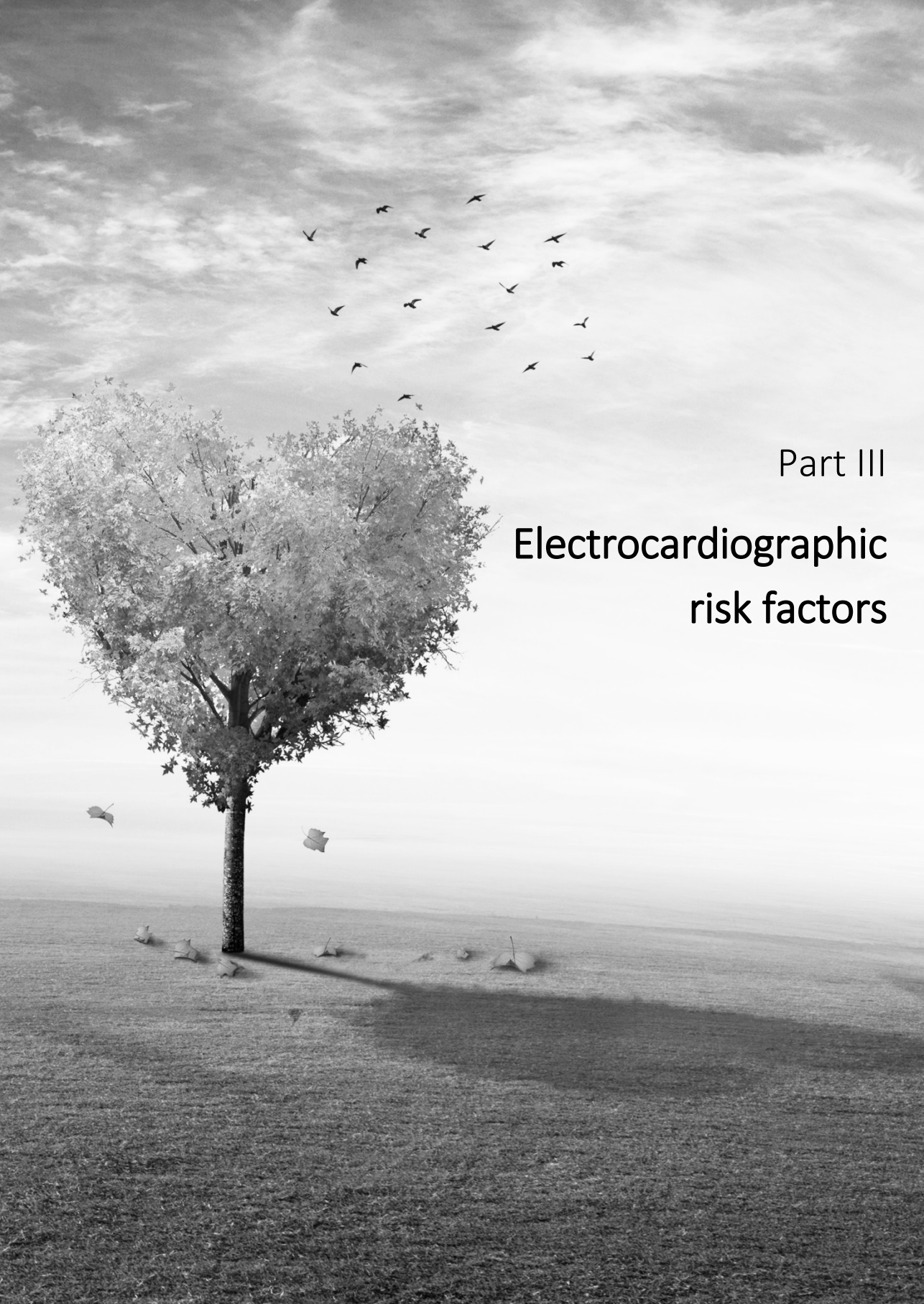

Part III

Electrocardiographic risk factors

Chapter 3.1

QT variability markers for the prediction of ventricular arrhythmias and sudden cardiac death

Maartje N Niemeijer*, Marten E van den Berg*, Mark Eijgelsheim, Gerard van Herpen, Bruno H Stricker, Jan A Kors, Peter R Rijnbeek

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Abstract

Background 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.

Conclusion QT variability markers are potential determinants of ventricular arrhythmias and cardiac mortality. However, it is unclear which marker and methodology are clinically most useful as well as what reference values are reliable. 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 syndrome (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 implantable cardioverter defibrillator (ICD) implantation. Berger et al. hypothesised that cardiac abnormalities lead to changes in ventricular repolarization[24]. However, these changes will not necessarily lead to arrhythmias, but it will lower the repolarization reserve[25,26]. Repolarization reserve is the concept that the complexity of the repolarization 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 February 4th 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[14]. 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 2, a full overview of all included studies is shown. We could not retrieve the full text of 9 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].

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

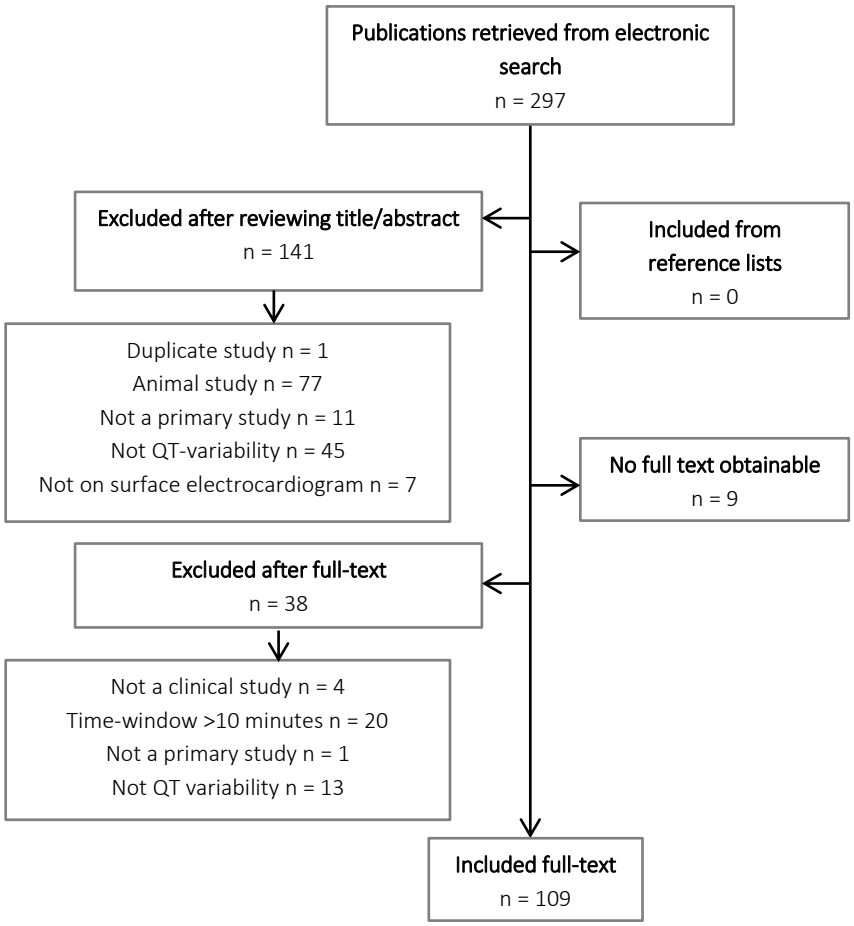


Figure 1. Overview of selection process

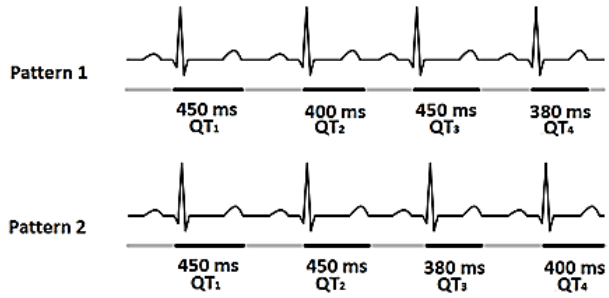
measured on consecutive beats, the differences in QT interval between the first and second beat, the second and third beat, and so on are the basis for the variability measure (consecutive QT variability). Alternatively, beat order is not taken into account and QT intervals of all beats in a certain 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 patterns 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

Table 1. Overview of markers and studies included

		Marker	Formula	n studies	Total n participants 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 59 (24-134) 11-396
		QTVN	$\frac{QT_{var}}{QT_m^2}$	31	4209 54 (29-106) 16-805
		SDqt	$\sqrt{QT_{var}}$	13	369 20 (10-48) 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 52 (27-128) 9-152
		RMSSDqt	$\sqrt{\frac{1}{n-1}\sum_{i=1}^{n-1}(QT_{i+1} - QT_i)^2}$	7	312 53 (20-68) 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	8,526 49 (24-99) 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 61.5±9.3* 55-68

* Mean±SD. The sum of studies exceeds 109 because several papers cover more than one marker.
Abbreviations: HRm: mean heart-rate; HRvar: heart-rate variance; MADqt: median absolute difference; n: number; QTm: mean QT interval; QTvar: QT variance; QTVI: QT variability index; QTVN: QT variance normalised for mean QT interval; RMSSDqt: root mean square of the successive QT interval differences; SDqt: standard deviation of QT intervals; STVqt: short-term variability of QT interval



	Pattern 1	Pattern 2
Non-consecutive, e.g. standard deviation of QT (SDqt)		
$\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(QT_i - QT_m)^2}$	$\sqrt{(1/3\ ((450-420)^2 + (400-420)^2 + (450-420)^2 + (380-420)^2))} =$ **35.59**	$\sqrt{(1/3\ ((450-420)^2 + (450-420)^2 + (380-420)^2 + (400-420)^2))} =$ **35.59**
Consecutive, e.g. short-term variability of QT (STVqt)		
$\sum_{i=1}^{n}\frac{\lvert QT_{i+1} - QT_i\rvert}{n\sqrt{2}}$	$(\lvert 400-450\rvert + \lvert 450-400\rvert + \lvert 380-450\rvert) / (4\sqrt{2}) =$ **30.05**	$(\lvert 450-450\rvert + \lvert 380-450\rvert + \lvert 400-380\rvert) / (4\sqrt{2}) =$ **15.91**

Figure 2. Effect of QT interval patterns on QT variability measures
Two hypothetical ECG patterns consisting of four beats are shown. The duration of QT intervals are identical in patterns 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.

whether or not QT variability is normalised 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

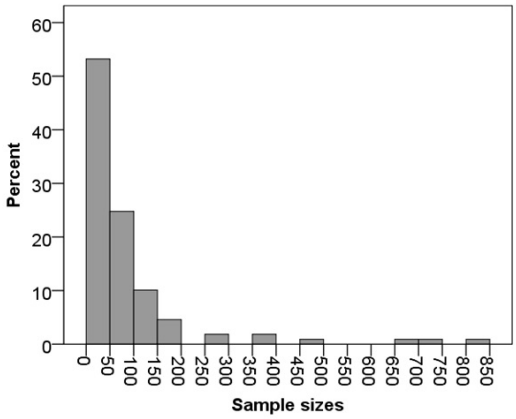


Figure 3. The distribution of population sizes across studies

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[61-63,66,70-102], e.g. congenital LQTS[78,95,96,101,102], drug-induced LQTS[77,97] 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, 3 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 resynchronisation 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 10ms)[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].

In Table 2 the effect of drugs on QT variability is shown[53,63,68,77,89,110,116,127,131,135,151-158]. 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 QT variability normalised for heart rate (variability)

Drug	Effect	Drug	Effect
β-Blockers		Antidepressants	
Carvedilol[63]	↓	Clonidine[151]	↑
Metoprolol[63]	↓	Pemoline[152]	↑
Sotalol[53,77,110,157]	↑/=	Venlafaxine[135]	↑
Antiarrhythmics		Duloxetine[135]	↑
Digoxin[157]	↑	Nortriptyline[131]	↑
Amiodarone[110]	=	Yohimbine[151]	↑
Ibutilide[156]	↑	Fluoxetine[152]	=
Atorvastatin[155]	=	Fluvoxamine[116]	=
β-Sympaticomimetics		Sertraline[116]	=
Dobutamine[89]	↑	Citalopram[116,135]	↑/=
Epinephrine[158]	↑	Paroxetine[131,135]	↑/=
Isoproterenol[127]	↑	Magnesium sulfate[157]	↓
Sevoflurane[154]	↑	Sildenafil[153]	↑

↑ indicates an increase in QT variability, ↓ indicates a decrease in QT variability, = indicates no influence on QT variability

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.
Abbreviations: QTn: QT variability normalised for heart rate (variability)

Comparison between QT variability marker groups

As described earlier, QT variability markers can be divided into 4 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

A total of 70 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.

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 among 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 milliseconds, 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, perform 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) normalisation 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 was 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 1 corresponds to the sudden i.e. high-frequency changes, and pattern 2 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 were made, QT variability discriminated between cases with an increased risk of 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 epidemiological 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 SCD and arrhythmias.

Supplements

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 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 Filters activated: Journal Article, English

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

#7 #5 NOT #6

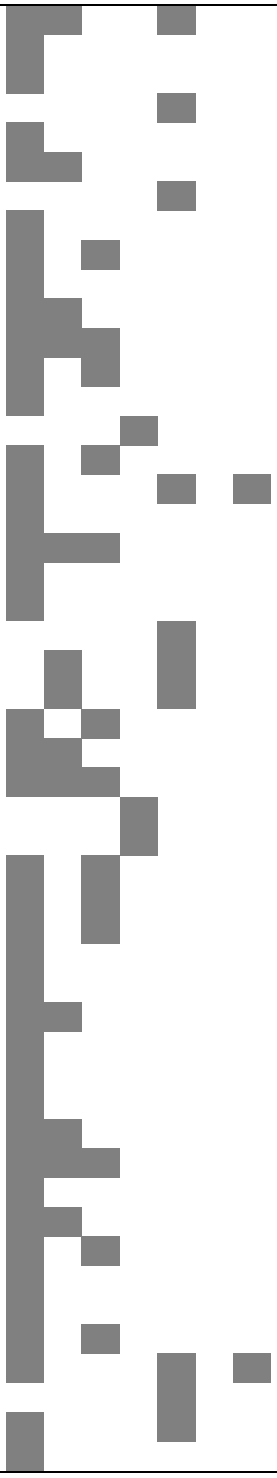
→268 publications

((qt OR repolarization OR 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" Filters activated: Journal Article [or review], English

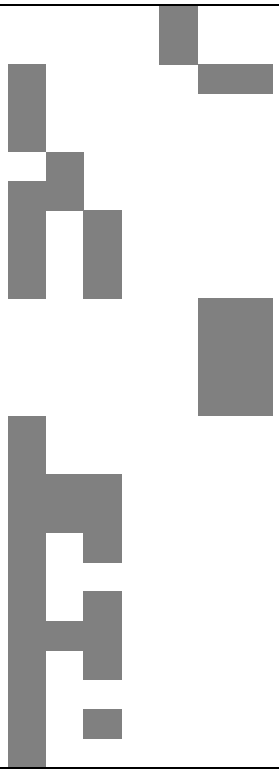
Supplement 2. Overview of included studies

Author and year of publication	Number of participants		Topic	QT variability markers						
	Total	Cases		QTVI	QTvar	QTVN	STVqt	SDqt	SDNNqt	RMSSDqt	MADqt
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 shizophrenics	■							
Baumert 2008	12/5		Depression/panic disorder	■	■						
Baumert 2008	20		Obstructive sleep apnea	■	■						
Baumert 2011	25	13	Postural tachycardia syndrome	■	■						
Baumert 2011	32	23	Essential hypertension	■							
Berger 19997	147	83	DIlated CM	■	■						
Berger 2003	30	22	Dilated CM	■	■						
Boettger 2010	23		Sport students	■							
Boettger 2010	131		Age, sexe differences	■							
Bonnet 2005	15		Young adults	■							
Carney 2003	36	18	Depression in patients with MI	■	■	■	■				
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	MI, β-blocker therapy	■		■		■			
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, CM, ICD	■		■					
Haigney 2009	70	47	Coronary artery disease, ICD	■		■					
Hasan 2012	72		Healthy subjects	■							
Hinterseer 2008	40	20	Drug-induced LQTS				■				
Hinterseer 2009	64	40	Congential 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
Jie 2010	33	16	Drug-induced LQTS
Jindal 2009	50	24	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		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		Elderly
Magri 2007	60	30	B-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
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, hypertrophic CM, ICD
Piccirillo 2006	190	44	Heart failure
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		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	6-8 weeks post MI



Singh 1997	49		Sleep, awaking
Singh 1997	1		Cardiac arrest
Solaimazadeh 2008	28	14	Familial dysautonomia
Spears 2012	21	11	SCD, epinephrine
Sullivan 2004	16		Panic disorder
Svernhage 1998	12	4	Torsades de Pointes
Tereshchenko 2009	298		VF/VT, ICD
Tereshchenko 2011	69	22	VF/VT, cardiac resynchronisation
Tereshchenko 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 CM
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	Averanxious disorder
Yeragani 2002	54	22	Panic disorder
Yeragani 2003	31	19	Panic disorder
Yeragani 2005	49		Diurnal changes
Yeragani 2006	48	25	Anxiety, panic disorder



* studied also QTRR

Abbreviations: CABG: coronary artery bypass graft; CM: cardiomyopathy; ICD: Implantable Cardioverter Defibrillator; ICU: intensive care unit; LQTS: long-QT syndrome; MI: myocardial infarction; SCD: sudden cardiac death; VF: ventricular fibrillation; VT: ventricular tachycardia

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Chapter 3.2

Consistency of heart-rate corrected QT interval prolongation and risk of sudden cardiac death

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Abstract

Background A prolonged heart-rate corrected QT (QTc) interval is a well-known risk indicator for sudden cardiac death (SCD) and a contraindication for drugs with potentially arrhythmogenic adverse effects. We aimed to study the consistency of QTc interval prolongation and whether a consistent QTc interval prolongation correlates differently with SCD compared than does an inconsistently prolonged QTc.

Methods We used a population-based cohort study of persons aged 55 years and older. We excluded participants using QTc-prolonging drugs or with a bundle branch block. QT was corrected for heart rate using Bazett's and Fridericia's formulas. Using a Cox' regression model, we assessed the association between QTc interval prolongation consistency and the occurrence of SCD.

Results A total of 3,484 participants had electrocardiograms (ECG) recorded on two consecutive visits. In 96-98% of participants with a normal QTc interval on the first ECG, the QTc interval remained normal, but only in 27-35% of those with a prolonged QTc interval, the QTc interval was prolonged on the second ECG after a median of 1.8 years. A consistently prolonged QTc interval was associated with an increased risk of SCD compared to a consistently normal QTc interval (Bazett: HR 2.23; 95%CI 1.17;4.24, Fridericia: HR 6.67; 95%CI 2.96;15.06). A prolonged QTc interval preceded or followed by a normal QTc interval was not significantly associated with an increased risk of SCD.

Conclusion Persons with an inconsistently prolonged QTc interval did not have a higher risk of SCD than those with a consistently normal QTc interval. Persons with a consistently prolonged QTc interval did have a higher risk of SCD. Our results suggest that repeated measurements of the QTc interval could enhance risk stratification.

Introduction

The QT interval on the electrocardiogram (ECG) represents the ventricular depolarization and repolarization. Since QT interval duration is highly dependent on RR interval duration, it is common to apply a heart-rate correction method to the QT (QTc) interval as proposed by Bazett[1] or Fridericia[2]. A prolonged QTc interval is a well-known ECG-derived marker for the risk of sudden cardiac death (SCD)[3,4], with a 2.5-fold increased risk of SCD in persons with a prolonged QTc interval in the Rotterdam Study[5]. SCD is one of the most common causes of cardiovascular death, with an estimated annual 4-5 million deaths worldwide[6]. It is primarily caused by ventricular arrhythmias such as ventricular fibrillation, ventricular tachycardia, and torsade de pointes[3]. Arrhythmogenic drugs are contraindicated in persons with a prolonged QTc interval[4]. A recent study by Aro et al. showed that a prolonged PR interval, another risk factor for cardiovascular mortality and SCD, normalized in a substantial part of the population after a median of 6 years[7]. Since this could also hold for QTc interval prolongation, it is important to determine consistency of a prolonged QTc interval and whether this relates to the risk of SCD. After all, one incidental finding of a prolonged QTc interval is nowadays used as contraindication and might withhold patients from being treated with relevant medicines. However, the consistency of QTc interval prolongation over time has never been studied in the general population. We aimed to study the consistency of QTc interval prolongation between two consecutive ECG recordings in a middle-aged and elderly general population and the association between QTc interval prolongation consistency and the occurrence of SCD to determine the usefulness of one incidental finding of a prolonged QTc interval.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study in the city of Rotterdam, the Netherlands. Details regarding design, objectives, and methods of the Rotterdam Study have been described previously[8,9]. In short, all inhabitants of the Ommoord district, aged 55 years and older were invited to participate. At baseline (1990-1993), 7,983 participants (response rate 78%) were included. A second visit took place from 1993-1995 and a third from 1997-1999. Besides visits to the research center, participants are continuously and actively monitored for major morbidity and mortality through linkage of general practitioners' and municipality records. 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)". All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Study population

We included all participants with ECG measurements on the first and second visit, and not using known (Supplement 1) or possible (Supplement 2) QTc-prolonging drugs[10] during ECG recording. Participants with a pacemaker rhythm or bundle branch block on one of the ECGs were excluded. In addition, we used the third visit to construct a flowchart of long-term consistency. Exposure to QTc-prolonging drugs was determined through pharmacy-dispensing data, which was available for more than 99% of participants from January 1st 1991 onwards, and included Anatomical Therapeutic Chemical-codes, dispensing date, total number of tablets/capsules per prescription, and the daily-prescribed number of tablets/capsules. Dispensing episodes were calculated by dividing the total number of tablets/capsules by the daily-prescribed number, with a carry-over period of seven days. If the date of one of the ECG measurements fell within a dispensing episode of one of the selected drugs, the participant was considered as being exposed.

QTc interval measurement

Standard 12-lead ECGs were recorded, by experienced research assistants, after approximately 20 minutes of rest, with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hertz and stored digitally. All ECGs were processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurements[11]. MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, by using template matching techniques[11-13]. MEANS determines the QT interval from the start of the QRS complex until the end of the T wave. To correct for heart rate, Bazett's formula[1], $QTcB=QT/\sqrt{RR}$, and Fridericia's formula[2], $QTcF=QT/\sqrt[3]{RR}$, were used (QT in milliseconds (ms), RR in seconds). A prolonged QTc interval was defined as an interval above 450ms in men and above 470ms in women[14]. The consistency of a normal or prolonged state on two measurements was classified into three categories: normal-normal, inconsistent (either normal-prolonged or prolonged-normal), and prolonged-prolonged.

Outcome definition

SCD was defined according to Myerburg's definition endorsed by the European Society of Cardiology: "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"[15,16]. Identification of SCD cases was done blinded to QT/QTc interval durations by two research physicians and subsequently confirmed by an experienced cardiologist after reviewing the medical files[17,18]. Follow-up was complete for almost all (96%) deaths until January 1st 2011.

Covariables

Body mass index was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressure were measured in sitting position at the right upper arm. The average of two consecutive measurements was taken. Smoking status was assessed during the home interview. Because of the high percentage of missing values for this covariable (11%), we carried the last observation forward, as this factor is relatively stable over time. Diabetes mellitus was defined as a fasting glucose above 6.9 mmol/l, a non-fasting glucose above 11.0 mmol/l, use of blood-glucose lowering medication, a previous diagnosis of diabetes mellitus, or a positive response on the interview. A history of coronary heart disease was defined as a previous diagnosis of myocardial infarction or a coronary revascularization procedure[17]. Heart failure diagnosis was based on typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction, usually echocardiography[17,19]. Heart rate was calculated as 60,000/RR interval in ms. RR interval was determined as the average of all RR intervals between consecutive normal beats on the ECG. All covariables were determined at the date of the second ECG measurement.

Data-analysis

We created 2x2 tables for the number of participants with a normal and prolonged QTc interval on the first and second ECG, separately for men and women. We assessed the consistency of the normal and prolonged QTc interval on two ECGs with kappa values. We calculated Pearson correlation coefficients for the continuous QTc interval of two ECGs. We used the third ECG to construct a flowchart of the QTc interval prolongation consistency over a longer time period. We compared characteristics of the second ECG of the normal-normal category with the ECG with a normal QTc interval in the inconsistently prolonged category, as well as of the second ECG of the prolonged-prolonged category with the ECG with a prolonged QTc interval in the inconsistently prolonged category. We calculated p-values using independent-samples t-tests for continuous variables and chi-square tests for dichotomous variables. We calculated the incidence rate of SCD for each category per 1,000 person-years with 95% confidence interval (CI) according to a Poisson distribution. We used a Cox regression model to estimate the hazard ratio (HR) of a prolonged QTc interval at baseline for occurrence of SCD. We used the data augmentation method and unstratified model described by Lunn and McNeil to estimate the HR of SCD for the different categories of consistency, taking into account the competing risk of deaths from other causes[20]. Follow-up time was calculated from date of the second ECG until date of death, loss to follow-up (n=58) or the end of the study period (January 1st 2011), whichever came first. The proportional hazards assumption was assessed using log survival curves. Sex and age were included as covariables in the crude model. In the multivariable adjusted model we further included covariables as mentioned before and the time between the first and second ECG. To eliminate residual confounding by heart rate, we also adjusted for

heart rate in a third model. We performed sensitivity analyses with additional adjustment for QRS duration and limiting follow-up to 10 years. A two-sided p-value below 0.05 was considered statistically significant. Data were analysed using IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, USA) and R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

Results

General characteristics

Figure 1 provides a flowchart of the selection of the study population, which eventually consisted of 3,484 participants. Baseline characteristics of the study population are shown in Table 1. The mean age was 69.1±8.1 years and 2044 (59%) were women. The time between the first and second ECG ranged from 0.7 to 4.4 years (median 1.8). During a median follow-up of 15.5 years (interquartile range 9.4-16.5), 1,690 (49%) persons died, of whom 189 (11%) were SCD cases.

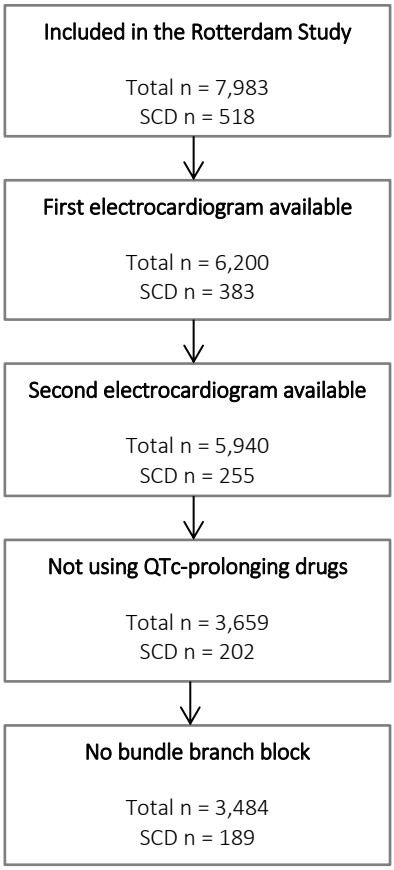

Figure 1. Flowchart of the selection of the study population
Abbreviations: n: number; QTc: heart-rate corrected QT interval; SCD: sudden cardiac death

Consistency of QTc interval prolongation

Table 2 shows 2x2 tables for consistency of QTc interval prolongation. A total of 96-98% of participants with a normal QTc interval on the first ECG also had a normal QTc interval on the second ECG. However, around two-third of the participants with a prolonged QTc interval on the first ECG presented with a normal QTc interval on the second. Inter-measurement consistency was fair, with kappa values ranging from 0.19 to 0.37 (Table 2). The correlation for the continuous QTcB interval between the two ECGs was r=0.59, and r=0.62 for QTcF interval. A flowchart for the consistency on three consecutive visits is shown in Figure 2, which shows that these results are approximately the same over a longer time period.

Table 1. Baseline characteristics of the study population (at date of the second electrocardiogram measurement; 1993-1995)

	Total study population (n=3,484)	Men (n=1,440)	Women (n=2,044)	Missing
Sudden cardiac death	189 (5.4%)	93 (6.5%)	96 (4.7%)	-
Age, years	69.1±8.1	68.1±7.4	69.8±8.6	-
Height, cm	168±9	176±7	162±7	118 (3.4%)
Body mass index, kg/m^2	26.4±3.7	25.9±2.9	26.7±4.1	120 (3.4%)
Heart rate, bpm	69±12	68±12	70±12	-
QRS duration, ms	97±13	102±13	94±12	-
Blood pressure, mmHg				
Systolic	141±22	140±22	141±23	32 (0.9%)
Diastolic	77±11	77±12	77±11	33 (0.9%)
History of heart failure	91 (2.6%)	40 (2.8%)	51 (2.5%)	-
History of coronary heart disease	247 (7.1%)	181 (12.6%)	66 (3.2%)	-
History of diabetes mellitus	334 (9.6%)	136 (9.4%)	198 (9.7%)	-
Smoking				388 (11.1%)
Current	719 (23.2%)	380 (28.2%)	339 (16.6%)	
Past	1,431 (46.2%)	863 (64.1%)	568 (32.5%)	

Data presented as mean±standard deviation or number (percentage)
Abbreviations: bpm: beats per minute; ms: milliseconds; n: number

Table 2. Consistency of heart-rate corrected QT interval prolongation, according to Bazett and Fridericia, separate for men and women in each category of change between a normal and prolonged heart-rate corrected QT interval, with kappa's for inter-measurement consistency

1st ECG	Bazett		Fridericia	
	2nd ECG			
All participants (n=3,484)				
	Normal	Prolonged	Normal	Prolonged
Normal	3,118 (95.9%)	132 (4.1%)	3,347 (98.4%)	56 (1.6%)
Prolonged	152 (65.0%)	82 (35.0%)	59 (72.8%)	22 (27.2%)
Kappa (95%CI)	0.32 (0.26;0.38)		0.26 (0.17;0.35)	
Men (n=1,440)				
	Normal	Prolonged	Normal	Prolonged
Normal	1,214 (94.1%)	76 (5.9%)	1,356 (97.8%)	31 (2.2%)
Prolonged	87 (58.0%)	63 (42.0%)	37 (69.8%)	16 (30.2%)
Kappa (95%CI)	0.37 (0.30;0.45)		0.30 (0.17;0.42)	
Women (n=2,044)				
	Normal	Prolonged	Normal	Prolonged
Normal	1,904 (97.1%)	56 (2.9%)	1,991 (98.8%)	25 (1.2%)
Prolonged	65 (77.4%)	19 (22.6%)	22 (78.6%)	6 (21.4%)
Kappa (95%CI)	0.21 (0.12;0.30)		0.19 (0.05;0.33)	

Percentages are of people with a normal heart-rate corrected QT interval on the first ECG that stay normal or change to prolonged interval, and vice versa
The first ECG was made between 1991-1993, the second ECG between 1993-1995
Abbreviations: CI: confidence interval; ECG: electrocardiogram; n: number of participants

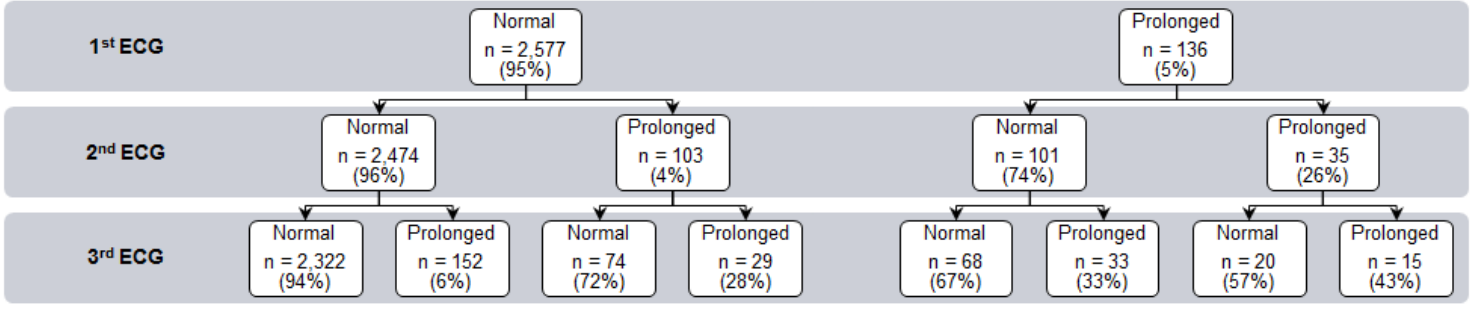

Figure 2. Flowchart of normal and prolonged heart-rate corrected QT intervals according to Bazett's formula, on three consecutive electrocardiogram measurements

The first electrocardiogram was made between 1991-1993, the second between 1993-1995 and the third between 1997-1999

Abbreviations: ECG: electrocardiogram; n: number of participants

Table 3. Characteristics of various categories of the QTc prolongation consistency

		Normal-normal (n=3,118)		Inconsistent* (n=284)				Prolonged-prolonged (n=82)	
		1st ECG	2nd ECG	ECG with normal QTc interval	p†	ECG with prolonged QTc interval	p‡	1st ECG	2nd ECG
Gender, women		1,904 (61%)				121 (43%)		19 (23%)	
Age (years)		66.9±7.9	68.8±8.0	70.4±8.9	0.003	702±8.8	0.006	71.5±8.8	73.3±8.9
Body mass index (kg/m2)		26.3±3.6	26.3±3.6	26.9±3.7	0.021	26.9±3.8	0.412	26.3±3.4	26.5±3.6
QTc interval (ms)	Men	415±19	414±18	430±15	<0.001	462±14	0.077	468±21	465±14
	Women	429±19	427±19	445±18	<0.001	483±12	0.895	484±11	482±10
Heart rate (bpm)		70±11	69±11	71±12	<0.001	80±16	0.273	80±16	78±15
QRS duration (ms)		97±11	96±12	103±13	<0.001	104±17	0.006	113±22	112±23
Blood pressure (mmHg)	Systolic	140±22	138±21	144±23	0.004	144±22	0.430	146±23	146±21
	Diastolic	77±11	74±11	78±13	0.178	78±12	0.998	78±12	77±10
History of heart failure		41 (1%)	59 (2%)	15 (5%)	<0.001	16 (6%)	<0.001	10 (12%)	14 (17%)
History of coronary heart disease		170 (5%)	190 (6%)	35 (12%)	<0.001	35 (12%)	0.003	20 (24%)	21 (26%)
History of diabetes mellitus		277 (9%)	284 (9%)	40 (14%)	0.006	38 (13%)	0.779	10 (12%)	10 (12%)
Smoking	Current	696 (22%)	701 (22%)	66 (23%)	0.173	61 (21%)	0.545	25 (30%)	23 (28%)
	Past	1,312 (42%)	1,349 (43%)	137 (48%)	0.033	143 (50%)	0.057	43 (52%)	45 (55%)

Data presented as mean±standard deviation or number (percentage)

* People with a prolonged heart-rate corrected QT interval on one electrocardiogram and a normal heart-rate corrected QT interval on the other. Characteristics are shown separately for the electrocardiogram with the normal and prolonged heart-rate corrected QT interval

† p value for the difference between the 2nd electrocardiogram of the normal-normal category and the electrocardiogram with a normal heart-rate corrected QT interval in the inconsistently prolonged category

‡ p value for the difference between the 2nd electrocardiogram of the prolonged-prolonged category and the electrocardiogram with a prolonged heart-rate corrected QT interval in the inconsistently prolonged category

Abbreviations: bpm: beats per minute; ECG: electrocardiogram; ms: milliseconds; n: number of participants; QTc: heart-rate corrected QT interval according to Bazett's formula

Table 4. Number of sudden cardiac death cases and incidence rates in each category of change between a normal and prolonged heart-rate corrected QT interval (n=3,484)

	Normal-normal	Inconsistent*	Prolonged-prolonged
Bazett	152 (4.9%)	24 (8.5%)	13 (15.9%)
Incidence rate†	3.7 (3.2;4.4)	7.4 (4.8;11.1)	18.5 (9.9;31.7)
Fridericia	170 (5.1%)	12 (10.4%)	7 (31.8%)
Incidence rate†	3.9 (3.4;4.6)	10.0 (5.1;17.4)	44.1 (17.7;90.9)

* People with a prolonged heart-rate corrected QT interval on one electrocardiogram and a normal heart-rate corrected QT interval on the other

† Incidence rate per 1,000 person-years with 95% confidence interval according to a Poisson distribution

Characteristics of the various categories of QTc interval prolongation consistency are shown in Table 3. On average, the normal QTc intervals in the inconsistently prolonged category were higher than those in the normal-normal category, while the mean QTc interval on the prolonged ECG in the inconsistently prolonged category was similar compared to the consistently prolonged category. Heart rate was significantly higher during ECGs on which a prolonged QTc interval was detected than on ECGs with a normal QTc interval. The proportion of people with a history of coronary heart disease or heart failure was highest in the consistently prolonged category.

QTc interval prolongation consistency and risk of SCD

A prolonged QTc interval at baseline was associated with a higher risk of SCD (QTcB: HR 1.47; 95%CI 1.04;2.07; QTcF: HR 2.36; 95%CI 1.55;3.60). After the first ECG recording but before the ECG of the second visit, 45 participants died of SCD, who were therefore not included in the study population. Men in this group (n=27) had a mean QTcB interval of 428±29ms, and n=6 (22%) had a prolonged QTcB interval. Women (n=18) had a mean QTcB interval of 444±36ms, and n=3 (17%) had a prolonged QTcB interval. The number of SCD cases and incidence rates per category are shown in Table 4. The proportional hazards assumption was not violated. The association between consistency and occurrence of SCD is presented in Table 5, separately for the formulas of Bazett and Fridericia. Using both heart-rate correction methods, the risk of SCD was found to be not significantly increased in participants with an inconsistently prolonged QTc interval in the multivariable adjusted model. However, participants with a consistently prolonged QTc interval did have an increased risk of SCD (model 3: Bazett: HR 2.23; 95%CI 1.17;4.24; Fridericia: HR 6.67; 95% CI 2.96;15.06). Additional adjustment for QRS duration, did not substantially change the results. A sensitivity analysis limiting follow-up to 10 years gave similar results.

Table 5. Association between heart-rate corrected QT-prolongation consistency on two consecutive electrocardiograms and risk of sudden cardiac death taking into account competing risk of deaths from other causes

Change of heart-rate corrected QT interval between two electrocardiogram measurements			
Bazett	Normal-normal	Inconsistent*	Prolonged-prolonged
Model 1 (n=3,484)	Reference	**1.65 (1.07;2.56)**	**3.28 (1.84;5.86)**
Model 2 (n=3,329)	Reference	1.33 (0.79;2.25)	**3.00 (1.62;5.53)**
Model 3 (n=3,329)	Reference	1.14 (0.67;1.93)	**2.23 (1.17;4.24)**
Model 4 (n=3,329)	Reference	1.08 (0.63;1.85)	**1.97 (1.01;3.85)**
Fridericia	Normal-normal	Inconsistent*	Prolonged-prolonged
Model 1 (n=3,484)	Reference	**1.87 (1.03;3.38)**	**8.51 (3.98;18.20)**
Model 2 (n=3,329)	Reference	1.51 (0.75;3.05)	**6.17 (2.75;13.82)**
Model 3 (n=3,329)	Reference	1.49 (0.74;3.02)	**6.67 (2.96;15.06)**
Model 4 (n=3,329)	Reference	1.36 (0.66;2.79)	**6.02 (2.65;13.68)**

Data presented as hazard ratio (95% confidence interval). Bold font indicates statistical significance ($p<0.05$)

* People with a prolonged heart-rate corrected QT interval on one electrocardiogram and a normal heart-rate corrected QT interval on the other

Model 1: adjusted for sex and age at date of the second electrocardiogram

Model 2: additionally adjusted for height, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, a history of diabetes mellitus, coronary heart disease and heart failure at date of the second ECG, and time between the first and second electrocardiogram

Model 3: additionally adjusted for heart rate at the second electrocardiogram

Model 4: additionally adjusted for QRS duration

Abbreviation: n: number

Discussion

We showed that a prolonged QTc interval persists only in around one-third of the persons after a median of two years, while a normal QTc interval persists in 96-98% of the subjects in a normal state. Furthermore, we demonstrated that the risk of SCD is significantly increased when a prolonged QTc interval is present at two measurements, but to a lesser degree when this is only a single observation preceded or followed by a normal QTc interval measurement. This could hold important consequences for the usefulness of this marker as a long-term risk indicator for SCD.

This study confirms that a single baseline measurement of a prolonged QTc interval is associated with a higher risk of SCD[3-5]. Interestingly, our results also show that prediction of SCD may be further improved when multiple QTc interval measurements are taken into account. An inconsistently prolonged QTc interval proved not to be associated with a significantly higher risk of SCD than a consistently normal QTc interval, while a consistently prolonged QTc interval was associated with a higher risk of SCD. Thus, a single QTc interval measurement may be of value in risk stratification for SCD, but based on the consistency analysis, prediction of SCD may be

improved by re-measuring the QTc interval after some time. Future research should comprise serial measurements at fixed time points, to determine the optimal time-window for repeated measurements. The consistency of a prolonged QTc interval could be important for the inclusion of a prolonged QTc interval as a high-risk indicator for SCD in clinical guidelines. Note however, that we only investigated persons not using QTc-prolonging drugs. Persons with an inconsistently prolonged QTc interval might have a different response to QTc-prolonging drugs than persons with a consistently normal QTc interval, and therefore they might still have a higher risk when exposed to QTc-prolonging drugs. Further research is needed to establish whether inconsistency of QTc interval prolongation is associated to differences in drug response.

Changes between a normal and prolonged QTc interval were accompanied by changes in RR intervals, suggesting that the change between a normal and prolonged QTc interval can be partly explained by a change in heart rate. The proportion of participants with a history of coronary heart disease or heart failure was significantly higher among the persons with a consistently prolonged QTc interval. A possible explanation could be that persons who have more structural abnormalities have a more consistently prolonged QTc interval. Overestimation of the QTc interval at higher heart rates is a known problem when using Bazett's formula[21], as shown by the diminishing risk of SCD after additional adjustment for heart rate. The differences in effect estimates we found between Bazett and Fridericia are not directly comparable since the reference categories are different.
Additional adjustment for QRS duration lowered the HRs, indicating that the increased risk of SCD through QTc interval prolongation is a result of prolonged ventricular depolarization as well as repolarization.

The high level of completeness of follow-up in this study is an important strength. We had access to detailed information on morbidity and mortality events through the medical records. Besides that, within the Rotterdam Study, ECG measurements are collected following standardized protocols.
A limitation of our study is that for the association with risk of SCD, we only studied two ECG measurements with a median interval of two years. Preferably, we would use more ECG measurements, however the number of categories would become unmanageably large and the number of events in each category unreliably small. We showed data for a third ECG to demonstrate that the consistency of the QTc interval prolongation based on three ECGs is similar to the consistency based on two ECGs. Second, for Fridericia's formula there is no general consensus on a cut-off value, and therefore we used Bazett's formula as the primary method for heart-rate correction. We used the same cut-off values for Bazett and Fridericia corrected QT intervals which could introduce bias in the effect estimates obtained using

Fridericia's formula. Because measurement of QT intervals is influenced by measurement error[4,21], we cannot fully claim that all changes between normal and prolonged QTc interval are genuine changes in repolarization duration. However, we measured the intervals using an automated method, which has been shown to perform as good as other methods[11-13,22,23]. The binary classification we used (normal vs. prolonged) may be considered artificial to some extent, because a male with a QTc interval of 449ms is classified differently from a male with a QTc interval of 451ms, while the clinical relevance of this 2ms difference is questionable[4]. However, this classification is often used in clinical practice and our intention was to demonstrate the value of this risk factor as it is used in daily practice. Another limitation of our study is the heterogeneity of SCD cases that is introduced through the definition we used. However, this definition has been widely endorsed for years and the incidence rate in our cohort was comparable to other studies[6,18]. Since 45 SCD cases occurred before a second ECG was made, these persons were not included in the study population. The prevalence of a prolonged QTc interval was higher in these cases than in the SCD cases included in the analysis (20% vs. 13%, respectively), thus excluding them could have introduced bias in the consistency analyses. The population-based setting hampered ECG recording under controlled circumstances, such as a non-fasting state and at the same time of the day. However, our measurement setting does reflect measurements in daily clinical practice. Finally, our results were obtained in an older and predominantly white population (98%), and may therefore not necessarily be generalizable to younger and non-white individuals.

In conclusion, we found that a prolonged QTc interval only persists in around one-third of the persons on a second recording after a median of 1.8 years. This study shows that persons with an inconsistently prolonged QTc interval had a lower risk of SCD compared to persons with a consistently prolonged QTc interval. These results suggest that repeated measurements of the QTc interval might improve its use as a risk indicator for SCD, however the optimal frequency of these measurements remains unknown and should be subject of future research.

Supplements

Supplement 1. List of known heart-rate corrected QT-prolonging drugs according to the list of the University of Arizona with Anatomical Therapeutic Chemical code

Name	Anatomical Therapeutic Chemical code
Amiodarone	C01BD01
Anagrelide	L01XX35
Arsenic trioxide	L01XX27
Astemizole	R06AX11
Azithromycin	J01FA10/S01AA26
Bepridil	C08EA02
Chloroquine	P01BA01
Chlorpromazine	N05AA01
Cisapride	A03FA02
Citalopram	N06AB04
Claritromycin	J01FA09
Cocaine	R02AD03/N01BC01/S01HA01/S02DA02
Disopyramide	C01BA03
Dofetilide	C01BD04
Domperidone	A03FA03
Dronedarone	C01BD07
Droperidol	N05AD08
Erytromycin	J01FA01/D10AF02/S01AA17
Escitalopram	N06AB10
Flecainide	C01BC04
Halofantrine	P01BX01
Haloperidol	N05AD01
Ibutilide	C01BD05
Levomethadyl	N07BC05 (LEVOMETHADONE)
Mesoridazine	N05AC03
Methadone	N07BC02
Moxifloxacin	J01MA14/S01AE07
Ondansetron	A04AA01
Pentamidine	P01CX01 (pentamidine isethionate)
Pimozide	N05AG02
Probucol	C10AX02
Procainamide	C01BA02
Quinidine	C01BA01
Sevoflurane	N01AB08
Sotalol	C07AA07
Sparfloxacin	J01MA09
Sulpiride	N05AL01
Terfenadine	R06AX12
Thioridazine	N05AC02
Vandetanib	L01XE12

Supplement 2. List of possible heart-rate corrected QT-prolonging drugs according to the list of the University of Arizona with Anatomical Therapeutic Chemical code

Name	Anatomical Therapeutic Chemical code
Alfuzosin	G04CA01
Apomorphine	N04BC07G04BE07
Atazanavir	J05AE08
Bedaquiline	J04AK05
Bortezomib	L01XX32
Bosutinib	L01XE14
Clozapine	N05AH02
Crizotinib	L01XE16
Dabrafenib	L01XE23
Dasatinib	L01XE06
Dexmedetomidine	N05CM18
Dihydroartemisinin+piperaquine	P01BF05
Dolasetron	A04AA04
Eribulin	L01XX41
Famotidine	A02BA03
Felbamate	N03AX10
Fingolimod	L04AA27
Foscarnet	J05AD01
Fosphenytoin	N03AB05
Gatifloxacin	J01MA16/S01AE06
Gemifloxacin	J01MA15
Granisetron	A04AA02
Iloperidone	N05AX14
Isradipine	C08CA03
Lapatinib	L01XE07
Levofloxacin	J01MA12/S01AE05/J01RA05
Lithium	N05AN01
Mifepristone	G03XB01
Mirabegron	G04BD12
Mirtazipine	N06AX11
Moexipril/HCTZ	C09AA13
Nicardipine	C08CA04
Nilotinib	L01XE08
Norfloxacin	J01MA06/S01AE02
Ofloxacin	J01MA01/S01AE01/S02AA16
Olanzapine	N05AH03
Oxytocin	H01BB02
Paliperidone	N05AX13
Pasireotide	H01CB05
Pazopanib	L01XE11
Perflutren lipid microspheres	CONTRAST
Promethazine	R06AD02/D04AA10
Quetiapine	N05AH04
Ranolazine	C01EB18
Rilpivirine	J05AG05
Risperidone	N05AX08

Roxithromycin	J01FA06
Saquinavir	J05AE01
Sertindole	N05AE03
Sorafenib	L01XE05
Sunitinib	L01XE14
Tacrolimus	L04AD02/D11AH01
Tamoxifen	L02BA01
Telavancin	J01XA03
Telithromycin	J01FA15
Tetrabenazine	N07XX06
Tizanidine	M03BX02
Tolterodine	G04BA07
Toremifene	L02BA02
Vardenafil	G04BE09
Vemurafenib	L01XE15
Venlafaxine	N06AX16
Voriconazole	J02AC03
Vorinostat	L01XX38
Ziprasidone	N05AE04

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Chapter 3.3

PR interval, P-wave indices, and sudden cardiac death

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Abstract

Background Prediction of sudden cardiac death (SCD) remains difficult due to a limited knowledge on direct risk factors.PR interval has been suggested as a potential risk factor for mortality, and for SCD specifically. P-wave indices have been associated with total mortality in previous studies. We aimed to study the association between PR interval, P-wave indices and occurrence of SCD in a large population-based cohort.

Methods We used data from the Rotterdam Study, a population-based cohort study of persons aged 45 years and older. In individuals without atrial fibrillation or pacemaker rhythm, we studied the association of the PR interval and the duration, amplitude, area, and terminal force of the P wave, with the occurrence of SCD in a time-dependent Cox regression model. We adjusted for known cardiovascular risk factors, and stratified by sex and age below or above 67 years.

Results In the study population of 11,370 participants with 19,289 available electrocardiograms, 276 SCDs occurred during a median follow-up of 8.8 years. We did not find significant associations between PR interval or P-wave duration and SCD. However, irrespective of sex and age, we found significant associations between the amplitude (HR 1.04; 95%CI 1.01;1.08), area (HR 1.07; 95%CI 1.02;1.14), and terminal force (HR 1.10; 95%CI 1.05;1.15) of the P wave and occurrence of SCD.

Conclusion In this study, we showed that the amplitude, area, and terminal force of the P wave were associated with SCD. Further research should determine the value of these indices for the prediction of SCD.

Introduction

Sudden cardiac death (SCD) forms a substantial part of cardiovascular mortality, which is the leading cause of death globally[1]. SCD is primarily caused by ventricular arrhythmias[2], and risk factors for SCD include male sex, increasing age, a history of heart failure or cardiac ischemia, and alcohol and drug use[1]. SCD is a heterogeneous outcome with multiple etiological pathways of which many have not been established. This complicates adequate risk assessment and successful prevention strategies for SCD, and more knowledge about additional risk factors is needed to improve risk stratification[3]. Electrocardiograms (ECGs) are non-invasive and can be obtained relatively easy and cheap in clinical practice, and several ECG markers have already been identified as risk factors for SCD, e.g. a prolonged QT interval[2]. Another ECG marker, PR interval, denotes the time from atrial depolarization to the onset of ventricular depolarization. A prolonged PR interval, also called a first-degree atrioventricular block, has long been thought to be a benign condition[4]. However, several studies showed that a prolonged PR interval was associated with an increased risk of morbidity, e.g. atrial fibrillation, and mortality[4,5], but results across studies were not consistent. The pathophysiological mechanism behind this could be that a prolonged PR interval precedes a higher-degree atrioventricular block or that a prolonged atrial depolarization disturbs left ventricular filling which causes diastolic mitral regurgitation, and eventually might cause left ventricular dysfunction and lead to a higher mortality rate[4,5]. Because of this mechanism, a prolonged PR interval might also be associated with an increased risk of SCD. However, the only previous study regarding this association did not find a significant difference in occurrence of SCD between people with a prolonged and a normal PR interval[5]. This study only used a baseline measurement of PR interval with 35-41 years of follow-up, which could have masked the association. Regarding the contradictory results of the association between PR interval and mortality endpoints, it has been hypothesized that instead of PR interval duration, in fact the duration of the P wave is associated with mortality[6]. Previous studies on characteristics of the P-wave focused mostly on their association with atrial fibrillation[7]. We aimed to study whether a prolonged PR interval is a risk factor for SCD in a general population of middle-aged and elderly persons with more than 20 years of follow-up, using a time-dependent model, and whether P-wave indices (duration, amplitude, area, and terminal force) were associated with occurrence of SCD. Furthermore, we evaluated whether this associations might differ by gender. This is the first study to assess the association between P-wave indices and SCD.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study in the city of Rotterdam,

the Netherlands. Details regarding design, objectives, and methods of the Rotterdam Study have been described elsewhere[8]. In short, all inhabitants of the Ommoord district aged 55 years and older were invited to participate. At baseline (1990-1993), 7,983 participants (response rate 78%) were included. In 2000, an additional 3,011 participants were enrolled (response rate 67%). This extension consisted of all persons living in the study district who had become 55 years of age or who had moved into the study district. A second extension of the cohort was initiated in 2006, in which 3,932 participants (response rate 65%) aged 45 years and older were included. Follow-up examinations were conducted periodically, which include a home interview and an extensive set of tests at a study center. Besides this, participants are continuously and actively monitored for major morbidity and mortality through linkage of general practitioners' and municipality records to the study base. 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)". This study complies with the Declaration of Helsinki.

Study population

The study population for this analysis consisted of all participants who gave informed consent for follow-up monitoring and for whom ECG and covariable data were available. ECGs on which a pacemaker rhythm was detected were excluded (n=123). Persons with atrial fibrillation at baseline were excluded.

ECG measurement

Standard 12-lead resting ECGs were recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hertz and stored digitally. All ECGs were processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurements[9]. MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques, and has been evaluated extensively[9,10]. A schematic representation of an ECG with the PR interval and P-wave indices in shown in Figure 1. MEANS determines the PR interval from the start of the P wave until the onset of the QRS complex. We visually checked ECGs on which an extremely prolonged PR interval (> 300 milliseconds (ms)) was detected, and we excluded ECGs on which MEANS detected the PR interval incorrectly (n=19). The duration of the P wave (P duration) was measured from the start until the end of the P wave. The amplitude of the P wave (P amplitude) was taken as the maximum (absolute) amplitude of the P wave in all 12 leads. For the area under the P wave (P area), we computed the sum of areas of the positive and negative P-wave deflections in each lead. We subsequently selected the maximum area over the 12 leads. The terminal force (P terminal force) was defined as the product of the duration and amplitude of the negative component of the P wave in lead V1.

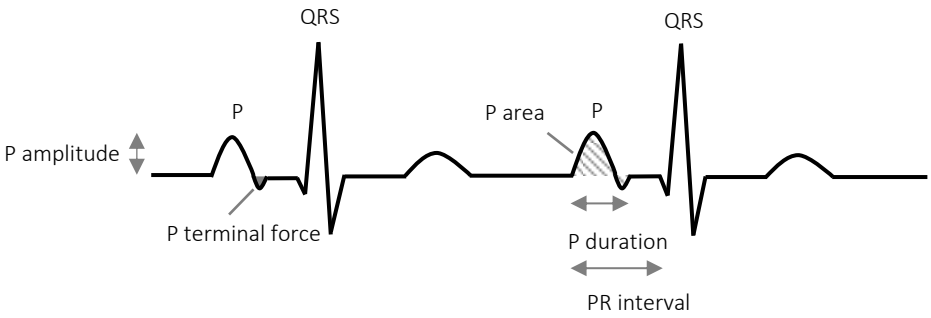

Figure 1. Electrocardiogram with the PR interval and P-wave indices

Outcome definition

SCD was defined according to Myerburg's definition endorsed by the European Society of Cardiology: "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"[11,12]. Unwitnessed deaths were coded as SCD if death was unexpected in persons found death, while they were in a stable medical condition 24 hours before they were found and without evidence of a noncardiac cause. SCD cases were identified independent of the current research question by two research physicians and confirmed by an experienced cardiologist after reviewing the medical files as described in more detail previously[13]. Follow-up was complete for 96% of deaths until January 1st 2011.

Covariables

As research into associated factors with P-wave indices is limited to date, we selected covariables mainly on previous associations with SCD, cardiovascular disease, and ECG disturbances described in the literature. Height and weight were measured during the center visits. Body mass index was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressure were measured in sitting position at the right upper arm. The average of two consecutive measurements was taken. Information on smoking status was ascertained through structured interviews. Participants were divided into three categories: never, past or current smokers. Heart rate in beats per minute was calculated from the ECG as 60,000/RR interval in ms. Left ventricular hypertrophy was determined on the ECG using a scoring system based on voltage-criteria (Sokolow-Lyon) and repolarization disturbances. Diabetes mellitus was defined as a fasting glucose above 6.9 mmol/l, a non-fasting glucose above 11.0 mmol/l, use of blood-glucose lowering medication, or a previous diagnosis of diabetes mellitus. Heart failure diagnosis was adjudicated in accordance with the guidelines of the European Society of Cardiology and included typical signs or symptoms of heart failure

confirmed by objective evidence of cardiac dysfunction[14]. A history of coronary heart disease was defined as a history of myocardial infarction or a percutaneous or surgical coronary revascularization procedure. Chronic obstructive pulmonary disease (COPD) status was based on obstructive spirometry, medical history, physical examination and drug use[15]. Medication dispensing data was obtained from all seven fully computerized pharmacies in the study district. Information on all filled prescriptions from January 1st 1991 onwards were available and included information on the product name, the Anatomical Therapeutic Chemical (ATC) code of the World Health Organisation, the dispensing date, the prescribed dosing regimen and the amount dispensed. For every filled prescription, dispensing episodes were calculated by dividing the total number of tablets/capsules by the daily prescribed number, plus a standard carry-over period of 7 days. A person was considered exposed if the date of ECG recording fell within a dispensing episode. We determined use of beta-blockers (C07), digoxin (C01AA05), class I/III antiarrhythmics (C01B), and diltiazem or verapamil (C08DA01/C08DB01). Data on all covariables was included as time-dependent covariable on the date of ECG recording.

Data analysis

We used a time-dependent Cox proportional hazards model to analyze the association of PR interval and P-wave indices with the occurrence of SCD. Follow-up time was calculated from date of the first available ECG, until date of death, loss to follow-up (n=83), date of diagnosis of atrial fibrillation, or the end of the study period (January 1st 2011), whichever came first. Cases of atrial fibrillation were censored because of the strong association of P-wave indices with atrial fibrillation[7] and of atrial fibrillation with SCD[16]. Age and sex were included in the crude model. The other

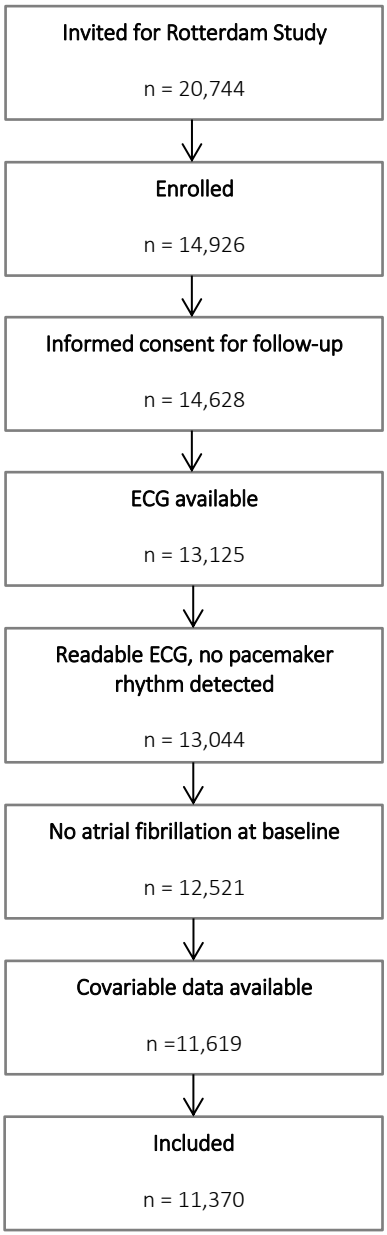

Figure 2. Selection of the study population
Abbreviations: ECG: electrocardiogram; n: number

covariables (body mass index, systolic blood pressure, diastolic blood pressure, smoking, heart rate, left ventricular hypertrophy, diabetes mellitus, heart failure, coronary heart disease, COPD, and use of beta-blockers, digoxin, class I/III antiarrhythmics, and diltiazem or verapamil) were included in the multivariable adjusted model. PR interval and P duration were analysed per 10ms, P amplitude was analysed per 10μV, and P area and P terminal force were analysed per ms·mV. PR interval was also analysed as a dichotomous variable, with a prolonged PR interval defined as a duration above 200ms. We stratified the analyses by sex and by age below or above 67 years of age (the median age of all ECGs recorded in the study population). In a sensitivity analysis for the association of PR interval with SCD, we censored participants when a bundle branch block was detected on the ECG, as this can influence the duration of the PR interval. In another sensitivity analysis, we censored participants when they developed coronary heart disease. A two-sided p-value below 0.05 was considered statistically significant. Measures of association are presented as hazard ratios (HRs) for the risk of SCD with 95% confidence intervals (CIs). Data were analysed using IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, U.S.).

Table 1. Baseline characteristics of the study population

Characteristics		Total study population (n=11,370)
Men		4,771 (42%)
Age (years)		64.6±9.4
Follow-up (years)		8.8 [6.1;15.0]
Number of electrocardiograms		19,289
Number of electrocardiograms per participant		1 (1;4)
Sudden cardiac deaths		276 (2%)
Height (cm)		168±10
Body mass index (kg/m^2)		27.0±4.1
Systolic blood pressure (mmHg)		139±22
Diastolic blood pressure (mmHg)		78±12
Smoking	Past	5,089 (45%)
	Current	2,553 (22%)
Heart rate (beats per minute)		70±11
Left ventricular hypertrophy		325 (3%)
Prevalent diabetes mellitus		1,139 (10%)
Prevalent heart failure		175 (2%)
Prevalent coronary heart disease		703 (6%)
Prevalent chronic obstructive pulmonary disease		498 (4%)
Beta-blocker use		1,463 (13%)
Digoxin use		78 (<1%)
Class I and III antiarrhythmic use		51 (<1%)
Diltiazem or verapamil use		195 (2%)

Data presented as number (percentage), median [quartile 1;quartile 3], median (minimum;maximum), or mean±standard deviation

Results

General characteristics

The study population consisted of 11,370 participants (Figure 2) with 19,289 ECG recordings available. During a median follow-up of 8.8 years (interquartile range 8.9 years), 276 SCDs occurred. The baseline characteristics of the study population are shown in Table 1. The mean age of the population was 64.6±9.4 years, and 42% of the study population were men. During follow-up, 770 (7%) participants developed atrial fibrillation. The median PR interval duration and P-wave indices are shown in Table 2, separate for men and women.

PR interval and SCD

The association of the PR interval with SCD is shown in Figure 3. Neither PR interval as a continuous variable, nor a prolonged PR interval was associated with an increased risk of SCD in both models. No large differences were seen between men and women (men: SCD n=144, women: SCD n=132; data not shown). Censoring participants when a bundle branch block was detected (remaining SCD n=251) did not change the results (data not shown).

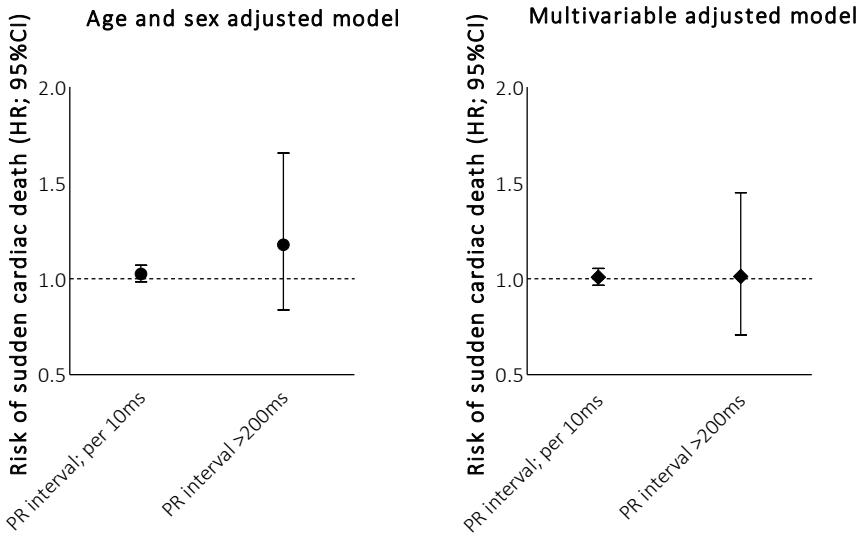

Figure 3. Association of the PR interval and a prolonged PR interval with occurrence of sudden cardiac death

Multivariable model adjusted for sex, age, body mass index, systolic and diastolic blood pressure, smoking, heart rate, left ventricular hypertrophy, diabetes mellitus, heart failure, coronary heart disease, chronic obstructive pulmonary disease, and use of beta-blockers, digoxin, class I/III antiarrhythmics, and diltiazem or verapamil at date of electrocardiogram recording

Abbreviations: CI: confidence interval; HR: hazard ratio; ms: milliseconds

Table 2. PR interval and P-wave indices on the total number of electrocardiogram recordings (n=19,289)

	Men (n=8,008)	Women (n=11,281)
PR interval (ms)	170 [154;186]	162 [148;178]
Prolonged PR interval (>200ms)	1,000 (12%)	881 (8%)
P duration (ms)	122 [114;130]	116 [108;124]
P amplitude (µV)	118 [99;141]	122 [100;148]
P area (ms·µV)	7,274 [5,896;8,702]	7,167 [5,696;8,672]
P terminal force (ms·µV)	1,900 [780;3,224]	1,748 [624;2,880]

Data presented as number (percentage) or median [quartile 1;quartile 3]
Abbreviations: ms: milliseconds; µV: microvolts

P-wave indices and SCD

The association of P-wave indices with SCD is shown in Figure 4. P-wave duration was not associated with occurrence of SCD, adjusted for sex, age, body mass index, systolic and diastolic blood pressure, smoking, heart rate, left ventricular hypertrophy, diabetes mellitus, heart failure, coronary heart disease, COPD, and use of beta-blockers, digoxin, class I/III antiarrhythmics, and diltiazem or verapamil. However, after adjustment for the relevant covariables, every 10µV higher amplitude of the P wave was associated with a 4% increase in hazard of SCD (HR 1.04, 95%CI 1.01;1.08). Both a 10ms·mV larger P area and P terminal force

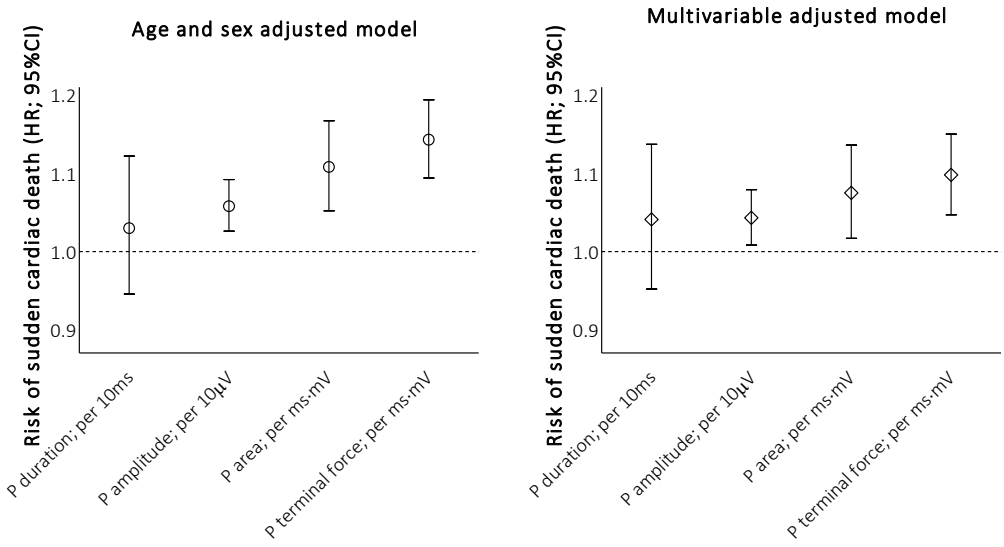

Figure 4. Association of the P-wave indices with occurrence of sudden cardiac death
Multivariable model adjusted for sex, age, body mass index, systolic and diastolic blood pressure, smoking, heart rate, left ventricular hypertrophy, diabetes mellitus, heart failure, coronary heart disease, chronic obstructive pulmonary disease, and use of beta-blockers, digoxin, class I/III antiarrhythmics, and diltiazem or verapamil at date of electrocardiogram recording
Abbreviations: CI: confidence interval; HR: hazard ratio; ms: milliseconds; mV: millivolts; µV: microvolts

were associated with a 7% and 10% increased hazard of SCD, respectively (HR1.07, 95%CI 1.02;1.14 and HR 1.10, 95%CI 1.05;1.15). Figure 5 shows that the associations found in men and women separately reflect the association found in the total study population. The analyses stratified by age below or above 67 years showed comparable results for both strata (data not shown). Censoring participants when they developed CHD (remaining SCD n=212) did not substantially changed the effect estimates, however the association between P amplitude and occurrence of SCD was no longer significant (data not shown).

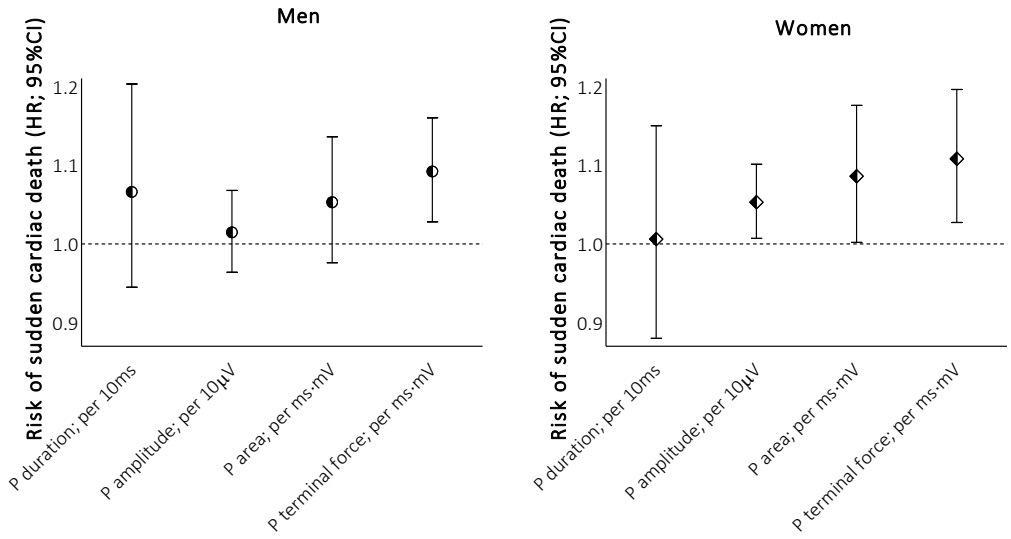

Figure 5. Association of the P-wave indices with occurrence of sudden cardiac death, stratified by gender
Adjusted for age, body mass index, systolic and diastolic blood pressure, smoking, heart rate, left ventricular hypertrophy, diabetes mellitus, heart failure, coronary heart disease, chronic obstructive pulmonary disease, and use of beta-blockers, digoxin, class I/III antiarrhythmics, and diltiazem or verapamil at date of electrocardiogram recording
Abbreviations: CI: confidence interval; HR: hazard ratio; ms: milliseconds; mV: millivolts; µV: microvolts

Discussion

In this study, we showed that a higher amplitude, area and terminal force of the P wave were associated with an increased risk of SCD in this middle-aged and elderly general population. We did not find significant associations of the PR interval or P-wave duration with SCD.

We found a significant association of the P-wave amplitude, area, and terminal force with occurrence of SCD. A large amplitude of the P wave, and thus a large P area, is mostly caused by

enlargement of the right atrium, resulting from increased pulmonary artery pressure, also called P pulmonale[17]. Previous studies have shown that pulmonary hypertension is associated with occurrence of SCD, but the mechanism for this association is not well understood[18]. Pulmonary hypertension can be caused by various diseases, such as a pulmonary embolism and COPD. A previous pulmonary embolism increases the risk of a new embolism. As the validation of SCD cases in a population-based study is based on the information that is available, fatal pulmonary embolism might, incorrectly, be coded as SCD, which might influence our analyses. We recently showed that COPD is associated with the occurrence of SCD[15]. In the current study, we included COPD status as covariable, however we were not able to correct for severity of COPD, while this might impact the degree of right atrial enlargement. This might also partly explain the association between P amplitude and P area and SCD that remains despite the correction for COPD. An increased amplitude of the P wave can also be caused by valvular disease and heart failure, and as these factors have been associated with SCD previously[19], this could also distort our analyses. We adjusted for a history of heart failure at date of the ECG, but we did not have data available on valvular disease. An interesting topic for further research would be a combination of echocardiographic parameters concerning these factors, P-wave indices and occurrence of SCD in a population-based setting. However, this combination is not available in the Rotterdam Study, with sufficient follow-up time. Although the association between pulmonary hypertension, measured with imaging techniques, and occurrence of SCD has been described before[18], our study shows that characteristics of this phenomenon on the ECG are also associated with SCD. Since an ECG is easier and cheaper to use in practice than imaging techniques, this association could provide new, useful and practical markers for the risk of SCD. A negative deflection in lead V1, i.e. a large P terminal force and thus also a larger P area, is a sign of left atrial enlargement, which is also expressed by a notched P wave in lead II, called a P mitrale. This can be a result of mitral valve insufficiency. However, in a previous study we did not find an association between left atrial diameter and occurrence of SCD[20]. In patients with pulmonary emphysema, right atrial enlargement might present as a large terminal force through right atrial displacement caused by anatomical changes in the diaphragm and mediastinum[21].

This study has several strengths and limitations. Our time-dependent analysis provides more accurate information regarding the determinants in this study than only a baseline measurement. We also had access to detailed information on covariables on every date an ECG was recorded, lowering the bias and confounding in our analyses and providing more precise estimates of the associated risk of SCD. Thirdly, within the Rotterdam Study, ECGs are recorded by experienced research assistants following a standardized protocol. Although the population-based setting hampered ECG recording under controlled circumstances, our setting reflects the measurement setting in clinical practice, enhancing the direct value of these results for daily practice. We used an automated algorithm to determine the ECG intervals and amplitudes,

ensuring absence of intra- and inter-observer variability. We censored incident cases of atrial fibrillation during follow-up, because atrial fibrillation precludes measurement of the P wave and because of the strong association or P-wave indices with atrial fibrillation[7] and the association between atrial fibrillation and SCD[16] could distort our analyses. Besides that, in persons with atrial fibrillation during the center visit, P-wave indices cannot be measured. A limitation of this study is the heterogeneity of SCD cases that occurs in every population-based cohort. As we included both witnessed and unwitnessed cases, we cannot be completely sure about the cardiac origin in the cases. However, the definition we applied has been widely endorsed for years and the incidence rate in our cohort is comparable to other studies[1,11-13]. We did not determine the predictive value of the P-wave indices in this study, as determining the predictive value in the same cohort as the discovery cohort would lead to an overestimation of the predictive value. Therefore, we recommend a validation of these findings to be done in an independent cohort. Furthermore, we studied an older and predominantly white population, and therefore our results might not be generalizable to younger and non-white populations.

In conclusion, we showed that amplitude, area and terminal force of the P wave were associated with an increased risk of SCD, whereas PR interval and P-wave duration were not. P-wave indices might aid in the prediction of SCD in addition to already known risk factors. Further studies in independent cohorts are needed to establish the potential mechanisms for this association and the role of P-wave indices in the prediction of SCD.

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Part IV

Clinical risk factors

Chapter 4.1

Subclinical cardiac dysfunction and sudden cardiac death

Maartje N Niemeijer, Maarten J G Leening, Marten E van den Berg, Albert Hofman, Oscar H Franco, Jaap W Deckers, Peter R Rijnbeek, Bruno H Stricker, Mark Eijgelsheim

Abstract

Background Subclinical cardiac dysfunction has been associated with increased mortality, and heart failure increases the risk of sudden cardiac death (SCD). Less well-known is whether subclinical cardiac dysfunction is also a risk factor for SCD. Our objective was to assess the association between echocardiographic parameters and SCD in a community-dwelling population free of heart failure.

Methods We computed hazard ratios (HRs) for left atrium diameter, left ventricular (LV) end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD), LV mass, qualitative LV systolic function, LV fractional shortening, and diastolic function.

Results During a median follow-up of 6.3 years in 4,686 participants, 68 participants died because of SCD. Significant associations with SCD were observed for qualitative LV systolic function and LV fractional shortening. For moderate/poor qualitative LV systolic function, the HR for SCD was 2.54 (95%CI 1.10;5.87). Each standard deviation decrease in LV fractional shortening was associated with a HR of 1.36 (95%CI 1.09;1.70).

Conclusion Subclinical abnormalities in LV systolic function were associated with SCD risk in this general population. Although prediction of SCD remains difficult and traditional cardiovascular risk factors are of greatest importance, this knowledge might guide future directions to prevent SCD in persons with subclinical cardiac dysfunction.

Introduction

Sudden cardiac death (SCD) is one of the most common lethal manifestations of heart disease, accounting for an estimated annual of 4-5 million deaths worldwide[1]. Manifest heart failure is a major risk factor for SCD[2]. To reduce SCD-related mortality, current guidelines recommend prophylactic implantable cardiac defibrillator therapy in patients with heart failure when left ventricular (LV) ejection fraction is 35% or lower[3]. However, before heart failure becomes clinically evident, subclinical cardiac dysfunction is often present[4] with an increased risk of morbidity and mortality[5,6]. Impaired LV function, as reflected by hypertrophy and impaired ejection fraction, has previously been demonstrated to be associated with an increased incidence of SCD in high risk populations, e.g. athletes[7], patients with hypertrophic cardiomyopathy[8], and patients with coronary heart disease (CHD)[9]. However, because almost all studies are performed in high-risk patient populations, population-based data are limited. Therefore, the objective of the present study was to investigate the association between subclinical abnormalities in echocardiographic parameters and the occurrence of SCD in a general population of older adults free of heart failure.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study, which started in 1990 in the city of Rotterdam, the Netherlands. Details have been described elsewhere[10]. In short, all inhabitants of the Ommoord district, aged 55 years of age and older were invited to participate. At baseline (1990-1993) 7,983 participants (response rate 78%) were enrolled. In 2000, an additional 3,011 participants were enrolled (response rate 67%). Follow-up examinations are conducted every 4-5 years and consist of a home interview and an extensive set of tests at a research facility. Participants are continuously and actively monitored for major morbidity and mortality through access to general practitioners and municipality records. The Rotterdam Study was 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. All participants provided written informed consent.

Study population

Echocardiographic measurements were obtained from 2002-2005 during the fourth examination of the first cohort and the identical second examination of the second cohort. We excluded participants with poor quality echocardiograms and with a history of heart failure. Heart failure diagnosis was adjudicated in accordance with the guidelines of the European Society of Cardiology and included typical signs or symptoms of heart failure confirmed by

objective evidence of cardiac dysfunction[11]. Adjudication of heart failure diagnosis was done without knowledge of the echocardiography results.

Outcome definition

SCD was adjudicated according to Myerburg's definition endorsed by the European Society of Cardiology: "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"[12]. Identification and validation of SCD cases was done independently by two research physicians and subsequently confirmed by an experienced cardiologist after reviewing the medical files as described in more detail previously[13]. Follow-up was complete for almost all deaths (96%) until January 1st 2011.

Echocardiographic measurements

Resting transthoracic echocardiograms were obtained by certified experienced echo-cardiographers according to a standardized protocol as described in detail previously[14]. In short, left atrium (LA) diameter, LV end-diastolic dimension (LVEDD) and LV end-systolic dimension (LVESD) were measured in the parasternal long-axis view using M-mode with 2-dimensional guidance. LV mass in grams was calculated as 0.80(1.04*(intraventricular septum thickness + LVEDD + posterior wall thickness)^3-(LVEDD)^3)+0.6[15]. LV fractional shortening at the endocardium was calculated as (LVEDD-LVESD)/LVEDD*100%[16]. Global qualitative LV systolic function was assessed by means of subjective visual grading, in four established categories (normal, fair, moderate, or poor) from the two-dimensional echocardiogram. The E/A ratio was assessed with the use of pulsed Doppler recordings of transmittal filling velocity in the apical four-chamber view, with the sample volume placed in the mitral valve orifice near the tips of the leaflets. Doppler peak E and peak A velocities were averaged over three cycles. E/A ratio was computed by dividing Doppler peak E velocity by Doppler peak A velocity[17]. Mitral valve inflow deceleration time (MVDT) was measured as the time between the peak E wave and the upper deceleration slope extrapolated to the zero baseline. LVEDD, fractional shortening, LV mass, and LA diameter were categorized into four categories; normal, fair, moderate, and poor, based on guideline-derived reference values[18], but because of the small number of participants in the moderate and poor categories, those were combined in the analyses. LVESD was categorized into two categories; normal and abnormal[18,19]. Diastolic function was defined into three categories: normal, impaired relaxation and a restrictive pattern[18,19] using E/A ratio and MVDT[4,20]. Because we did not measure the early diastolic longitudinal velocity of the mitral annulus, we could not categorize all participants in these diastolic function categories and classified those that we could not as indeterminate[14]. Reference values for the categorization are shown in Table 1, separately for men and women when appropriate[18-21]. Intra-reader and inter-reader reproducibility measurement have been described by Kardys et al[14].

Table 1. Reference values, separate for men and women when appropriate, and number of participants for categories of echocardiographic parameters

Echocardiographic parameter	Sex	Grade				Missing
		Normal	Fair	Moderate*	Poor*	
LVEDD (mm)	Men	42-59	60-63	64-68	≥69	
	Women	39-53	54-57	58-61	≥62	
	n	3,935(84%)	554(12%)	153(3%)	37(<1%)	7(<1%)
Fractional shortening (%)	Men	25-43	20-24	15-19	≤14	
	Women	27-45	22-26	17-21	≤16	
	n	4,439(95%)	164(4%)	42(<1%)	23(<1%)	18(<1%)
LV mass (g)	Men	88-224	225-258	259-292	≥293	
	Women	67-162	163-186	187-210	≥211	
	n	4,062(87%)	316(7%)	153(3%)	99(2%)	56(1%)
LA diameter (mm)	Men	≤40	41-46	47-52	≥53	
	Women	≤38	39-42	43-46	≥47	
	n	2,148(46%)	1,442(31%)	746(16%)	339(7%)	11(<1%)
Qualitative LV systolic function	n	2,734(58%)	1,701(36%)	180(4%)	63(1%)	8(<1%)
		Normal	Abnormal			
LVESD (mm)	All	<45	≥45			
	n	4,546(97%)	122(3%)			18(<1%)
		Normal	Impaired relaxation	Restrictive pattern	Indeter-minate	
Diastolic function	All	E/A 0.75-1.50 & MVDT 150-280	E/A <0.75 & MVDT <150	E/A >1.50 & MVDT>280	Other combi-nations	
	n	2,726(58%)	207(4%)	18(<1%)	1,494 (32%)	241(5%)

Due to rounding percentages do not always exactly add up to 100%.
* Because of the small number of participants, the moderate and poor category were combined in the analyses
Abbreviations: E/A: E/A ratio; LA: left atrium; LV: left ventricular; LVEDD: LV end-diastolic dimension; LVESD: LV end-systolic dimension; MVDT: mitral valve inflow deceleration-time; n: number of persons per category

Potential covariables

Body mass index was calculated as weight in kilograms divided by height in squared meters. Systolic and diastolic blood pressures were measured in sitting position at the right upper arm. The average of two consecutive measurements was taken. Hypertension was defined as systolic blood pressure above 140 mmHg, diastolic blood pressure above 90 mmHg, or use of blood pressure lowering medication for the indication hypertension. Total and high-density lipoprotein cholesterol were measured with the use of standardized laboratory techniques. Diabetes mellitus was defined as fasting glucose above 6.9 mmol/L, non-fasting glucose above 11.0 mmol/L, use of blood glucose lowering medication, or a previous diagnosis of diabetes mellitus.

A history of CHD was defined as a myocardial infarction or coronary revascularization procedure[11]. Smoking status was assessed during the interview. Heart-rate corrected QT (QTc) interval according to Bazett's formula was derived from standard resting electrocardiograms.

Data analysis

We used Cox proportional hazards models to analyze the association between multiple echocardiographic parameters and SCD as outcome. Follow-up time was calculated from the date of echocardiography until date of death, lost to follow-up (n=11), or the end of the study period (January 1st 2011). Echocardiographic measurements were analyzed both continuously and categorically. For the continuous analysis, we analyzed per sex-specific, age-adjusted standard deviation (SD), derived through linear regression by saving standardized residuals. Sex and age at date of echocardiography were included as covariables in the basic model for the analysis of categories. The covariables in the multivariable model were selected by means of

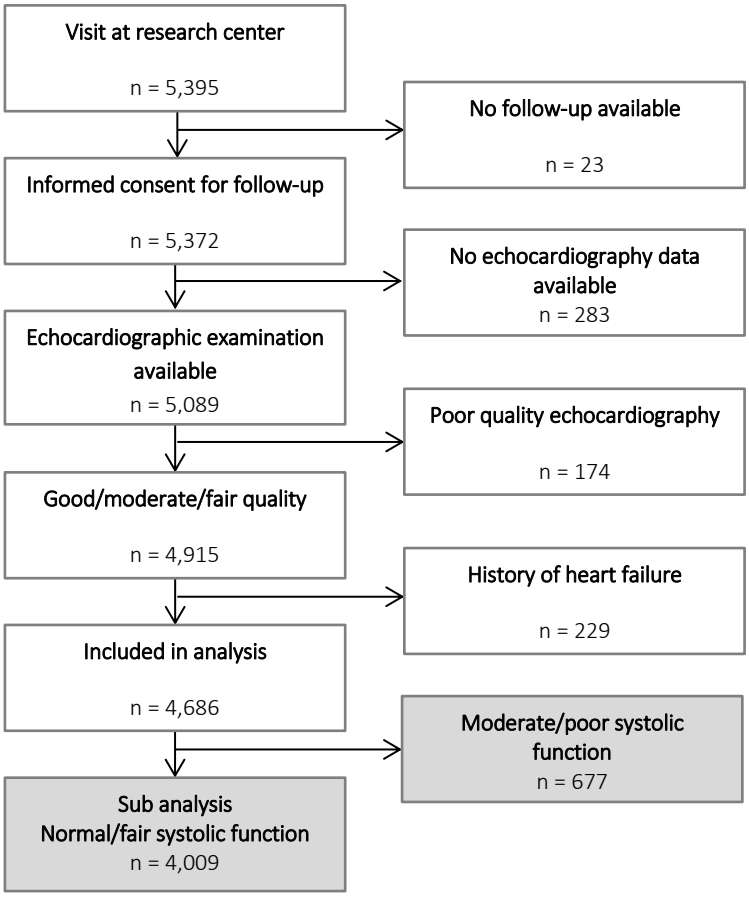

Figure 1. Selection of the study population

backward selection with the use of the likelihood ratio test with a cut-off p-value of 0.1. We also used QT and RR interval in our model instead of QTc, to investigate whether there is residual confounding by heart rate, as is known for Bazett's formula. We constructed cumulative survival curves for categorized echocardiographic parameters. Because we were interested in cause-specific hazard ratios (HRs), we used traditional Cox models that censor participants at the time of death from causes other than SCD[22]. Nevertheless, we performed sensitivity analyses on the categorical echocardiographic parameters by means of the data augmentation method and unstratified model described by Lunn and McNeil to estimate the hazard ratio of SCD compared to non-SCD in the multivariable model to account for competing risk of deaths from other causes[23]. We repeated all analyses after exclusion of persons with a history of CHD and persons with moderate or poor values of the systolic parameters (LVEDD, LVESD, fractional shortening and qualitative LV systolic function). We also analyzed the association while censoring persons with incident CHD during follow-up. We combined significantly associated parameters into different models, for which we assessed discriminative ability by internally validated (i.e. optimism-corrected) concordance statistic (c-statistic).

A two-sided p-value below 0.05 was considered to be statistically significant. All measures of association are presented as HR with 95% confidence intervals (CI). We used IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, USA), R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), and SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Table 2. Baseline characteristics of the population for analysis (2002-2005)

		Total population n=4,686
Age (years)		71.8±7.4
Sex	Men	1,949 (41.6%)
	Women	2,737 (58.4%)
Body mass index (kg/m^2)		27.4±3.9
Blood pressure (mmHg)	Systolic	150±21
	Diastolic	80±11
Hypertension		3,760 (80.2%)
Total cholesterol (mmol/l)		5.7±2.1
High-density lipoprotein cholesterol (mmol/l)		1.5±2.0
Diabetes mellitus		685 (14.6%)
Current smoking		716 (15.3%)
Heart rate (beats per minute)		69±11
History of coronary heart disease		387 (8.3%)
History of myocardial infarction		267 (5.7%)*
History of revascularization		247 (5.3%)*
Atrial fibrillation		224 (4.8%)
Atrioventricular block		239 (5.1%)
Heart-rate corrected QT interval (milliseconds)		433±26

Data presented as mean±standard deviation or number (percentage)
* Numbers overlap
Abbreviations: n: number

Results

General characteristics

A total of 4,686 participants were eligible for analysis (Figure 1). Their baseline characteristics are shown in Table 2. The total study population comprised 2,737 women (58%) and the overall mean age of the participants was 71.8 years. During a median follow-up of 6.3 years (interquartile range 1.4) 68 of a total of 804 deaths were attributed to SCD. All multivariable models were adjusted for systolic and diastolic blood pressure, diabetes mellitus, smoking, CHD, and QTc interval. Other covariables did not contribute to the model. Using QT and RR instead of QTc did not affect the results.

Continuous analysis

Results for the analysis on echocardiographic parameters, analyzed per sex-specific, age-adjusted SD increase or decrease are shown in Table 3. Per 1 SD decrease in fractional shortening, the risk of SCD increased (HR 1.36; 95%CI 1.09;1.70). Increase of LVEDD, LVESD, LV mass, and LA diameter were not significantly associated with a higher risk of SCD.

Categorical analysis

The number of participants for each category of the echocardiographic parameters are presented in Table 1. The risk of SCD increased with deteriorating cardiac function (Figure 2). In the multivariable model, the risk of SCD was higher only in the moderate/poor qualitative LV systolic function category (HR 2.54; 95%CI 1.10;5.87) compared to normal (Table 4). An abnormal LVESD (HR 1.42; 95%CI 0.55;3.65) did not significantly affect the risk of SCD.

Table 3. Continuous echocardiographic parameters and sudden cardiac death risk

Echocardio-graphic measurement	Mean±SD		Model 1		Model 2	
	Men	Women	HR (95%CI)	p	HR (95%CI)	p
LVEDD*	53±5	49±5	1.05 (0.83;1.33)	0.68	0.91 (0.72;1.14)	0.41
LVESD*	34±6	30±5	1.41 (1.17;1.69)	<0.001	1.13 (0.93;1.39)	0.23
Fractional shortening†	37±7	40±6	1.66 (1.35;2.03)	<0.001	1.36 (1.09;1.70)	0.006
LV mass*	162±45	131±35	1.15 (0.92;1.43)	0.23	0.96 (0.76;1.22)	0.75
LA diameter*	42±6	39±5	1.07 (0.84;1.36)	0.58	0.94 (0.73;1.20)	0.60

* Analyzed continuously, hazard ratios per sex-specific, age-adjusted standard deviation increase
† Analyzed continuously, hazard ratios per sex-specific, age-adjusted standard deviation decrease
Model 1: crude model
Model 2: adjusted for systolic blood pressure, diastolic blood pressure, diabetes mellitus, current smoking, history of coronary heart disease, and heart-rate corrected QT interval
Abbreviations: CI: confidence interval; HR: hazard ratio; LA: left atrium; LV: left ventricular; LVEDD: LV end-diastolic dimension; LVESD: LV end-systolic dimension; SD: standard deviation

Table 4. Categorical echocardiographic parameters and sudden cardiac death risk

Echocardiographic measurement	Model 1				Model 2			
	Fair		Moderate/poor		Fair		Moderate/poor	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
LVEDD	1.03 (0.47;2.27)	0.94	2.42 (1.04;5.63)	0.04	0.93 (0.42;2.07)	0.86	1.60 (0.66;3.91)	0.30
Fractional shortening	2.10 (0.90;4.90)	0.08	5.42 (2.17;13.55)	<0.001	1.66 (0.70;3.95)	0.25	2.48 (0.89;6.93)	0.08
Qualitative LV systolic function	1.57 (0.90;2.74)	0.11	4.16 (2.05;8.43)	<0.001	1.38 (0.77;2.50)	0.28	2.54 (1.10;5.87)	0.030
LV mass	1.95 (0.92;4.15)	0.08	1.66 (0.70;3.92)	0.25	1.49 (0.67;3.32)	0.33	1.14 (0.44;2.94)	0.78
LA diameter	1.33 (0.76;2.34)	0.32	1.25 (0.67;2.32)	0.48	1.37 (0.76;2.44)	0.29	0.98 (0.51;1.91)	0.96

Normal is the reference category.
For sex-specific categorization of the echocardiographic parameter refer to Table 1.
Model 1: adjusted for age and sex
Model 2: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, diabetes mellitus, current smoking, history of coronary heart disease, and heart-rate corrected QT interval
Abbreviations: CI: confidence interval; HR: hazard ratio; LA: left atrium; LV: left ventricular; LVEDD: LV end-diastolic dimension

Diastolic function, as measured by the E/A ratio and MVDT, did not reach statistical significance (impaired relaxation: HR 1.22; 95%CI 0.46;3.25; restrictive pattern: HR 5.59; 95%CI 0.72;43.51; indeterminate: HR 0.70; 95%CI 0.38;1.30). Analyzed separately, E/A ratio and MVDT also provided non-significant results. Results from the sex-stratified analyses and sensitivity analyses by exclusion of persons with CHD were consistent with the results in the overall population (data not shown), but the number of SCD cases limited our ability to draw firm conclusions. The results of the competing risk model are shown in Table 5. A higher LV mass, LA diameter, and better qualitative LV systolic function were significantly associated with non-SCD.

Continuous analysis in persons with normal and fair systolic function

After exclusion of 677 participants with a moderate or poor value on any of the systolic parameters (Figure 1; remaining SCDs n=49), only the effects of fractional shortening and LVEDD was statistically significant. Greater LVEDD was protective from SCD (HR 0.76; 95%CI 0.59;0.99). Per SD decrease in fractional shortening the HR was 1.32 (95%CI 1.01;1.73) in the adjusted model. An analysis while censoring persons with incident CHD, showed similar HRs, although not significant owing to the low number of cases (data not shown).

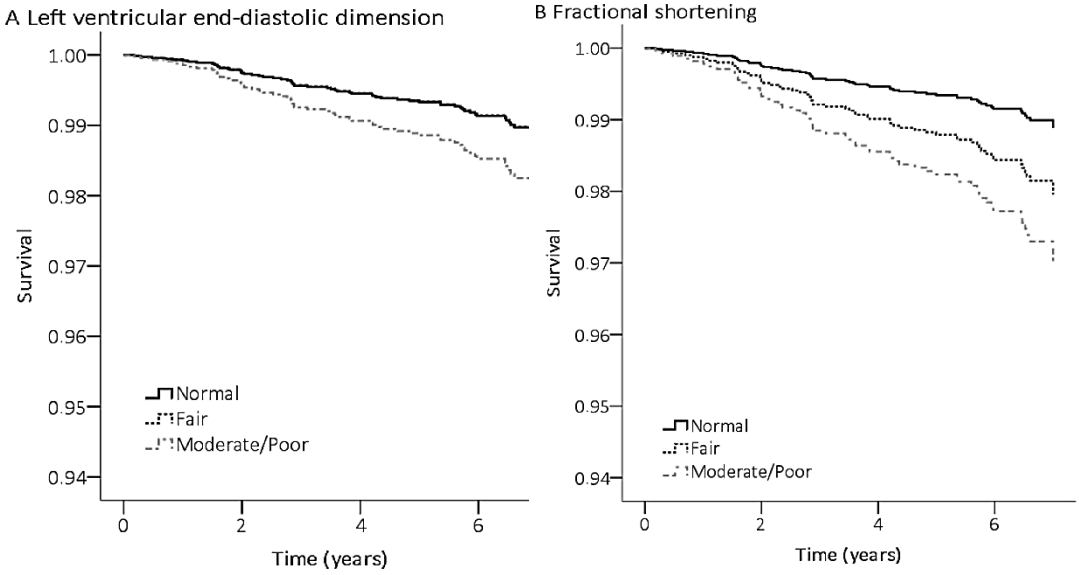

Figure 2. Cumulative survival curves for occurrence of sudden cardiac death with regard to echocardiographic measurements. Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, diabetes mellitus, current smoking, history of coronary heart disease, and heart-rate corrected QT interval

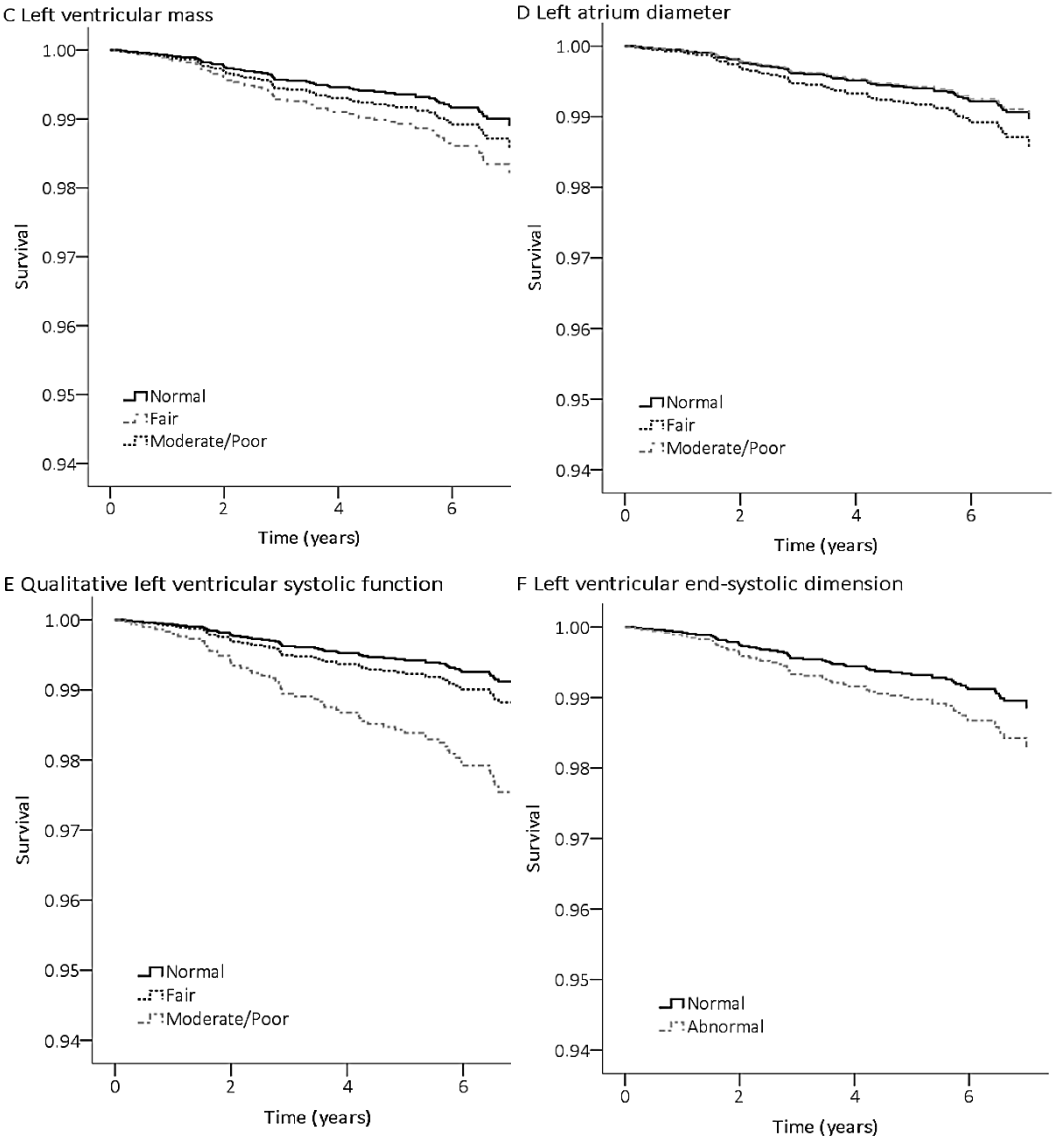

Figure 2. (continued) Cumulative survival curves for occurrence of sudden cardiac death with regard to echocardiographic measurements. Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, diabetes mellitus, current smoking, history of coronary heart disease, and heart-rate corrected QT interval

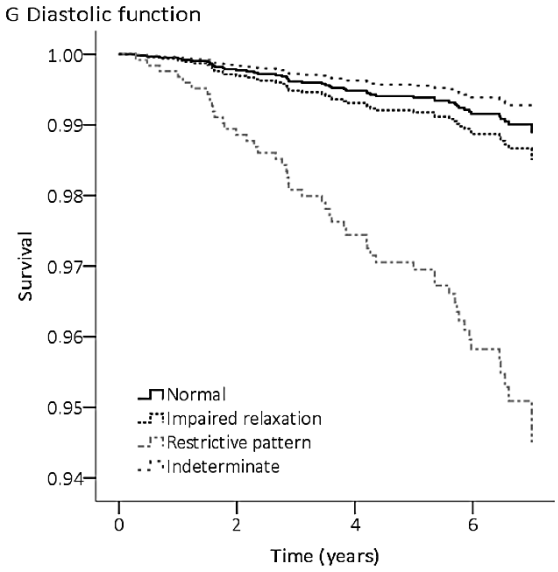

Figure 2. (continued) Cumulative survival curves for occurrence of sudden cardiac death with regard to echocardiographic measurements. Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, diabetes mellitus, current smoking, history of coronary heart disease, and heart-rate corrected QT interval

Discrimination analysis in persons with normal and fair systolic function

The addition of fractional shortening (the most consistently associated parameter) yielded a small increase in c-statistic when added to a model of the other covariables that are known to be associated with SCD; 0.78 (95%CI 0.71;0.86) for the model with known covariables; 0.79 (95%CI 0.72;0.87) after addition of fractional shortening.

Discussion

We demonstrated that subclinical abnormalities in LV systolic function are associated with an increased risk of SCD in the general population. However, the increase in discriminative value, when added to a model with well-known clinical risk factors, is limited.

A previous study investigated this association in the general population, showing that a lower fractional shortening in 802 men aged 42-60 years was associated with an increased risk of SCD[24]. Our study adds to this knowledge as it was performed in a somewhat older population comprising both sexes.

There are several pathways through which a decreased LV systolic function can increase the risk of SCD. First, a low-normal fractional shortening could indicate progression towards a subclinical

Table 5a. Categorical echocardiographic parameters and risk of sudden cardiac deaths, taking into account the competing risk of death from other causes

Echocardiographic measurement	Fair		Moderate/Poor	
	HR (95%CI)	p	HR (95%CI)	p
LVEDD	0.93 (0.42;2.07)	0.86	1.59 (0.65;3.87)	0.31
Fractional shortening	1.65 (0.69;3.92)	0.26	2.46 (0.88;6.90)	0.09
Qualitative LV systolic function	1.37 (0.76;2.48)	0.29	2.50 (1.08;5.78)	0.032
LV mass	1.48 (0.66;3.32)	0.34	1.14 (0.44;2.94)	0.78
LA diameter	1.36 (0.76;2.42)	0.30	0.98 (0.51;1.91)	0.96
LVESD	Abnormal			
	HR (95%CI)		P	
	1.40 (0.54;3.61)		0.48	
Diastolic function	Impaired relaxation		Restrictive pattern		Indeterminate	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
	1.21 (0.46;3.22)	0.70	6.48 (0.84;50.20)	0.07	0.70 (0.38;1.29)	0.25

Table 5b. Categorical echocardiographic parameters and risk of death from other causes

Echocardiographic measurement	Fair		Moderate/Poor	
	HR (95%CI)	p	HR (95%CI)	p
LVEDD	1.08 (0.86;1.37)	0.51	0.95 (0.64;1.41)	0.81
Fractional shortening	1.07 (0.77;1.50)	0.68	1.55 (0.94;2.55)	0.09
Qualitative LV systolic function	1.25 (1.06;1.48)	0.008	1.36 (1.01;1.82)	0.041
LV mass	1.21 (0.92;1.59)	0.17	1.37 (1.04;1.80)	0.023
LA diameter	0.92 (0.77;1.11)	0.38	1.08 (0.90;1.30)	0.40
LVESD	Abnormal			
	HR (95%CI)		P	
	1.35 (0.93;1.96)		0.12	
Diastolic function	Impaired relaxation		Restrictive pattern		Indeterminate	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
	1.43 (1.07;1.92)	0.016	4.74 (2.09;10.74)	<0.001	1.16 (0.98;1.38)	0.09

Normal is the reference category.
For sex-specific categorization of the echocardiographic parameter refer to Table 1.
Analyses adjusted for age, sex, systolic blood pressure, diastolic blood pressure, diabetes mellitus, current smoking, history of coronary heart disease, and heart-rate corrected QT interval
Abbreviations: CI: confidence interval; HR: hazard ratio; LA: left atrium; LV: left ventricular; LVEDD: LV end-diastolic dimension; LVESD: LV end-systolic dimension

stage of heart failure. It has previously been shown that ventricular dysfunction is often present before heart failure becomes clinically evident[4] and gives rise to increased morbidity and mortality[5,6]. Alternatively, a low-normal fractional shortening and SCD could have a shared etiology, such as hypertension, CHD, or genetic variations. Systolic dysfunction is primarily caused by CHD[19], which is also an important risk factor for SCD[25]. However, after adjustment for a history of CHD in our analysis or exclusion of persons with manifest CHD, the association between fractional shortening and SCD remained statistically significant. Large-scale genetic studies identified genetic variations associated with QT and RR intervals, both well recognized SCD risk factors, which were annotated within genes associated with Mendelian disorders resulting in heart failure[26-29].
Previous studies showed that in patients with CHD, LV mass predicted SCD[30] and studies in athletes demonstrated a higher proportion of persons with an "athlete's heart"[31] among the SCD cases. This leads to the hypothesis that SCD has a different origin in persons with CHD, athletes, and the general population where SCD has a heterogeneous patho-etiology[30].

The high level of completeness of follow-up in this cohort is an important asset. We had access to very detailed information on morbidity and mortality events through direct access to the medical records. Besides that, data are collected within the Rotterdam Study according to standardized protocols. Because the Rotterdam Study has no exclusion criteria for enrollment, our results are generalizable to the general population.
This study has some limitations we should address. First is the heterogeneity of SCD cases which follows from the definition of SCD. However, this definition has been widely endorsed for several years and is in accordance with formal cardiologic guidelines. The incidence rate in our study was comparable to other studies[1,13], indicating adequate case validation. Second, the echocardiographic data we had available did not enable us to determine LV volumes and thereby LV ejection fraction. However, fractional shortening and ejection fraction are highly correlated. Furthermore, assessment of diastolic function in our study was complicated by the fact that we did not measure the early diastolic longitudinal velocity of the mitral annulus. Finally, because we had a limited number of cases, we could include only a limited number of confounders. But because we adjusted for the most important confounders, we expect minimal residual confounding.

In conclusion, in this general population, subclinical abnormalities in LV systolic function were associated with an increased risk of SCD. Because the discriminative ability of fractional shortening was limited, prediction remains difficult and established clinical factors are most important. However, our findings might lead to a better understanding of the negative outcomes associated with subclinical (stage B) heart failure and the pathophysiology of SCD and might guide future directions to prevent SCD in persons with subclinical cardiac dysfunction.

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Chapter 4.2

Chronic obstructive pulmonary disease and sudden cardiac death

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Abstract

Background Both sudden cardiac death (SCD) and chronic obstructive pulmonary disease (COPD) are common conditions in the elderly. Previous studies have identified an association between COPD and cardiovascular disease, and with SCD in specific patient groups. Our aim was to investigate whether there is an association between COPD and SCD in the general population.

Methods The Rotterdam Study is a population-based cohort study among 14,926 subjects aged 45 years and older with up to 24 years of follow-up. Analyses were performed with a (time-dependent) Cox proportional hazard model adjusted for age, sex, and smoking.

Results Of the 13,471 persons included in the analysis; 1,615 had a diagnosis of COPD and there were 551 cases of SCD. COPD was associated with an increased risk of SCD (age and sex adjusted hazard ratio (HR) 1.34, 95%CI 1.06;1.70). The risk particularly increased in the period 2,000 days (5.48 years) after the diagnosis of COPD (age and sex adjusted HR 2.12, 95%CI 1.60;2.82) and increased further to a more than three-fold higher risk in COPD subjects with frequent exacerbations during this period (age- and sex adjusted HR 3.58, 95%CI 2.35;5.44). Analyses restricted to persons without prevalent myocardial infarction or heart failure yielded similar results.

Conclusion COPD is associated with an increased risk for SCD. The risk especially increases in persons with frequent exacerbations 5 years after the diagnosis of COPD. This risk indicator could provide new directions for better-targeted actions to prevent SCD.

Introduction

Sudden cardiac death (SCD) forms a substantial part of cardiovascular mortality, which is the leading cause of death globally[1], with a currently estimated incidence of 4-5 million cases worldwide per year[2]. SCD is primarily caused by ventricular arrhythmias[3] and risk factors for SCD include male sex, increasing age[2], a history of heart failure or cardiac ischemia, cardiomyopathies, channelopathies, and alcohol and drug use[4]. However, SCD is a heterogeneous outcome with multiple etiological pathways of which many have not been established. This complicates an adequate risk assessment and prevention plan for SCD.

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death[5]. COPD is characterized by a progressive airflow limitation and is associated with a chronic inflammatory response in the airways and lungs[6]. Besides these local pulmonary manifestations, systemic effects and comorbidities are frequently present and determine the prognosis of patients with COPD[7,8]. Patients with COPD have a two to threefold higher risk of developing cardiovascular disease and half of COPD deaths can be attributed to cardiovascular disease[9]. Although the underlying mechanisms for this high cardiovascular morbidity and mortality in patients with COPD are not fully established to date, a role for the accompanied hypoxia and hypoxemia[6], higher heart rate[10], systemic inflammation[6] or recurrent exacerbations has been suggested. Moreover, patients with COPD suffer more frequently from ventricular arrhythmias than controls[11,12]. In patients hospitalized for an exacerbation of COPD, atrial fibrillation and ventricular arrhythmias are independent predictors of death at 1 year[13]. Furthermore, COPD has also been shown to be an independent predictor of SCD in high-risk patients after a percutaneous coronary intervention and coronary artery bypass graft[13,14] and in patients with myocardial infarction[15]. However, this association has not yet been assessed in the general population. Therefore, our objective was to assess whether COPD is an independent risk factor for SCD in the general population, and whether COPD, exacerbations and systemic inflammation modify the association between COPD and SCD.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study, which started in 1990 in the Ommoord district, in the city of Rotterdam, the Netherlands[16,17]. All inhabitants of the Ommoord district, aged 55 years of age and older were invited to participate (n=10,215). At baseline, from 1990 through 1993, 7,983 participants (response rate 78%) were included. In 2000, an additional 3,011 participants, out of 4,472 invitees, were enrolled (response rate 67%). This extension consisted of all persons living in the study district who had become 55 years of age or had moved into the study district. A second similar extension of the cohort was initiated

in 2006, in which 3,932 participants (out of 6,057 invitees; response rate 65%), aged 45 years and older were included. Follow-up examinations are conducted periodically, approximately every 4 to 5 years. Examinations consist of a home interview and an extensive set of tests at a specially built research facility in the study district. Besides this, participants are continuously monitored for major morbidity and mortality through linkage of general practitioners and municipality records to the study base. The Rotterdam Study was approved by the medical ethics committee according to the Population Study Act Rotterdam Study executed by the Ministry of Health, Welfare and Sport of the Netherlands. The study population consisted of participants who gave informed consent for follow-up.

COPD

COPD was diagnosed by an obstructive spirometry (FEV_1/FVC < 70%) performed at the research center visit or, if not available, by a physician based on the combination of clinical history, physical examination and spirometry[18]. The severity of COPD was determined by the forced expiratory volume in one second (FEV1), expressed as percentage predicted, according to the modified global initiative for chronic obstructive lung disease (GOLD) criteria (2007): mild COPD (GOLD I, $FEV_1 \geq 80\%$), moderate COPD (GOLD II, $50\% \leq FEV_1 < 80\%$), and severe COPD (GOLD III, $FEV_1 < 50\%$)[19]. Asthma patients were excluded. The date of incident COPD was defined as the date of obstructive lung function measurement, the date of COPD diagnosis in the medical records or the date of a first COPD medication prescription in someone with established COPD, whichever came first. Medication use was obtained through pharmacy-filled prescription data from 7 pharmacies in the region which are all on one computer network. Moderate exacerbations were defined as needing a course of steroids and/or antibiotics and severe exacerbations as needing hospitalization. Frequent exacerbators were defined as COPD participants who had during follow-up on average two or more moderate or severe exacerbations a year[20].

SCD

SCD cases were validated independently by two researchers and confirmed by a cardiologist through details from the medical records. In witnessed deaths, SCD was diagnosed according to Myerburg's definition endorsed by the European Society of Cardiology: "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"[21,22]. Unwitnessed deaths were coded as SCD if death was unexpected in persons found dead, while they were in a stable medical condition 24 hours before they were found and without evidence of a noncardiac cause[23].

High-sensitivity C-reactive protein (hsCRP) serum level measurement

HsCRP serum levels were measured as previously described[24]. Non-fasting blood was collected at baseline and stored at -80°C until CRP measurement. Storing temperature did not affect stability of CRP. HsCRP measurements were performed on all blood samples using a rate near-infrared particle immunoassay (Immage Immunochemistry System; Beckman Coulter, San Diego, CA).

Covariables

Information on covariables was obtained through interview (i.e. smoking and alcohol use), laboratory, medical files, or physical examination (i.e. height, weight, systolic and diastolic blood pressure, myocardial infarction, heart failure, revascularization procedures, diabetes mellitus, atrial fibrillation, heart-rate corrected QT (QTc) interval and statin use) at the regular visits of the study participants to the Rotterdam Study research center. Pack years of cigarette smoking were computed as duration of self-reported smoking (years) multiplied by the number of daily smoked cigarettes, divided by 20. Body mass index was calculated as weight in kilograms divided by height in meters squared. Systolic (SBP) and diastolic blood pressure (DBP) were measured in sitting position at the right upper arm. The average of 2 consecutive measurements was taken. Hypertension was defined as a SBP >140 mmHg, DBP >90 mmHg, or use of blood pressure lowering medication for the indication hypertension. Myocardial infarction was adjudicated based on a combination of symptoms, electrocardiogram measurements and enzyme markers. Heart failure diagnosis was adjudicated in accordance with the guidelines of the European Society of Cardiology and included typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction, as described in more detail previously[25,26]. A revascularization procedure is either a percutaneous coronary intervention (PCI) or a coronary artery bypass graft (CABG). Diabetes mellitus was defined as fasting glucose above 6.9 mmol/L, non-fasting glucose above 11.0 mmol/L, the use of blood-glucose lowering medication, or a previous diagnosis of diabetes mellitus. Atrial fibrillation is ascertained from electrocardiogram measurements as well as medical records. QTc interval according to Bazett's formula was derived from a standard electrocardiogram. Medication dispensing data was obtained from all 7 fully computerized pharmacies in the study district. Information on all filled prescriptions from January 1st 1991 onwards were available and included information on the product name, the Anatomical Therapeutic Chemical code (ATC-code) of the World Health Organisation, the dispensing date, the prescribed dosing regimen and the amount dispensed. More detailed information on the methods of data collection and definitions of cardiac outcomes have been previously described[27].

Statistical analysis

Differences between subjects with and without COPD were studied using Mann-Whitney U and Chi-Square tests. Time-dependent Cox proportional hazards models were performed to assess the association between COPD and SCD. Follow-up time was determined as the time between study entry or incident COPD date and death or end of study (January 1st 2011). This definition includes the time at risk for sudden cardiac death. However, we also performed a sensitivity analysis adding the time between cohort entry and incident COPD to the non-COPD follow-up time. All models were adjusted for age and sex, and additionally for all biologically plausible covariates according to previous literature which changed the risk estimate by more than 10%. Potential confounders were age, sex, height, weight, body mass index, smoking behaviour, pack-years, myocardial infarction, heart failure, coronary revascularisation procedures, systolic blood pressure, diastolic blood pressure, hypertension, total serum cholesterol, diabetes mellitus, atrial fibrillation and heart-rate corrected QT interval according to Bazett's formula. As high-sensitivity C-reactive protein (hsCRP) might be an important effect modifier, we investigated this variable in a separate analysis. HsCRP serum levels were categorized as high versus moderate/low, based on the American Heart Association classification[28]. We performed a sensitivity analysis by exclusion of persons with prevalent heart failure and myocardial infarction. We constructed cumulative survival curves using the Kaplan-Meier method. Since we were interested in how COPD affects SCD in general terms, Cox models for the hazard on SCD that treat other death as censored were perfectly valid[29]. Nevertheless, with regard to the competing risk of non-SCD, we performed a sensitivity analysis using the data augmentation method and unstratified model described by Lunn & McNeil to estimate the hazard ratio (HR) of

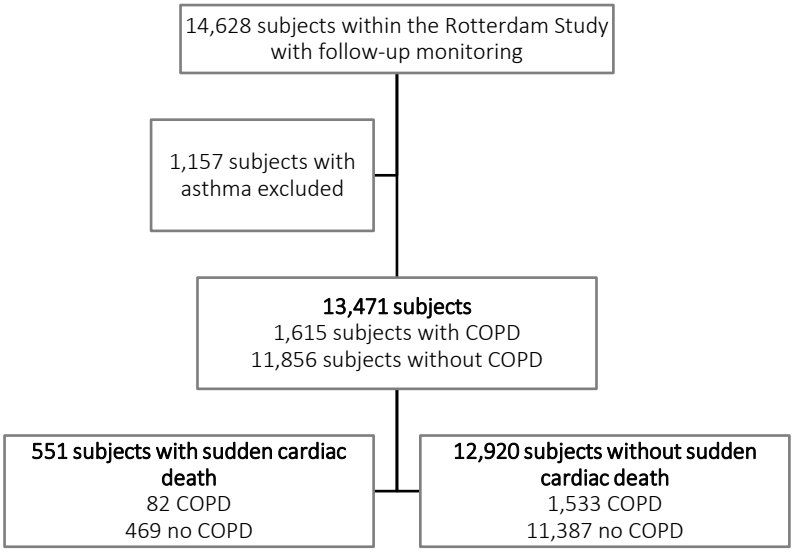

Figure 1. Flowchart of the selection of the study population

SCD in comparison with non-SCD[30]. Although it should be mentioned that if the proportionality assumption is not satisfied, then the stratified Lunn-McNeil model should be used, which is identical to the Cox cause-specific model[31]. Furthermore, since we were also interested in the incidence of SCD, we estimated the cumulative incidence function (CIF)[32] from the competing risk data and performed the Gray's test for equality of CIFs[33,34]. The CIF is a summary curve, showing the cumulative event rates over time due to a particular cause[32,35]. The Gray's test is developed for the comparison of the cumulative incidence of events among different groups[33]. A two-sided p-value below 0.05 was considered statistically significant. Statistical analyses were performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria) and SPSS, version 20.0 (IBM Corp., Somers, NY, USA).

Results

General characteristics

Table 1 shows the baseline characteristics of the study population (n=13,471) with a median age of 64 years (interquartile range (IQR)=16) at the start of follow-up. COPD subjects were older, more often male, more (current) smokers, had a slightly lower body mass index, higher levels of hsCRP at baseline and had more frequently a medical history of myocardial infarction and coronary artery bypass graft. During a median follow-up of 3,229 days (8.84 years; IQR: 3,651 days; total of 123,024 person years of follow-up), 5,197 (39%) of the participants died of whom 551 died suddenly. 82 (5%) COPD subjects and 469 (4%) subjects without COPD died due to SCD (Figure 1). The SCDs were witnessed in about half of the cases (54%) and the ratio of witnessed versus unwitnessed SCD was not different for COPD subjects versus subjects without COPD (p=0.975). The time of SCD was divided into 3 equal parts of 8 hours and registered in 380 (69%) of 551 SCDs. The data show that SCD subjects with COPD died more frequently during night time(0h-8h; 53%) than SCD subjects without COPD (36%; p=0.023; Figure 2).

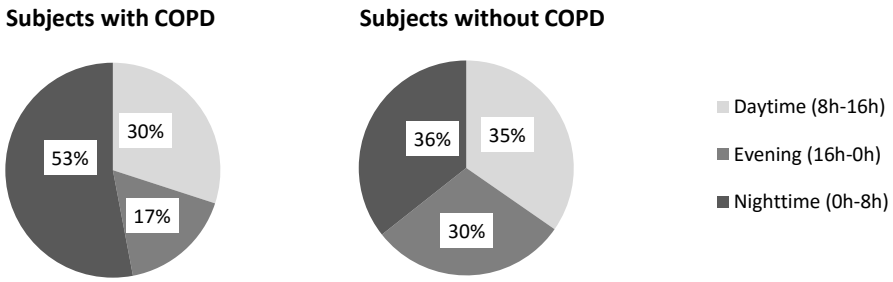

Figure 2. Time of sudden cardiac death in subjects with and without chronic obstructive pulmonary disease (COPD)

Table 1. Baseline characteristics of the study population at cohort entry (n=13,471)

		COPD (n=1,615)	No COPD (n=11,856)	p-value
Age (years)		70 [13]	63 [15]	<0.001
Males		909 (56%)	4,727 (40%)	<0.001
Smoking	Past	689 (44%)	4,903 (43%)	<0.001
	Current	632 (40%)	2,209 (19%)	
Pack-years cigarette smoking		26 [37]	3 [23]	<0.001
Height (cm)		170 [13]	167 [14]	<0.001
Weight (kg)		74.9 [17.0]	74.6 [17.8]	0.773
Body mass index (kg/m^2)		25.8 [4.7]	26.4 [4.9]	<0.001
Blood pressure	Systolic	137 [29]	137 [29]	0.399
(mmHg)	Diastolic	75 [16]	77 [16]	<0.001
Hypertension		741 (55%)	5,344 (56%)	0.499
Myocardial infarction		118 (7%)	581 (5%)	<0.001
Heart failure		52 (3%)	235 (2%)	0.002
Coronary revascularization		57 (4%)	334 (3%)	0.141
Coronary artery bypass graft*		41 (3%)	212 (2%)	0.048
Percutaneous transluminal coronary angioplasty*		19 (1%)	155 (1%)	0.608
Atrial fibrillation		71 (5%)	438 (4%)	0.268
Diabetes mellitus		154 (10%)	1,196 (10%)	0.488
QTc interval (milliseconds)		430 [31]	429 [30]	0.618
hsCRP (mmol/l)		1.9 [3.0]	1.5 [2.5]	<0.001
Total serum cholesterol (mmol/l)		6.2 [1.6]	6.1 [1.6]	0.036
Serum potassium (mmol/l)		4.2 [0.4]	4.2 [0.4]	0.948
GFR (sex-specific ml/min/1.73m^2)		74.8 [19.5]	73.6 [19.2]	<0.001
Diuretic use		511 (32%)	1,966 (17%)	<0.001

Data presented as number (percentage) or median [interquartile range] * Numbers overlap
There were missings in baseline smoking status (n=426), pack-years (n=728), height (n=1,447), weight (n=1,438), BMI (n=1,455), hsCRP (n=1,922), myocardial infarction (n=161), heart failure (n=297), coronary revascularization (n=583), systolic/ diastolic blood pressure (n=1,317), cholesterol (n=1,439), hypertension (n=2,547), atrial fibrillation (n=1,876), QTc interval (n=1,659), potassium (n=3,282) and GFR (n=3,325).
Abbreviations: COPD: chronic obstructive pulmonary disease; GFR: glomerular filtration rate; hsCRP: high-sensitivity C-reactive protein; : number of participants; QTc: heart-rate corrected QT interval according to Bazett's formula

Association between COPD and SCD

Figure 3 illustrates the higher probability of SCD for COPD subjects compared to subjects without COPD. Adjusted for age and sex, COPD was significantly associated with an increased risk of dying due to SCD (HR 1.34, 95%CI 1.06;1.70). A stratified analysis in patients without prevalent myocardial infarction nor heart failure nor CABG nor PCI resulted in a comparable age and sex adjusted HR of 1.38 (95%CI 1.03;1.85). An additional analysis only including subjects with a study acquired spirometry resulted in an age and sex adjusted HR of 3.32 (95%CI 1.77;6.22) of SCD for COPD subjects with an obstructive spirometry compared to subjects with

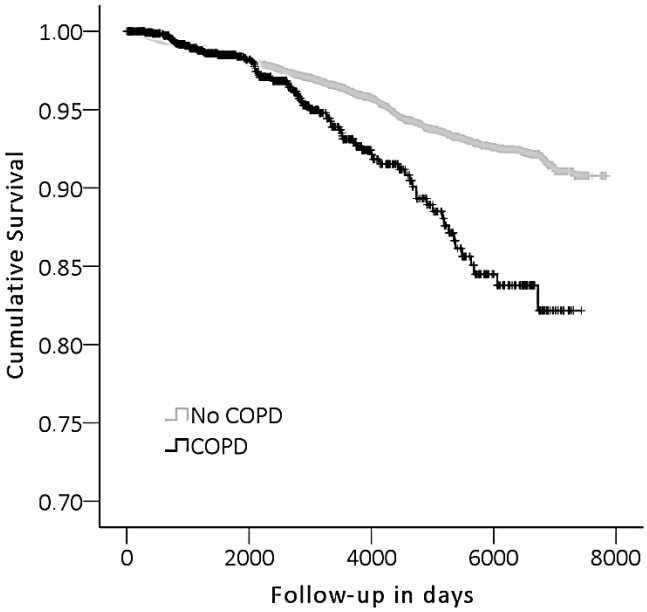

Figure 3. Kaplan-Meier curve of sudden cardiac death according to chronic obstructive pulmonary disease status (COPD; Log-rank p<0.001)

normal spirometry. Table 2 demonstrates the risk of SCD according to the severity of airflow limitation. A sensitivity analysis using the stratified Lunn-McNeil method to model the competing risk effect of other causes of death, resulted in an age and sex adjusted HR of COPD on SCD of 1.43; 95%CI 1.19;1.67. The CIF represents the higher incidence of SCD and other causes of death for subjects with COPD compared to subjects without COPD (Figure 4). The CIFs for COPD versus no COPD were statistically significant for SCD (Gray's test; p=0.0006) and for non-SCD (Gray's test; p<0.0001). Another sensitivity analysis adding the time between cohort entry and incident COPD to the control follow-up time did not substantially change the estimate.

Table 2. Chronic obstructive pulmonary disease and the hazard of sudden cardiac death according to severity of airflow limitation (n=5,661)

	HR (95%CI)	p-value
Normal lung function	Reference	
Mild COPD (GOLD I, n=393)	3.25 (1.48;7.18)	0.003
Moderate COPD (GOLD II, n=385)	3.31 (1.52;7.22)	0.003
Severe COPD (GOLD III, n=74)	3.76 (0.86;16.43)	0.079

Abbreviations: CI: confidence interval; COPD: chronic obstructive pulmonary disease; GOLD: global initiative for chronic obstructive lung disease; HR: hazard ratio; n: number

Figure 3 further illustrates that the distinction of SCD risk according to COPD status occurred about 2,000 days (5.48 years) after the study start (incident COPD date or cohort entry for participants without incident COPD), while the difference for other causes of death was seen much earlier (Figure 4). Since the proportional hazard assumption was violated, we evaluated the effect of COPD on SCD using a time-dependent Cox regression analysis stratifying time in more or less than 2,000 days after COPD diagnosis. Within these strata, the proportional hazards assumption was satisfied and adjusted for age and sex, the HR for the period before 2,000 days was non-significant (HR 0.66, 95%CI 0.43;1.02), whereas the HR for the period after 2,000 days was significantly increased (HR 2.12, 95%CI 1.60;2.82; Table 3). We additionally adjusted the model for covariables which changed the risk estimate by more than 10%, i.e. only pack years of cigarette smoking. COPD was associated with an almost two-fold increased risk to develop SCD, independent of age, sex, and pack years of cigarette smoking (HR 1.93, 95%CI 1.44;2.59; Table 3). The other potential confounders (i.e. height, weight, body mass index, myocardial infarction, heart failure, coronary revascularisation procedure, blood pressure, hypertension, total cholesterol, diabetes mellitus, atrial fibrillation, and heart-rate corrected QT interval) did not significantly influence the effect. We did not observe any significant interactions with sympathomimetic respiratory drugs or with cardiac treatment.

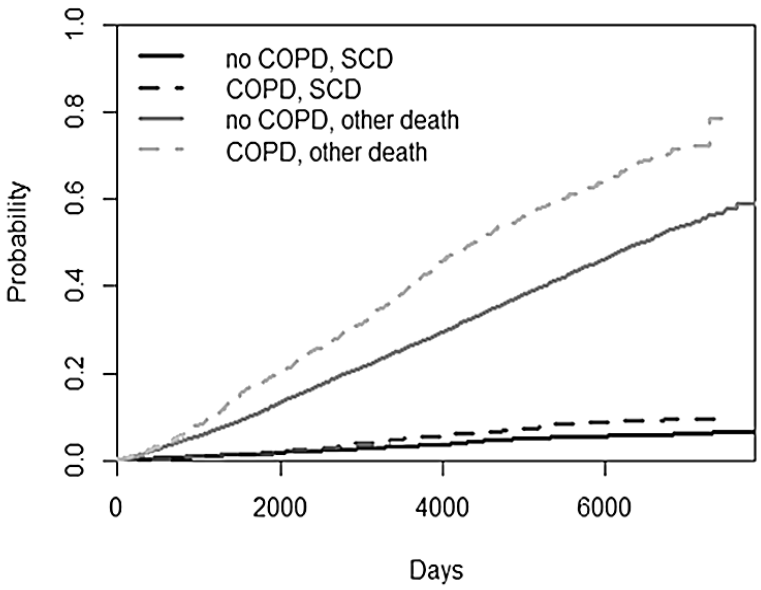

Figure 4. Cumulative incidence function of sudden cardiac death (SCD; Gray's test p=0.0006) and other death (Gray's test p<0.0001) according to chronic obstructive pulmonary disease (COPD) status

Table 3. Chronic obstructive pulmonary disease and the hazard of sudden cardiac death for the period >2,000 days (5.48 years) of follow-up

	Model 1	Model 2
	HR (95%CI)	HR (95%CI)
No COPD	Reference	Reference
COPD	2.12 (1.60;2.82)	1.93 (1.44;2.59)
COPD without frequent exacerbations	1.66 (1.16;2.37)	1.52 (1.06;2.19)
COPD with frequent exacerbations	3.58 (2.35;5.44)	3.21 (2.08;4.95)

Model 1: age and sex adjusted.
Model 2: adjusted for age, sex and pack years of cigarette smoking.
Frequent exacerbations were defined as having at least two moderate or severe exacerbations a year, averaged over the total years of follow-up.
Abbreviations: CI: confidence interval; COPD: chronic obstructive pulmonary disease; HR: hazard ratio

Influence of exacerbations

Regarding the influence of COPD exacerbations, 366 (23%) of 1,615 COPD subjects had frequent exacerbations. 33 (9%) frequent exacerbators compared to 49 (4%) COPD subjects without frequent exacerbations died due to SCD. The Kaplan-Meier curve for SCD in Figure 5 illustrates the poorer survival for COPD subjects with frequent exacerbations (Log-rank p<0.001). Adjusted for age, sex and pack years of cigarette smoking, the COPD frequent exacerbator phenotype was significantly associated with a more than three-fold increased risk of developing SCD (HR 3.21; 95%CI: 2.08;4.95; Table 3). A sensitivity analysis using the stratified Lunn-McNeil method to

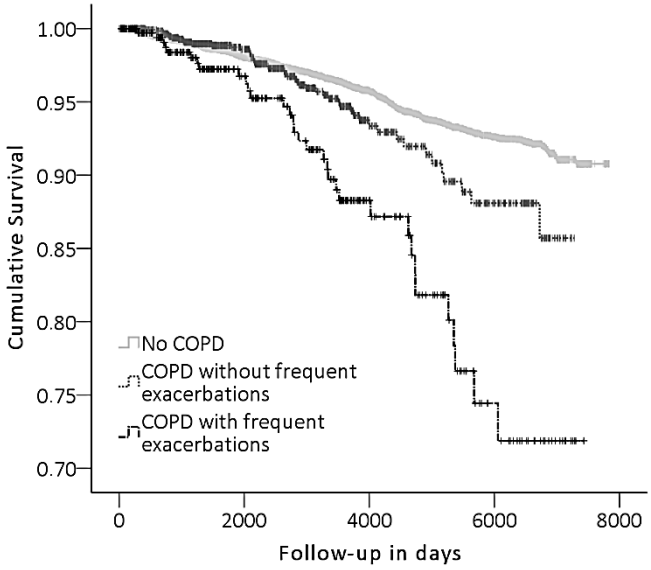

Figure 5. Kaplan-Meier curve of sudden cardiac death according to chronic obstructive pulmonary disease (COPD) status with or without frequent exacerbations (Log-rank p<0.001)

model the competing risk effect of other causes of death, resulted in an age and sex adjusted HR of the frequent exacerbators on SCD of 2.51 (95%CI: 2.15;2.86). Finally, the cumulative incidence of SCD was significantly higher when COPD subjects had frequent exacerbations (Figure 6).

Influence of baseline systemic inflammation (hsCRP)

Of the 13,471 subjects, 11,549 (85.7%) had a baseline hsCRP measurement. A linear regression model adjusted for age and sex, demonstrated that the natural logarithm of hsCRP concentration was significantly increased in subjects with COPD and SCD compared to subjects with COPD without SCD ($p<0.05$). When stratified according to the level of baseline systemic inflammation, the HR for SCD was significantly increased in COPD subjects with frequent exacerbations having a hsCRP level >3 mg/l (HR 3.67; 95% CI 1.97;6.85; Table 4). In contrast, in all subjects with a low-to-moderate degree of systemic inflammation, the HR for SCD was significantly increased in COPD subjects without frequent exacerbations (HR 1.84; 95% CI 1.16;2.91; Table 4).

Table 4. Chronic obstructive pulmonary disease and the hazard of sudden cardiac death for the period >2,000 days (5.48 years) of follow-up, stratified according to the serum level of C-reactive protein

		Model 1	Model 2
		HR (95%CI)	HR (95%CI)
hsCRP ≤3 mg/l	No COPD	Reference	Reference
	COPD	1.98 (1.32;2.98)	1.76 (1.15;2.68)
	COPD without frequent exacerbation	2.09 (1.34;3.26)	1.84 (1.16;2.91)
	COPD with frequent exacerbations	1.60 (0.65;3.90)	1.48 (0.60;3.64)
hsCRP >3 mg/l	No COPD	Reference	Reference
	COPD	1.88 (1.16;3.05)	1.93 (1.18;3.16)
	COPD without frequent exacerbations	1.21 (0.62;2.35)	1.24 (0.63;2.42)
	COPD with frequent exacerbations	3.51 (1.90;6.49)	3.67 (1.97;6.85)

Model 1: age and sex adjusted.
Model 2: adjusted for age, sex and pack years of cigarette smoking.
Frequent exacerbations were defined as having at least two moderate or severe exacerbations a year, averaged over the total years of follow-up.
Abbreviations: CI: confidence interval; COPD: chronic obstructive pulmonary disease; hsCRP: high-sensitivity C-reactive protein; HR: hazard ratio

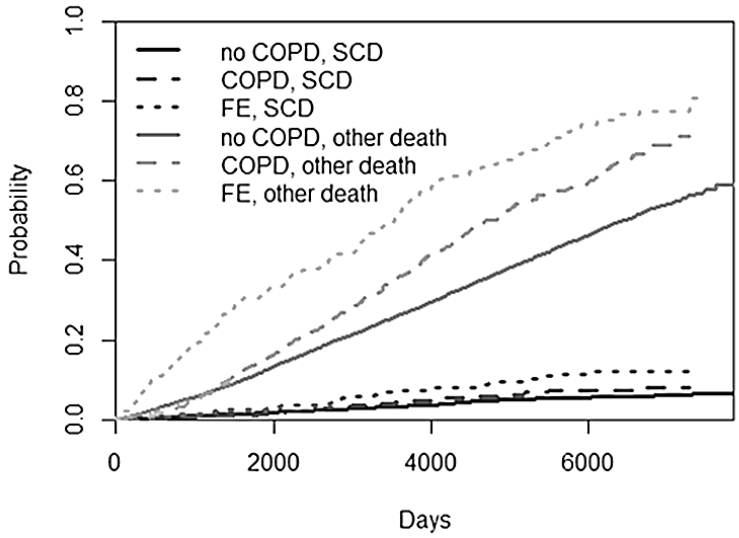

Figure 6. Cumulative incidence function of sudden cardiac death (SCD; Gray's test p=0.0002) and other death (Gray's test p<0.0001) according to chronic obstructive pulmonary disease (COPD) exacerbator status (no COPD, COPD without frequent exacerbations, COPD with frequent exacerbations)

Discussion

In this population-based study in community dwelling middle-aged and elderly persons, we showed an approximately 30% increased risk of sudden cardiac death (SCD) in subjects with COPD. The risk almost doubled in the period 2,000 days (5.48 years) after the diagnosis of COPD, a trend which was not observed for other causes of death. Among subjects with a higher degree of baseline systemic inflammation, COPD patients with frequent exacerbations had a 3.7-fold increase in the risk of SCD.

An increased risk of SCD in patients with COPD has been previously described in specific patient populations at high risk of SCD[13]. Our prospective cohort study demonstrated for the first time that COPD is associated with an increased risk of SCD in the general population, even when the already increased risk of COPD on all-cause mortality was taken into account. There are several factors in persons with COPD that could play a role in the mechanism which ultimately leads to SCD. COPD has been associated with an increased risk of ventricular arrhythmias[11,12] and a prolongation of the QTc interval[36], currently the most well-known electrocardiogram-derived risk indicator for SCD[37]. QTc prolongation in COPD patients can originate from different mechanisms. First, COPD is associated with autonomic neuropathy, which is associated with QTc prolongation[36]. Also, common comorbidities in COPD patients, such as coronary heart disease and heart failure are associated with a prolonged QTc[38]. Other factors that are present in persons

with COPD are a higher resting heart rate[10], hypoxia and hypoxemia[6], cardiac ischemia[4,9], and heart failure[4,39], which are all known risk factors for SCD. Although information on time of sudden cardiac death was incomplete, our data suggest that patients with COPD die more frequently during night time, potentially due to the reduced ventilation and blunted responses to hypercapnia resulting in more ventricular ectopic episodes while they are sleeping[40,41]. Since baseline cardiovascular diseases did not have a substantial impact on the association between COPD and SCD, our data suggest that preexisting cardiovascular disease in patients with COPD does not explain the observed association. Moreover, our data suggest that mechanisms through which COPD might raise the risk of SCD act in a severity or duration-dependent manner since the estimate increased by duration of COPD. One mechanism could be that COPD leads to hyperinflation, followed by remodelling, pulmonary hypertension, cardiac arrhythmias and subsequently SCD.

Exacerbations could provide another mechanism through which SCD is caused. Suissa et al. recently described that with every exacerbation both the risk of subsequent COPD exacerbations and the risk of mortality increased[42]. Elevated biomarkers of cardiovascular morbidity, such as troponine[43], midregional-pro atrial natriuretic peptide[44], and N-terminal-pro brain natriuretic peptide[45] (the latter two are both indicators of heart failure) are associated with exacerbation mortality and a higher risk of myocardial infarction and stroke after a COPD exacerbation has been observed[46]. Wedzicha et al. described that COPD exacerbations may lead to increased cardiovascular morbidity through systemic inflammation[47]. This hypothesis is endorsed by another study that showed increased levels of CRP and interleukin-6 following COPD exacerbations[48]. Systemic inflammation in patients with COPD can be evaluated using CRP levels, an acute phase protein[49]. CRP is strongly associated with cardiovascular disease in general[50,51], and SCD[52] and COPD specifically[18,49]. Our results demonstrate that the effect of frequent exacerbations was modified by the baseline degree of systemic inflammation. Similar to the subgroup of COPD patients with persistent inflammation which demonstrated high incidence of exacerbations and worse survival, the risk of SCD was minimally affected by the degree of airflow limitation[53]. Possibly, COPD patients with substantial systemic inflammation develop more atherosclerosis, increasing the risk of coronary heart disease whereby each exacerbation further increases the risk of SCD.

The association between COPD and SCD provides various directions for further research. The first is to unravel the underlying mechanisms. Secondly, effective strategies to reduce the risk of SCD in COPD patients are needed. In addition to increased awareness of COPD as a risk factor for SCD, preventive options such as medical treatment (e.g. β-blockers), placement of Implantable Cardioverter Defibrillator (ICD) or withdrawing QTc-prolonging drugs, should be investigated.

Our study has several strengths and some limitations. First, in previous studies, the association between COPD and SCD has been assessed only in specific patient populations who already had an increased risk of SCD, but not in a general population setting with adjustment for all known confounders. Second, SCD was validated by research physicians blinded to the COPD status of the subject. Another strength of this study is that we have prospectively gathered data on morbidity and mortality events, and on covariables, limiting the risk of information bias. However, some potential misclassification may have occurred by inclusion of unwitnessed deaths, though the percentage of unwitnessed deaths in our study was not different for COPD subjects compared to subjects without COPD. A first limitation is that autopsies and electrocardiography at time of death were rarely performed[2], hampering the evaluation of exact causes of death. However, we applied a universal definition, which is the best method for identification of SCD cases in a population-based study and indicates similar data validity[23]. By exclusion of non-cardiac causes in all sudden deaths, we refined sudden deaths to only those of cardiac origin. Secondly, we do not have information on successful resuscitations. Importantly, numbers of subjects with ICD placement were very low, implicating that use of ICD could not distort our results. Another limitation is that lung function measurements were not performed in all study participants at baseline. However, a sensitivity analysis in subjects with spirometry, confirmed the increased risk of SCD in subjects with an obstructive lung function compared to subjects with a normal lung function. Finally, it has previously been suggested that participants in a prospective cohort study are more healthy than non-participants[54]. The Rotterdam Study has an overall response rate of 72%. Persons with more severe COPD could have had lower participation rates. However, in our study this would have only resulted in an underestimation of the effect.

In conclusion, our study demonstrated that COPD is a risk factor for SCD in the general population, especially in the period more than 5 years after the diagnosis of COPD. The risk further increases in COPD patients with frequent exacerbations having a higher degree of baseline systemic inflammation. These data could facilitate accurate risk stratification and pave the path to targeted preventive actions for patients with COPD.

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Chapter 4.3

Serum magnesium and the risk of death from coronary heart disease and sudden cardiac death

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Abstract

Background Few studies have analyzed the associations between serum magnesium, coronary heart disease (CHD) mortality and sudden cardiac death (SCD). Results are conflicting and the pathway is unclear. We therefore analyzed the association of serum magnesium with CHD mortality and SCD within the prospective population-based Rotterdam Study, with adjudicated endpoints and long-term follow-up.

Methods We used multivariable Cox proportional hazard models and analyzed quartiles of serum magnesium, with the second and third quartile combined as reference group (0.81-0.88 mmol/l).

Results 9,820 participants (mean age 65.1 years, 56.8% female) were included with a median follow-up of 8.7 years. Low serum magnesium (≤0.80 mmol/l) was associated with an increased risk of CHD mortality (n=431, HR 1.36, 95%CI 1.09;1.69) and SCD (n=217, HR 1.54, 95%CI 1.12;2.11). Low serum magnesium was associated with accelerated subclinical atherosclerosis, as indicated by increased carotid intima-media thickness (cIMT, +0.013mm, 95%CI 0.005;0.020). Low serum magnesium was also associated with an increased heart-rate corrected QT interval, mainly through an effect on heart rate (RR interval -7.1ms, 95%CI -13.5;-0.8). Additional adjustments for cIMT and heart rate did not change the associations with CHD mortality and SCD.

Conclusion Low serum magnesium is associated with an increased risk of CHD mortality and SCD. Although low magnesium was associated with both cIMT and heart rate, this did not explain the relationship between serum magnesium and CHD mortality or SCD. Future studies should focus on why magnesium associates with CHD mortality and SCD and whether intervention reduces these risks.

Introduction

Magnesium is the second most abundant intracellular cation and it plays a key role in a wide range of cellular functions[1]. Total body magnesium depends on dietary intake and recent studies showed that the vast majority of elderly do not consume the average dietary requirement for magnesium[2]. In addition to a magnesium insufficient diet, elderly are also at risk for hypomagnesemia due to comorbidities and medication that increase urinary excretion of magnesium[1]. The prevalence of hypomagnesemia in the general population is estimated at 2%[3], but it may be as high as 53% in specific high risk groups, such as patients with chronic heart failure[4]. Although hypomagnesaemia may have acute and chronic complications, serum magnesium is still measured relatively infrequently[1].

In recent studies, low serum magnesium has been associated with inflammation[5] and disturbances in the regulation of vascular tone and endothelial function[6-9]. These mechanisms are thought to contribute to the development and progression of atherosclerosis, potentially worsening coronary heart disease (CHD)[10,11]. Magnesium is also known for its role in the electrical stability and energy balance of cardiomyocytes[12]. Hypomagnesemia has been associated with atrial and ventricular arrhythmias and low serum magnesium could therefore also be a risk factor for sudden cardiac death (SCD)[2,4,13].

Previous studies have addressed the relationship between serum magnesium and CHD[11,14] and the relationship with SCD[15,16], but with conflicting results for both outcomes[2]. In addition, these studies had few cases or lacked adjudicated endpoints, which could have resulted in misclassification. We therefore aimed to study the association of serum magnesium levels with both CHD mortality and SCD within the Rotterdam Study, a prospective population-based cohort study among middle aged and elderly persons with adjudicated endpoints and long-term follow-up. Low serum magnesium has been associated with accelerated atherosclerosis[17]. We therefore hypothesized that this could be the potential mechanism through which serum magnesium increases the risk of CHD mortality. Since carotid intima media thickness (cIMT) is considered a proxy for accelerated atherosclerosis, we explored the association between serum magnesium and cIMT as a potential mediator for the relationship with CHD mortality. QT prolongation is a well-established risk factor for SCD[18] and serum magnesium was shown to influence QT interval in a clinical setting[19]. We therefore hypothesized that QT prolongation could be the pathway through which low serum magnesium is associated with SCD.

Methods

Study design, setting, and population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study, ongoing since 1990 in a suburb of the city of Rotterdam, the Netherlands. The rationale

and design of this study have been described in detail elsewhere[20]. In summary, the original cohort consisted of 7,983 unselected inhabitants of the study area, aged 55 years or over. This cohort was extended in 2000 with 3,011 persons who had reached the age of 55 years, or who had moved into the research area. In 2006, the cohort was extended again with 3,932 persons aged 45 years and older living in the research area who had not yet been invited. This resulted in a total study population of 14,926 individuals, aged 45 years and older. All eligible participants provided written informed consent to participate in the study. Separate consent was asked for follow-up data collection on clinical outcomes and vital status. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Dutch Ministry of Health, Welfare and Sport, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Screening Act: Rotterdam Study)".

Assessment of serum magnesium

For our study, we used blood collections from the third visit of the first cohort (1997-1999), and the first visits of the second cohort (2000-2001) and third cohort (2006-2008). Of the 11,740 eligible participants, serum magnesium was available for 9,882 participants. Of these 9,882 participants, 59 did not provide consent to collect follow-up information from their treating physicians and three participants were lost to follow-up at the date of blood collection and therefore did not contribute to our study. This resulted in a study population of 9,820 participants. Magnesium was measured in serum by the Department of Clinical Chemistry of the Erasmus Medical Center using a Roche/Hitachi Cobas c501 analyser. We modelled the association between serum magnesium and CHD mortality, using restricted cubic splines and found it to be linear. We therefore analyzed serum magnesium as a continuous parameter when studying the association with CHD mortality. The association with SCD, was also modeled using restricted cubic splines and found to be U-shaped. Therefore, we also divided serum magnesium into quartiles and analyzed serum magnesium as a categorical variable in all our analyses. We combined the second and third quartile into a reference group to best fit the observed U-shape, yielding a low, high, and reference group[21].

Assessment of covariables

The assessment of anthropometrics in the Rotterdam Study has been described previously[22]. Body mass index (BMI) was calculated by dividing body weight in kilograms by height in meters squared. Diabetes mellitus was defined as the use of glucose-lowering drugs, a fasting glucose level above 6.9mmol/l or a non-fasting glucose level above 11.0mmol/l when fasting levels were not available[23]. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[24] and was expressed as ml/min/1.73m^2 based on serum creatinine values that were measured at the same visit as the serum magnesium levels using an enzymatic assay method. Total cholesterol and high-density

lipoprotein (HDL) cholesterol were measured at the same visit as the serum magnesium levels using the Roche Modular P800. Information on history of stroke, myocardial infarction, and heart failure was obtained through linkage with medical records kept by general practitioners working in the study area, and subsequently adjudicated by two research physicians and confirmed by a neurologist or cardiologist[25]. Information on smoking habits and alcohol consumption was obtained during a home interview. Smoking was categorized into three categories: non-smoker, former smoker, and current smoker. Alcohol consumption was categorized into yes or no. Drug exposure has been monitored continuously since January 1st 1991 through linkage with digital pharmacy records of the pharmacies in the study district. The following ATC codes were used to retrieve relevant drug exposure for all participants: verapamil (C08DA01), diltiazem (C08DB01), beta-blocking agents (C07), digoxine (C01AA05), class I/III antiarrhythmic drugs (C01B), and diuretics (C03)[26]. The use of definite hart-rate corrected QT (QTc)-prolonging drugs was determined using the University of Arizona list of QTc-prolonging drugs[27]. Participants were classified as being exposed if the date of the blood draw fell within the prescription period. Physical activity was assessed using a validated adapted version of the Zutphen Physical Activity Questionnaire, and expressed in metabolic equivalent of task hours per week[28].

Assessment of outcomes

Follow-up time was calculated from the date that blood was drawn until the date of death, loss to follow-up (n=594), or the end of the study period (January 1st 2012 for CHD mortality and January 1st 2011 for SCD). Methods for outcome data collection and definitions have previously been described in more detail[25,29]. In short, CHD mortality was defined as definite fatal myocardial infarction, definite fatal CHD and possible fatal CHD. Information on the vital status of all participants was obtained on a weekly basis from the central registry of the municipality in Rotterdam and through digital linkage with records from general practitioners working in the study area. The cause of death was established by abstracting information from the medical records of the general practitioners or nursing home physicians and hospital discharge letters. SCD was defined as "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", according to Myerburg's definition endorsed by the European Society of Cardiology[30,31]. Unwitnessed deaths were coded as SCD if death was unexpected in persons found dead, while they were in a stable medical condition 24 hours before they were found in the absence of evidence of a non-cardiac cause[29]. Each outcome was adjudicated by two research physicians who independently classified information on occurrence, certainty and date using standardized criteria and subsequently validated by an experienced cardiologist. The classification of CHD mortality (as a cause of death) and SCD (as a mode of death) overlap and results on CHD mortality could be driven by

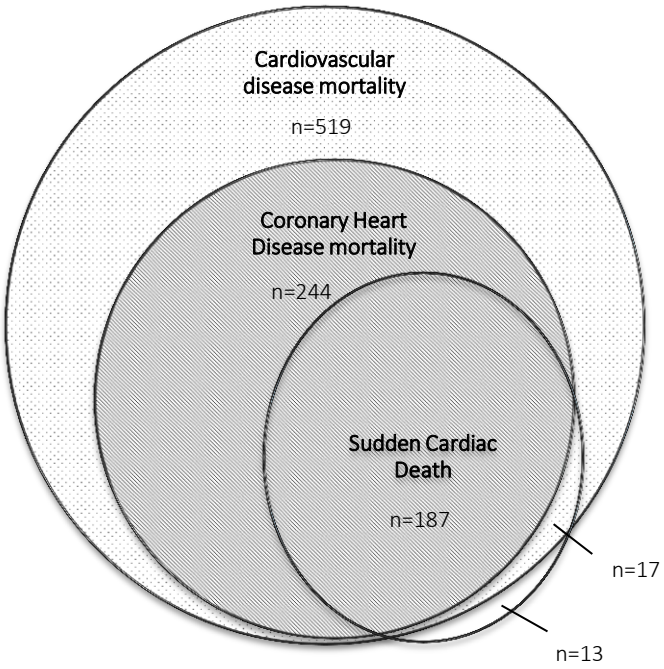


Figure 1. Overlap between the classification of coronary heart disease mortality (as a cause of death) and sudden cardiac death (as a mode of death)
Among all 2,303 participants who died during follow-up, 780 died due to cardiovascular disease of which 431 participants were classified as coronary heart disease mortality. Of these 431 participants, 187 participants were also classified as sudden cardiac death. 17 participants were classified as sudden cardiac death and cardiovascular deaths (but not coronary heart disease) and 13 participants were classified as sudden cardiac death but could otherwise not be classified as cardiovascular deaths.

the effect on SCD (Figure 1). Therefore, we also defined a 'non-sudden CHD mortality' category including participants who were only classified as death from CHD but not as SCD.

Carotid intima-media thickness and electrocardiogram (ECG) measurements

cIMT correlates with future cardiovascular events and was therefore used as a subclinical marker of atherosclerosis[32]. We used longitudinal two-dimensional ultrasound images of the common carotid artery for a 1-cm length that was proximal to the bulb, obtained with a 7.5 MHz linear array transducer and UltraMark IV (Advanced Technology Laboratories, Bethel, WA, U.S.A), to measure the distance between the lumen intima interface and the media-adventitia interface, which indicates the cIMT. The maximal cIMT, summarized as the mean of the maximal measurements from the near and far walls on both the left and right sides, was used for analysis[33].

Each participant also had a standard 12-lead resting ECG recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hertz which was stored digitally. All ECGs were processed by the standardized Modular ECG Analysis System (MEANS) to obtain ECG measurements[34]. The QT interval represents the interval between the start of ventricular depolarization to the end of ventricular repolarization. MEANS determines this interval from the start of the QRS complex until the end of the T-wave. Heart rate modifies this interval, therefore QT-interval measurements are corrected for the individuals' RR interval to allow comparison by using Bazett's correction[35]. For all participants, cIMT measurements and ECG measurement were performed during the same visit during which blood was drawn for serum magnesium measurements.

Statistical analyses

We plotted Kaplan-Meier curves for CHD mortality, non-sudden CHD mortality, and SCD adjusted for competing risk of death by other causes as proposed by Fine and Grey, using the cmprsk package in 'R'[36]. We used Cox proportional hazard regression models to examine the relationship between the different groups of serum magnesium and CHD mortality, non-sudden CHD mortality, and SCD. We built different models to analyze these associations. In the first model we adjusted for age and sex. The second model also included eGFR, BMI, systolic and diastolic blood pressure, diabetes mellitus, history of myocardial infarction, stroke, or heart failure, smoking status, alcohol consumption, use of diuretics and total cholesterol/HDL cholesterol ratio. We studied the association between serum magnesium and QTc interval in a multivariable linear model using the same covariables and additionally adjusted for drugs that are known to affect the QT and/or RR interval (see above). Because Bazett's formula is known to overestimate at short RR intervals and underestimate at long RR intervals, we also additionally adjusted for RR interval[37]. The association between serum magnesium and heart rate (expressed as RR interval) was also studied using the same covariables. We tested for potential pathways by additionally adjusting the second model for QT and RR interval to account for a potential arrhythmogenic pathway and by including cIMT as marker for subclinical atherosclerosis. The proportional hazard assumption was tested by plotting log-minus-log survival curves and visually examining the curves, which did not indicate that the assumption was violated[38]. Results are presented as hazard ratios (HRs) with 95% confidence intervals (95% CI). Missing data in covariables (0 to 9.1%) were handled by single imputation using an expectation-maximization algorithm[39]. All analyses were repeated on complete cases to check for potential differences between results based on imputed data and those based on complete cases. With the exception of the baseline characteristics, results are reported for imputed data. We considered a two-sided p-value <0.05 as statistically significant. Data were analyzed using SPSS version 21.0 (IBM Corp., Somers, NY, USA) and R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

Sensitivity analyses

To further distinguish between an effect of serum magnesium on accelerated atherosclerosis or serum magnesium having an anti-arrhytmogenic effect, we studied the association between serum magnesium and incident myocardial infarction (MI). If the effect of serum magnesium on CHD mortality is mediated through accelerated atherosclerosis, an effect on incident MI would also be expected. We studied the association with incident MI only with serum magnesium as a continuous parameter, as there were only a limited number of MI events and we expected the shape of the association to be similar to CHD mortality. We expected physical activity to be a potential confounder of the association between serum magnesium and CHD mortality. However, data on physical activity was only available for a subset of 6,385 participants. We therefore performed a sensitivity analysis on this subset to check if inclusion of physical activity would have influenced our results. To test the robustness of our findings we also performed two additional analyses where we restricted our analysis to participants free of diabetes mellitus and with normal kidney function (defined as eGFR >60ml/min/1.73m^2) in order to explore potential effect modification by diabetes mellitus or chronic kidney disease. Furthermore, we additionally adjusted for serum sodium, potassium, and calcium levels to test whether the association of magnesium with any of the outcomes could be explained by a concomitant electrolyte disorder.

Table 1. Characteristics of the study population

	Total n = 9,820	Low n = 2,351	Reference n = 5,170	High n = 2,299
Age, years	65.1±9.9	65.1±10.2	64.9±9.7	65.6±10.0
Women	5,575 (57%)	1,368 (58%)	2,908 (56%)	1,299 (57%)
Body mass index, kg/m^2	27.3±4.2	27.8±4.6	27.2±4.1	26.8±4.0
Systolic blood pressure, mmHg	140±21	141±21	139±21	140±22
Diastolic blood pressure, mmHg	79±12	79±12	79±11	79±12
Smoking				
Never	2,991 (31%)	661 (28%)	1,600 (31%)	730 (32%)
Past	4,549 (47%)	1,094 (47%)	2,406 (47%)	1,049 (46%)
Current	2,175 (22%)	574 (25%)	1,112 (22%)	489 (22%)
Alcohol use	8,274 (85%)	1,919 (82%)	4,430 (87%)	1,925 (85%)
History of diabetes mellitus	1,021 (10%)	466 (20%)	433 (8%)	122 (5%)
History of myocardial infarction	494 (5%)	134 (6%)	237 (5%)	123 (5%)
History of stroke	340 (3%)	92 (4%)	160 (3%)	88 (4%)
History of heart failure	257 (3%)	69 (3%)	118 (2%)	70 (3%)
eGFR, ml/min/1.73m^2	81.5±17.3	83.2±20.0	81.8±16.0	79.0±17.0
Serum magnesium, mmol/l	0.84±0.06	0.77±0.04	0.85±0.02	0.92±0.04
Total cholesterol, mmol/l	5.7±1.0	5.6±1.0	5.7±1.0	5.8±1.1
HDL cholesterol, mmol/l	1.40±0.41	1.38±0.42	1.41±0.40	1.40±0.41
Diuretic use	1,017 (11%)	325 (14%)	418 (8%)	233 (11%)

Data presented as mean±standard deviation or number (percentage)
Data are shown for non-imputed data
Low magnesium group 0.34-0.80 mmol/l, reference group 0.81-0.88 mmol/l, high magnesium group 0.89-1.74mmol/l
Abbreviations: eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; n: number

Results

Baseline characteristics

Baseline characteristics of our study population are shown in Table 1. Among the 9,820 participants the mean age was 65.1 years, 56.8% were women and 96% were of European descent. Serum magnesium levels ranged from 0.34 to 1.74 mmol/l. Participants in the low magnesium group (n=2,351) more often used diuretics, and more often had diabetes mellitus. During a median follow-up of 8.7 years (80,750 person-years in total), 2,303 of the 9,820 participants died (23.5%). In 2,213 of the 2,303 participants who died, a cause of death could be adjudicated. Among these participants, 780 died of cardiovascular disease of which 431 were classified as death from CHD. Of the 2,303 participants who died, 217 were classified as SCD. Figure 1 shows how the classifications CHD mortality and SCD relate.

Magnesium and mortality

Figure 2 shows the Kaplan-Meier curves for CHD mortality, non-sudden CHD mortality and SCD

Table 2. Association between magnesium groups and different types of mortality

Serum magnesium groups*	Number of events	Crude incidence rate per 1000 person-years (95%CI)	Model 1	Model 2
		Coronary Heart Disease mortality		
Low	134	7.1 (6.0;8.4)	**1.53 (1.23;1.90)**	**1.36 (1.09;1.69)**
Reference	206	4.8 (4.1;5.5)	Reference	Reference
High	91	4.9 (3.9;6.0)	0.97 (0.76;1.24)	0.94 (0.73;1.21)
		Non-sudden Coronary Heart Disease mortality†		
Low	74	3.9 (3.1;4.9)	**1.39 (1.04;1.85)**	1.27 (0.95;1.70)
Reference	127	2.9 (2.4;3.5)	Reference	Reference
High	43	2.3 (1.7;3.1)	0.73 (0.52;1.04)	**0.69 (0.48;0.98)**
		Sudden Cardiac Death		
Low	70	4.2 (3.2;5.3)	**1.76 (1.29;2.39)**	**1.54 (1.12;2.11)**
Reference	92	2.4 (1.9;2.9)	Reference	Reference
High	55	3.3 (2.5;4.3)	1.32 (0.94;1.84)	1.35 (0.96;1.89)

Data presented as hazard ratio (95% confidence interval), bold font indicates statistical significance ($p<0.05$)

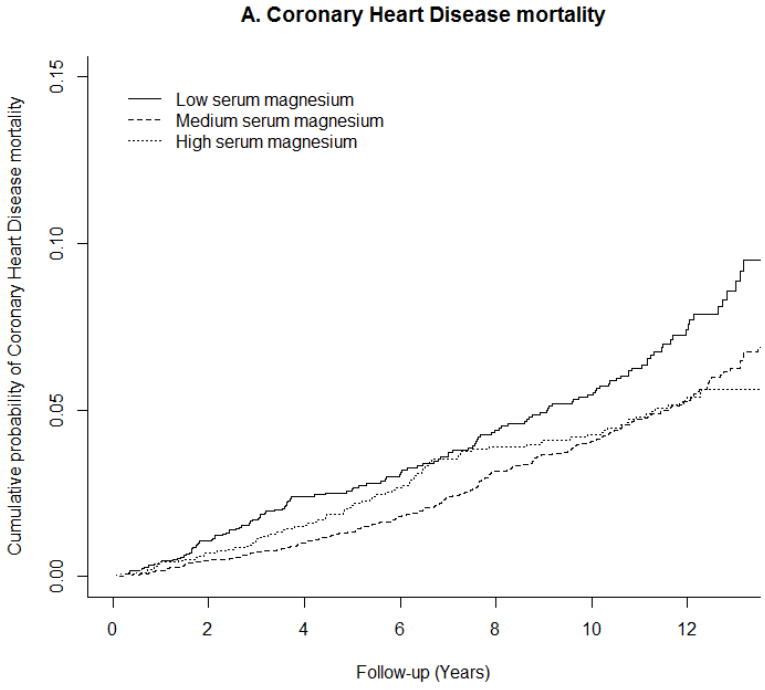
* Low magnesium group 0.34-0.80 mmol/l, reference group 0.81-0.88mmol/l, high magnesium group 0.89-1.74mmol/l

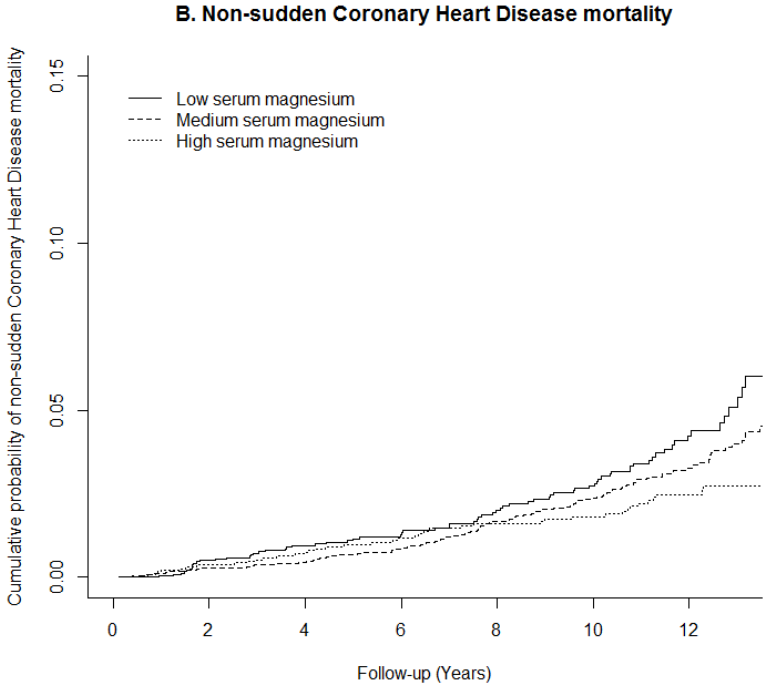
† This category includes participants who died from coronary heart disease, but who were not classified as sudden cardiac deaths

Model 1: adjusted for age and sex

Model 2: additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use

Abbreviations: CI: confidence interval


A. Coronary Heart Disease mortality
Low serum magnesium
Medium serum magnesium
High serum magnesium
Cumulative probability of Coronary Heart Disease mortality
0.00
0.05
0.10
0.15
0
2
4
6
8
10
12
Follow-up (Years)


B. Non-sudden Coronary Heart Disease mortality
Low serum magnesium
Medium serum magnesium
High serum magnesium
Cumulative probability of non-sudden Coronary Heart Disease mortality
0.00
0.05
0.10
0.15
0
2
4
6
8
10
12
Follow-up (Years)

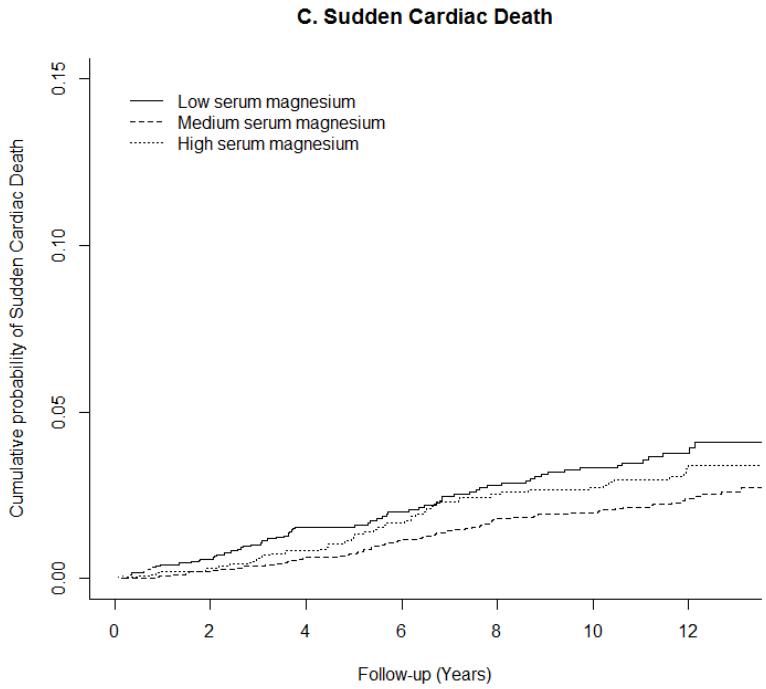


Figure 2. Estimated cumulative probability curves for coronary heart disease mortality (A), non-sudden coronary heart disease mortality (B), and sudden cardiac death (C) between different groups of serum magnesium levels, taking into account the competing risk of death by other causes. Low serum magnesium defined as 0.34-0.80 mmol/l, medium serum magnesium as 0.81-0.88 mmol/l and high serum magnesium as 0.89l1.74 mmol/l.

between the different groups of serum magnesium, taking competing risk into account. Table 2shows the associations between the three magnesium groups and mortality. Participants in the low magnesium group had a 36% increased risk for CHD mortality (HR 1.36, 95%CI 1.09;1.69), compared with the reference group. Participants in the low magnesium group had a significantly increased risk for SCD (HR 1.54, 95%CI 1.12;2.11), but no association was found in the high group (HR 1.35, 95%CI 0.96;1.89). Excluding SCD cases from the CHD outcome resulted in an attenuated risk in the low magnesium group (HR 1.27, 95%CI 0.95;1.70) and a lower risk in the high magnesium group (HR 0.69, 95%CI 0.48;0.98), compared with the reference group. We also tested for a linear association with CHD mortality and non-sudden CHD mortality and found that per 0.1mmol/l increase in serum magnesium the risk for CHD mortality decreased (HR 0.82, 95%CI 0.70;0.96), as well as the risk for non-sudden CHD mortality (HR 0.72, 95%CI 0.58;0.88). We found no evidence of effect modification by gender (P for interaction=0.436), and therefore did not stratify by gender.

Table 3. Association between different magnesium groups and markers of potential pathways

Serum magnesium groups*	Model 1	Model 2
Carotid Intima-Media Thickness (in millimeters)		
Low	**0.023 (0.015;0.030)**	**0.013 (0.005;0.020)**
Reference	Reference	Reference
High	-0.005 (-0.013;0.003)	-0.003 (-0.010;0.004)
QTc interval (in milliseconds)†		
Low	**2.7 (1.6;3.9)**	**1.8 (0.7;2.9)**
Reference	Reference	Reference
High	**2.0 (0.9;3.1)**	**2.2 (1.1;3.3)**
QTc interval (in milliseconds), additionally adjusted for RR interval†		
Low	**2.0 (1.0;3.0)**	**1.3 (0.3;2.3)**
Reference	Reference	Reference
High	**1.5 (0.5;2.5)**	**1.6 (0.6;2.6)**
RR interval (in milliseconds)†		
Low	**-10.1 (-16.7;-3.5)**	**-7.1 (-13.5;-0.8)**
Reference	Reference	Reference
High	**-7.1 (-13.8;-0.4)**	**-7.8 (-14.1;-1.4)**

Data presented as mean difference (95% confidence interval), bold font indicates statistical significance (p<0.05)

* Low magnesium group 0.34-0.80 mmol/l, reference group 0.81-0.88mmol/l, high magnesium group 0.89-1.74mmol/l

†In the analysis on ECG parameters, model 2 also includes adjustment for verapamil use, diltiazem use, beta-blocker use, digoxin use, anti-arrhythmic drug use, QT-prolonging drug use

Model 1: adjusted for age and sex

Model 2: additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use

Abbreviations: QTc: heart-rate corrected QT interval

Intima media thickness, QT-interval, and heart rate

Table 3 shows the associations between the different magnesium groups and markers of potential pathways through which magnesium may mediate CHD mortality and SCD. We observed a significantly higher cIMT in the low magnesium group (+0.013mm, 95%CI 0.005;0.020), but not in the high magnesium group. The QTc interval was significantly longer in both the low and high magnesium groups (+1.8ms 95%CI 0.7;2.9 and +2.2ms 95%CI 1.1;3.3, respectively). When we additionally adjusted for heart rate, we found that the association remained significant, but the effect sizes became smaller. A separate, multivariable analysis on heart rate (expressed as RR interval) as the outcome of interest showed significant associations in both the low group (-7.1ms, 95%CI -13.5;-0.7) and the high group (-7.8ms, 95%CI -14.1;-1.4). Additional adjustment for QT interval and heart rate did not change the association with SCD (before adjustment HR 1.54, 1.12;2.11; after adjustment HR 1.50, 1.09;2.06). Furthermore, additional adjustment for cIMT did not change the association with non-sudden CHD (before adjustment HR 1.27, 0.95;1.70; after adjustment HR 1.26, 0.94;1.69). Table 4 shows the

Table 4. Influence of potential pathway markers on the association between magnesium groups and cardiovascular outcomes

Serum magnesium groups*	Model 1	Model 2	Model 3
Coronary Heart Disease mortality			
Low	**1.36 (1.09;1.69)**	**1.34 (1.08;1.68)**	**1.35 (1.08;1.68)**
Reference	Reference	Reference	Reference
High	0.94 (0.73;1.21)	0.94 (0.73;1.20)	0.95 (0.74;1.22)
Non-sudden Coronary Heart Disease mortality			
Low	1.27 (0.95;1.70)	1.27 (0.95;1.71)	1.26 (0.94;1.69)
Reference	Reference	Reference	Reference
High	**0.69 (0.48;0.98)**	**0.69 (0.48;0.97)**	**0.70 (0.49;0.99)**
Sudden Cardiac Death			
Low	**1.54 (1.12;2.11)**	**1.50 (1.09;2.06)**	**1.53 (1.11;2.10)**
Reference	Reference	Reference	Reference
High	1.35 (0.96;1.89)	1.32 (0.94;1.85)	1.35 (0.96;1.89)

Data presented as hazard ratio (95% confidence interval), bold font indicates statistical significance ($p<0.05$)

* Low magnesium group 0.34-0.80 mmol/l, reference group 0.81mmol/l to 0.88mmol/l, high magnesium group 0.89-1.74mmol/l

Model 1: adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use (similar to model 2 Table 2)

Model 2: model 1 additionally adjusted for QT interval and RR interval

Model 3: model 1 additionally adjusted for carotid intima-media thickness

influence of these potential pathway markers on all associations between serum magnesium and death from CHD, non-sudden CHD mortality, and SCD.

Sensitivity analyses

When studying the association between serum magnesium and incident MI, we found that in our age and sex adjusted model a 0.1 mmol/l increase in serum magnesium level was associated with a decreased risk of MI (HR 0.84, 0.72;0.99). However this was no longer statistically significant after further adjustment for potential confounders (HR 0.90, 0.76;1.06, data not shown). In the subset of 6,385 participants where physical activity was available, the inclusion of physical activity to the full model did not change the association between serum magnesium and CHD mortality, non-sudden CHD mortality and SCD (data not shown). When restricting all analyses to participants free of diabetes mellitus and chronic kidney disease (eGFR >60 ml/min) the relationship found with CHD mortality and SCD was not altered (Table 5). Additional adjustments for serum sodium, potassium, and calcium to investigate whether the effect of serum magnesium on mortality is mediated through other electrolytes, did not alter the point estimates.

Table 5. Sensitivity analysis on subset of participants free of diabetes mellitus and with normal kidney function and analysis on the influence of other electrolytes

Serum magnesium groups*	Model 1 n = 9,280	Model 2 n = 7,843	Model 3 n = 9,280
	Coronary Heart Disease mortality		
Low	**1.36 (1.09;1.69)**	**1.44 (1.06;1.95)**	**1.33 (1.06;1.66)**
Reference	Reference	Reference	Reference
High	0.94 (0.73;1.21)	0.93 (0.67;1.29)	0.97 (0.76;1.25)
	Sudden Cardiac Death		
Low	**1.54 (1.12;2.11)**	**1.67 (1.11;2.51)**	**1.56(1.13;2.14)**
Reference	Reference	Reference	Reference
High	1.35 (0.96;1.89)	1.19 (0.77;1.85)	1.35 (0.96;1.90)

Data presented as hazard ratio (95% confidence interval), bold font indicates statistical significance ($p<0.05$)

* Low magnesium group ≤0.80 mmol/L, reference group 0.81mmol/L to 0.88mmol/L, high magnesium group ≥0.89mmol/L

Model 1: adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use (similar to model 2 Table 2)

Model 2: model 1 in a subset of participants free of diabetes mellitus and with normal kidney function (defined as an eGFR >60 ml/min/1.73m^2)

Model 3: model 1 additionally adjusted for serum sodium, serum potassium and serum calcium in all participants

Discussion

In this prospective population-based cohort study among 9,820 participants with a median follow-up of 8.7 years, we found that low serum magnesium was associated with an increased risk of CHD mortality and SCD. When we excluded SCD from the CHD mortality end point, we found an inverse relationship between serum magnesium and non-sudden CHD mortality. Even though serum magnesium was associated with QTc, heart rate, and cIMT, this did not explain the relationship between serum magnesium and CHD mortality or SCD.

We observed a 36% increased risk of CHD mortality in participants with low serum magnesium. The association between serum magnesium and CHD mortality has been studied previously in a cohort of Finnish men but no significant association was found, possible due to a low number of cases (n=230)[14]. The National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (NHFES) did find a significant association, but only when comparing the third quartile of serum magnesium to the first quartile[11]. The Nurses' Health Study (NHS) analyzed the association between dietary magnesium and CHD mortality[40]. A significant association was identified when comparing the highest with the lowest quintile of dietary magnesium intake, but they were unable to study the association with serum magnesium due to lack of cases[40]. A meta-analysis on the association between low serum magnesium and fatal CHD, which included the

two studies discussed above[11,14] found a non-significant trend towards an increased risk[9]. However, this meta-analysis also included two studies with SCD as outcome of interest which, judging from the forest plot, were mainly responsible for this observed inverse effect[2].

We analyzed SCD independently of CHD and found an increased risk for SCD in participants with low serum magnesium levels. Investigators from the Framingham Heart Study did not observe an association between low serum magnesium and SCD[16]. However, this study included only 29 cases of SCD (compared to 217 cases in our study), and may therefore have lacked power. Investigators from the Atherosclerosis Risk in Communities Study (ARIC) and from the NHS also analyzed the association between serum magnesium and SCD and both reported a lower risk for SCD when comparing the highest magnesium quartile with the lowest magnesium quartile[15,41]. The difference in risk in participants with high serum magnesium between our study and the other two studies could be the result of differences in kidney function. In comparison to baseline kidney function from the ARIC Study and the NHS, participants within the Rotterdam Study had on average a 10 ml/min/1.73m^2 lower eGFR at baseline than the participants from the ARIC Study and NHS. The increased risk of SCD in the high serum magnesium category could therefore still be the result of residual confounding by kidney function, assuming that a single eGFR measurement does not capture all changes associated with a decreased kidney function. We considered QT prolongation as a potential pathway through which serum magnesium may affect SCD risk, since prolongation and shortening of the QT interval is associated with SCD[42,43]. However, adjustments for QT and RR interval did not fully explain the observed associations. An explanation for this limited change in effect estimate may be that a single measurement of QT interval does not fully capture all the electrophysiological changes caused by a low serum magnesium. Another possibility could be that the effect of serum magnesium on QT interval is too small to have impact on the risk of SCD, as it is currently assumed that a minimal increase of five milliseconds is necessary to increase the risk of torsade de pointes[44].

A substantial overlap between participants classified as CHD mortality and SCD exists. CHD mortality is a mixture of modes of death ranging from ischemia induced ventricular arrhythmia to chronic ischemic heart failure. SCD is a particular mode of death which is predominantly caused by arrhythmias[31]. When we limited our outcome to non-sudden CHD deaths, we still observed a trend toward increased risk in the low magnesium group. We also observed a potential protective effect of high serum magnesium on non-sudden CHD mortality, which has been observed previously in NHEFS[11]. Although this observation combined with the significant effect on cIMT supports a role for magnesium in atherosclerosis, it does not appear to be the main pathway as the association between serum magnesium and non-sudden CHD death remained after adjustment for cIMT. This could be due to the small effect size of serum magnesium on cIMT. In previous studies it was shown that per 0.17mm increase in cIMT the risk

for acute myocardial infarction increased with 17%[45]. However in our study, we only found an increase of 0.013mm when comparing participants with low serum magnesium with the reference group. It is therefore unlikely that this small increase would have a large influence on our outcome. Furthermore, no association was found between serum magnesium and incident MI which would have been expected if the main effect of serum magnesium is through accelerated atherosclerosis. This lack of association with incident ischemic heart disease has been observed previously in the NHANES study[11]. The discrepancy between an effect on CHD mortality but not on incident MI, argues against a direct role of serum magnesium on accelerated atherosclerosis, making an (acute) arrhythmogenic effect of serum magnesium more plausible.

Our study has several strengths. First of all, we did not use total cardiovascular mortality but studied CHD mortality, non-sudden CHD mortality and SCD separately. The classification of cardiovascular mortality is heterogeneic and often also includes non-atherosclerotic outcomes like hemorrhagic stroke. This heterogeneity makes it difficult to study associations, since there is also heterogeneity in the underlying pathophysiology of the various types of cardiovascular disease. Another strength of our study is the high case load, and, more importantly, the availability of adjudicated endpoints which were blinded from the laboratory results, reducing potential bias due to differential misclassification. The main limitation of our study was the fact that we only have a single serum magnesium measurement at baseline and no follow-up serum magnesium measurements. We therefore cannot adjust for intra-individual variability over time. However, a recent study showed that there is a strong correlation between serum magnesium concentrations measured one year apart[5], suggesting the possibility of an individual setpoint for serum magnesium, similar to what has been shown for serum sodium[46,47]. In observational studies, reversed causality remains a limitation. However, the long-term follow-up after the serum magnesium measurement decreases the probability of reversed causality in our study. Another limitation of our study is the potential heterogeneity of SCD cases which is inherent to the definition of SCD[48]. Generalization to other ethnic groups is also a limitation, as the participants in the Rotterdam Study are predominantly of European descent (96%). Finally, residual confounding can always play a role in observational studies. We tried to address this by adjusting for a large number of potential confounders and performing various sensitivity analyses, which did not indicate substantial amounts of confounding. A potential confounder for which we were unable to adjust is diet. Higher dietary magnesium intake could be a proxy for a healthy diet, therefore resulting in a lower risk of CHD mortality. Unfortunately, valid dietary measurements were only available for a subset of 2,504 participants making it difficult to adjust for this potential confounder.

In conclusion, low serum magnesium is associated with an increased risk of CHD mortality and SCD. Although low magnesium has a significant effect on subclinical atherosclerosis and heart rate, this did not explain the observed associations with CHD or SCD. The results from this and previous studies may provide a rationale to design intervention studies to analyze whether magnesium supplementation could prove to be effective in lowering the burden of CHD mortality and SCD.

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Part V

Pharmacological risk factors

Chapter 5.1

Concomitant prescriptions of QT-prolonging drugs between 1996 and 2014

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Abstract

Background Regulatory authorities recommend avoiding concomitant use of QT-prolonging drugs, because of a potentially increased risk of arrhythmias. However, it is unclear whether physicians adhere to this advice. We aimed to study the frequency of concomitant prescriptions of QT-prolonging drugs in a general population.

Methods We used the IPCI database which contains electronic healthcare records of more than 600 general practitioners in the Netherlands, with data of approximately 1.7 million patients between 1996 and 2014, including drug prescriptions. We calculated the prevalence rate and incidence rate per 1,000 person-years of concomitant prescriptions of known and possible QT-prolonging drugs per age category, sex, and calendar year. To account for switching between drugs, we analysed the prescriptions per drug class and we applied a minimum duration of overlap.

Results The prevalence rate of concomitant prescriptions of QT-prolonging drugs was 4.24 per 1,000 person-years and the incidence rate was 3.95 per 1,000 person-years. Combinations were more often prescribed in women than in men and occurrence increased with age. The frequency of concomitant prescriptions has increased over the past two decades. Up to seven drugs were concomitantly prescribed, but the vast majority comprised combinations of two QT-prolonging drugs. Antipsychotics, antidepressants, anti-infectives, promethazine, and propulsives (cisapride or domperidone) were frequently prescribed in varying combinations.

Conclusion In this study, we show that the frequency of concomitant prescriptions of QT-prolonging drugs has increased over the past decades despite the advice of regulatory authorities to avoid concomitant prescriptions of such drugs. Reasons for non-adherence should be further investigated.

Introduction

Drug-induced prolongation of the QT interval[1] is a well-known risk factor for ventricular arrhythmias and sudden cardiac death[2-5]. Drugs that prolong the QT interval are mentioned in lists, for example the University of Arizona, Center for Education and Research on Therapeutics list[1], which distinguishes three categories according to the level of evidence that is available. For defining the 'known' QT-prolonging drugs, a positive temporal concordance was documented between drug intake and QT prolongation. For defining the 'possible' category, less evidence was available. For defining 'conditional' QT-prolonging drugs, evidence was considered inadequate or contradictory[6]. Due to ageing of the population polypharmacy is an emerging problem[7]. Using QT-prolonging drugs concomitantly could lead to an increased risk of arrhythmias and sudden cardiac death. However evidence to support this is lacking. Nevertheless, regulatory authorities recommend to avoid concomitant use of QT-prolonging drugs[4,5]. It is unclear whether physicians adhere to this advice. A study in a claims database in the United States showed that in 1999, 2.1% of the population filled prescriptions of multiple QT-prolonging drugs with overlapping use in calendar time[8]. But since then, no other studies have investigated this and the trend over calendar time has not been studied to date. We aimed to determine the occurrence of concomitant prescriptions of QT-prolonging drugs in primary care using healthcare records from 1996 to 2014.

Methods

Setting and study population

Data were retrieved from the Integrated Primary Care Information (IPCI) database. The IPCI database forms a longitudinal, observational, dynamic cohort which contains the electronic medical records of a group of more than 600 general practitioners (GPs) in the Netherlands. In the Dutch healthcare system, the GP has a pivotal role and functions as a gatekeeper of medical care. Almost all inhabitants of the Netherlands are registered with a GP. The database contains the medical records of approximately 1.7 million patients. This is the cumulative number of patients who have ever been part of the dynamic cohort of patients who have been registered with one of the collaborating GPs. The medical records in the database are anonymized and contain data on demographics, symptoms and diagnoses (coded and in free text), referrals, laboratory findings, hospitalizations, discharge letters, and drug prescriptions. The drug prescriptions are coded using the Anatomical Therapeutic Chemical (ATC) code[9] and dosing regimen. Details of the IPCI database have been described elsewhere[10,11]. The system complies with European Union guidelines on the use of electronic healthcare data for medical research and has been proven valid for pharmacoepidemiologic studies[11].

The study population comprised all subjects within the IPCI database between 1996 and 2014, with at least one year of valid records in the database. This study was approved by the IPCI review board.

Co-factors

Age, sex, and calendar year were studied as determinants of concomitant prescriptions of two or more QT-prolonging drugs. Age was studied in the following strata: 0-18, 18-30, 30-50, 50-70, 70-85, and 85+.

Study outcome

Exposure to QT-prolonging drugs was obtained from prescription data. We selected known (Supplement 1) and possible (Supplement 2) QT-prolonging drugs according to the University of Arizona list[1], based on the full (5th level; 7 digits) ATC code, which indicates individual (international non-proprietary name) drugs. To determine the periods during which an individual drug was prescribed, we calculated the duration of each prescription. We primarily used the duration as provided in the database by the GP. When this information was missing, we calculated the duration according to the total number per prescription divided by the prescribed daily number. If both total number per prescription and the prescribed daily number was missing we imputed the duration in four subsequent steps until all missing durations were imputed; (i) imputed with the median per drug name (which also contains the method of administration and dosage) for each patient, (ii) imputed with the median per ATC code for each patient, (iii) imputed with the median per drug name for the total population, and (iv) imputed with the median per ATC code for the total population. We determined periods of concomitant use with Jerboa, a software tool to construct prescription episodes and to calculate prevalence and incidence rates[12,13]. A period of concomitant prescriptions was defined as a period during which the durations of two or more QT-prolonging drugs were overlapping in calendar time. To account for switching between different drugs, i.e. the prescription periods overlap but people have in fact stopped using one of the drugs, we analysed the periods of concomitant use according to 4th level (5 digits) ATC codes, i.e. at the level of drug classes. We did this because it is unlikely that a person gets a prescription for two drugs from the same pharmacotherapeutic class concomitantly (e.g. the beta-blockers propranolol and sotalol, or the ACE-inhibitors captopril and enalapril) and this probably indicates switching to another drug for the same indication because of limited effectiveness or adverse effects instead of overlapping use. To further account for switching we applied a minimum required overlap period between two or more prescriptions, i.e. two or more drugs should be prescribed during an overlapping period of a minimum number of days to be counted as concomitant use. For chronic medication we used a minimum overlap between two prescriptions of 30 days, for non-chronic medication, e.g. antibiotics, we used smaller minimum overlaps, as shown in Table 1. The minimum overlap is

Table1. Minimum overlap periods used to determine periods of concomitant use

1 day	
Dermatological agents	D04AA10, D10AF02, D11AH01
Mifepristone	G03XB01
Oxytocin	H01BB02
Cocaine	N01BC01
Eye drops	S01AA17, S01AA26, S01AE01, S01AE07, S01HA01
3 days	
Anti-infectives	J01FA01, J01FA06, J01FA09, J01FA10, J01MA01, J01MA06, J01MA11, J01MA12, J01MA14, J02AC03, J05AE01, J05AE08, J05AG05
5 days	
Antimalarials	P01BA01, P01BX01, P01CX01
Antihistamines	R06AD02, R06AX11, R06AX12
20 days	
Antipsychotics	N05AH02, N05AH03, N05AH4
30 days	
Other	

based on commonly prescribed durations of these drugs or the median prescribed duration of individual drugs (N05AH) because the median was lower than the normal duration of a prescription of 30 days. If the combination comprised a non-chronic and a chronic drug, the shortest minimum overlap was applied, e.g. if sotalol and an antibiotic were prescribed concomitantly, the overlapping period of the two prescriptions should be at least three days. Jerboa constructs episodes of concomitant prescriptions of the selected ATC codes, while taking into account these minimum overlaps.

Data-analysis

We counted the total number of episodes of concomitant prescriptions. First, we studied the prevalence and incidence rates of all the types of concomitantly prescribed drugs. Subsequently, we studied the prevalence and incidence separate for the number of drugs prescribed concomitantly, i.e. combinations of two, three, four, etc. drugs, regardless of the type of drug prescribed. The prevalence rate of concomitant prescriptions was calculated as the total number of patients with at least one period of concomitant prescriptions divided by the total number of person-years (py) in the period under analysis; Prevalence per 1,000py = number of patients with a prescription for multiple QT-prolonging drug/(py/1000). The incidence rate was calculated as the number of patients with a newly prescribed specific combination of QT-prolonging drugs divided by the total py at risk in the period under analysis; Incidence per 1,000py = number of patients with a newly prescribed specific combinations of multiple QT-prolonging drugs/(py at risk/1000). A set of concomitant prescriptions was defined as incident if no identical number or set of concomitant prescriptions were identified in the one-year prior. People were censored for one year from the onset of a specific combination of QT-prolonging drugs. The prevalence rate was calculated until 2013, as data in 2014 was not

Table 2. Distribution of person-years of the study population by sex and age categories

Age category	Total (n=1,786,598)	Men (n=873,580; 49%)	Women (n=913,018; 51%)
0-18	1,183,322	604,283	579,039
18-30	794,895	399,086	395,809
30-50*	1,585,812	780,838	804,975
50-70	1,388,418	686,269	702,149
70-85	503,953	223,274	280,679
85+	96,472	29,762	66,710

* Numbers do not add up due to rounding

complete for the whole year. Incomplete data over the course of a year influences prevalence, as the number of existing users is highest at the beginning of a year, while the sum of py over that year is not complete. Therefore, the prevalence will be spuriously high when calculated over 2014. This influences the incidence rate to a lesser extent as the number of patients with a new prescription for QT-prolonging drugs is likely to be relatively stable.

Results

General characteristics

The total study population consisted of 1,786,598 subjects of whom 51% were women. In total, the study population contributed 5,552,872py with a mean duration of follow-up per patient of 3 years. The distribution of py by sex and age categories is shown in Table 2. The percentage of missing durations per drug as provided by the GP are listed in Supplement 3. The median percentage of missing durations was 12% (Q1 7%;Q3 27%).

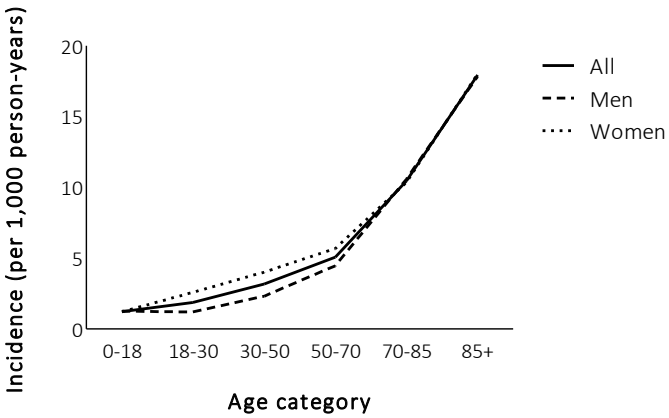

Figure 1. Incidence rate of any concomitant prescription of QT-prolonging drugs per age category for the overall population and stratified by sex

Figure 2. Incidence rate of any concomitant prescription of QT-prolonging drugs per calendar year for the overall population and stratified by sex

Prevalence and incidence rates of concomitant use

During the study period, a total of 23,563 persons (1.3%) received concomitant prescriptions of QT-prolonging drugs. The prevalence rate of any concomitant prescription in the total study population was 4.24 per 1,000py, the incidence rate was 3.95 per 1,000py. Combinations of QT-prolonging drugs were more frequently prescribed to women than to men (incidence rate 4.59 per 1,000py vs. 3.29 per 1,000py, respectively). The incidence of concomitant prescriptions increased with age, from 1.23 per 1,000py for subjects aged 0-18 years to 17.94 per 1,000py for subjects aged 85 years and older (Figure 1). The frequency has increased over time, as shown in Figure 2. The incidence rate increased from 2.98 per 1,000py in 1996 to 3.63 per 1,000py in 2014. The prevalence rate showed a comparable trend with an increase from 3.63 per 1,000py in 1996 to 7.12 per 1,000py in 2013. The incidence rate of concomitant prescriptions per combination of two, three, four, five, six, or seven drugs prescribed concomitantly is shown in Figure 3. The prevalence rate of concomitant prescriptions per number of drugs was comparable to the incidence rate (data not shown). The majority of concomitant prescriptions consisted of combinations of two QT-prolonging drugs (prevalence 4.16 per 1,000py, incidence 3.92 per 1,000py; Figure 3A).

Type of drugs prescribed concomitantly

Eighteen of the 25 (64%) most frequently prescribed combinations of two QT-prolonging drugs comprised antibiotics (J01) and in the total number of combinations of two drugs, 23% comprised an anti-infective drug (class J). Table 3 shows the ten most frequently prescribed combinations of two or three QT-prolonging drugs without antibiotics. Drugs most often

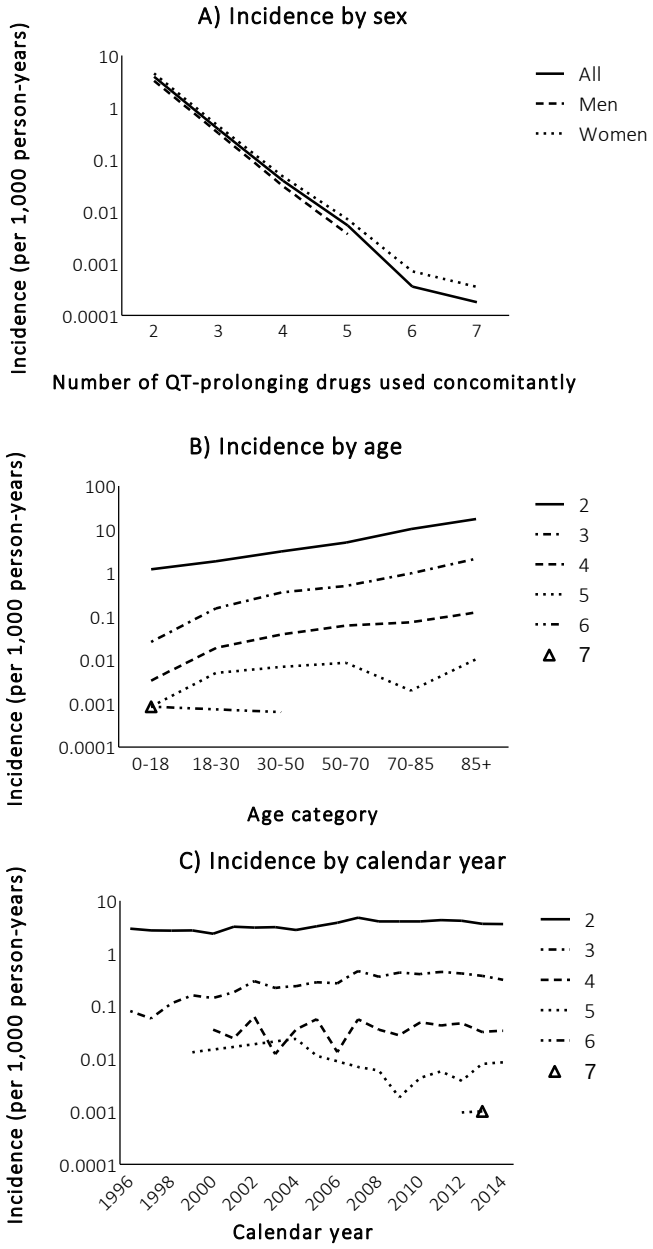

Figure 3. Incidence rate of different numbers of concomitant prescription of QT-prolonging drugs

A) For the overall population and separate for men and women

B) By age category

C) By calendar year

Y axis has a logarithmic scale

Table 3. Most prescribed combinations of two or three QT-prolonging drug classes without antibiotics, with the specific drugs in the drug classes

Combinations of two QT-prolonging drugs			
1	Clozapine, olanzapine, quetiapine	Mirtazapine, venlafaxine	
2	Clozapine, olanzapine, quetiapine	Citalopram, escitalopram	
3	Clozapine, olanzapine, quetiapine	Lithium	
4	Flecainide	Sotalol	
5	Citalopram, escitalopram	Mirtazapine, venlafaxine	
6	Mirtazapine, venlafaxine	Promethazine	
7	Citalopram, escitalopram	Promethazine	
8	Clozapine, olanzapine, quetiapine	Promethazine	
9	Droperidol, haloperidol, pipamperone	Promethazine	
10	Topical erythromycin	Mirtazapine, venlafaxine	
Combinations of three QT-prolonging drugs			
1	Clozapine, olanzapine, quetiapine	Mirtazapine, venlafaxine	Promethazine
2	Clozapine, olanzapine, quetiapine	Citalopram, escitalopram	Promethazine
3	Clozapine, olanzapine, quetiapine	Lithium	Citalopram, escitalopram
4	Clozapine, olanzapine, quetiapine	Citalopram, escitalopram	Mirtazapine, venlafaxine
5	Clozapine, olanzapine, quetiapine	Lithium	Promethazine
6	Clozapine, olanzapine, quetiapine	Lithium	Mirtazapine, venlafaxine
7	Clozapine, olanzapine, quetiapine	Aripiprazole, paliperidone, risperidone	Mirtazapine, venlafaxine
8	Clozapine, olanzapine, quetiapine	Aripiprazole, paliperidone, risperidone	Citalopram, escitalopram
9	Clozapine, olanzapine, quetiapine	Mirtazapine, venlafaxine	Promethazine
10	Droperidol, haloperidol, pipamperone	Mirtazapine, venlafaxine	Promethazine

* QT-prolonging drugs in the drug class that are used in the study population

prescribed concomitantly besides antibiotics are antipsychotics (N05A; especially clozapine, olanzapine, and quetiapine), antidepressants (N06A), and phenothiazine derivatives (R06AD; promethazine). We did not show the most frequently prescribed drug classes for combinations of four or more drugs, because of too low numbers. These combinations mainly comprise the same drug classes most often prescribed in combinations of two or three drugs with the addition of propulsives (cisparide or domperidone), drugs for urinary incontinence, and dermatological agents.

Discussion

In this study, we showed that the frequency of concomitant prescriptions of QT-prolonging drugs has increased over the past 19 years, with an overall incidence rate of 3.95 per 1,000py. Combinations of QT-prolonging drugs are more frequently prescribed in women and in the elderly. The most frequent concomitantly prescribed drugs are anti-infectives, antipsychotics, antidepressants, promethazine, and propulsives.

Regulatory authorities such as the Food and Drug Administration and the European Medicines Agency recommend to avoid concomitant use of QT-prolonging drugs, because of a potentially increased risk of arrhythmias[4,5]. In a previous study, we investigated the association between concomitant use of QT-prolonging drugs and the duration of the heart-rate corrected QT (QTc) interval[14]. In that study, we could not demonstrate an additive QTc-prolongation in users of two or three known or possible QT-prolonging drugs compared to users of one such drug. This might indicate that the advice to avoid concomitant use in all people might be too strict. However, further research into the association between concomitant use of QT-prolonging drugs and the occurrence of arrhythmias is needed since the duration of the QT interval is an intermediate outcome.

Many frequently prescribed drug classes were anti-infectives, which are normally prescribed for a short period. This is also reflected by the fact that the prevalence and incidence rates do not differ much, which indicates short periods of concomitant prescriptions. Other combinations mostly include drugs for several psychiatric indications, such as antipsychotics, antidepressants, lithium, and drugs used as sedatives.

A previous study by Curtis et al., showed that in 1999 2.1% of the general population used combinations of QT-prolonging drugs[8]. We showed that 1.3% of our study population was exposed to concomitant prescriptions at any time during follow-up. There are two factors which could explain the difference between the two studies; (i) Curtis et al. included people aged 18 years and older, while we also included subjects aged below 18 years and in this group concomitant use is less prevalent, which lowers the overall percentage, and (ii) Curtis et al. also included drugs that could influence the pharmacokinetics of QT-prolonging drugs, but which are not QT-prolonging by itself. In the current study, we showed that the prevalence and incidence of concomitant prescriptions have increased over the past decades. The reasons for non-adherence to the advice of regulatory authorities should be studied further. Possibly, physicians are not aware of this advice or they assess the QT interval in every patient in whom they consider prescribing multiple QT-prolonging drugs and only prescribe combinations to people without substantial QT prolongation.

This study has several strengths and limitations. An important strength is the size of our study population and the population-based character. In the Netherlands, virtually every inhabitant is registered with a GP. Therefore, no selection has been made from the general population. The use of prescription data is a strength in this study, because our aim was to study prescription patterns. However, we cannot ascertain whether a subject is actually using the prescribed medication and we have no information regarding advice physicians might give to stop using other medication when prescribing another QT-prolonging drug. A limitation is the absence of prescriptions of drugs in the hospital and the outpatient clinic, which causes an underestimation of the number of concomitant prescriptions in this study. Second, certain drugs that have QT-prolonging properties are prescribed "as needed" to some patients. As we do not have accurate information regarding this issue, we have calculated durations of these drugs as if people used these drugs chronically. We have also included drugs for topical use, such as crèmes and eye drops, because we cannot be sure that these medications do not have systemic effects. Third, we tried to prevent misclassification from switching by (i) applying a minimum overlap, and (ii) analysing concomitant prescriptions based on drug classes. However, it is possible that this leads to under or overestimations. In the Netherlands, people normally receive their medication for a maximum duration of three months. Thirty days is an arbitrary cut-off for minimum overlap, but with a shorter minimum overlap we would probably include many switchers or patients who have been advised to stop using their other QT-prolonging drug. With a longer duration, we would underestimate the number of concomitant prescriptions. Finally, the list of QT-prolonging drugs is a dynamic list, which is adapted whenever knowledge on QT-prolonging properties increases. Drugs can be added or removed from the list. We did not account for this in the analysis.

In conclusion, we showed that concomitant prescription of QT-prolonging drugs have increased over the past two decades despite the advice of regulatory authorities to avoid concomitant use of these drugs because of a possibly increased risk of arrhythmias. Reasons for non-adherence should be further investigated.

Supplements

Supplement 1. List of known QT-prolonging drugs according to the list of the University of Arizona with Anatomical Therapeutic Chemical code

Name	Anatomical Therapeutic Chemical code
Amiodarone	C01BD01
Anagrelide	L01XX35
Arsenic trioxide	L01XX27
Astemizole	R06AX11
Azithromycin	J01FA10/S01AA26
Bepridil	C08EA02
Chloroquine	P01BA01
Chlorpromazine	N05AA01
Cisapride	A03FA02
Citalopram	N06AB04
Claritromycin	J01FA09
Cocaine	N01BC01/ R02AD03/S01HA01
Disopyramide	C01BA03
Dofetilide	C01BD04
Domperidone	A03FA03
Dronedarone	C01BD07
Droperidol	N05AD08
Erytromycin	D10AF02/J01FA01/S01AA17
Escitalopram	N06AB10
Flecainide	C01BC04
Grepafloxacin	J01MA11
Halofantrine	P01BX01
Haloperidol	N05AD01
Ibutilide	C01BD05
Levoflaxacin	J01MA12/S01AE05
Levomethadyl	N07BC05 (levomethadone)
Mesoridazine	N05AC03
Methadone	N07BC02
Moxifloxacin	J01MA14/S01AE07
Ondansetron	A04AA01
Pentamidine	P01CX01 (pentamidine isethionate)
Pimozide	N05AG02
Probucol	C10AX02
Procainamide	C01BA02
Quinidine	C01BA01
Sevoflurane	N01AB08
Sotalol	C07AA07
Sparfloxacin	J01MA09
Sulpiride	N05AL01
Terfenadine	R06AX12
Thioridazine	N05AC02
Vandetanib	L01XE12

Supplement 2. List of possible QT-prolonging drugs according to the list of the University of Arizona with Anatomical Therapeutic Chemical code

Name	Anatomical Therapeutic Chemical code
Alfuzosin	G04CA01
Apomorphine	G04BE07/N04BC07
Aripiprazole	N05AX12
Atazanavir	J05AE08
Bedaquiline	J04AK05
Bortezomib	L01XX32
Bosutinib	L01XE14
Clozapine	N05AH02
Crizotinib	L01XE16
Dabrafenib	L01XE23
Dasatinib	L01XE06
Dexmedetomidine	N05CM18
Dihydroartemisinin+piperaquine	P01BF05
Dolasetron	A04AA04
Eribulin	L01XX41
Famotidine	A02BA03
Felbamate	N03AX10
Fingolimod	L04AA27
Foscarnet	J05AD01
Fosphenytoin	N03AB05
Gatifloxacin	J01MA16/S01AE06
Gemifloxacin	J01MA15
Granisetron	A04AA02
Iloperidone	N05AX14
Isradipine	C08CA03
Lapatinib	L01XE07
Lithium	N05AN01
Mifepristone	G03XB01
Mirabegron	G04BD12
Mirtazapine	N06AX11
Moexipril/HCTZ	C09AA13
Nicardipine	C08CA04
Nilotinib	L01XE08
Norfloxacin	J01MA06/S01AE02
Ofloxacin	J01MA01/S01AE01/S02AA16
Olanzapine	N05AH03
Oxytocin	H01BB02
Paliperidone	N05AX13
Pasireotide	H01CB05
Pazopanib	L01XE11
Perflutren lipid microspheres	Contrast
Pipamperone	N05AD05
Promethazine	D04AA10/R06AD02
Quetiapine	N05AH04
Ranolazine	C01EB18
Rilpivirine	J05AG05

Risperidone	N05AX08
Roxithromycin	J01FA06
Saquinavir	J05AE01
Sertindole	N05AE03
Sorafenib	L01XE05
Sunitinib	L01XE14
Tacrolimus	D11AH01/L04AD02
Tamoxifen	L02BA01
Telavancin	J01XA03
Telithromycin	J01FA15
Tetrabenazine	N07XX06
Tizanidine	M03BX02
Tolterodine	G04BA07
Toremifene	L02BA02
Vardenafil	G04BE09
Vemurafenib	L01XE15
Venlafaxine	N06AX16
Voriconazole	J02AC03
Vorinostat	L01XX38
Ziprasidone	N05AE04

Supplement 3. Number of durations missing as provided by the general practitioner per Anatomical Therapeutic Chemical code

Anatomical Therapeutic Chemical code	N total	N missing	% missing
A02BA03	30,784	4,478	14.5%
A03FA02	66,530	15,630	23.5%
A03FA03	425,254	78,479	18.5%
A04AA01	26,661	5,522	20.7%
A04AA02	4,420	589	13.3%
C01BA01	2,627	674	25.7%
C01BA02	86	0	0%
C01BA03	9,980	989	9.9%
C01BC04	117,620	8,721	7.4%
C01BD01	111,567	6,869	6.2%
C07AA07	442,604	36,934	8.3%
C08CA03	27,640	2,693	9.7%
C08CA04	7,172	854	11.9%
D04AA10	236	9	3.8%
D10AF02	214,257	127,553	59.5%
D11AH01	28,712	19,568	68.2%
G03XB01	6	2	33.3%
G04BD07	83,742	5,732	6.8%
G04BD12	90	0	0%
G04BE07	1,199	627	52.3%
G04BE09	9,188	3,618	39.4%
G04CA01	188,969	12,661	6.7%
H01BB02	11,741	6,231	53.1%
J01FA01	83,152	15,787	19.0%
J01FA06	20,959	3,444	16.4%
J01FA09	386,241	52,133	13.5%
J01FA10	495,730	52,136	10.5%
J01MA01	33,019	4,545	13.8%
J01MA06	168,394	19,856	11.8%
J01MA11	99	8	8.1%
J01MA12	18,856	2,101	11.1%
J01MA14	11,263	718	6.4%
J02AC03	814	94	11.5%
J05AE01	355	86	24.2%
J05AE08	2,464	63	2.6%
J05AG05	13	0	0%
L01XE05	125	11	8.8%
L01XE06	264	3	1.1%
L01XE07	25	4	16.0%
L01XE08	141	2	1.4%
L01XE11	37	2	5.4%
L01XE15	31	0	0%
L01XX32	12	9	75.0%
L01XX35	359	26	7.2%
L02BA01	63,046	3,351	5.3%
L04AA27	288	6	2.1%

L04AD02	22,277	1,314	5.9%
M03BX02	10,188	1,115	10.9%
N01BC01	21	19	90.5%
N03AX10	239	29	12.1%
N04BC07	242	177	73.1%
N05AA01	2,012	766	38.1%
N05AC02	11,573	3,227	27.9%
N05AD01	134,062	18,212	13.6%
N05AD05	91,740	16,399	17.9%
N05AD08	350	111	31.7%
N05AE03	380	93	24.5%
N05AG02	42,948	4,799	11.2%
N05AH02	75,527	11,431	15.1%
N05AH03	182,470	18,242	10.0%
N05AH04	228,539	19,451	8.5%
N05AL01	20,293	2,201	10.8%
N05AN01	162,168	15,731	9.7%
N05AX08	191,635	18,864	9.8%
N05AX12	35,971	810	2.3%
N05AX13	2,533	153	6.0%
N06AB04	542,917	32,682	6.0%
N06AB10	114,573	3,109	2.7%
N06AX11	375,163	42,612	11.4%
N06AX16	577,334	31,491	5.5%
N07BC02	54,327	8,735	16.1%
N07XX06	506	23	4.5%
P01BA01	4,479	1,391	31.1%
P01BX01	95	38	40.0%
P01CX01	136	53	39.0%
R06AD02	211,791	40,428	19.1%
R06AX11	4,050	1,265	31.2%
R06AX12	62,619	16,160	25.8%
S01AA17	347	167	48.1%
S01AA26	930	480	51.6%
S01AE01	60,497	27,832	46.0%
S01AE07	151	24	15.9%
S01HA01	43	41	95.3%

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Chapter 5.2

Drugs and ventricular repolarization

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Abstract

Background Prolonged ventricular repolarization (measured as heart-rate corrected QT (QTc) prolongation or JT-interval prolongation) is a risk factor for ventricular arrhythmias and can be drug-induced. Drugs can be classified as having known or possible QTc-prolonging properties. Regulatory agencies recommend avoiding concomitant use of multiple QTc-prolonging drugs, but evidence is lacking to what degree ventricular repolarization is influenced by concomitant use of these drugs.

Methods Within a population-based cohort of persons aged 45 years and older, with up to 5 electrocardiograms recorded per participant between 1991 and 2010, we used generalized estimating equations to study the association between concomitant use of multiple QTc-prolonging drugs and repolarization duration.

Results The study population consisted of 13,009 participants with 26,908 electrocardiograms. With the addition of a second or third QTc-prolonging drug there was no substantial increase in QTc and JT interval and no increased risk of a prolonged QTc interval, compared to use of 1 QTc-prolonging drug. There was a large difference between the effect of 1 known or 1 possible QTc-prolonging drugs on QTc interval: 15ms for known, and 3ms for possible QTc-prolonging drugs.

Conclusion In this study, the added prolongation in users of 2 or 3 QTc-prolonging drugs on QTc was small. There was a large difference in QTc prolongation between known and possible QTc-prolonging drugs. Further research in larger or high-risk populations is needed to establish whether it is safe to use multiple QTc-prolonging drugs concomitantly to prevent that the current advice might unnecessarily withhold beneficial drugs from patients.

Introduction

A prolonged heart-rate corrected QT (QTc) interval is a risk factor for ventricular arrhythmias[1-3], and has been associated with a three-fold increased risk of sudden cardiac death[4]. QTc prolongation can be congenital, caused by certain diseases such as coronary heart disease, or drug-induced[5]. Drugs that prolong QTc are mentioned in different lists, for example, the University of Arizona list[6], which distinguishes 3 categories. For drugs in the known category, a positive temporal concordance was documented between intake and QTc prolongation. For the possible category, less evidence is available. For the conditional category evidence is inadequate or contradictory[7].

We aimed to study 3 questions. First, since this classification does not indicate the degree of QTc prolongation in the different categories, we aimed to compare the degree of QTc prolongation between known and possible QTc-prolonging drugs as a class. Second, the QT interval consists of the QRS complex, representing ventricular depolarization, and the JT interval, representing ventricular repolarization. Recently, there has been a shift towards using JT instead of QTc interval, as this is thought to be a more specific risk factor for arrhythmias[8]. Therefore, we aimed to study the association with JT interval. Finally, regulatory agencies recommend avoiding concomitant use of multiple QTc-prolonging drugs[2,3], in order to prevent additional QTc prolongation and arrhythmias. However, there is no evidence that concomitant use is associated with additive QTc prolongation in the general population and therefore we aimed to determine the effect of concomitant use on repolarization duration.

Methods

The Rotterdam Study is a prospective population-based cohort study, which started in 1990, with periodic re-examinations, among 14,926 participants (response rate 72%) aged 45 years and older[9]. 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)". All participants provided written informed consent. The study population consisted of all participants with pharmacy data and at least 1 electrocardiogram (ECG) available.

Exposure to QTc-prolonging drugs was determined through pharmacy-dispensing data, available from January 1st 1991 onwards. We selected known (Supplement 1) and possible (Supplement 2) QTc-prolonging drugs[6]. Dispensing episodes were calculated by dividing the total number of tablets or capsules by the prescribed daily number. If the date of an ECG measurement fell within a dispensing episode of the selected drugs, the participant was considered exposed during that ECG recording.

Standard resting ECGs were recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) and processed by the Modular ECG Analysis System (MEANS)[10]. MEANS determines the QT from the start of the QRS complex until the end of the T wave with the use of template matching techniques, a method that has been validated extensively[11]. To correct for heart rate, Bazett's formula, QTc=QT/√RR, was used. A prolonged QTc was defined as an interval >450 milliseconds (ms) in men and >470ms in women[12]. JT interval was defined as QT-QRS interval.

Potential covariables were determined at the date of ECG recording. Body mass index was calculated as weight/height^2. Systolic and diastolic blood pressure were measured in sitting position at the right upper arm. Hypertension was defined as a systolic blood pressure >140mmHg, diastolic blood pressure >90mmHg, or use of blood-pressure lowering medication. Smoking status was assessed during an interview. Coronary heart disease was defined as a myocardial infarction or a coronary revascularization procedure[13]. Heart failure diagnosis included typical signs or symptoms confirmed by objective evidence of cardiac dysfunction[13]. Diabetes mellitus was defined as a fasting glucose >6.9mmol/l, a non-fasting glucose >11.0mmol/l, or the use of blood-glucose lowering medication. Heart rate in beats per minute was calculated as 60,000/RR interval in ms.

We analysed the association between QTc-prolonging drug use and QTc and JT interval as continuous variables, and a prolonged QTc as a dichotomous variable with generalized estimating equations (GEE) to correct for correlation between repeated measurements in 1 participant, using a varying between-measurement time-window, with non-use as reference. We used the independent working correlation matrix because it had the lowest quasi likelihood under independence model criterion[14]. We first analysed known and possible QTc-prolonging drugs combined, with 3 categories: nonuse, use of 1, and use of ≥2 QTc-prolonging drugs. Subsequently, we created 6 categories: nonuse, use of 1 possible, use of 2 or 3 possible, use of 1 known, use of 2 known, and concomitant use of known and possible QTc-prolonging drugs. Covariables which changed the effect estimate of the age and sex adjusted model with more than 10% were added to a multivariable model. Data were analysed using IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, USA).

Results

The study population consisted of 13,009 participants with 26,908 ECGs. Baseline characteristics are shown in Table 1. The distribution of ECGs over the different categories of exposures is shown in Figure 1 and Figure 2.

Table 1. Baseline characteristics of the study population

	Total study population n = 13,009
Total number of ECGs	26,908
Number of ECGs per participant	2 [1-5]
Age (years)	65.4±10.0
Women	7,550 (58%)
Body mass index (kg/m^2)	26.9±4.1
Height (cm)	168±10
Past smokers	5,701 (45%)
Current smokers	2,891 (22%)
QTc interval (milliseconds)	431±26
Heart rate (beats per minute)	70±12
Prevalent coronary heart disease	868 (7%)
Prevalent heart failure	322 (2%)
Prevalent diabetes mellitus	1,363 (10%)
Systolic blood pressure (mmHg)	139±22
Diastolic blood pressure (mmHg)	78±12
Hypertension	7,385 (57%)

Data presented as number (percentage), median [range], or mean±standard deviation
Abbreviations: ECG: electrocardiogram; n: number; QTc: heart-rate corrected QT interval according to Bazett's formula

Only heart rate contributed to the model as covariable. Figure 1A shows that with the addition of a second or third QTc-prolonging drug no substantial QTc prolongation occurs compared to use of 1 QTc-prolonging drug: 11.3ms (95%CI 9.5;13.1) in users of 1 and 12.4ms (95%CI 2.5;22.3) in users of ≥2 QTc-prolonging drugs. For JT interval comparable results were found (Figure 1B). There was no increased risk of a prolonged QTc interval (Figure 1C). When distinguishing known and possible QTc-prolonging drugs, use of known QTc-prolonging drugs was associated with a substantially larger increase in QTc interval than possible QTc-prolonging drugs: 15.4ms (95%CI 13.1;17.7 for 1 known and 3.3ms (95%CI 0.7;5.9) for 1 possible QTc-prolonging drug (Figure 2A). Results for JT interval and a prolonged QT interval were comparable (Figure 2B-2C). Only a small number of ECGs (n=68) were recorded during concomitant use of multiple QTc-prolonging drugs. Exposure to 2 known QTc-prolonging drugs was present at the time of 15 ECGs (Supplement 3). Concomitant use of multiple known drugs does not seem to be associated with an additional increase in the duration of these intervals compared to use of 1 drug (Figure 2A-2B). However, people using 2 known QTc-prolonging drugs had a higher risk estimate of a prolonged QTc interval compared to users of 1 known QTc-prolonging drug, however the confidence interval was wide given the low number of exposed ECGs (Figure 2C). The trends over the categories were statistically significant for all analyses ($p<0.001$).

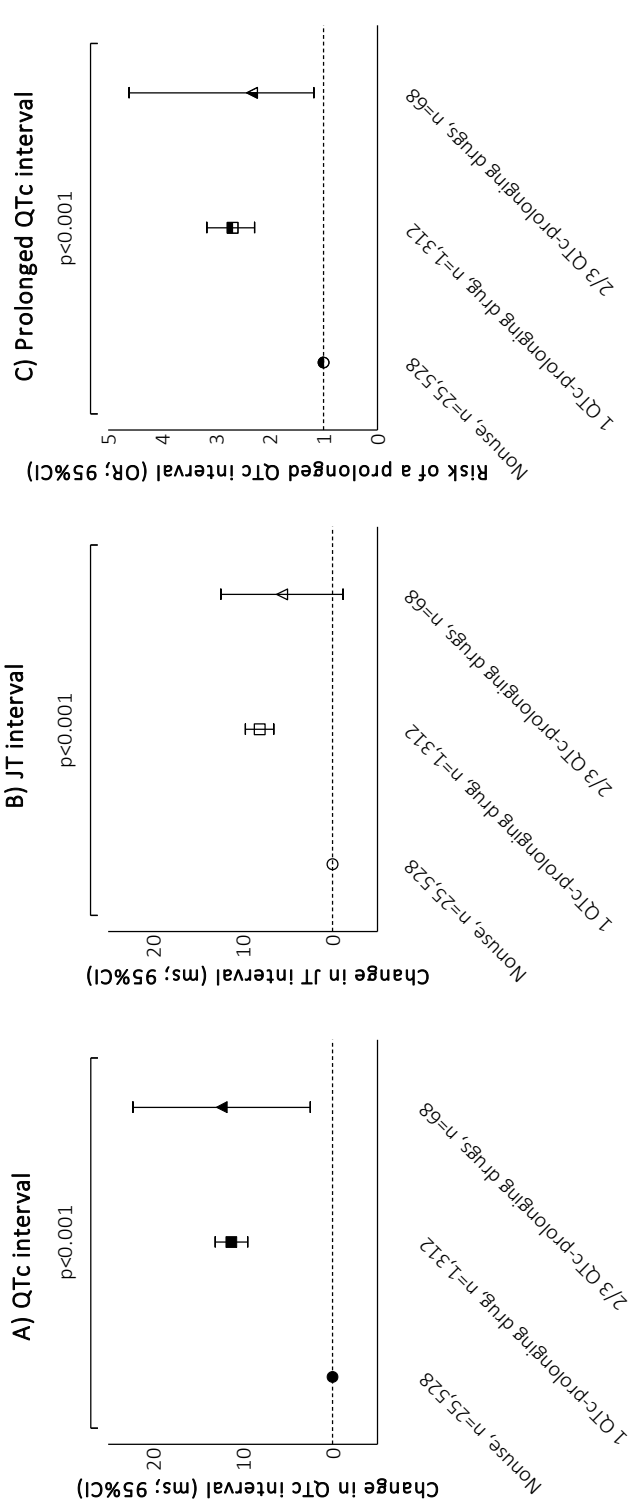

Figure 1. Association between the number of QTc-prolonging drugs used concomitantly and the QTc interval (A), JT interval (B), and a prolonged QTc interval (C)

Adjusted for age, sex and heart rate

Abbreviations: CI: confidence interval; ms: milliseconds; OR: odds ratio; p: p-value for trend; QTc: heart-rate corrected QT interval

A prolonged QTc interval was defined as a QTc above 450 milliseconds for men and above 470 milliseconds for women

Known: use of known QTc-prolonging drugs (Supplement 1)

Possible: use of possible QTc-prolonging drugs (Supplement 2)

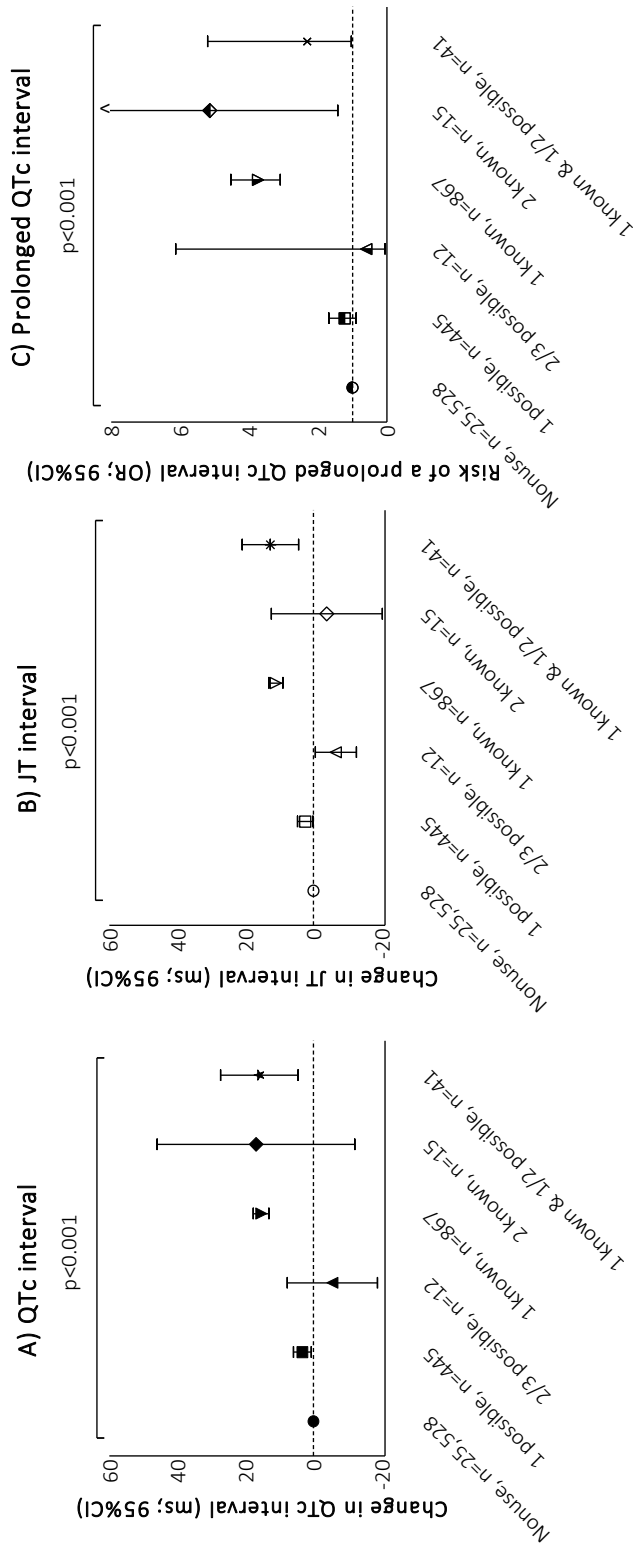

Figure 2. Association between the different categories of QTc-prolonging drug use and the QTc interval (A), JT interval (B), and a prolonged QTc interval (C)

Adjusted for age, sex and heart rate

Abbreviations: CI: confidence interval; ms: milliseconds; OR: odds ratio; p: p-value for trend; QTc: heart-rate corrected QT interval

A prolonged QTc interval was defined as a QTc above 450 milliseconds for men and above 470 milliseconds for women

Known: use of known QTc-prolonging drugs (Supplement 1)

Possible: use of possible QTc-prolonging drugs (Supplement 2)

Discussion

In this study, the added effect of a second or third QTc-prolonging drug on QTc-interval duration was small. Currently, it is recommended to avoid concomitant use of multiple QTc-prolonging drugs[2,3]. However, if the added effect of a second QTc-prolonging drug is only small and not associated with an increased risk of arrhythmias, beneficial drugs that are on this list could be withheld from patients unnecessarily if they already use another QTc-prolonging drug. Since our results are based on only 68 exposed ECGs, interpretation of these findings is limited and did not allow for assessment of arrhythmia risk. However, another study found comparable results for concomitant use of amiodarone and haloperidol in combination with other QTc-prolonging drugs in 16 patients admitted to a tertiary referral center[15].

Use of possible QTc-prolonging drugs was associated with a JT and QTc increase of only 2-3ms. Regulatory agencies consider an increase of less than 5ms in QTc interval as not relevant with regard to the risk of arrhythmias and sudden cardiac death[2,3]. As we found an increase of 3ms in users of possible QTc-prolonging drugs, this could indicate that these drugs are safe with regard to arrhythmias. However, this is an average effect of the drugs in this category and individual drugs could have larger QTc-prolonging effects. Given the large difference in QTc interval between known and possible QTc-prolonging drugs we cannot recommend to combine the 2 categories into 1 exposure class in studies on QTc-prolonging drugs. Since there has been a shift towards using JT interval for drug safety instead of using QTc, it is important to notice that use of QTc-prolonging drugs is also associated with JT-interval prolongation. There was no significant difference in JT intervals comparing use of 2 known QTc-prolonging drugs with nonuse of QTc-prolonging drugs. This may be partly explained by the small sample size, as in the analyses with possible and known QTc-prolonging drugs combined we see comparable effect estimates for 1 and for 2 or 3 QTc-prolonging drugs. However, to establish the role of JT interval further study is necessary.

Our study probably suffers from confounding by contraindication: people with a prolonged QTc will not get a prescription for a second QTc-prolonging drug, while persons with no substantial QTc prolongation are more likely to get a second drug. Therefore, we cannot state that concomitant use is not associated with QTc prolongation in general. This study might also suffer from confounding by indication: the underlying diseases for which the QTc-prolonging drugs are prescribed may be associated with ECG disturbances. However, we added the most important indications associated with QTc prolongation as potential covariables, which did not change the results. Another option would be to analyse the consecutive ECGs in one person, to study the association between initiating and stopping drug therapy and repolarization duration. However, the small number of consecutive ECGs in one person in the different categories limited the

possibility for this analysis. That a second known QTc-prolonging drug has almost no added effect on QTc interval, while it does increase the risk of a prolonged QTc, is probably due to the fact that with use of 1 QTc-prolonging drug, the QTc interval almost reaches the cut-off value of a prolonged QTc. With addition of a second drug a small increase pushes the QTc past this cut-off, and a person is considered to be at high risk of arrhythmias. However, it is questionable whether this small increase in QTc interval is clinically relevant.

In conclusion, this study shows that known and possible QTc-prolonging drugs as a class have a different effect on QTc and JT interval and it suggests that concomitant use of 2 QTc-prolonging drugs is not necessarily associated with a substantial increase in QTc interval compared to use of 1 such drug. Further research in larger or high-risk populations is needed to assess whether concomitant use of multiple QTc-prolonging drugs should be avoided in the entire population.

Supplements

Supplement 1. List of known QTc- prolonging drugs according to the list of the University of Arizona with Anatomical Therapeutic Chemical code and the number of exposed ECGs

Name	Anatomical Therapeutic Chemical code	Number of exposed ECGs
Amiodarone	C01BD01	143
Anagrelide	L01XX35	0
Arsenic trioxide	L01XX27	0
Astemizole	R06AX11	3
Azithromycin	J01FA10/S01AA26	2/0
Bepridil	C08EA02	0
Chloroquine	P01BA01	6
Chlorpromazine	N05AA01	0
Cisapride	A03FA02	113
Citalopram	N06AB04	33
Claritromycin	J01FA09	21
Cocaine	R02AD03/N01BC01/S01HA01	0/0/0
Disopyramide	C01BA03	27
Dofetilide	C01BD04	0
Domperidone	A03FA03	80
Dronedarone	C01BD07	0
Droperidol	N05AD08	0
Erytromycin	J01FA01/D10AF02/S01AA17	6/6/0
Escitalopram	N06AB10	4
Flecainide	C01BC04	113
Grepafloxacin	J01MA11	0
Halofantrine	P01BX01	0
Haloperidol	N05AD01	16
Ibutilide	C01BD05	0
Levoflaxacin	J01MA12/S01AE05	1/0
Levomethadyl	N07BC05 (levomethadone)	0
Mesoridazine	N05AC03	0
Methadone	N07BC02	0
Moxifloxacin	J01MA14/S01AE07	0/0
Ondansetron	A04AA01	2
Pentamidine	P01CX01 (pentamidine isethionate)	0
Pimozide	N05AG02	2
Probucol	C10AX02	0
Procainamide	C01BA02	0
Quinidine	C01BA01	10
Sevoflurane	N01AB08	0
Sotalol	C07AA07	319
Sparfloxacin	J01MA09	0
Sulpiride	N05AL01	1
Terfenadine	R06AX12	24
Thioridazine	N05AC02	6
Vandetanib	L01XE12	0

Supplement 2. List of possible QTc- prolonging drugs according to the list of the University of Arizona with Anatomical Therapeutic Chemical code and the number of exposed ECGs

Name	Anatomical Therapeutic Chemical code	Number of exposed ECGs
Alfuzosin	G04CA01	126
Apomorphine	N04BC07/G04BE07	0/0
Aripiprazole	N05AX12	0
Atazanavir	J05AE08	0
Bedaquiline	J04AK05	0
Bortezomib	L01XX32	0
Bosutinib	L01XE14	0
Clozapine	N05AH02	3
Crizotinib	L01XE16	0
Dabrafenib	L01XE23	0
Dasatinib	L01XE06	0
Dexmedetomidine	N05CM18	0
Dihydroartemisinin+piperaquine	P01BF05	0
Dolasetron	A04AA04	0
Eribulin	L01XX41	0
Famotidine	A02BA03	33
Felbamate	N03AX10	0
Fingolimod	L04AA27	0
Foscarnet	J05AD01	0
Fosphenytoin	N03AB05	0
Gatifloxacin	J01MA16/S01AE06	0/0
Gemifloxacin	J01MA15	0
Granisetron	A04AA02	0
Iloperidone	N05AX14	0
Isradipine	C08CA03	57
Lapatinib	L01XE07	0
Lithium	N05AN01	40
Mifepristone	G03XB01	0
Mirabegron	G04BD12	0
Mirtazipine	N06AX11	33
Moexipril/HCTZ	C09AA13	0
Nicardipine	C08CA04	19
Nilotinib	L01XE08	0
Norfloxacin	J01MA06/S01AE02	19/0
Ofloxacin	J01MA01/S01AE01/S02AA16	4/0/0
Olanzapine	N05AH03	5
Oxytocin	H01BB02	0
Paliperidone	N05AX13	0
Pasireotide	H01CB05	0
Pazopanib	L01XE11	0
Perflutren lipid microspheres	Contrast	0
Pipamperone	N05AD05	17
Promethazine	R06AD02/D04AA10	18/0
Quetiapine	N05AH04	5
Ranolazine	C01EB18	0

Rilpivirine	J05AG05	0
Risperidone	N05AX08	6
Roxithromycin	J01FA06	1
Saquinavir	J05AE01	0
Sertindole	N05AE03	0
Sorafenib	L01XE05	0
Sunitinib	L01XE14	0
Tacrolimus	L04AD02/D11AH01	0/0
Tamoxifen	L02BA01	52
Telavancin	J01XA03	0
Telithromycin	J01FA15	0
Tetrabenazine	N07XX06	0
Tizanidine	M03BX02	4
Tolterodine	G04BD07	26
Toremifene	L02BA02	0
Vardenafil	G04BE09	1
Vemurafenib	L01XE15	0
Venlafaxine	N06AX16	44
Voriconazole	J02AC03	0
Vorinostat	L01XX38	0
Ziprasidone	N05AE04	0

Supplement 3. Concomitant use of known QTc-prolonging drugs

Drug 1	Drug 2	Number of electrocardiograms		
		Total	Men	Women
Sotalol	Flecainide	3	2*	1
Sotalol	Citalopram	2	1	1
Sotalol	Cisapride	2	0	2*
Sotalol	Domperidone	1	0	1
Flecainide	Amiodarone	1	0	1
Flecainide	Citalopram	1	1	0
Amiodarone	Cisapride	2	0	2*
Amiodarone	Domperidone	1	1	0
Domperidone	Disopyramide	1	0	1
Escitalopram	Haloperidol	1	1	0

* Both electrocardiograms in the same person at a different measurement round

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Part VI

Pharmacogenetic risk factors

Chapter 6.1

Genetics of drug-induced QT interval prolongation

Maartje N Niemeijer, Marten E van den Berg, Mark Eijgelsheim, Peter R Rijnbeek, Bruno H Stricker

Abstract

A prolonged QT interval is an important risk factor for ventricular arrhythmias and sudden cardiac death. QT prolongation can be caused by drugs. There are multiple risk factors for drug-induced QT prolongation, including genetic variation. QT prolongation is one of the most common reasons for withdrawal of drugs from the market, despite the fact that these drugs may be beneficial for certain patients and not harmful in every patient. Identifying genetic variants associated with drug-induced QT prolongation might add to tailored pharmacotherapy and prevent beneficial drugs from being withdrawn unnecessarily. In this review, our objective was to provide an overview of the genetic background of drug-induced QT prolongation, distinguishing pharmacokinetic and pharmacodynamic pathways. Pharmacokinetic-mediated genetic susceptibility is mainly characterized by variation in genes encoding drug-metabolizing cytochrome P450 enzymes or drug transporters. For instance, the P-glycoprotein drug transporter plays a role in the pharmacokinetic susceptibility of drug-induced QT prolongation. The pharmacodynamic component of genetic susceptibility is mainly characterized by genes known to be associated with QT interval duration in the general population and genes in which the causal mutations of congenital long QT syndromes are located. Ethnicity influences susceptibility to drug-induced QT interval prolongation, with Caucasians being more sensitive than other ethnicities. Research on the association between pharmacogenetic interactions and clinical endpoints such as sudden cardiac death is still limited. Future studies in this area could enable us to determine the risk of arrhythmias more adequately in clinical practice.

Introduction

The QT interval is measured on the electrocardiogram (ECG) and represents the ventricular depolarization and repolarization. The QT interval is usually corrected for heart rate, often using the formula of Bazett, as heart rate influences the duration of the QT interval. Normally, the average heart-rate corrected QT interval is approximately 410-430 milliseconds (ms) for adult men and 420-430ms for adult women[1]. A prolonged heart-rate corrected QT interval is usually defined as above 450ms in men, and above 470ms in women[2]. A prolonged QT interval is a well-known risk factor for ventricular arrhythmias and sudden cardiac death[3-6], and can be congenital or acquired[7,8]. Congenital QT prolongation is caused by mendelian genetic disorders, called the long QT syndromes. The long QT syndromes can be divided into subtypes according to the gene in which the causal mutation is located[9]. Acquired QT prolongation can be caused by cardiac diseases, such as coronary heart disease and heart failure, but it is usually caused by certain drugs[8,10]. The susceptibility to acquired QT interval prolongation can also be influenced by genetic variation[9]. Two things point to this: first, the heritability of QT interval duration in the general population (excluding congenital long QT syndrome patients) is estimated to be around 35%[11,12], and second, first-degree relatives of patients with congenital long QT syndrome have a higher risk of drug-induced QT prolongation than non-related individuals[13]. In genome-wide association studies (GWAS), a great number of genes associated with QT interval duration have been identified[14-16]. The gene with the strongest signal related to QT interval duration is the nitric oxide synthase 1 adaptor protein gene (*NOS1AP*), located on chromosome 1[14-20], and is influencing impulse propagation[21]. Other findings from GWAS included polymorphisms within genes known to be mutated in congenital long QT syndromes, genes associated with intracellular calcium handling, as well as genes previously not known to influence cardiac repolarization[9,15,16]. Because drug use is the most common cause of acquired QT prolongation[7], QT interval prolongation is an important outcome in drug-safety research[5,6]. In drug-safety studies, QT interval prolongation is often combined with torsade de pointes, a rare ventricular arrhythmia associated with QT prolongation. Drugs can lead to a relatively high frequency of torsade de pointes, of up to 5%[22]. QT interval prolongation is one of the leading causes of drug relabeling or withdrawal of drugs from the market[23,24]. Drugs that are associated with either QT prolongation or torsade de pointes are mentioned in various lists, such as the University of Arizona list[25,26]. These lists include antiarrhythmic drugs, such as flecainide and amiodarone, but also non-cardiac drugs[25]. These non-cardiac drugs are a diverse set with various indications and working mechanisms and include antidepressant drugs (e.g. citalopram), antibiotics (e.g. erythromycin and fluoroquinolones), and drugs against nausea (e.g. domperidone)[25]. Regulatory authorities state that these drugs should not be administered to patients already using another drug with QT-prolonging properties or to patients who have an increased risk of QT prolongation[5,6].

As QT interval duration is influenced by genetic variants, the individual risk of drug-induced QT interval prolongation may also be influenced by genetic factors. Pharmacogenetics describes the role of genetic variation in drug response, which can relate to both pharmacokinetic and pharmacodynamic properties. Pharmacokinetics constitute the effect of the body on the drug, which is usually categorized into effects on absorption, distribution, metabolism, and elimination of the drug. Pharmacodynamics reflect the effect of the drug, in a certain concentration, on the body. Both pharmacokinetics and pharmacodynamics influence efficacy and effectiveness of the drug, and the occurrence of adverse drug reactions. As genotyping has become less expensive over the past years, larger populations can be investigated and more and more variants determining susceptibility to drug-induced QT interval prolongation could possibly be detected. This might bring us closer to the goal of tailored pharmacotherapy[27]. Therefore, our objective was to provide an overview of the pharmacogenetics of drug-induced QT interval prolongation. Hereto, we distinguished pharmacokinetic and pharmacodynamic genetic susceptibility and ethnic differences in QT interval prolongation. We searched PubMed from inception until April 8th 2015 using the search terms: (pharmaco)genetic, (pharmaco)genomic, QT/QTc, prolong*, long, drug-induced, drug related, drug, medication, medicine, ethnic, race. We applied a restriction for articles in the English language.

An overview of the pharmacogenetics discussed in this review is shown in Figure 1 and an overview of the knowledge on ethnic differences in susceptibility to QT prolongation is given in Figure 2.

Pharmacokinetic genetic susceptibility

A description of the pharmacokinetics of all QT-prolonging drugs and interactions with other drugs is beyond the scope of this review. We focused on the influence of genes related to pharmacokinetics on drug-induced QT interval prolongation.

Cytochrome P450 substrates

Many drugs are metabolized in the liver by cytochrome P450 (*CYP*), a family of enzymes. These enzymes are encoded by various genes named according to their family (e.g. "*2*") and subfamily (e.g. "*C*"), based on the amino acid structure. A full code indicates a specific gene (e.g. *CYP2C9)*. A large proportion of the QT-prolonging drugs are metabolized by *CYP2D6*, *CYP3A4/5*, and *CYP2C9*[28]. Several cytochrome P450 genes have been investigated in relation to drug-induced QT interval prolongation.

CYP2D6 is the most extensively studied gene of the *CYP* family in relation to drug-induced QT prolongation. Genetic variation within *CYP2D6* was associated with QT prolongation in users of risperidone and iloperidone[29-31]. However, in users of haloperidol and thioridazine, *CYP2D6*

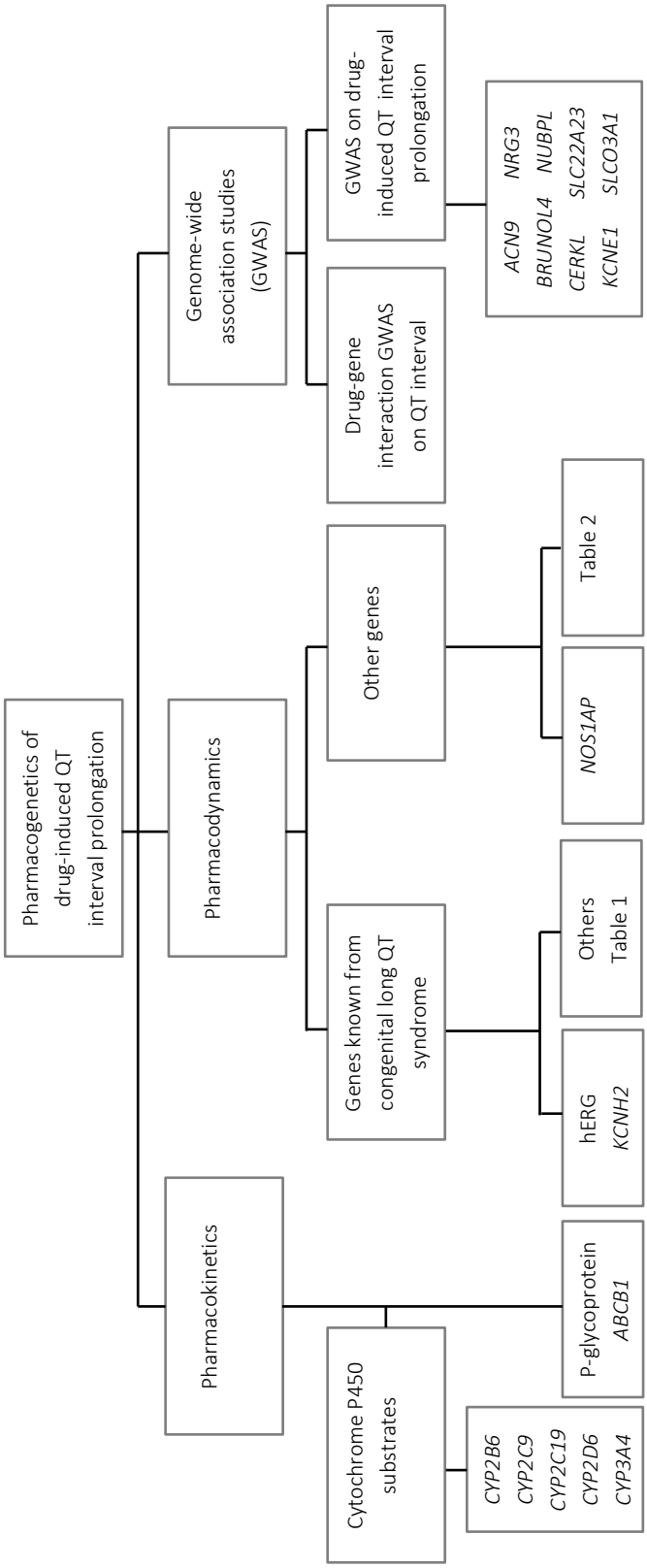

Figure 1. Overview of the genes associated with drug-induced QT interval prolongation

status was not associated with QT prolongation[32-35]. The proportion of poor metabolizers was not different among users of the antispasmodic terodiline surviving ventricular tachycardia or torsade de pointes than in the total population[36].

Another often studied gene of the *CYP* family is *CYP2C19*. *CYP2C19* status was a predisposing factor for QT prolongation in antimicrobial (except quinolones) users[37] and a higher proportion of poor metabolizers was found among terodiline users who survived ventricular tachycardia or torsade de pointes compared with the normal population[36]. *CYP2C19* status did not influence QT interval in users of nelfinavir or thioridazine[35,38].

Some other *CYP* family members were investigated. Methadone users who were *CYP2B6* poor metabolizers had an increased QT interval duration and a 4.5-fold increased risk of a prolonged QT interval compared with methadone users who were normal or extensive metabolizers[39]. *CYP2C9* was not associated with QT prolongation in risperidone users[29]. *CYP3A4* and *CYP2C9* variants predisposed to an increased risk of QT prolongation or torsade de pointes induced by antimicrobial agents, with the exception of quinolones[37].

P-glycoprotein

P-glycoprotein is a transmembrane efflux pump and influences bioavailability of several drugs. P-glycoprotein is encoded by the ATP-binding cassette B1 gene (*ABCB1*)[40]. The 3 most frequently investigated polymorphisms within this gene are C1236T, G2677T, and C3435T[41-43]. In a study in 66 schizophrenia patients using risperidone, the QT interval was significantly longer in patients carrying a T allele of the C3435T polymorphism than homozygous C allele carriers[44]. A study on romidepsin, a potentially QT-prolonging anticancer drug, showed that mice lacking P-glycoprotein had higher intracardiac concentrations of the drug. This study also showed that carriers of genetic variants in *ABCB1* have less QT interval prolongation following a dose of romidepsin[45]. Thus, P-glycoprotein might also be an effect modifier of other potential QT-prolonging drugs.

Pharmacodynamic genetic susceptibility

Genes known from congenital long QT syndrome

Five to nineteen percent of patients with drug-induced torsade de pointes carry mutations in genes involved in congenital long QT syndrome[46,47]. These genes are listed in Table 1, which also shows the number of studies that did or did not find an association between these genes and drug-induced QT interval prolongation or torsade de pointes[31,46-57]. Since the human ether-a-go-go (*eag*)-related gene (*hERG*) channel and its associated gene, the potassium channel, voltage gated *eag*-related subfamily H, member 2 gene (*KCNH2*), were investigated most frequently and are currently a topic of special interest in drug safety research, it will be discussed separately.

Table 1. Genes known from congenital long QT syndrome, investigated in drug-induced long QT syndrome or torsade de pointes

cLQTS type	Gene abbreviation	Gene name[58]	Gene protein product[9]	Association found*	
				Yes	No
cLQTS1	*KCNQ1*	Potassium channel, voltage gated KQT-like subfamily Q, member 1 gene	IKs channel alpha-subunit	3	4
cLQTS2	*KCNH2*	Potassium channel, voltage gated eag-related subfamily H, member 2	IKr channel alpha-subunit	9	4
cLQTS3	*SCN5A*	Sodium channel, voltage gated, type V alpha-subunit	INa channel alpha-subunit	3	4
cLQTS4	*ANK2*	Ankyrin 2, neuronal		1	1
cLQTS5	*KCNE1*	Potassium channel, voltage gated subfamily E regulatory beta-subunit 1	IKs channel beta-subunit	4	3
cLQTS6	*KCNE2*	Potassium channel, voltage gated subfamily E regulatory beta-subunit 2	IKr channel beta-subunit	2	6
cLQTS7	*KCNJ2*	Potassium channel, inwardly rectifying subfamily J, member 2	IKl alpha-subunit	0	3
cLQTS8	*CACNA1C*	Calcium channel, voltage-dependent, L type, alpha-1C subunit	ICaL channel alpha-subunit	2	1
cLQTS9	*CAV3*	Caveolin-3		0	2
cLQTS10	*SCN4B*	Sodium channel, voltage gated, type IV beta-subunit	INa channel beta-subunit 4	1	1
cLQTS11	*AKAP9*	A kinase anchor protein 9		1	2
cLQTS12	*SNTA1*	Syntrophin, acidic 1		1	1
cLQTS13	*KCNJ5*	Potassium channel, inwardly rectifying subfamily J, member 5	IKACh channel alpha-subunit	0	1

***** Number of studies that did or did not find an association with the corresponding gene
Abbreviation: cLQTS: congenital long QT syndrome

hERG channel encoded by KCNH2

The *hERG* channel has received much attention in the past decades. In congenital long QT syndrome type 2, the causal mutation is located in the *KCNH2* gene, which encodes this channel[59]. *KCNH2* encodes the α subunit of the channel mediating the rapidly activating component of the delayed rectifier K^+ (Ikr) current[60]. The *hERG* channel can be blocked by drugs in two different ways: (i) direct blocking of the channel; (ii) indirect blockade by disrupting channel protein trafficking through a reduction of the number of channels in the membrane[59,61]. The resulting inhibition of the Ikr current in turn leads to an excessive lengthening of the action potential. This can lead to early afterdepolarizations and subsequently to arrhythmias and sudden cardiac death[62]. Almost all drugs that have been associated with torsade de pointes or QT prolongation block the Ikr current, but not all Ikr-blocking drugs have QT-prolonging properties[63]. In a study of 14 antipsychotics, a significant correlation was reported between the estimated *hERG* blockade and the increase in QT interval for 5 Ikr-blocking drugs (haloperidol,

olanzapine, risperidone, thioridazine, and ziprasidone)[64]. Several studies identified mutations in the *KCNH2* gene in persons with drug-induced prolonged QT interval or torsade de pointes, which were not present in controls[46,47,50,52,65]. These were *KCNH2* mutations not related to congenital long QT syndrome type 2. Several *KCNH2* mutations were identified in persons with drug-induced arrhythmias in a database of a spontaneous reporting system for adverse drug reactions[49]. However, in one study, persons with drug-induced torsade de pointes had a similar proportion of *hERG* mutations as controls[54] and three studies did not find an association between *KCNH2* and drug-induced QT prolongation[48,51,53]. In a study on dofetilide-induced *hERG* blockade, it was reported that a blockade of 10% was associated with a 20ms (95% confidence interval [CI] 12;32) increase in QT interval[66]. A study in methadone users showed that persons with a *KCNH2* mutation had a 15ms (95%CI 5;26) longer QT interval[57].

Other genes known from congenital long QT syndrome

Several studies have assessed genes involved in congenital long QT syndrome, to identify variants, other than the causal ones in congenital long QT syndrome, that are associated with drug-induced QT interval prolongation. The design of these studies is very heterogeneous[31,46-57,61]. For example, studies included methadone users[57], cases of drug-induced arrhythmias from a database of spontaneous reports of adverse drug reactions[49], and a case report[51]. Results from these studies are ambiguous, as not one gene has consistently been associated with drug-induced QT prolongation. The fact that the study populations were small, hampers the possibility to draw firm conclusions.

Other genes having pharmacodynamic effects

NOS1AP

The *NOS1AP* gene encodes the nitric oxide synthase 1 activating protein and regulates the enzyme neuronal nitric oxide synthase (*nNOS*), a regulator of intracellular calcium levels and cardiomyocyte contraction[67,68]. *nNOS* inhibits L-type calcium channels and influences cardiac repolarization[69] and impulse propagation[21]. Previous studies have shown that *NOS1AP* is associated with QT interval duration[14-16,70], and common variants within this gene have been associated with an increased risk of QT prolongation, induced by amiodarone, sotalol or diuretics[71]. As the *NOS1AP* gene is associated with inhibition of L-type calcium channels, which are blocked by certain calcium channel blockers (i.e. diltiazem and verapamil) it can be expected that this gene modifies response to these drugs. Two variants within the *NOS1AP* gene were associated with a modified effect of the drug on QT interval duration in users of verapamil, and to a lesser degree, diltiazem[72]. *NOS1AP* has also been associated with antipsychotic-induced QT prolongation[48] and drug-induced torsade de pointes[56]. Heterozygous and homozygous major allele carriers of the rs10494366 variant had an increased risk of a prolonged QT interval

following granisetron or dolasetron administration in the perioperative setting, compared with homozygous minor allele carriers[73].

Table 2. Genes investigated in candidate-gene studies on drug-induced QT prolongation

Gene abbreviation	Gene name[58]	Association found*
AKAP6	A kinase anchor protein 6	Yes[52]
AKAP7	A kinase anchor protein 7	Yes[52]
APLP2	Amyloid beta precursor-like protein 2	Yes[52]
ATP1B1	ATPase, Na^+/K^+ transporting, beta 1 polypeptide	No[48]
ATP2A2	ATPase, CA^{++} transporting, cardiac muscle, slow twitch 2	Yes[52]
-	Beta-adrenergic receptor genes	No[74]
BRUNOL4	Bruno-like 4	No[48]
CACNB2	Calcium channel, voltage-dependent, beta 2 subunit	Yes[52]/ No[53]
CALR	Calreticulin	Yes[52]
CASQ2	Calsequestrin 2	Yes[56]
CERKL	Ceramide kinase-like	No[48]
CNOT1	CCR4-NOT transcription complex, subunit 1	No[48]
FKBP1B	FK506 binding protein 1b	No[56]
GINS3	GINS complex subunit 3	No[48]
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like	Yes[52]/ No[53,56]
JPH2	Junctophilin 2	Yes[52]
JPH3	Junctophilin 3	Yes[52]
KCND3	Potassium channel, voltage gated Shal related subfamily D, member 3	Yes[52]
KCNN3	Potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 3	Yes[52]
LIG3	Ligase III, DNA, ATP-dependent	No[48]
LITAF	Lipopolysaccharide-induced TNF factor	No[48]
NDRG4	NDRG family member 4	No[48]
NRG3	Neuregulin 3	No[48]
NUBPL	Nucleotide-binding protein-like	Yes[48]
PALLD	Palladin, cytoskeletal associated protein	No[48]
PLN	Phospholabam	No[48]
PPP2R3A	Protein phosphatase 2, regulatory subunit beta, alpha	Yes[52]
RNF207	Ring finger protein 207	No[48]
RYR2	Ryanodine receptor 2	Yes[52,56]/ No[53]
SCN1B	Sodium channel, voltage gated, type I beta subunit	Yes[56]/ No[53]
SCN4A	Sodium channel, voltage gated, type IV alpha subunit	Yes[56]
SETD6	SET domain containing 6	No[48]
SLC35F1	Solute carrier family 35, member F1	No[48]
SLCO3A1	Solute carrier organic anion transporter family, member 3A1	No[48]
ZFHX3	Zinc finger homeobox 3	Yes[52]

* Association, enrichment or predictive value detected for this gene

Other candidate-gene studies

Several other genes with suspected pharmacodynamic effects were investigated for their association with drug-induced QT prolongation or drug-induced torsade de pointes. Selection of these genes was based on known association with arrhythmias or cardiac diseases or because of a suggestive or significant signal in a GWAS. The results of the candidate-gene studies are summarized in Table 2[48,52,53,56,74].

Genome-wide association studies (GWAS) on drug-induced QT interval prolongation

In GWAS, associations are studied between a phenotype and an extensive set of polymorphisms throughout the whole genome. Generally, only a subset of all polymorphisms of a person are genotyped, after which the other polymorphisms are imputed with a reference panel. The most frequently used reference panels are HapMap[75,76] and 1000 Genomes[77-79]. HapMap contains around 2.5 million common polymorphisms, 1000 Genomes contains around 30 million polymorphisms and includes both common and rare variants. The choice of reference panel can influence the number of findings and whether rare variants can be identified. We describe this type of research separately, because both pharmacokinetic and pharmacodynamic polymorphisms can be detected with GWAS. We also describe a whole-exome sequencing study, which is technically not a GWAS, but has a similar aim and methodology.

There are 2 possible ways to investigate the genetic basis of drug-induced QT prolongation in the framework of GWAS[80]. The first is to investigate the association between the polymorphism and a drug-induced phenotype. The phenotype can be either a dichotomous clinically relevant endpoint (e.g. a heart-rate corrected QT interval above 450ms in men and above 470ms in women)[2] or a continuous variable (e.g. the delta QT in ms before and after exposure)[2]. The second is to perform drug-gene interaction GWAS. In these analyses, the interactions between the use of certain drugs and all genetic polymorphisms available in the reference panel are investigated in relation to QT interval as continuous outcome or a prolonged QT interval or torsade de pointes as dichotomous outcome. However, for drug-gene interaction GWAS, very large study populations are required[80]. Moreover, because multiplicative models are tested, biologically relevant interactions, which are usually additive, may be missed.

GWAS on drug-induced QT interval prolongation

A GWAS in 216 torsade de pointes cases caused by amiodarone, sotalol, and quinidine and 771 controls, using both HapMap and 1000 Genomes imputed data, did not show genome-wide significant associations[81]. In another GWAS in 738 subjects included in a randomized clinical trial using approximately 600,000 polymorphisms, an association was found between the solute

carrier family 22 member 23 gene (*SLC22A23*) and antipsychotic-induced (specifically quetiapine) QT prolongation[48]. *SLCO3A1*, another member of this family, was associated with QT interval prolongation induced by the antipsychotic iloperidone in 183 subjects included in a randomized trial[82]. This solute carrier family encodes organic ion transporters that shuttle drugs across the cell membrane and are expressed in cardiac tissue[48,82]. Five other genes were identified in the GWAS on iloperidone-induced QT prolongation using about 300,000 polymorphisms. These included the ceramide kinase-like gene (*CERKL*), the palladin, cytoskeletal associated protein gene (*PALLD*), the bruno-like 4 gene (*BRUNOL4*), the neuregulin 3 gene (*NRG3*), and the nucleotide-binding protein-like gene (*NUBPL*)[82]. *CERKL* regulates currents of various potassium channels, including the *hERG* channel. *PALLD* has been associated with myocardial infarction. *BRUNOL4* is expressed in cardiac tissue and has been associated with cardiac structure and function. *NRG3* has been found to play a role in cardiac development. The role of *NUBPL* is unclear[82]. Of these 6 genes, only the association between *NUBPL* and drug-induced QT prolongation could be replicated in another study[48]. A whole-exome sequencing study in 65 cases and 148 controls revealed 2 genes, *KCNE1* and *ACN9*, associated with antiarrhythmic and antipsychotic drug-induced QT interval prolongation[53].

Drug-gene interaction GWAS

To our knowledge, there is only 1 drug-gene interaction GWAS on QT interval duration. This study investigated the interaction between genome-wide genetic variation using HapMap imputed data and four types of exposure (thiazide diuretics, tri/tetracyclic antidepressants, sulfonylurea hypoglycemic agents, and QT-prolonging drugs) in 33,781 participants from 10 cohort studies. It did not show a genome-wide significant association with QT interval. There were also no significant interactions with 26 polymorphisms previously reported to be related to QT interval duration[83].

Ethnic differences in susceptibility to drug-induced QT interval prolongation

As ethnic differences ultimately reflect genetic variation, it is useful to study ethnicity with regard to susceptibility to drug-induced QT interval prolongation. However, the role of ethnic differences has not been well established in studies on drug-induced QT interval prolongation. In 20 thorough QT/QTc studies, only 10% of the total study populations was African American and only 7% was Asian[84]. We will discuss studies that have investigated the association between ethnicity and QT interval duration, ethnicity and genes associated with QT interval, the influence of ethnicity on associations between drugs and QT interval, and the influence of ethnicity on drug-gene interaction associated with QT interval. This is summarized in Figure 2.

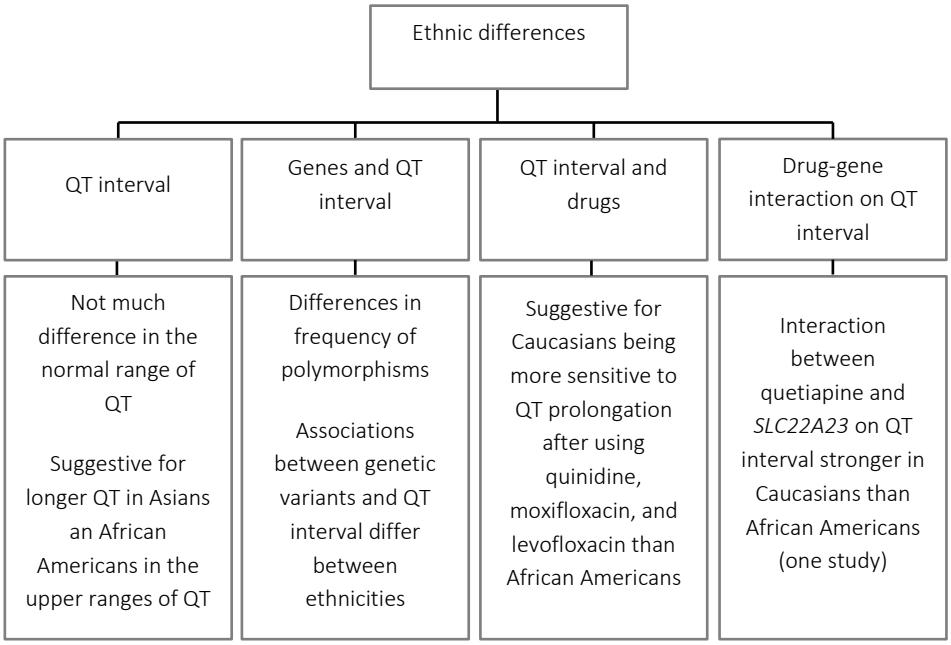

Figure 2. Overview of the available knowledge on ethnic differences in susceptibility to drug-induced QT interval prolongation

Ethnic differences in QT interval duration

Data on baseline differences of QT interval between different ethnicities is sparse. The available data indicates that there are no large differences in the normal range[24]. A difference of 6-7ms was demonstrated for Asian women in the normal range of the QT interval compared with other ethnicities, and this was a difference of 10ms in the upper range of the QT interval[85]. Among 41 African Americans and 3,456 Caucasians with a prolonged QT interval, African Americans had a 29ms longer heart-rate corrected QT interval[86].

Ethnic differences in genetic variation associated with QT interval

The frequency of polymorphisms in genes known from congenital long QT syndrome had varying distributions among ethnic groups[87-90]. A study on *SCN5A* showed that out of the 39 variants found, 20 were only found in African Americans, 7 only in Caucasians, 4 only in Asians, and 3 only in Hispanics. Only 1 polymorphisms was found in all 4 ethnic groups[87,89]. Of the 49 polymorphisms identified in *KCNQ1*, *KCNH2*, *KCNE1*, and *KCNE2*, 27 were only found in African Americans, 13 only in Caucasians, 2 only in Asians, and 2 only in Hispanics. Only 2 polymorphisms were identified in all 4 ethnic groups[88,90]. A study on *KCNH2* in 100 African Americans and 100 Caucasians, showed that the minor alleles of 2 polymorphisms were more frequent among Caucasians than among African Americans[90]. These studies only described the

frequency of variants, but not a potentially causal role of these genes with regard to QT interval prolongation.

Contradictory results were published on the effect of genetic variants on QT between different ethnicities. Among Caucasians more variants of 28 tagging SNPs of the *NOS1AP* gene were significantly associated with QT interval than in African Americans, Hispanics, and Chinese[91]. However, other studies reported comparable associations between *NOS1AP* gene variants and QT interval among Caucasians, Hispanics, and African Americans[92,93], but also among African Americans of both European and African ancestry[94]. Comparable associations in different ethnic groups were also reported between genetic variants of *PLN1*, *KCNQ1*, *NDRG4*, and *ATP1B1* and QT interval[93,94]. However, 1 study identified variants within *NOS1AP* and *ATP1B1* among African Americans that had not been reported in Caucasians[93].

Ethnic differences in drug response with regard to QT prolongation

African Americans had the highest risk of a prolonged QT interval after acute overdose of QT-prolonging drugs (odds ratio [OR] 2.01; 95%CI 1.03;3.91) while Hispanics had the lowest risk (OR 0.49; 95%CI 0.26;0.92) compared to all other ethnicities[95]. For the other ethnicities no statistically significant differences were observed[95]. Another study showed that Caucasians using quinidine, a powerful QT-prolonging drug, were more sensitive to QT prolongation than African Americans, even though baseline values of the QT interval were comparable[96,97]. Moxifloxacin is often used as positive control in thorough QT/QTc studies and is a moderately powerful QT-prolonging drug. Studies showed that there were no ethnic differences in QT prolongation following moxifloxacin and levofloxacin administration[98-101], however the trend suggested that Caucasians may be more sensitive to QT prolongation than Asians[101].

Ethnic differences in drug-gene interaction on QT interval

One study compared the interaction between quetiapine use and polymorphisms of the *SLC22A23* gene on QT interval and found a stronger effect of this drug-gene interaction in Caucasians than in African Americans[48].

Discussion and conclusion

Nowadays, QT interval prolongation is one of the most common reasons to withdraw drugs from the market. Many risk factors for QT interval prolongation and associated arrhythmias have been identified, including both non-genetic and genetic risk factors[3,9,10]. As QT-prolonging effects might only occur in people with certain genetic variations[24], drugs can be completely withdrawn from the market, while they could be safe and beneficial for certain patients. With more knowledge on the genetic variation associated with drug-induced QT interval prolongation, we might be able to identify which patients would benefit, and which patients

would be at risk, and subsequently not have to withdraw drugs with QT-prolonging properties from the market completely[102]. Pharmacovigilance studies focus on drugs that have come onto the market and thus do not have extreme QT-prolonging properties. Therefore, we mostly detect genetic variants with small effects. However, studies into the genetic variation associated with extreme or frequent adverse effects in early-phase studies could tell us more about genetic variants with large effects. Genetic variants associated with large adverse drug effects might be an important aid in risk stratification, give insight into the genes and proteins relevant for drug-induced QT interval prolongation and arrhythmias, or ultimately result in genetic targeted marketing (in accordance with cancer pharmacogenetics) if the effect is fully dependent on a genotype. However, other independent risk factors besides genetic variants play a role in the development of a prolonged QT interval, which are important for risk stratification in individual patients.

GWAS have been instrumental in the discovery of a wide range of associations between genetic variants and many different phenotypes. However, gene-environment interaction GWAS are challenging, because of the large sample size that is required for these kind of studies[80]. Therefore, in the field of pharmacogenetics, candidate-gene studies are probably more feasible. These studies are used to elaborate on the possible mechanisms through which drug-induced QT prolongation occurs. They may also shed light on the large interpersonal variability in drug response and therefore add to safer use of many drugs. However, GWAS will remain an important tool for hypothesis generation. Power can be gained by improving the outcome and exposure definition, leading to a reduction in misclassification. Nowadays, QT-prolonging drugs are investigated as one exposure group, often because of the low number of exposed persons; for example, in the drug-gene interaction GWAS by Avery et al[83]. If the pathways through which these drugs cause QT prolongation comprise the same genetic variants, this approach works. However, if the working mechanisms are heterogeneous, exposure is misclassified and this will reduce power to detect meaningful variants.

Future research could focus on identifying which polymorphisms within a gene have the best predictive value for adverse effects, the so-called fine mapping. With the emergence of whole-genome sequencing and whole-exome sequencing in large cohort studies[103], we will be able to study genotypes in more detail and we will have data available on more rare variants[103]. This might enable us to discover more polymorphisms associated with adverse drug reactions, which might also increase the predictive value of polymorphisms that are important in pharmacogenetic interactions. The increasing availability of expression and methylation data in cohort studies might enable us to determine epigenetic variants influencing the occurrence of disease. Expression and methylation variants might also be associated with the age at which QT prolongation occurs.

Future pharmacogenetic research should also focus on direct clinical endpoints, such as arrhythmias, overall mortality, and sudden cardiac death. This will tell us more about the direct value of pharmacogenetic interactions for daily clinical practice. A research method which is currently emerging is the molecular autopsy[104,105]. In cases of sudden death, without obvious causes identified with autopsy, the pathologist can only assume that death had an arrhythmogenic origin[104]. In these cases, genotyping family members or the deceased can shed light on the genetic variation which might be the underlying factor that caused the arrhythmia and death[104]. This research method could provide more insight into genetic variations associated with sudden deaths.

Concerns about the value of the QT interval in the prediction of drug-induced arrhythmias and sudden cardiac death have been highlighted in recent years, because some drugs are associated with QT prolongation but not with an increased risk of arrhythmias[106,107]. In addition to identifying genetic variation contributing to drug-induced QT prolongation, we should also focus on identifying other measurable markers, such as heart-rate variability[108] and QT variability[109], and their genetic determinants of drug-induced arrhythmia in order to improve risk prediction.

Since ethnic differences might be important for individual-specific risk of drug-induced QT prolongation, more research is needed to establish which drugs can be used safely in the different ethnic groups. The studies mentioned thus far suggest that Caucasians are more sensitive to drug-induced QT interval prolongation than other ethnicities. However, studies do not provide consistent results, possibly because other risk factors besides genetic variants play an important role in QT interval prolongation. Shah hypothesizes that given the fact that the differences between ethnicities in these studies are generally small, ethnic differences are probably relatively most important in users of mild QT-prolonging drugs[24]. If a drug has strong QT-prolonging properties in general, it is not likely that the small difference related to ethnicity will make much difference. If a drug has no QT-prolonging properties in general, it is not likely that it will be a cause of strong QT prolongation in one ethnic group. However, if a drug has moderate QT-prolonging properties, the small differences could mean that a drug can be used safely in some ethnic groups, but not in others, because the degree of QT prolongation is either just above or just below the safety cut-off value[24]. The Food and Drug Administration considers an increase below 5ms as not clinically relevant[6]. If, for example, a drug prolongs the QT interval with 3ms in one ethnicity, while it causes an extra 3ms increase in another ethnicity, the added prolongation is small, but it will be just above the safety cut-off value, meaning that the drug should be contraindicated in one ethnicity, but not in the other. One might question whether such a far-reaching consequence would be justified considering the limited amount of available information. In addition, within each ethnic group, genetic modifiers can be different and pharmacogenetic studies should therefore not be limited to Caucasians only.

As genotyping has become less expensive over the last couple of years, genotyping patients for genetic variants that influence their risk of adverse drug effects has become more feasible. This will allow us to investigate larger populations, enabling us to find more, and also rare, genetic variants. If we know the genetic profile of a patient with regard to adverse drug effects, we might be able to prescribe drugs more safely. This would also allow us to be more accurate in the dosage that a patient needs[102]. However, clinical validity has to be tested for specific drugs, as this has often been done insufficiently to date[110]. Of course, many ethical, legal, and financial problems have to be solved before this can be applied in clinical practice. But as it becomes more and more clear that genetic variation is an important effect modifier when prescribing several drugs, we should continue to identify pharmacogenetic interactions and study their clinical consequences.

In conclusion, we provided an overview of the most important pharmacogenetic background of drug-induced QT interval prolongation, which includes pharmacokinetic and pharmacodynamic variants. GWAS have been proven challenging because of the required large sample size. However, with the availability of genetic data in more and more large study populations, this method remains important for hypothesis generation. Findings from these studies can then be used for candidate-gene studies to further elaborate on possible drug-gene interactions. Not much data is available on ethnic differences in susceptibility to drug-induced QT interval prolongation, although Caucasians seem to be more sensitive to QT prolongation than other ethnicities. Future research could focus on fine mapping of genetic regions, epigenetics, clinical endpoints, or use of alternative markers for drug-induced arrhythmias. Knowledge on the genetic variants associated with drug-induced QT interval prolongation is important as this will provide more accurate predictions as to which patients will experience the adverse drug effect and to whom the drugs can be prescribed relatively safely. This may also prevent unnecessary withdrawal of drugs from the market.

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Chapter 6.2

ABCB1 gene variants, digoxin, and risk of sudden cardiac death

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Abstract

Background The ATP-Binding Cassette B1 (*ABCB1)* gene encodes P-glycoprotein, a transport protein, which plays an important role in the bioavailability of digoxin. We aimed to investigate the interaction between variants within the *ABCB1* gene and digoxin on the risk of sudden cardiac death (SCD).

Methods Within the Rotterdam Study, a population-based cohort study in persons 45 years of age and older, we used Cox regression to analyze the association between 3 polymorphisms that have been associated with digoxin bioavailability, extracted from 1000-Genomes imputed *ABCB1* genotypes and the risk of SCD, stratified by digoxin use.

Results In a total study population of 10,932 persons, 419 SCDs occurred during a median follow-up of 9.8 years. In nonusers of digoxin the risk of SCD was not different across genotypes. In digoxin users, homozygous T allele carriers of C1236T (HR 1.90; 95%CI 1.09;3.30; allele frequency 0.43), G2677T (HR 1.89; 95%CI 1.10;3.24; allele frequency 0.44) and C3435T (HR 1.72; 95%CI 1.03;2.87; allele frequency 0.53) had a significantly increased risk of SCD in a recessive model. Interaction between the ABCB1 polymorphisms and digoxin use was significant for C1236T and G2677T in the age and sex adjusted model.

Conclusion In this study, we showed that in digoxin users, variant alleles at each of the three loci in the *ABCB1* gene were associated with an increased risk of SCD compared to digoxin users with none or one T allele. If replicated, the findings imply that the *ABCB1* genotype modifies the risk of cardiac digoxin toxicity.

Introduction

The ATP-binding cassette B1 (*ABCB1)* gene, formerly known as the Multidrug Resistance 1 (*MDR1)* gene, encodes P-glycoprotein[1]. This transport protein influences resistance to and bioavailability of several drugs[1-3], including digoxin[4,5]. Digoxin is the oldest cardiac drug still in use today[6] and is prescribed for atrial fibrillation and mild to moderate heart failure[7]. Digoxin has a narrow therapeutic range and its use is therefore associated with a high risk of intoxications[8,9]. Digoxin causes shortening of the heart-rate corrected QT (QTc) interval[10] and especially in high concentrations, digoxin can lead to arrhythmias and sudden cardiac death (SCD)[8,9]. From a meta-analysis in 2005 no conclusions could be drawn on the potentially modifying effect of *ABCB1* single nucleotide polymorphisms (SNPs) on digoxin serum concentrations[11]. However, more recent studies did show that variant allele carriers have higher serum concentrations[5,12]. Preliminary results showed that genetic variants within the *ABCB1* gene have a modifying effect on the risk of SCD amongst digoxin users[13]. The objective of this study was to further investigate whether *ABCB1* SNPs modify SCD risk during use of digoxin in a general population of middle-aged and elderly persons.

Methods

Setting and study population

The Rotterdam Study is a prospective population-based cohort study in the city of Rotterdam, the Netherlands. Details regarding design, objectives, and methods of the Rotterdam Study have been described elsewhere[14,15]. In short, all inhabitants of the Ommoord district, ≥55 years were invited to participate. At baseline (1990-1993) 7,983 participants (response rate 78%) were included. In 2000, an additional 3,011 participants were enrolled (response rate 67%). This extension consisted of all persons living in the study district who had become 55 years of age or had moved into the study district. A second extension of the cohort was initiated in 2006, in which 3,932 participants (response rate 65%), ≥45 years were included. Follow-up examinations were conducted periodically, which consist of a home interview and an extensive set of tests at a research facility. Participants are continuously monitored for morbidity and mortality through linkage of general practitioners and municipality records to the study base. The Rotterdam Study was 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.

The study population for this analysis consisted of all participants who gave informed consent for follow-up monitoring and had pharmacy, genetic, and covariable data available. At every

date a SCD case occurs, i.e. the index date, all participants still in the cohort are sampled as controls and exposure is assessed for cases and controls on that date[16].

Exposure

Medication-dispensing data was obtained from all seven fully computerized pharmacies in the study district. Information on all filled prescriptions from January 1st 1991 onwards were available and included information on the product name, the Anatomical Therapeutic Chemical (ATC) code[17], the dispensing date, the prescribed dosing regimen, and the amount dispensed. For every filled prescription of digoxin (ATC-code C01AA05), dispensing episodes were calculated by dividing the total number of tablets/capsules by the daily prescribed number with a carry-over period of 7 days. A person was considered exposed if the index date fell within a dispensing episode. The defined daily dose (DDD) of digoxin is 0.25mg[17].

Genotyping and imputation

A total of 12,453 subjects were genotyped with Illumina 500 (+duo) and Illumina 610 quad and 11,496 subjects passed genotyping quality control. Exclusion criteria were a call rate <98%, Hardy-Weinberg p-value $<10^{-6}$, minor allele frequency <0.01%, excess autosomal heterozygosity >0.336, sex mismatch, and outlying identity-by-state clustering estimates. Data was imputed with the 1000-Genomes reference panel (phase 1, version 3) using MACH version 1.0.15/1.0.16. We selected 3 SNPs within the *ABCB1* gene, which have been associated with digoxin bioavailability: C1236T, G2677T/A and C3435T[5,18]. Imputation quality for all three SNPs was high (>0.99). The SNPs are in relatively strong linkage disequilibrium: D'=0.930, R^2=0.865 between C1236T and G2677T, D'=0.891, R^2=0.449 between C1236T and C3435T, and D'=0.946, R^2=0.506 between G26777T and C3435T[19]. Since the allele frequency of the A allele within the triallelic G2677T/A is low in the general population (around 2%[19]), this SNP was imputed as a biallelic SNP with G as the major and T as the minor allele. We rounded the imputed SNP dosages to 0 (CC for C1236T and C3435T, GG for G2677T), 1 (CT for C1236T and C3435T, GT for G2677T) and 2 (TT).

Outcome definition

SCD was defined according to Myerburg's definition endorsed by the European Society of Cardiology: "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"[20,21]. Identification of SCD cases was done independent of the current research question by two research physicians and confirmed by an experienced cardiologist after reviewing the medical files as described in more detail previously[22,23]. Follow-up was complete for 96% of deaths until January 1st 2011.

Covariables

Information on smoking status was ascertained through structured interviews. Participants were divided into three categories: never, past or current smokers. Standard, 12-lead ECGs were made during the center visits and QT and RR interval were measured using the Modular ECG Analysis System (MEANS)[24,25]. To correct for heart rate, Bazett's formula, $\mathrm{QTc} = \mathrm{QT}/\sqrt{\mathrm{RR}}$, was used[26]. On each index date, data on these covariables from the most recent examination or interview was included as time-dependent covariable[16]. Heart failure diagnosis was adjudicated in accordance with the guidelines of the European Society of Cardiology and included typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction[22,27]. A history of coronary heart disease was defined as a history of myocardial infarction or a coronary revascularization procedure[22]. Atrial fibrillation was ascertained through ECG measurements and medical records[22]. Serum digoxin concentrations were determined through linkage to the "Star-Medisch Diagnostisch Centrum", which performs all outpatient laboratory assessments for the General Practitioners in the area of Rotterdam from April 1st 1997 onwards, and were thus only available in a subset of participants.

Data-analysis

To estimate the effect of *ABCB1* SNPs on digoxin concentration in our study, we used linear regression adjusted for age, sex and digoxin dose. We used only the first available serum measurement of each subject, to limit the effect of digoxin dose titration.

We used Cox proportional hazards models to analyze the association between digoxin use and the occurrence of SCD and the association between the genotypes and risk of SCD, stratified by time-dependent digoxin use[16]. We fitted an additive, recessive, or dominant model, based on the outcome from the general genotype models, i.e. hazard ratios (HRs) for GT/CT and for TT with CC/GG as reference. We tested for interaction between digoxin use and genotypes using the Wald test. Follow-up time was calculated from January 1st 1991 until date of death, loss to follow-up (n=93) or the end of the study period (January 1st 2011), whichever came first. Subjects who entered the study after January 1st 1991 were counted at risk only during the time at which they were under observation. Age at baseline and sex were included in model 1. The other covariables were added in model 2. Digoxin dose was subsequently added (model 3).

A two-sided p-value below 0.05 was considered statistically significant. Measures of association were presented as mean differences in digoxin serum concentration or HRs for the risk of SCD with 95% confidence intervals (CIs). Data were analysed using IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, USA).

Subsample and sensitivity analyses

Haplotype data was available in a subsample of the study population. Genotyping was done using Taqman allelic discrimination assays as previously described[5]. Haplotypes were estimated using HaploStats 1.3.0 package for R 2.5.0, using haplo.em[28,29]. We excluded haplotypes with a posterior probability below 0.95. The association between the main variant diplotype TTT-TTT and the occurrence of SCD was tested similar to the genotype analyses, with the CGC-CGC diplotype as reference. The other diplotypes were included as a dummy variable.

As digoxin elimination is mainly by renal excretion and involves P-glycoprotein, kidney function could be an important covariable. However, kidney function was only available at baseline for the second and third subcohort, and on two visits for the first subcohort. Therefore we included kidney function as covariable in a second subsample analysis. Kidney function (estimated glomerular filtration rate) was calculated according to the abbreviated modification of diet in renal disease formula[30]. For the first subcohort, the baseline measurement (if available) was used for index dates until March 1997 (start of the visit with a new kidney function measurement). After that, the kidney function of that visit was used (if available).

To account for possible limitations of Bazett's formula, in a sensitivity analysis we included QT and RR together in model 2, instead of using QTc. We also analyzed model 2 without adjustment for QTc or QT interval as this could be an intermediate factor.

Results

General characteristics

The study population consisted of 10,932 participants (Figure 1). General characteristics of the total study population are shown in Table 1. The baseline characteristics stratified on ever digoxin use are shown in Supplement 1. The mean age was 65.2±9.6 years and 42% of the participant were male.

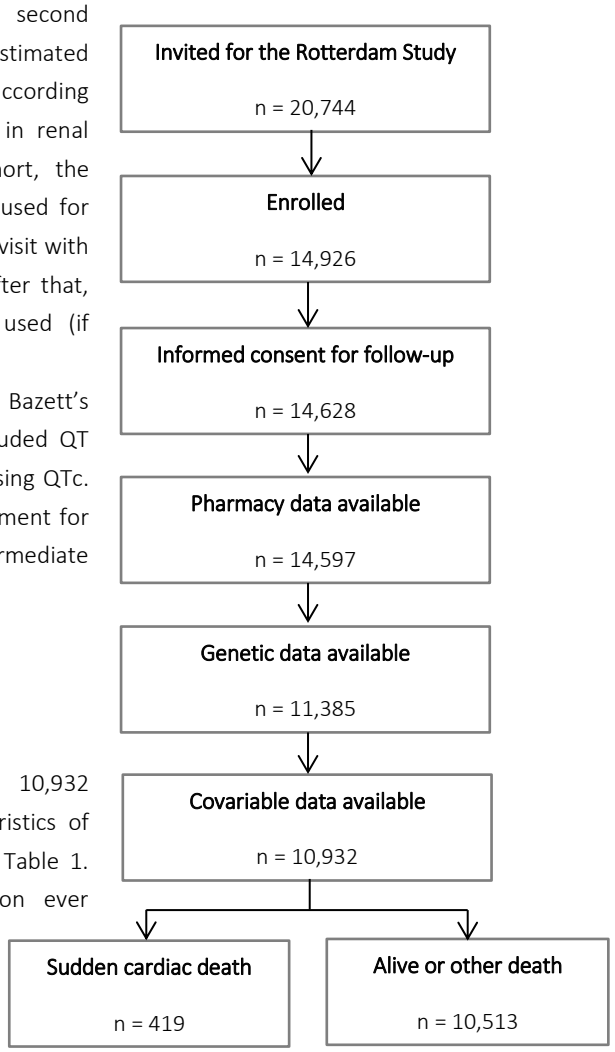

Figure 1. Selection of the study population

Table 1. Baseline characteristics of the total study population

		Total population
n		10,932
Sudden cardiac death cases		419 (4%)
Age (years)		65.2±9.6
Men		4,638 (42%)
Body mass index (kg/m^2)		26.9±4.1
Blood pressure (mmHg)	Systolic	138±21
	Diastolic	77±12
Smoking	Past	4,942 (45%)
	Current	2,461 (23%)
Total cholesterol (mmol/l)		6.2±1.2
QTc interval (milliseconds)		432±25
Heart rate (beats per minute)		70±12
Prevalent coronary heart disease		751 (7%)
Prevalent heart failure		257 (2%)
Prevalent atrial fibrillation		449 (4%)
Estimated glomerular filtration rate (ml/min/1.73m^2)*		79.4±16.4
Digoxin use at baseline		229 (2%)
Digoxin use during follow-up		971 (9%)
Allele frequencies	1236 C	0.57
	2677 G	0.56
	3435 C	0.47
Haplotype frequencies†	CGC	0.41
	TTT	0.41
	Other	0.18

* Total study population for this variable n=10,051, sudden cardiac death cases n=356
† Total study population for this variable n=5,089, sudden cardiac death cases n=332
Data presented as number (percentage) or mean±standard deviation
Abbreviations: n: number; QTc: heart-rate corrected QT interval according to Bazett's formula

During a median follow-up of 9.8 years (interquartile range 4.1-17.0), 419 SCDs occurred; 351 during nonuse of digoxin, 68 during digoxin use. The mean digoxin dose in the total study population of digoxin users was 0.55±0.25 DDD.

Serum digoxin concentration and ABCB1

Dosage information and serum digoxin measurements were available in 175 people, who used a mean DDD of 0.59±0.33 and had a mean serum concentration of 1.0±0.6µg/l. *ABCB1* gene SNPs were associated with an increased digoxin serum concentration (Table 2). The results of C1236T and G2677T were most consistent with an additive model. Per additional T allele for C1236T, the mean serum digoxin concentration was 0.20µg/l (95%CI 0.08;0.33) higher, for G2677T 0.19µg/l (95%CI 0.06;0.32) and for C3435T 0.12µg/l (95%CI 0.00;0.24).

Table 2. Mean difference in digoxin serum concentration (µg/l; with 95% confidence interval) per *ABCB1* genotype according to a genotype, additive, dominant and recessive model

		Genotype			Additive	Dominant		Recessive	
C1236T		CC	CT	TT	Per T allele	CC	CT/TT	CC/CT	TT
	n	51	89	35	175	51	124	140	35
		Ref	**0.28 (0.07;0.48)**	**0.39 (0.14;0.64)**	**0.20 (0.08;0.33)**	Ref	**0.31 (0.11;0.51)**	Ref	0.22 (-0.01;0.44)
G2677T		GG	GT	TT	Per T allele	GG	GT/TT	GG/GT	TT
	n	50	92	33	175	50	125	142	33
		Ref	**0.29 (0.09;0.50)**	**0.35 (0.09;0.61)**	**0.19 (0.06;0.32)**	Ref	**0.31 (0.11;0.51)**	Ref	0.16 (-0.07;0.39)
C3435T		CC	CT	TT	Per T allele	CC	CT/TT	CC/CT	TT
	n	36	85	54	175	36	139	121	54
		Ref	**0.27 (0.04;0.50)**	**0.27 (0.02;0.52)**	0.12 (0.00;0.24)	Ref	**0.27 (0.05;0.49)**	Ref	0.07 (-0.12;0.27)

Bold font indicates statistically significant result ($p<0.05$)
Adjusted for sex, age and digoxin dose
Abbreviation: n: number; ref: reference category

Table 3. Effect of ABCB1 genotype on the risk of sudden cardiac death, stratified on digoxin use

	Nonusers of digoxin (351 sudden cardiac deaths)			Digoxin users (68 sudden cardiac deaths)			P for interaction*
C1236T	CC	CT	TT	CC	CT	TT	
%†/n‡	18%/66	50%/170	32%/115	17%/19	49%/28	34%/21	
Model 1	Reference	0.95 (0.75;1.20)	0.97 (0.72;1.32)	Reference	0.94 (0.53;1.66)	**1.92 (1.03;3.58)**	0.12
Model 2	Reference	0.96 (0.76;1.22)	0.99 (0.73;1.34)	Reference	0.92 (0.52;1.63)	1.64 (0.87;3.08)	0.22
Model 3§	NA	NA	NA	Reference	0.97 (0.53;1.78)	1.86 (0.96;3.60)	0.22
G2677T	GG	GT	TT	GG	GT	TT	
%†/n‡	19%/68	50%/176	31%/107	18%/20	49%/27	34%/21	
Model 1	Reference	1.01 (0.79;1.28)	1.03 (0.76;1.40)	Reference	0.88 (0.50;1.56)	**1.92 (1.04;3.55)**	0.13
Model 2	Reference	1.03 (0.81;1.31)	1.07 (0.79;1.45)	Reference	0.88 (0.49;1.57)	1.58 (0.85;2.93)	0.29
Model 3§	NA	NA	NA	Reference	0.92 (0.49;1.70)	1.79 (0.94;3.42)	0.29
C3435T	CC	CT	TT	CC	CT	TT	
%†/n‡	29%/110	49%/167	22%/74	28%/27	47%/23	25%/18	
Model 1	Reference	1.03 (0.78;1.35)	1.11 (0.83;1.50)	Reference	0.67 (0.36;1.24)	1.33 (0.73;2.42)	0.42
Model 2	Reference	1.07 (0.81;1.41	1.17 (0.87;1.58)	Reference	0.66 (0.35;1.23)	1.19 (0.65;2.18)	0.89
Model 3§	NA	NA	NA	Reference	0.61 (0.31;1.18)	1.27 (0.67;2.38)	0.90

Data presented as hazard ratio with 95% confidence intervals; bold font indicates statistically significant result (p<0.05)

* p-value for interaction between digoxin use and the polymorphism

† As we used a time-dependent model, persons could be sampled as user and nonuser multiple times. Therefore, we provided percentages of sampling moments for each genotype, stratified on digoxin use. Due to rounding, numbers do not add up to 100% exactly.

‡ Number of sudden cardiac death cases per genotype

§ 63 sudden cardiac death cases

Model 1: Adjusted for sex and age

Model 2: Model 1 + time-dependent smoking status (current/past/never), time-dependent heart-rate corrected QT interval, and prevalent heart failure, atrial fibrillation and coronary heart disease

Model 3: Model 2 + digoxin dose

NA: not applicable

ABCB1 and risk of SCD

Digoxin use was associated with an increased risk of SCD in model 1 and 2 (HR 3.81; 95%CI 2.92;4.98 and HR 1.95; 95%CI 1.44;2.64, respectively). Results for the analyses on the effect of *ABCB1* variants stratified on digoxin use are presented in Table 3. In nonusers of digoxin the risk of SCD was not associated with the genetic variants. In digoxin users, homozygous minor allele carriers for the C1236T and G2677T SNPs had a significantly higher risk of SCD in model 1 (HR 1.92; 95%CI 1.03;3.58 and HR 1.92; 95%CI 1.04;3.55, respectively). In model 2 and model 3, this was no longer significant.

Based on the results from the genotype model, we additionally analyzed the SNPs according to a recessive model (Table 4). According to model 2, the risk of SCD was significantly increased in homozygous minor allele carriers of C1236T and G2677T. In model 3, all three SNPs were associated with a significantly increased risk of SCD; C1236T HR 1.90 (95%CI 1.09;3.30), G2677T

Table 4. Effect of *ABCB1* genotype on the risk of sudden cardiac death according to the recessive model, stratified on digoxin use

	Nonusers of digoxin (351 sudden cardiac deaths)		Digoxin users (68 sudden cardiac deaths)		P for interaction*
C1236T	*CC/CT*	*TT*	*CC/CT*	*TT*	
%†/N‡	68%/236	32%/115	66%/47	34%/21	
Model 1	Reference	1.01 (0.77;1.32)	Reference	**1.99 (1.17;3.39)**	**0.04**
Model 2	Reference	1.02 (0.78;1.33)	Reference	**1.72 (1.00;2.96)**	0.16
Model 3§	NA	NA	Reference	**1.90 (1.09;3.30)**	0.17
G2677T	*GG/GT*	*TT*	*GG/GT*	*TT*	
%†/N‡	69%/244	31%/107	67%/47	34%/21	
Model 1	Reference	1.03 (0.79;1.34)	Reference	**2.06 (1.22;3.49)**	**0.03**
Model 2	Reference	1.05 (0.80;1.37)	Reference	**1.70 (1.00;2.88)**	0.14
Model 3§	NA	NA	Reference	**1.89 (1.10;3.24)**	0.15
C3435T	*CC/CT*	*TT*	*CC/CT*	*TT*	
%†/N‡	78%/277	22%/74	75%/50	25%/18	
Model 1	Reference	1.09 (0.87;1.37)	Reference	**1.71 (1.05;2.78)**	0.09
Model 2	Reference	1.12 (0.89;1.40)	Reference	1.53 (0.93;2.52)	0.30
Model 3§	NA	NA	Reference	**1.72 (1.03;2.87)**	0.30

Data presented as hazard ratio with 95% confidence intervals; bold font indicates statistically significant result (p<0.05)

* p-value for interaction between digoxin use and the polymorphism

† As we used a time-dependent model, persons could be sampled as user and nonuser multiple times. Therefore, we provided percentages of sampling moments for each genotype, stratified on digoxin use. Due to rounding, numbers do not add up to 100% exactly.

‡ Number of sudden cardiac death cases per genotype

§ 63 sudden cardiac death cases

Model 1: Adjusted for sex and age

Model 2: Model 1 + time-dependent smoking status (current/past/never), time-dependent heart-rate corrected QT interval, and prevalent heart failure, atrial fibrillation and coronary heart disease

Model 3: Model 2 + digoxin dose

NA: not applicable

Table 5. Subsample analysis: Risk of sudden cardiac death associated with *ABCB1* diplotype, according to the diplotype and recessive model, stratified on digoxin use (n=5,089)

	Nonusers of digoxin			Digoxin users		
Diplotype	CGC-CGC	TTT-other	TTT-TTT	CGC-CGC	TTT-other	TTT-TTT
%*/n†	25%/48	51%/81	24%/47	30%/13	49%/12	21%/13
Model 1	Reference	0.90 (0.63;1.28)	1.03 (0.69;1.55)	Reference	0.62 (0.28;1.37)	1.58 (0.73;3.43)
Model 2	Reference	0.96 (0.67;1.37)	1.12 (0.75;1.68)	Reference	0.71 (0.32;1.58)	1.48 (0.68;3.23)
Model 3	NA	NA	NA	Reference	0.73 (0.31;1.71)	1.66 (0.73;3.78)
Recessive	CGC-CGC/TTT-other		TTT-TTT	CGC-CGC/TTT-other		TTT-TTT
%*/n†	76%/129		24%/47	79%/25		21%/13
Model 1	Reference		1.11 (0.79;1.55)	Reference		**2.04 (1.04;4.01)**
Model 2	Reference		1.15 (0.82;1.61)	Reference		1.76 (0.88;3.50)
Model 3	NA		NA	Reference		1.97 (0.97;3.99)

Data presented as hazard ratio with 95% confidence intervals; bold font indicates statistically significant result (p<0.05)
* As we used a time-dependent model, persons could be sampled as user and nonuser multiple times. Therefore, we provided percentages of participants for each genotype, stratified on digoxin use.
† Number of sudden cardiac death cases per genotype
‡ 35 sudden cardiac death cases
Model 1: Adjusted for sex and age
Model 2: Model 1 + time-dependent smoking status (current/past/never), time-dependent heart-rate corrected QT interval, and prevalent heart failure, atrial fibrillation and coronary heart disease
Model 3: Model 2 + digoxin dose
NA: not applicable

HR 1.89 (95%CI 1.10;3.24) and C3435T HR 1.72 (95%CI 1.03;2.87). When testing interaction between digoxin use and genetic variants according to the recessive model, the interaction was significant only for C1236T and G2677T in model 1. We did not adjust for serum digoxin concentration, as we had concentration measurements available in only 12 SCD cases.

Subsample and sensitivity analyses

In 5,572 participants haplotypes were estimated, after exclusion of participants with a posterior probability below 0.95, 5,089 remained with a mean posterior probability of 0.99. The haplotype analyses showed comparable results with slightly higher HRs than the genotype analyses, especially in the recessive model (Table 5).

Additional adjustment for estimated glomerular filtration rate gave comparable results (Table 6). Leaving QTc interval out of the model or adjusting for QT and RR interval together instead of using QTc did not change the estimates substantially (Table 7).

Table 6. Subsample analysis: Effect of *ABCB1* genotype and haplotype on the risk of sudden cardiac death according to the recessive model, stratified on digoxin use, with additional adjustment for estimated glomerular filtration rate (n=10,051)

	Nonusers of digoxin 297/228 sudden cardiac deaths*		Digoxin users 59/47 sudden cardiac deaths*	
C1236T	*CC/CT*	*TT*	*CC/CT*	*TT*
Model 1	Reference	0.97 (0.72;1.30)	Reference	**1.80 (1.02;3.18)**
Model 2†	NA	NA	Reference	**1.94 (1.08;3.48)**
G2677T	*GG/GT*	*TT*	*GG/GT*	*TT*
Model 1	Reference	1.00 (0.75;1.34)	Reference	1.69 (0.96;2.98)
Model 2†	NA	NA	Reference	**1.83 (1.02;3.27)**
C3435T	*CC/CT*	*TT*	*CC/CT*	*TT*
Model 1	Reference	1.09 (0.85;1.40)	Reference	1.65 (0.98;2.80)
Model 2†	NA	NA	Reference	**1.84 (1.07;3.18)**
Diplotype	*CGC/TTT*	*TTT-TTT*	*CGC/TTT*	*TTT-TTT*
Model 1	Reference	1.11 (0.75;1.63)	Reference	1.79 (0.85;3.76)
Model 2‡	NA	NA	Reference	2.05 (0.95;4.45)

Data presented as hazard ratio with 95% confidence intervals; bold font indicates statistically significant result (p<0.05)

* The first number relates to the number of cases in the genotype analyses, the second number the number of cases in the haplotype analyses (model 1)

† 55 sudden cardiac deaths

‡ 34 sudden cardiac deaths

Model 1: Adjusted for sex, age, time-dependent smoking status (current/past/never), time-dependent heart-rate corrected QT interval, and prevalent heart failure, atrial fibrillation and coronary heart disease and estimated glomerular filtration rate

Model 2: Model 1 + digoxin dose

NA: not applicable

Table 7. Sensitivity analysis: Effect of *ABCB1* genotype on the risk of sudden cardiac death according to the recessive model, stratified on digoxin use

	Nonusers of digoxin		Digoxin users	
C1236T	*CC/CT*	*TT*	*CC/CT*	*TT*
Model 1	Reference	1.02 (0.78;1.33)	Reference	1.71 (1.00;2.94)
Model 2	Reference	1.01 (0.77;1.32)	Reference	**1.76 (1.03;3.02)**
G2677T	*GG/GT*	*TT*	*GG/GT*	*TT*
Model 1	Reference	1.05 (0.80;1.37)	Reference	1.69 (0.99;2.86)
Model 2	Reference	1.04 (0.80;1.35)	Reference	**1.73 (1.02;2.94)**
C3435T	*CC/CT*	*TT*	*CC/CT*	*TT*
Model 1	Reference	1.12 (0.89;1.41)	Reference	1.51 (0.92;2.49)
Model 2	Reference	1.09 (0.87;1.37)	Reference	1.53 (0.93;2.52)

Data presented as hazard ratio with 95% confidence intervals; bold font indicates statistically significant result ($p<0.05$)
Model 1: Adjusted for sex, age, time-dependent smoking status (current/past/never), time-dependent QT and RR interval, and prevalent heart failure, atrial fibrillation and coronary heart disease
Model 2: Adjusted for sex, age, time-dependent smoking status (current/past/never), and prevalent heart failure, atrial fibrillation and coronary heart disease

Discussion

In this study, we showed that amongst users of digoxin, homozygous T allele carriers of *ABCB1* gene SNPs C1236T, G2677T and C3435T, have an increased risk of SCD. In nonusers of digoxin, this effect is not present. These findings might be useful for informed decision-making in personalized pharmacotherapy.

We showed an increase in digoxin serum concentration of 0.12-0.20μg/l per T allele of all three SNPs, or an increase of 0.27-0.39μg/l in homozygous T allele carriers. As for most people a serum concentration between 0.8-2.0μg/l is considered therapeutic[7], this is a substantial increase.

A remaining question is whether the SNPs have an effect independent of serum concentrations in digoxin users. The results for the association between *ABCB1* SNPs and serum digoxin concentration suggest an additive or dominant model, while the association of the SNPs with SCD suggests a recessive effect of the variant alleles. Given these different results it is possible that the effect on the risk of SCD is not completely explained by the higher serum concentrations. Another possibility is that the serum concentration has to exceed a certain threshold before it increases the risk of SCD, which is possibly mainly reached in persons homozygous for the T allele. Unfortunately, we could not adjust for serum concentrations as only a limited number of participants had serum concentration measurements available. The low number of available serum concentration measurements could be explained by the fact that

we only have these measurements if they are requested by the general practitioners and not during hospital or outpatient clinic visits. Therefore, further studies are needed to establish whether the effect of the *ABCB1* SNPs on SCD is fully explained by higher serum digoxin concentrations or that the SNPs also have an independent effect on the risk of SCD in digoxin users. If this is the case, serum concentration measurements alone will probably not be the most adequate and safe monitoring method. As genotyping has become less expensive over the past years, genotyping new digoxin users might become a clinically useful addition to enhance safe use of digoxin. The p-values for interaction between digoxin and C1236T and G2677T were significant only in the age and sex adjusted model. For C3435T there was no significant interaction. This could be due to the small number of exposed cases who were homozygous major allele carriers. However, this limits the possibility to draw firm conclusions on the modifying effect of *ABCB1* variants. The finding of a significant association and interaction for C1236T and G2677T probably reflect the same association signal given the high degree of linkage disequilibrium. C3435T has been used in earlier studies that used haplotype analyses that showed stronger associations compared to single SNP analysis. In our subsample with haplotype data available, participants with a TTT-TTT diplotype had a slightly higher risk compared to the genotype analyses.

Our study has strengths and limitations. The high level of completeness of follow-up in this cohort is an important asset. We had access to detailed information on morbidity and mortality through access to the medical records. We also had data available on a large number of time-dependent covariables, limiting the confounding in our analyses. Unfortunately, kidney function measurements were not available for all participants and for participants who did have measurements available, the majority only had a baseline measurement, which could introduce misclassification in the time-dependent analyses. Therefore, we adjusted for this covariable in subsample analyses, which showed comparable results to the main analyses. Within the Rotterdam Study, data is collected according to standardized protocols. We stratified on digoxin use, since digoxin is used in subjects who already have a higher risk of SCD and in such patients confounding by indication is usually difficult to exclude.

A limitation of our study is the heterogeneity of SCD cases that is introduced through the definition of SCD. However, this definition has been widely endorsed for several years and incidence rates in our study are comparable to other studies[23]. Moreover, misclassification would probably be random for the variant alleles and mean that our estimates are conservative and the risk of SCD may be even higher. Second, since we do not have genotype data for all participants, we rounded imputed dosages to obtain categorical estimates of the genotypes. Although this could lead to misclassification, imputation quality was high for all three SNPs. Since the use of imputed data hampered haplotype analysis, we analyzed haplotypes within a subsample of the population with genotype data available. We only studied three SNPs, but with

the emergence of whole-genome and sequencing data in large cohorts, more variants within genes could be studied, to find the variants with the best predictive value. Another limitation is that our results are obtained in an older and white population. Therefore our results may not necessarily be generalizable to younger and non-white individuals. Fourth, the number of exposed cases within our study population was low, especially for serum digoxin concentrations. Finally, we did not correct for multiple testing as this is an *a-priori* defined candidate-gene study based on previous findings on the associations between digoxin, *ABCB1* gene variants, and SCD, and second because the three SNPs are highly correlated. The choice for the recessive model was not pre-hypothesized, but based on the results of the genotype model. Our findings should be replicated in an independent cohort before this knowledge can be applied to clinical practice.

In conclusion, we showed that homozygous T allele carriers of the *ABCB1* SNPs C1236T, G2677T and C3435T using digoxin have an increased risk of SCD compared to digoxin users with none or one T allele, while there was no effect in nonusers of digoxin. As these variant alleles are common in the general population, this might influence a substantial part of the digoxin users. If these findings can be replicated in an independent cohort, testing genetic variants in new users of digoxin might add to safe use of digoxin if drug concentration monitoring is insufficient to reduce the associated risk in a specific group of patients who are at high risk of the adverse event on the basis of underlying morbidity.

Supplement

Supplement 1. Baseline characteristics of the total study population, stratified on ever digoxin use

		Never users*	Ever users*
n		9,961 (91%)	971 (9%)
Age (years)		64.5±9.3	73.2±8.7
Men		4,202 (42%)	436 (45%)
Body mass index (kg/m^2)		26.9±4.1	26.8±4.0
Blood pressure (mmHg)	Systolic	138±21	143±23
	Diastolic	78±12	74±13
Smoking	Past	4,480 (45%)	462 (48%)
	Current	2,270 (23%)	191 (20%)
Total cholesterol (mmol/l)		6.1±1.2	6.3±1.2
QTc interval (milliseconds)		431±24	436±34
Heart rate (beats per minute)		70±11	74±16
Prevalent coronary heart disease		585 (6%)	166 (17%)
Prevalent heart failure		142 (1%)	115 (12%)
Prevalent atrial fibrillation		214 (2%)	235 (24%)
Estimated glomerular filtration rate (ml/min/1.73m^2)†		80.0±16.2	72.8±17.5
Allele frequencies	1236 C	0.57	0.57
	2677 G	0.56	0.56
	3435 C	0.47	0.47
Haplotype frequencies‡	CGC	0.41	0.43
	TTT	0.41	0.40
	Other	0.18	0.17

* The proportion of ever users overestimates the actual proportion of currently exposed moments in the time-dependent analyses, as persons could be sampled as user or nonuser multiple times.
† Total study population for this variable n=10,051, sudden cardiac death cases n=356
‡ Total study population for this variable n=5,089, sudden cardiac death cases n=332
Data presented as number (percentage) or mean±standard deviation
Abbreviations: n: number; QTc: heart-rate corrected QT interval according to Bazett's formula

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Chapter 6.3

CACNA1C variant rs2238018, calcium channel blockers, and heart rate

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Abstract

Background We aimed to study whether a *CACNA1C* variant, rs2238018, modifies the effect of diltiazem and verapamil on heart rate.

Methods We included 9,424 participants from a population-based cohort of persons aged >45 years with 18,450 electrocardiograms: 269 electrocardiograms were recorded during diltiazem and 108 during verapamil use.

Results The polymorphism showed significant interaction with diltiazem and verapamil on heart rate (p=0.04). Each T allele (frequency 19.6%) was associated with lower heart rates in users: -2.52 bpm (95%CI -5.44;0.40) in diltiazem users and -4.29 bpm (95%CI -7.96;-0.61) in verapamil users. No effect was seen in nonusers.

Conclusion Knowledge of rs2238018 status might be relevant as heart-rate reduction can be the treatment objective or an adverse effect.

Introduction

Calcium channel blockers are drugs which block the current of calcium ions into the cell through the L-type calcium channels[1]. In cardiomyocytes, free calcium ions are needed for sinus and atrioventricular node function[2]. Blocking the calcium channels delays conduction in cardiomyocytes and subsequently reduces heart rate[1,2]. There are, however, considerable differences in structure and activity between the individual calcium channel blockers[3,4]. Only diltiazem and verapamil were shown to decrease heart rate in dosages that are clinically used[1,2]. These drugs are prescribed for several indications, including angina pectoris and hypertension[1]. In the case of angina pectoris they are prescribed partly to reduce heart rate, because this lowers oxygen demand of the heart and therefore decreases the chance of major cardiovascular events[1,5,6]. However, a decrease in heart rate can also be an adverse effect and influence adherence. Therefore, factors that modify the response to diltiazem and verapamil are relevant for clinical decisions as they can indicate which patients will benefit most from treatment, who will have adverse effects, or who will benefit minimally and may need a higher dosage or an alternative treatment. One of these factors could be genetic variation. The L-type calcium channel is encoded by the *CACNA1C* gene[7], and this gene could be a modifying factor in response to calcium channel blockers. In a previous study, we found that a specific single nucleotide polymorphism (SNP), rs2238018, within the *CACNA1C* gene, explains part of the variability in resting heart rate[8] and therefore we selected this SNP for the present study. Our primary aim was to study whether the rs2238018 polymorphism is associated with a modified effect on the response of heart rate and occurrence of bradycardia during exposure to diltiazem or verapamil. The L-type calcium channels in the cardiomyocytes are under hormonal regulation by testosterone[9,10] and basal heart-rate levels differ between men and women and vary with testosterone levels among men[11]. Therefore, the effects of this pharmacogenetic interaction on heart rate may differ by sex. For that reason, our secondary aim was to study whether the modifying effect of the rs2238018 polymorphism on heart rate in users of diltiazem and verapamil is different in men and women.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study in the city of Rotterdam, the Netherlands. Details regarding design, objectives, and methods of the Rotterdam Study have been described elsewhere[12,13]. In short, all inhabitants of the Ommoord district, aged 55 years and older were invited to participate. At baseline (1990-1993) 7,983 participants (response rate 78%) were included. In 2000, an additional 3,011 participants were enrolled (response rate

67%). This extension consisted of all persons living in the study district who had become 55 years of age or had moved into the study district. A second extension of the cohort was initiated in 2006, in which 3,932 participants (response rate 65%), aged 45 years and older were included. Follow-up examinations were conducted periodically (currently a maximum of 5 visits per participants), which consist of a home interview and an extensive set of tests at a research facility. 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)". All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Study population

The study population for this analysis consisted of all participants for whom we had pharmacy, genetic and covariable data available. Electrocardiograms (ECGs) were excluded if P waves were absent or if people were using beta-blocking agents, digoxin or class I/III antiarrhythmic drugs during ECG recording.

Exposure

Medication-dispensing data was obtained from all seven fully computerized pharmacies in the study district. Information on all filled prescriptions from January 1st 1991 onwards were available and included information on the product name, the Anatomical Therapeutic Chemical code (ATC-code)[14], the dispensing date, the prescribed dosing regimen and the amount dispensed. For every dispensing of diltiazem (ATC-code C08DB01) and verapamil (C08DA01), dispensing episodes were calculated by dividing the total number of tablets/capsules by the daily prescribed number. A person was considered exposed if the ECG recording date fell within the dispensing episode. The defined daily dose (DDD) of both diltiazem and verapamil is 240 mg[14].

Genotyping and imputation

A total of 12,453 subjects were genotyped with Illumina 550(+duo) and Illumina 610 quad and 11,496 subjects passed genotyping quality control. Exclusion criteria were a call rate <98%, Hardy-Weinberg p-value <10^{-6} and minor allele frequency <0.01%, autosomal heterozygosity, sex mismatch and outlying identity-by-state clustering estimates. Data was imputed with the 1000-Genomes reference panel (phase 1, version 3) using MACH version 1.0.15/1.0.16. For the genotype model, we rounded the imputed SNP dosages to 0 (CC), 1 (CT) and 2 (TT).

Outcome

Standard 12-lead resting ECGs were recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hertz and stored digitally. All ECGs were

processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurements[15]. MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques, and has been evaluated extensively[15-17]. MEANS determines the median RR interval after exclusion of beats that immediately precede or follow any premature ventricular complex. Heart rate in beats per minute (bpm) was calculated as 60,000/RR interval in milliseconds (ms). We defined bradycardia as a heart rate equal or below 60 bpm.

Potential covariables

All covariables were determined at the date of ECG recording. Height and weight were measured during the center visits. Body mass index was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressure were measured in sitting position at the right upper arm. The average of two consecutive measurements was taken. Hypertension was defined as a systolic blood pressure above 140 mmHg, diastolic blood pressure above 90 mmHg, or use of blood pressure lowering medication for the indication hypertension. Information about smoking status was ascertained through structured interviews. Participants were divided into three categories: never, past or current smokers. Diabetes mellitus was defined as a fasting glucose above 6.9 mmol/l, a non-fasting glucose above 11.0 mmol/l, the use of blood-glucose lowering medication, or a previous diagnosis of diabetes mellitus. Heart failure diagnosis was adjudicated in accordance with the guidelines of the European Society of Cardiology[18] and included typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction[19]. A history of coronary heart disease was defined as a history of myocardial infarction or a percutaneous or surgical coronary revascularization procedure[19].

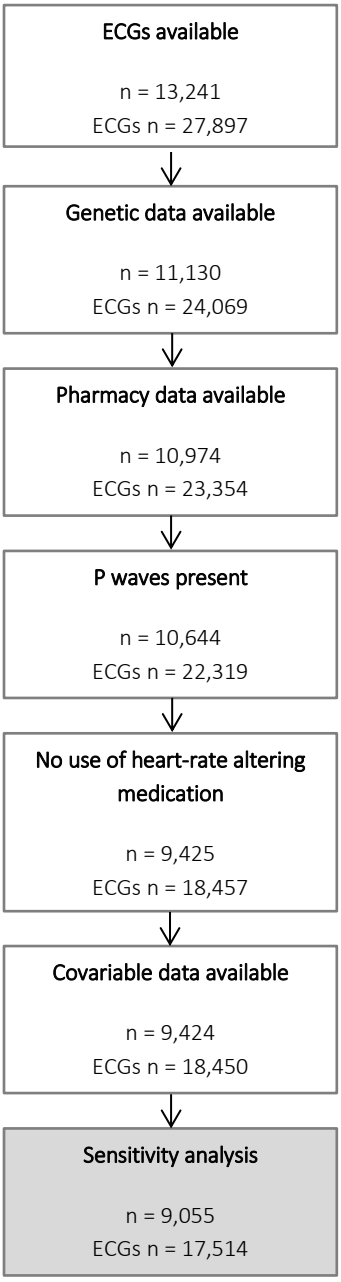

Figure 1. Selection of the study population
Abbreviations: ECG: electrocardiogram, n: number

Data-analysis

We used generalized estimating equations (GEE) to correct for correlation between repeated measurements in the same participants, using a varying between-measurement time window[20,21]. We selected the working correlation matrix based on which model had the lowest quasi likelihood under independence model criterion[22]. First, we studied the association between use of diltiazem or verapamil and heart rate. We tested interaction by including an interaction term between use of either diltiazem or verapamil and the SNP. Subsequently, we assessed the effect of the rs2238018 polymorphism on heart rate and bradycardia stratified by drug use. Age, sex, duration and dosage (per DDD) were included in the crude model. Covariables that changed the effect estimate from the age, sex, duration and dosage adjusted model in any of the analyses by more than 10% were included in the multivariable adjusted model for all analyses. We also analysed the association stratified by sex. In a sensitivity analysis we excluded ECGs where a heart rate above 100 bpm or below 40 bpm or a left or right bundle branch block was detected. A two-sided p-value below 0.05 was considered statistically significant. Measures of association are presented as betas for the change in heart rate or odds ratios (ORs) for the risk of bradycardia with 95% confidence intervals (CIs). We used IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, U.S.) for all analyses.

Table 1. General characteristics of the study population at the first eligible electrocardiogram recording

	Total study population n = 9,424	Men n = 3,920	Women n = 5,504
Total number of electrocardiograms	18,450	7,593	10,857
Number of electrocardiograms per participant	1 [1-5]	1 [1-5]	1 [1-5]
Age (years)	65.0±9.7	64.2±9.0	65.6±10.1
Height (cm)	168±10	176±7	163±6
Body mass index (kg/m^2)	26.7±4.0	26.5±3.4	26.9±4.4
Smoking Past	4,123 (44%)	2,225 (57%)	1,898 (34%)
Current	2,145 (23%)	1,055 (27%)	1,090 (20%)
Systolic blood pressure (mmHg)	138±21	139±20	138±22
Diastolic blood pressure (mmHg)	78±12	79±12	77±12
Hypertension	4,947 (52%)	2,051 (52%)	2,896 (53%)
Prevalent coronary heart disease	446 (5%)	324 (8%)	122 (2%)
Prevalent heart failure	139 (1%)	48 (1%)	91(2%)
Prevalent diabetes mellitus	862 (9%)	412 (11%)	450 (8%)
Left bundle branch block	119 (1%)	41 (1%)	78 (1%)
Right bundle branch block	217 (2%)	130 (3%)	87 (2%)
rs2238018 minor (T) allele frequency	19.6%	19.5%	19.6%
Heart rate (beats per minute)	71±11	69±12	72±11
Bradycardia (heart rate ≤60 beats per minute)	1,638 (17%)	932 (24%)	706 (13%)

Data presented as number (percentage), median [range] or mean±standard deviation

Results

General characteristics

The study population consisted of 9,424 participants, in whom 18,450 ECGs have been recorded (Figure 1), with a median of 1 ECG recording per person (range 1-5). General characteristics of the study population at the first eligible ECG are shown in Table 1. The mean age of the study population at baseline was 65.0±9.7 years and 58% was female. Of the 18,450 ECGs, 269 were recorded during use of diltiazem and 108 during use of verapamil. The minor (T) allele frequency of the rs2238018 polymorphism was 19.6%. Imputation quality was >0.99.

rs2238018, diltiazem and verapamil and heart rate

For the GEE, the independent working correlation structure had the best fit. Additional to age, sex, duration and dosage, we included a history of coronary heart disease, and diabetes mellitus as covariable. Other potential covariables did not contribute to the model. Overall, diltiazem was associated with a lower heart rate: -3.77 bpm (95%CI -7.09;-0.45). Verapamil had a smaller, non-significant effect: -2.14 bpm (95%CI -5.89;1.60). The distribution of ECG recordings by drug use, rs2238018 status and sex are shown in Table 2. The rs2238018 polymorphism significantly modified the effect of diltiazem and verapamil on heart rate (p=0.04 for interaction). Results of the analyses on the effect of the SNP, stratified on diltiazem and verapamil use, are shown in Figure 2. In nonusers no differences in heart rate were seen between different genotypes (p=0.59 for trend). A significant progressively lower heart rate was seen in users of verapamil with 1 or 2 T alleles. In this group, each additional T allele was associated with a lower heart rate of -4.29 bpm (95%CI -7.96;-0.61). In diltiazem users, heart rate was -2.52 bpm (95%CI -5.44;0.40) lower per additional T allele, although not statistically significant.

The effect of the rs2238018 polymorphism was stronger in women (Figure 3). In men there was no significant interaction between use of diltiazem or verapamil and the SNP (p=0.82), while interaction was significant in women (p=0.01).

Table 2. Distribution of 18,450 available electrocardiograms by drug use, rs2238018 status and gender

	Nonusers		Diltiazem		Verapamil	
	Men	Women	Men	Women	Men	Women
Overall	7,454	10,619	109	160	30	78
CC	4,839 (65%)	6,888 (65%)	74 (68%)	110 (69%)	19 (63%)	45 (58%)
CT	2,336 (31%)	3,382 (32%)	31 (28%)	41 (26%)	10 (33%)	29 (37%)
TT	279 (4%)	349 (3%)	4 (4%)	9 (6%)	1 (3%)	4 (5%)

Due to rounding percentages do not always exactly add up to 100%

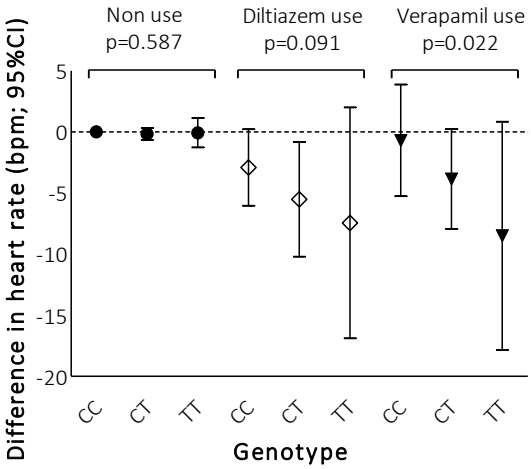

Figure 2. Difference in heart rate by rs2238018 status with nonusers carrying two major alleles as reference, stratified on use of diltiazem or verapamil
Adjusted for age, sex, duration and dosage, a history of coronary heart disease and diabetes mellitus
Abbreviation: bpm: beats per minute, CI: confidence interval; p: p-value for trend

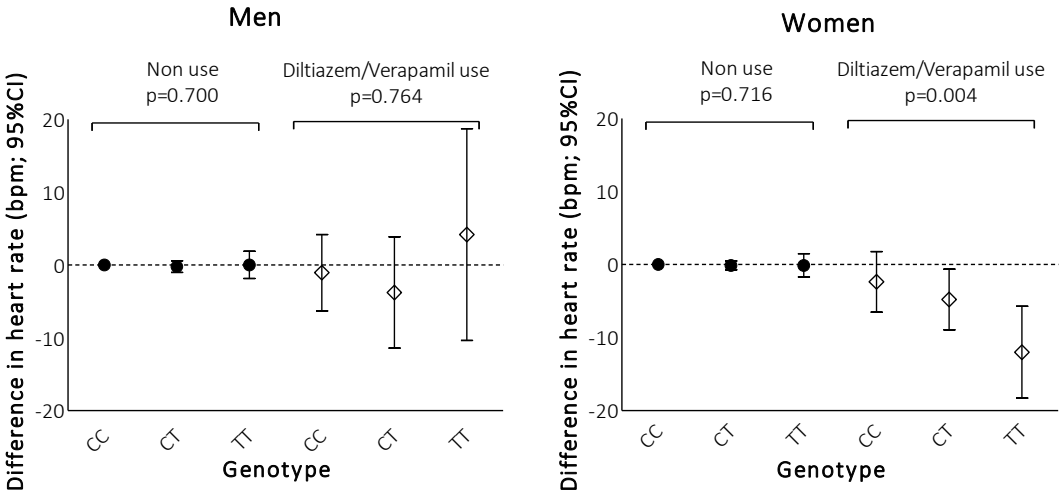

Figure 3. Difference in heart rate by rs2238018 status and diltiazem or verapamil use with nonusers carrying two major alleles as reference, stratified by gender
Adjusted for age, duration and dosage, a history of coronary heart disease and diabetes mellitus
Abbreviation: bpm: beats per minute, CI: confidence interval; p: p-value for trend over genotypes

rs2238018, diltiazem and verapamil and bradycardia

Bradycardia was detected on 3,485 ECGs (18.9%), 91 of those ECGs were recorded during use of either diltiazem or verapamil. Because of the low number of ECGs recorded during use, ECGs of participants using diltiazem and verapamil were combined. The distribution of ECG recordings by drug use, the rs2238018 polymorphism and presence of bradycardia are shown in Table 3. Interaction between the rs2238018 polymorphism and diltiazem and verapamil on risk of bradycardia was borderline non-significant (p=0.06 for interaction). In nonusers no significant trend for an increased risk of bradycardia was seen over different genotypes (p=0.13 for trend). In users of diltiazem or verapamil, the risk of bradycardia was increased for each additional T allele (p=0.02 for trend) as can be seen in Figure 4. Homozygous minor allele carriers using either diltiazem or verapamil had a 4.64-fold increased risk (95%CI 1.40;15.41) of bradycardia compared to homozygous major allele carriers.

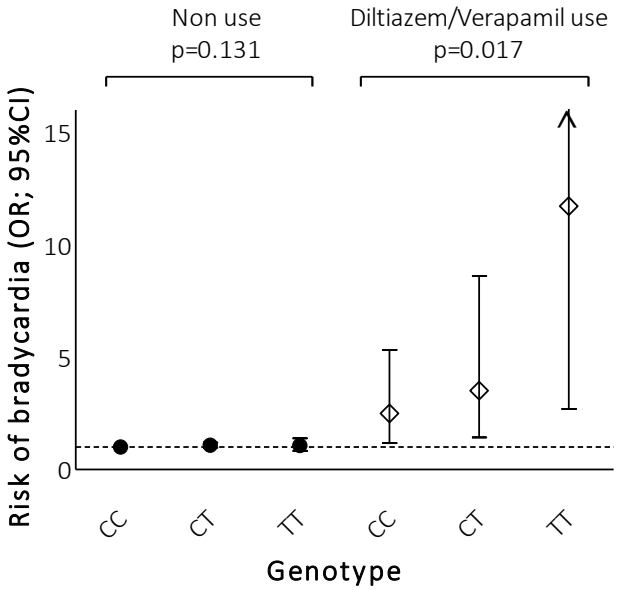

Figure 4. Risk of bradycardia (heart rate ≤60 beats per minute) by rs2238018 status with nonusers carrying two major alleles as reference, stratified on use of diltiazem and verapamil
Adjusted for age, sex, duration and dosage, a history of coronary heart disease and diabetes mellitus
Abbreviation: CI: confidence interval; p: p-value for trend

Table 3. Distribution of 18,450 available electrocardiograms by drug use, rs2238018 status and presence of bradycardia

	Nonusers		Diltiazem/Verapamil users	
	No bradycardia	Bradycardia	No bradycardia	Bradycardia
Overall	14,679	3,394	286	91
CC	9,574 (65%)	2,153 (63%)	197 (69%)	51 (56%)
CT	4,600 (31%)	1,118 (33%)	80 (28%)	31 (34%)
TT	505 (3%)	123 (4%)	9 (3%)	9 (10%)

Sensitivity analyses

After exclusion of ECGs where a heart rate above 100 bpm or below 40 bpm or a bundle branch block was detected (remaining ECGs n=17,514 in n=9,055 participants), similar results were found, although with smaller effect estimates, but results were not statistically significant (Figure 5).

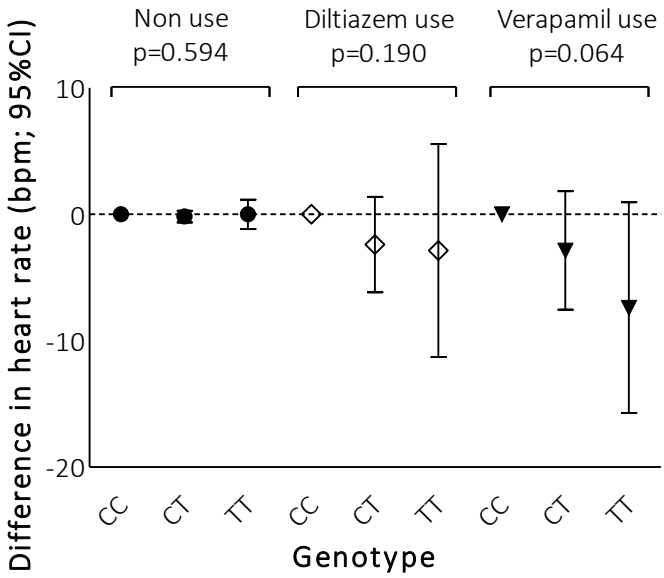

Figure 5. Sensitivity analysis after exclusion of electrocardiograms where a heart rate above 100 bpm or below 40 bpm or a bundle branch block was detected: difference in heart rate by rs2238018 status with nonusers carrying two major alleles as reference, stratified on use of diltiazem or verapamil

Adjusted for age, sex, duration and dosage, a history of coronary heart disease and diabetes mellitus

Abbreviation: bpm: beats per minute, CI: confidence interval; p: p-value for trend

Discussion

In this population-based study we showed that the effect of diltiazem and verapamil on heart rate is significantly modified by rs2238018, a polymorphism within the *CACNA1C* gene. This may have clinical consequences, as a decreased heart rate can be either one of the main objectives in treatment with these drugs or an adverse effect when the treatment is given for other indications.

As calcium channel blockers specifically block the channel that is encoded by the *CACNA1C* gene it is not surprising that both calcium channel blockers and SNPs of the *CACNA1C* gene have an influence on the function of this pore, i.e. letting calcium flow into the cell and enabling cell function. Both calcium channel blockers and *CACNA1C* have been associated with heart rate[1,8], but to our knowledge, the interaction between the two has not been described previously. The underlying mechanism for this association could be through direct effects on the affinity of the drug for binding the L-type calcium channel or indirectly through decreased L-type calcium channel density. We believe this interaction is clinically relevant as one of the indications for calcium channel blockers is angina pectoris, because these drugs can lower heart rate and thus lower oxygen demand of the heart[1]. Based on our results, minor allele carriers may have a better response to treatment than homozygous major allele carriers. Conversely, in patients who take diltiazem or verapamil for other indications, a decreased heart rate can be an adverse effect. Homozygous minor allele carriers might experience a higher burden of this adverse effect, which could influence adherence to treatment.
In a large meta-analysis of 15 genome-wide association studies, the rs2238018 polymorphism explained part of the variability in heart rate[8], but was not genome-wide significantly associated with heart rate. We only found a very small effect of the rs2238018 polymorphism on heart rate and bradycardia in nonusers, which supports this non-significant finding in the previous study, which included only nonusers of diltiazem, verapamil, β-blocking agents, and digoxin. That this interacting effect is mainly present in women might be related to differences in ion channel density between men and women due to hormonal effects[9-11]. The L-type calcium channels are regulated by testosterone[9,10] and heart rate varies with testosterone levels in men[11]. Furthermore, basal heart rate levels differ between men and women[11]. Therefore, we expected to find a different effect in men and women. It would be interesting to correct these analyses for testosterone levels, but these data were not available during all ECG recordings. Because of the low number of exposed men among the homozygous T allele carriers, these results have to be interpreted cautiously.

Previous pharmacogenetic studies have focused on clinical endpoints in relation to calcium channel blocker use and *CACNA1C* genotype[23-25]. One study showed that people who are

homozygous for the major allele of a different SNP within the *CACNA1C* gene, have a better outcome (based on death, non-fatal myocardial infarction and stroke) on verapamil than on atenolol treatment[24]. This is in line with our findings, but the comparison is limited due to the relatively heterogeneously defined outcome in that study. Two other studies investigated the interaction of *CACNA1C* variation and calcium channel blocker use on blood pressure response[23,25]. However, these studies investigated dihydropyridine calcium channel blockers, which are different from diltiazem and verapamil in both structure and working mechanism. Our study has focused on the mechanism through which a lower morbidity and mortality could be achieved. Further research should assess whether the effect on heart rate is relevant with regard to clinical endpoints such as adherence to drugs and occurrence of coronary heart disease. Many people could possibly benefit from these findings when implemented in clinical practice, as this polymorphism is common in the general population (20% in our population, 26% in the 1000-Genomes reference panel[26]). The rs2238018 polymorphism lies in an intronic region[26]. This variant has been associated with drug-induced long QT syndrome[27]. Mutations in the *CACNA1C* gene have been associated with arrhythmias[28], and are found in some types of familial sudden cardiac death syndromes[29], which indicates an influence on cardiac conduction.

Our study has several strengths. First, the Rotterdam Study is a prospective population-based study. Data has been collected according to standardized protocols and ECGs have been assessed using a standardized automatic method with MEANS. This program has been evaluated extensively[15-17], ensuring valid heart-rate measurement. Since the Rotterdam Study has no exclusion criteria for enrollment, our results are generalizable to the general middle-aged and elderly population. We excluded ECGs where P waves were absent, because this could be the result of atrial fibrillation or atrial flutter, which are associated with both exposure and outcome. In a sensitivity analysis, we have excluded ECGs where extreme heart rates and bundle branch blocks were detected to minimize the impact of extreme RR intervals. Finally, we had access to pharmacy data, which provided us with dispensing data instead of prescription data and details about the daily dose. There are also limitations we should address. We used imputed genotypes because we did not have full genotype data for all participants of the Rotterdam Study. We rounded imputed dosages to obtain estimates of the genotypes, however the additive model was based on imputed dosages. This rounding could have led to misclassification, however imputation quality was high. Second, the association is tested cross-sectionally at different points in time and not in a controlled clinical study. Finally, we did not replicate our findings in an independent cohort. However, the chance of false-positive errors is low, since this is a candidate-gene study based on an association we previously found between the rs2238018 polymorphism and heart rate, previous findings on the association between calcium channel blockers and heart rate, and the biological plausibility of the relationship between the *CACNA1C* gene and the working mechanism of calcium channel blockers. Therefore we believe our study

gives reliable estimates of the modifying effect of *CACNA1C* variants in diltiazem and verapamil users.

In conclusion, our study showed that T allele carriers of rs2238018 within the *CACNA1C* gene have a stronger response to diltiazem and verapamil with respect to heart rate. This knowledge is important as a lower heart rate can either be an objective of treatment or it can be seen as an adverse effect. In the first case, genotyping could help in identifying patients who will respond insufficiently, in the second case it could influence adherence to treatment. Knowledge of rs2238018 status might add to the future ambitions of tailored pharmacotherapy with diltiazem and verapamil, however further studies on clinically relevant endpoints are needed.

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Part VII

General discussion & Summary

Chapter 7.1

Discussion

Maartje N Niemeijer

In this chapter, the main findings of the work presented in this thesis will be discussed, followed by an overview of methodological considerations. Finally, the implications for clinical practice are reviewed, and future perspectives are outlined. In the individual chapters, we already discussed some of these issues pertaining to the individual studies. In this chapter, we aim to provide a more general view by connecting the chapters and by discussing more general epidemiologic concepts and describing these in the context of the studies in this thesis.

Aim

Sudden cardiac death (SCD) is one of the most important manifestations of cardiovascular mortality and therefore a major health burden, especially in the elderly[1-4]. Preventive options are available, such as implantable cardioverter defibrillators, drugs, and avoidance of certain medications, but are not completely successful[5,6]. Moreover, determining which persons are at risk of SCD, and thus will benefit most from these options, remains difficult[7,8]. This is partly because not all risk factors are known[8]. Furthermore, characteristics of the known risk factors are not fully studied and these determinants might influence the validity and usefulness of these markers. For example, nothing is known about the consistency of prolongation of the heart-rate corrected QT (QTc) interval, and therefore it is unclear if and for how long the result of one QTc measurement is valid for SCD risk stratification. The overall goal of the work described in this thesis is to improve risk stratification and to be able to employ better-targeted preventive measures. This is divided into three specific aims:

i. To study the current and past incidence of SCD in order to determine the magnitude of the problem;
ii. To identify new risk factors for SCD in the general population;
iii. To study characteristics of already known risk factors for SCD.

Main findings

Magnitude of the problem

The incidence of cardiovascular disease is increasing worldwide, but this increase is predominantly attributable to low and middle-income countries[3,4,9]. In high-income countries, cardiovascular mortality is declining[4]. As SCD forms a substantial part of cardiovascular mortality[2], it is expected that the incidence rate of SCD in high-income countries has also declined. In **chapter 2.1**, we aimed to study the incidence of SCD and whether the incidence has indeed declined in the Rotterdam Study. We found an age-standardised incidence rate of 4.2 per 1,000 person-years of SCD over the total follow-up from 1990 until 2010, with a higher rate in men than in women. The incidence rate increased with higher age. The incidence rate has declined over the past two decades, from 4.7 per 1,000 person-years between 1990 and 2000,

to 2.1 per 1,000 person-years between 2001 and 2010. This decline is possibly attributable to better diagnosis and treatment of cardiac diseases[10,11], especially by a higher frequency of coronary revascularisation procedures[12-14]. However, worldwide SCD remains a major health problem. First, because the incidence will probably increase in low and middle-income countries in the coming years[3]. And second, because SCD is still a health burden and a psychological burden to relatives of those struck by SCD because of the unexpected nature of death[8], also in high-income countries.

New risk factors

In **chapter 3.1**, we systematically reviewed the literature regarding QT variability markers, relatively novel electrocardiogram (ECG) derived risk factors. These markers have been proposed because in recent years concerns have been raised regarding the most well-known ECG-derived risk factor, a prolonged QTc interval[15-18], especially in drug safety studies[19]. Studies have shown that some QTc-prolonging drugs do not increase the incidence of arrhythmias and SCD[20,21], while some drugs that do carry an arrhythmogenic risk do not always show QTc prolongation[22]. Therefore, the concept of repolarization reserve was introduced[23-26]. This concept refers to the idea that the complexity of the repolarization includes some redundancy[26], and arrhythmias will occur when the repolarization reserve is lowered[27]. QT interval variability is thought to be a better measure of repolarization reserve than the QTc interval alone[21]. Constant changes in the duration of the repolarization, and thus a high QT interval variability, are indicative of a low reserve[21,23,24]. We identified nine different QT variability markers in the literature, which can be classified into four groups, according to two characteristics; (i) whether QT variability was measured between consecutive beats, or over a number of beats, and (ii) whether the QT variability was normalized for heart rate. In the retrieved articles, significant associations were shown between QT variability markers and arrhythmias or SCD, indicating that QT variability markers are potentially useful risk factors for arrhythmias and SCD, but these markers have not been systematically compared. In **chapter 3.3**, we studied the ECG-derived PR interval and P-wave indices as risk factors for SCD. We did not find significant associations of the PR interval duration and the P-wave duration with occurrence of SCD. However, we found significant associations between the amplitude, area, and terminal force of the P wave and SCD. These P-wave indices have previously been associated with pulmonary hypertension and atrial enlargement. Although the association of pulmonary hypertension and atrial enlargement with risk of SCD has been shown before[28], the ECG is easier and cheaper to record in clinical practice than an echocardiogram. Therefore, this study might provide risk factors that are more useful and efficient for clinical practice, but further research into the relation between P-wave indices, echocardiography, and occurrence of SCD is needed.

In **chapter 4.3**, we studied serum magnesium levels as a potential risk factor for SCD. Previous studies have investigated the association between serum magnesium levels and risk of cardiovascular mortality. However, results are conflicting and it remains unclear through which pathway this effect is mediated[29]. Magnesium is known for its role in the electrical stability and energy balance of myocytes[30], and a low serum magnesium level has been associated with atrial and ventricular arrhythmias[29,31,32]. Possibly, a low serum magnesium is also associated with atherosclerosis[33,34] and a prolonged QTc interval[35], both pathways through which SCD can arise. In this study, we found that a low serum magnesium was associated with coronary heart disease mortality (hazard ratio 1.4) and SCD specifically (hazard ratio 1.5). A low level of serum magnesium was also associated with a prolonged QTc interval and with an increased carotid intima media thickness, a measure of atherosclerosis. However, the mean differences associated with a low serum magnesium were small, and adjustment for these factors in the analysis on SCD did not substantially change the results. Thus, a low serum magnesium is associated with occurrence of SCD, but the main pathway is probably not only through a prolonged QTc interval and atherosclerosis.

Two risk factors that we investigated in this thesis have been studied before in high-risk patient populations, but not in the general population, namely subclinical cardiac dysfunction and chronic obstructive pulmonary disease (COPD). It has been shown before that people with heart failure, and thus a decreased cardiac function, have an increased risk of SCD[36-38]. Subclinical cardiac dysfunction has been associated with SCD in specific high-risk patient populations, such as athletes[39], and patients with hypertrophic cardiomyopathy[40] and coronary heart disease[41]. In **chapter 4.1**, we aimed to study the association between subclinical cardiac dysfunction and the occurrence of SCD in the general population. We excluded all persons with a diagnosis of heart failure at the date of echocardiography, and estimated the risk of SCD associated with changes in cardiac function in the remaining population. This study showed that a lower fractional shortening was associated with an increased risk of SCD. Fractional shortening is the relative difference between the end-diastolic and the end-systolic dimension of the left ventricle[42], and values above 25% are regarded as normal[43]. Each decrease in fractional shortening of 7% in men and 6% in women was associated with a 36% higher hazard of SCD after adjustment for relevant confounders. However, when combined with other known risk factors for SCD, the predictive value did not improve much, indicating that the association between a lower fractional shortening and SCD provides insight into possible etiological pathways, but is not useful in the prediction of SCD.

COPD has previously been associated with a prolonged QTc interval and an increased risk of cardiac arrhythmias[44-46]. COPD has also been associated with a higher risk of SCD in specific high-risk populations, e.g. patients with coronary heart disease[47-49]. In **chapter 4.2**, we showed that

having a diagnosis of COPD increased the risk of SCD by 34%. In the period 5.5 years after the diagnosis of COPD the hazard increased by 93% compared to persons without COPD. The risk was higher in COPD patients with frequent exacerbations than in COPD patients without frequent exacerbations (hazard ratio 3.2 vs. 1.5), and slightly higher in COPD patients with high baseline levels of systemic inflammation markers, measured by C-reactive protein levels, than without high systemic inflammation (hazard ratio 1.9 vs. 1.8). In patients with both frequent exacerbations and high levels of systemic inflammation markers the hazard ratio increased to 3.7. The fact that the risk was increased in the period 5.5 years after the diagnosis of COPD, and in COPD patients with frequent exacerbations, and a high grade of systemic inflammation, shows that a more severe grade of COPD is associated with a higher risk of SCD.

Characteristics of known risk factors

In this thesis, several studies aimed to determine characteristics of some known risk factors for SCD, which can influence the usefulness and validity of these risk factors for clinical practice. One of the most well-known risk factors for SCD in the general population and an important outcome in drug-safety studies is the QTc interval[18,50-53]. Several drugs have been shown to prolong the QTc interval in the so-called thorough QT/QTc studies, which are required in the pre-marketing phase of drug development by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA)[51,52,54-56]. A prolonged QTc interval is defined as above 450 milliseconds in men, and above 470 milliseconds in women[57]. An increase up to 5 milliseconds is not considered as clinically relevant, an increase of 5 to 20 milliseconds as possibly clinically relevant, and above 20 milliseconds as clinically relevant[51,52]. If a drug has QTc-prolonging properties it can still be marketed, if the benefits outweigh the adverse effects[37]. However, a prolonged QTc interval is one of the most common reasons to withdraw a drug from the market[22,37,58,59]. When a physician wants to prescribe a QTc-prolonging drug to a patient, the QTc interval should be measured to determine the baseline risk of arrhythmias and SCD[51,52]. Nowadays, one measurement of a prolonged QTc interval might be seen as sufficient to withhold the drug from a patient for a long time. However, the consistency of a prolonged QTc interval over time has never been studied. In **chapter 3.2**, we found that approximately one-third of the persons with a prolonged QTc interval on a baseline measurement had a normal QTc interval on a subsequent measurement, after a median interval of 1.8 years. We further showed that people with an inconsistently prolonged QTc interval did not have a significantly increased risk of SCD compared to people with a normal QTc interval on both measurements. People with a prolonged QTc interval on both measurements did have an increased risk of SCD, with a hazard ratio of 2.2-6.7, depending on the correction formula used for the QTc interval, after adjustment for relevant confounders in a population of people not using QTc-prolonging drugs during ECG recording. Apparently, the decision to treat or abstain in such persons may differ over time and a single QTc interval prolongation should not be seen as an ever-lasting

contraindication. We studied measurements with a median of 1.8 years apart. Further research is needed to establish the short-term consistency of QTc interval prolongation.

Furthermore, the FDA and EMA advise that concomitant use of multiple QTc-prolonging drugs should be avoided[51,52]. No distinction is made between drugs with known, possible, or conditional QTc-prolonging properties. However, evidence to support this advice is lacking. In **part 5** of this thesis, we aimed to study whether physicians adhere to the advice not to prescribe multiple QTc-prolonging drugs concomitantly and whether this advice is justified, with regard to the effect on the QTc interval. In **chapter 5.1**, we showed that in a general population of approximately 1.7 million persons, combinations of QTc-prolonging drugs are often prescribed, with up to seven QTc-prolonging drugs. A previous study showed that in 1999, 2% of people in an outpatient population in the USA received concomitant prescriptions of multiple QTc-prolonging drugs[60]. This indicates that either physicians are not aware of this advice, or they choose not to adhere to it, possibly because they measure the QTc interval in every patient and prescribe combinations only to persons with a normal QTc interval or because they instruct patients to stop using their other medication. In **chapter 5.2**, we did not find an additively prolonged QTc interval when people used two or three QTc-prolonging drugs concomitantly compared to the use of one such drug (12 vs. 11 milliseconds). However, the number of ECGs during which persons were using a combination of QTc-prolonging drugs was low (n=68), which limits the possibility to draw firm conclusions regarding the safety of using multiple drugs concomitantly and further research into this topic is needed. Our results might indicate that the advice to avoid concomitant use of multiple QTc-prolonging drugs in all persons might be too strict. However, in this study we only investigated the association with prolongation of the QTc interval. Further research should demonstrate whether there is no increased risk of arrhythmias in persons using multiple QTc-prolonging drugs concomitantly.

In **part 6**, we studied the genetic background of drug-induced QTc prolongation and we studied the association of pharmacogenetic interactions with SCD and heart rate. In **chapter 6.1**, we studied the genetic background of drug-induced QTc prolongation with a review of the available literature. Most studies used a candidate-gene approach, i.e. a gene is known for its action on either drug response or on the QTc interval, and is then tested for its association with drug-induced QTc interval prolongation by studying potential interaction by drug and gene. In the retrieved articles, we identified genes that were associated with both pharmacokinetic and pharmacodynamic effects of drugs on QTc prolongation. Pharmacokinetics-related genetic susceptibility is mainly characterized by variation in genes encoding drug-metabolizing cytochrome P450 enzymes or drug transporters. The pharmacodynamic component of genetic susceptibility is mainly characterized by genes known to be associated with QT interval duration in the general population and genes in which the causal mutations of congenital long QT

syndromes are located. It is expected that with larger sample sizes and better exposure and outcome definitions more variants can be detected.

In the two candidate-gene studies in this thesis, we were able to show drug-gene interactions, i.e. the modifying effect of genetic variants in drug users, while the effect is absent in nonusers. In **chapter 6.2**, we studied the interaction between digoxin use and *ABCB1* gene variants, encoding P-glycoprotein, on the risk of SCD. Digoxin use has been associated with an increased risk of SCD[61,62], and the *ABCB1* gene variants have been shown to influence serum concentration levels of digoxin[63-65]. In this study, we found that homozygous variant allele carriers of three polymorphisms within the *ABCB1* gene using digoxin had a 72-90% increased risk of SCD compared to users of digoxin with none or one variant allele. In nonusers of digoxin, we did not find a significant effect of the *ABCB1* gene variants. This increased risk can either originate through an independent effect of the gene variants in digoxin users, or because the risk of SCD only increases above a certain threshold of the digoxin serum concentration which is only reached in homozygous variant allele carriers. In **chapter 6.3**, we studied the association between a drug-gene interaction and heart rate. Lowering the heart rate can be the objective in treatment with calcium channel blockers, especially diltiazem and verapamil, for example to decrease the oxygen demand of the heart in patients with angina pectoris, but it can also be an adverse effect, for example in patients treated for hypertension[66-68]. Calcium channel blockers act by blocking the L-type calcium channels in the cardiomyocytes[68]. These channels are encoded by the *CACNA1C* gene[69]. A mutation in the cardiac L-type calcium channels was associated with arrhythmias[70] and a variant in this gene, rs2238018, has been shown to explain part of the variability in resting heart rate[71]. A higher heart rate has been associated with occurrence of SCD[72-75]. Therefore, we studied the interaction between calcium channel blocker use and rs2238018 status on heart rate, and found that in users of diltiazem and verapamil, heart rate was significantly lower in carriers of the rs2238018 variant allele. In nonusers, there was no association between the genetic variant and heart rate. The association between SCD and *CACNA1C* variants, possibly modified by drug use, may be an interesting topic for further research.

Methodological considerations

Setting

Most studies in this thesis were part of the Rotterdam Study, a prospective population-based cohort study in 14,926 persons aged 45 years and older[76,77]. This cohort consists of three subcohorts, which were consecutively included at fixed points in time, namely in 1990 (Rotterdam Study I), 2000 (Rotterdam Study II), and 2006 (Rotterdam Study III). People could be included into the study population if they lived in Ommoord, a district in Rotterdam. People

leave the study when they die or when they indicate that they do not want to participate in the study anymore. As the Rotterdam Study has a fixed entry time and no people enter beyond these entry times, this is called a fixed cohort[78]. In **chapter 5.1**, we used the Integrated Primary Care Information (IPCI) project, a database in which the medical files of patients of 600 general practitioners (GPs) are gathered[79,80]. This is also a prospective, population-based cohort study but in a dynamic patient population. In a dynamic patient population, people can move in and out of the study population at any given moment, in this case when they enter or leave a GP practice, which is called a dynamic cohort[78]. When looking at incidences over calendar time, the difference between a fixed and a dynamic cohort is important. In a fixed cohort, people leave the cohort at any time, but no people enter the cohort during follow-up unless a new subcohort is enrolled. As a consequence, during follow-up there will be fewer people left that are susceptible to the outcome, as those who are vulnerable will develop the outcome while those who are not vulnerable will remain in follow-up. This phenomenon is called depletion of susceptibles, and its consequence is that calculating the incidence rate over calendar time in a fixed cohort is difficult. In a dynamic cohort, new people enter the study continuously, and new susceptible people will enter the study during follow-up, which makes it more likely that the number of susceptible people during the complete follow-up period is fairly constant. In **chapter 2.1**, we intended to investigate whether the incidence of SCD had declined over the past twenty years in the Rotterdam Study. In order to account for depletion of susceptibles, we determined the incidence rate of SCD in two independent subcohorts of the Rotterdam Study, namely Rotterdam Study I and Rotterdam Study II. We calculated the incidence rate for both subcohorts per ten years of follow-up, to ensure a comparable grade of depletion of susceptibles. We did not use the IPCI database to calculate the incidence of SCD over time, because this study started in 1996, and thus had less follow-up time available. In **chapter 5.1**, we used the IPCI database to calculate the incidence and prevalence of concomitant prescriptions of multiple QTc-prolonging drugs over time per calendar year.

Drug exposure

To determine drug exposure in a population-based cohort study, there are three possibilities: (i) use interview data in which the participants are asked about their medication use, (ii) use data from the physician to determine which drugs are prescribed to participants, or (iii) use data from the pharmacies to determine which prescriptions have been filled at the pharmacies. The advantage of the second and third method is that the data come from a database, instead of asking the participant, which reduces the risk of differential and non-differential information bias in the data. However, the disadvantage of the second and third method is that one cannot ascertain whether a participant really used the drug. With the second method, participants can choose not to go to the pharmacy to get their prescriptions filled. With the third method we know that a participant got the prescription filled, but we cannot be sure that he or she actually

took the drug. In the Rotterdam Study, we have interview and pharmacy data available. In the IPCI database, which is used for **chapter 5.1**, drug exposure is determined according to the prescriptions by the GP. In this chapter, we aimed to determine whether physicians adhere to the advice not to prescribe multiple QTc-prolonging drugs concomitantly, and therefore using prescription data was a valid method. In the other chapters regarding drug exposure (**chapter 5.2**, **chapter 6.2**, and **chapter 6.3**), we used pharmacy data. This might lead to some misclassification, because as stated earlier, participants might get their prescriptions filled, but not use the drug. However, this misclassification would probably be the same for cases and controls, and therefore be non-differential, leading to an underestimation of the true effect. The misclassification might be random if in a pharmacogenetic study, the polymorphism is associated with an adverse effect which leads to discontinuation of drug use. However, this would also lead to an underestimation of the effect in the two pharmacogenetic studies in this thesis (**chapter 6.2** and **chapter 6.3**), as the polymorphisms had no effect in nonusers.

In several studies in this thesis, use of QTc-prolonging drugs plays an important role. In **chapter 3.2** we excluded users of QTc-prolonging drugs, in **chapter 5.1** we investigated the patterns of use of these drugs, in **chapter 5.2** we studied the association between concomitant use of multiple of these drugs and repolarization duration, and in **chapter 6.1** we studied the available knowledge on the genetic background of drug-induced QT interval prolongation. The QTc-prolonging drugs are usually divided into three categories; known, possible, and conditional[54,81]. This categorization does not indicate the degree of QTc prolongation associated with use of the drugs[81], but indicates the degree of scientific evidence for a causal association with the suspected drug. In studies done to date, the known and possible categories are often combined into one exposure class[82]. Sometimes two possible QTc-prolonging drugs are counted as one known QTc-prolonging drug[82], an approach which is not scientifically subtle. In **chapter 5.2**, we showed that there is a large difference in degree of QTc prolongation associated with known and possible QTc-prolonging drugs. Use of one known QTc-prolonging drug was associated with a 15 milliseconds increase in QTc interval, while use of possible QTc-prolonging drugs was associated with an increase of only 3 milliseconds. Therefore, we recommend that future studies do not combine the two categories into one exposure class, as this leads to misclassification of exposure.

Sudden cardiac death

The validation of SCD cases in a population-based cohort study is difficult, because ideally for all SCD cases the arrhythmogenic origin should be certain, but participants within a population-based study rarely die while being monitored. Therefore, we used a definition based on symptoms and time from onset of symptoms until death. The definition was established in 1997 by Myerburg and endorsed by the European Society of Cardiology in 2001. It defines SCD as “a

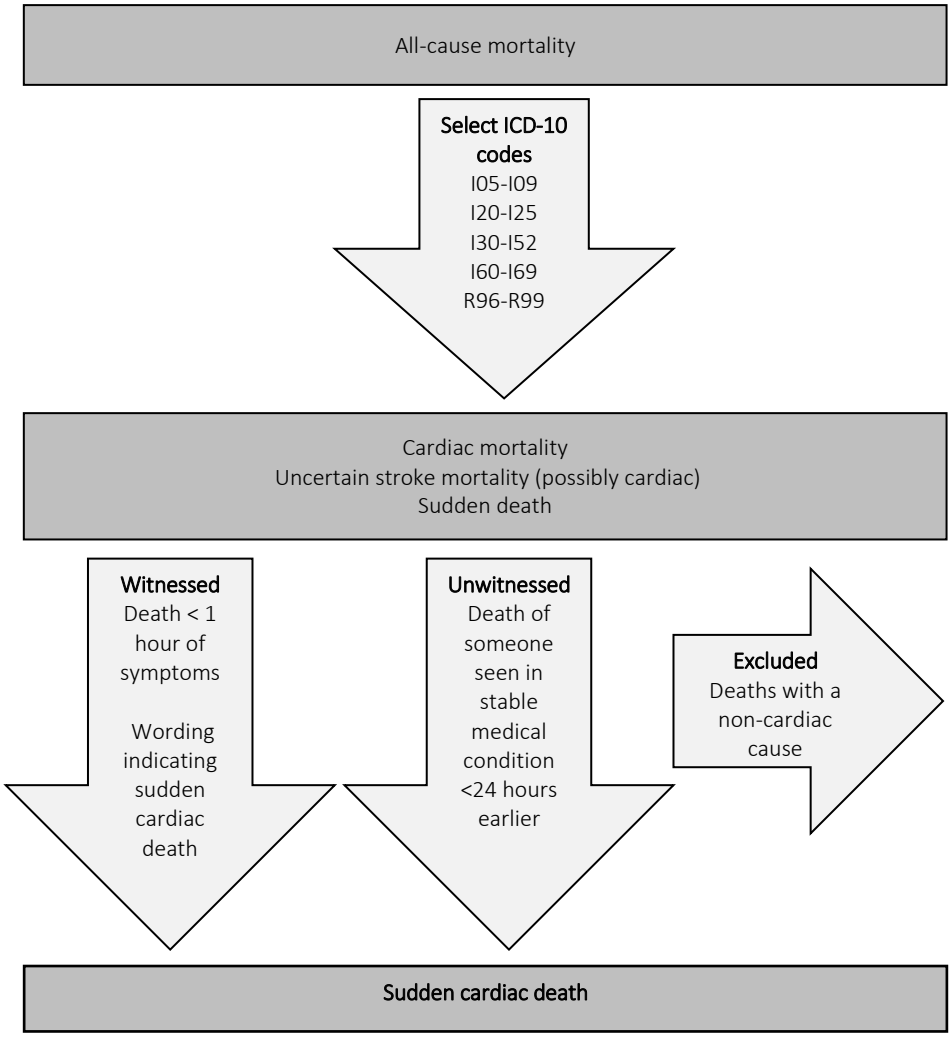

Figure 1. Validation of sudden cardiac death cases within the Rotterdam Study

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"[83,84]. This definition applies only to witnessed deaths. We also included unwitnessed deaths, if someone was found dead while he or she was in a stable medical condition 24 hours earlier, in accordance with previous studies[1,84-86] (Figure 1). By using this definition, potential heterogeneity was introduced in our case set. For instance, other acute causes of death, such as a fatal pulmonary embolism, an intracerebral hemorrhage, or an aortic rupture can be wrongly classified as SCD. By selecting the cardiac causes of death through the ICD-10 classification system[87] and by exclusion of deaths of non-cardiac causes, we

aimed to refine sudden deaths to only those of cardiac origin. The incidence rate of SCD in the Rotterdam Study, as described in **chapter 2.1**, was similar to the rate found in other studies, indicating similar validity as other population-based studies[1,2,88].

ECG measurements

The studies on ECG measurements were all performed within the Rotterdam Study. All ECG parameters are measured using the Modular ECG Analysis System[89]. This system has been used and validated extensively[90-92]. Using such a system ensures absence of intra-observer variability, which otherwise would occur if the ECGs were to be analyzed manually. Since approximately 30,000 ECGs are recorded in total in the Rotterdam Study, manual measurement would be a cumbersome task that probably would have to be done by multiple persons, with the risk of inter-observer variability.

The QT interval represents the ventricular depolarization (QRS complex) and ventricular repolarization (JT interval). Especially a prolonged repolarization is thought to be a cause of arrhythmias and SCD. Therefore, recently a shift has occurred towards using the JT interval to determine the safety of drugs[18]. However, it is unclear whether the JT interval is a true representation of an increased risk of arrhythmias. In **chapter 5.2**, we showed that in people using one or two known QTc-prolonging drugs the QTc interval was prolonged. The JT interval was increased in users of one QTc-prolonging drug, but not in persons using two QTc-prolonging drugs concomitantly. This might be a false-negative finding because of the low number of exposed ECGs. Another possibility could be that the addition of a second drug causes an additive prolongation of the ventricular depolarization (QRS complex). If the ventricular depolarization is prolonged, the ventricular repolarization might start while the depolarization is not completely finished and thus while the QRS complex has not ended yet. In that case, the JT interval may not be a valid representation of the total duration of the repolarization. Furthermore, in **chapter 3.2**, we showed that additional adjustment for QRS duration lowered the risk estimates obtained from the analyses with QTc according to the formulas of both Bazett and Fridericia. This might indicate that including measurement of the QRS complex (by using the QT interval) better predicts a high risk of arrhythmias. Further research into the QT and JT interval and QRS complex is needed to establish which ECG-derived factor or combination of factors has the best predictive value.

Bias

In epidemiology, three sources of bias are distinguished: (i) information bias, (ii) selection bias, and (iii) confounding bias[93]. In this thesis, we used prospective cohort studies, which means that a group of individuals with one or more shared characteristics (e.g. in the Rotterdam Study: being 55 years of age or older at study entry, and living in Ommoord) is prospectively followed over time. Data are gathered from the medical files and from examinations at the research

center. Therefore, we do not have to rely on the memory of the participants or physicians. Having to rely on the memory of participants can cause bias, as the recall can be different for cases and controls. For example, people with certain diseases are more likely to remember risky behavior or an unhealthy lifestyle, and people with severe disease tend to have a better recall of drug exposure data than healthy controls[94]. This bias is called recall bias, a form of information bias. Using prospectively-gathered data lowers the likelihood of information bias.

In **chapter 4.2**, we investigated the association between COPD and SCD. In this study, persons are considered at risk during follow-up, and exposed after they are diagnosed as having COPD. A potential problem in this study, which relates to the determination of the follow-up time, is immortal time bias[95]. Since we know of some of the participants that they develop COPD during follow-up, we know that they cannot die before the date of COPD diagnosis, this is called immortal time (Figure 2). There are four methods to determine the unexposed (non-COPD) and exposed (COPD) time at risk in a study with immortal time (Figure 3)[95]. First, the diagnosis of COPD during follow-up can be seen as a diagnosis at baseline (Figure 3A). Thus, time until this diagnosis is also analyzed as COPD time. This gives misclassification of exposure and thus information bias, but is a relatively acceptable method because of the insidious onset of COPD. Second, the time until diagnosis can be excluded from the time at risk (Figure 3B). This will give immortal time bias, a form of selection bias, as the selection of time at risk is different for persons with COPD and without COPD. Third, a time-varying analysis can be used, where the time until diagnosis of COPD is added to the non-COPD time at risk, while the time after the

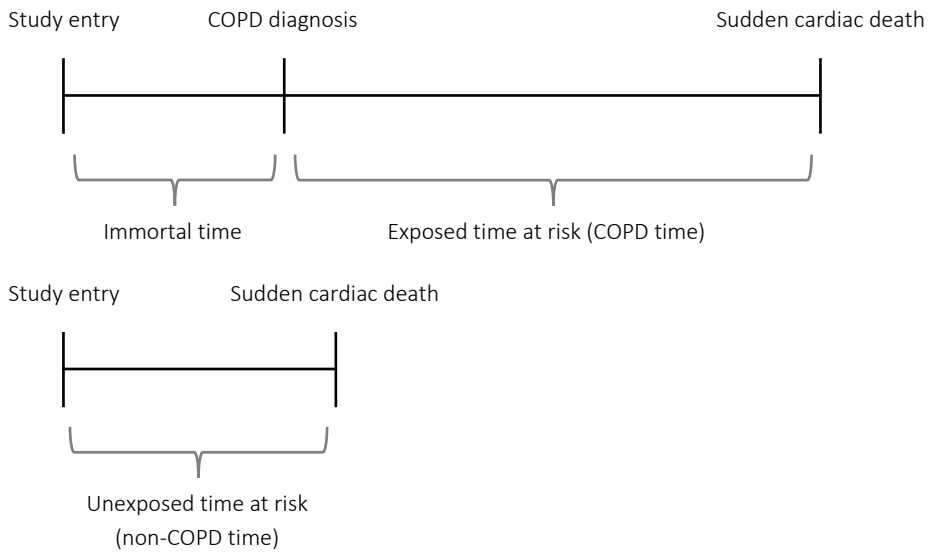

Figure 2. Immortal time, and exposed and unexposed time at risk

diagnosis is added to the COPD time at risk (Figure 3C). This provides the most accurate determination of the exposed and unexposed follow-up time if there is no latent period[95]. The latent period refers to the time before COPD is diagnosed, while the disease is already

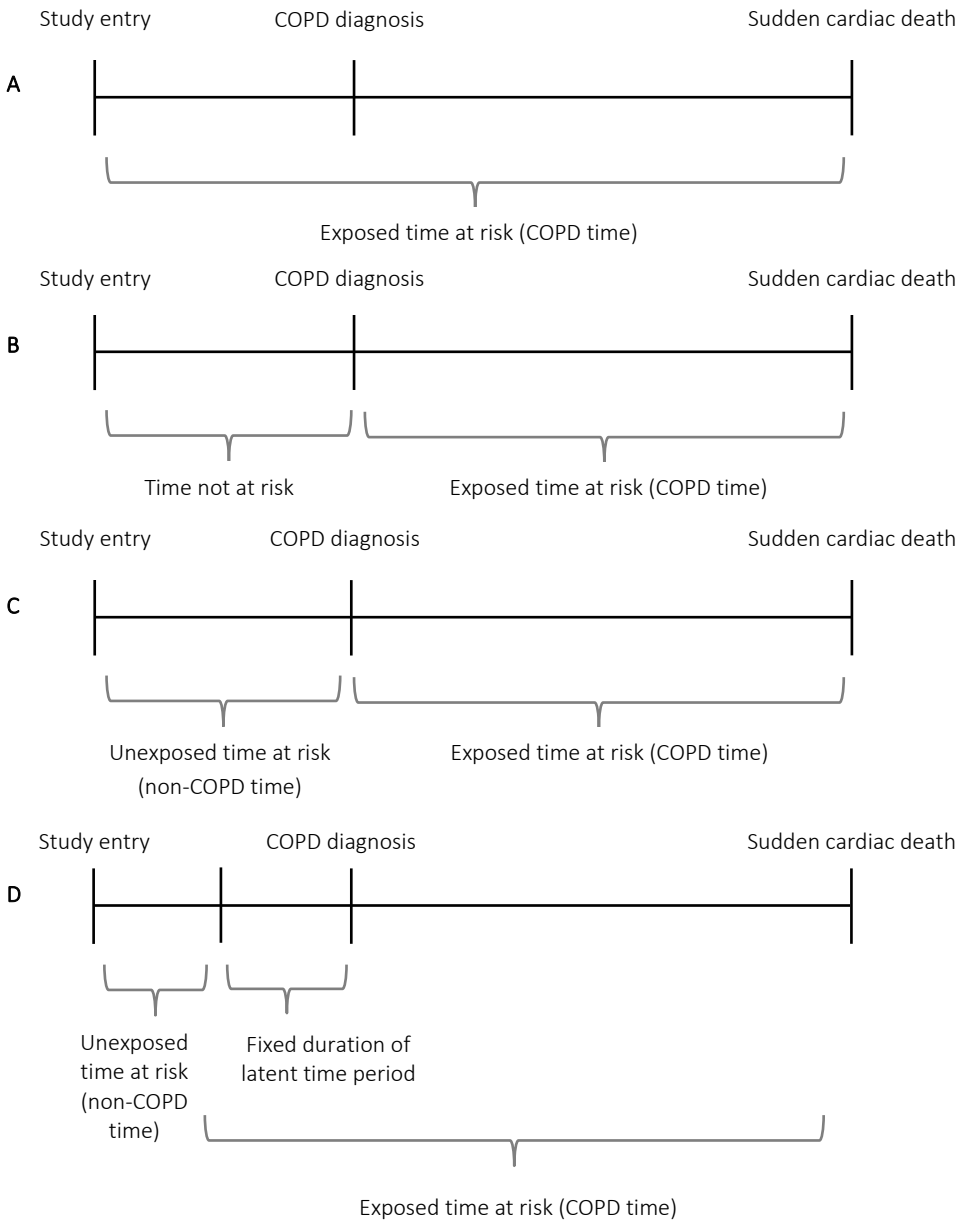

Figure 3. Four analysis methods to deal with immortal time bias

developing. In this period, adverse effects of the developing disease possibly already increase the risk of SCD. This might be the case in COPD, as patients have progressive airflow limitation and systemic inflammation, also before the disease is diagnosed. If the time until diagnosis of COPD is used as non-COPD time, this would therefore misclassify exposure. Since this misclassification would only occur in participants who develop COPD during follow-up, this misclassification would be non-random and would thus lead to information bias. A fourth option would be to determine the time at risk by taking into account a fixed latent time period, which is deducted from the date of COPD diagnosis, and is added to the COPD time (Figure 3D). However, since we cannot be certain about the duration of the latent period, this method will also introduce misclassification and we do not know whether this will lead to an underestimation or overestimation, because the duration of the latent period can differ per COPD patient. In our analysis, we used the second method as our main method. This method is more conservative than the third method, as in that method more non-COPD time at risk is added, while the number of SCD cases is the same. This results in a lower incidence rate of SCD in the non-COPD group compared to the second method, and thus a higher hazard ratio for SCD associated with COPD. However, as described previously, the second method has a large disadvantage, as it introduces immortal time bias in the analysis. Therefore, we did a sensitivity analysis using the third method, which indeed showed that with the second method we underestimated the associated risk of SCD in people with COPD. In conclusion, this analysis would either suffer from information bias or selection bias. By using two different methods, we aimed to provide a range of the most likely results if we had complete information about the latent period.

A third form of bias is confounding bias, which is a common problem in observational studies. Confounding refers to the possibility that the association between the exposure and outcome can be explained by a third variable, the confounder[96], as shown in Figure 4. In this thesis, all analyses were adjusted for multiple covariables and sometimes time-dependent covariables (which ideally requires marginal structural modelling[97]) to account for this problem. Despite our efforts to minimize bias, residual confounding is always possible in observational studies. The statistical methods to adjust for confounding are discussed below in the statistical analyses section.

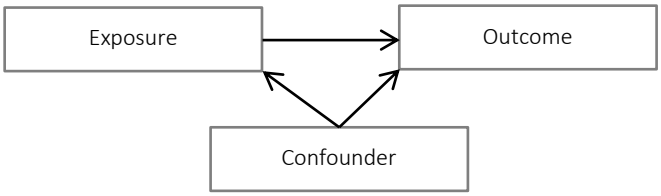

Figure 4. A schematic representation of confounding

Two special forms of confounding in pharmacoepidemiological studies are confounding by indication and confounding by contraindication. Confounding by indication refers to the situation in which the indication for treatment is associated with the outcome of interest independently from the drug [96,98]. That means, the indication is also a risk factor for the outcome in those who are not treated with the drug. This is a potential problem in **chapter 5.2**, **chapter 6.2**, and **chapter 6.3**. In **chapter 5.2**, the association between concomitant use of multiple QTc-prolonging drugs and ventricular repolarization duration was studied. But the indications for some of the drugs, such as atrial fibrillation and heart failure, are also a risk factor for prolonged repolarization[36,38]. A possible method to adjust for this would be to analyze the change in repolarization duration in the same person who starts or stops using multiple of these drugs concomitantly. Since measurements within the same person are compared, some of the confounding by indication will be controlled for. However, since the characteristics of a person (and sometimes also the indication) also change over time, this method cannot be seen as the Holy Grail. In this chapter, we were not able to use this method, because of the low number of users of multiple QTc-prolonging drugs in our population. In **chapter 6.2** and **chapter 6.3**, we stratified the analyses on use of the drug of interest. This is a good method to control for confounding by indication when studying effect modification by a drug. In an analysis of the effect of a specific drug it is not helpful, as one cannot observe the effect of the drug relative to nonusers. By stratifying on exposure, the confounding by indication is controlled for, as all persons in the exposed stratum have a similar indication for treatment.

Confounding by contraindication is also specific for pharmacoepidemiological studies, and it refers to the situation when drugs are not prescribed to patients with a particular contraindication, which is associated with the outcome[99]. Contrary to confounding by indication, which often leads to a spurious overestimation of the true risk, confounding by contraindication mostly leads to an underestimation of the true population risk. In **chapter 5.2**, we studied the association between concomitant use of multiple QTc-prolonging drugs. However, if a patient responds to use of one QTc-prolonging drug with a QTc prolongation of 20 milliseconds, the physician will probably not prescribe a second QTc-prolonging drug. But if a patient did not have QTc prolongation when using one QTc-prolonging drug, the physician might decide that it is safe to prescribe a second QTc-prolonging drug. This patient might not be susceptible to QTc prolongation at all, and will also not have QTc prolongation while using multiple QTc-prolonging drugs concomitantly. This might explain why we did not find an additive QTc-prolonging effect during use of multiple QTc-prolonging drugs. However, our study indicates that the recommendation to avoid concomitant use of multiple QTc-prolonging drugs might be too strict to apply to all patients.

Statistical analyses

In this thesis, we used three main statistical analyses: (i) a regular Cox proportional hazards model, (ii) a time-dependent Cox regression model, and (iii) repeated measurement analysis using generalized estimating equations (GEE). All these models can be used in a study population that is followed for a longer time period (Figure 5). The first model is used to study the association of a measurement or exposure at one point in time with the outcome after a period of follow-up[100] (Figure 5A). We used this method in **chapter 3.2**, **chapter 4.1**, and **chapter 4.3**. In **chapter 3.2**, we aimed to study the association between consistency of two QTc interval measurements and occurrence of SCD. The consistency was determined at one point in time, namely at the second ECG measurement. A disadvantage of this analysis is the introduction of survival bias, i.e. people have to survive until the second ECG measurement, and the survival is probably different for people with a normal or a prolonged QTc interval at the first measurement. This is further described in the discussion of this chapter. In **chapter 4.1**, we studied the association between echocardiographic parameters and SCD. The echocardiographic parameters were measured at two time points during follow-up. However, the second round of echocardiograms were made between 2009 and 2013, and since follow-up of SCD cases was done until January 1st 2011 we did not have enough follow-up and thus not enough cases to analyze these data. Therefore, we only used the first round of measurements, which were made between 2002 and 2005. In **chapter 4.3**, we studied the association between serum magnesium levels and SCD. For the first subcohort of the Rotterdam Study we had two magnesium measurements available, however for the second and third subcohort, we only had a baseline measurement available. Therefore, we chose to analyze only the second measurement of magnesium for the first subcohort, to ensure comparable follow-up time in the three subcohorts. Besides that, these magnesium measurements are analyzed using the same technique, which limits the inter-measurement variability in the analyses. A limitation of this statistical method is that exposure and covariables might be misclassified during a long follow-up time. The data are analyzed as if the exposure and covariables remain the same during the complete follow-up. However, most covariables are time-varying characteristics. For example, a participant might have a normal serum magnesium level at baseline, but a low serum magnesium five years later. With this analysis method, the person is considered to have a normal serum magnesium level during the entire follow-up time, which introduces misclassification. This misclassification is probably often non-differential, i.e. random, and it will most likely give an underestimation of the true effect. However, also random misclassification is a source of non-validity and an underestimated finding or a false-negative finding of an adverse event might sometimes be even more harmful than an overestimated finding or false-positive finding of a beneficial effect.

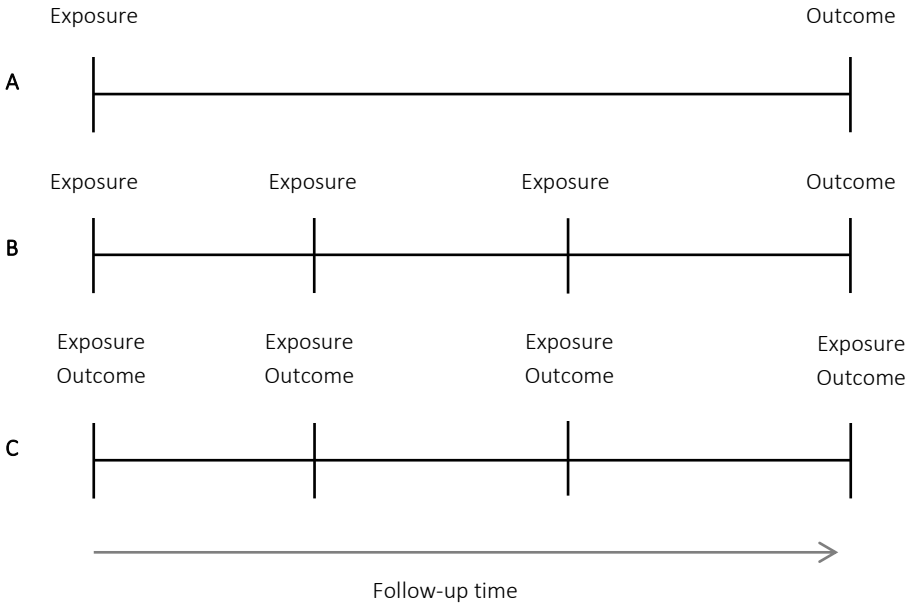

Figure 5. Three statistical analysis methods for studies with follow-up

In **chapter 3.3**, **chapter 4.2**, and **chapter 6.2**, we used a time-varying regression model[94]. With this method, at each time the event of interest occurs, i.e. the index date, all persons still in the cohort are sampled as control[94]. Subsequently at every index date, the exposure and covariables are determined again or data are retrieved from the measurement closest to the index date (Figure 5B). By using this method, the data on exposure and covariables are updated with the most recent information. This ensures a better classification of the exposure and covariables than the previous method with only a baseline measurement. However, this method might also introduce bias because the covariables may be influenced by the change from unexposed to exposed during follow-up.

In **chapter 5.2** and **chapter 6.3**, we used a repeated measurement analysis[101,102]. In these analyses, follow-up time is not taken into account, but at each time the outcome is assessed, e.g. QTc interval or heart rate, the association is assessed with the exposure at the same point in time, and the association is adjusted for covariables which are usually, but not necessarily, also measured at various time points during follow-up (Figure 5C). With this method the association between exposure and outcome is assessed cross-sectionally, but at different points in time during follow-up. Multiple measurements within one person are correlated, and the GEE corrects the results for the intra-personal correlation of measurements[102]. With this statistical method, reversed causality is a potential problem, i.e. since exposure and outcome are determined at the same point in time, we cannot be sure about the direction of the effect.

However, in these two chapters, the biological mechanisms point to an effect of the drug use on repolarization duration and heart rate, and not the other way around.

Significant association vs. prediction

In this thesis, we found significant associations between several risk factors and occurrence of SCD. However, the presence of a significant association does not necessarily mean that the factor is causal and/or can be used in prediction of the event. In order to assess the predictive value, the concordance statistic (c-statistic) or another measure of discriminative value has to be calculated[103-106]. The c-statistic is a value between 0.5 and 1.0, indicating the relationship between the sensitivity and specificity of a certain risk factor for the occurrence of SCD. Sensitivity refers to the proportion of true-positives, whereas specificity refers to the proportion of true-negatives. With a c-statistic of 0.5, the predictive factor has no more value than flipping a coin, whereas a c-statistic of 1.0 indicates perfect discrimination of high-risk vs. low-risk subjects. The reason that we did not calculate c-statistics in most studies in this thesis, is that determining the c-statistic on the same study population as in which the association is detected, will provide overestimated c-statistics values[107]. Therefore, the discriminative value of the investigated risk factors in this thesis should be determined in an independent cohort. We calculated the c-statistic in only one study, namely in **chapter 4.1**, about echocardiographic parameters and SCD. We were especially interested to see what fractional shortening would add to the predictive value, as we had high hopes that it would improve the predictive value. We acknowledged that it would provide overestimated results, but we intended to show an estimation of the added predictive value. However, there was almost no added predictive value of this marker to already known risk factors (0.78 vs. 0.79). This showed that fractional shortening is significantly associated with SCD, which can improve our understanding of the etiology, but this marker is not useful for the prediction of SCD.

Clinical implications

In this thesis, we studied several characteristics of the QTc interval, the most well-known ECG-derived risk factor for SCD and an important outcome in drug safety studies. In **chapter 3.2**, we showed that a prolonged QTc interval is not a consistent measurement in the overall population not using QTc-prolonging drugs, and that people with an inconsistently prolonged QTc interval do not have a significantly increased risk of SCD. In **chapter 5.2**, we showed that concomitant use of multiple QTc-prolonging drugs is not associated with an additively prolonged QTc interval compared to the use of one such drug. As a consequence of this, beneficial drugs may be withheld from patients unnecessarily. However, further research is needed to establish the safety of prescribing QTc-prolonging drugs to people with an inconsistently prolonged QTc interval or to people already using one QTc-prolonging drug. Since in the study on the

consistency of a prolonged QTc interval we excluded users of QTc-prolonging drugs, we did not investigate the response to drugs. Possibly, people with an inconsistently prolonged QTc interval do have a lower repolarization reserve, making them more susceptible to arrhythmias and SCD when using QTc-prolonging drugs. In the study on concomitant drug use and duration of the repolarization, the number of ECGs during use of multiple QTc-prolonging drugs was low. Future studies should point out whether it would be useful to re-measure the QTc interval after some time when a patient is treated with one or multiple QTc-prolonging drugs, but also when QTc-prolonging drugs are not prescribed because a prolonged QTc interval was measured before treatment was considered. As we have shown in **chapter 6.1**, the risk of drug-induced QTc-prolongation is also dependent on genetic variants. Studies into genetic variants might be helpful in further unravelling the individual response to QTc-prolonging drugs with regard to the risk of arrhythmias and SCD.

Two chapters in this thesis concerned pharmacogenetic studies. In **chapter 6.2**, we showed that the risk of SCD in digoxin use is modified by *ABCB1* gene variants, and in **chapter 6.3** we showed that the heart-rate lowering effect of diltiazem and verapamil is modified by a *CACNA1C* variant. These studies indicate that it might be useful to determine the genetic profile of patients in order to prescribe drugs more safely, or to be able to estimate which patients will not respond well to treatment and might benefit more from a different drug or a higher dosage. As genotyping has become less expensive over the last couple of years and more genetic variants are detected that are important for individualized treatment, genotyping patients for clinical practice has become more feasible. However, clinical validity has to be tested for specific drugs[108] and many ethical, legal, and financial issues have to be solved before this can be widely applied in clinical practice. Besides the value for clinical practice, identifying genetic variants associated with drug response is an important method to identify biological pathways through which the drug has its mechanism of action and through which adverse effects are caused. This enhances our biological knowledge and may be favorable for the development of new therapeutic approaches.

Three studies in this thesis aimed to study characteristics linked to atrial enlargement. In **chapter 3.3**, we determined significant associations between an increased amplitude, area, and terminal force of the P wave, and occurrence of SCD. An increased amplitude and area of the P wave originate from enlargement of the right atrium[109]. This could be the consequence of pulmonary hypertension, which has been associated with SCD previously, but the pathway of this association is not well understood[28]. Pulmonary hypertension can be caused by, for example COPD. In **chapter 4.2**, we showed that COPD was independently associated with occurrence of SCD. This could indicate that pulmonary hypertension and right atrial enlargement are intermediate factors in the association between COPD and SCD. An enlarged right atrium can be

measured on an echocardiogram, however during the echocardiographic measurements that we studied in chapter 4.1, no measures of the right atrium were taken. A higher P terminal force was also associated with SCD in chapter 3.3. A higher P terminal force is normally caused by enlargement of the left atrium. However, in chapter 4.1, we did not find a significant association between left atrium diameter and occurrence of SCD. A possible explanation could be that in patients with pulmonary emphysema, right atrial enlargement might present as a large terminal force through right atrial displacement caused by anatomical changes in the diaphragm and mediastinum[110]. In chapter 3.3, we adjusted for presence of COPD, thereby reducing confounding by this disease, after which we still found a significant association between a larger terminal force and occurrence of SCD. However, as we have shown in chapter 4.2, the severity of COPD is associated with SCD, and we could not adjust for this aspect. These studies seem to indicate that an enlarged right atrium is associated with occurrence of SCD. However, further studies with echocardiographic parameters of the right atrium are needed to investigate this association in more detail. As the right atrium was measured in the second echocardiographic measurement round in the Rotterdam Study (from 2009-2013), in the future we will be able to study this association. However, we need more follow-up time to gather enough SCD cases.

An important thing to remember is that in epidemiological studies, the outcome is a mean difference for the exposed vs. unexposed subjects in the total study population, while the individual effect might be larger. In chapter 5.2, we showed that the mean difference in QTc interval associated with use of one or multiple QTc-prolonging drugs is small, but the individual differences may be much larger. The same is valid for chapter 6.3, where we studied the association of the interaction between calcium channel blockers and a *CACNA1C* variant with heart rate. Per T allele in average users of verapamil, heart rate was decreased with four beats per minute. Again, the effect is likely to be stronger or weaker for individuals. The same holds for chapter 4.3, where only small mean differences in QTc interval and carotid intima media thickness were associated with a low serum magnesium.

Future perspectives

With the identification and characterization of risk factors, the ultimate goal is to be able to develop patient-level prediction models and subsequently treat who is at a high risk of arrhythmias and SCD. In general, population screening should meet several criteria: (i) the disease should be an important health problem, (ii) there should be an adequate treatment option, (iii) sufficient facilities for diagnosis and treatment should be available, (iv) there should be a recognizable latent period or early symptomatic phase, (v) there should be an adequate detection method, (vi) the detection method should be acceptable to the population, (vii) the natural course of the disease should be known, (viii) there should be consensus on who needs to

be treated, (ix) the screening, diagnosis, and treatment should be cost-effective, and (x) the process of detection should be a continuous process and not a one-time project[111-113]. Although population screening is not feasible for SCD, as only the first two criteria are met, these criteria reveal topics that should be subject to further study in order to improve prediction in high-risk patient populations, such as persons with cardiac disease.

The studies in this thesis were mostly done in a middle-aged and elderly population. Studies in an ageing population can provide us with insights that could also be useful in younger populations, however validity of these findings in younger populations has to be determined before these can be applied to clinical practice.

Final remarks

Although the incidence of SCD declined in our population over the past two decades, awareness and prevention should remain an important issue in clinical care as well as at the public health level. Additional preventive care, further improvement of cardiovascular disease management and better risk assessment will most likely lead to a further reduction in the health burden of SCD in the future. For a better risk assessment, an adequate screening method for an increased risk of SCD is needed. Many risk factors have been established, but an optimal prediction method still has to be developed. In this thesis, we aimed to identify new risk factors, but we also aimed to identify characteristics of known risk factors, to improve their validity and usefulness for clinical practice. With this thesis a step towards further improvement of the prediction of SCD is taken, in order to identify those patients with a truly increased risk of SCD and in this way increase the success rate of preventive measures.

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Chapter 7.2

Summary

Part 1 of this thesis gives a general introduction to risk factors for sudden cardiac death (SCD) and describes the main aims of this thesis. SCD is a common cause of death, with around four to five million cases annually worldwide. Determining which persons are at high risk for SCD remains difficult, due to lack of knowledge on individual risk factors and because in the majority of cases, SCD is the first manifestation of cardiac disease. Therefore, this thesis had three aims: (i) to study the current and past incidence of SCD, (ii) to identify new risk factors for SCD in the general population, and (iii) to study characteristics of already known risk factors for SCD. We studied electrocardiographic (ECG) risk factors, clinical risk factors, pharmacological risk factors, and pharmacogenetic risk factors. All studies (unless otherwise specified) in this thesis were performed as part of the Rotterdam Study, a prospective population-based cohort study of 14,926 middle-aged and elderly persons, followed from 1990-2010. Participants visit the research center every four to five years for several tests, they are continuously monitored for major morbidity and mortality, their genetic variants are determined, and their medication-dispensing data is available.

In part 2, we showed that the incidence rate of SCD was 4.2 per 1,000 person-years between 1990 and 2010 in people aged 45 years and older. This is comparable to other studies. We further showed that the incidence rate has declined over the past two decades, from 4.7 per 1000 person-years between 1990 and 2000, to 2.1 per 1000 person-years from 2001 until 2010. This is consistent with the observation that the incidence of cardiovascular mortality has declined over the past years in high-income countries. The decline in SCD incidence is probable due to better diagnosis and treatment of cardiovascular diseases.

In part 3, we studied several ECG-derived risk factors. In chapter 3.1, we systematically reviewed the literature concerning markers of QT interval variability; a relatively novel ECG-derived risk factor. Nine different markers were described in the literature which could be divided into four groups according to two characteristics: (i) whether the variability was measured between consecutive beats or over a number of beats, and (ii) whether QT variability was normalized for heart rate. Significant associations were found between QT variability markers and the occurrence of arrhythmias and SCD, indicating that these markers could be useful determinants to identify people at risk of arrhythmias and SCD. Which marker and methodology is optimal is not yet clear. Chapter 3.2 shows that a prolonged heart-rate corrected QT (QTc) interval is an inconsistent finding, as one-third of the people with a prolonged QTc interval on a baseline ECG measurement have a normal QTc interval duration on a subsequent measurement after a median of 1.8 years. Moreover, an inconsistently prolonged QTc interval was not associated with an increased risk of SCD compared to a consistently normal QTc interval. People with a consistently prolonged QTc interval did have an increased risk of SCD. None of these persons used QTc-prolonging drugs during ECG recording. Currently, a prolonged QTc interval is a

contraindication for the use of QTc-prolonging drugs, as these might cause an additive QTc prolongation, and subsequently may give rise to arrhythmias. This study demonstrates that the decision to treat or abstain may differ over time and that a single QTc prolongation should not be seen as an ever-lasting contraindication. In chapter 3.3, we showed that a higher amplitude, area, and terminal force of the P wave were associated with a higher risk of SCD. These are markers of left and right atrial enlargement. The duration of the PR interval and the P wave were not associated with occurrence of SCD. Although the association between causes of atrial enlargement and occurrence of SCD has been shown before, an ECG is an easier and cheaper method for risk stratification than an echocardiogram. This study may therefore provide risk factors that are more useful and efficient for clinical practice.

Part 4 gives a description of studies on clinical risk factors for SCD. In chapter 4.1, we studied the association between echocardiographic parameters and SCD. It is already known that persons with heart failure have an increased risk of SCD. However, the association between subclinical decrease in cardiac function and SCD in the general population has not been studied before. We found that a lower fractional shortening, i.e. the relative difference between the end-diastolic and the end-systolic dimension of the left ventricle on the echocardiogram, was associated with a higher risk of SCD in a population free of heart failure. Despite the fact that the added value of this factor to already known risk factors for prediction was small, these findings increase our understanding of the etiology of SCD. In chapter 4.2, we showed that chronic obstructive pulmonary disease (COPD) was an independent risk factor for SCD. This association has already been described in high-risk patient populations, such as COPD patients with a history of myocardial infarction, but not yet in the general population. Furthermore, our study showed that a more severe grade of COPD, measured by a longer duration of the disease, a higher frequency of exacerbations, and higher levels of systemic inflammation, was associated with a higher risk of SCD. These results could enhance risk stratification in the general population and pave the path to targeted preventive actions for patients with COPD. In chapter 4.3, we showed that a low serum magnesium was associated with occurrence of SCD. Magnesium is known for its role in the electrical stability and energy balance of myocytes, and a low serum magnesium has been associated with atrial and ventricular arrhythmias. We found significant associations of low serum magnesium with an increased risk of arrhythmias and atherosclerosis. We used a prolonged QTc interval as a marker for an increased risk of arrhythmias, and carotid intima media thickness as a marker for atherosclerosis. However, we found that the association between a low serum magnesium and SCD is not fully explained by a prolonged QTc interval and an increased carotid intima media thickness.

In part 5, we studied a pharmacological risk factor, namely concomitant use or prescriptions of QTc-prolonging drugs. Regulatory agencies recommend to avoid concomitant use of these

drugs, as this might give rise to additive QTc prolongation and an increased risk of arrhythmias, however evidence to support this advice is lacking. In chapter 5.1, we studied whether physicians adhere to the advice of regulatory authorities to avoid concomitant prescriptions of QTc-prolonging drugs. We studied this in the Integrated Primary Care Information (IPCI) project, a database of more than 600 general practitioners' databases, in which data on approximately 1.7 million patients is collected. We found that combinations of QTc-prolonging drugs are often prescribed, with up to seven drugs concomitantly. This indicates that either physicians are not aware of this advice, they choose not to adhere to it, they measure the QTc interval in every patient and prescribe combinations only to persons with a normal QTc interval, or that the patients are instructed to stop using their other medication. Chapter 5.2 describes that concomitant use of QTc-prolonging drugs is not necessarily associated with an additive QTc prolongation. However, only few ECGs were recorded during concomitant use of multiple of these drugs in the Rotterdam Study. Whether the advice to avoid concomitant use of multiple QTc-prolonging drugs is too strict and has to be adapted, should be subject to further research. We also showed that there is a large difference in the grade of QTc prolongation between two classes of QTc-prolonging drugs: for known QTc-prolonging drugs 15 milliseconds, for possible QTc-prolonging drugs 3 milliseconds. Therefore, we recommend that future studies do not combine the two categories into one exposure class, as this leads to misclassification of exposure.

Part 6 describes the pharmacogenetic background of QTc prolongation, SCD, and heart rate. In chapter 6.1, we systematically reviewed the literature regarding the genetic background of drug-induced QTc prolongation. Both genes with pharmacokinetic and pharmacodynamic effects of drugs on QTc prolongation were identified in the literature. Pharmacokinetics-related genetic susceptibility is mainly characterized by variation in genes encoding drug-metabolizing cytochrome P450 enzymes or drug transporters. The pharmacodynamic component of genetic susceptibility is mainly characterized by genes known to be associated with QT interval duration in the general population and genes in which the causal mutations of congenital long QT syndromes are located. It is expected that with larger sample sizes, and better exposure and outcome definitions, more variants can be detected. In chapter 6.2, we studied the association of the interaction between *ABCB1* gene variants and digoxin with occurrence of SCD. Digoxin use is associated with an increased risk of SCD, and the *ABCB1* gene variants have been associated with serum digoxin levels. We showed that in users of digoxin, the risk of SCD was significantly higher in homozygous variant allele carriers of the *ABCB1* variants. In non-users of digoxin, there was no association between the genetic variant and occurrence of SCD. The increased risk of SCD can either originate from an independent effect of the gene variants in digoxin users, or because the risk of SCD only increases above a certain threshold of the digoxin serum concentration which is only reached in homozygous variant allele carriers. If the

increased risk of SCD associated with the genetic variants is independent of the serum digoxin concentrations, testing genetic variants in new users of digoxin might add to safe use of digoxin with regard to the risk of SCD. In chapter 6.3, we investigated the association of the interaction between a *CACNA1C* variant and diltiazem and verapamil use with heart rate. Diltiazem and verapamil are known for their heart-rate lowering effect and work through the calcium channels in the cardiomyocytes that are encoded by the *CACNA1C* gene. A higher heart rate has been associated with a higher risk of SCD. We showed that in users of verapamil or diltiazem, the number of variant alleles was significantly associated with a lower heart rate. In nonusers there was no significant association between the genetic variants and heart rate. Although the mean differences associated with the variant allele in verapamil and diltiazem users were small, individual differences might be larger, and this might influence effectiveness of and adherence to treatment.

Lastly, part 7 provides an overview of the main findings of this thesis, the methodological considerations of the studies, the clinical implications, and future perspectives of this thesis. Although the incidence of SCD declined in our population over the past two decades, awareness and prevention should remain an important issue in clinical care as well as at public health level. Additional preventive care, further improvement of cardiovascular disease management and better risk assessment will most likely lead to a further reduction in the health burden of SCD in the future. The studies described in this thesis have identified new risk factors for SCD and have described characteristics of already known risk factors. A next step towards a better risk stratification for SCD would be the development of a prediction model. The findings from this thesis can be used for the basis of such a prediction model.

Chapter 7.3

Samenvatting

Deel 1 van dit proefschrift geeft een introductie op risicofactoren voor acute hartdood en beschrijft de hoofddoelen van dit proefschrift. Acute hartdood is een veel voorkomende doodsoorzaak, met ongeveer vier tot vijf miljoen gevallen wereldwijd per jaar. Bepalen wie een hoog risico lopen op acute hartdood blijft moeilijk, omdat er een gebrek is aan kennis over specifieke risicofactoren en omdat acute hartdood in de meerderheid van de gevallen de eerste uiting van een hartziekte is. Daarom had dit proefschrift drie doelen: (i) het vóórkomen van acute hartdood nu en in het verleden te bestuderen, (ii) het identificeren van nieuwe risicofactoren voor acute hartdood in de algemene populatie, en (iii) het bestuderen van eigenschappen van reeds bekende risicofactoren. We hebben onderzoek gedaan naar risicofactoren bepaald op het electrocardiogram (ECG, ook wel hartfilmpje genoemd), klinische risicofactoren, farmacologische risicofactoren en farmacogenetische risicofactoren. Alle studies (tenzij anders aangegeven) in dit proefschrift werden uitgevoerd binnen de Rotterdam Studie (ook wel het Erasmus Rotterdam Gezondheid Onderzoek of ERGO genoemd). Dit is een prospectieve cohortstudie in de algemene populatie van 14.926 mensen van middelbare en hogere leeftijd, die gevolgd zijn van 1990 tot 2010. Deelnemers aan dit onderzoek komen elke vier tot vijf jaar op het onderzoekscentrum waar ze verschillende onderzoeken ondergaan. Ze worden daarnaast voortdurend gevolgd met betrekking tot het vóórkomen van ziekte en overlijden. Hun genetische variatie is bepaald en de gegevens van de apotheek aangaande geneesmiddel-verstrekkingen zijn beschikbaar.

In **deel 2** beschrijven we dat de incidentie van acute hartdood tussen 1990 en 2010 4,2 per 1.000 persoonsjaren was bij personen van 45 jaar en ouder. Dit is vergelijkbaar met andere studies. We laten verder zien dat de incidentie is afgenomen over de afgelopen 20 jaar, van 4,7 per 1.000 persoonsjaren tussen 1990 en 2000 naar 2,1 per 1.000 persoonsjaren tussen 2001 en 2010. Dit is een bevinding die strookt met het gegeven dat de mortaliteit door hart- en vaatziekten in de afgelopen jaren gedaald is in ontwikkelde landen. De afname van acute hartdood komt waarschijnlijk door betere diagnose en behandeling van hart- en vaatziekten.

In **deel 3** hebben we verschillende risicofactoren bestudeerd die worden afgeleid van het ECG. In **hoofdstuk 3.1** hebben we de beschikbare literatuur samengevat met betrekking tot indicatoren voor QT interval variabiliteit, een relatief nieuwe risicofactor. We vonden negen verschillende indicatoren, die we konden indelen in vier groepen aan de hand van twee kenmerken; (i) of de variabiliteit gemeten werd op opeenvolgende slagen of over een vast aantal slagen, en (ii) of QT variabiliteit werd gecorrigeerd voor de hartfrequentie. Significante associaties werden beschreven tussen indicatoren voor QT interval variabiliteit en het optreden van hartritmestoornissen of acute hartdood. Dit geeft aanwijzingen dat deze indicatoren nuttig zouden kunnen zijn om mensen met een hoog risico op hartritmestoornissen en acute hartdood te identificeren. Welke indicator daarvoor het beste is, en welke methode moet worden

gebruikt om die te meten is nog niet duidelijk. Hoofdstuk 3.2 laat zien dat een verlengd voor hartfrequentie gecorrigeerd QT (QTc) interval een inconsistente bevinding is. Eén-derde van de mensen met een verlengd QTc interval op een ECG gemaakt aan het begin van de studie heeft namelijk een normaal QTc interval op een daaropvolgend ECG, dat na 1,8 jaar (mediaan) gemaakt werd. Verder laten we zien dat een inconsistent verlengd QTc interval (op één meting een verlengd QTc interval, terwijl er op het andere ECG een normale QTc tijd wordt gemeten) niet geassocieerd is met een verhoogd risico op acute hartdood. Mensen met een consistent verlengd QTc interval hadden daarentegen wel een verhoogd risico op acute hartdood. Niemand van de studiepersonen in deze analyse gebruikte QTc-verlengende middelen. Tegenwoordig is een verlengd QTc interval een contra-indicatie voor het gebruik van QTc-verlengende middelen, omdat deze een additieve verlenging van het QTc interval kunnen veroorzaken en daardoor een verhoogd risico op hartritmestoornissen geven. Deze studie laat zien dat de beslissing om een middel wel of niet voor te schrijven kan veranderen over de tijd, en dat een eenmalige meting van een verlengd QTc interval niet mag gelden als een levenslange contra-indicatie. In hoofdstuk 3.3 laten we zien dat een hogere amplitude, een groter oppervlak en een grotere negatieve deflectie van de P golf geassocieerd is met een hoger risico op acute hartdood. Dit zijn indicatoren van linker en rechter atrium vergroting. De lengte van het PR interval en de P golf waren niet geassocieerd met het vóórkomen van acute hartdood. Hoewel de associatie tussen oorzaken van atriale vergroting en het vóórkomen van acute hartdood eerder aangetoond is, is een ECG een gemakkelijkere en goedkopere manier om in de dagelijkse praktijk een inschatting te maken van het risico op acute hartdood dan het maken van een echo van het hart. Deze studie voorziet daarmee mogelijk in kennis over risicofactoren die nuttiger en efficiënter zijn voor de klinische praktijk.

Deel 4 beschrijft verschillende studies naar klinische risicofactoren voor acute hartdood. In hoofdstuk 4.1 bestudeerden we de associatie tussen parameters op de echo van het hart en acute hartdood. Het is al bekend dat mensen met hartfalen een verhoogd risico op acute hartdood hebben, maar de associatie tussen subklinische verlaging in hartfunctie (dus voordat mensen klachten krijgen van hartfalen) en het vóórkomen van acute hartdood is nog niet bestudeerd in de algemene populatie. We vonden dat een lagere fractional shortening, dat is het relatieve verschil tussen de eind-diastolische en eind-systolische dimensie van het linker ventrikel, was geassocieerd met een hoger risico op acute hartdood in een groep van mensen zonder bekend hartfalen. Hoewel de toegevoegde waarde van deze factor op het voorspellen van acute hartdood laag is, vertelt deze bevinding ons wel meer over het optreden van acute hartdood. In hoofdstuk 4.2 laten we zien dat chronische obstructieve longziekte (ook COPD genoemd) een onafhankelijke risicofactor is voor acute hartdood. Deze associatie is al beschreven in hoog-risico patiënten, zoals mensen met een voorgeschiedenis van een hartinfarct, maar nog niet in de algemene populatie. Verder laat deze studie ook zien dat een

ernstigere vorm van COPD, bepaald aan de hand van een langere duur van de ziekte, een hogere frequentie van exacerbaties (verergeringen) en een hoger niveau van systemische ontstekingsmarkers, zoals C-reactief proteïne (CRP), geassocieerd is met een hoger risico op acute hartdood. In **hoofdstuk 4.3** tonen we aan dat een laag magnesium gehalte in het bloed geassocieerd is met acute hartdood. Magnesium speelt een rol in de elektrische stabiliteit en energiebalans in de hartspiercellen. Daarnaast is een laag magnesium geassocieerd met atriale en ventriculaire ritmestoornissen. Wij vonden significante associaties van laag magnesium met een verhoogd risico op hartritmestoornissen en aderverkalking (atherosclerose). We gebruikten een verlengd QTc interval als marker voor een verhoogd risico op hartritmestoornissen, en de dikte van de intima media van de arteria carotis als marker voor atherosclerose. Maar de associatie tussen een laag magnesium in het bloed en acute hartdood werd niet volledig verklaard door verlenging van het QTc interval en een toegenomen intima media dikte van de arteria carotis.

In **deel 5** bestudeerden we een farmacologische risicofactor, namelijk gelijktijdige prescripties van meerdere QTc-verlengende middelen. Geneesmiddelautoriteiten adviseren om gelijktijdige prescripties van meerdere van deze middelen te vermijden, omdat dit een additieve QTc verlenging kan geven en daardoor een verhoogd risico op hartritmestoornissen. Momenteel is er echter geen bewijs om dit advies te ondersteunen. In **hoofdstuk 5.1** hebben we bestudeerd of artsen zich aan dit advies houden om deze middelen niet gelijktijdig voor te schrijven. We hebben dit bestudeerd in het Integrated Primary Care Information (IPCI) project. Dit is een database van meer dan 600 huisartsbestanden in Nederland. Daarin is geanonimiseerde informatie van ongeveer 1,7 miljoen patiënten verzameld. We vonden dat combinaties van QTc-verlengende geneesmiddelen regelmatig worden voorgeschreven, tot wel zeven geneesmiddelen tegelijkertijd. Dit geeft aan dat artsen zich niet bewust zijn van dit advies, ervoor kiezen zich er niet aan te houden, bijvoorbeeld omdat ze het QTc interval meten in alle patiënten en combinaties alleen voorschrijven aan personen met een normale QTc tijd, of omdat ze patiënten instrueren te stoppen met hun andere medicatie. **Hoofdstuk 5.2** beschrijft dat gelijktijdig gebruik van meerdere QTc-verlengende middelen niet noodzakelijkerwijs geassocieerd is met een additieve verlenging van het QTc interval. Echter, in de Rotterdam Studie zijn maar weinig ECGs gemaakt tijdens gelijktijdig gebruik van meerdere QTc-verlengende middelen. Of het advies om gelijktijdig gebruik te vermijden te strikt is en zou kunnen worden aangepast, moet verder onderzocht worden. Voorts lieten we in deze studie zijn dat er een groot verschil in QTc verlenging is tussen twee categorieën geneesmiddelen: voor de bekende QTc-verlengende middelen 15 milliseconden en voor de mogelijk QTc-verlengende middelen 3 milliseconden. Daarom raden we aan dat deze twee categorieën in toekomstig onderzoek niet worden gecombineerd tot één categorie, omdat dit leidt tot misclassificatie.

Deel 6 beschrijft de farmacogenetische achtergrond van QTc verlenging, acute hartdood en de hartfrequentie. In hoofdstuk 6.1 hebben we de literatuur samengevat betreffende de genetische achtergrond van geneesmiddel-geïnduceerde QTc verlenging. We identificeerden zowel genen met farmacokinetische als farmacodynamische effecten op QTc verlenging. Farmacokinetiek-gerelateerde effecten worden voornamelijk gekarakteriseerd door variatie in genen die coderen voor enzymen die medicijnen metaboliseren of transporteren. De farmacodynamische component wordt gekarakteriseerd door genen die geassocieerd zijn met de lengte van het QTc interval in de algemene populatie en door genen waarin de causale mutaties liggen van congenitale QT verlengingssyndromen. Het is te verwachten dat met grotere studiepopulaties en met betere definities van de determinanten en uitkomsten in een studie meer varianten kunnen worden ontdekt. In hoofdstuk 6.2 hebben we de associatie bestudeerd tussen een interactie van varianten in het *ABCB1* gen met digoxine en het vóórkomen van acute hartdood. Gebruik van digoxine is geassocieerd met een verhoogd risico op acute hartdood, en varianten in het *ABCB1* gen zijn geassocieerd met digoxinespiegels in het bloed. Wij laten zien dat bij gebruikers van digoxine, het risico op acute hartdood significant hoger was in personen die het variant allel van de *ABCB1* varianten dragen. Bij personen die geen digoxine gebruiken, is er geen associatie tussen de genetische varianten en het vóórkomen van acute hartdood. Het hogere risico kan komen doordat er in digoxinegebruikers een effect is onafhankelijk van de bloedconcentratie, maar het kan ook dat het risico alleen verhoogd is in mensen die een spiegel boven een bepaalde waarde hebben, die alleen bereikt wordt in mensen met twee variant allelen. Als het effect in digoxinegebruikers onafhankelijk van de digoxinespiegels optreedt, kan het genotyperen van patiënten bij wie digoxine als behandeling wordt overwogen bijdragen aan een veiliger gebruik van dit geneesmiddel. In hoofdstuk 6.3 hebben we de associatie tussen een interactie van een variant in het *CACNA1C* gen met diltiazem en verapamil en de hartfrequentie onderzocht. Van diltiazem en verapamil is bekend dat ze de hartfrequentie beïnvloeden. Deze geneesmiddelen werken via de L-type calcium kanalen in de hartspiercellen. Deze kanalen worden gecodeerd door het *CACNA1C* gen. Een hogere hartfrequentie is geassocieerd met een hoger risico op acute hartdood. Wij laten zien dat in gebruikers van diltiazem en verapamil een hoger aantal variant allelen geassocieerd is met een lagere hartfrequentie. In personen die deze middelen niet gebruiken zagen we geen effect van de genetische variatie. Hoewel de gemiddelde verschillen klein zijn, kunnen individuele verschillen wel relatief groot zijn en dit kan de effectiviteit en therapietrouw beïnvloeden.

Ten slotte geven we in deel 7 een overzicht van de belangrijkste bevindingen in dit proefschrift, de methodologische overwegingen van de studies, de klinische implicaties en de perspectieven voor de toekomst. Hoewel de incidentie van acute hartdood gedaald is in de afgelopen 20 jaar, blijft aandacht en preventie belangrijk, in zowel de klinische setting als in de algemene volksgezondheidszorg. Additionele preventieve maatregelen, verdere verbetering van de

behandeling van hart- en vaatziekten en een betere inschatting van het risico op acute hartdood zullen waarschijnlijk kunnen leiden tot een verdere afname van de kosten van de gezondheidszorg in de toekomst. De studies die beschreven staan in dit proefschrift hebben nieuwe risicofactoren voor acute hartdood geïdentificeerd en kenmerken van reeds bekende risicofactoren beschreven. Een volgende stap in de richting van een betere inschatting van het risico op acute hartdood is de ontwikkeling van een voorspellend model. De bevindingen van dit proefschrift kunnen daarvoor gebruikt worden.

Part VIII

Appendices

Dankwoord

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PhD portfolio

Name PhD student:	Maartje Nieske den Besten - Niemeijer
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PhD training	Year
General courses	
- Master of Science in Clinical Epidemiology, Netherlands Institute of Health Sciences, Rotterdam, the Netherlands	2012-2015
Specific courses	
- SNPs and Human Diseases, Molmed, Rotterdam, the Netherlands	2012
- Intensive Care Research, COEUR, Erasmus MC, Rotterdam, the Netherlands	2014
Seminars and workshops	
- Research seminars, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands	2012-2015
- PhD day, Erasmus MC, Rotterdam, the Netherlands	2014-2015
National and international conferences	
- CHARGE Investigators Meeting, Rotterdam, the Netherlands	2013
- CHARGE Investigators Meeting, Los Angeles, USA	2014
- 31st International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Boston, USA	2015
Presentations	
- Poster presentation "Variability of the QT interval: a promising new marker on the ECG. A systematic review." ICPE, Montreal, Canada	2013
- Oral presentation "A *CACNA1C* variant is associated with a modified response of heart rate in users of calcium channel blockers: the Rotterdam Study" ICPE, Boston, USA	2015
- Oral presentation "*ABCB1* gene variants, digoxin and risk of sudden cardiac death in a general population" ICPE, Boston, USA	2015
- Poster presentation "Drugs and ventricular repolarization in a general population: the Rotterdam Study" ICPE, Boston, USA	2015

- Poster presentation "*ABCB1* gene variants, digoxin and risk of sudden cardiac death in a general population" ESC, London, UK 2015

Teaching activities

Lecturing

- "Een wetenschapper op bezoek" as part of a series of lessons: "Van piep tot stok" on primary schools in Rotterdam, Wetenschapsknooppunt Erasmus University Rotterdam 2014-2015

Supervising practicals and excursions, Tutoring

- Pharmaco-epidemiology and drug safety, NIHES, Rotterdam, the Netherlands 2014-2015
- Principles of research in medicine, NIHES, Rotterdam, the Netherlands 2014-2015
- Grading essays Bachelor 3 Medicine, Erasmus MC, Rotterdam, the Netherlands 2014-2015
- Biostatistical methods I, NIHES, Rotterdam, the Netherlands 2014

Supervising Master thesis

- Juan Carlos Sanchez Calvo, Masterstudent NIHES, Rotterdam, the Netherlands. Diuretic use and sudden cardiac death in the Rotterdam Study: is there a role for systolic function parameters? 2013-2014

List of publications

Manuscripts that form the basis of this thesis

Chapter 2.1

Maartje N Niemeijer, Marten E van den Berg, Maarten JG Leening, Albert Hofman, Oscar H Franco, Jaap W Deckers, Jan Heeringa, Peter R Rijnbeek, Bruno H Stricker, Mark Eijgelsheim. Declining incidence of sudden cardiac death from 1990-2010 in a general middle-aged and elderly population: the Rotterdam Study. *Heart Rhythm* 2015 Jan; 12(1):123-129. Epub 2014 Sep 30.

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Chapter 3.2

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Chapter 3.3

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Chapter 4.2

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Chapter 4.3

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Chapter 5.1

Maartje N Niemeijer, Ingrid Leal, Marten E van den Berg, Mark Eijgelsheim, Miriam CJM Sturkenboom, Bruno H Stricker, Peter R Rijnbeek. Patterns of concomitant prescriptions of multiple QT-prolonging drugs in a general population in the Netherlands between 2007 and 2013. *Submitted*

Chapter 5.2

Maartje N Niemeijer, Marten E van den Berg, Oscar H Franco, Albert Hofman, Jan A Kors, Bruno H Stricker, Mark Eijgelsheim, Peter R Rijnbeek. Drugs and ventricular repolarization in a general population: the Rotterdam Study. *Pharmacoepidemiology and Drug Safety* 2015 Oct; 24(10):1036-1041. Epub 2015 Aug 6.

Chapter 6.1

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Layal Chaker*, Marten E van den Berg*, **Maartje N Niemeijer**, Oscar H Franco, Albert Hofman, Peter R Rijnbeek, Jaap W Deckers, Mark Eijgelsheim, Bruno H Stricker, Robin R Peeters. Thyroid function and sudden cardiac death: a population-based cohort study. *Submitted*

Honghuang Lin, **Maartje N Niemeijer***, Martina Mueller-Nurasyid*, et al. Gene-gene analyses for atrial fibrillation reveals an interaction between *HCN4* and *SLC28A1*. *Submitted*

Marten E van den Berg, **Maartje N Niemeijer**, Jaap W Deckers, Oscar H Franco, Albert Hofman, Jan A Kors, Bruno H Stricker, Mark Eijgelsheim, Peter R Rijnbeek. QT variability as risk factor for total mortality and sudden cardiac death in a population-based cohort. *Submitted*

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Marten E van den Berg, **Maartje N Niemeijer**, Mark Eijgelsheim, Jaap W Deckers, Albert Hofman, Oscar H Franco, Bruno H Stricker, Jan A Kors, Peter R Rijnbeek. Clinical and electrocardiographic risk factors combined can improve the prediction of sudden cardiac death in an older population: the Rotterdam Study. *In preparation*

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Other projects

Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE)

- Analysis on occurrence of atrial fibrillation using exome chip
- Genome-wide association study using 1000-Genomes imputed data on atrial fibrillation
- Mendelian randomization on the association between body mass index and atrial fibrillation

Other

- Genome-wide association study using Genome of the Netherlands imputed data on electrocardiogram traits
- Genome-wide association study using 1000-Genomes imputed data on liver fibrosis

About the author

Marieke Niemeijer was born on February 28th 1988 in Dordrecht, the Netherlands. She graduated in 2006 from "Studiehuis Molenplein" in Den Helder and started medical school at the VU Medical Centre in Amsterdam. In 2009 she received her Bachelor of Science degree in Medicine. After receiving her Master of Science and Medical degree in October 2012, she started the work described in this thesis under the supervision of prof.dr. BH Stricker at the department of Epidemiology, Erasmus MC (head: prof.dr. A Hofman). In August 2015 she obtained a Master of Health Sciences in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES). As of October 2015 she is in training to become a medical doctor specialized in obstetric medicine in "Medisch Centrum Haaglanden" in the Hague.