

Pharmacological and Biological Risk Factors for Cardiac Arrhythmias

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Pharmacological and Biological Risk Factors for Cardiac Arrhythmias

Pharmacologische en biologische risicofactoren voor cardiale aritmieën

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Chapter 1

General introduction

Sudden death is among the most common causes of death in developed countries. Sudden death from cardiac causes accounts for approximately 50% of all deaths from cardiovascular diseases and 20% of all deaths.[1-3] The majority (80-85%) of sudden cardiac deaths are caused by acute ventricular arrhythmia.[4] An important potential cause of ventricular arrhythmia is prolongation of ventricular repolarization, for instance, as is observed in the rare and genetically-determined 'congenital long QT syndrome'.[5, 6] Prolongation of ventricular repolarization may result in early after depolarizations (EAD), which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmia.[7-10]

Ventricular repolarization disturbances

The QT interval on the electrocardiogram (ECG), measured from the Q-top, the beginning of the QRS complex, until the end of the T wave, is the traditional measurement for assessing the duration of ventricular de- and repolarization. Although there are several formulas to adjust for heart rate (e.g. according to Fridericia or RR-adjusted QT interval), Bazett's formula ($QT_c = QT/\sqrt{RR}$) is most often used since decades (figure 1).[11] According to European regulatory guidelines, QTc prolongation can be distinguished into 3

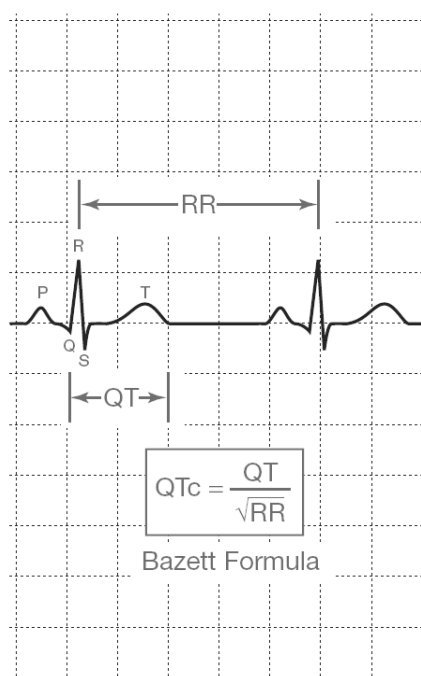


Figure 1 Corrected QT interval using Bazett's formula. Source: Al-Khatib SM et al. JAMA 2003;289:2120-2127.

clinically-relevant categories. For men, the cut-off points are less than 430 milliseconds (ms) (normal), 430-450 ms (borderline) and more than 450 ms (prolonged), and for women less than 450 ms (normal), 450-470 ms (borderline), and more than 470 ms (prolonged).[12]

The electrical activity of the heart is mediated through channels that regulate the flow of ions in and out of cardiac cells. The inward depolarizing currents, mainly through Na^+ and Ca^{2+} channels, result in normal depolarization, and outward repolarizing currents, mainly through K^+ channels, result in repolarization (figure 2). Malfunction of ion channels may lead to an intracellular excess of positively charged ions, i.e. either by an inadequate outflow of potassium ions or by an excessive inflow of sodium ions. The

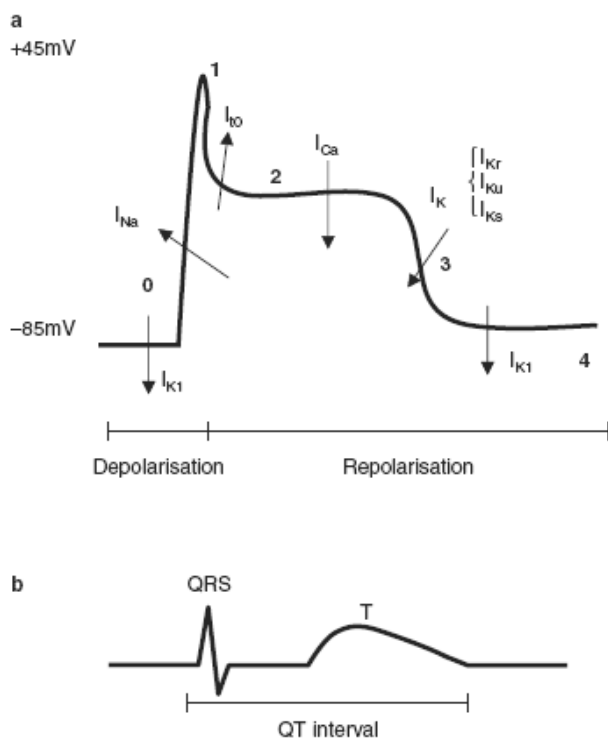


Figure 2 The cardiac action potential. (a) action potential showing the five phases of cardiac depolarisation and repolarisation with ion current directions during activation of the different ion channels; (b) ECG.

Phase 0: Rapid depolarisation is caused by a large inward current of sodium ions (I_{Na}). Repolarisation consists of three phases (1-3): **Phase 1:** Rapid repolarisation phase is caused by inactivation of I_{Na} and the transient efflux of potassium ions (I_{to}); **Phase 2:** Plateau phase, which is a reflection of a balance between the influx of calcium ions through L-type calcium channels (I_{Ca}) and outward repolarising potassium currents (I_{K}); **Phase 3:** Late repolarisation phase results from the efflux of potassium (I_{Kr} , I_{Ku} , I_{Ks}). **Phase 4:** Resting potential is maintained by the inward rectifier potassium current (I_{K1}).

I_{Ca} = calcium current; I_{K} = potassium current; I_{K1} = inwardly rectifying potassium current; I_{Na} = depolarising sodium current; I_{to} = transient outward potassium current; I_{Kr} = rapidly activating delayed rectifier potassium current; I_{Ks} = slowly activating delayed rectifier potassium current; I_{Ku} = ultra rapidly activating delayed rectifier potassium current. Source: Titier K et al. *Drug Safety* 2005;28:35-51.

intracellular excess of positively charged ions prolongs ventricular repolarization and results in QT interval prolongation on the ECG.[13]

Risk factors for repolarization disturbances

Although risk factors of QTc prolongation can be divided into two main categories, i.e. congenital and acquired abnormalities, repolarization disturbances often occur due to gene-environment interactions.[9]

Congenital Long QT syndrome

The congenital long QT syndrome (LQTS) is an inherited disease characterized by prolongation of ventricular repolarization, which is manifested by QT prolongation, syncopal episodes, malignant ventricular tachycardia and fibrillation.[6] The prevalence of LQTS is estimated to be approximately 1 in 2000 to 2500 live births.[14, 15] The autosomal dominant form (Romano-Ward syndrome) is far more common than the recessive form, which is associated with deafness (Jervell and Lange-Nielsen syndrome).[16] The majority of LQTS patients is asymptomatic and are either discovered incidentally on an ECG, by family history or after they survived an episode of syncope or severe ventricular arrhythmia. The prognosis of untreated patients is considered to be quite poor. It is estimated that approximately 20% of untreated patients presenting with syncope die within 1 year and 50% within 10 years.[17]

LQTS is genetically heterogeneous and is caused by several genes (table 1). The most prevalent forms are LQT1 (*KCNQ1*) and LQT2 (*KCNH2*) due to mutations in potassium channels, and LQT3 (*SCN5A*) due to a sodium channel mutation. Most LQT1 events are triggered by exercise or stress, LQT2 events by emotional stress such as auditory stimuli, while LQT3 events most often occur during sleep or at rest.[18] Although the risk of cardiac events is higher among patients with LQT1 and LQT2, the frequency of lethal cardiac events is higher in LQT3 patients.[19]

Also in those without congenital LQTS, the QT interval is a quantitative trait with approximately 30% heritability in the general population.[20-22] Several genetic

Table 1 Common forms of the long QT syndrome (LQTS)

	Genetic subtype		
	LQT1	LQT2	LQT3
Disease-associated gene	<i>KCNQ1</i>	<i>KCNH2/HERG</i>	<i>SCN5A</i>
Ion current affected	I_{Ks}	I_{Kr}	I_{Na}
Pathophysiology	Decreased potassium outward current	Decreased potassium outward current	Excessive sodium inward current
Trigger of arrhythmia	Exercise (diving, swimming), stress	Emotional stress (acoustic)	Rest, sleep
Occurrence	> 50%	35-40%	10-15%

I_{Ks} = rectifier potassium current, slow component; I_{Kr} = rectifier potassium current, rapid component; I_{Na} = inward sodium current.

variants that are associated with QT interval duration have been identified: *NOS1AP*, *KCNQ1*, *KCNE1*, *KCNH2* (*hERG*), *SCN5A*, and loci near *NDRG4* and *GINS3*, *PLN*, *RNF207*, *LITAF*, *LIG3* and *RFFL*. These genetic variants explain 5.4-6.5% of the variation in the QT interval.[23-28]

Acquired QTc interval prolongation

In clinical medicine, QT interval prolongation is often not the primary scope of interest and QTc prolongation usually attracts more attention. The use of this indicator might complicate pathogenetic research since the QTc interval can increase due to QT interval prolongation, due to RR interval shortening, or both concomitantly. There are many factors that predispose to QTc prolongation including e.g. age, female gender, left ventricular hypertrophy, heart failure, myocardial ischemia, hypertension, diabetes mellitus, elevated serum cholesterol, high body mass index, slow heart rate and electrolyte abnormalities (including hypokalemia and hypomagnesemia).[9, 29-32] However, one of the most frequent causes of acquired QTc prolongation is the use of specific drugs.[33]

QTc prolonging drugs

In the past decade, one of the most frequent causes of withdrawal or restriction of marketed drugs has been prolongation of the QTc interval.[7, 34] An increasing number of antipsychotic, antihistaminic, gastrointestinal and anti-infective drugs (e.g. thioridazine, astemizole, cisapride, grepafloxacin) has been withdrawn from the market due to delay of cardiac repolarization and reports of Torsade de Pointes. Several other drugs (e.g. terfenadine, haloperidol, sertindole) were restricted in use because of this potential adverse reaction.[8, 35] Some QTc prolonging drugs which were withdrawn, were associated with a QTc interval prolongation of only 5 to 10 ms in patient populations.[8] However, this was an average and the possibility of more extreme values should be taken into account. If such drugs are used on a large scale, it becomes evident that non-cardiac drugs associated with a pro-arrhythmogenic potential can be identified as a considerable public health problem.[10]

Virtually all QTc prolonging drugs act by blocking the rapid component of the delayed rectifier potassium channel (I_{kr}) encoded by the human ether a-go-go related gene (*hERG*) (figure 3).[8] Previously unrecognized LQTS can be identified in 5 to 20% of patients with drug-induced Torsade de Pointes.[36-38] The clinical implications of drug-induced QTc prolongation are not completely clear. It is known that some drugs associated with QTc prolongation are devoid of torsadogenic effects, whereas others seem to be associated with cardiac arrhythmia without QTc prolongation.[8, 39, 40] Blocking of the I_{kr} current is not specific, since drugs that also block this current do not always cause Torsade de Pointes (e.g. amiodarone). On the other hand some drugs that

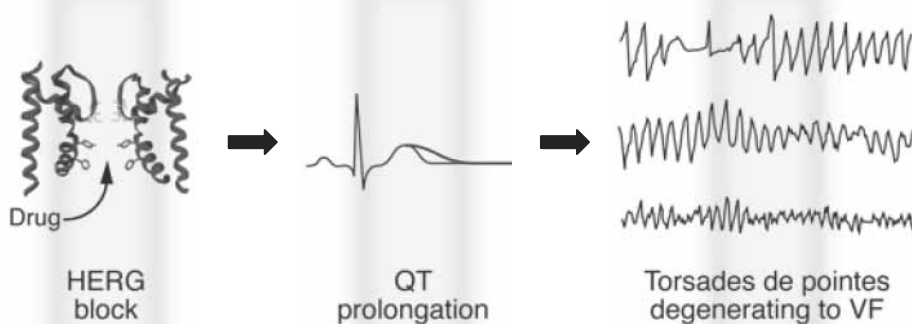


Figure 3 Mechanisms of sudden cardiac death with hERG blockade. Drug blockade of the hERG channel (left) can result in QT interval prolongation (middle) and torsade de pointes (right; upper panel), which can degenerate to ventricular fibrillation (VF) (right; lower panel).
Source: Roden DM et al. *Journal of Clinical Investigation* 2005;115:2025-32.

prolong the QTc interval with only a few milliseconds were nevertheless implicated in the occurrence of cardiac arrhythmias and Torsade de Pointes (e.g. terfenadine).[39, 40] However, terfenadine is a potent I_{kr} blocker but usually does not prolong the QTc interval because it is readily transformed into a metabolite with no effect on the QT interval. Therefore, it is likely that other additional pharmacological actions are required to cause Torsade de Pointes.[8] These actions may be mediated by other ion channels, e.g. Ca^{2+} or Na^{+} channels.[41]

The internet based registry of R.L.Woosley (<http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm>) contains drugs that are known to prolong the QTc interval.[33] The QTc prolonging drugs are classified into 4 categories, varying from drugs that are generally accepted to have a risk of causing Torsade de Pointes (list 1) (table 2) to drugs that, in some reports, have a weak association with Torsade de Pointes and are unlikely to increase the risk when used in therapeutic dosages (list 4).

Sudden cardiac death

According to the most recent definition, sudden cardiac death is defined as 1) a witnessed natural death attributable to cardiac causes, heralded by abrupt loss of consciousness, within one hour of onset of acute symptoms, or 2) an unwitnessed, unexpected death of someone seen in a stable medical condition <24 hours previously with no evidence of a non-cardiac cause.[42, 43]

Table 2 Drugs that may cause Torsade de Pointes. Source: <http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm> [33]**Anti-arrhythmics**

Amiodarone
 Disopyramide
 Dofetilide
 Ibutilide
 Procainamide
 Quinidine
 Sotalol

Anti-histamines

Astemizole
 Terfenadine

Anti-infectives

Clarithromycin
 Erythromycin
 Pentamidine
 Sparfloxacin

Anti-malarials

Chloroquine
 Halofantrine

Anti-psychotics

Chlorpromazine
 Haloperidol
 Mesoridazine
 Pimozide
 Thioridazine

Gastro-intestinal drugs

Cisapride
 Domperidone

Opiate agonists

Levomethadyl
 Methadone

Other

Arsenic trioxide
 Bepridil
 Droperidol
 Probucol

Sudden cardiac death accounts for more deaths each year than the total number of deaths from AIDS, breast cancer, lung cancer and stroke together. It is estimated that more than 3 million people die yearly from sudden cardiac death worldwide.[4]

The incidence of sudden cardiac arrest in the general Dutch population was 9.2 per 10,000 inhabitants.[3] This can be extrapolated to approximately 40 sudden cardiac arrests per day in the Netherlands. The incidence of sudden cardiac death in the general

Dutch population was almost 1 per 1,000 person-years per year.[44] In Dutch patients who had undergone 24-hour ambulatory electrocardiography for various indications, 3.7% of the patients experienced sudden death within 2 years after electrocardiography.[45]

Most sudden cardiac arrests occur out-of-hospital with a survival rate of less than 1%.[4] The majority (80%) of cardiac arrests occur at home and approximately 40% of sudden cardiac deaths are unwitnessed.[44, 46] Since a large proportion (33-50%) of sudden cardiac death victims have no warning symptoms and are not identified as being at high risk before the event, it may be important to improve the outcome of resuscitation attempts outside the hospital.[47] The short time frame after cardiac arrest during which circulation has to be restored to prevent death or irreversible cerebral damage is essential.[48] In recent years, automated external defibrillators (AEDs) are used more frequently, allowing non-physicians to defibrillate shortly after the onset of ventricular fibrillation.[49]

Etiology

The majority (80-85%) of sudden cardiac deaths are caused by acute ventricular arrhythmias. Preceding acute myocardial infarction is present in 20% of the cases. Sudden cardiac death associated with bradyarrhythmias (15%) usually represents end-stage heart failure.[4]

Coronary artery disease is the most common underlying disease in all cases of sudden cardiac death (75-80%). Dilated and hypertrophic cardiomyopathy is the second most common underlying cause (10-15%). Primary electrical heart disease and genetic ion-channel abnormalities (<5%) account for only a small proportion of sudden deaths from cardiac causes.[4, 50]

Brugada syndrome is the most common cause of sudden cardiac death in individuals without structural heart disease. Brugada syndrome is characterized by a history of syncope and a typical ECG pattern of ST-segment elevation in leads V_1 to V_3 . It is claimed to be responsible for up to 12% of all sudden deaths and approximately 20% of deaths occurring in patients with structurally normal hearts. In the Western world, the prevalence is estimated at 1 in 5000 individuals. This syndrome exhibits an autosomal dominant pattern of inheritance. All identified mutations have been in a subunit of the sodium cardiac channel (*SCN5A*).[17]

Prevention of sudden cardiac death

In all LQTS patients, it is important to avoid drugs known to prolong the QTc interval and electrolyte abnormalities. The major therapeutic options for LQTS are beta-blockers and implantable cardioverter-defibrillators (ICDs). The aim of beta-blockade is to decrease the heart rate at exercise; beta-blockers do not substantially shorten the QT interval.[51]

Beta-blocking agents represent the first choice therapy in symptomatic LQTS patients, unless specific contraindications are present.[18]

The use of ICDs is indicated as primary prevention in patients at high risk for sudden cardiac death, including LQTS patients with symptoms before puberty and those with very long QTc intervals (e.g. >500 ms). ICD implantation should be considered as secondary prevention in patients who have survived a life-threatening arrhythmic event. [50-52]

As has been illustrated in this introduction, sudden cardiac death is a major clinical and public health problem. Many causes of QTc prolongation and thereby sudden cardiac death have been identified, however, not all risk factors are known yet. The occurrence of ventricular arrhythmias is a multifactorial process involving several factors.[53] In order to take effective preventive measures, persons at risk for sudden cardiac death should be identified. It is therefore important to identify risk factors that will predict fatal arrhythmic events.[54]

Scope and outline of this thesis

In this thesis, the effect will be studied of certain drugs and endocrine factors on QTc prolongation and sudden cardiac death. The studies presented in this thesis used data from the Rotterdam Study and the Integrated Primary Care Information (IPCI) project. [55, 56]

The Rotterdam Study is a large prospective population-based cohort among 7,983 inhabitants of Ommoord, a suburb of Rotterdam, who were 55 years or older. Since the start of the study in 1989, participants have visited the research center up to 4 times. In 2000 the first extended cohort was enrolled, which included 3011 inhabitants aged 55 years or older at that time, who visited the research center up to 2 times. The IPCI project is a general practice research database, containing the complete medical records on approximately 1 million patients.

In the first studies, the association is investigated between QTc interval prolongation and sudden cardiac death and several types of drug classes (psychotropic drugs, calcium channel blockers, *hERG*-encoded potassium channel blocking drugs, domperidone and anticonvulsants. In the second part of this thesis, we evaluated the effects of endocrine factors (thyroid hormones, testosterone, glucose and insulin) on the QT(c) interval.

In chapter 2.1, we study the association between use of psychotropic drugs and the duration of the QTc interval. In chapter 2.2 we study the effect of the interaction of calcium channel blockers with *NOS1AP* variants on QTc interval duration. In chapter 3.1 we explore the association between use of *hERG*-encoded potassium channel blocking

drugs and sudden cardiac death. Chapter 3.2 describes the association between use of domperidone and ventricular arrhythmias and sudden cardiac death. Chapter 3.3 focuses on the association between anticonvulsants and sudden cardiac death.

In chapter 4.1 we study the effect of thyroid hormones on QTc duration. In chapter 4.2 we assess the association between use of antithyroid drugs (as proxy for underlying hyperthyroidism) and sudden cardiac death. In chapter 5.1 we explore the effect of serum levels of testosterone on QTc and RR length in males. Chapter 5.2 describes the association of glucose and insulin with the QTc and RR interval. In chapter 6, we discuss the main findings of this thesis and we provide suggestions for future research.

In addition, in chapter 7 we present recently discovered genes involved in atrial fibrillation and the PR interval during studies which were performed at the last stage of the research period for this thesis. Atrial fibrillation is an electrical disorder of the heart's upper chambers characterized by an irregular heart rhythm. The overall lifetime risk of AF is almost 25% in the US and Europe.[57, 58] In chapter 7.1 we have replicated variants on chromosome 4 associated with atrial fibrillation in 4 independent cohorts. The primary aim of genome-wide association (GWA) studies is to identify novel genetic loci associated with interindividual variation in the levels of risk factors and the degree of clinical disease. In chapter 7.2 we conducted meta-analyses of genome-wide association studies for atrial fibrillation in collaboration with the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium [59] and identified the new locus *ZFHX3* for atrial fibrillation. The electrocardiographic PR interval reflects atrial and atrioventricular nodal conduction, disturbances of which increase risk of atrial fibrillation. In chapter 7.3 we conducted meta-analyses of GWA studies for the PR interval in 7 community-based studies and identified 9 loci. Five of these 9 loci were also associated with atrial fibrillation.

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Chapter 2.1

Psychotropic drugs associated with QTc prolongation

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Abstract

Background

To study whether listed putative QTc-prolonging psychotropic drugs indeed prolong the QTc interval under everyday circumstances, and to evaluate whether this is a class effect or an individual drug effect, we conducted a prospective population-based cohort study.

Methods

This study was conducted as part of the Rotterdam Study, and included 3377 men and 4845 women (≥ 55 years), who had triennial ECGs. The primary endpoints of the study were the length of the QTc interval at each ECG, the difference in QTc interval between consecutive ECGs within one person and the risk of an abnormally prolonged QTc interval. Drug use at the index date was obtained from automated dispensing records. The associations were examined by means of a repeated measurement analysis, adjusted for age, gender, diabetes mellitus, hypertension, myocardial infarction, heart failure and use of class 1 QTc prolonging drugs.

Results

Of the 8,222 participants, 813 participants developed QTc prolongation during follow-up (9.9%) and 492 participants (74.4% female) used psychotropic drugs at the time of an ECG. Starting tricyclic antidepressants increased the QTc interval significantly with 6.9 ms (95%CI 3.1;10.7) between consecutive ECGs in comparison with consecutive ECGs of participants not using tricyclic antidepressants, in particular starting amitriptyline (8.5 ms (95%CI 2.8;14.2)), maprotiline (13.9 ms (95%CI 3.6;24.3)) and nortriptyline (35.3 ms (95%CI 8.0;62.6)). Starting lithium also increased the QTc interval significantly (18.6 ms (95%CI 4.8;32.4)).

Conclusions

In this population-based prospective cohort study, we confirmed the importance of antidepressants and antipsychotics as potential contributors to QTc-prolongation. Especially starting of tricyclic antidepressant drugs (as a class) is associated with a significant intra-individual increase in the QTc interval in comparison to the change in non-users. The tricyclic antidepressants appear to prolong the QTc interval as a class effect.

Introduction

In the past decade, one of the most frequent causes of withdrawal or restriction of marketed drugs has been prolongation of the heart-rate corrected QT (QTc) interval which is the traditional measurement for assessing the duration of ventricular repolarization. Prolongation of ventricular repolarization may result in early after depolarizations (EAD), which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmias.[1-4]

An increasing number of drugs, especially non-cardiac drugs, are known to delay cardiac repolarization and to induce Torsade de Pointes.[4] The issue of non-cardiac drugs associated with a pro-arrhythmogenic potential is identified as a considerable public health problem.[4] In recent years, several lists have been published of non-cardiac drugs associated with QTc prolongation and cardiac arrhythmias.[5-8] A limitation of these lists is that they are mostly based on empirical data and incomplete evidence. In addition, it is often unknown whether non-listed drugs of similar therapeutic or chemical classes do not also provoke QTc prolongation or whether they are not listed due to insufficient data. For example, because many, but not all psychotropic drugs are known to prolong the QTc interval, the question is whether QTc prolongation is a therapeutic and chemical class effect or an individual drug effect.[9-11]

Current guidelines, intended to predict whether a new drug carries an increased risk of serious cardiac arrhythmias, place much emphasis on the risk of QTc interval prolongation as a proxy indicator for the risk of ventricular arrhythmias.[12] We conducted a population-based prospective cohort study, with serial ECG measurements over time to study whether listed as well as non-listed psychotropic drugs are indeed associated with QTc prolongation and to evaluate whether this is a class effect or an individual drug effect.

Methods

Setting and study design

The Rotterdam Study is a prospective population-based cohort study, which started with a baseline visit between 1990 and 1993. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. All inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years and over, were invited to participate (n=10,275). Of them, 7983 (78%) gave their written informed consent and took part in the baseline examination (Rotterdam Study). Objectives and methods of the Rotterdam Study have been described in detail elsewhere.[13] At baseline, all participants were visited at home for a standardized questionnaire, and 7151 were subsequently

examined at the research center. Since the start of the study, follow-up visits took place in the period 1993 through 1996 for the second visit, in the period between 1997 through 1999 for the third visit and in the period between 2002 through 2004 for the fourth visit. Furthermore, in 2000 a second cohort (first extended cohort) was enrolled. This included all inhabitants of Ommoord, at that time aged 55 years and over and not enrolled in the Rotterdam Study, who were invited to participate ($n = 4504$). Of them, 3011 (67%) entered the study and took part in the baseline examination. The second visit of the first extended cohort took place in the period between 2004 and 2005. In addition to follow-up examinations, both cohorts are continuously monitored for major morbidity and mortality through linkage of general practitioner and municipality records. Furthermore, all drug prescriptions dispensed to participants by automated pharmacies are routinely stored in the database since 1 January 1991.

Study population

All cohort members who had at least one ECG were enrolled in the study. Participants could contribute up to four ECGs to the analyses. Overall, 20,596 ECGs were available, 8586 in men and 12,010 in women. Digitally stored ECGs were available for 5397 participants at the time of the first visit, of 4798 participants at the time of the second visit, of 3818 participants at the time of the third visit and of 3118 participants at the time of the fourth visit. There were 2273 ECGs available of the participants of the first extended cohort at the time of the first visit and of 1190 at the time of the second visit. Missing ECGs were mainly due to temporary technical problems with ECG recording. Participants left the cohort mainly due to mortality while a minority was lost to follow-up (figure 1). For the present study, the visit during which the first ECG was made was defined as baseline. Follow-up lasted until reaching one of the censoring dates (death or transferring out) or the end of the study period (1 January 2006).

ECG

The primary endpoint of this study was the length of the QTc interval in milliseconds (ms). A 12-lead resting ECG was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECGs were processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurements, in agreement with the FDA guidance for clinical evaluation of QT/QTc interval prolongation[14], the MEANS program has been evaluated and validated extensively.[15-18] In one of these validation studies, ECGs with selected abnormalities were analyzed by 5 cardiologists and 11 different computer programs of which MEANS performed as one of the best.[18] In another validation study in which QT intervals by manual measurement were compared with those generated by ECG machines, manual and automated measurements generated similar numerical results in 3 studies in healthy volunteers, which

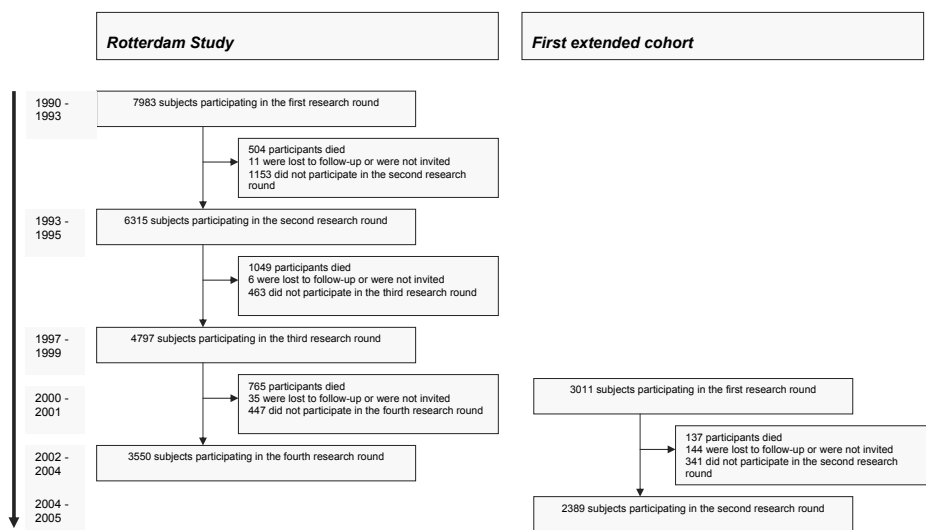


Figure 1 Diagram of study population of the Rotterdam Study

all included a positive control.[19] MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques.[16] The MEANS program determines the QT interval from the start of the QRS complex until the end of the T wave. To adjust for heart rate, Bazett's formula ($QTc = QT/\sqrt{RR}$) was used.[20] European regulatory guidelines were used to categorize QTc prolongation into 3 categories. For men, the cut-off points were less than 430 ms (normal), 430-450 ms (borderline) and more than 450 ms (prolonged), and for women less than 450 ms (normal), 450-470 ms (borderline), and more than 470 ms (prolonged). [21] Furthermore, the MEANS program determines left ventricular hypertrophy and left and right bundle branch block.

ECGs of participants who used cardiovascular drugs that are listed as QTc altering agents (amiodarone, diltiazem, disopyramide, flecainide, indapamide, isradipine, ketanserin, quinidine, losartan, nicardipine, sotalol, triamterene and verapamil) or QTc shortening agents (digoxin) at the index date were excluded. In addition, the ECGs of persons with a pacemaker were excluded, as well as of persons with evidence of left ventricular hypertrophy, left and right bundle branch block, since these conditions are associated with a prolonged QTc interval.[22, 23]

Medication

In this study, the exposure of interest included antidepressant and antipsychotic drugs selected from the following sources: drugs specified in lists 1 through 4 from a commonly consulted internet based registry of QTc prolonging drugs (<http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm>); drugs in the list with QTc prolonging drugs

from De Ponti et al. [5, 6, 8, 24]; and all other antidepressants and antipsychotics that are not listed in one of these two lists. The QTc prolonging drugs from the internet based registry are classified into 4 categories, varying from drugs that are generally accepted by authorities to have a risk of causing Torsade de Pointes (list 1) to drugs that, in some reports, have a weak association with Torsade de Pointes and are unlikely to increase the risk when used in therapeutic dosages (list 4). In addition, De Ponti et al. have published a list of non-anti-arrhythmic drugs with pro-arrhythmogenic effects, based on a structured literature search including published (non-) clinical evidence and official warnings in the labelling.[5, 6]

In our analyses, we included antipsychotics that are listed in the internet based registry and the list from De Ponti et al. as QTc prolonging drugs: clozapine, haloperidol, lithium, prochlorperazine, quetiapine, thioridazine, and antipsychotics that do not appear as QTc prolonging drugs on either list: benperidol, bromperidol, levomepromazine, penfluridol, perphenazine, pipamperone, zuclopenthixol. Furthermore, we included all antidepressants that are listed as QTc prolonging drugs: amitriptyline, citalopram, clomipramine, doxepin, fluoxetine, imipramine, maprotiline, mianserin, nortriptyline, paroxetine, sertraline, trazodone, venlafaxine, and all antidepressants that do not appear as QTc prolonging drug on either list: fluvoxamine, moclobemide, tranylcypromine.

The index date was the date of the ECG, psychotropic drugs were considered to be current if the duration of the prescription covered the index date. The duration was the total number of units issued per prescription divided by the prescribed daily number of units.

Covariates

Diabetes mellitus, hypertension, myocardial infarction and heart failure are considered to be risk factors for QTc prolongation and presence of these conditions at each index date was included as a covariate.[25-28] Clinical measures were obtained during the visits at the Rotterdam Study research center. Diabetes mellitus was defined as the use of blood glucose-lowering medication and/or a non-fasting serum glucose level of 11.1 mmol/l or higher and/or serum glucose levels ≥ 7 mmol/l (1997-2000).[29] Hypertension was defined according to World Health Organization (WHO) criteria.[30] Myocardial infarction at baseline and during follow-up was assessed by hospital discharge diagnosis or in case a patient was not hospitalized, when signs and symptoms, analysis of the ECG and cardiac enzyme data were diagnostic of a myocardial infarction.[31, 32] Heart failure at baseline and during follow-up were assessed by the presence of suggestive signs and symptoms and by the use of medication for the indication heart failure.[33, 34] Class 1 QTc prolonging drugs were defined as drugs of list 1 of the website based registry, which contains drugs that are generally accepted to have a risk to cause Torsade de Pointes.[8] Potassium at baseline was measured by means of a Microlyte device.

Statistical analysis

Three types of analyses were conducted to assess the association between exposure to psychotropic drug use and QTc prolongation. The first analysis was cross-sectional and comprised all ECG measurements (up to four ECGs per individual). Because QTc measurements in subsequent ECGs of the same subject are correlated, the association between exposure to psychotropic drugs and the length of the QTc-interval was examined by means of linear regression repeated measures analyses as implemented in PROC MIXED (SAS software). Analyses were adjusted for gender and the following time-depending covariates: age, diabetes mellitus, hypertension, myocardial infarction, heart failure and use of class 1 QTc prolonging drugs. Several sensitivity analyses were performed, first, we adjusted for the dosage of antidepressant drugs. Second, we adjusted for potassium levels in participants with an ECG at baseline. Third, we excluded all class 1 QTc prolonging drugs. Fourth, since Bazett's formula tends to under-correct for lower heart rates and over-correct for higher heart rates we also included heart rate in the model and used Fridericia's correction ($QTc = QT/RR^{0.33}$).

The second analysis was longitudinal and analyzed the within person change in QTc between two subsequent ECGs ($QTc[t_x] - QTc[t_{x-1}]$). Four exposure categories were distinguished: non-users of a particular drug at t_{x-1} who were users at t_x (I); users at both ECGs (II); users at t_{x-1} who were non-users at t_x (III); and non-users at both ECGs (IV). By means of linear regression repeated measures analyses, we compared the QTc length in category I with category IV as a reference. For the longitudinal analysis, participants were censored after first development of QTc prolongation. For the longitudinal study design, analyses were adjusted for QTc at the previous measurement in addition to the other co-variates.

The overall effect of each psychotropic class was estimated, both in the cross-sectional and longitudinal analysis. The estimates were obtained averaging, within classes, over the drug-specific estimates, using random effect models.[35] Likelihood (REML) method, implemented in the SAS procedure PROC MIXED.

The third analysis modeled the association between exposure to psychotropics and the risk of a prolonged QTc interval by means of logistic regression repeated measures analyses (PROC GENMOD). For the third analysis participants were censored after first development of QTc prolongation. Analyses were adjusted for the same covariates as in the cross-sectional analysis and the QTc interval at baseline. All repeated measurements analyses were performed using SAS software, version 9.1.

Results

Study subjects

The baseline characteristics of all participants after exclusion of left ventricular hypertrophy (538 ECGs), left and right bundle branch block (387 and 665 ECGs), use of cardiovascular QTc prolonging drugs (1334 ECGs), and participants with a pacemaker (53 ECGs) are presented in table 1. Overall, 17,516 ECGs in 8,222 participants, remained for analysis.

Table 1 Characteristics of the study population

	Rotterdam study (baseline) (1990 – 1993)	2nd research round (1993 – 1996)	3rd research round (1997 – 1999)	4th research round (2002 – 2004)	First extended cohort (baseline) (2000 – 2002)	2nd research round (2004 – 2005)
Number of participants	4562	4140	3193	2531	2035	1055
Gender (female)	60.2 %	59.7 %	58.8 %	60.9 %	55.6 %	57.8 %
Mean age in years (SD)	67.8 (8.8)	68.6 (8.2)	71.4 (6.9)	74.6 (6.1)	64.1 (7.7)	65.7 (5.8)
Mean QTc interval (SD) (ms)	427.7 (23.6)	425.6 (23.0)	429.1 (23.1)	432.0 (23.6)	429.7 (21.9)	427.8 (22.8)
ECGs with QTc prolongation	6.6 %	5.6 %	7.6 %	9.5 %	6.8 %	6.7 %

SD= standard deviation

The mean age of the study population at the first ECG in the Rotterdam Study and the first extended cohort was 66.7 years (Standard Deviation 8.6 years). Women were significantly older than men.

The mean QTc interval at study entry was significantly lower in males (422.4 ms) than in females (432.1 ms). 73.5% had normal QTc durations at baseline and 19.8% had borderline QTc prolongation, using the abovementioned gender-specific cut-off points.

Overall, 813 participants who were free of baseline QTc prolongation developed QTc prolongation during follow-up (9.9%), with mean QTc levels of 476.5 ms.

Psychotropic drugs and QTc prolongation, cross-sectional analysis

Current use of psychotropic drugs at the time of any ECG was relatively low (n=492, 6.0%). The use of QTc-prolonging psychotropic medications was significantly higher in females (4.4%) than in males (1.5%). Of the current antipsychotic drug users 5.1% had QTc prolongation, whereas 4.1% of the current antidepressant drug users had QTc prolongation.

The use of several psychotropic drugs was associated with a significantly increased QTc interval in the cross-sectional analyses (table 2). Among the antipsychotics, olanzapine and thioridazine were associated with a significantly higher QTc interval. There was no evidence that other phenothiazines, that are not listed as QTc prolonging drugs increased the QTc interval, but power was limited for several drugs. Among the antidepressants only the tricyclic antidepressants were associated with a significant increase in QTc interval. Not all of the individual drugs showed a significant higher QTc interval, but adjustment for frequency of use showed that the class as a whole was associated with a

Table 2 Psychotropic medication and QTc interval prolongation (cross-sectional analysis on any ECG)

Medication	De Ponti ^A	Lists QTc/ TdP ^B	Users	QTc (Bazett) increase (ms) in users compared to non-users (95% CI) ^C
Antipsychotics			84	
<i>Phenothiazines</i>			17	-5.4 (-24.2 ; 13.4)
Levomepromazine	-	-	6	-3.1 (-18.0 ; 11.8)
Periciazine	-	-	1	NA
Perphenazine	-	-	4	-35.7 (-54.9 ; -16.6)
Prochlorperazine	x	-	1	NA
Thioridazine	x	1, 3	4	28.3 (5.9 ; 50.8)
Trifluoperazine	x	-	1	NA
<i>Butyrophenones</i>			29	-0.4 (-4.3 ; 3.6)
Benperidol	-	-	1	NA
Bromperidol	-	-	5	1.8 (-15.6 ; 19.2)
Haloperidol	x	1, 3	8	1.2 (-13.5 ; 15.9)
Pipamperone	-	-	15	-0.6 (-10.8 ; 9.6)
<i>Thioxanthenes</i>			9	-12.2 (-25.5 ; 1.1)
Zuclopenthixol	-	-	9	-12.2 (-25.5 ; 1.1)
<i>Miscellaneous antipsychotics</i>			29	6.5 (-2.2 ; 15.1)
Clozapine	X	2, 3	2	NA
Lithium	-	2, 3	18	10.1 (0.7 ; 19.4)
Olanzapine	X	-	3	22.9 (0.1 ; 45.7)
Penfluridol	-	-	4	-6.1 (-25.5 ; 13.3)
Quetiapine	X	2, 3	1	NA
Sulpiride	-	-	1	NA
Antidepressants			408	
<i>Tricyclic antidepressants</i>			177	6.9 (3.1 ; 10.7)
Amitriptyline	X	3, 4	106	5.1 (1.4 ; 8.9)
Clomipramine	X	3, 4	19	6.6 (-1.6 ; 14.7)
Doxepin	X	3, 4	4	-7.9 (-27.3 ; 11.5)
Imipramine	X	3, 4	9	12.8 (0.3 ; 25.3)
Maprotiline	X	-	33	9.6 (3.1 ; 16.1)
Nortriptyline	X	3, 4	6	23.3 (7.7 ; 38.9)

Table 2 continued

Medication	De Ponti ^A	Lists QTc/TdP ^B	Users	QTc (Bazett) increase (ms) in users compared to non-users (95% CI) ^C
<i>Selective serotonin reuptake inhibitors</i>			186	0.1 (-1.7 ; 1.8)
Citalopram	X	3, 4	4	6.5 (-13.7 ; 26.7)
Fluoxetine	X	3, 4	25	0.0 (-7.7 ; 7.8)
Fluvoxamine	-	-	30	-0.1 (-7.2 ; 7.0)
Paroxetine	X	3, 4	111	0.6 (-3.1 ; 4.2)
Sertraline	-	3, 4	16	-5.2 (-15.1 ; 4.7)
<i>MAO inhibitors</i>			4	-0.7 (-2.1 ; 0.7)
Moclobemide	-	-	3	-0.3 (-19.6 ; 19.0)
Tranylcypromine	-	-	1	NA
<i>Other antidepressants</i>			41	2.21 (-2.7 ; 7.1)
Mianserin	X	-	20	-1.7 (-10.3 ; 6.9)
Mirtazapine	-	-	12	8.1 (-4.2 ; 20.4)
Trazodone	X	-	3	1.1 (-19.6 ; 21.7)
Venlafaxine	X	2, 3	6	6.3 (-9.5 ; 22.2)

A: Present in De Ponti list. TdP= Torsade de Pointes

B: Present in lists from internet based registry.

CI=Confidence Interval; QTc interval in ms.

C: Adjusted for age and gender, diabetes mellitus, hypertension, myocardial infarction, heart failure and use of class 1 QTc prolonging drugs.

NA: not assessable, measures of association are not calculated for categories with less than 3 users.

higher QTc interval. SSRIs, MAO inhibitors and miscellaneous antidepressants were not associated with a QTc increase.

Additional adjustment for daily dose and potassium did not substantially change the estimates. After exclusion of all class 1 QTc prolonging drugs, the results for the classes as a whole did not change substantially. In two separate analyses, we adjusted for heart rate and used Fridericia's correction as outcome. There were no considerable changes, however, the point estimates for the tricyclic antidepressants decreased slightly.

Psychotropic drugs and QTc prolongation, longitudinal analysis

After censoring of ECGs when prolongation occurred during follow-up (361 ECGs) and exclusion of participants with QTc elevation at the first ECG (1794 ECGs), 16,389 ECGs in 7,667 participants, remained. Starting a psychotropic drug between two consecutive ECGs occurred in 193 participants (2.5%). There were too few starters of antipsychotics to draw firm conclusions using intra-individual QTc changes from non-user to user, but antidepressants could be analysed. The longitudinal analysis showed that starting tricyclic antidepressants was associated with an increase in the QTc interval in comparison to participants not starting tricyclic antidepressants in two subsequent ECGs (table 3).

Table 3 Difference in QTc interval length between two consecutive ECGs (longitudinal analysis regarding intra-individual change from non-user to user)

Medication	Number of participants starting the psychotropic drug	QTc change in persons starting psychotropic drugs compared to non-users (95% CI) ^A
Antipsychotics	34	
<i>Phenothiazines</i>	4	-8.0 (-32.6 ; 16.5)
Levomepromazine	4	-8.0 (-32.6 ; 16.5)
<i>Butyrophenones</i>	12	2.0 (-3.2 ; 7.2)
Benperidol	1	NA
Bromperidol	2	NA
Haloperidol	2	NA
Pipamperone	7	3.7 (-11.8 ; 19.1)
<i>Thioxanthenes</i>	6	-13.5 (-33.9 ; 6.9)
Zuclopenthixol	6	-13.5 (-33.9 ; 6.9)
<i>Miscellaneous antipsychotics</i>	12	12.7 (-5.1 ; 30.4)
Clozapine	2	NA
Lithium	8	18.6 (4.8 ; 32.4)
Penfluridol	1	NA
Quetiapine	1	NA
Antidepressants	159	
<i>Tricyclic antidepressants</i>	66	10.4 (3.5 ; 17.4)
Amitriptyline	37	8.5 (2.8 ; 14.2)
Clomipramine	11	6.0 (-4.9 ; 17.0)
Doxepin	1	NA
Imipramine	1	NA
Maprotiline	12	13.9 (3.6 ; 24.3)
Nortriptyline	4	35.3 (8.0 ; 62.6)
<i>Selective serotonin reuptake inhibitors</i>	82	-2.4 (-10.1 ; 5.4)
Citalopram	1	NA
Fluoxetine	8	-5.6 (-18.4 ; 7.2)
Fluvoxamine	12	-19.4 (-31.0 ; -7.8)
Paroxetine	54	2.6 (-2.1 ; 7.2)
Sertraline	9	-12.1 (-25.6 ; 1.4)
<i>MAO inhibitors</i>	1	NA
Moclobemide	1	NA
<i>Other antidepressants</i>	10	-0.5 (-2.9 ; 2.0)
Mianserin	5	0.3 (-16.3 ; 16.9)
Trazodone	1	NA
Venlafaxine	3	-2.6 (-44.7 ; 39.4)

CI=Confidence Interval; QTc interval in ms.

A: Difference in QTc prolongation between two consecutive ECGs from patients starting psychotropic drugs compared to patients not using psychotropic drugs in two consecutive ECGs. Adjusted for age and gender, diabetes mellitus, hypertension, myocardial infarction, heart failure, QTc of previous measurement and use of class 1 QTc prolonging drugs.

NA: not assessable, measures of association are not calculated for categories with less than 3 starters.

Start of each of the individual drugs was associated with an increase of the QTc interval (amitriptyline 8.5 ms (95%CI 2.8;14.2), maprotiline (13.9 ms (95%CI 3.6;24.3)) and nortriptyline (35.3 ms (95%CI 8.0;62.6)).

Risk of an abnormally prolonged QTc interval

Use of some psychotropic drugs was associated with a significantly higher odds of a prolonged QTc interval. Of the individual drugs, current use of penfluridol (odds ratio 1.27 (95%CI 1.08-1.48)), lithium (OR 1.07 (95%CI 1.01-1.15)), amitriptyline (OR 1.05 (95%CI 1.02-1.08)), and maprotiline (OR 1.13 (95%CI 1.07-1.19)) was associated with a significantly higher risk of a prolonged QTc interval.

Discussion

To our knowledge, this is the first large longitudinal prospective cohort study assessing the association between psychotropic drugs and QTc-prolongation in a population of older adults. The cross-sectional analyses and within person longitudinal analyses showed that use of some antipsychotics (especially lithium, olanzapine and thioridazine) and almost all tricyclic antidepressants are associated with a significant increase in QTc interval. Most of these drugs were listed by either de Ponti or the internet-based registry. Maprotiline, which was clearly associated with an increase in QTc interval, is not listed on the internet based registry. For many of the listed drugs we could not confirm a significant increase in the QTc interval. However, this may have been due to limited power from small sample sizes. In general, the direction of change was as expected. One of our original study questions was to see whether QTc prolongation was associated with the therapeutic/chemical class rather than with individual drugs. The only class that was associated with an overall increase of the QTc interval were tricyclic antidepressant drugs. In this class most of the individual drugs (amitriptyline, imipramine and maprotiline) were associated with a significant increase of the QTc interval. Although for clomipramine, nortriptyline and doxepin no such association was demonstrated (although they are on the list), this might be explained by a lack of power. We cannot find a logical explanation for the significant QTc interval shortening of fluvoxamine; chance could underlie this observation but a genuine effect cannot be excluded.

Our results are in agreement with previously reported information on these drugs. Amitriptyline and maprotiline are the tricyclic antidepressant drugs which have been implicated most frequently in case reports of Torsade de Pointes and QTc prolongation is considered a risk factor for Torsade de Pointes.[10] Previously, we have demonstrated that QTc prolongation is associated with an increased risk of sudden cardiac death.[36] In elderly patients using antipsychotic drugs, QTc prolongation is common.[37]

Lithium has been reported in the literature as a cause of QTc interval prolongation.[6, 38] Thioridazine, which was associated with a significant increase in QTc interval in our cross-sectional analyses has been withdrawn in 2005 due to QTc prolongation, cardiac arrhythmias and sudden death.[39] Some QTc prolonging drugs which were withdrawn from the market because of Torsade de Pointes, were associated with a QTc interval prolongation of only 5 to 10 ms in patient populations.[2]

Although all listed drugs have been reported to be associated with Torsade de Pointes or cardiac arrhythmias, in our study no significant associations with QTc prolongation were found for a number of these listed drugs, which may be explained by between patient variation in susceptibility and the small numbers of users of some specific drugs.

Our study has several strengths. First, the cross-sectional study design allowed us to find associations between psychotropic drugs and QTc prolongation. Because of the well-known limitation of cross-sectional designs, i.e. the fact that it is often not possible to assess whether exposure really preceded the outcome, we also performed a longitudinal analysis which gave more insight into the question whether the drugs were the cause of the QTc prolongation. Second, class effects were calculated using random effect models to adjust for the number of users of a specific drug within a class. To minimize bias by simultaneous use of other QTc prolonging drugs, we adjusted for use of other listed QTc prolonging drugs. The population-based design of the study probably limited the chance of selection bias. If exposure misclassification occurred it is probably non-differential. There is no information bias, since we used pharmacy data, which are registered prospectively and irrespective of disease status. One major advantage in our study was the availability of data on a large group of participants, including up to four ECGs per subject at regular intervals during follow-up, which allowed us to obtain more precise long-term ECG measures for each individual. Furthermore, the use of digital ECG recordings, all measured using the MEANS system, likely reduced intra- and interobserver variability in the assessment of the QTc interval. Confounding was minimized by adjusting for all known risk factors of QTc prolongation. An advantage of the Rotterdam Study is the prospective ascertainment of risk factors over a long period of follow-up. However, our study has also some potential limitations. Some participants were lost to follow-up, however, since only 196 out of 10,994 participants of the Rotterdam Study were lost to follow-up, loss-to-follow-up bias is unlikely. The association between psychotropic drugs and sudden cardiac death was not assessed directly, due to a low number exposed cases. However, we demonstrated in an earlier study that QTc prolongation is an important risk factor for sudden cardiac death.[36]

In conclusion, in the longitudinal analysis of this large population-based prospective cohort, we confirmed that all drugs that demonstrated QTc prolongation are already listed. Although not all tricyclic antidepressants are listed, QTc prolongation following starting of tricyclic antidepressants seems to be a class effect.

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Chapter 2.2

Calcium channel blockers, *NOS1AP* and QTc prolongation

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Abstract

Background

Common variation in the *NOS1AP* gene has been associated with QT interval variation in several large population samples. *NOS1* is presumed to influence intracellular calcium. We aimed to study whether *NOS1AP* single nucleotide polymorphisms rs10494366 T>G and rs10918594 C>G modify the QTc prolonging effect of calcium channel blockers.

Methods

We included 16,603 ECGs from 7565 participants (≥ 55 yr), who were genotyped, from the prospective population-based Rotterdam Study after exclusion of patients with left ventricular hypertrophy, left and right bundle branch block, as well as carriers of pacemakers. The endpoint was the length of the QTc interval in calcium channel blocker users and non-users with the minor alleles compared to the major alleles (wild type). We used a repeated measurement analysis, adjusted for all known confounders.

Results

Use of verapamil was associated with a significant QTc interval prolongation (6.0 ms (95%CI 1.7;10.2)) compared to non-users. Furthermore, users of verapamil with the rs10494366 GG genotype showed significantly more QTc prolongation than users with the TT genotype (25.4 ms (95%CI 5.9;44.9) (P-value for multiplicative interaction 0.0038). Users of isradipine with the GG genotype showed more QTc prolongation than users with the TT genotype (19.8 ms (95%CI 1.9;37.7)), however, SNP rs10494366 did not modify the effect on QTc interval on a multiplicative scale ($p=0.3563$). SNP rs10918594 showed similar results.

Conclusions

In conclusion, we demonstrated that the minor alleles of both *NOS1AP* SNPs significantly potentiate the QTc prolonging effect of verapamil.

Introduction

In the past decade, one of the most frequent causes of withdrawal or restriction of marketed drugs was prolongation of the heart-rate corrected QT (QTc) interval in combination with case reports of sudden cardiac death.[1-4]

The QTc interval is the traditional measurement for assessing the duration of ventricular repolarization. QTc prolongation may result in early after depolarizations (EAD) and re-entry, and thereby provoke Torsade de Pointes and fatal ventricular arrhythmias.[1-5] The QTc interval is influenced by factors such as gender, age and use of certain drugs. An increasing number of drugs has been recognized to delay cardiac repolarization and to induce Torsade de Pointes.[4] However, it is mostly unknown which underlying risk factors may modify the risk of drug-induced QTc-prolongation. As the QT interval is a quantitative trait with approximately 30% heritability,[6-8] it is likely that such genetic effect modifiers exist.

Recently, one of us with others reported the finding from a genome-wide association study showing that a common variant (rs10494366, minor allele frequency 38%) in the *NOS1AP* gene is associated with QT interval variation in several large population samples, which was replicated in the Rotterdam Study.[9-12] The *NOS1AP* gene encodes the nitric oxide synthase 1 activating protein.[9] The mechanism by which common variation in *NOS1AP* affects the QTc interval is presently unknown. *NOS1AP* is a regulator of neuronal nitric oxide synthase which forms a ternary complex with PSD95 (membrane-associated guanylate kinase) [13] and Dexas 1 (member of the Ras family of small monomeric G proteins).[14] NOS1 has a role in cardiac contractility [15, 16], and it is hypothesized that nitric oxide signalling may be involved in cardiac repolarization.

Since both NOS1 and calcium channel blockers suppress L-type calcium channels, we conducted a population-based prospective cohort study to investigate whether *NOS1AP* single nucleotide polymorphisms rs10494366 T>G and rs10918594 C>G modify the QTc prolonging effect of calcium channel blockers.

Methods

Setting and study design

The Rotterdam study is a prospective population-based cohort study, which started with a baseline visit between 1990 and 1993. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. All inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years and over, were invited to participate (n=10,275). Of them, 7983 (78%) gave their written informed consent and took part in the baseline examination. Objectives and methods of the Rotterdam Study have been

described in detail elsewhere.[17, 18] At baseline, all participants were visited at home for a standardized questionnaire, and 7151 were subsequently examined at the research center. Since the start of the study, follow-up visits took place in the period 1993 through 1996 for the second visit, in the period between 1997 through 1999 for the third visit and in the period between 2002 through 2004 for the fourth visit. Furthermore, in 2000 a second cohort (first extended cohort) was enrolled. This included all inhabitants of Ommoord, at that time aged 55 years and over, who were invited to participate ($n = 4504$). Of them, 3011 (67%) entered the study and took part in the baseline examination. The second visit of the first extended cohort took place in the period between 2004 and 2005. In addition to follow-up examinations, the total cohort is continuously monitored for major morbidity and mortality through linkage of general practitioner and municipality records. Furthermore, all drug prescriptions dispensed to participants by automated pharmacies are routinely stored in the database since 1 January 1991.

Study cohort

All cohort members of the Rotterdam Study and the first extended cohort, who had at least one ECG and genotyping, were enrolled in the study population. Participants could contribute up to four ECGs to the analyses. Overall, 20,596 ECGs were available, 8586 in men and 12,010 in women. Digitally stored ECGs were available for 5397 participants at the time of the first visit (75% of 7151 participants visiting the research center), of 4798 participants at the time of the second visit (76% of 6315 participants visiting the research center), of 3818 participants at the time of the third visit (91% of 4215 participants visiting the research center), and of 3118 participants at the time of the fourth visit (99% of 3145 participants visiting the research center). There were 2273 ECGs available of the participants of the first extended cohort at the time of the first visit (84% of 2722 participants who visited the research center), and of 1190 at the time of the second visit (53% of 2249 participants who visited the research center). Missing ECGs were mainly due to temporary technical problems with ECG recording. For the present study, the visit during which the first ECG was made was defined as baseline. ECGs of participants who used digoxin, which is a QTc shortening agent, were excluded. In addition, the ECGs were excluded of persons with a pacemaker, as well as of persons with evidence of left ventricular hypertrophy, or left and right bundle branch block, since these conditions are associated with a prolonged QTc interval.[19, 20] Consequently, 7565 participants (4404 women and 3161 men) were included.

QTc interval

The primary endpoint of the study was the length of the QTc interval in ms. A 12-lead resting ECG was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECGs were processed by the

Modular ECG Analysis System (MEANS) to obtain ECG measurements, in agreement with the FDA guidance for clinical evaluation of QT/QTc interval prolongation.[21] The MEANS program has been evaluated and validated extensively.[22-25] In one of the validation studies, ECGs with selected abnormalities were analyzed by 5 cardiologists and 11 different computer programs of which MEANS performed as one of the best.[25] In a validation study in which QT intervals by manual measurement were compared with those generated by ECG machines, manual and automated measurements generated similar numerical results in 3 studies in healthy volunteers, which all included a positive control.[26] MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques.[23] The MEANS program determines the QT interval from the start of the QRS complex until the end of the T wave. To adjust for heart rate, Bazett's formula ($QTc = QT/\sqrt{RR}$) was used.[27] European regulatory guidelines were used to categorize QTc prolongation into 3 categories. For men, the cut-off points were less than 430 ms (normal), 430-450 ms (borderline) and more than 450 ms (prolonged), and for women less than 450 ms (normal), 450-470 ms (borderline), and more than 470 ms (prolonged).[28] Additionally, the MEANS program determines left ventricular hypertrophy and left and right bundle branch block.

Drug exposure

In this study, the exposure of interest included calcium channel blockers. In our analyses, we included the following drugs that are available in the Netherlands and are used by at least 40 participants in the Rotterdam Study: calcium channel blockers with mainly vascular effects: amlodipine, isradipine and nifedipine; and calcium channel blockers with direct cardiac effects: diltiazem and verapamil.

Isradipine is included in a commonly consulted internet based registry of QTc prolonging drugs[29]; diltiazem, isradipine and verapamil are included in the list with QTc prolonging drugs from De Ponti et al.[30] The lists with QTc prolonging drugs are based on the medical literature and on the FDA database for reported adverse events.

Participants were classified as current users of calcium channel blockers if the duration of the prescription covered the date of the ECG (index date). The duration was the total number of units issued per prescription divided by the prescribed daily number of units.

Covariates

Diabetes mellitus, hypertension, myocardial infarction and heart failure are considered to be risk factors for QTc prolongation and presence of these conditions at each index date was included as a covariate.[31-34] Clinical measures were obtained during the visits at the Rotterdam Study research center. In 1990-1993 non-fasting blood samples were

obtained, while in 1997-2000 blood samples were obtained after overnight fasting. Diabetes mellitus was defined as the use of blood glucose-lowering medication and/or a non-fasting serum glucose level of 11.1 mmol/l or higher and/or serum glucose levels ≥ 7 mmol/l (1997-2000).[35] Hypertension was defined as a systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 100 mm Hg and/or use of antihypertensive medication, encompassing grade 2 and grade 3 hypertension according to World Health Organization (WHO) criteria.[36] Myocardial infarction at baseline and during follow-up was assessed by hospital discharge diagnosis or in case a patient was not hospitalized, when signs and symptoms, analysis of the standard 12-lead electrocardiogram and cardiac enzyme data were diagnostic of a myocardial infarction.[37, 38] Heart failure at baseline and during follow-up were assessed by the presence of suggestive signs and symptoms and by the use of medication for the indication heart failure.[39, 40]. Use of QTc prolonging drugs (list 1) of the website based registry at the index date was considered as a covariate.[29]

Genotyping

All participants were genotyped for the *NOS1AP* SNP rs10494366 T>G which was previously shown to be associated with the QT interval in 3 independent samples.[9] The partially correlated SNP rs10918594 C>G, which was associated with QT interval in one of the samples,[9] was also genotyped. Both were genotyped using Taqman assays C_1777074_10 and C_1777009_10 (Applied Biosystems, Foster City, Ca., USA) in 1 ng of genomic DNA extracted from leukocytes, as previously reported.[41]

Statistical analysis

The association between exposure to calcium channel blockers and the length of the QTc interval was examined by means of linear regression repeated measures analyses implemented in PROC MIXED (SAS software, version 9.1), since QTc measurements in subsequent ECGs in the same subject are correlated. Analyses were adjusted for gender and the following time-depending covariates: age, diabetes mellitus, hypertension, myocardial infarction, heart failure and use of list 1 QTc prolonging drugs.

Genotype frequencies were tested for Hardy-Weinberg equilibrium using a Chi-square test. In a second analysis, users with the minor alleles were compared with users with the major alleles. For all calcium channel blockers, we tested whether the 2 *NOS1AP* variant alleles modified the QT prolonging effect on a multiplicative scale. Subsequently, analyses were performed by adjusting for dosage and duration of use. All analyses were performed with SAS, version 9.1.

Results

Study subjects

The baseline characteristics of all 7565 participants (16,603 ECGs) of the study population remaining after exclusion of left ventricular hypertrophy (538 ECGs), left and right bundle branch block (387 and 665 ECGs), use of digoxin (557 ECGs), participants with a pacemaker (53 ECGs) and missing genotypes (2055 ECGs) are presented in table 1. At the time of an ECG, 750 participants used calcium channel blockers with mainly vascular effects and 429 participants used other calcium channel blockers with direct cardiac effects.

Table 1 Characteristics of the study population

	Rotterdam study (baseline) (1990 – 1993)	First extended cohort (baseline) (2000 – 2002)
Number of participants	5233	2332
Gender (female)	3108 (59.4%)	1296 (55.6%)
Mean age in years (SD)	68.5 (8.7)	64.4 (7.6)
Mean QTc interval (SD) (ms)	428.3 (24.1)	429.9 (22.3)
ECGs with QTc prolongation	389 (7.4%)	170 (7.3%)
<i>NOS1AP</i> rs10494366		
TT	2136 (40.8%)	930 (39.9%)
TG	2380 (45.5%)	1094 (46.9%)
GG	717 (13.7%)	308 (13.2%)
<i>NOS1AP</i> rs10918594		
CC	2442 (46.7%)	1091 (46.8%)
CG	2258 (43.1%)	1006 (43.1%)
GG	533 (10.2%)	235 (10.1%)
Diabetes mellitus	502 (9.6%)	261 (11.2%)
Heart failure	133 (2.5%)	44 (1.9%)
Hypertension	1397 (26.7%)	683 (29.3%)
Myocardial infarction	584 (11.2%)	121 (5.2%)
Use of QTc prolonging drugs	110 (2.1%)	46 (2.0%)

SD= standard deviation

Genotyping

The G-allele (minor) frequency of rs10494366 T>G was 36.5% and of rs10918594 C>G was 31.4%. Successful genotype calls were made in 96.3% and 97.3% of subjects, respectively. Both SNPs were in Hardy-Weinberg equilibrium ($p=0.53$ for rs10494366 and $p=0.78$ for rs10918594). The two SNPs were in linkage disequilibrium with an r^2 of 0.63 and D' of 0.88 (95%CI 0.87 – 0.90). Upon phasing, we observed two common two-SNP haplotypes: TC (61.2%) and GG (29.2%), consisting of the two major and two minor alleles, respectively, and two minor haplotypes containing one major and one

minor allele each: GC (7.2%) and TG (2.3%). Genotype distributions were similar for men and women and there were no age differences among genotypes.

QTc interval

The mean QTc interval at study entry was significantly lower in males (421.8 ms) than in females (431.5 ms). 74.3% had normal QTc durations at baseline and 19.3% had a borderline QTc interval and 6.4% a prolonged QTc interval, using previously described gender specific cut-off points. Overall, 559 participants developed QTc prolongation during follow-up, with mean QTc levels of 473.9 milliseconds.

Current use of verapamil was associated with a significant QTc interval prolongation (6.0 ms (95%CI 1.7 ; 10.2)), after adjustment for age, gender, diabetes mellitus, heart failure, hypertension, myocardial infarction and use of list 1 QTc prolonging drugs (table 2).

Table 2 Calcium channel blockers and QTc prolongation

	Non-users	Prolongation QTc interval non-users	Users¹	Prolongation QTc interval (95% CI)²
Calcium channel blockers with mainly vascular effects	6815	Reference	750	0.0 (-1.6 ; 1.7)
Amlodipine	7327	Reference	238	0.9 (-1.7 ; 3.6)
Isradipine	7521	Reference	44	0.8 (-6.0 ; 7.6)
Nifedipine	7206	Reference	359	-0.5 (-2.9 ; 1.9)
Calcium channel blockers with direct cardiac effects	7136	Reference	429	2.0 (-0.1 ; 4.1)
Diltiazem	7269	Reference	296	1.7 (-0.8 ; 4.3)
Verapamil	7453	Reference	112	6.0 (1.7 ; 10.2)

CI=Confidence Interval; QTc interval in ms

1: Since some specific drugs were used by <40 participants, numbers do not add up

2: QTc prolongation of calcium channel blocker users compared to non-users, adjusted for age and sex, diabetes mellitus, hypertension, myocardial infarction, heart failure and use of list 1 QTc prolonging drugs.

Calcium channel blockers, *NOS1AP* variant alleles and QTc interval

Minor alleles of both *NOS1AP* SNPs were associated with a significant QTc interval prolongation, after complete adjustment (approximately 3.4 ms per additional G-allele (95%CI 2.4 ; 4.4) for rs10494366) (table 3).

SNP rs10494366 T>G in combination with current use of isradipine was associated with a significant QTc prolongation for the GG genotype compared to users with the TT genotype (19.8 ms (1.9 ; 37.7)). However, SNP rs10494366 T>G did not modify the effect on QTc interval on a multiplicative scale in association with isradipine (p=0.3563). Users of verapamil with the GG-genotype had significantly more QTc prolongation than

Table 3 Calcium channel blockers, *NOS1AP* and QTc prolongation

	Users ¹	rs 10494366 TT	Users ¹	rs 10494366 TG ²	Users ¹	rs 10494366 GG ²	P-value multi- plicative test
Non-users	2581	Reference	2953	3.4 (2.4 ; 4.4)	852	7.3 (5.8 ; 8.7)	
Calcium channel blockers with mainly vascular effects	314	Reference	328	2.8 (-1.5 ; 7.0)	108	5.2 (-0.8 ; 11.2)	0.1849
Amlodipine	92	Reference	110	3.5 (-3.6 ; 10.7)	36	1.9 (-7.9 ; 11.7)	0.4640
Isradipine	22	Reference	17	5.3 (-8.6 ; 19.2)	5	19.8 (1.9 ; 37.7)	0.3563
Nifedipine	151	Reference	156	1.4 (-5.1 ; 7.8)	52	5.6 (-3.5 ; 14.6)	0.5463
Calcium channel blockers with direct cardiac effects	171	Reference	193	1.8 (-4.2 ; 7.8)	65	10.8 (2.5 ; 19.1)	0.9209
Diltiazem	120	Reference	129	-0.7 (-7.8 ; 6.4)	47	4.3 (-5.2 ; 13.7)	0.1986
Verapamil	47	Reference	47	5.0 (-8.4 ; 18.3)	18	25.4 (5.9 ; 44.9)	0.0038

CI=Confidence Interval; QTc interval in ms

1: Since some specific drugs were used by <40 participants, numbers do not add up

2: QTc prolongation of users with TG or GG genotype compared to users with the TT genotype, adjusted for age and sex, diabetes mellitus, hypertension, myocardial infarction, heart failure and use of list 1 QTc prolonging drugs.

users with the TT-genotype. Moreover, SNP rs10494366 T>G modified the effect on the QTc interval on a multiplicative scale in association with verapamil ($p=0.0038$). This association is still significant after Bonferroni correction ($0.05/5=0.01$). We observed no difference in effect on QTc prolongation of the SNPs between men and women. Similar effects were observed for the rs10918594 C>G SNP.

Nine of the 18 verapamil users with the GG genotype were male, the mean age was 69.4 years (SD 7.8). The mean defined daily dosage (DDD) was 1.0 (SD 0.5) and the mean duration of verapamil use was 89 days (SD 70). None of the 18 participants had diabetes mellitus or a myocardial infarction, all participants were diagnosed with heart failure, 4 with hypertension and none of the participants used QTc prolonging drugs.

Additional adjustment for daily dose and duration of use did not substantially change the estimates.

Discussion

We demonstrated in this large prospective cohort study of an elderly population, that the minor alleles of both *NOS1AP* SNPs significantly potentiated the QTc interval prolonging effect of verapamil. Furthermore, regression coefficients indicated that a similar effect

might exist for isradipine, although SNP rs10494366 T>G did not modify the effect on QTc interval on a multiplicative scale in association with isradipine.

Current use of verapamil was associated with a significant increase in the QTc interval. For amlodipine, isradipine, nifedipine and diltiazem we could not confirm a significant increase in the QTc interval. This might be due to the fact that verapamil causes a high-affinity blockage of the *hERG*-current (IC_{50} range 0.14 – 0.83 μ M), whereas amlodipine (IC_{50} unknown), diltiazem (IC_{50} range 10 – 17.3 μ M), isradipine (after 10 μ M $3.1 \pm 1.8\%$ inhibition), and nifedipine (IC_{50} 275 μ M) weakly or do not block the *hERG*-current. [42-45] The concentration of verapamil needed to block the *hERG*-encoded potassium channels overlaps with the concentration needed to block the calcium channel (IC_{50} range 0.25 – 15.5 μ M), whereas the concentrations of the other calcium channel blockers needed to block the calcium channels are much lower than the concentration needed to block the *hERG*-encoded potassium channels (amlodipine, isradipine and nifedipine (IC_{50} range 0.1 – 1.0 μ M) and diltiazem (IC_{50} range 0.63 – 5.0 μ M)).[45] Although a QTc interval prolongation of 6 ms in one individual usually remains without clinical consequences, an average shift of 6 ms in a Gaussian distribution on a population level will inevitably push more individuals into the upper percentiles of the QTc interval with its increased risk of Torsade de Pointes and sudden cardiac death.

Recently, it was found that SNP rs10494366 T>G was associated with an increase in the adjusted QTc interval.[10] In participants with minor alleles of SNP rs10494366 T>G the effect of verapamil on the QTc interval is potentiated. The effect we found was significant on a multiplicative scale which suggests interaction on one common pathway. Although the mechanism by which *NOS1AP* influences the QT interval and interacts with verapamil is not known, it may involve calcium and potassium currents in the cardiomyocyte. *NOS1AP* has been found to activate *NOS1*. [13] *NOS1* stimulates sarcoplasmic reticulum (SR) Ca^{2+} release, which leads to increased intracellular calcium.[46] The elevation of intracellular Ca^{2+} suppresses the Ca^{2+} entry pathway, the L-type calcium channels.[47] Furthermore, elevations in intracellular calcium selectively enhances the delayed rectifier current, which leads to increased outflow of potassium. [47] Verapamil suppresses L-type calcium channels, which are also suppressed by *NOS1*. [45] Moreover, verapamil blocks the *hERG*-encoded potassium channels.[42-44] Thus, the physiological feedback system, i.e. the Ca^{2+} sensitivity of the potassium current, is disturbed due to the high-affinity *hERG*-current blockage caused by verapamil. This suggests a model in which increased intracellular calcium and slow outflow of potassium in participants with minor alleles of *NOS1AP* using verapamil, leads to additional QTc prolongation.

Our study has several strengths. First, the availability of data on a large group of participants, including up to four ECGs per subject at regular intervals during follow-up, which allowed us to obtain more precise long-term ECG measures for each individual.

Furthermore, the use of digital ECG recordings all measured using the MEANS system likely reduced intra- and interobserver variability in the assessment of the QTc interval. Second, the prospective ascertainment of risk factors over a relatively long period of follow-up. Third, the availability of genetic material and complete coverage of drug dispensing records allowed us to study the association between calcium channel blockers, *NOS1AP* variant alleles and QTc prolongation. The population-based design of the study probably limited the chance of selection bias. Also information bias is unlikely since we used pharmacy data, which are registered prospectively and irrespective of disease status, while the QTc-intervals were digitally recorded. Confounding was minimized by adjusting for all known risk factors of QTc prolongation. However, our study has also some potential limitations. The limited exposure to calcium channel blockers at the time of sudden cardiac death events, prohibited us from examining the effect modification of *NOS1AP* variant alleles on sudden cardiac death. However, the *NOS1AP* variant alleles may modify the risk of sudden cardiac death associated with QTc prolonging drugs. Furthermore, in this study we used QTc prolongation as a surrogate marker of sudden cardiac death. However, we demonstrated in an earlier study that QTc prolongation itself was associated with a three-fold increased risk of sudden cardiac death.[48]

In conclusion, we demonstrated that the minor alleles of both *NOS1AP* SNPs significantly potentiate the QTc prolonging effect of verapamil. Because QTc prolongation is associated with an increased risk of sudden cardiac death, this gene-drug interaction may be of clinical importance.

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Chapter 3.1

Non-cardiovascular drugs which inhibit *hERG*-encoded potassium channels and risk of sudden cardiac death

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Abstract

Background

Virtually all QTc prolonging drugs act by blocking the *hERG* (human ether a go-go related gene) encoded potassium channels, whereas not all QTc prolonging drugs are associated with an increased risk of serious cardiac arrhythmias. To investigate whether binding capacity to *hERG*-encoded potassium channels can actually predict hard clinical endpoints, we studied whether non-cardiovascular drugs, which are known to block the *hERG*-encoded potassium channels, are associated with an increased risk of sudden cardiac death and whether the ratio between therapeutic plasma concentrations and the concentration which inhibits 50% of the potassium channels is an indicator of the risk of sudden cardiac death.

Methods

We studied the risk of sudden cardiac death associated with use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs in the Integrated Primary Care Information (IPCI) database, a longitudinal general practice research database. We performed a population-based case-control study, matched for age, gender, GP practice and calendar time. We calculated odds ratios with conditional logistic regression analysis, multivariably adjusted. In addition, we compared *hERG*-encoded potassium channel inhibiting capacity of the different drugs, defined as the effective free therapeutic plasma concentration ($ETCP_{unbound}$) divided by the concentration which inhibits 50% of the potassium channels (IC_{50}), with the risk of sudden cardiac death.

Results

We identified 1424 cases of sudden cardiac death and 14,443 controls. Current use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs was associated with an increased risk of sudden cardiac death (OR_{adj} 1.67 (95%CI 1.19-2.33)). The risk of sudden cardiac death was significantly increased in users of antipsychotics (OR_{adj} 3.90 (2.06-7.37)). Patients using *hERG*-encoded potassium channels inhibiting drugs with a high $ETCP_{unbound}/IC_{50}$ ratio (≥ 0.033) had a higher risk of sudden cardiac death (OR_{adj} 2.03 (1.21-3.40)) than patients using drugs with a low $ETCP_{unbound}/IC_{50}$ ratio (< 0.033) (OR_{adj} 1.47 (0.96-2.27)).

Conclusions

We confirmed that current use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs was associated with an increased risk of sudden cardiac death in the general population. In addition, we demonstrated that drugs with a high *hERG*-encoded potassium channels inhibiting capacity had a higher risk of sudden cardiac death than patients using drugs with a low potassium channels inhibiting capacity.

Introduction

In the past decade, one of the most frequent causes of withdrawal of or restriction in use of marketed drugs was prolongation of the heart-rate corrected QT (QTc) interval. The QTc interval is the traditional measurement for assessing the duration of ventricular repolarization.[1] Prolongation of ventricular repolarization may result in early after depolarizations (EAD), which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmias.[1-4] The issue of non-cardiac drugs associated with a pro-arrhythmogenic potential is identified as a considerable public health problem.[1]

Virtually all drugs that prolong the QTc interval block a specific potassium current, the rapid component of the delayed rectifier (I_{Kr}).[5] I_{Kr} is generated by expression of the **human Ether-à-go-go Related Gene (*hERG*)**, which is also known as *KCNH2*. I_{Kr} is often used interchangeably with the term *hERG*-encoded potassium channel, and conducts K^+ ions out of cardiomyocytes and is responsible for timely repolarization.[6]

The exact clinical implications of drug-induced QTc prolongation are not quite clear. It is known that some drugs associated with QTc prolongation are devoid of torsadogenic effects, whereas others seem to be associated with cardiac arrhythmia with just marginal QTc prolongation.[3, 7, 8] In 2003, collaborating researchers from several pharmaceutical industries published an extensive review on the ability of QTc prolonging drugs to bind to *hERG*-encoded potassium ion channels in relation to free plasma concentrations.[7] Drugs with a small margin (i.e. drugs that block the potassium channels in concentrations close to therapeutic plasma concentration) had a high risk of serious cardiac arrhythmias. Drugs with a high margin (i.e. those that block the potassium channel only at high-therapeutic plasma concentrations) seemed to have a lower risk. In this review, e.g. sertindole and thioridazine had a small margin, suggesting a high risk of cardiac arrhythmias. Amiodarone had a very high margin, which is in line with its known low risk of Torsade de Pointes.[7] However, most of these predictions were based on a literature survey of non-clinical and clinical data. This ratio between therapeutic plasma concentrations and the concentration which inhibits 50% of the potassium channels might be a helpful tool in the prediction of the risk of ventricular arrhythmias.

To investigate whether binding capacity to *hERG*-encoded potassium channels can actually predict hard clinical endpoints, we studied whether non-cardiovascular drugs, which are known to block the *hERG*-encoded potassium channels, are associated with an increased risk of sudden cardiac death and whether the ratio between therapeutic plasma concentrations and the concentration which inhibits 50% of the potassium channels is an indicator of the risk of sudden cardiac death.

Methods

Setting and study design

Data were retrieved from the Integrated Primary Care Information (IPCI) project, a longitudinal observational database, containing data from computer-based medical patient records of a large group of general practitioners (GPs) in the Netherlands, for a population based case-control study. In the Dutch health care system, the GP has a pivotal role by acting as a gatekeeper for all medical care. Details of the database have been described elsewhere.[9, 10] Briefly, the database contains the complete medical records on approximately 1 million patients. The electronic records contain coded and anonymous data on patient demographics, symptoms (in free text), diagnoses (using the International Classification for Primary Care (ICPC) [11] and free text) from GPs and specialists, referrals, laboratory findings, hospitalisations, and drug prescriptions, including their indications and dosage regimen. To guarantee completeness of the data, GPs participating in the IPCI project are not allowed to maintain a system of paper-based records besides the electronic medical records. The system complies with European Union guidelines on the use of medical data for medical research and was considered as valid for pharmaco-epidemiological research in several validation studies that evaluated the quality of the available information.[9] The Scientific and Ethical Advisory Board of the IPCI project approved this study.

The source population comprised all patients of 18 years and older in the IPCI database with a valid database history (date of registration with GP) of at least one year. The study population comprised all cases with sudden cardiac death occurring in the source population during the study period plus their matched controls (see below). The study period started on 1 January 1995 and ended on 1 May 2007. All subjects were followed until death, transferring out of the GP practice, last data draw down or end of the study period, whichever came first.

Definition of sudden cardiac death

Sudden cardiac death was defined as: 1) a witnessed natural death attributable to cardiac causes, heralded by abrupt loss of consciousness, within one hour of onset of acute symptoms, or 2) an unwitnessed, unexpected death of someone seen in a stable medical condition <24 hours previously with no evidence of a non-cardiac cause.[12, 13]

The computerised medical and demographic data were screened for all deaths, which occurred during the study period. The medical records of all identified cases of death were reviewed manually to assess whether death could be classified as sudden cardiac death. Validation was performed independently by two medically trained persons blinded to exposure and in case of discrepancy, a specialized physician arbitrated. To each case of sudden cardiac death, up to twenty controls were randomly drawn from

the source population matched on age (year of birth), gender, and practice (GP). The index date was defined as the date on which sudden cardiac death occurred in cases. This date was also the index date for matched controls.

Use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs

In this study, the exposure of interest included all non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs [7, 14] which are prescription drugs in the study population and are not available as over-the-counter drugs. Since IPCI contains GP records with prescription information, information about use of over-the-counter drugs is not available. The following drugs were included: the antidepressants amitriptyline, fluoxetine, and imipramine; the anticonvulsant phenytoin; the antihistamines chlorpheniramine, ebastine, mizolastine; the antimicrobials ciprofloxacin, clarithromycin, erythromycin, and ketoconazole; the antipsychotics haloperidol, olanzapine, pimozide, risperidone, and thioridazine; the gastrointestinal drug cisapride; and the hormone antagonist tamoxifen.

The duration of each prescription was calculated by dividing the total number of units issued per prescription by the prescribed daily number of units. Use was defined as current if the index date fell within a period of use or within a maximum of 7 days after the end of the last prescription (to deal with carry-over effects and/or irregular use by patients). Past use was defined as discontinuation of the drug, more than 7 days before the index date. If patients did not receive a prescription in the study period prior to the index date they were considered as non-exposed.

Covariates

Known risk factors and other covariates for sudden cardiac death were gathered from the medical records through computerized searches and manual validation. Myocardial infarction, arrhythmias, transient ischemic attack, stroke and heart failure were assessed, based on the diagnoses provided by the general practitioner and by specialists in the medical records. Hypertension was identified through the diagnoses in the medical records, the use of antihypertensive medication and/or blood pressure measurements above 140/90 mmHg.[15] Diabetes mellitus and hypercholesterolemia were identified through diagnoses in the medical records from GPs and specialists and/ or the use of antidiabetic or lipid lowering medication. Use of QTc prolonging drugs, antiarrhythmic drugs, digoxin, CYP3A4 affecting drugs, laxatives, diuretics, oral corticosteroids and beta agonists at the index-date were considered as a covariate.[16]

Statistical analysis

The relative risk of sudden cardiac death associated with use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs was estimated by calculation of the adjusted odds ratios using conditional logistic regression analyses. Covariates which were univariately associated with sudden cardiac death (at a $p < 0.1$ level) were included in the regression analyses if they changed the point estimate of the association between use of these drugs and sudden cardiac death by more than 5%. [17]

First, we assessed the association of use of any non-cardiovascular *hERG*-encoded potassium channels inhibiting drug with sudden cardiac death. Among current users we evaluated the effect of duration (< 30 days; ≥ 30 days and < 90 days; ≥ 90 days) of continuous use which was defined as the delay between first intake and the index date. In addition, we evaluated the effect of anti-*hERG*-encoded potassium channels inhibiting capacity, defined as the effective free therapeutic plasma concentration ($ETCP_{unbound}$) divided by the concentration which inhibits 50% of the potassium channels (IC_{50}), among current users. Redfern et al. suggested that the “safe” margin of the $IC_{50}/ETCP_{unbound}$ ratio is 30, since drugs with a ratio smaller than 30 have a high torsadogenic propensity, whereas drugs with a ratio greater than 30 appeared to have a low torsadogenic propensity.[7] Therefore, we used an $ETCP_{unbound}/IC_{50}$ ratio of 0.033 corresponding to an $IC_{50}/ETCP_{unbound}$ ratio of 30, as a cut-off point. We investigated potential effect modification by age and gender. Second, we assessed separately per drug whether current use was associated with an increased risk of sudden cardiac death. We assessed the effect of dosage among current users (< 1 DDD; ≥ 1 DDD). All analyses were performed using SPSS for Windows version 15.0 (Chicago, Illinois, USA).

Results

Subject characteristics

The source population for this study comprised 478,661 subjects with at least one year of valid history during the study period. The total number of person-years of follow-up was 1,905,382 years, 14,259 persons died, 926 persons were classified as definite sudden cardiac death, 498 as probable sudden cardiac death. Overall, there were 1424 cases and 14,443 matched controls. The mean age of the cases was 72.9 years and 58.4 % were male.

Table 1 Baseline characteristics, demographics, distribution of covariates

Characteristic	Sudden cardiac death cases (n = 1424)	Controls (n = 14,443)	OR (95% CI) ¹
Gender			
Male	831 (58.4%)	8739 (60.5%)	
Female	593 (41.6%)	5704 (39.5%)	
Age (mean, SD) (years)	72.9 (13.9)	66.7 (13.8)	
≤ 55	170 (11.9%)	3008 (20.8%)	
55 – 65	193 (13.6%)	2853 (19.8%)	
66 – 75	366 (25.7%)	4274 (29.6%)	
> 75	695 (48.8%)	4308 (29.8%)	
Co-morbidities			
Myocardial infarction	77 (5.4%)	271 (1.9%)	2.75 (2.06 – 3.67)*
Transient ischemic attack	65 (4.6%)	383 (2.7%)	1.18 (0.88 – 1.59)
Stroke	59 (4.1%)	259 (1.8%)	1.66 (1.21 – 2.28)*
Arrhythmia	141 (9.9%)	837 (5.8%)	1.45 (1.18 – 1.79)*
Hypertension	268 (18.8%)	2284 (15.8%)	1.18 (1.04 – 1.34)*
Diabetes mellitus	284 (19.9%)	1274 (8.8%)	2.43 (2.09 – 2.84)*
Heart failure	272 (19.1%)	615 (4.3%)	4.09 (3.42 – 4.90)*
Hypercholesterolemia	204 (14.3%)	1619 (11.2%)	1.60 (1.35 – 1.91)*
Smoking	283 (19.9%)	2857 (19.8%)	1.28 (1.10 – 1.50)*
Alcohol abuse	30 (2.1%)	107 (0.1%)	3.59 (2.31 – 5.58)*
Concomitant medication			
QTc prolonging drugs	58 (4.1%)	283 (2.0%)	2.01 (1.48 – 2.75)*
Antiarrhythmic drugs	19 (1.3%)	104 (0.7%)	1.76 (1.03 – 2.99)*
Digoxin	118 (8.3%)	317 (2.2%)	3.52 (2.76 – 4.48)*
CYP3A4 inducing drugs	10 (0.7%)	45 (0.3%)	2.05 (0.97 – 4.30)
CYP3A4 inhibiting drugs	65 (4.6%)	302 (2.1%)	1.78 (1.32 – 2.38)*
Laxatives	89 (6.3%)	431 (3.0%)	1.65 (1.27 – 2.14)*
Diuretics	241 (16.9%)	901 (6.2%)	3.09 (2.58 – 3.69)*
Oral corticosteroids	44 (3.1%)	137 (0.9%)	2.71 (1.87 – 3.91)*
Beta agonists	144 (10.1%)	851 (5.9%)	1.83 (1.50 – 2.24)*

1: Odds Ratios matched for age, gender, practice and calendar time

SD Standard Deviation

* P<0.05

Association between non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs and sudden cardiac death

Of all cases, 54 patients were current users at the index date and 227 patients were past users (table 2). There was a significant association between current use and sudden cardiac death (adjusted OR 1.67 (95% CI 1.19-2.33)). Past use was not associated with an increased risk of sudden cardiac death (OR_{adj} 1.00 (0.85-1.19)). Patients using

Table 2 Use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs and risk of sudden cardiac death

Non-cardiovascular <i>hERG</i>-encoded potassium channels blocking drugs	Cases (n=1424)	Controls (n = 14,443)	OR (95% CI)¹	OR (95% CI)²
<i>Never use</i>	1 143 (80.3)	12099 (83.8)	Reference	Reference
<i>Past use</i>	227 (15.9)	2088 (14.5)	1.08 (0.92 – 1.27)	1.00 (0.85 – 1.19)
<i>Current use</i>	54 (3.8)	256 (1.8)	1.81 (1.30 – 2.53)	1.67 (1.19 – 2.33)
Duration of use				
< 30 days	38 (2.7)	162 (1.1)	2.00 (1.35 – 2.96)	1.86 (1.25 – 2.77)
≥ 30 days	16 (1.1)	94 (0.7)	1.47 (0.81 – 2.67)	1.30 (0.70 – 2.40)
< 90 days	52 (3.7)	254 (1.8)	1.77 (1.27 – 2.49)	1.64 (1.17 – 2.31)
≥ 90 days	2 (0.1)	2 (0)	NA	NA
ETCP _{unbound} /IC ₅₀ ratio				
< 0.033	28 (2.0)	176 (1.2)	1.61 (1.05 – 2.47)	1.47 (0.96 – 2.27)
≥ 0.033	26 (1.8)	80 (0.6)	2.19 (1.31 – 3.64)	2.03 (1.21 – 3.40)
<i>Effect modification</i>				
Gender				
Females	33 (5.6)	143 (2.5)	2.10 (1.37 – 3.22)	2.02 (1.32 – 3.10)
Males	21 (2.5)	113 (1.3)	1.46 (0.85 – 2.50)	1.23 (0.71 – 2.12)
Age				
< 65 years	13 (3.6)	66 (1.1)	3.07 (1.63 – 5.76)	2.68 (1.40 – 5.13)
≥ 65 years	41 (3.9)	190 (2.2)	1.55 (1.06 – 2.28)	1.44 (0.98 – 2.12)

1: Odds Ratios matched for age, gender, practice and calendar time

2: Odds Ratios matched for age, gender, practice and calendar time, adjusted for use of diuretics.

ETCP_{unbound}/IC₅₀ ratio: Measure of anti-*hERG*-encoded potassium channels inhibiting activity.[14]

hERG-encoded potassium channels inhibiting drugs less than 30 days had a higher risk of sudden cardiac death (OR_{adj} 1.86 (1.25-2.77)) than patients using these drugs more than 30 days (OR_{adj} 1.30 (0.70-2.40)). Patients using *hERG*-encoded potassium channels inhibiting drugs with a high ETCP_{unbound}/IC₅₀ ratio (≥0.033) had a higher risk of sudden cardiac death (OR_{adj} 2.03 (1.21-3.40)) than patients using drugs with a low ETCP_{unbound}/IC₅₀ ratio (<0.033) (OR_{adj} 1.47 (0.96-2.27)).

Stratified analyses showed that the risk of sudden cardiac death was higher in women (OR_{adj} 2.02 (1.32-3.10)) than in men (OR_{adj} 1.23 (0.71-2.12)) and higher in patients younger than 65 years (OR_{adj} 2.68 (1.40-5.13)) than in patients older than 65 years (OR_{adj} 1.44 (0.98-2.12)), but these differences were not statistically significant.

Association between individual drug (groups) and sudden cardiac death

The risk of sudden cardiac death was significantly increased in users of antipsychotics (OR_{adj} 3.90 (2.06–7.37)), predominantly in users of haloperidol and risperidone (table 3). The risk was not increased in users of antidepressants, anticonvulsants, antihistamines, antimicrobials, gastrointestinal drugs and hormone antagonists, probably due to the limited number of exposed cases. The risk of sudden cardiac death tended to be higher in users of antimicrobials and the hormone antagonist tamoxifen, although non-significantly (OR_{adj} 2.22 (0.89 – 5.49) and OR_{adj} 2.78 (0.91–8.50), respectively). Patients using higher dosages (≥ 1 DDD) of antidepressants, antimicrobials and antipsychotics had a higher risk of sudden cardiac death than patients using lower dosages (< 1 DDD) (table 3).

Table 3 Current use of individual non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs and risk of sudden cardiac death

	ETCP _{unbound} / IC ₅₀ ratio	Cases (n=1424)	Controls (n = 14,443)	OR (95% CI) ¹	OR (95% CI) ²
Antidepressants					
Current use		12	126	0.98 (0.53 – 1.81)	1.01 (0.54 – 1.90)
Amitriptyline	0.00880	9	100	0.90 (0.44 – 1.83)	0.91 (0.44 – 1.87)
Fluoxetine	0.00935	3	24	1.42 (0.40 – 5.05)	1.62 (0.45 – 5.78)
Imipramine	0.0312	0	2	NA	NA
<1 DDD		10	112	0.88 (0.44 – 1.74)	0.89 (0.44 – 1.79)
≥ 1 DDD		2	14	2.69 (0.58 – 12.47)	2.44 (0.50 – 12.00)
Anticonvulsants					
Current use		3	14	1.36 (0.31 – 6.04)	1.15 (0.25 – 5.21)
Phenytoin	0.0436	3	14	1.36 (0.31 – 6.04)	1.15 (0.25 – 5.21)
Antihistamines					
Current use		0	15	NA	NA
Chlorpheniramine	0.00688	0	7	NA	NA
Ebastine	0.0170	0	3	NA	NA
Mizolastine	0.0249	0	5	NA	NA
Antimicrobials					
Current use		10	18	2.99 (1.24 – 7.24)	2.22 (0.89 – 5.49)
Ciprofloxacin	0.00547	3	5	2.75 (0.58 – 12.91)	2.35 (0.48 – 11.65)
Clarithromycin	0.0367	5	12	2.30 (0.67 – 7.87)	1.50 (0.42 – 5.35)
Erythromycin	0.00235	1	1	NA	NA
Ketoconazole	0.0932	1	0	NA	NA
<1 DDD		5	13	2.22 (0.65 – 7.55)	1.79 (0.50 – 6.35)
≥ 1 DDD		5	5	4.06 (0.80 – 20.51)	3.52 (0.72 – 17.18)

Table 3 continued

	ETCP _{unbound} / IC ₅₀ ratio	Cases (n=1424)	Controls (n = 14,443)	OR (95% CI) ¹	OR (95% CI) ²
Antipsychotics					
Current use		20	35	3.90 (2.06 – 7.37)	3.90 (2.06 – 7.37)
Haloperidol	0.133	11	16	3.49 (1.43 – 8.53)	3.49 (1.43 – 8.53)
Olanzapine	0.0225	3	1	NA	NA
Pimozide	0.0287	0	7	NA	NA
Risperidone	0.0121	5	9	3.90 (1.13 – 13.53)	3.90 (1.13 – 13.53)
Thioridazine	29.7	1	2	NA	NA
<1 DDD		12	19	3.66 (1.59 – 8.45)	3.66 (1.59 – 8.45)
≥1 DDD		8	16	5.20 (2.10 – 12.91)	5.20 (2.10 – 12.91)
Gastrointestinal drugs					
Current use		7	37	1.56 (0.63 – 3.86)	1.27 (0.51 – 3.20)
Cisapride	2.45	7	37	1.56 (0.63 – 3.86)	1.27 (0.51 – 3.20)
Hormone antagonists					
Current use		5	15	3.24 (1.11 – 9.52)	2.78 (0.91 – 8.50)
Tamoxifen	0.0210	5	15	3.24 (1.11 – 9.52)	2.78 (0.91 – 8.50)

ETCP_{unbound}/IC₅₀ ratio: Measure of anti-*hERG*-encoded potassium channels inhibiting activity.[14]

Effects of DDDs are presented for classes with at least 10 exposed cases.

1: Odds Ratios matched for age, gender, practice and calendar time

2: Odds Ratios matched for age, gender, practice and calendar time. Antidepressants adjusted for diabetes mellitus and heart failure; anticonvulsants adjusted for hypertension, heart failure and use of steroids and antiarrhythmics; antimicrobials adjusted for diabetes mellitus, heart failure, QTc prolonging drugs (other than drugs studied), use of antipsychotics, gastrointestinal drugs, CYP3A4 inhibitors, digoxin, diuretics, steroids and beta agonists; gastrointestinal drugs adjusted for diabetes mellitus, heart failure and diuretics and hormone antagonists adjusted for heart failure, diabetes mellitus and use of digoxin, diuretics and steroids.

Discussion

In this study, we demonstrated that current use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs in the general population was associated with an increased risk of sudden cardiac death. The risk tended to be higher in women than in men, which is in line with earlier findings that women seem to be more susceptible to drug-induced cardiac arrhythmias than men.[3, 8]

In line with regulatory recommendations, most new drugs are tested for their ability to block *hERG*-encoded potassium channels.[18] However, some QTc prolonging drugs that do not seem to cause Torsade de Pointes, also block this current, whereas on the other hand some drugs that prolong the QTc interval with only few milliseconds are implicated in the occurrence of cardiac arrhythmias.[7, 8, 19, 20] Since the amount of QTc prolongation is not a specific predictor of symptomatic cardiac rhythm disorders, it is important to have another measure to predict arrhythmias.

We were able to demonstrate that drugs with a high *hERG*-encoded potassium channels inhibiting capacity (drugs that block the potassium channels in concentrations close to therapeutic plasma concentrations) had a higher risk of sudden cardiac death than patients using drugs with a low potassium channels inhibiting capacity (drugs that block the potassium channels only at high-therapeutic plasma concentrations). This suggests that the ratio between therapeutic plasma concentrations and the concentration which inhibits 50% of the potassium channels might be a helpful tool in the prediction of fatal ventricular arrhythmias.

In a previous study, it was demonstrated that *hERG*-encoded potassium channels inhibiting drugs were associated with an increased number of reports of serious ventricular arrhythmias and sudden death in the adverse drug reactions database of the World Health Organization.[14] These reports are voluntarily submitted to pharmacovigilance centres and often do not contain a full medical history whereas data on other risk factors may be incomplete or lacking. Moreover, since reporting of adverse drug reactions is voluntarily, not all adverse drug reactions are reported and not all adverse reactions are recognized by the doctor. Earlier, we demonstrated in IPCI that use of non-cardiac QTc prolonging drugs specified in list 1 from a commonly consulted internet based registry of QTc prolonging drugs was associated with an increased risk of sudden cardiac death.[16, 21] This study, however, was smaller than the current one (700 cases are in common), focused on QTc prolonging drugs in general, and did not take *hERG*-encoded potassium channels inhibiting activity into account. The increased risk of sudden cardiac death in users of antipsychotics has been described before.[22, 23]

In our population, we were able to take advantage of the fact that in the Dutch health care system, all medical information (including specialist and hospital care) is collected at practices that cover the general population instead of selected socio-economic groups. As a consequence, there was extensive information available on drug use, potential confounders, and all circumstances surrounding death. Nevertheless, our study has some potential limitations. First, we cannot exclude that some misclassification of outcome occurred. We may have missed some deaths although this will be minimal, since death is consistently registered by GPs. Second, not all acute deaths may have been of cardiac origin. We determined sudden cardiac death, however, on the basis of the full medical records and all circumstances surrounding the death were available. Recently, an evaluation comparing different methods to determine the incidence of sudden cardiac death suggested that this method provides a very reliable way of determining sudden cardiac death cases.[24] Misclassification of exposure may have occurred for various reasons. First, we used outpatient prescription data and we had no information as to whether the prescription was actually filled and taken. Second, the legend duration for a calculated prescription may not reflect actual use. Third, *hERG*-encoded potassium channels inhibition might be only one out of several mechanisms of drug-induced QTc prolongation

and sudden cardiac death. Recently, several new genetic loci were found.[25] Possibly, some of these might also modify the magnitude of QTc prolongation by drugs.

In conclusion, we confirmed that current use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs was associated with an increased risk of sudden cardiac death in the general population. In addition, we demonstrated that drugs with a high *hERG*-encoded potassium channels inhibiting capacity had a higher risk of sudden cardiac death than patients using drugs with a low potassium channels inhibiting capacity. This suggests that the ratio between therapeutic plasma concentrations and the concentration which inhibits 50% of the potassium channels might be a helpful tool in the prediction of fatal ventricular arrhythmias.

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Chapter 3.2

Domperidone and ventricular arrhythmia or sudden cardiac death

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Abstract

Background

Recently a 4-fold increase in risk of sudden cardiac death (SCD) was reported for domperidone in a study that focused on QTc prolonging drugs as a class and sudden cardiac death. We wanted to evaluate the association between use of domperidone and serious non-fatal ventricular arrhythmia (VA) and SCD in the general population.

Methods

Design: Population-based case-control study during 1996-2007.

Setting: Integrated Primary Care Information (IPCI) database, a longitudinal general practice research database in the Netherlands.

Population: All patients ≥ 18 years without cancer were included in the source population.

Exposure: Use of domperidone (current, past and none) and daily dose of use.

Outcome: Serious non-fatal VA or SCD.

Analysis: Controls matched to cases on age, gender, practice and index date. Comparison of exposure odds for SCD alone and ventricular arrhythmia plus SCD by means of conditional logistic regression, multivariate adjusted. In addition, we stratified by insurance since there was differential misclassification due to insurance (since the drug is mostly used OTC and reimbursed).

Results

The study population comprised 1366 cases (62 VA and 1304 SCD) and 14,114 matched controls. Of all cases, 10 patients were current domperidone users at the index date, all with SCD. The matched unadjusted OR of domperidone and SCD was 3.72 (95%CI 1.72 – 8.08). Daily doses >30 mg were associated with a significant increased risk of SCD (OR_{adj} 11.4 (1.99 - 65.2)). Since there was a near interaction with health insurance ($P=0.050$), all analyses were stratified by insurance. In publicly insured patients, 7 cases were current users at the index date. Current use was associated with a significant increased risk of SCD (OR_{adj} 4.17 (1.33- 13.1)). In privately insured patients there was 1 exposed case and in non-insured 2.

Conclusions

Current use of domperidone, especially high doses, is associated with an increased risk of SCD. We could not demonstrate an effect of domperidone on non-fatal VA due to absence of exposed cases.

Introduction

Domperidone is a peripheral dopamine D₂-receptor antagonist with gastrokinetic and anti-emetic properties and has been marketed since March 1978 as a prescription drug for the following indications: nausea, vomiting, and dyspepsia associated with motility disorders.[1] In many countries, such as the Netherlands, domperidone is also available as over the counter product for treatment of nausea and dyspepsia associated with motility disorders. In the Netherlands, domperidone was available as over the counter drug, but reimbursement was possible when prescribed. As of January 2004, domperidone was no longer reimbursed, even when prescribed. As of January 2005, the drug was reimbursed in case of chronic use (> 6 months).

A recent epidemiological study conducted in the Netherlands evaluated the use of non-cardiac QTc-prolonging drugs and the risk of sudden cardiac death (SCD). In a sub-analysis it was found that domperidone increased the risk of SCD almost 4-fold.[2] Since the study was not primarily focused on individual drugs and modeling was not specific for the individual drugs, the marketing authorization holder asked for a more specific study that would focus on domperidone and would include not only SCD but also non-fatal ventricular arrhythmias (VA's).

Prolongation of the heart-rate corrected QT (QTc) interval, which is the traditional measurement for assessing the duration of ventricular repolarization, life-threatening ventricular tachyarrhythmias and even cardiac arrests have been reported after intravenous use of domperidone, however often confounded by concomitant medications and co-morbidity.[3] Prolongation of ventricular repolarization may result in early after depolarizations (EAD), which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmias.[4-8]

Inhibition of the human ether-a-go-go-related gene (*hERG*), which encodes a delayed rectifier K⁺ current, leads to prolongation of the QTc interval.[9] Domperidone has been shown to inhibit the *hERG* current and to cause a significant prolongation of cardiac repolarization.[3] Domperidone is mainly metabolised via the cytochrome P450 3A4 (CYP3A4) isoenzyme, *in vitro* data indicate that the concomitant use of drugs that significantly inhibit this enzyme may result in increased plasma concentrations of domperidone. Even though no effect on QTc was seen with oral domperidone as monotherapy, prolongation of the QTc interval was observed when combined with ketoconazole, a CYP3A4 inhibitor.[10]

The primary purpose of this study was to evaluate the association between use of domperidone and serious fatal (SCD) and non-fatal VA, while controlling specifically for confounding factors in this association. This was done in the same database as was used for the initial study [2], but was extended to include ventricular arrhythmia and updated until 2007.

Methods

Setting

All data were retrieved from the Integrated Primary Care Information (IPCI) database, a longitudinal database of electronic medical records from general practitioners (GPs) in the Netherlands. In the Dutch health care system, the GP has a pivotal role by acting as a gatekeeper for all medical care. Nearly every citizen is enrolled in the practice of a GP independent of health status.[11] Details of the database have been described elsewhere.[11, 12] Briefly, the database currently contains the complete medical records of 1 million citizens. The electronic records contain coded (using the International classification for Primary Care[13] and free text) and narrative data on demographics, health care insurance (public or private as a proxy for income), symptoms, and diagnoses from GPs and specialists, referrals, laboratory findings, hospitalizations, and drug prescriptions, including their indications and dosage regimen. To maximize completeness of the data, GPs participating in the IPCI project are not allowed to maintain a system of paper-based records besides the electronic medical records. The project complies with European Union guidelines on the use of medical data for medical research and has been proven valid for pharmaco-epidemiological research in several validation studies that evaluated the quality of the available information.[12] The Scientific and Ethics Advisory Board of the IPCI project approved this study (project number: 07/07).

Source population

The source population comprised all patients of 18 years and older in the IPCI database with a valid database history of at least one year. Follow-up started whenever all entrance criteria (one year of valid database capture, age of 18 years, and 1 January 1995) were reached. All subjects in the source population were followed until the occurrence of the outcome of interest (SCD, non-fatal VA or the earliest of both), cancer (exclusion criterion), death, transferral out of the practice, date of last data collection from the GP or end of the study (1 May 2007), whichever came first. The study population comprised all cases with serious VA or SCD occurring in the source population during the study period and their matched controls.

Case and control definition

The primary outcome of interest was serious idiopathic VA (ventricular fibrillation (VF) or Torsade de Pointes (TdP)) and SCD. Potential cases were identified through a sensitive search on codes and narratives. Subsequently, the medical records of all potential cases were reviewed manually to assess whether death could be classified as SCD or whether non-fatal VA could be classified as VF or TdP. Initial validation was performed independently by two medically trained persons who were blinded to exposure and classified

cases as potential or none cases. All potential cases were re-assessed by a specialized physician and classified as probable or definite.

Case assessment of SCD was based on the most recent definition of SCD: a natural death due to cardiac causes heralded by abrupt loss of consciousness within 1 h after the onset of acute symptoms or an unwitnessed, unexpected death of someone seen in a stable medical condition < 24 h previously with no evidence of a noncardiac cause. [14] Cases were classified as SCD if the medical record indicated that death occurred within 1 h after the onset of symptoms, and if the following wording was found in the free text: 'sudden cardiac death', 'acute cardiac death', 'mors subita', 'sudden death', 'died suddenly', 'died unexpectedly', or if this was an unwitnessed, unexpected death of someone seen in 'good health' or in a stable medical condition, 24 h previously and without evidence of a non-cardiac cause (e.g. pneumonia, convulsion, choking, stroke or suicides). The assessment of SCD was done independently of the previous assessment of SCD in the same database (Straus et al.) [2] and more strict with regards to any prior symptoms in the unwitnessed cases to avoid protopathic bias (specifically for domperidone) due to chest pain.

Non-fatal VA cases were identified by broad searching on the following codes or text words/abbreviations: collapse, VES, VF, ventricular & arrhythmia, PVC, ventricle, tachycardia, Torsade, VT, rhythm disturbance, flutter, cardioversion, defibrill*, QRS, QTc, ECG abnormalities, death and ICPC diagnosis codes: K79, K80, K84. All potential cases either had been referred or seen by a cardiologist or an ECG was performed. All non-fatal VA cases were divided in TdP (diagnosis made by a specialist with the help of an ECG) or VF (diagnosis by an ECG). For all VA's it was assessed whether they occurred as primary or secondary disorder (i.e. as primary disorder or after myocardial infarction). The date of onset of VA was assessed based on the information in the electronic medical record (including free text). All assessments were done while being blinded to exposure.

To each case, up to 20 (SCD) or 40 (non-fatal VA) controls were randomly drawn from the source population matched on age (year of birth), gender and practice. The index date was defined as the date on which VA or SCD occurred in the cases. This date was also the index date for matched controls.

Exposure definition

The exposure of interest was use of domperidone. In order to classify use at the index date, we calculated the duration of each prescription, by dividing the total number of units issued per prescription by the number of units prescribed daily. Exposure at the index date was categorized into three groups of current-, past-, and never use. Since domperidone is normally prescribed for shorter periods of time, use was defined as current if the index date fell within a period of use or within a maximum of 7 days after the end of the last prescription to account for carry-over effects. Past use was defined

as discontinuation of domperidone for more than 7 days. If patients had no prescription prior to the index date they were considered non-exposed.

To better study the effect of stopping and potential confounding by indication, we subcategorized past exposure into recent past exposure (stopping between 8 days and 3 months), moderate past exposure (3-6 months), distant past exposure (6-12 months previous) and very distant past exposure (> 1 year prior).

If domperidone was prescribed 'as needed', the GP estimated duration was taken if available, otherwise a default of 30 days was taken. Among current users, we evaluated the effect of daily dose (<30, 30 mg (1 DDD) and >30 mg), which allows for dose response assessment.

Covariates

Known risk factors for VAF SCD and other covariates were gathered from the medical records. Cerebrovascular ischaemia, cardiovascular ischaemia, heart failure, depression, schizophrenia, epilepsy, neuropathy, dyspepsia and COPD were assessed, based on the diagnoses provided by the GP and specialists in the medical records. Hypertension was identified through the diagnoses in the medical records, use of antihypertensive medication and/or blood pressure measurements, according to the guidelines of the World Health Organization (a blood pressure exceeding 140 mmHg systolic and/or 90 mmHg diastolic).[15] Diabetes mellitus and hypercholesterolaemia were identified through diagnoses in the medical records from GPs and specialists and/or use of antidiabetic or lipid-lowering medication. Diabetic gastropathy (nausea, vomiting, heartburn, abdominal bloating and early fullness in persons with diabetes mellitus) was assessed through manual review of the patient's files in the year prior to the index date. Information on smoking and alcohol abuse was obtained from the codes and narratives in the medical records. As concomitant medication, we considered QTc prolonging drugs as specified in the most recent version of list 1 (drugs that are generally accepted by authorities to have a risk of causing Torsades de Pointes) (<http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm>).[16] Furthermore, we considered drugs that may interact with domperidone metabolism (CYP3A4 inhibiting and inducing drugs) and drugs that affect *hERG*, digoxin, diuretics, laxatives, systemic beta-agonists and oral corticosteroids. Covariate drug use was classified as current (prescription duration covers the index date or stopped less than 8 days ago), past (stopped at index date between 7-365 days) or never use. As a measure of health status, the number of GP office visits in the year prior to the index date was assessed by the total number of visits during the one year prior.

Statistical analysis

Prescription rates (number of users per year) of domperidone were calculated in the source population and stratified by health care insurance to inspect potential differential

misclassification due to insurance (since the drug is mostly OTC and reimbursed). The association between VA/ SCD and the covariates was assessed through conditional logistic regression analysis separately for SCD as well as together. All covariates that were univariately associated with the outcome ($p < 0.10$) were considered as potential confounders. Subsequently the multivariate models were built to include all covariates that changed the association between current use of domperidone and the outcome by more than 5% (i.e. $0.95 > \text{OR}_{\text{adjusted}} / \text{OR}_{\text{unadjusted}} > 1.05$). Analyses were conducted with and without adjustment for the number of visits in the previous year, since GP visits have no etiological relationship with the outcome. Interaction of the association between domperidone exposure and specific covariates (age, gender, insurance type) was tested on the basis of multiplicative interaction using logistic regression (with the matching factors as covariates). In addition, we stratified for type of health care insurance.

Sensitivity analyses were conducted to investigate various sources of bias and residual confounding. First, the effect of residual confounding by diabetic gastroparesis was addressed by excluding all diabetes patients. In addition we conducted an analysis that excluded all patients with prior cardiovascular disease (heart failure and myocardial infarction) to avoid residual confounding due to severity of underlying cardiovascular disease. Additional misclassification of exposure due to unmeasured OTC use of domperidone was inspected by censoring at 1/1/2004 which was the date that prescribed domperidone was no longer reimbursed, thereby taking away any incentive to get the drug on prescription.

Results

The source population for this study comprised 478,661 subjects with at least one year of valid history during the study period. Figure 1 shows the prevalence of domperidone use in the source population over calendar time by type of health care insurance. Publicly insured persons had a higher prevalence of recorded domperidone use than privately insured patients, but in 2004 (when reimbursement was lifted for all OTC drugs even when prescribed) the prevalence dropped in publicly insured patients and remained stable in privately insured patients, pointing to differential misclassification by insurance type.

Overall, 926 persons were classified as definite SCD and 498 as probable SCD (table 1). After exclusion of 120 cases with cancer prior to the index date, the study population comprised 1304 cases of SCD and 13,480 matched controls. The mean age of the cases was 72.5 years and 58 % were male.

Of the potential VA cases, 287 were classified as definite VF, and 6 as Torsade de Pointes, however only 57 and 6 were not preceded by other disease (such as myocardial

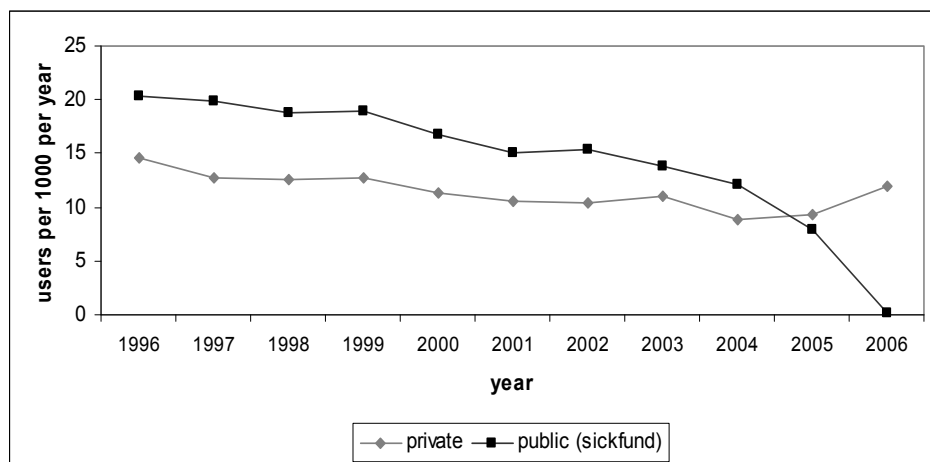


Figure 1 Prevalence of domperidone use by calendar year

Table 1 Baseline characteristics, demographics, distribution of covariates

Characteristic	Sudden cardiac death			Serious ventricular arrhythmia		
	Cases (n = 1304)	Controls (n = 13480)	OR (95%CI)	Cases (n=62)	Controls (n=634)	OR (95%CI)
Gender						
Male	755 (57.9)	8211 (60.9)		41 (66.1)	439 (69.2)	
Age (mean, SD) (years)						
≤ 55	72.5 (14.1)	66.3 (13.9)		64.9 (15.2)	61.6 (14.1)	
55 – 65	166 (12.7)	2938 (21.8)		13 (21.0)	212 (33.4)	
66 – 75	180 (13.8)	2733 (20.3)		14 (22.6)	146 (23.0)	
> 75	330 (25.3)	3948 (29.3)		20 (32.3)	171 (27.0)	
	628 (48.2)	3861 (28.6)		15 (24.2)	105 (16.6)	
Co-morbidities						
Ischaemic cerebro-/cardiovascular disease	385 (29.5)	1916 (14.2)	2.01 (1.74 - 2.33)	22 (35.5)	62 (9.8)	5.43 (2.77 - 10.66)
Angina	294 (22.5)	1408 (10.4)	2.04 (1.74 - 2.39)	19 (30.6)	46 (7.3)	6.44 (3.13 - 13.25)
Myocardial infarction	64 (4.9)	234 (1.7)	2.64 (1.92 - 3.64)	5 (8.1)	11 (1.7)	5.14 (1.39 - 19.03)
Transient ischemic attack	59 (4.5)	339 (2.5)	1.22 (0.90 - 1.66)	2 (3.2)	11 (1.7)	1.54 (0.31 - 7.77)
Cerebrovascular accident	51 (3.9)	215 (1.6)	1.76 (1.25 - 2.48)	1 (1.6)	3 (0.5)	2.75 (0.28 - 27.29)
Hypertension	487 (37.3)	4103 (30.4)	1.24 (1.09 - 1.42)	20 (32.3)	167 (26.3)	1.01 (0.51 - 1.99)
Diabetes mellitus	258 (19.8)	1165 (8.6)	2.47 (2.10 - 2.91)	10 (16.1)	51 (8.0)	1.77 (0.73 - 4.27)
Diabetic gastropathy	30 (2.3)	138 (1.0)	2.06 (1.34 - 3.17)	1 (1.6)	4 (0.6)	2.76 (0.31 - 24.85)
Heart failure	237 (18.2)	536 (4.0)	4.00 (3.29 - 4.85)	9 (14.5)	9 (1.4)	7.37 (2.35 - 23.06)
Hypercholesterolemia	178 (13.7)	1459 (10.8)	1.56 (1.30 - 1.88)	16 (25.8)	82 (12.9)	4.41 (2.08 - 9.35)
Depression	91 (7.0)	699 (5.2)	1.40 (1.10 - 1.79)	5 (8.1)	33 (5.2)	1.40 (0.42 - 4.62)
Schizophrenia	11 (0.8)	20 (0.1)	6.14 (2.74 - 13.8)	0 (0)	0 (0)	NA
Chronic obstructive lung disease	183 (14.0)	1118 (8.3)	1.58 (1.31 - 1.90)	11 (17.7)	63 (9.9)	1.80 (0.77 - 4.24)
Epilepsy	15 (1.2)	63 (0.5)	2.39 (1.29 - 4.43)	0 (0)	1 (0.2)	NA

Table 1 continued

Characteristic	Sudden cardiac death			Serious ventricular arrhythmia		
	Cases (n = 1304)	Controls (n = 13480)	OR (95%CI)	Cases (n=62)	Controls (n=634)	OR (95%CI)
Neuropathy	23 (1.8)	260 (1.9)	0.84 (0.53 - 1.32)	2 (3.2)	14 (2.2)	1.45 (0.30 - 7.10)
Dyspepsia	319 (24.5)	2741 (20.3)	1.18 (1.02 - 1.38)	13 (21.0)	126 (19.9)	1.13 (0.56 - 2.27)
Life style						
Smoking	256 (19.6)	2631 (19.5)	1.33 (1.13 - 1.56)	16 (25.8)	134 (21.1)	1.64 (0.81 - 3.31)
Alcohol abuse	24 (1.8)	97 (0.7)	3.31 (2.04 - 5.36)	0 (0)	7 (1.1)	NA
Public insurance	831 (63.7)	5932 (44.0)	2.66 (2.30 - 3.08)	28 (45.2)	366 (57.7)	1.97 (0.96 - 4.05)
Mean GP visits (SD)	6.7 (7.8)	4.3 (5.0)	1.05 (1.04 - 1.07)	5.4 (5.2)	3.6 (4.1)	1.08 (1.01 - 1.16)
Concomitant medication						
QTc prolonging drugs	53 (4.1)	260 (1.9)	2.11 (1.52 - 2.92)	7 (11.3)	13 (2.1)	8.30 (2.43 - 28.31)
hERG inhibiting drugs	139 (10.7)	813 (6.0)	1.82 (1.48 - 2.24)	10 (16.1)	43 (6.8)	2.92 (1.19 - 7.18)
CYP 3A4 inducing drugs	10 (0.8)	40 (0.3)	2.33 (1.08 - 5.05)	0 (0)	0 (0)	NA
CYP 3A4 inhibiting drugs	59 (4.5)	277 (2.1)	1.83 (1.34 - 2.50)	1 (1.6)	13 (2.1)	NA
Drugs for dyspepsia and GERD (H2RAs and PPIs)	97 (7.4)	840 (6.2)	1.08 (0.85 - 1.37)	2 (3.2)	40 (6.3)	0.28 (0.04 - 2.09)
Laxatives	81 (6.2)	378 (2.8)	1.77 (1.33 - 2.34)	1 (1.6)	16 (2.5)	NA
Digoxin	112 (8.6)	287 (2.1)	3.74 (2.91 - 4.82)	5 (8.1)	13 (2.1)	3.19 (0.89 - 11.44)
Diuretics	220 (16.9)	810 (6.0)	3.23 (2.67 - 3.89)	8 (12.9)	33 (5.2)	2.86 (1.12 - 7.26)
Corticosteroids	36 (2.8)	115 (0.1)	2.49 (1.65 - 3.75)	0 (0)	0 (0)	NA
Beta agonists	134 (10.3)	775 (5.7)	1.94 (1.26 - 2.21)	0 (0)	0 (0)	NA

CI=Confidence Interval

OR=Odds Ratio matched for age, gender, index date and practice

SD=Standard Deviation

infarction). 21 of these 63 persons died within 30 days after the onset of VA. One case of the 63 serious VA cases had cancer and was excluded. There were no currently exposed cases to domperidone, which hampers the possibility to assess the association between VA and prescribed domperidone.

Domperidone and sudden cardiac death

Of all SCD cases, 10 patients were current domperidone users at the index date and 94 patients were past users (table 2). The matched unadjusted relative risk of domperidone and SCD was 3.72 (95%CI 1.72 - 8.08). Current use of domperidone was associated with SCD in the entire population although this was not significant anymore when adjusting also for GP visits. This effect of GP visits was mostly due to the strong correlation between GP visits and type of health care insurance ($P=5.8 \times 10^{-32}$).

There was a near interaction with health insurance ($P=0.050$), but not with age ($P=0.547$) and gender ($P=0.491$). Because of the interaction with insurance, all subsequent analyses were stratified by type of health care insurance. Seven of the currently exposed domperidone cases were publicly insured, 1 privately and 2 were not insured.

Table 2 Risk of sudden cardiac death

Use of domperidone	Cases	Controls	OR (95% CI) ¹	OR (95% CI) ²	OR (95% CI) ³
Overall population	1304	13480			
<i>Never use</i>	1200	12781	1.0 (reference)	1.0 (reference)	1.0 (reference)
<i>Past use</i>	94	671	1.56 (1.23 - 1.98)	1.36 (1.05 - 1.75)	1.28 (0.99 - 1.65)
recent past	10	54	1.91 (0.95 - 3.86)	1.56 (0.73 - 3.33)	1.39 (0.65 - 2.99)
moderate past	7	34	2.30 (0.98 - 5.38)	2.24 (0.93 - 5.41)	2.00 (0.83 - 4.86)
distant past	13	83	1.59 (0.86 - 2.96)	1.26 (0.67 - 2.39)	1.03 (0.53 - 2.00)
very distant past	64	500	1.46 (1.09 - 1.94)	1.29 (0.95 - 1.75)	1.26 (0.93 - 1.70)
<i>Current use</i>	10	28	3.72 (1.72 - 8.08)	2.44 (1.01 - 5.89)	1.99 (0.80 - 4.96)
<30 mg	2	10	NA	NA	NA
30 mg	4	15	2.57 (0.79 - 8.36)	1.41 (0.38 - 5.32)	1.02 (0.23 - 4.42)
>30 mg	4	3	16.0 (3.49 - 73.6)	11.2 (2.02 - 62.45)	11.4 (1.99 - 65.2)
Stratified by insurance					
Publicly insured	831	5932			
<i>Never use</i>	759	5615	1.0 (reference)	1.0 (reference)	1.0 (reference)
<i>Past use</i>	65	305	1.77 (1.28 - 2.44)	1.47 (1.05 - 2.08)	1.34 (0.94 - 1.90)
<i>Current use</i>	7	12	4.46 (1.46 - 13.7)	4.98 (1.59 - 15.6)	4.17 (1.33 - 13.1)
<30 mg	1	5	NA	NA	NA
30 mg	3	7	3.02 (0.67 - 13.7)	3.27 (0.70 - 15.3)	2.57 (0.54 - 12.2)
>30 mg	3	0	NA	NA	NA
Privately insured	412	7289			
<i>Never use</i>	387	6918	1.0 (reference)	1.0 (reference)	1.0 (reference)
<i>Past use</i>	24	358	1.47 (0.87 - 2.48)	1.26 (0.72 - 2.20)	1.22 (0.70 - 2.12)
<i>Current use</i>	1	13	NA	NA	NA
Not insured	61	259			
<i>Never use</i>	54	248	1.0 (reference)	1.0 (reference)	1.0 (reference)
<i>Past use</i>	5	8	2.05 (0.40 - 10.4)	1.53 (0.25 - 9.40)	1.11 (0.15 - 8.47)
<i>Current use</i>	2	3	NA	NA	NA

1 Odds ratios matched for age, gender, practice and index date.

2 Overall population: Odds ratios adjusted for heart failure, insurance type, CYP3A4 inhibitors, *hERG* inhibiting drugs, laxatives, digoxin, diuretics, steroids, beta agonists; Publicly insured: Odds ratios adjusted for heart failure, *hERG* inhibiting drugs, laxatives, diuretics, steroids and digoxin; Privately insured: Odds ratios adjusted for heart failure, diuretics, steroids, beta agonists and digoxin; Not insured: Odds ratios adjusted for heart failure, diuretics, steroids, CYP3A4 inhibitors, *hERG* inhibiting drugs, digoxin and beta agonists

3 Additionally adjusted for GP visits

In publicly insured persons, domperidone was associated with a significantly increased risk of SCD after adjustment for heart failure, *hERG* inhibiting drugs, laxatives, diuretics, steroids and digoxin (OR 4.98 (1.59-15.6)). The association between current use of domperidone and SCD decreased slightly after further adjustment for GP visit frequency (4.17 (1.33-13.1)). Past use of domperidone was not associated with SCD. In privately and non-insured patients we could not estimate the adjusted effect of current use of domperidone due to small numbers of exposed persons. The matched unadjusted odds

ratio's were 0.78 (0.09 – 6.81) for privately insured patients and 1.73 (0.10 – 30.8) for none-insured patients.

Dose-response analyses showed that a high daily prescribed dose (>30 mg for total population) was associated with a significant increased risk of SCD after complete adjustment (11.4 (1.99-65.2)), whereas normal (30 mg) or low dose (< 30 mg) were not associated (1.02 (0.23-4.42) and 1.24 (0.19-8.12) respectively). After stratification for insurance, dose response could not be assessed in the separate categories because of small numbers. Since there were null exposed controls in the high dose category we aggregated high and normal dose for publicly insured patients (OR_{adj} 7.21 (2.03 – 25.6)).

Domperidone and ventricular arrhythmia/ sudden cardiac death

In order to assess the association between VA/SCD and domperidone, we combined the SCD and VA datasets, censoring 17 SCD cases at the moment of VA, as they were also included in the VA set (as they had confirmed VA). High dose of domperidone (>30 mg) was associated with a significantly increased risk of SCD/VA (11.4 (1.99 – 64.9)) in the total population after complete adjustment (table 3), however this was in fact the SCD effect.

Sensitivity analyses

In view of the possibility of residual confounding by diabetic gastroparesis, we excluded diabetes patients (table 4). This increased the association between current use of domperidone and SCD up to 5.12 (2.01 - 13.0) and also the dose response relationship (high daily prescribed dose (>30 mg) 54.2 (4.95 - 592.6)).

Exclusion of cardiovascular diseases increased the association between current use of domperidone and SCD after complete adjustment (4.05 (1.60 – 10.2)) and increased the dose response relationship (high daily prescribed dose (>30 mg) 35.8 (3.68 – 347.5)).

Exclusion of all case and matched controls sets (matched on index date) with index dates after the first of January 2004 (when domperidone was not reimbursed anymore if prescribed) did not change the association of current use of domperidone and SCD (2.03 (0.77 – 5.36)) and the dose response relationship (high daily prescribed dose (>30 mg) 11.6 (2.08 – 64.6)).

Discussion

This study was conducted to investigate the association between use of domperidone and the occurrence of serious ventricular arrhythmias. We demonstrated that current use of domperidone, and in particular high dose, was associated with a substantial increased risk of SCD. We could not assess the risk of ventricular arrhythmia since

Table 3 Risk of sudden cardiac death and non-fatal ventricular arrhythmia

Use of domperidone	Cases	Controls	OR (95% CI) ¹	OR (95% CI) ²	OR (95% CI) ³
Overall population	1366	14114			
Never use	1258	13384	1.0 (reference)	1.0 (reference)	1.0 (reference)
Past use	98	700	1.57 (1.24 – 1.99)	1.35 (1.05 – 1.73)	1.26 (0.98 – 1.62)
Current use	10	30	3.54 (1.64 – 7.64)	2.35 (0.99 – 5.62)	1.92 (0.78 – 4.73)
<30 mg	2	11	NA	NA	NA
30 mg	4	16	2.45 (0.76 – 7.86)	1.36 (0.37 – 5.04)	0.99 (0.23 – 4.23)
> 30 mg	4	3	16.0 (3.48 – 73.4)	11.2 (2.02 – 62.3)	11.4 (1.99 – 64.9)
Publicly insured	865	6194			
Never use	790	5858	1.0 (reference)	1.0 (reference)	1.0 (reference)
Past use	68	323	1.76 (1.28 – 2.41)	1.46 (1.04 – 2.04)	1.32 (0.94 – 1.86)
Current use	7	13	4.45 (1.45 – 13.6)	4.13 (1.32 – 13.0)	4.92 (1.58 – 15.4)
<30 mg	1	5	NA	NA	NA
30 mg	3	8	3.03 (0.67 – 13.7)	3.24 (0.69 – 15.1)	2.56 (0.54 – 12.1)
> 30 mg	3	0	NA	NA	NA
Privately insured	440	7655			
Never use	414	7274	1.0 (reference)	1.0 (reference)	1.0 (reference)
Past use	25	367	1.45 (0.87 – 2.42)	1.24 (0.72 – 2.14)	1.20 (0.70 – 2.05)
Current use	1	14	NA	NA	NA
Not insured	61	265			
Never use	54	252	1.0 (reference)	1.0 (reference)	1.0 (reference)
Past use	5	10	2.05 (0.10 – 30.8)	1.53 (0.25 – 9.40)	1.11 (0.15 – 8.47)
Current use	2	3	NA	NA	NA

1 Odds ratios matched for age, gender, practice and index date.

2 Overall population: Odds ratios adjusted for heart failure, insurance type, CYP3A4 inhibitors, *hERG* inhibiting drugs, laxatives, digoxin, diuretics, steroids, beta agonists; Publicly insured: Odds ratios adjusted for heart failure, *hERG* inhibiting drugs, laxatives, diuretics, steroids and digoxin; Privately insured: Odds ratios adjusted for heart failure, diuretics, steroids, beta agonists and digoxin; Not insured: Odds ratios adjusted for heart failure, diuretics, steroids, CYP3A4 inhibitors, *hERG* inhibiting drugs, digoxin and beta agonists.

3 Additionally adjusted for GP visits

there were no exposed VA cases. Past use of domperidone was not associated with an increased risk of SCD.

Inhibition of the *hERG*-encoded potassium channels leads to prolongation of the QTc interval, which can degenerate into ventricular arrhythmias and eventually lead to SCD.[9] Domperidone has been shown to inhibit the *hERG*-encoded potassium channels and thereby cause a significant prolongation of cardiac repolarization.[3] The main metabolic pathway of domperidone is via the cytochrome P450 3A4 (CYP3A4) isoenzyme. Concomitant use of drugs that inhibit this enzyme may result in increased plasma concentrations of domperidone. Studies in healthy volunteers have demonstrated prolongation of the QTc interval when domperidone was combined with ketoconazole, a CYP3A4 inhibitor. When oral domperidone was used as monotherapy, no effect on QTc was seen.[10]

Table 4 Risk of sudden cardiac death, sensitivity analyses

	Exclusion of diabetes mellitus			Exclusion of cardiovascular diseases			Censoring at 01/01/2004		
	Cases	Controls	OR (95% CI) ¹	Cases	Controls	OR (95% CI) ¹	Cases	Controls	OR (95% CI) ¹
Total population	1046	12315		1027	12753		1044	10892	
Never use	969	11701	1.0 (reference)	953	12125	1.0 (reference)	956	10351	1.0 (reference)
Past use	67	592	1.23 (0.90 - 1.67)	65	605	1.26 (0.93 - 1.71)	79	518	1.40 (1.05 - 1.85)
Current use	10	22	5.12 (2.01 - 13.0)	9	23	4.05 (1.60 - 10.2)	9	23	2.03 (0.77 - 5.36)
<30 mg	2	9	NA	1	9	NA	2	7	NA
30 mg	4	11	4.07 (1.06 - 15.7)	4	12	2.45 (0.67 - 8.96)	3	13	0.70 (0.12 - 4.03)
>30 mg	4	2	54.2 (4.95 - 592.6)	4	2	35.8 (3.68 - 347.5)	4	3	11.6 (2.08 - 64.6)
Publicly insured	661	5354		652	5574		676	4995	
Never use	608	5078	1.0 (reference)	602	5287	1.0 (reference)	615	4727	1.0 (reference)
Past use	46	265	1.25 (0.81 - 1.93)	44	275	1.38 (0.91 - 2.11)	54	257	1.37 (0.94 - 2.01)
Current use	7	11	6.07 (1.81 - 20.4)	6	12	4.11 (1.25 - 13.6)	7	11	4.17 (1.33 - 13.0)
<30 mg	1	5	NA	0	5	NA	1	4	NA
30 mg	3	6	4.79 (0.86 - 26.9)	3	7	2.15 (0.43 - 10.7)	3	7	2.55 (0.54 - 12.1)
>30 mg	3	0	NA	3	0	NA	3	0	NA
Privately insured	338	6727		325	6951		325	6951	
Never use	318	6397	1.0 (reference)	306	6618	1.0 (reference)	306	6618	1.0 (reference)
Past use	19	320	1.38 (0.72 - 2.66)	18	323	1.35 (0.72 - 2.55)	18	323	1.35 (0.72 - 2.55)
Current use	1	10	NA	1	10	NA	1	10	NA

¹ Overall population: Odds ratios matched for age, gender, practice and index date and adjusted for heart failure, insurance type, CYP3A4 inhibitors, *hERG* inhibiting drugs, laxatives, digoxin, diuretics, steroids, beta agonists and GP visits; Publicly insured: Odds ratios matched for age, gender, practice and index date and adjusted for heart failure, *hERG* inhibiting drugs, laxatives, digoxin and GP visits; Privately insured: Odds ratios matched for age, gender, practice and index date and adjusted for heart failure, diuretics, steroids, beta agonists, digoxin and GP visits.

The current study is different from the study reported by Straus et al. for various reasons.[2] First, the model was specifically built around domperidone alone and not around use of non-cardiac QTc prolonging agents in general. Second, because of the potential of protopathic bias due to chest pain and stomach ache, cases of SCD were validated more strictly, excluding all those who might have vague chest symptoms. Third, the number of cases was larger since the study by Straus et al. ended in 2003. Fourth, the analysis now also looked at dose response. Despite the fact that the modelling was specific for domperidone, conclusions were the same although more refined, namely that domperidone in high dosages is associated with sudden cardiac death in the general population.

In our population, we were able to take advantage of the fact that in the Dutch health care system, all medical information (including specialist and hospital care) is collected at GP practices that cover the general population instead of selected socio-economic groups. As a consequence, there was extensive information available on drug use, potential confounders, and all the circumstances surrounding death.

Nevertheless, our study has some potential limitations. First, we cannot exclude that some misclassification of outcome occurred. We may have missed some deaths although this will be minimal, since death is consistently registered by GPs. Second, not all acute deaths may have been of cardiac origin. We determined SCD, however, on the basis of the full medical records and all circumstances surrounding the death were available. Recently, an evaluation comparing different methods to determine the incidence of SCD suggested that this method provides a very reliable way of determining SCD cases.[17] Third, we may have missed some ventricular arrhythmias, although this will be minimal due to the broad search criteria we used. All VA cases were determined based on the full medical records and needed to have an ECG confirmation. We may have missed all cases who died without ECG evidence but these will be included as SCD. Finally, since we only included definite VA cases not preceded by other disease, such as myocardial infarction, misclassification will be minimal.

Misclassification of exposure may have occurred for various reasons. First, we used outpatient prescription data and we had no information as to whether the prescription was actually filled and taken. Second, the legend duration for a calculated prescription (indicating the theoretical duration) may not reflect actual use. However, as in most cases (7) use was not on an as needed basis, the legend duration probably well matches the actual duration of use. Third, since 1/1/2004 domperidone is no longer reimbursed even when prescribed (from 1/1/2005 reimbursed again in case of chronic use). Publicly insured persons had a higher prevalence of recorded domperidone use than privately insured patients, but in 2004 the prevalence dropped in publicly insured patients and remained stable in privately insured patients, pointing to differential misclassification by insurance type, which can be seen in figure 1. Since we observed near interaction

with insurance we stratified by this variable. Fourth, potential non-continuous use may explain why the risk for SCD was elevated in some past use categories.

Although we adjusted for all known confounders, residual confounding may exist, but is unlikely that this would explain the strong association for high doses of domperidone. Adjustment for GP visits showed an effect as confounder which may be explained by the fact that prodromal symptoms of SCD, such as nausea and vomiting, can be mistakenly diagnosed as symptoms due to gastric pathology. Diabetic gastropathy was considered to be a potential confounder. However, in patients without diabetes mellitus, the association between current use and SCD increased, which excludes the possibility of residual confounding by diabetic gastropathy. In patients without cardiovascular disease, current use of domperidone was associated with an increased risk, which avoids residual confounding due to severity of underlying cardiovascular disease.

In conclusion, this study underscores our prior conclusion that domperidone use is associated with an increased risk of SCD[2], however it refines it to the extent that mainly high dosages are associated with an increased risk, and it excludes the possibility of diabetic gastropathy and protopathic bias. This study was specifically designed to investigate the association between domperidone and VA and this association could not be demonstrated due to the lack of exposed and few confirmed VA cases.

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Chapter 3.3

Anticonvulsants and risk of sudden cardiac death

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Abstract

Background

Although sudden cardiac death has been frequently reported as the most common direct epilepsy-related cause of death in epilepsy and the indications of anticonvulsants (ACs) prescriptions have widened to include chronic pain syndromes, the association of ACs with sudden cardiac death is not systematically studied in a population-based study. Therefore, we studied whether use of ACs is associated with an increased risk of sudden cardiac death in a population-based database.

Methods

We used the Integrated Primary Care Information (IPCI) database, which is a longitudinal general practice research database. Drug use at the index date was assessed with drug prescriptions from general practices. We used a conditional logistic regression analysis in a case-control analysis, matched for age, gender, practice and calendar time, multivariate adjusted.

Results

We identified 926 cases of sudden cardiac death and 9832 matched controls. Current use of ACs was associated with a 2.5-fold increased risk of sudden cardiac death (adjusted OR 2.5 (95%CI 1.5-4.2)), in particular among carbamazepine users (OR_{adj} 3.4 (95%CI 1.5-7.7)). Past use was not associated with sudden cardiac death (OR_{adj} 1.4 (95%CI 0.9-2.3)). The highest risk was observed among users of sodium channel blocking ACs (OR_{adj} 2.9 (95%CI 1.5-5.5)). The risk among users of non-sodium channel blocking ACs was lower and not significant (OR_{adj} 1.6 (95%CI 0.8-3.5)).

Conclusions

We demonstrated that current use of ACs is associated with an increased risk of sudden cardiac death in the general population, even in persons who use ACs for other indications than epilepsy. The risk of sudden cardiac death seems to be higher among users of carbamazepine and other ACs with sodium channel blocking properties.

Introduction

Epilepsy is a common chronic neurologic disease affecting over 50 million people worldwide.[1] The disease is associated with recurrent seizures and an increased mortality rate compared to the general population.[2] Although prevalent, death in epilepsy remains difficult to predict, because a substantial proportion of deaths is sudden and unexpected.[3] Various descriptive conditions have been related to sudden cardiac death in epilepsy, including age, type and frequency of seizures and severity of epilepsy.[4-7] However, a clear pathophysiologic mechanism for sudden cardiac death in epilepsy is lacking. The main proposed mechanisms for sudden cardiac death in epilepsy are cardiac arrhythmias [8] and central apnoea.[9] There is an emerging interest in cardiac arrhythmias in epilepsy [10-14] and the pro-arrhythmic potential of anticonvulsants (ACs).[15-18]

So far, there has been only sparse evidence for the role of ACs in sudden cardiac death in epilepsy, and no individual AC has been associated with sudden cardiac death. Yet, ACs could potentially contribute to sudden cardiac death in epilepsy as they may disturb heart rhythm in two ways: 1) ACs may influence cardiac conduction directly by blocking cardiac sodium current, while blockade of cardiac sodium channels may evoke lethal arrhythmias, as large-scale randomized placebo-controlled studies have indicated [19, 20]; 2) ACs act centrally in the brain and thereby influence the autonomic control of the heart, while autonomic dysfunction is associated with lethal cardiac arrhythmias.[21]

Although sudden cardiac death has been frequently reported as the most common direct epilepsy-related cause of death in epilepsy and the indications of AC prescriptions have widened to include chronic pain syndromes, the association of ACs with sudden cardiac death is not systematically studied in a population-based study. Therefore, we studied whether use of ACs is associated with an increased risk of sudden cardiac death in a population-based database.

Methods

Setting and study design

Data were retrieved from the Integrated Primary Care Information (IPCI) project, a longitudinal observational database, containing data from computer-based medical patient records of a large group of general practitioners (GPs) in the Netherlands, for a population based case-control study. In the Dutch health care system, the GP has a pivotal role by acting as a gatekeeper for all medical care. Details of the database have been described elsewhere.[22, 23] Briefly, the database contains the complete medical records on approximately 1 million patients. The electronic records contain coded and anonymous data on patient demographics, symptoms (in free text), diagnoses (using

the International Classification for Primary Care (ICPC) [24] and free text) from GPs and specialists, referrals, laboratory findings, hospitalisations, and drug prescriptions, including their indications and dosage regimen. To guarantee completeness of the data, GPs participating in the IPCI project are not allowed to maintain a system of paper-based records besides the electronic medical records. The system complies with European Union guidelines on the use of medical data for medical research and was considered as valid for pharmaco-epidemiological research in several validation studies that evaluated the quality of the available information.[22] The Scientific and Ethical Advisory Board of the IPCI project approved this study.

The source population comprised all patients of 18 years and older in the IPCI database with a valid database history (date of registration with GP) of at least one year. The study population comprised all cases with sudden cardiac death occurring in the source population during the study period plus their matched controls (see below). The study period started on 1 January 1995 and ended on 1 May 2007. All subjects were followed until death, transferring out of the GP practice, last data draw down or end of the study period, whichever came first.

Definition of sudden cardiac death

Sudden cardiac death was defined as: 1) a witnessed natural death attributable to cardiac causes, heralded by abrupt loss of consciousness, within one hour of onset of acute symptoms, or 2) an unwitnessed, unexpected death of someone seen in a stable medical condition <24 hours previously with no evidence of a non-cardiac cause.[25, 26]

The computerised medical and demographic data were screened for all deaths, which occurred during the study period. The medical records of all identified cases of death were reviewed manually to assess whether death could be classified as sudden cardiac death. Validation was performed independently by two medically trained persons blinded to exposure and in case of discrepancy, a specialized physician arbitrated. To each case of sudden cardiac death, up to twenty controls were randomly drawn from the source population matched on age (year of birth), gender, and practice (GP). The index date was defined as the date on which sudden cardiac death occurred in cases. This date was also the index date for matched controls.

Use of anticonvulsants

In this study, the exposure of interest included all available ACs. The following drugs were included: phenytoin, carbamazepine, levetiracetam, vigabatrin, ethosuximide, clonazepam, phenobarbital, primidone, lamotrigine, oxcarbazepine, gabapentin, and valproic acid. The ACs were divided into two groups based on their documented ability to block the sodium channel (neural and/or cardiac). Sodium channel blocking drugs: phenytoin, carbamazepine, oxcarbazepine, lamotrigine and gabapentin.[27-29]

Non-sodium channel blocking drugs included: valproic acid, levetiracetam, primidone, phenobarbital, clonazepam, ethosuximide and vigabatrin.[27-32]

The duration of each prescription was calculated by dividing the total number of units issued per prescription by the prescribed daily number of units. Use of ACs was defined as current if the index date fell within a period of use or within a maximum of 28 days after the end of the last prescription (to deal with carry-over effects). Past use was defined as discontinuation of an AC >28 days before the index date. If patients had no prescription for an AC prior to the index date, they were considered as non-exposed. Among current users, we evaluated the effect of duration (≤ 30 and >30 days continuous use).

Covariates

Known risk factors and other covariates for sudden cardiac death were gathered from the medical records through computerized searches and manual validation. Myocardial infarction, transient ischemic attack, stroke, arrhythmia and heart failure were assessed, based on the diagnoses provided by the general practitioner and by specialists in the medical records. Hypertension was identified through the diagnoses in the medical records, the use of antihypertensive medication and/or blood pressure measurements above 140/90 mmHg.[33] Diabetes mellitus, and hypercholesterolemia were identified through diagnoses in the medical records from GPs and specialists and/ or the use of antidiabetic, or lipid lowering medication. Use of QTc prolonging drugs, antiarrhythmic drugs, digoxin, diuretics, calcium channel blockers and beta blockers at the index-date were considered as a covariate.[34]

Statistical analysis

The relative risk of sudden cardiac death associated with use of ACs was estimated by calculation of the adjusted odds ratios using conditional logistic regression analysis. Covariates which were univariately associated with sudden cardiac death (at a $p < 0.1$ level) were included in the regression analyses if they changed the point estimate of the association between use of ACs and sudden cardiac death by $>10\%$.[35]

First, we studied whether use of ACs was associated with an increased risk of sudden cardiac death. Among current users we evaluated the effect of duration of continuous use which was defined as the delay between first intake and the index date. We investigated potential effect modification by age and gender with interaction terms and subsequent stratifications.

Second, we examined whether use of sodium channel blocking and non-sodium channel blocking ACs was associated with an increased risk of sudden cardiac death. We conducted separate analyses for the individual ACs with at least 3 current users. All analyses were performed using SPSS for Windows version 15.0 (Chicago, Illinois, USA).

Results

Subject characteristics

The source population for this study comprised 478,661 subjects with at least one year of valid history during the study period. During a follow-up of 1,905,382 person-years, 14,259 persons died, including 926 definite sudden cardiac death cases. We identified 9,832 matched controls. The mean age of cases was 71.7 years and 62.0% were male (table 1).

Table 1 Baseline characteristics, demographics, distribution of covariates

Characteristic	Sudden cardiac death cases (n = 926)	Controls (n = 9832)	OR (95% CI) ¹
Gender			
Male	574 (62.0)	6319 (64.3)	
Female	352 (38.0)	3513 (35.7)	
Age (mean, SD) (years)	71.7 (13.7)	66.1 (13.6)	
≤ 55	121 (13.1)	2145 (21.8)	
55 – 65	145 (15.7)	2147 (21.8)	
66 – 75	248 (26.8)	2818 (28.7)	
> 75	412 (44.5)	2722 (27.7)	
Co-morbidities			
Ischemic cerebro- or cardiovascular disease	273 (29.5)	1448 (14.7)	2.0 (1.7 – 2.3)
Arrhythmia	83 (9.0)	553 (5.6)	1.3 (1.0 – 1.7)
Hypertension	364 (39.3)	3005 (30.6)	1.3 (1.1 – 1.5)
Diabetes mellitus	169 (18.3)	833 (8.5)	2.3 (1.9 – 2.8)
Heart failure	162 (17.5)	397 (4.0)	4.0 (3.2 – 5.0)
Hypercholesterolemia	137 (14.8)	1085 (11.0)	1.7 (1.3 – 2.0)
Smoking	197 (21.3)	2024 (20.6)	1.3 (1.1 – 1.5)
Alcohol abuse	22 (2.4)	84 (0.9)	3.5 (2.1 – 5.9)
Concomitant medication			
QTc prolonging drugs	41 (4.4)	186 (1.9)	2.3 (1.6 – 3.3)
Antiarrhythmic drugs	15 (1.6)	76 (0.8)	1.9 (1.0 – 3.5)
Digoxin	76 (8.2)	221 (2.2)	3.2 (2.4 – 4.4)
Diuretics	168 (18.1)	597 (6.1)	3.3 (2.7 – 4.1)
Calcium channel blockers	87 (9.4)	604 (6.1)	1.4 (1.1 – 1.7)
Beta blockers	141 (15.2)	1082 (11.0)	1.4 (1.1 – 1.7)

1: Odds Ratios matched for age, gender, practice and calendar time
SD Standard Deviation

Association anticonvulsants and sudden cardiac death

Of all cases, 23 were current users at the index date and 24 patients were past users (table 2). Current use of ACs was associated with a 2.5-fold increased risk of sudden cardiac death (adjusted OR 2.5 (95%CI 1.5-4.2)). Past use was not associated with sudden cardiac death (OR_{adj} 1.4 (95%CI 0.9-2.3)). Patients using ACs more than 30 days had a higher risk of sudden cardiac death (OR_{adj} 3.2 (95%CI 1.5-6.8)) than patients using ACs less than 30 days (OR_{adj} 1.8 (95%CI 0.9-3.6)).

Table 2 Use of anticonvulsants and risk of sudden cardiac death

	Cases (n=926)	Controls (n = 9832)	OR (95% CI)¹	OR (95% CI)²
<i>Never use</i>	879	9583	1.0 (reference)	1.0 (reference)
<i>Past use</i>	24 (2.6)	166 (1.7)	1.5 (1.0 – 2.4)	1.4 (0.9 – 2.3)
<i>Current use</i>	23 (2.5)	83 (0.8)	2.8 (1.7 – 4.6)	2.5 (1.5 – 4.2)
Duration of use				
≤ 30 days	11 (1.2)	52 (0.5)	2.3 (1.1 – 4.6)	1.8 (0.9 – 3.6)
> 30 days	12 (1.3)	31 (0.3)	3.5 (1.7 – 7.2)	3.2 (1.5 – 6.8)
Effect modification				
Gender				
Females	9 (2.6)	34 (1.0)	2.7 (1.2 – 6.1)	2.4 (1.1 – 5.5)
Males	14 (2.4)	49 (0.8)	2.8 (1.5 – 5.3)	2.2 (1.1 – 4.4)
Age				
< 65 years	7 (2.6)	27 (0.6)	4.4 (1.9 – 10.4)	2.9 (1.1 – 7.3)
≥ 65 years	16 (2.4)	56 (1.0)	2.2 (1.2 – 4.1)	1.9 (1.0 – 3.7)

1: Odds Ratios matched for age, gender, practice and calendar time

2: Odds Ratios matched for age, gender, practice and calendar time, adjusted for diabetes mellitus, heart failure, hypertension, hypercholesterolemia, alcohol abuse, cerebro- and cardiovascular ischemia and use of diuretics, calcium channel blockers, beta blockers and antiarrhythmic drugs.

Stratified analyses among current users showed a comparable risk in males and females (OR_{adj} males: 2.2 (95%CI 1.1-4.4); OR_{adj} females: 2.4 (95%CI 1.1-5.5)). The risk of sudden cardiac death was higher in patients younger than 65 years (OR_{adj} 2.9 (95%CI 1.1-7.3)) than in patients older than 65 years (OR_{adj} 1.9 (95%CI 1.0-3.7)), but these differences were not statistically significant.

Association of individual ACs and sudden cardiac death

The highest risk of sudden cardiac death was observed among users of sodium channel blocking ACs (OR_{adj} 2.9 (95%CI 1.5-5.5)) (table 3). The risk among users of non-sodium channel blocking ACs was lower and not significant (OR_{adj} 1.6 (95%CI 0.8-3.5)).

Table 3 Current use of individual anticonvulsants and risk of sudden cardiac death

	Cases (n=926)	Controls (n = 9832)	OR (95% CI)¹	OR (95% CI)²
<i>Sodium channel blocking ACs*</i>	15 (1.6)	43 (0.4)	3.5 (1.9 – 6.6)	2.9 (1.5 – 5.5)
Carbamazepine	10 (1.1)	26 (0.3)	4.0 (1.8 – 8.9)	3.4 (1.5 – 7.7)
Gabapentin	3 (0.3)	6 (0.1)	5.0 (1.1 – 22.3)	3.0 (0.6 – 14.6)
Lamotrigine	0	3 (0.0)	NA	NA
Oxcarbazepine	0	1 (0.0)	NA	NA
Phenytoin	3 (0.3)	10 (0.1)	2.0 (0.4 – 9.1)	2.9 (0.5 – 15.3)
<i>Non-sodium channel blocking ACs*</i>	10 (1.1)	51 (0.5)	2.0 (1.0 – 4.1)	1.6 (0.8 – 3.5)
Clonazepam	4 (0.4)	14 (0.1)	2.9 (0.9 – 9.1)	2.4 (0.7 – 8.2)
Levetiracetam	0	1 (0.0)	NA	NA
Phenobarbital	2 (0.2)	14 (0.1)	NA	NA
Primidone	0	4 (0.0)	NA	NA
Valproic acid	4 (0.4)	20 (0.2)	2.1 (0.7 – 6.7)	1.8 (0.6 – 5.8)

1: Odds Ratios matched for age, gender, practice and calendar time.

2: Odds Ratios matched for age, gender, practice and calendar time, adjusted for diabetes mellitus, heart failure, hypertension, hypercholesterolemia, alcohol abuse, cerebro- and cardiovascular ischemia and use of diuretics, calcium channel blockers, beta blockers and antiarrhythmic drugs.

ACs anticonvulsants

* Since few patients used 2 AEDs concomitantly, numbers do not add up.

Although the numbers of the individual ACs were relatively small, separate analyses of individual ACs showed an increased and significant risk of sudden cardiac death in carbamazepine users (OR_{adj} 3.4 (95%CI 1.5-7.7)). In addition, use of gabapentin tended to be associated with an increased risk of sudden cardiac death, however, non-significant (OR_{adj} 3.0 (95%CI 0.6-14.6)).

Discussion

We found that current use of ACs is associated with a 2.5-fold increased risk of sudden cardiac death. Current use of sodium-channel blocking ACs was associated with an almost 3-fold increased risk of sudden cardiac death. Notably, carbamazepine was associated with an almost 3.5-fold increased risk of sudden cardiac death.

The relation between sudden cardiac death and the use of ACs has so far been reported in small cohorts of patients that are treated at specialized epilepsy institutions. The small size of these cohorts, their severity of epilepsy, and the fact that they often use multiple ACs has made it difficult so far to determine the role of ACs in sudden cardiac death.[5, 15, 36-38] In this large-scale population-based study, we studied the role of ACs in sudden cardiac death in the general population. Besides patients who used

ACs for epilepsy, we also included patients who used ACs for other indications, e.g., neuropathic pain. As our cases and controls were derived from the general population, most of the included epilepsy patient had stable epilepsy and used a single AC, thereby reducing confounding by severity of epilepsy and polytherapy of ACs. Our epilepsy cases may thus represent a yet unrecognized category of sudden cardiac death in epilepsy. Sudden death in epilepsy is frequently described in the literature under the acronym SUDEP (Sudden Unexplained Death in EPilepsy). Although the design and confounding factors in these studies did not allow for conclusions on a possible association between sudden death risk and the use of ACs, these studies have provided important information about sudden cardiac death in young epilepsy patients. From these studies, we learned that SUDEP is common in young patients with severe therapy-resistant epilepsy, and that SUDEP is probably a seizure-related event.[39] However, the literature tends to ignore the much larger group of epilepsy patients with more stable epilepsy, suggesting that the risk for sudden death does not exist in this group.

In this study, we show that epilepsy is also strongly related with sudden cardiac death in elderly patients. In contrast to the young SUDEP cases described in the literature, most (78%) of our epilepsy cases used a single AC, implicating stable and non-severe epilepsy. None of the 6 witnessed epilepsy cases had a seizure-related death. These observations suggest that the disease epilepsy plays not such a prominent role in sudden cardiac death in elderly subjects in the general population as it does in young SUDEP cases. Sudden cardiac death in elderly patient with non-severe epilepsy might be more related to the use of ACs. This hypothesis is further supported by the fact that use of ACs is also associated in our population with sudden cardiac death in patients who used these drugs for other indications than epilepsy.

To further investigate the role of ACs in sudden cardiac death, the effects of ACs with sodium channel blocking properties were tested. Previous studies have associated cardiac drugs with sodium-channel blocking properties with sudden cardiac death. [19, 20] Moreover, patients with congenitally reduced cardiac sodium current (e.g., Brugada syndrome) also have an increased risk for ventricular arrhythmias and sudden cardiac death. Reduced cardiac sodium current is believed to increase the risk for cardiac arrhythmias by causing conduction delay in the heart.[40] Although this same mechanism is proposed in the literature as a possible mechanism by which ACs may increase the risk of sudden cardiac death, it has not been studied before.[21] Here, we show that use of ACs with sodium channel blocking properties is associated with an almost 3-fold increased risk of sudden cardiac death, even after correction for all confounding factors. Conversely, ACs without sodium channel blocking properties exhibited a lower and non-significant association with sudden cardiac death. To our knowledge, this is the first study to show that sodium channel blocking ACs are associated with sudden cardiac death.

A sensible dose-response analysis could not be performed since ACs were prescribed for different indications (epilepsy and non-epileptic diseases). The prescribed doses for epilepsy were higher than for the non-epileptic indications. As the disease epilepsy probably also affects the risk of sudden cardiac death and interacts with AC use (gene variants of sodium channels co-expressed in the brain predisposing for epilepsy and cardiac arrhythmias). In patients with such a genetic susceptibility low doses are sufficient to block the genetically impaired channels. Therefore, a dose-response analysis is only possible in stratified groups of epileptic patients (preferably subdivided in mutation carriers and non-mutation carriers) and non-epileptic patients. The available numbers of cases were not sufficient to perform such an analysis.[41, 42]

Experimental studies show that phenytoin, phenobarbital and lamotrigine have the ability to block cardiac *hERG*-encoded potassium channels.[43, 44] Sufficient blockade of cardiac *hERG*-encoded potassium channels leads to QT prolongation on the ECG and is associated with ventricular arrhythmias and sudden cardiac death.[45] However, these ACs are not known to prolong the QT interval on the ECG, suggesting that they block cardiac *hERG*-encoded potassium channels only minimally and clinically non-significantly. To further rule out the possibility that *hERG*-encoded potassium channels inhibition may contribute to the increased risk for sudden cardiac death which is associated with the use of sodium channel blocking ACs, we conducted a sub-analysis, in which we excluded phenytoin and lamotrigine. Even with the exclusion of these drugs, the use of sodium channel blocking ACs was still strongly associated with sudden cardiac death.

Although group sizes were small when we studied each AC individually, we still found that carbamazepine was associated with an increased risk of sudden cardiac death. To our knowledge, this is the first study which associates carbamazepine with sudden cardiac death in stable epilepsy and non-epilepsy patients. Timmings described a larger proportion of carbamazepine users among 14 patients who died suddenly compared to patients who attended the general epilepsy clinic. However, this was not a case-control study and no attempts were made to correct for confounding risk factors of sudden cardiac death.[36] The Norwegian and The Swedish study showed no correlation between specific AC use and sudden cardiac death.[6, 38] An association between high serum levels of carbamazepine ($>40\mu\text{mol/L}$) at the last visit and sudden death was suggested in a sub-analysis of the Swedish study.[18] However, this risk occurred especially among patients who used polytherapy of ACs. The authors were therefore cautious in concluding that high levels of carbamazepine are associated with an increased risk of sudden cardiac death, as polytherapy of ACs and high serum levels of carbamazepine are both markers of severe and unstable epilepsy. A case-control study of 154 SUDEP cases showed an odds ratio of 2 for carbamazepine. These authors, too, warn against concluding that carbamazepine is causally related with SUDEP, as carbamazepine use may reflect epilepsy severity in this cohort of patients with severe

epilepsy, as also suggested by the use of multiple ACs: among cases, 50% had at one point used 3 or more ACs compared to 29% of controls.[17] In comparison, among cases in our cohort, none had used ≥ 3 ACs.

The association of carbamazepine with sudden cardiac death may be mediated by the sodium channel blocking properties of this drug. Cardiac sodium channel blockade may increase the risk for ventricular arrhythmias, but also the risk for atrial arrhythmias. [19, 20, 40] Recently, we showed that sodium channels are also expressed in the sinus node of the heart.[46] The sinus node initiates each cardiac contraction and is responsible for the heart rate. Blockade of sodium channels in the sinus node may slow impulse generation and heart rate. Potentially serious slow heart rates are often described in carbamazepine users in the literature.[47-51] As further evidence for a role of sodium channel blockade, two other ACs with sodium channel blocking properties (phenytoin and gabapentin) showed a trend of increased sudden cardiac death risk, similar to carbamazepine.

The major strength of our study lies in its population-based character, minimizing selection bias. This enabled us to study patients who used ACs for different indications. Furthermore, our access to the databases of the general practitioners allowed us to collect extensive information on drug use, concomitant diseases, potential confounders, and circumstances surrounding death.

However, our study has also some limitations, comparable to other studies that address sudden cardiac death in the general population. First, although general practitioners register death consistently, we may have missed some deaths. Second, as the definition of sudden cardiac death depends largely on the available information regarding the circumstances surrounding death, misclassifications may have occurred. To reduce misclassification, we only included cases with sufficient and clear information on the circumstances surrounding death. Third, misclassification of drug exposure may have occurred, as we used prescription data by the general practitioner. No information was available on the compliance of medication intake. However, it is likely that the misclassification will be randomly distributed among cases and controls.

In conclusion, we found that use of ACs is associated with an increased risk of sudden cardiac death in the general population, even in persons who use ACs for other indications than epilepsy. The risk of sudden cardiac death seems to be higher among users of carbamazepine and other ACs with sodium channel blocking properties.

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Chapter 4.1

High free thyroxine levels are associated with QTc prolongation in males

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Abstract

Background

The literature on the effect of excess thyroid hormone on ventricular repolarization is controversial. Our aim was to examine whether free T4 and TSH are associated with QTc prolongation.

Methods

Setting: A population-based cohort study.

Participants: 365 men and 574 women aged 55 years and older from the Rotterdam Study cohort with an electrocardiogram (ECG) who were randomly sampled for assessment of thyroid status (free T4/TSH) at baseline, after exclusion of participants with hypothyroidism, use of antithyroid drugs, thyroid hormones or digoxin, left ventricular hypertrophy and left and right bundle branch block.

Endpoints: Length of the QTc interval and risk of borderline QTc prolongation.

Analysis: Linear and logistic regression analysis, adjusted for age and gender, diabetes mellitus, myocardial infarction, hypertension and heart failure.

Results

Overall, there was no significant association between TSH and QTc interval (0.8 ms (95%CI -3.5;5.2) in the first quintile compared to the fifth quintile). Subjects in the fifth quintile of free T4 did not have an increased QTc interval (3.2 ms (95%CI -1.1;7.6)); stratification on gender showed an increment of 10.9 ms (95%CI 3.4;18.3) in the fifth quintile in men and 1.1 ms (95%CI -4.2;6.3) in the fifth quintile of free T4 in women. Compared to subjects in the first quintile, male subjects in the fifth quintile of free T4 had a significantly increased risk of a borderline QTc interval and QTc prolongation (Odds Ratio 2.40 (95%CI 1.20-4.80)).

Conclusions

High levels of free T4 are associated with substantial QTc prolongation in men of up to 10 ms. The fact that free T4 is also associated with a significantly increased risk of borderline and prolonged QTc values with its risk of sudden cardiac death, endorses the clinical importance of our findings.

Introduction

An excess of thyroid hormone exerts a major effect on the cardiovascular system, and the influence of thyroid disorders on heart rhythm, output and contractility has been widely studied.[1] Relatively little, however, is known about the effect of thyroid hormone on ventricular repolarization. It has been reported that hypothyroidism is associated with prolongation of the heart-rate corrected QT (QTc) interval [2, 3], a measure of duration of ventricular repolarization. In hyperthyroidism, the situation is more controversial, since prolonged and shortened QTc interval both have been reported.[4-12] Decreased repolarization times have been reported in several animal studies [4-7] and in one human study [8] and at the same time QTc prolongation has been reported in patients with hyperthyroidism. [9-12] A potential explanation for an association with prolongation of the QTc interval is an increased activity of cardiac Na⁺/K⁺ ATPase in thyroid hormone excess, leading to increased intracellular K⁺ with subsequent membrane hyperpolarization and an increase in QTc duration.[1, 11] A prolonged QTc interval may be clinically relevant, since an increase of ventricular repolarization time may result in early after depolarizations (EAD), which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmias.[13-17]

Because of these contradictory results and limited data concerning hyperthyroidism and the duration of the QTc interval, we studied the association of TSH and free T4 with the QTc interval in a prospective, population-based study of elderly.

Methods

Setting and study design

The Rotterdam study is a population-based cohort study, which started with a baseline visit between 1990 and 1993. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. All inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years and over, were invited to participate (n=10,275). Of them, 7983 (78%) gave their written informed consent and took part in the baseline examination. Objectives and methods of the Rotterdam Study have been described in detail elsewhere.[18, 19] At baseline, all participants were visited at home for a standardized questionnaire, and 7151 were subsequently examined at the research center. The cohort is continuously being monitored for major morbidity and mortality through linkage of the Rotterdam Study database with general practitioner and municipality records.

Study population

Thyroid status was assessed in a random selection of 2000 participants from the Rotterdam Study at baseline, as has been described previously.[20] After exclusion of subjects for whom no blood was available or who used amiodarone at baseline (due to the effects of this drug on thyroid function)[21], TSH was assessed in 1843 participants. Due to technical and logistical reasons, free T4 could only be assessed in 1544 of these participants. Electrocardiograms (ECGs) were available for 1126 of these participants at the time of the first visit.

We excluded participants with clinical hypothyroidism (TSH > 4.3 mU/l and free T4 < 11 pmol/l) or subclinical hypothyroidism (TSH > 4.3 mU/l and free T4 \geq 11 pmol/l) (n=72), since hypothyroidism is associated with QTc prolongation.[2, 3] Furthermore, we excluded participants with ECG evidence of left ventricular hypertrophy (n=44), left and right bundle branch block (n=17 and 30, respectively) and/or participants using digoxin (n=21), antithyroid drugs (n=6) and thyroid hormones (n=11), since these conditions can alter the QTc interval.[22, 23] Consequently, the study population consisted of 939 participants (figure 1).

Assessment of thyroid status

At baseline, non-fasting serum samples were obtained, which were put on ice directly and processed within 30 minutes after which they were kept frozen at -20°C . TSH levels were measured with TSH Lumitest (Henning, Berlin, Germany).[24] The reference range of serum TSH levels was 0.4 - 4.3 mU/l. Serum free T4 was measured by a chemoluminescence assay (Vitros, ECI Immunodiagnostic System, Ortho-Clinical Diagnostics, Amersham, UK), the reference range of serum free T4 levels was 11–25 pmol/l. Participants with TSH levels lower than 0.4 mU/l and free T4 levels higher than 25 pmol/l were considered to have clinical hyperthyroidism, participants with TSH levels lower than 0.4 mU/l and free T4 levels between 11 and 25 pmol/l were considered to have subclinical hyperthyroidism.

ECG

A 12-lead resting ECG was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. The ECGs and serum samples were obtained on the same day. All ECGs were processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurements. The MEANS program has been evaluated extensively and has been validated.[25-28] In one of these validation studies, ECGs with selected abnormalities were analyzed by 5 cardiologists and 11 different computer programs of which MEANS performed as one of the best [28] In another validation study in which QT intervals by manual measurement were compared with those generated by ECG machines, manual and automated measurements generated

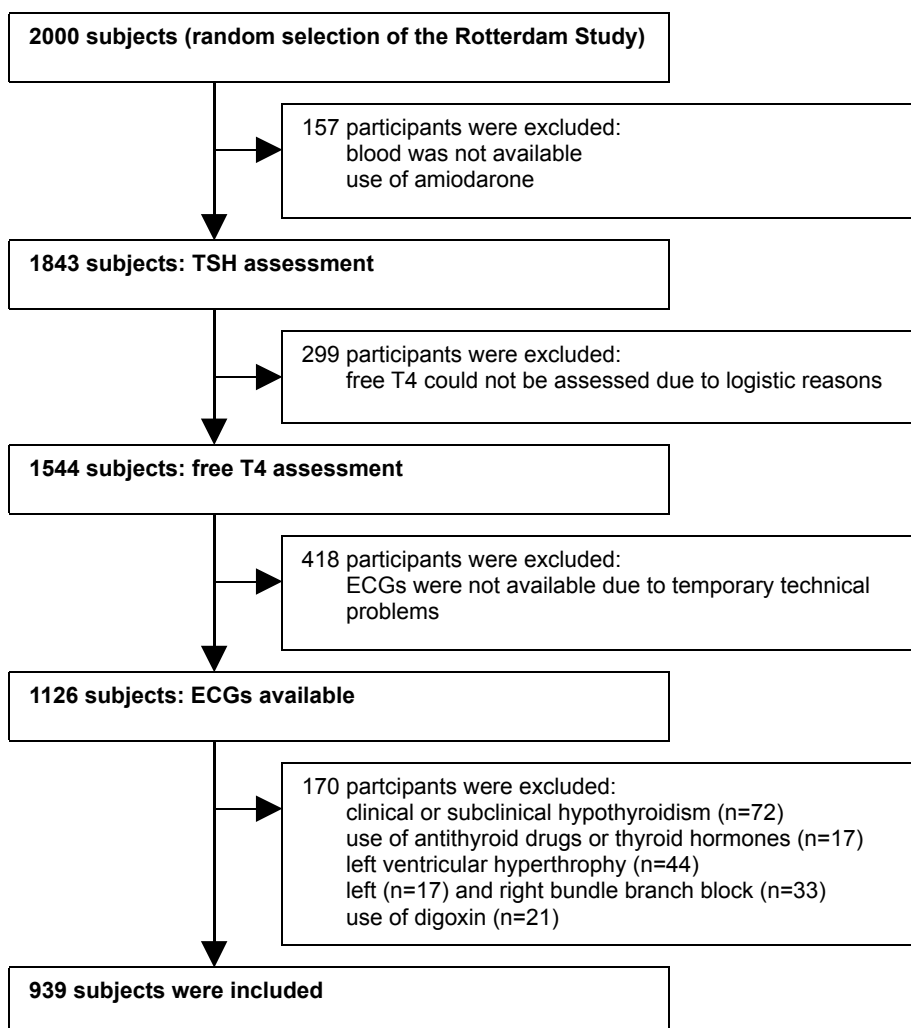


Figure 1 Diagram of study population

similar numerical results in 3 studies in healthy volunteers, which all included a positive control.[29] MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques. [26] The MEANS program determines the QT interval from the start of the QRS complex until the end of the T wave. To adjust for heart rate, Bazett's formula ($QT_c = QT / \sqrt{RR}$) was used.[30] European regulatory guidelines were used to categorize the QTc interval into 3 categories: normal, borderline and prolonged. For men, the cut-off points were < 430 ms (normal), 430-450 ms (borderline) and > 450 ms (prolonged), and for women < 450 ms (normal), 450-470 ms (borderline), and > 470 ms (prolonged).[31] Missing ECGs

were mainly due to temporary technical problems with ECG recording. The index date was the date of the ECG at baseline, at the same date blood samples were collected.

Covariates

Diabetes mellitus, hypertension, myocardial infarction and heart failure are considered to be risk factors for QTc prolongation and presence of these conditions at each index date was included as a covariate.[32-35] Clinical measures were obtained during the visits at the Rotterdam Study research center. Diabetes mellitus and hypertension were defined according to the World Health Organization criteria.[36, 37] Prevalence of myocardial infarction was assessed by hospital discharge diagnosis or in case a patient was not hospitalized, when signs and symptoms, analysis of the standard 12-lead ECG and cardiac enzyme data were diagnostic of a myocardial infarction.[38, 39] Prevalence of heart failure was assessed by the presence of suggestive signs and symptoms as previously described.[40, 41]

Statistical analysis

Two analyses were conducted. First, a linear regression analysis was conducted with QTc as outcome and thyroid status (TSH and free T4 levels divided in quintiles) as determinants. Second, logistic regression analysis was conducted to assess the risk of a borderline QTc interval or QTc prolongation. All analyses were adjusted for age and gender and additionally for diabetes mellitus, myocardial infarction, hypertension and heart failure. In a separate analysis, we have examined the association between TSH and QTc prolongation using log TSH, since TSH is logarithmically distributed.

Because women in general have a longer QTc interval than men, a separate analysis was conducted with stratification for gender. In a separate analysis, we also adjusted for potassium, calcium and use of class 1 QTc prolonging drugs of list 1 of the website based registry at the index-date, since these drugs are generally accepted to have a risk of causing Torsade de Pointes.[42] Furthermore, we have performed a sensitivity analysis by excluding participants with clinical or subclinical hyperthyroidism. All analyses were performed using SPSS for Windows version 11.0 (Chicago, Illinois, USA).

Results

Subject characteristics

The baseline characteristics of the participants are presented in table 1. Overall, 939 participants were included, of whom 574 were female and 365 male. The mean age of the study population at baseline was 68.0 years (standard deviation (SD) 7.6 years). Women were significantly older than men. Mean TSH at baseline was 1.60 mU/l (SD=0.90),

Table 1 Baseline characteristics

	Total	(Sub) clinical hyperthyroidism	No hyperthyroidism
Number of participants	939	59	880
Age (years, mean, SD)	68.0 (7.6)	67.9 (7.4)	68.0 (7.6)
Gender (male)	365 (38.9%)	19 (32.2%)	346 (39.3%)
Mean QTc interval (ms) (SD)	429.4 (21.7)	429.3 (23.4)	429.4 (21.6)
Borderline QTc prolongation	192 (20.4%)	14 (23.7%)	178 (20.2%)
Abnormal QTc prolongation	56 (6.0%)	1 (1.7%)	55 (6.3%)
Body Mass Index (mean, SD)	26.3 (3.7)	26.3 (4.1)	26.3 (3.7)
Diabetes mellitus (n, %)	93 (9.9%)	7 (11.9%)	86 (9.8%)
Myocardial infarction (n, %)	101 (10.8%)	1 (1.7%)*	100 (11.4%)*
Hypertension (n, %)	282 (30.0%)	17 (28.8%)	265 (30.1%)
Heart failure (n, %)	9 (1.0%)	1 (1.7%)	8 (0.1%)
Use of β -blocking drugs (n, %)	12 (1.3%)	0	12 (1.4%)
Use of QTc prolonging drugs (n, %)	13 (1.4%)	1 (1.7%)	12 (1.4%)
Mean TSH (mU/l) (SD)	1.60 (0.90)	0.29 (0.36)*	1.69 (0.86)*
Mean Free T4 (pmol/l) (SD)	16.5 (3.0)	19.8 (5.0)*	16.3 (2.6)*

SD = Standard Deviation

*: $P < 0.05$ Use of β -blocking drugs and QTc prolonging drugs was defined as use at the index date.

mean free T4 was 16.5 pmol/l (SD=3.0). Ten participants had clinical hyperthyroidism and 49 participants had subclinical hyperthyroidism.

QTc prolongation

The mean duration of the QTc interval at baseline was significantly shorter in males (424.0 ms) than in females (432.9 ms) ($p < 0.0001$). Overall, 73.6% of the participants had normal QTc duration and 20.4% had a borderline QTc interval, using the above-mentioned gender specific cut-off points. Furthermore, 56 participants had QTc prolongation, with mean QTc levels of 473.7 milliseconds.

Overall, there was no significant association between TSH and QTc interval with a decrease of 0.6 ms (95%CI -2.1 ; 0.9) per mU/l. The first quintile of TSH was not associated with an increase of the QTc interval (0.8 ms (95%CI-3.5 ; 5.2)) compared to the fifth quintile. This effect fluctuated among the other quintiles. TSH was not associated with the risk of borderline QTc interval or QTc prolongation (table 2). Additional analyses using log TSH did not change the results.

In contrast, there was a significant age- and gender-adjusted association between free T4 levels and QTc interval with an increase of 0.6 ms (95%CI 0.1;1.0) per pmol/l. The highest quintile of free T4 was not associated with an increase of the QTc interval (3.2 ms (95%CI -1.1 ; 7.6)) in comparison to the first quintile. The P-value for linear

Table 2 Association of thyroid status with QTc interval

	QTc prolongation in ms (95% CI) ¹	QTc prolongation in ms (95% CI) ²	Risk of borderline or abnormal QTc prolongation (95%CI) ¹	Risk of borderline or abnormal QTc prolongation (95%CI) ²
TSH quintiles				
≤ 0.86	0.8 (-3.5 ; 5.2)	0.8 (-3.5 ; 5.1)	1.13 (0.70 ; 1.81)	1.13 (0.70 ; 1.83)
0.87 – 1.25	2.1 (-2.2 ; 6.4)	1.9 (-2.3 ; 6.2)	1.17 (0.74 ; 1.88)	1.16 (0.72 ; 1.88)
1.26 – 1.66	1.0 (-3.3 ; 5.3)	1.1 (-3.2 ; 5.4)	1.03 (0.64 ; 1.66)	1.04 (0.64 ; 1.70)
1.67 – 2.31	0.5 (-3.8 ; 4.9)	0.6 (-3.7 ; 4.9)	0.97 (0.60 ; 1.57)	0.98 (0.60 ; 1.60)
≥ 2.32	Reference	Reference	Reference	Reference
<i>P</i> value for linear trend	0.514	0.857	0.424	0.448
Free T4 quintiles				
≤ 13.9	Reference	Reference	Reference	Reference
14.0 – 15.3	-1.2 (-5.6 ; 3.1)	-1.2 (-5.6 ; 3.1)	0.93 (0.56 ; 1.54)	0.93 (0.56 ; 1.55)
15.4 – 16.7	0.9 (-3.5 ; 5.3)	1.0 (-3.4 ; 5.3)	1.30 (0.79 ; 2.13)	1.33 (0.81 ; 2.19)
16.8 – 18.6	2.7 (-1.6 ; 7.1)	2.1 (-2.2 ; 6.4)	1.56 (0.97 ; 2.52)	1.43 (0.88 ; 2.34)
≥ 18.7	3.2 (-1.1 ; 7.6)	2.6 (-1.7 ; 6.9)	1.72 (1.07 ; 2.77)	1.59 (0.98 ; 2.58)
<i>P</i> value for linear trend	0.031	0.081	0.003	0.014

CI = Confidence Interval

1 : adjusted for age and gender

2 : adjusted for age, gender, diabetes, hypertension, myocardial infarction and heart failure

TSH in mU/l and free T4 in pmol/l

trend was 0.031 with a gradual increase of the QTc interval among the quintiles. The fifth quintile of free T4 was associated with a significantly higher risk of a borderline QTc interval or QTc prolongation (Odds Ratio 1.72 (95%CI 1.07-2.77)) compared to the lowest quintile (figure 2). The P-value for linear trend was 0.003. After adjustment for diabetes mellitus, hypertension, myocardial infarction and heart failure, the results changed minimally.

Stratified analysis for gender

After stratification for gender, the effects were significant in men but not in women (table 3). The fifth quintile of free T4 was associated with a significant age-adjusted increase of the QTc interval of 10.9 ms (95%CI 3.4 ; 18.3) in men, with a significant linear trend (P-value 0.004). The fifth quintile of free T4 was associated with a significantly increased risk of a borderline QTc interval in men (OR 2.40 (95%CI 1.20 -4.80) with a gradual increase (P-value for linear trend 0.003). In women, there was a non-significant trend towards a longer QTc-interval in the highest quintiles of free T4.

Additional adjustment for calcium, potassium and use of class 1 QTc prolonging drugs resulted in higher point estimates. The highest quintile of free T4 in males was

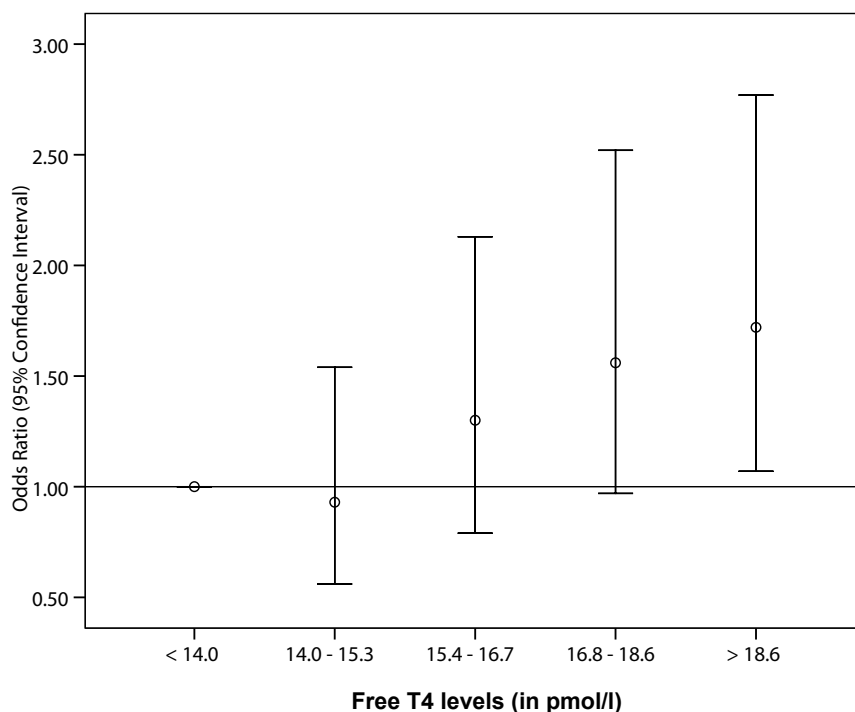


Figure 2 Differences between the risks of borderline or abnormal QTc prolongation in the free T4 quintiles compared to the lowest quintile

associated with a significant adjusted increase of the QTc interval of 12.7 ms (95%CI 5.6 ; 19.9) with a gradual increase (P-value for linear trend <0.0001). The fifth quintile of free T4 in males was associated with a significantly higher risk of a borderline QTc interval or QTc prolongation (OR 3.29 (95%CI 1.50-7.22)) compared to the first quintile. The P-value for linear trend was 0.001.

Exclusion of the 59 participants with clinical or subclinical hyperthyroidism did not change the results substantially. The highest quintile of free T4 in males was associated with a significant adjusted increase of the QTc interval of 9.7 ms (95%CI 2.1 ; 17.2) with a gradual increase (P-value for linear trend 0.008). The fifth quintile of free T4 in males was associated with a significantly higher risk of a borderline QTc interval or QTc prolongation (OR 2.12 (95%CI 1.02-4.40)) compared to the first quintile. The P-value for linear trend was 0.009.

Discussion

In this population-based study, we found an association between free T4 levels and prolongation of the QTc interval. As far as we know, this is the first time that this was

Table 3 Association of free T4 with QTc interval stratified for men and women

	QTc prolongation in ms (95% CI) ¹	QTc prolongation in ms (95% CI) ²	Risk of borderline or abnormal QTc prolongation (95%CI) ¹	Risk of borderline or abnormal QTc prolongation (95%CI) ²
Free T4 quintiles				
Male				
≤ 13.8	Reference	Reference	Reference	Reference
13.9 – 15.1	1.5 (-5.9 ; 8.9)	0.6 (-6.8 ; 7.9)	0.93 (0.45 ; 1.94)	0.77 (0.36 ; 1.65)
15.2 – 16.6	6.2 (-1.3 ; 13.7)	6.3 (-1.2 ; 13.7)	1.56 (0.77 ; 3.17)	1.63 (0.79 ; 3.37)
16.7 – 18.3	4.2 (-3.1 ; 11.5)	2.8 (-4.5 ; 10.1)	1.66 (0.83 ; 3.31)	1.40 (0.68 ; 2.88)
≥ 18.4	10.9 (3.4 ; 18.3)	10.0 (2.6 ; 17.5)	2.40 (1.20 ; 4.80)	2.16 (1.06 ; 2.88)
<i>P value for linear trend</i>	0.004	0.008	0.003	0.009
Free T4 quintiles				
Female				
≤ 14.1	Reference	Reference	Reference	Reference
14.2 – 15.5	-1.9 (-7.3 ; 3.5)	-2.0 (-7.31 ; 3.4)	1.13 (0.56 ; 2.30)	1.11 (0.55 ; 2.27)
15.6 – 16.8	-0.8 (-6.0 ; 4.4)	-1.0 (-6.2 ; 4.2)	1.28 (0.65 ; 2.52)	1.29 (0.65 ; 2.55)
16.9 – 18.9	-0.6 (-5.9 ; 4.7)	-0.9 (-6.2 ; 4.4)	1.38 (0.70 ; 2.73)	1.34 (0.68 ; 2.66)
≥ 19.0	1.1 (-4.2 ; 6.3)	0.3 (-5.0 ; 5.5)	1.55 (0.80 ; 3.01)	1.42 (0.73 ; 2.78)
<i>P value for linear trend</i>	0.564	0.799	0.154	0.252

CI = Confidence Interval

1 : adjusted for age

2 : adjusted for age, diabetes, hypertension, myocardial infarction and heart failure
free T4 in pmol/l

demonstrated in a large cohort study of an elderly population. The QTc interval increases gradually among the quintiles and the prolongation appears to be strongest in men with a prolongation of approximately 10 ms in the highest quintile. Even after exclusion of participants with hyperthyroidism, free T4 levels are still associated with prolongation of the QTc interval. We did not find an association between TSH and the QTc interval. A potential explanation for this finding is that an association between TSH and QTc would be indirect, while free T4 is more directly related to thyroid hormone action on the heart. TSH binds to the TSH-receptor on thyroid cells, resulting in stimulation of thyroid hormone production but has probably not an effect on QTc of its own. [43]

Thyroid hormone may affect ventricular repolarization but the literature differs with respect to the direction of this alteration. Hypothyroidism has been associated with prolongation of the QTc interval.[2, 3] The mechanism behind this association might be an enhanced sympathetic activity.[11] Hyperthyroidism has been found to be associated with decreased as well as increased repolarization times. In animal studies [4-7] and in one human study [8] decreased repolarization times have been found, however,

in several small human studies [9-12] and in an animal study [44] QTc prolongation has been reported. In a prospective study comparing 16 patients with Graves' disease with a matched reference group, the 24-hour average QTc in the Graves' patients was significantly prolonged and returned to normal after treatment of thyrotoxicosis. QTc has also been shown to be positively correlated with free T3 and free T4.[11]

A prospective study comparing patients with subclinical hyperthyroidism with healthy individuals, demonstrated that QTc intervals were significantly longer in patients with subclinical hyperthyroidism.[12] This prolongation might have clinical consequences since we demonstrated in earlier studies that QTc prolongation was associated with a three-fold increased risk of sudden cardiac death.[17] It is assumed, that even a minor average increase of the QTc interval in a population may enhance the risk of Torsade de Pointes in a small group of susceptible patients, if large numbers of patients are exposed. Some QTc prolonging drugs which were withdrawn from the market because of Torsade de Pointes, were associated with a QTc interval prolongation of only 5 to 10 ms in patient populations.[14]

A possible explanation for the association of hyperthyroidism and QTc prolongation can be provided by an increase in the activity of cardiac Na^+/K^+ ATPase due to thyroid hormone excess, leading to increased intracellular K^+ with subsequent membrane hyperpolarization.[1, 11] The effect appeared to be strongest in men. Women are known to have on average a longer QTc interval than men.[31] The shorter QTc interval in men is attributed to the role of testosterone on the duration of the action potential. In hyperthyroid men, impaired sexual function, gynaecomastia, asthenospermia and low testicular volume are attributed to lowered bio-available testosterone and a decreased Free Androgen Index despite an increase in total and SHBG-bound testosterone.[45, 46] The decreased bio-available testosterone level in hyperthyroid men results in less shortening of the QTc interval. Bio-available testosterone decreases with age, and this could explain the relative QTc prolongation in men with high free T4 levels in this study of an elderly population.[47, 48] Therefore, the difference in the QTc interval between male and female disappears in males with high free T4 levels.

Our study has several strengths. An advantage of the Rotterdam Study is its population-based character which decreases the risk of selection bias. The baseline characteristics of our subcohort were comparable to the baseline characteristics of the whole population of the Rotterdam Study. Furthermore, the use of digital ECG recordings, all measured using the MEANS system, likely reduced intra- and interobserver variability in the assessment of the QTc interval. Confounding was minimized by adjusting for all known risk factors for QTc prolongation, although we cannot exclude the possibility of unknown confounders. However, our study has also some limitations. Because of the cross-sectional design we cannot exclude that QTc prolongation was already present in some patients before the increase of free T4. Therefore, the results from this study

should be confirmed with longitudinal data from other large cohorts. Thyroid status was only assessed in a subgroup of the entire cohort of the Rotterdam Study. Validity is nevertheless unaffected since the selection was at random. Free T3 measurements are not available in the Rotterdam Study, however, since T4 is a pro-hormone of T3 and T3 exhibits greater activity, we expect that the results might have been more pronounced, if we would have used free T3 as exposure. Finally, our study population consisted of participants aged 55 years and older. Whether our findings can be generalized to other age groups requires further study.

In conclusion, we demonstrated in this cohort of an elderly population, that high levels of free T4 are associated with QTc prolongation in men. Although a QTc interval prolongation of 10 ms in one individual usually remains without clinical consequences, an average shift of 10 ms in a Gaussian distribution on a population level will inevitably push more individuals into the upper percentiles of the QTc interval with its increased risk of Torsade de Pointes and sudden cardiac death.

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Chapter 4.2

Population-based studies of antithyroid drugs and sudden cardiac death

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Abstract

Background

Thyroid free T4 is associated with QTc-interval prolongation which is a risk factor for sudden cardiac death (SCD). Hyperthyroidism has been associated with SCD in case reports but there are no population-based studies confirming this. Therefore we investigated whether use of antithyroid drugs (as a direct cause or as an indicator of poorly controlled hyperthyroidism) is associated with an increased risk of SCD.

Methods

We studied the occurrence of SCD in a two-step procedure in two different Dutch populations. First, the prospective population-based Rotterdam Study including 7898 participants (≥ 55 yr). Second, we used the Integrated Primary Care Information (IPCI) database which is a longitudinal general practice research database to see whether we could replicate results from the first study. Drug use at the index date was assessed with prescription information from automated pharmacies (Rotterdam Study) or drug prescriptions from general practices (IPCI). We used a Cox proportional hazards model in a cohort analysis, adjusted for age, gender and use of QTc prolonging drugs (Rotterdam Study) and conditional logistic regression analysis in a case-control analysis, matched for age, gender, practice and calendar time and adjusted for arrhythmia and cerebrovascular ischaemia (IPCI).

Results

In the Rotterdam Study, 375 participants developed SCD during follow-up. Current use of antithyroid drugs was associated with SCD (HR_{adj} 3.9; 95%CI 1.7-8.7). IPCI included 1424 cases with SCD and 14,443 controls. Also in IPCI, current use of antithyroid drugs was associated with SCD (OR_{adj} 2.9; 95%CI 1.1-7.4).

Conclusion

Use of antithyroid drugs was associated with a threefold increased risk of SCD. Although this might be directly caused by antithyroid drug use, it might be more readily explained by underlying poorly controlled hyperthyroidism, since treated patients who developed SCD still had low TSH levels shortly before death.

Introduction

Hyperthyroidism is a common disease in the elderly with an estimated prevalence between 0.5% and 6%. [1-3] Thyroid hormone excess in the elderly affects the cardiovascular system. [4] A strong positive correlation between free T3 and the QTc interval has been reported. [5, 6] Recently, we demonstrated that free T4 is associated with QTc prolongation in males. [7] Prolongation of ventricular repolarization may result in early after depolarizations (EAD), which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmias. [8-12]

Cardiovascular disease is the leading cause of death in the western world and sudden cardiac death accounts for almost half of these cardiovascular deaths. [13] Clinical or subclinical hyperthyroidism is associated with increased overall mortality, in particular mortality due to circulatory and cardiovascular diseases. Even subclinical hypo- and hyperthyroidism have been associated with an increased risk of mortality in patients with cardiac disease. [14-16] In a previous study, no difference was demonstrated in mortality or serious vascular events in users of antithyroid drugs. [17] No effects of subclinical hyperthyroidism on cardiovascular events were demonstrated in two prospective studies. [18, 19] Two meta-analyses resulted in different conclusions. [20, 21]

There have been several case reports describing an association between undiagnosed hyperthyroidism and ventricular arrhythmias. [22, 23] Sudden cardiac death in patients with undiagnosed hyperthyroidism has been described in a few case reports, often associated with a thyrotoxic crisis. [24, 25] The association of hyperthyroidism with sudden cardiac death has not been described in large epidemiologic studies.

Since the association between hyperthyroidism and ventricular arrhythmias or sudden cardiac death has been reported in several case reports, but not in large epidemiologic studies, we investigated in a prospective population-based cohort study whether use of antithyroid drugs (as a direct cause or as an indicator of poorly controlled hyperthyroidism) is associated with an increased risk of sudden cardiac death. Subsequently, we performed a case-control study in a separate population to see whether we could replicate findings from the first study.

Methods

Setting and study design

Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study, which started with a baseline visit between 1990 and 1993. The Medical Ethics Committee of the Erasmus

Medical Center, Rotterdam, the Netherlands, approved the study. All inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years and over, were invited to participate ($n=10,275$). Of them, 7983 (78%) gave their written informed consent and took part in the baseline examination. Objectives and methods of the Rotterdam Study have been described in detail elsewhere.[26, 27] At baseline, all participants were visited at home for a standardized questionnaire, and 7151 were subsequently examined at the research center. Since the start of the study, three follow-up visits took place approximately every 3 years. In addition to follow-up examinations, the cohort is continuously monitored for major morbidity and mortality through linkage with general practitioner and municipality records. Drug prescriptions dispensed to participants by automated pharmacies with computerized records and a history of several years, are routinely stored in the database since 1 January 1991. To have at least four months of medication history, the study period started on 1 May 1991. The study ended on one of the censoring dates (death or transferring out) or the end of the study period (1 January 2006). Overall, 7898 participants were included.

Integrated Primary Care Information (IPCI)

Data were retrieved from the Integrated Primary Care Information (IPCI) project, a longitudinal observational database, containing data from computer-based medical patient records of a large group of general practitioners (GPs) in the Netherlands, for a population based case-control study. In the Dutch health care system, the GP has a pivotal role by acting as a gatekeeper for all medical care. Details of the database have been described elsewhere.[28, 29] Briefly, the database contains the complete medical records on approximately 1 million patients. The electronic records contain coded and anonymous data on patient demographics, symptoms (in free text), diagnoses (using the International Classification for Primary Care (ICPC) [30] and free text) from GPs and specialists, referrals, laboratory findings, hospitalisations, and drug prescriptions, including their indications and dosage regimen. To maximise completeness of the data, GPs participating in the IPCI project are not allowed to maintain a system of paper-based records besides the electronic medical records. The system complies with European Union guidelines on the use of medical data for medical research and has been proven valid for pharmaco-epidemiological research in several validation studies that evaluated the quality of the available information.[28] The Scientific and Ethical Advisory Board of the IPCI project approved this study.

The source population comprised all patients of 18 years and older in the IPCI database with a valid database history (date of registration with GP) of at least one year. The study population comprised all cases with sudden cardiac death occurring in the source population during the study period plus their matched controls (see below). The study period started on 1 January 1995 and ended on 1 May 2007. All subjects were followed

until death, transferral out of the GP practice, last data draw down or end of the study period, whichever came first.

Sudden cardiac death definition

In both the Rotterdam Study and in IPCI, sudden cardiac death was defined as: 1) a witnessed natural death attributable to cardiac causes, heralded by abrupt loss of consciousness, within one hour of onset of acute symptoms, or 2) an unwitnessed, unexpected death of someone seen in a stable medical condition <24 hours previously with no evidence of a non-cardiac cause.[31, 32]

Rotterdam Study

The ascertainment of sudden cardiac death cases in the Rotterdam Study has been described previously.[12] In short, information on vital status is obtained from municipal health authorities in Rotterdam and GPs. Two research physicians independently coded all reported events, blinded to exposure, and judged the likelihood of sudden cardiac death according to the definition above. In cases of disagreement, consensus was sought and finally, a cardiologist reviewed all events. The index date was the date of death.

IPCI: Case and control definition

The computerised medical and demographic data were screened for all deaths, which occurred during the study period. The medical records of all identified cases of death were reviewed manually to assess whether death could be classified as sudden cardiac death. Validation was performed independently by two medically trained persons blinded to exposure and in case of discrepancy, a specialized physician arbitrated. To each case of sudden cardiac death, up to twenty controls were randomly drawn from the source population matched on age (year of birth), gender, and practice (GP). The index date was defined as the date on which sudden cardiac death occurred in cases. This date was also the index date for matched controls.

Use of antithyroid drugs

In this study, the exposure of interest included the available antithyroid drugs carbimazole, thiamazole, and propylthiouracil. The duration of each prescription was calculated by dividing the total number of units issued per prescription by the prescribed daily number of units. Use of antithyroid drugs was defined as current if the index date fell within a period of use or within a maximum of 40 days after the end of the last prescription (to deal with carry-over effects and/or irregular use by patients). Past use was defined as discontinuation of an antithyroid drug, more than 40 days before the index date. If patients had no prescription for an antithyroid drug prior to the index date

they were considered as non-exposed. Among current users we evaluated the effect of duration (≤ 365 days; > 365 days) of use which was defined as the delay between first intake and the index date, and the effect of dosage (< 1 defined daily dosage (DDD); ≥ 1 DDD). The DDD is the recommended average maintenance dose per day for a drug used for its main indication in adults.[33]

Covariates

Rotterdam Study

Clinical measures were obtained during the visits at the Rotterdam Study research center. Diabetes mellitus was defined as the use of blood glucose-lowering medication and/or a non-fasting serum glucose level of 11.1 mmol/l or higher and/or serum glucose levels ≥ 7 mmol/l (1997-2000).[34] Hypertension was identified through the use of anti-hypertensive medication and/or the assessment of blood pressure measurements.[35] Prevalence and incidence of myocardial infarction, heart failure, stroke and transient ischemic attacks were assessed as previously described.[36-40] Hypercholesterolemia was defined as a total serum cholesterol level above 6.2 mmol/l or the use of cholesterol-lowering drugs.[41] Use of QTc prolonging drugs (list 1) of the website based registry, use of β -blocking drugs and use of non-dihydropyridine calcium channel blockers at the index-date were considered as a covariate.[42]

IPCI

Known risk factors and other covariates for sudden cardiac death were gathered from the medical records through computerised searches and manual assessment. Cerebro- and cardiovascular ischemia and heart failure were assessed, based on the diagnoses provided by the general practitioner and by specialists in the medical records. Hypertension was identified through the diagnoses in the medical records, the use of antihypertensive medication and/or the assessment of blood pressure measurements.[35] Diabetes mellitus, arrhythmias and hypercholesterolemia were identified through diagnoses in the medical records from GPs and specialists and/ or the use of antidiabetic, anti-arrhythmic or lipid lowering medication. Use of QTc prolonging drugs, use of β -blocking drugs and use of non-dihydropyridine calcium channel blockers at the index-date were considered as a covariate.[42]

Statistical analysis

The relative risk of sudden cardiac death associated with use of antithyroid drugs was estimated by calculation of the hazard ratios using Cox proportional hazards models in the Rotterdam Study, and by calculation of the odds ratios using conditional logistic regression analyses in IPCI.

Covariates which were univariately associated with sudden cardiac death (at a $p < 0.1$ level) were included in the regression analyses if they changed the point estimate of the association between use of antithyroid drugs and sudden cardiac death by more than 10%.[43] We assessed the effect of duration of use and dosage and we investigated potential effect modification by age and gender, with interaction terms and subsequent stratifications. We performed several sensitivity analyses: by excluding users of amiodarone, participants with atrial fibrillation and adjusted for heart rate at baseline. The analyses were performed using SPSS for Windows version 15.0 (Chicago, Illinois, USA).

Results

Subject characteristics

Rotterdam Study

The baseline characteristics of all participants are presented in table 1. The mean age of the study population (7,898 participants) at baseline was 70.5 years (Standard Deviation 9.7 years), 38.9% was male.

The mean follow-up period for men was 9.6 years, the mean follow-up period for women was 10.0 years. During the follow-up period, 3,589 participants died of all causes. Overall, 297 persons were classified as definite sudden cardiac death and 78 as probable sudden cardiac death. Of the 375 sudden cardiac death events, 173 were in males. The mean age at baseline of the cases was 74.2 years (SD 8.5).

IPCI

The source population for this study comprised 478,661 subjects with at least one year of valid history during the study period. The total number of person-years of follow-up was 1,905,382 years, 14,259 persons died, 926 persons were classified as definite sudden cardiac death, 498 as probable sudden cardiac death. Overall, there were 1424 cases and 14,443 matched controls. The mean age of the cases was 72.9 years and 58.4 % were male.

Association between antithyroid drugs and sudden cardiac death

Rotterdam Study

Of all sudden cardiac death cases, 6 participants were current users of antithyroid drugs at the index date and 5 participants had used antithyroid drugs in the past but were no longer using them (table 2). Two of these currently exposed cases were classified

Table 1 Baseline characteristics, demographics, distribution of covariates

Characteristic	Rotterdam Study (cohort study)		IPCI (case-control study)	
	Current use of antithyroid drugs at baseline (n = 61)	No use of antithyroid drugs at baseline (n = 7837)	Sudden cardiac death cases (n = 1424)	Controls (n = 14443)
Gender				
Male	11 (18.0%)*	3064 (39.1%)*	831 (58.4%)	8739 (60.5%)
Female	50 (82.0%)*	4773 (60.9%)*	593 (41.6%)	5704 (39.5%)
Age (mean, SD) (years)	76.7 (9.0)*	70.6 (9.7)*	72.9 (13.9)*	66.7 (13.8)*
≤ 55	-	-	170 (11.9%)	3008 (20.8%)
55 – 65	10 (16.4%)	3001 (38.3%)	193 (13.6%)	2853 (19.8%)
66 – 75	17 (27.9%)	2614 (33.3%)	366 (25.7%)	4274 (29.6%)
> 75	34 (55.7%)	2222 (28.4%)	695 (48.8%)	4308 (29.8%)
Co-morbidities				
Myocardial infarction	5 (8.2%)	868 (11.1%)	77 (5.4%)*	271 (1.9%)*
Transient ischemic attack	1 (1.6%)*	416 (5.3%)*	65 (4.6%)*	383 (2.7%)*
Cerebrovascular accident	10 (16.4%)	941 (12.0%)	59 (4.1%)*	259 (1.8%)*
Arrhythmia	-	-	141 (9.9%)*	837 (5.8%)*
Hypertension	27 (44.3%)	2549 (32.5%)	268 (18.8%)*	2284 (15.8%)*
Body Mass Index (mean, SD)	26.1 (3.7)	26.3 (3.7)	-	-
Diabetes mellitus	15 (24.6%)*	839 (10.7%)*	284 (19.9%)*	1274 (8.8%)*
Smoking	-	-	283 (19.9%)	2857 (19.8%)
Current smoking	7 (11.5%)	1385 (17.7%)	-	-
Past smoking	11 (18.0%)*	2579 (32.9%)*	-	-
Alcohol abuse	(0%)*	176 (2.2%)*	30 (2.1%)*	107 (0.1%)*
Heart failure	3 (4.9%)	271 (3.5%)	272 (19.1%)*	615 (4.3%)*
Hypercholesterolemia	18 (29.5%)*	2995 (38.2%)*	204 (14.3%)*	1619 (11.2%)*
Use of list1 QTc prolonging drugs	17 (27.9%)*	491 (6.3%)*	58 (4.1%)*	283 (2.0%)*
Use of β-blocking drugs	18 (29.5%)*	1467 (18.7%)*	209 (14.7%)*	1608 (11.1%)*
Use of non-dihydropyridine calcium channel blockers	4 (6.6)*	259 (3.3%)*	58 (4.1%)*	279 (1.9%)*

* P<0.05

SD Standard Deviation

as definite sudden cardiac death and four as probable. The age ranged from 66 until 87 years. One of the patients was diagnosed with multinodular goitre, from the other patients the underlying cause of the hyperthyroidism was unknown. One of the patients had episodes of ventricular tachycardia and ventricular fibrillation in the recent history, possibly due to underlying hyperthyroidism. One patient used amiodarone at the time of sudden cardiac death. One patient had a TSH of 0.13 (6 days before sudden cardiac death), one a TSH<0.01 (15 days before sudden cardiac death). In the third case, the treating physician required blood test (including TSH) 11 days before sudden cardiac

Table 2 Risk of SCD

Use of antithyroid drugs	Rotterdam Study				IPCI			
	SCD	No SCD	HR (95% CI) ¹	HR (95% CI) ²	Cases	Controls	OR (95% CI) ³	OR (95% CI) ⁴
Number	375	7523			1424	14443		
Never use	364	7383	Reference	Reference	1415	14386	Reference	Reference
Past use	5	81	2.0 (0.8 - 4.8)	2.0 (0.8 - 4.8)	2	40	0.5 (0.1 - 1.9)	0.5 (0.1 - 1.9)
Current use	6	59	4.3 (1.9 - 9.6)	3.9 (1.7 - 8.8)	7	17	3.3 (1.3 - 8.5)	2.9 (1.1 - 7.4)

SCD = sudden cardiac death

1 Hazard ratios adjusted for gender and time-depending age.

2 Hazard ratios adjusted for gender and time-depending age and use of list1 QTc prolonging drugs.

3 Odds ratios matched for age, gender, practice and calendar time.

4 Odds ratios matched for age, gender, practice and calendar time and adjusted for arrhythmia and cerebrovascular ischemia.

death. The conclusions some days later was: "hyperthyroidism" but no values were given in the medical records. Thyroid function was not known for the other three cases.

Current use of antithyroid drugs was associated with a significantly increased risk of sudden cardiac death, adjusted for gender and age and use of list 1 QTc prolonging drugs (HR 3.9 (95% CI 1.7-8.8)). Past use was not associated with an increased risk of sudden cardiac death (HR 2.0 (0.8-4.8)).

After exclusion of users of amiodarone, the point estimates did not change substantially (risk of sudden cardiac death (HR 3.7 (1.5-8.9))). There was effect modification by gender ($p=0.005$), men had a higher risk of sudden cardiac death (HR 10.4 (2.6-42.5)) than women (HR 2.9 (1.1-7.7)). There was no effect modification by age ($p=0.74$). The risk of sudden cardiac death in current users of antithyroid drugs remained unchanged, after adjusting for age, gender, list 1 QTc prolonging drugs and heart rate at baseline (HR 3.1 (1.0-9.7)). After exclusion of participants with prevalent atrial fibrillation, the HR for current use slightly increased (4.3 (1.9-9.6)). Participants using ≥ 1 DDD had a higher risk of sudden cardiac death (HR 4.1 (1.3-12.7)) than participants using < 1 DDD (HR 1.5 (0.2-10.8)). Participants using antithyroid drugs less than 1 year had a higher risk of sudden cardiac death (HR 4.5 (1.1-18.3)) than participants using antithyroid drugs more than 1 year (HR 2.3 (1.2-4.6)).

IPCI

Of all cases, 7 patients were current users of antithyroid drugs at the index date and 2 patients were past users. Three of these cases were classified as definite sudden cardiac death and four as probable. The age ranged from 60 until 90 years. Two patients were diagnosed with toxic multinodular goitre, one with diffuse toxic goitre and one with multinodular goitre with degenerate changes, from the other 3 patients the cause of hyperthyroidism was unknown. None of the patients used amiodarone at the time of

sudden cardiac death. Six patients died within a few weeks after start of antithyroid drugs, when they probably still had low TSH levels and increased free T4 levels.

There was a significant association between sudden cardiac death and use of antithyroid drugs, adjusted for arrhythmia and cerebrovascular ischaemia (OR 2.9 (1.1-7.4)). Past use was not associated with an increased risk of sudden cardiac death.

Sensitivity analysis showed, that after exclusion of users of amiodarone, point estimates did not change substantially (OR 3.3 (1.3-8.5)). Stratified analyses showed that the association between sudden cardiac death and use of antithyroid drugs tended to be higher in patients younger than 70 years (OR 16.1 (1.0-262.0)) than in patients older than 70 years (OR 1.8 (0.5-7.3)), but this difference was not statistically significant. Effect modification by gender could not be demonstrated.

Discussion

In this study, we demonstrated in two independent populations that use of antithyroid drugs was associated with an increased risk of sudden cardiac death. There are two potential explanations for our findings. First, sudden cardiac death might be an adverse reaction to the antithyroid drugs. However this seems unlikely since different antithyroid drugs were involved and it lacks a biologically plausible mechanism. Second, patients may still have had inadequately treated hyperthyroidism with increased levels of free T4. Since TSH and free T4 levels were only available in a minority of patients, we could not verify this. Validation of the exposed sudden cardiac death cases demonstrated that the exposed cases with known TSH measurements, had low TSH levels a few days before the index date. This could indicate that these patients were still hyperthyroid at that time. Our findings could suggest that these deaths were caused by undertreatment of hyperthyroidism.

Sudden deaths have been described after therapy with radioactive iodine. However, in the Rotterdam Study none of the exposed patients had received radioactive iodine. In IPCI, one of the exposed controls received radioactive iodine before the index-date and none of the exposed cases. Amiodarone may cause iodine-induced thyrotoxicosis (AIT), which might worsen arrhythmias and increase mortality.[44] However, excluding all users of amiodarone did not change the point estimates substantially. The association between antithyroid drugs and sudden cardiac death in our study tended to be higher in younger persons in the case-control study. We did not find effect modification by age in the Rotterdam Study, possibly since in a study of an elderly population there is less age variability. Participants who were treated with a higher dosage had a higher risk of sudden cardiac death, probably since these participants had more severe hyperthyroid-

ism. Furthermore, participants who were treated less than 1 year had an increased risk, possibly due to depletion of susceptibles.

Subclinical hyperthyroidism is associated with increased cardiovascular mortality among persons of 60 years or older.[14] Hyperthyroidism has been associated with ventricular arrhythmias and sudden cardiac death in several case reports.[17, 22-25, 45] Recently, we found an association of free T4 with a higher risk of QTc prolongation in males.[7] A potential explanation for this association is an increased activity of cardiac Na⁺/K⁺ ATPase in thyroid hormone excess, leading to increased intracellular K⁺ with subsequent membrane hyperpolarization and an increase in QTc duration.[5, 6] In the Rotterdam Study, the association of antithyroid drugs and sudden cardiac death tended to be higher in males, which supports the association of free T4 and QTc prolongation in males, we found earlier. As far as we know, this is the first time that use of antithyroid drugs has been associated with sudden cardiac death in two large epidemiologic studies.

Our study has several strengths. First, the availability of data on a large group of participants in two independent study populations. We were able to take advantage of the fact that in most cases of sudden cardiac death extensive information of the facts surrounding the event was available, which allowed rigorous adjudication of sudden cardiac death events. Second, the complete coverage of drug dispensing records in the Rotterdam Study and of prescription data in IPCI allowed us to study the association between antithyroid drugs and sudden cardiac death. The population-based design of the Rotterdam Study probably limited the chance of selection bias. Selection bias is also unlikely in IPCI which covers the complete population in a circumscribed area and both cases and controls were selected from the same source population. There is no information bias, since we used pharmacy data and GP data which are automatically registered, prospectively and irrespective of disease status. Confounding was minimized by adjusting for all known cardiovascular risk factors of sudden cardiac death. However, our study has also some potential limitations. Not all acute deaths may have been of cardiac origin. We determined sudden cardiac death, however, on the basis of the full medical records and all circumstances surrounding the death were available. Recently, an evaluation comparing different methods to determine the incidence of sudden cardiac death suggested that this method provides a very reliable way of determining sudden cardiac death cases.[46] Because of the small number of users of antithyroid drugs in our analysis in the Rotterdam Study, we tested the validity of our findings by replication in the IPCI database. The fact that the association was not only demonstrated and subsequently replicated in two independent study populations but also that the point estimates were quite similar, confirms the validity of the association between current use of antithyroid drugs and sudden cardiac death. Second, we could not investigate the mechanism of the association between hyperthyroidism and sudden cardiac death. The patients using antithyroid drugs could have hyper-, hypo- or normothyroid values.

However, since the exposed sudden cardiac death cases with a thyroid measurement, had a decreased TSH measurement shortly before the index date, it is more likely due to the underlying hyperthyroidism than due to the antithyroid drugs. Earlier, we demonstrated that free T4 is associated with QTc prolongation in males [7] and also that QTc prolongation is associated with an increased risk of sudden cardiac death.[12]

In conclusion, we demonstrated that use of antithyroid drugs seems to be associated with a threefold increased risk of sudden cardiac death. Although this might be due to antithyroid drug use, it could be more readily explained by underlying hyperthyroidism, since increased free T4 levels are associated with QTc prolongation and treated patients who developed sudden cardiac death still had low TSH levels shortly before death. This suggests that hyperthyroidism may be a risk factor for sudden cardiac death.

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Chapter 5.1

The association of serum testosterone levels and ventricular repolarization

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Abstract

Background

It is assumed that testosterone is an important regulator of gender-related differences in ventricular repolarization. Therefore, our aim was to study whether serum levels of testosterone are associated with QTc, QT and RR interval variation.

Methods

Setting: Two independent population-based cohort studies.

Participants: 445 male participants (≥ 55 yr) from the Rotterdam Study cohort and 1428 male participants from the Study of Health in Pomerania (SHIP) with an electrocardiogram who were randomly sampled for assessment of serum testosterone at baseline, after exclusion of participants with testosterone altering drugs, QTc prolonging drugs or dig(it)oxin, left ventricular hypertrophy and left and right bundle branch block.

Endpoints: Length of the QTc, QT and RR intervals.

Analysis: Linear regression model, adjusted for the two individual studies and a pooled analysis of both studies.

Results

The pooled analysis of the Rotterdam Study and SHIP showed that the QTc interval gradually decreased among the tertiles (P value for trend 0.024). The third tertile of serum testosterone was associated with a lower QTc interval compared to the first tertile (-3.4 ms (-6.5;-0.3)). However, the third tertile of serum testosterone was not associated with a lower RR-adjusted QT interval compared to the first tertile (-0.7 ms (-3.1;1.8)). The RR interval gradually increased among the tertiles (P value for trend 0.002) and the third tertile of serum testosterone showed an increased RR interval compared to the first tertile (33.5 ms (12.2;54.8)).

Conclusions

In the pooled analysis of two population-based studies, serum testosterone levels were not associated with the RR-adjusted QT interval. Lower QTc intervals in men with higher serum testosterone levels were probably due to the association of serum testosterone with prolongation of the RR interval.

Introduction

There are well known gender-related differences in human cardiac repolarization.[1, 2] This is demonstrated by a longer heart-rate corrected QT (QTc) interval in women, which is the traditional clinical measurement for assessing the duration of ventricular repolarization.[3] Prolongation of ventricular repolarization may result in early after depolarizations, which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmias.[4-7]

The gender differences in the QTc interval are not present in young children, whereas at the time of onset of puberty the duration of the QTc interval in boys shortens, which results in a longer QTc interval in adult women compared to men.[8-10] These gender differences decrease with age.[10] Since the period between puberty and approximately 50 years of age coincides with the highest circulating levels of androgens in males, male sex hormones may play a role in the shorter QTc interval in men. Furthermore, virilized women exhibit QTc intervals similar to those of healthy men, whereas castrated men had QTc intervals similar to those of normal women.[11] Testosterone therapy also reduces QT dispersion in men with congestive heart failure.[12]

These data suggest that testosterone might be an important regulator of ventricular repolarization, which might explain the gender-related differences in ventricular repolarization. Therefore, our aim was to study whether testosterone is associated with QTc, QT and RR interval duration. Since bio-available testosterone decreases with age [13], we studied this association in two cohorts. First, in a cohort of elderly (the Rotterdam Study) and second, in a cohort with a younger mean age (the Study of Health in Pomerania (SHIP)).

Methods

Setting and study design

Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study, which started with a baseline visit between 1990 and 1993. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. All inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years and over, were invited to participate (n=10,275). Of them, 7983 (78%) gave their written informed consent and took part in the baseline examination. Objectives and methods of the Rotterdam Study have been described in detail elsewhere.[14, 15] At baseline, all participants were visited at home for a standardized questionnaire, and 7151 were subsequently examined at the research

center. The cohort is continuously monitored for major morbidity and mortality through linkage with general practitioner and municipality records. Drug prescriptions dispensed to participants by automated pharmacies are routinely stored in the database since 1 January 1991.

All male cohort members of the Rotterdam Study ($n=3105$), who had an ECG as well as serum testosterone and dehydroepiandrosterone sulfate (DHEAS) measurements at baseline were enrolled in the study population. Digitally stored ECGs were available for 2200 male participants at the time of the first visit. Missing ECGs were mainly due to temporary technical problems with ECG recording. Androgen status was assessed in different random subsets of these males. Male participants with an ECG and with both a serum testosterone measurement and a DHEAS measurement ($n=540$) were included. Participants who received anabolic steroids (Anatomic Therapeutic Chemical (ATC) code A14A) ($n=1$) were excluded. None of the participants received sexual hormones (ATC code G03), testosterone 5 α -reductase inhibitors (ATC code G04CB), sexual hormone antagonists (ATC code L02B) or had a pacemaker. Participants who used digoxin ($n=25$), which is a QTc shortening agent, or QTc prolonging drugs ($n=6$) as well as persons with evidence of left ventricular hypertrophy ($n=36$) or left ($n=13$) and right bundle branch block ($n=36$) were excluded, since these conditions are associated with an altered QTc interval.[16, 17] Overall, 445 participants were included in the Rotterdam study population.

The Study of Health in Pomerania (SHIP)

SHIP is a cross-sectional population-based study in West Pomerania, a region in the north-east of Germany. The total population of West Pomerania selected for SHIP comprised 212,157 inhabitants. A two-stage cluster sampling method adopted from the WHO MONICA Project Augsburg, Germany yielded 12 five year age strata (20 to 79 years) for both genders, each including 292 individuals.[18] The sampling was performed from population registries where all German citizens are registered. Data collection started in October 1997 and was finished in March 2001. The net sample comprised 6267 eligible subjects. Finally, 4310 subjects (69%) participated. The study was monitored by a board of independent scientists. All participants gave written informed consent. The study conformed to the principles of the Declaration of Helsinki as reflected by an *a priori* approval of the Ethics Committee of the University of Greifswald. Use of medication at baseline was recorded by a computer-aided method using the ATC code.

All male cohort members of SHIP ($n=2118$) with ECG, testosterone and DHEAS measurements at baseline were enrolled in the study population. Digitally stored ECGs were available for 1826 male participants at the time of the first visit. Male participants with an ECG and both a testosterone and a DHEAS measurement ($n=1677$) were included. Participants who received sexual hormones (ATC code G03) ($n=3$), testosterone 5 α

reductase inhibitors (ATC code G04CB) (n=4) or sexual hormone antagonists (ATC code L02B) (n=3) were excluded. None of the participants reported to receive anabolic steroids (ATC code A14A). Furthermore, participants who used digoxin (n=5) or digoxin (n=153), QTc prolonging drugs (n=13), persons with a pacemaker (n=15), as well as persons with evidence of left ventricular hypertrophy (n=4) or left (n=1) and right bundle branch block (n=48) were excluded. Overall, 1428 participants were included in the SHIP study population.

QTc, QT and RR interval

The endpoints of the study were the lengths of the QTc, QT and RR intervals in ms. A 12-lead resting ECG was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECGs (both from the Rotterdam Study as well as from SHIP) were centrally processed in Rotterdam using the Modular ECG Analysis System (MEANS) to obtain ECG measurements, in agreement with the FDA guidance for clinical evaluation of QT/QTc interval prolongation.[19] The MEANS program has been evaluated and validated extensively.[20-23] In one of these validation studies, ECGs with selected abnormalities were analyzed by 5 cardiologists and 11 different computer programs of which MEANS performed as one of the best.[23] In a study in which QT intervals by manual measurement were compared with QT measurement by ECG machines, manual and automated measurements generated similar numerical results in 3 studies in healthy volunteers, which all included a positive control.[24] MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques.[21] The QT interval is determined from the start of the QRS complex until the end of the T wave. To adjust for heart rate, Bazett's formula ($QT_c = QT / \sqrt{RR}$) was used.[25] The RR interval was taken as the median of the RR intervals in the recording. Additionally, the MEANS program determines left ventricular hypertrophy and left and right bundle branch block.

Steroids

Rotterdam Study

At baseline, non-fasting blood samples were obtained. Time of sampling was recorded. Testosterone and DHEAS were estimated using coated tube or double antibody RIAs, respectively, purchased from Diagnostic Systems Laboratories.[26]

SHIP

At baseline, non-fasting blood samples were obtained. Time of sampling was recorded. Testosterone and DHEAS were measured using competitive chemiluminescent enzyme

immunoassays on an Immulite 2500 analyzer (DPC Biermann GmbH, Bad Nauheim, Germany).

Covariates

Rotterdam Study

Hypertension was identified through the use of antihypertensive medication and/or the assessment of blood pressure measurements, according to the guidelines of the World Health Organisation.[27] Prevalence and incidence of myocardial infarction were assessed as previously described.[28, 29] Diabetes mellitus was defined as the use of blood glucose-lowering medication and/or a non-fasting serum glucose level of 11.1 mmol/l or higher and/or fasting serum glucose levels ≥ 7 mmol/l.[30] Prevalence and incidence of heart failure were assessed by the presence of suggestive signs and symptoms as previously described.[31, 32] Potassium and calcium were measured by means of a Microlyte device. During the home interview, smoking status and use of alcohol were assessed. Creatinine clearance was computed with the Cockcroft Gault method. Renal failure was defined by the internationally accepted criterion of a GFR below 60 ml/min.[33] Gamma-glutamyl transferase (GGT), aspartate-amino transferase (ASAT), alanine-amino transferase (ALAT) levels were used to determine hepatic failure.

SHIP

Hypertension was identified through the use of antihypertensive medication and/or the assessment of blood pressure measurements, according to the guidelines of the World Health Organisation.[27] Diabetes mellitus and myocardial infarction were defined as self-reported physician's diagnosis. Determination of calcium was performed by a colorimetric assay and potassium by ion-selective electrodes (Roche/Hitachi 717; Roche Diagnostics GmbH, Mannheim, Germany). During the home interview, smoking status and use of alcohol were assessed. Creatinine clearance was computed with the Cockcroft Gault method. Renal failure was defined by the internationally accepted criterion of a GFR below 60 ml/min.[33] GGT, ALAT and ASAT were used to determine hepatic failure.

Statistical analysis

The association between the QTc, QT, RR intervals and testosterone was assessed through linear regression with log-transformed testosterone and testosterone measurements divided in tertiles. Since DHEAS was associated with both the QTc and the RR interval, we adjusted all analyses for DHEAS. Furthermore, all analyses were adjusted for age, time of blood withdrawal, hypertension, myocardial infarction, diabetes mellitus, potassium, calcium and in the Rotterdam Study also for heart failure.

First, a linear regression analysis was conducted with QTc, QT and RR interval as dependent and testosterone as independent variables for the Rotterdam Study and SHIP separately. Second, we conducted a pooled analysis of the Rotterdam Study and SHIP. Furthermore, we performed several sensitivity analyses: First, by stratification for age. Second, in additional analyses we adjusted for smoking, alcohol abuse, renal failure, hepatic failure and use of beta-blockers. All analyses were performed using SPSS for Windows version 15.0 (Chicago, Illinois, USA).

Results

Subject characteristics

The baseline characteristics of the participants are presented in table 1. The mean age of the study population of the Rotterdam Study was 68.0 years (standard deviation (SD) 7.8 years). The mean testosterone level was 11.3 nmol/l (SD=3.7). The mean age of the study population of SHIP was 49.1 years (SD 16.0 years). Mean testosterone levels were 17.0 nmol/l (SD=5.9).

Table 1 Baseline characteristics of study population

	Rotterdam Study	SHIP
Number of participants	445	1428
Age (years, mean, SD)	68.0 (7.8)	49.1 (16.0)
Mean QTc interval (ms) (SD)	423.2 (25.0)	423.5 (24.4)
Mean QT interval (ms) (SD)	398.2 (28.6)	394.1 (29.9)
Mean RR interval (ms) (SD)	896.2 (156.7)	876.0 (156.2)
Body Mass Index (kg/m ²) (SD)	26.6 (3.4)	27.7 (4.2)
Diabetes mellitus (n, %)	50 (11.2)	95 (6.7)
Myocardial infarction (n, %)	84 (18.9)	59 (4.1)
Hypertension (n, %)	111 (24.9)	594 (41.6)
Heart failure (n, %)	7 (1.6)	-
Mean potassium (mmol/l) (SD)	4.1 (0.3)	4.1 (0.3)
Mean calcium (mmol/l) (SD)	2.4 (0.1)	2.4 (0.1)
Mean DHEAS (μmol/l) (SD)	4.3 (2.7)	5.3 (3.3)
Mean testosterone (nmol/l) (SD)	11.3 (3.7)	17.0 (5.9)

SD=standard deviation

DHEAS= dehydroepiandrosterone sulfate

Androgens and QTc interval

The mean duration of the QTc interval was approximately similar in both studies, the mean duration of the QT and RR interval was slightly longer in the Rotterdam study than in SHIP (table 1). Comparison of the highest testosterone tertile with the lowest tertile showed no significant association between testosterone and QTc (-5.9 ms (95%CI -13.8 ; 2.1) and -2.9 ms (95%CI -5.9 ; 0.1) respectively) nor with the QT interval (-2.4 ms (95%CI -8.8 ; 4.0) and -0.6 ms (95%CI -3.0 ; 1.8) respectively) in the Rotterdam and SHIP study separately (table 2). The logarithmic transformation of testosterone was associated with the QTc interval in SHIP (-7.9 ms (95%CI -15.3 ; -0.6)).

Table 2 Association of testosterone with QTc, QT and RR interval

	Testosterone	QTc-interval in ms (95% CI) ¹	QT-interval in ms (95% CI) ²	RR-interval in ms (95% CI) ¹
Rotterdam Study (n=445)	Log-transformation	-6.5 (-23.7 ; 10.8)	5.9 (-8.2 ; 20.1)	149.8 (30.6 ; 269.1)
	Tertiles			
	1	Reference	Reference	Reference
	2	3.8 (-4.1 ; 11.7)	5.5 (-0.8 ; 11.9)	29.2 (-27.3 ; 85.8)
	3	-5.9 (-13.8 ; 2.1)	-2.4 (-8.8 ; 4.0)	42.4 (-14.3 ; 99.2)
	<i>P value for linear trend</i>	0.132	0.419	0.143
SHIP (n=1428)	Log-transformation	-7.9 (-15.3 ; -0.6)	-3.4 (-9.2 ; 2.4)	55.9 (5.2 ; 106.6)
	Tertiles			
	1	Reference	Reference	Reference
	2	-0.7 (-3.6 ; 2.2)	1.5 (-0.8 ; 3.9)	24.0 (3.8 ; 44.1)
	3	-2.9 (-5.9 ; 0.1)	-0.6 (-3.0 ; 1.8)	31.3 (10.3 ; 52.3)
	<i>P value for linear trend</i>	0.061	0.623	0.004

CI = Confidence Interval

1 : adjusted for age, time of blood withdrawal, diabetes, hypertension, myocardial infarction, potassium, calcium, BMI, dehydroepiandrosterone sulfate (DHEAS) and in the Rotterdam Study also for heart failure

2 : adjusted for age, time of blood withdrawal, diabetes, hypertension, myocardial infarction, potassium, calcium, BMI, DHEAS, RR interval and in the Rotterdam Study also for heart failure

Testosterone in nmol/l. For the Rotterdam Study the tertiles were defined as (1) ≤ 9.9; (2) 10.0 – 12.6; (3) ≥ 12.7. For SHIP the tertiles were defined as (1) ≤ 13.9; (2) 14.0 – 18.6; (3) ≥ 18.7.

In SHIP, the RR interval increased gradually with increasing testosterone levels (P value for trend 0.004) and the second and third tertiles of testosterone had longer RR intervals compared to the first tertile (24.0 ms (95%CI 3.8 ; 44.1) and 31.3 ms (95%CI 10.3 ; 52.3) respectively). The logarithmic transformation of testosterone was associated with the RR interval in both the Rotterdam Study and SHIP (149.8 ms (95%CI 30.6 ; 269.1) and 55.9 ms (95%CI 5.2 ; 106.6) respectively).

Pooled analysis

There was a gradual decrease of the QTc interval among the tertiles (P value for trend 0.024), while the third tertile of testosterone was significantly associated with the QTc interval compared to the first tertile (-3.4 ms (95%CI -6.5 ; -0.3)) (table 3). Compared to the first tertile of testosterone, the third tertile was not associated with the QT interval (-0.7 ms (95%CI -3.1 ; 1.8)).

Table 3 Association of testosterone with QTc, QT and interval: pooled analysis of the Rotterdam Study and SHIP (n=1873)

Testosterone	QTc-interval in ms (95% CI) ¹	QT-interval in ms (95% CI) ²	RR-interval in ms (95% CI) ¹
Log-transformation	-8.1 (-14.8 ; -1.4)	-3.1 (-8.4 ; 2.3)	62.5 (15.7 ; 109.2)
Tertiles			
1	Reference	Reference	Reference
2	-0.7 (-3.6 ; 2.1)	0.5 (-1.8 ; 2.8)	10.8 (-9.1 ; 30.8)
3	-3.4 (-6.5 ; -0.3)	-0.7 (-3.1 ; 1.8)	33.5 (12.2 ; 54.8)
<i>P value for linear trend</i>	0.024	0.529	0.002

CI = Confidence Interval

1: adjusted for age, time of blood withdrawal, diabetes, hypertension, myocardial infarction, potassium, calcium, BMI, dehydroepiandrosterone sulfate (DHEAS) and cohort

2: adjusted for age, time of blood withdrawal, diabetes, hypertension, myocardial infarction, potassium, calcium, BMI, RR interval, DHEAS and cohort

Testosterone in nmol/l. The tertiles were defined as (1) ≤ 12.5 ; (2) 12.6 – 17.3; (3) ≥ 17.4 .

There was a gradual increase of the RR interval among the tertiles (P value for trend 0.002), while the third tertile of testosterone was significantly associated with the RR interval compared to the first tertile (33.5 ms (95%CI 12.2 ; 54.8)). Sensitivity analyses revealed no effect modification by age. Additional adjustment for smoking, alcohol, renal failure, hepatic failure or use of beta-blockers did not change the estimates.

Discussion

In this pooled analysis of two population-based studies, we found that serum testosterone levels were associated with shortening of the QTc interval and prolongation of the RR interval in men, whereas we did not find an association between serum testosterone levels and the uncorrected QT interval. Earlier, it has been suggested that the shorter QTc interval in men than in women may be explained by the fact that testosterone influences repolarization by its effect on Ca^{2+} and K^{+} channels, as well as on *hERG*-encoded K^{+} channels.[34] Since the QT interval was not significantly associated with serum testosterone, our findings suggest that the difference in the QTc interval in men

is mainly due to the underlying association with the RR interval. Since QTc is calculated by Bazett's formula, an increase in RR interval will lead to a shorter QTc interval.

According to literature, the gender differences in the QTc interval decrease with age [10], this could be due to the fact that bio-available testosterone decreases with age. [13] It is well known that women have a longer QTc interval than men, [2] whereas the uncorrected QT intervals are rather similar in men and women. [10] Since women have a faster heart rate, and therefore shorter RR intervals compared to men, women have a prolonged QTc interval when the same heart rate correction formula is applied. The gender differences in the RR interval remain after autonomic blockade, which suggests a possible gender-related difference in sino-atrial node function. [35] However, this difference appears to be related to a difference in maximum exercise capacity between men and women rather than to intrinsic sinus node gender-related differences. [35] Since androgen levels increase in response to exercise [36], the increased RR interval in men with high testosterone levels could be due to increased exercise capacity, which affects the sino-atrial node function.

Our investigation has several strengths that include the population-based design of participating studies and the central ECG data management, which facilitates best comparability between both studies. Although we did not use the complete population in the Rotterdam Study, selection bias is unlikely because the characteristics of the study population were comparable to the baseline characteristics of the whole population of the Rotterdam Study. Furthermore, information bias is unlikely because data were gathered prospectively and because the use of digital ECG recordings all measured using the automatic MEANS system likely reduced intra- and interobserver variability in the assessment of the QTc, QT and RR intervals. Confounding was minimized by adjusting for all known risk factors. However, our study has also some limitations. Because of the cross-sectional design we cannot exclude that QTc prolongation or RR interval shortening was already present in some participants before the decrease of testosterone. Therefore, the results from this study should be confirmed with longitudinal data from other large cohorts. Another limitation of our study is that repeated blood sampling in men with initially low serum testosterone levels is recommended to confirm androgen deficiency [37], but this and other epidemiologic studies [38-41] were based on a single measurement of serum testosterone. However, misclassification of men with low serum testosterone levels would be expected to underestimate, not overestimate associations.

In conclusion, we demonstrated in a pooled analysis of two population-based studies that serum testosterone is not associated with a difference in the RR-adjusted QT interval and that the decreased QTc interval in men with higher levels is probably due to the association of serum testosterone with prolongation of the RR interval.

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Chapter 5.2

Serum glucose and insulin are associated with QTc and RR intervals in non-diabetic elderly

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Abstract

Background

Since diabetes mellitus has been associated with QTc and RR interval variation, and hyperglycemia is an independent risk factor for cardiovascular disease, our aim was to study whether non-diabetic persons with impaired fasting serum glucose and hyperinsulinemia have QTc/QT-interval prolongation and RR-interval shortening, and whether these were associated with an increased risk of sudden cardiac death.

Methods

First, a cross-sectional analysis was conducted as part of the population-based Rotterdam Study including 1050 men and 1520 women (≥ 55 yr), without diabetes mellitus. Participants for whom an ECG, fasting serum glucose and fasting insulin measurements were available, were eligible for the study. Participants using digoxin or QTc prolonging drugs, and participants with left ventricular hypertrophy and left and right bundle branch block were excluded. The endpoints of the study were the length of the QTc, QT and RR interval. The associations were examined by means of linear regression analysis. Second, in all 6020 participants of the Rotterdam Study with an ECG, the associations between the QTc, QT and RR interval and sudden cardiac death were examined by means of Cox regression analysis.

Results

Overall, there was a significant association between impaired fasting serum glucose and the QTc interval with an increase of 2.6 ms (95%CI: 0.3;5.0). Hyperinsulinemia was also associated with QTc prolongation (3.0 ms (0.8;5.3)). Impaired fasting glucose and hyperinsulinemia were significantly associated with a decrease of the RR interval (-33.7 ms (-48.8;-18.6) and -44.4 ms (-58.7;-30.0) respectively).

Participants in the fourth quartile of the QTc and QT interval had a significantly increased risk of sudden cardiac death compared to participants in the first quartile (HR 2.87 (95%CI 2.02 – 4.06); HR 3.05 (1.99 – 4.67) respectively). Furthermore, there was a significant inverse association between the fourth quartile of the RR interval compared to the first quartile and the risk of sudden cardiac death (HR 0.49 (0.34 – 0.80)).

Conclusions

We demonstrated in this population-based study that impaired fasting glucose and hyperinsulinemia are associated with a significantly increased QTc-interval and with significant shortening of the RR interval. We demonstrated that both a prolonged QTc as well as a shortened RR interval are associated with an increased risk of sudden cardiac death.

Introduction

Diabetes mellitus is a common disease in the elderly which adversely affects cardiac repolarization, probably by autonomic imbalance. It is associated with prolongation of the heart-rate corrected QT (QTc) interval which is the traditional measurement for assessing the duration of ventricular repolarization.[1, 2] QTc prolongation may result in early after depolarizations, which in turn may induce re-entry and thereby provoke torsade de pointes and fatal ventricular arrhythmias.[3-7]

In diabetes, hyperglycemia is a risk factor for cardiovascular disease. Non-diabetic participants with impaired fasting glucose or hyperinsulinemia may have an increased risk of cardiovascular disease as well. Glucose intolerance, high fasting serum glucose levels and insulin levels have been associated with prolongation of the QTc interval in non-diabetic persons.[8, 9] In a small study of 35 non-diabetic offspring of type 2 diabetic patients, it was demonstrated that during acute hyperinsulinemia, the QT interval remained unchanged, whereas the QTc interval increased.[10] Serum glucose and hyperinsulinemia have been demonstrated to be associated with the RR interval in non-diabetic patients.[11, 12]

To our knowledge, no large studies about the association between serum glucose and insulin, and the uncorrected QT interval in non-diabetic persons have been published. Since diabetes mellitus has been associated with QTc and RR interval variation, and hyperglycemia is an independent risk factor for cardiovascular disease, our aim was to study whether non-diabetic participants with an impaired fasting glucose measurement and hyperinsulinemia have an increased risk of QTc prolongation and RR shortening and thereby an increased risk of sudden cardiac death. Therefore, we studied whether impaired fasting glucose and hyperinsulinemia are associated with the QTc, QT and RR interval in non-diabetic participants of a large prospective population-based study in elderly. Subsequently, we examined the association between the QTc, QT and RR interval and sudden cardiac death in the same population.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study, which started with a baseline visit between 1990 and 1993. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. All inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years and over, were invited to participate (n=10,275). Of them, 7983 (78%) gave their written informed consent and took part in the baseline examination. Objectives and methods of the Rotterdam Study have been

described in detail elsewhere.[13, 14] At baseline, all participants were visited at home for a standardized questionnaire, and 7151 were subsequently examined at the research center. Since the start of the study, follow-up visits took place in the period 1993 through 1996 for the second visit, in the period between 1997 through 1999 for the third visit and in the period between 2002 through 2004 for the fourth visit. In addition to follow-up examinations, the cohort is continuously monitored for major morbidity and mortality through linkage with general practitioner and municipality records. Drug prescriptions dispensed to participants by automated pharmacies are routinely stored in the database since 1 January 1991.

Study designs

We performed two analyses in the Rotterdam Study. First, we used a cross-sectional design to analyse the association between serum insulin and glucose as assessed at the third visit to the research center, and QT-, QTc-, and RR-interval with data also from the third visit. Hereto, both the ECG and blood sampling occurred at the same day at the third visit to the research center. The reason was that during rounds 1 and 2, no data were available on both serum insulin and serum glucose. Second, for studying the association between QT-, QTc-, and RR-interval and sudden cardiac death, we used a prospective cohort design with the whole Rotterdam Study for whom an ECG was available. This analysis included the participants from the first analysis as described above. Since fasting glucose and fasting insulin measurements were only available at the third visit, we could not directly assess the effect between fasting glucose, fasting insulin and sudden cardiac death due to the limited number of sudden cardiac death events occurring during follow-up since the third visit.

Cross-sectional analysis of QT, QTc-, and RR-interval

All participants of the Rotterdam Study without diabetes mellitus, who had an ECG and a fasting glucose and fasting insulin measurement at the third visit were enrolled in our first analysis. Diabetes mellitus (type I or II) was defined as the use of blood glucose-lowering medication and/or a non-fasting serum glucose level of 11.1 mmol/l or higher and/or fasting serum glucose levels ≥ 7 mmol/l at the third investigation or any time prior to that moment.[15] Digitally stored ECGs were available for 3818 participants at the time of the third visit. Missing ECGs were mainly due to temporary technical problems with ECG recordings.

Participants with diabetes mellitus (n=357), participants without a fasting glucose measurement (n=168) or a fasting insulin measurement (n=270) or insulin outliers (n=1 (insulin=9292 pmol/l)), participants who were not fasting at blood sampling (n=160), participants who used QTc prolonging drugs as defined on list 1 of the website based registry (<http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm>) [16] (n=150), participants

who used digoxin (n=102), which is a QTc shortening agent, persons with a pacemaker (n=13), as well as persons with evidence of left ventricular hypertrophy (n=125) or left and right bundle branch block (n=73, n=131) were excluded, since these conditions are associated with an altered QTc interval.[17, 18] Overall, 2570 participants were included in this cross-sectional analysis.

Cohort study of sudden cardiac death

All cohort members of the Rotterdam Study with an ECG were enrolled in the study population. The study ended on one of the censoring dates (death or transferring out) or the end of the study period (1 January 2006). Overall, 6020 participants were included in the cohort analysis.

QTc, QT and RR interval

The endpoints of the study were the length of the QTc, QT and RR interval in milliseconds (ms). A 12-lead resting ECG was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECGs were processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurements, in agreement with the FDA guidance for clinical evaluation of QT/QTc interval prolongation.[19] The MEANS program has been evaluated and validated extensively.[20-23] In one of these validation studies, ECGs with selected abnormalities were analyzed by 5 cardiologists and 11 different computer programs of which MEANS performed as one of the best.[23] MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques.[21] The QT interval is determined from the start of the QRS complex until the end of the T wave. To adjust for heart rate, Bazett's formula ($QTc = QT / \sqrt{RR}$) was used.[24] Additionally, the MEANS program determines left ventricular hypertrophy and left and right bundle branch block.

Sudden cardiac death

According to the most recent definition, sudden cardiac death was defined as: 1) a witnessed natural death attributable to cardiac causes, heralded by abrupt loss of consciousness, within one hour of onset of acute symptoms, or 2) an unwitnessed, unexpected death of someone seen in a stable medical condition <24 hours previously with no evidence of a non-cardiac cause.[25, 26]

The ascertainment of sudden cardiac death cases has been described previously.[7] In short, information on vital status is obtained from municipal health authorities in Rotterdam and GPs. Two research physicians independently coded all reported events, blinded to exposure, and judged the likelihood of sudden cardiac death according to

the definition above. In cases of disagreement, consensus was sought and finally, a cardiologist reviewed all events. The index date was the date of death.

Fasting glucose and insulin status

At the third visit, fasting blood samples were drawn by venapuncture and the serum glucose levels were measured using the glucose hexokinase method (Instruchemie).[27] Serum insulin was determined by metric assay (Biosource Diagnostics). This assay has no cross-reactivity with either proinsulin or C-peptide.

Glucose status was classified into two categories: subjects with normal fasting glucose (NFG) and subjects with impaired fasting glucose (IFG). IFG is a recently defined diagnostic category based on fasting plasma glucose concentration.[28] Analogous to the World Health Organization criteria of impaired glucose tolerance, it represents a metabolic stage intermediate between normal glucose homeostasis and diabetes mellitus and is associated with insulin resistance syndrome.[28, 29] NFG was defined as a fasting glucose level below 6.1 mmol/L. IFG was defined as a fasting serum glucose level between 6.1 and 6.9 mmol/L.[28]

Insulin status was classified into two categories: subjects with normal insulin levels (<100 pmol/l) and subjects with hyperinsulinemia (≥ 100 pmol/l).

Covariates

Clinical measures were obtained during the visits at the Rotterdam Study research center. Hypertension was identified through the use of antihypertensive medication and/or the assessment of blood pressure measurements, according to the guidelines of the World Health Organization.[30] Prevalence and incidence of myocardial infarction were assessed by hospital discharge diagnosis or in case a patient was not hospitalized, when signs and symptoms, analysis of the standard 12-lead ECG and cardiac enzyme data were diagnostic of a myocardial infarction.[31, 32] Prevalence and incidence of heart failure were assessed by the presence of suggestive signs and symptoms as previously described.[33, 34] Hypercholesterolemia was defined as a total serum cholesterol level above 6.2 mmol/l or the use of cholesterol-lowering drugs, hypertriglyceremia was defined as a triglycerides level above 1.695 mmol/l.[35]

Statistical analysis

The associations between the determinants impaired fasting glucose and hyperinsulinemia, and the outcomes QTc, QT and RR interval, were assessed through linear regression analysis. The relative risk of sudden cardiac death associated with the QTc, QT and RR interval was estimated by calculation of the hazard ratios using Cox proportional hazards models. All analyses were adjusted for age and gender. In addition, all covariates that were associated with the outcome ($p < 0.10$) were considered as potential

confounders in both analyses. In the analyses assessing impaired fasting glucose and hyperinsulinemia, and QTc, QT, RR interval, the following covariates were considered as potential confounder: body mass index, waist-hip ratio, hypercholesterolemia, hypertriglyceremia, hypertension, myocardial infarction, heart failure and use of beta-blockers. In the analyses assessing the QTc, QT, RR interval and sudden cardiac death, the following covariates were considered as potential confounder: diabetes mellitus, hypercholesterolemia, hypertension, myocardial infarction, heart failure, body mass index, use of QTc prolonging drugs, use of beta-blockers and use of digoxin. The multivariate models were built to include all covariates that changed the association between exposure and the outcome by more than 5%.

First, a linear regression analysis was conducted with the QTc, QT, RR interval as outcomes and impaired fasting glucose and hyperinsulinemia as determinants. Second, in participants with a normal fasting glucose measurement, linear regression analysis was conducted with hyperinsulinemia as determinant. We performed a sensitivity analysis by additionally adjusting for determinants of the metabolic syndrome: hypertension, hypertriglyceremia, hypercholesterolemia, waist-hip ratio and BMI.

Third, a Cox regression was conducted with sudden cardiac death as outcome and the QTc, QT and RR interval divided in quartiles as determinants. All analyses were performed using SPSS for Windows version 15.0 (Chicago, Illinois, USA).

Results

Subject characteristics

The characteristics of the participants at the third visit are presented in table 1. Overall, 2570 participants were included, of whom 1520 were female and 1050 male. The mean age of the study population was 71.1 years (standard deviation (SD) 6.8 years). Mean fasting glucose was 5.5 mmol/l (SD=0.5), mean fasting insulin was 72.7 pmol/l (SD=41.9). Overall, 403 participants had impaired fasting glucose and 455 participants had hyperinsulinemia.

QTc, QT and RR interval

The mean duration of the QTc interval was significantly shorter in males (423.2 ms) than in females (432.5 ms) ($p<0.0001$). Moreover, the mean duration of the RR interval was significantly longer in males (933.4 ms) than in females (895.7 ms) ($p<0.0001$).

Overall, there was a significant association between impaired fasting glucose and the QTc interval with an increase of 2.6 ms (95%CI: 0.3 ; 5.0), after adjustment for age, gender and hypertension (table 2). Furthermore, hyperinsulinemia was also associated with QTc prolongation (3.0 ms (0.8 ; 5.3)). However, impaired fasting glucose and

Table 1 Characteristics of study population

	Total (n=2570)	Impaired fasting glucose (n=403)	Hyperinsulinemia (n=455)
Age (years, mean, SD)	71.1 (6.8%)	71.6 (6.9%)	71.6 (6.8)
Gender (female)	1520 (59.1%)	233 (57.8%)	290 (63.7%)
Mean QTc interval (ms) (SD)	428.3 (22.7%)	430.6 (24.0%)	431.4 (23.9%)
Mean QT interval (ms) (SD)	406.8 (29.7%)	403.8 (31.9%)	402.4 (29.3%)
Mean RR interval (ms) (SD)	910.2 (144.7%)	887.8 (153.1%)	877.2 (138.8%)
Body Mass Index kg/ m ² (mean, SD)	26.0 (3.4)	27.3 (3.6)	28.2 (3.6)
Waist-hip ratio (mean, SD)	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)
Hypercholesterolemia (n, %)	220 (8.6%)	38 (9.4%)	40 (8.8%)
Hypertriglyceremia (n, %)	671 (26.1%)	139 (34.5%)	210 (46.2%)
Hypertension (n, %)	776 (30.2%)	172 (42.7%)	188 (41.3%)
Myocardial infarction (n, %)	231 (9.0%)	35 (8.7%)	47 (10.3%)
Heart failure (n, %)	64 (2.5%)	9 (2.2%)	19 (4.2%)
Use of beta-blockers (n, %)	374 (14.6%)	90 (22.3%)	89 (19.6%)
Mean fasting glucose (mmol/l) (SD)	5.5 (0.5%)	6.4 (0.2%)	5.8 (0.5%)
Mean fasting insulin (pmol/l) (SD)	72.7 (41.9%)	97.9 (52.6%)	141.3 (46.5%)

SD=standard deviation

hyperinsulinemia were not associated with an increase of the QT interval, adjusted for age, gender, RR interval and hypertension (0.0 ms (-2.0 ; 2.1)) and -0.1 (-2.0 ; 1.9) respectively).

Impaired fasting glucose and hyperinsulinemia were associated with a significant decrease of the RR interval after adjustment for age, gender and hypertension (-33.7 ms (-48.8 ; -18.6) and -44.4 ms (-58.7 ; -30.0) respectively). After adjusting for hypertension, hypertriglyceremia, hypercholesterolemia, waist-hip ratio and BMI, the results did not change substantially.

Normal fasting glucose status

In participants with normal fasting glucose, hyperinsulinemia was not associated with the QTc or QT interval (table 3). However, in subjects with normal fasting glucose, hyperinsulinemia was significantly associated with a decrease of the RR interval after adjustment for age, gender and hypertension (-54.4 ms (-71.1 ; -37.7)).

QTc, QT, RR interval and sudden cardiac death

The baseline characteristics of all participants are presented in table 4. The mean age of the study population at baseline was 69.4 years, 59.6% was female. During the follow-up period, 209 persons were classified as definite sudden cardiac death and 54 as probable sudden cardiac death.

Table 2 Association of fasting glucose and insulin with the QTc, QT and RR interval in non-diabetic participants

	Number of participants	QTc prolongation in ms (95% CI) ¹	QT prolongation in ms (95% CI) ²	RR prolongation in ms (95% CI) ¹
Fasting glucose	2570			
Normal fasting glucose (≤ 6.0)	2167	Reference	Reference	Reference
Impaired fasting glucose (6.1–6.9)	403	2.6 (0.3 ; 5.0)	0.0 (-2.0 ; 2.1)	-33.7 (-48.8 ; -18.6)
Fasting insulin	2570			
Normal insulin (<100)	2115	Reference	Reference	Reference
Hyperinsulinemia (≥ 100)	455	3.0 (0.8 ; 5.3)	-0.1 (-2.0 ; 1.9)	-44.4 (-58.7 ; -30.0)

CI = Confidence Interval

1 : adjusted for age, gender, hypertension

2 : adjusted for age, gender, RR interval and hypertension

Glucose in mmol/l

Insulin in pmol/l

Participants in the fourth quartile of the QTc/ QT interval had a significant increased risk of sudden cardiac death compared to participants in the first quartile (HR 2.87 (95%CI 2.02 – 4.06); HR 3.05 (1.99 – 4.67) respectively). (table 5) Furthermore, there was a significant inverse association between the fourth quartile of the RR interval compared to the first quartile and the risk of sudden cardiac death (HR 0.49 (0.34 – 0.80)).

Table 3 Association of insulin with the QTc, QT and RR interval in non-diabetic participants with a normal fasting glucose measurement

Normal fasting glucose	Number of participants	QTc prolongation in ms (95% CI) ¹	QT prolongation in ms (95% CI) ²	RR prolongation in ms (95% CI) ¹
Fasting insulin				
Normal insulin (<100)	1855	Reference	Reference	Reference
Hyperinsulinemia (≥ 100)	312	2.5 (-0.2 ; 5.1)	-1.5 (-3.8 ; 0.7)	-54.4 (-71.1 ; -37.7)

CI = Confidence Interval

1 : adjusted for age, gender and hypertension

2 : adjusted for age, gender, RR interval and hypertension

Insulin in pmol/l

Discussion

In this population-based study, we demonstrated that non-diabetic participants with an impaired fasting glucose and hyperinsulinemia had a significantly increased QTc interval and a significantly shorter RR interval than participants with normal fasting glucose and insulin measurements. Fasting glucose and fasting insulin were not associated with the QT interval after adjustment for the RR interval. Furthermore, we demonstrated that an increased QTc/ QT interval and decreased RR interval are associated with a significantly increased risk of sudden cardiac death. Although a QTc interval prolongation of 2-3 ms in one individual usually remains without clinical consequences, an average shift of 2-3 ms in a Gaussian distribution on a population level will inevitably push more individuals into

Table 4 Baseline characteristics of sudden cardiac death study population

Characteristic	n=6020
Mean age (years) (SD)	69.4 (9.2)
Gender (female)	3585 (59.6%)
Diabetes mellitus (n, %)	652 (10.8%)
Hypercholesterolemia (n, %)	2539 (42.2%)
Hypertension (n, %)	2025 (33.6%)
Myocardial infarction (n, %)	715 (11.9%)
Heart failure (n, %)	203 (3.4%)
Mean Body Mass Index (kg/ m ²) (SD)	29.3 (15.1)
Use of QTc prolonging drugs (n, %)	348 (5.8%)
Use of beta-blockers (n, %)	1159 (19.3%)
Use of digoxin (n, %)	390 (6.5%)
Mean QTc interval (ms) (SD)	434.7 (28.5)
Mean QT interval (ms) (SD)	405.8 (34.7)
Mean RR interval (ms) (SD)	881.3 (156.1)

SD Standard Deviation

the upper percentiles of the QTc interval with its increased risk of ventricular arrhythmias and sudden cardiac death.

Our findings suggest that the association of glucose and insulin with the QTc interval is due to the underlying shortening of the RR interval, since QTc is calculated by Bazett's formula using the square root of the RR interval in the denominator. However, we confirmed that both an increased QTc as well as a shortened RR interval are associated with an increased risk of sudden cardiac death, as has been demonstrated before.[7, 36-38] Our findings cannot be explained by the metabolic syndrome, since additional adjustment for the determinants of the metabolic syndrome did not change the results substantially. We demonstrated that the association of hyperinsulinemia with the RR interval was even more pronounced in participants with a normal fasting glucose. This suggests that in subjects with nonsymptomatic insulin resistance syndrome, increased sympathetic nervous system activity and autonomic imbalance occur.

Impaired fasting glucose and hyperinsulinemia have been associated with the QTc interval in non-diabetic persons [8, 9]. However, a relation with the QT interval has only been demonstrated in a small study of 35 non-diabetic offspring of type 2 diabetic patients.[10] That study showed that during acute hyperinsulinemia, the QT interval remained unchanged, whereas the QTc interval increased. Impaired fasting glucose and hyperinsulinemia have been demonstrated to be associated with the RR interval in non-diabetic patients.[11, 12] Several mechanisms by which impaired fasting glucose and hyperinsulinemia may affect the QTc and RR interval have been reported, amongst others increased sympathetic activity, autonomic imbalance, impairment of the hERG channel,

Table 5 Association of QTc, QT and RR interval with sudden cardiac death

	Quartiles	SCD (n=263)	HR (95% CI) ¹	HR (95% CI) ²
QTc interval	1	51	Reference	Reference
	2	59	1.39 (0.95 - 2.03)	1.37 (0.94 - 2.00)
	3	57	1.53 (1.05 - 2.25)	1.48 (1.01 - 2.16)
	4	96	3.42 (2.42 - 4.84)	2.87 (2.02 - 4.06)
QT adjusted for RR interval	1	72	Reference	Reference
	2	56	1.21 (0.83 - 1.77)	1.14 (0.78 - 1.66)
	3	61	2.04 (1.36 - 3.07)	1.82 (1.21 - 2.75)
	4	74	3.67 (2.40 - 5.60)	3.05 (1.99 - 4.67)
RR interval	1	90	Reference	Reference
	2	62	0.58 (0.42 - 0.80)	0.63 (0.46 - 0.88)
	3	63	0.61 (0.44 - 0.84)	0.68 (0.49 - 0.94)
	4	48	0.46 (0.32 - 0.66)	0.49 (0.34 - 0.80)

SCD = sudden cardiac death; HR = Hazard Ratio

¹ Adjusted for gender and time-depending age.² QTc interval additionally adjusted for myocardial infarction, and use of QTc prolonging drugs and digoxin; QT interval additionally adjusted for time-depending myocardial infarction and use of QTc prolonging drugs; RR interval additionally adjusted for use of beta-blockers and digoxin.

Quartiles: QTc (1) ≤416 ms; (2) 417-431 ms; (3) 432-449; (4) ≥450 ms; QT (1) ≤382 ms; (2) 383-403 ms; (3) 404-425 ms; (4) ≥426 ms; RR (1) ≤770 ms; (2) 771-869 ms; (3) 870-979 ms; (4) ≥940 ms.

raised production of free radicals, disturbed myocardial membrane function or a reduction of Na⁺/K⁺-ATPase activity.[39-41] Although the exact underlying mechanism may be unknown, increased sympathetic activity caused by hyperinsulinemia may decrease the RR interval.[42] Furthermore, the insulin resistance syndrome can be considered as a pre-stage of diabetes mellitus, which suggests the early presence of autonomic nervous dysfunction.[43]

Our study has several strengths. An advantage of the Rotterdam Study is its population-based character. Although we limited our study population, selection bias is unlikely because the characteristics of the study population were comparable to the baseline characteristics of the whole population of the Rotterdam Study. Furthermore, information bias is unlikely because data were gathered prospectively and because of the use of digital ECG recordings. The digital ECGs were all automatically interpreted using the MEANS system which reduced intra- and interobserver variability in the assessment of the QTc, QT and RR intervals. Confounding was minimized by adjusting for all known risk factors. However, our study has also some limitations. Because of the cross-sectional design we cannot exclude that RR interval shortening was already present in some patients before the increase of glucose and insulin. Therefore, the results from this study should be confirmed with longitudinal data from other large cohorts. Finally, our study population consisted of participants aged 55 years and older. Whether our findings can be generalized to other age groups requires further study.

In conclusion, we demonstrated in this population-based study that impaired fasting glucose and hyperinsulinemia are significantly associated with an increased QTc-interval but not with an increase of the QT interval. This is explained by a shortening of the RR interval, probably due to an increased sympathetic activity. We demonstrated that both an increased QTc/ QT as well as a shortened RR interval are associated with an increased risk of sudden cardiac death.

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Chapter 6

General discussion

Sudden cardiac death is a major clinical and public health problem. Therefore, it is important to identify persons at risk for sudden cardiac death and to be aware of all factors associated with an increased risk. Although many causes of QTc prolongation and thereby sudden cardiac death have been identified, not all risk factors have been determined. The general objective of this thesis was to study the effect of certain drugs and endocrine factors on QTc prolongation and sudden cardiac death. In this chapter, the main findings of this research and the main methodological considerations of these studies are presented. Furthermore, potential clinical implications and suggestions for future research are discussed.

Main findings

Drugs and repolarization disturbances

Nowadays, an increasing number of drugs, especially non-cardiac drugs, are known to delay cardiac repolarization and to induce Torsade de Pointes.[1] Therefore, it is recommended that drugs are examined for their effect on ventricular repolarization before marketing. One of the non-clinical methods is to evaluate the inhibition of I_{kr} encoded by the human-ether a go-go related (*hERG*) gene, which is the most common mechanism responsible for drug-induced prolongation of the QT interval in humans.[2] In addition, the 'thorough QT/ QTc study' is conducted, which is a premarketing clinical trial, specifically requested by registration authorities to evaluate the effect on cardiac repolarization. Additional evaluation in subsequent clinical studies can be performed in later stages of development. In addition to non-clinical and clinical data, adverse event data can be another source of information on proarrhythmic potential, both during clinical studies as well as post-marketing. The gathered information can eventually lead to delayed or denied marketing authorization, changes in the labelling of the summary of product characteristics, restriction in use or withdrawal from the market.[3] Pre-registration assessment of the potential for QTc prolongation has several limitations, such as the limited size of the study population, selection of participants, who are relative healthy and often do not have comorbidities or use concomitant drugs, and the limited duration of trials.[4, 5] Since not all safety-relevant information is available before marketing, post-marketing information is important for the safe use of drugs.

In recent years, several lists have been published of non-cardiac drugs associated with QTc prolongation and cardiac arrhythmias.[6-9] An internet-based registry includes a list of drugs based on information from the International Registry of Drug-Induced Arrhythmias, literature, change in labels of (new) drugs and the Food and Drug Administration's (FDA) database for adverse drug event reports.[9] In addition, De Ponti et al. have published a list of non-anti-arrhythmic drugs with pro-arrhythmogenic effects,

based on a structured literature search including published (non-) clinical evidence and official warnings in the labelling.[6, 7]

It is often unknown whether non-listed drugs of similar therapeutic or chemical classes as listed ones do not also provoke QTc prolongation or whether they are not listed due to insufficient data. For instance, only some psychotropic drugs have been associated with QTc interval prolongation whereas others were not, maybe because it was never investigated. Therefore, we studied whether listed as well as non-listed psychotropic drugs are indeed associated with QTc prolongation and evaluated whether this is a class effect or an individual drug effect.

In the Rotterdam Study, we found that use of some antipsychotics (especially lithium, olanzapine and thioridazine) and almost all tricyclic antidepressants are associated with a significant increase in QTc interval (**chapter 2.1**). The results of our study are in agreement with previously reported information on these drugs.[7, 10-12] Most of these drugs were listed by either de Ponti or the internet-based registry. However, maprotiline, which was clearly associated with an increase in QTc interval, is not listed on the internet-based registry. On the other hand, for many of the listed drugs we could not confirm a significant increase in the QTc interval, which may have been due to limited power due to a low number of exposed persons per individual drug. In general, however, the direction of change was as expected. The only class that was associated with an overall increase of the QTc interval concerned tricyclic antidepressant drugs. In this class, most of the individual drugs that were prescribed (amitriptyline, imipramine and maprotiline) were associated with a significant increase of the QTc interval. These drugs not only block the human Ether-à-go-go Related Gene (*hERG*)-encoded potassium channels but also the cardiac sodium channels.[8, 13, 14] Most drugs that prolong the QTc interval act by blocking the rapid component of the delayed rectifier potassium channel (I_{Kr}) encoded by *hERG*; only few drugs are known to prolong the QTc interval by modifying I_{Na} . [15-17] Given the structural similarity between Na^+ channels and K^+ channels, it is not surprising that many Na^+ channel-blocking drugs also bind to K^+ channels to prevent K^+ efflux.[16] In conclusion, most of the drugs associated with QTc prolongation were listed by either de Ponti or the internet-based registry. However, maprotiline, which was clearly associated with an increase in QTc interval, is not listed on the internet-based registry. On the other hand, for many of the listed drugs we could not confirm a significant increase in the QTc interval, which may have been due to limited power by the low number of exposed persons per individual drug.

Drug-genetic interaction in QTc prolongation

Several calcium channel blockers are included in the internet based registry (isradipine) or the list of De Ponti et al. (diltiazem, isradipine and verapamil).[7, 9] In addition, it has been reported recently that common *NOS1AP* variants are associated with QTc interval

prolongation.[18, 19] The *NOS1AP* gene encodes the nitric oxide synthase 1 (NOS1) activating protein.[18] NOS1 has a role in cardiac contractility, and it is hypothesized that nitric oxide signalling may be involved in cardiac repolarization.[20, 21] Since both NOS1 and calcium channel blockers suppress L-type calcium channels, these QTc prolonging genetic variants and drugs might interact,[22-24] resulting in even further increased QTc prolongation. In the Rotterdam Study, we found that current use of verapamil was associated with a significant QTc interval prolongation. In addition, the minor alleles of both *NOS1AP* SNPs significantly potentiated the QTc interval prolonging effect of verapamil (**chapter 2.2**). Furthermore, regression coefficients indicated that a similar effect might exist for isradipine, although the SNPs did not modify the effect on QTc interval on a multiplicative scale in association with isradipine. Our findings might be explained by the fact that verapamil causes a high-affinity blockage of the *hERG*-current, whereas amlodipine, diltiazem, isradipine, and nifedipine block the *hERG* current weakly, or not at all.[8, 25-27] The concentration of verapamil which is required to block the *hERG*-encoded potassium channels overlaps with the concentration that blocks the calcium channel, whereas for the other calcium channel blockers this concentration is much lower than the concentration needed to block the *hERG*-encoded potassium channels.[27] Although the mechanism by which *NOS1AP* influences the QT interval and interacts with verapamil is not known, it may involve calcium and potassium currents in the cardiomyocyte. *NOS1AP* has been found to activate NOS1.[28] NOS1 stimulates Ca^{2+} release in sarcoplasmic reticulum, which leads to increased intracellular calcium.[29] The elevation of intracellular Ca^{2+} suppresses the Ca^{2+} entry pathway, the L-type calcium channels.[30] Furthermore, elevations in intracellular calcium selectively enhances the delayed rectifier current, which leads to increased outflow of potassium.[30] Verapamil suppresses L-type calcium channels, which are also suppressed by NOS1.[27] Moreover, verapamil blocks the *hERG*-encoded potassium channels.[8, 25, 26] Thus, the physiological feedback system, i.e. the Ca^{2+} sensitivity of the potassium current, is disturbed due to the high-affinity *hERG*-current blockade caused by verapamil. This suggests a model in which increased intracellular calcium and slow outflow of potassium in participants with minor alleles of *NOS1AP* using verapamil, leads to additional QTc prolongation.

Drugs and ventricular arrhythmias or sudden cardiac death

Virtually all QTc prolonging drugs act by blocking the rapid component of the delayed rectifier potassium channel (I_{kr}) encoded by *hERG*. [15] However, some QTc prolonging drugs that do not seem to cause sudden cardiac death, such as amiodarone [31], also block this current, whereas on the other hand some drugs that prolong the QTc interval with only few milliseconds were implicated in the occurrence of cardiac arrhythmias (e.g. terfenadine).[8, 32, 33] Terfenadine is a potent I_{kr} blocker but usually it does not

prolong the QTc interval because it is readily transformed into its metabolite which is without an effect on QT interval. Terfenadine has been withdrawn from the market in several countries because of the possible occurrence of interactions with diseases and drugs that inhibit metabolism by cytochrome P450 CYP3A4, and due to the availability of alternatives.[1, 15]

In 2003, it was shown that drugs with a small margin (i.e. drugs that block I_{kr} in concentrations close to the therapeutic plasma concentration) had a high risk of serious cardiac arrhythmia while drugs with a high margin (i.e. those of which only high-therapeutic plasma concentrations block potassium channels) had a lower risk. [8] This margin between *hERG*-encoded I_{kr} channels binding capacity and free plasma concentration might be a useful tool in the prediction of the risk of Torsade de Pointes, and might be more important as an indicator of risk for cardiac arrhythmias.

Although most QTc prolonging drugs act by blocking I_{kr} , few drugs are known to prolong the QTc interval by modifying I_{Na} . Sodium channel-blocking drugs can cause slowed intraventricular conduction, with the development of a re-entrant circuit, resulting in ventricular tachycardia or ventricular fibrillation. Examples of drugs with sodium channel-blocking activity are certain antihistamines, beta-blockers, tricyclic antidepressants and phenothiazines.[16, 17] In chapter 3, we studied the effect of various drug classes (I_{kr} and I_{Na} blocking) on ventricular arrhythmia and sudden cardiac death. We studied the I_{kr} blocking (non-cardiovascular *hERG*-encoded potassium channel inhibiting) drugs in general and domperidone in specific, and we studied the I_{Na} blocking anticonvulsants.

hERG-encoded potassium channel inhibiting drugs have been associated with an increased number of reports of serious ventricular arrhythmias and sudden death in the adverse drug reactions database of the World Health Organization.[34] Although important, this resource has limitations because reports are voluntarily submitted to pharmacovigilance centres and often do not contain a full medical history, whereas data on other risk factors may be incomplete or lacking. Moreover, since reporting of adverse drug reactions is voluntary, not all adverse drug reactions are reported and not all adverse reactions are recognized by the doctor. In this thesis, we demonstrated on the basis of IPCI data that current use of non-cardiovascular *hERG*-encoded potassium channel inhibiting drugs in the general population was associated with an increased risk of sudden cardiac death (**chapter 3.1**). Furthermore, we were able to confirm that drugs with a high *hERG*-encoded potassium channels inhibiting capacity had a higher risk of sudden cardiac death than patients using drugs with a low potassium channels inhibiting capacity.

A specific study was conducted on domperidone since this drug was available as over the counter drug, is widely used and was associated with a significant almost 4-fold

increase in risk of sudden cardiac death in a previous study that did, however, not focus on the drug and did not model the effect of the drug specifically. [35] The study on domperidone was stratified by insurance type to avoid differential misclassification of exposure and since there was heterogeneity between the effects in publicly and privately insured patients. In the IPCI database, we found that current use of domperidone and in particular high doses, were associated with a substantially increased risk of sudden cardiac death (**chapter 3.2**). This is in agreement with the relatively high $ETCP_{unbound}/IC_{50}$ ratio (0.119).[8] We were not able to identify cases of ventricular arrhythmia with current domperidone exposure, due to both the limited number of confirmed cases of ventricular arrhythmia and the low exposure prevalence to prescribed domperidone. Since prodromal symptoms of sudden cardiac death, such as nausea and vomiting, can be mistakenly diagnosed as symptoms due to gastric pathology, we considered GP visits to be a confounder in this study.

Anticonvulsants (ACs) may influence cardiac conduction directly by blocking the cardiac sodium current. In addition, ACs act centrally in the brain and thereby influence the autonomic control of the heart, while autonomic dysfunction is associated with lethal cardiac arrhythmias.[36] In collaboration with experts from the Academic Medical Center, we found in IPCI that current use of ACs in general (especially carbamazepine) is associated with a more than 2-fold increased risk of sudden cardiac death (**chapter 3.3**). Current use of sodium-channel blocking ACs was associated with an almost 3-fold increased risk of sudden death. Conversely, ACs without sodium channel blocking properties exhibited a lower and non-significant association with sudden cardiac death.

Experimental studies showed that phenytoin, fenobarbital and lamotrigine have the ability to block *hERG*-encoded potassium channels.[37, 38] However, the relatively low $ETCP_{unbound}/IC_{50}$ ratio (0.0436) of phenytoin, for instance, suggests that only high-therapeutic plasma concentrations block *hERG*-encoded potassium channels.[8] After exclusion of these drugs, the use of sodium channel blocking ACs was still strongly associated with sudden cardiac death. A substantial proportion of deaths in patients with epilepsy is sudden and unexpected.[39] However, besides patients using ACs for epilepsy, we also included patients using ACs for other indications, e.g., neuropathic pain. Since our cases and controls were derived from the general population, most of the included epilepsy patient had stable epilepsy and used a single AC, thereby reducing confounding by severity of epilepsy and polytherapy of ACs. The risk of sudden cardiac death in phenytoin users was higher in the study described in chapter 3.3 than in 3.1, since only definite cases of sudden cardiac death were presented in chapter 3.3 in contrast with the study described in chapter 3.1 presenting both definite and probable cases due to the limited number of users for some drugs.

Endocrine factors and repolarization disturbances and fatal ventricular arrhythmias

Gender-related differences in human cardiac repolarization are well known and illustrated by a longer QTc interval in women.[40, 41] In literature, it is suggested that testosterone might be an important regulator of ventricular repolarization, and this in turn might explain the gender-related differences in ventricular repolarization. In both the Rotterdam Study as well as in SHIP, we found that serum testosterone levels were associated with a shorter QTc interval and prolongation of the RR interval in men, whereas we did not find an association between serum testosterone levels and the uncorrected QT interval (**chapter 5.1**). Since the QT interval was not significantly associated with serum testosterone, our findings suggest that the difference in the QTc interval in men is mainly due to the underlying association with the RR interval. Since QTc is calculated by Bazett's formula, an increase in RR interval will lead to a shorter QTc interval. Our findings are in agreement with earlier findings.[42] According to literature, the gender differences in the QTc interval decrease with age which might be due to the fact that bio-available testosterone decreases with age.[43] In the Rotterdam Study, we detected a significant difference in the QTc interval between males and females until the age of 80 years (figure 1).

In the Rotterdam Study, we found an association between free T4 levels and prolongation of the QTc interval (**chapter 4.1**). We did not find an association between TSH and the QTc interval. A potential explanation for this finding is that an association between TSH and QTc would be indirect, while free T4 is more directly related to thyroid hormone activity on the heart. TSH binds to the TSH-receptor on thyroid cells, resulting in stimulation of thyroid hormone production but has probably not an effect on QTc of its own.[44] In this study, we did not present analyses with the QT interval adjusted for the RR interval as outcome, whereas we did show these analyses in chapters 5.1 and 5.2, which were conducted later. These additional analyses showed that both prolongation of the QTc-, as well as the QT interval adjusted for the RR interval were associated with higher free T4 levels.

Thyroid hormone may affect ventricular repolarization but the literature differs with respect to the direction of this change. Hypothyroidism has been associated with prolongation of the QTc interval,[45, 46] whereas hyperthyroidism has been found to be associated with decreased as well as increased repolarization times.[47-56] A possible explanation for the association of hyperthyroidism with QTc prolongation can be provided by an increase in the activity of cardiac Na⁺/K⁺ ATPase due to thyroid hormone excess, leading to increased intracellular K⁺ with subsequent increased repolarisation duration.[54, 57] The effect appeared to be strongest in men. In hyperthyroid men, impaired sexual function, gynaecomastia, asthenospermia and low testicular volume

are attributed to lowered bio-available testosterone.[58, 59] In chapter 5.1 we demonstrated that men with a higher level of testosterone have a shorter QTc interval. The decreased level of bio-available testosterone in hyperthyroid men results in less shortening of the QTc interval.

Since free T4 levels were associated with an increased QTc interval duration, we hypothesized that hyperthyroidism could be associated with an increased risk of sudden cardiac death. Since thyroid hormone levels were only available in a minority of patients, we could not verify this. Therefore, we investigated whether use of antithyroid drugs (as a direct cause or as an indicator of poorly controlled hyperthyroidism) is associated with an increased risk of sudden cardiac death. In both the Rotterdam Study as well as in the IPCI database, we found that current use of antithyroid drugs was associated with an increased risk of sudden cardiac death (**chapter 4.2**). There are two potential explanations for our findings. First, sudden cardiac death might be an adverse reaction to the antithyroid drugs themselves. However, this seems unlikely, since different types of antithyroid drugs were equally involved; also, this theory would be devoid of a biologically plausible mechanism. Second, patients may still have had inadequately treated hyperthyroidism early during treatment, consequently with increased levels of free T4. Validation of the exposed cases of sudden cardiac death demonstrated that the exposed cases with known TSH measurements had low TSH levels a few days before the index date. This confirmed the second explanation, i.e. that these patients were still

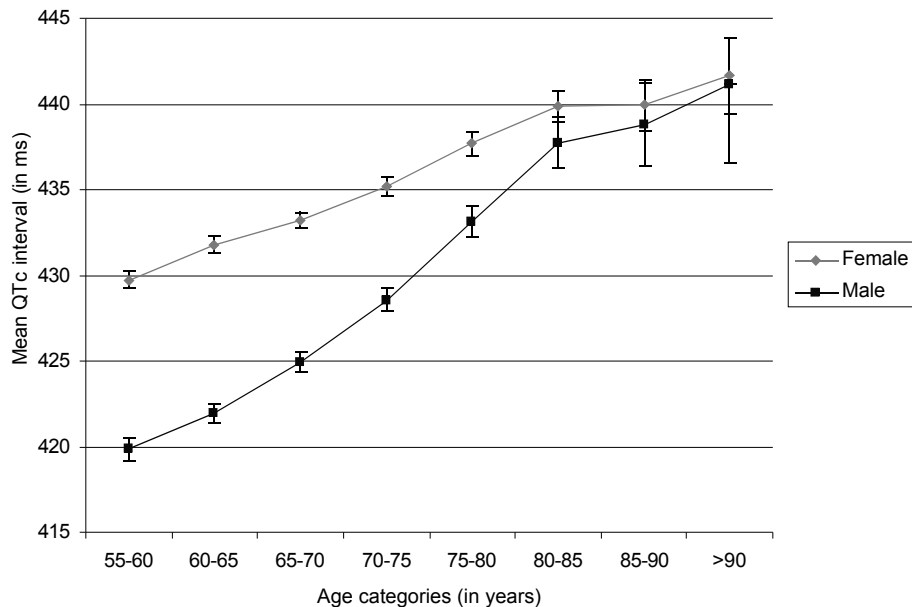


Figure 1 Gender difference in the QTc interval in the Rotterdam Study

hyperthyroid at the moment of sudden cardiac death. Our findings suggest that these deaths were caused by undertreatment of hyperthyroidism.

Hyperthyroidism has been associated with ventricular arrhythmias and sudden cardiac death in several case reports.[53, 60-64] In the Rotterdam Study, the association between antithyroid drugs and sudden cardiac death tended to be higher in males, which supports the association of free T4 and QTc prolongation in males which we described earlier. Because of the small number of users of antithyroid drugs in our analysis in the Rotterdam Study, we tested the validity of our findings by replication in the IPCI database. The fact that the association was observed in two independent study populations and that the point estimates were quite similar, confirms the validity of the association between current use of antithyroid drugs and sudden cardiac death.

Diabetes mellitus is associated with QTc prolongation.[65, 66] Since hyperglycemia is an independent risk factor for cardiovascular disease, we studied whether non-diabetic participants with an impaired fasting glucose measurement and hyperinsulinemia have an increased risk of QTc prolongation and RR shortening and thereby an increased risk of sudden cardiac death. In the Rotterdam Study, we demonstrated that non-diabetic participants with an impaired fasting glucose and hyperinsulinemia had a significantly increased QTc interval and shorter RR interval than participants with normal fasting glucose and insulin measurements (**chapter 5.2**). Fasting glucose and fasting insulin were not associated with the uncorrected QT interval. These findings suggest that the association of glucose and insulin with the QTc interval is due to the shortening of the RR interval. However, in the same population both the QTc as well as the RR interval were associated with an increased risk of sudden cardiac death, which is in line with earlier findings.[67-70] Although the exact underlying mechanism is unknown, it is likely that increased sympathetic activity caused by hyperinsulinemia may increase heart rate and therefore decrease the RR interval.[71]

Methodological considerations

In the following sections we will discuss some methodological considerations that apply to the internal and external validity of the studies described in this thesis.

Setting

All studies described in this thesis were performed using population-based data sources. Most studies were performed using data from the Rotterdam Study, a large population-based cohort study of elderly.[72, 73] The strengths of this study are its general population based setting, the large number of participants, the consistent assessment

of measurements for every subject, long-duration of follow-up, and the availability of extensive information about various clinical characteristics of all participants gathered at every follow-up round. In addition, through linkage with GP and municipal mortality records, the total cohort is continuously monitored for major morbidity and mortality. Furthermore, virtually all participants fill their prescriptions in automated pharmacies linked to one computer network. From 1 January 1991 onwards, data on all dispensed drugs, including the prescribed daily dose, is available in computerized format on a day-to-day basis. All information is gathered irrespective of the outcome under study. For the studies on thyroid hormone and testosterone levels, we used a subcohort of the Rotterdam Study. However, the samples were measured randomly and the baseline characteristics of these subcohorts were comparable to the baseline characteristics of the complete study cohort.

Most other studies were performed using data from the Integrated Primary Care Information (IPCI) project, a longitudinal observational database, containing data from computer-based medical patient records of a large group of general practitioners (GPs) in the Netherlands, for a population based case-control study.[74, 75] The database contains the complete medical records on more than 1,000,000 patients. The electronic records contain coded and anonymous data on patient demographics, symptoms (in free text), diagnoses (using the International Classification for Primary Care (ICPC) [76] and free text) from GPs and specialists, referrals, laboratory findings, hospitalisations, and drug prescriptions, including their indications and dosage regimens. The strength of the database is that it contains a representative sample of the Dutch general population. In the Dutch health care system, the GP has a pivotal role by acting as a gatekeeper for all medical care. Therefore, the medical records of each individual patient can be assumed to contain all relevant medical information.

One study was partly conducted with data from the Study of Health in Pomerania (SHIP), which is a population-based study in West Pomerania, a region in the northeast of Germany.[77] The strengths of this study are its general population based setting and the availability of extensive information about various clinical characteristics of all participants gathered at every follow-up round.

Design

Most studies described in this thesis were designed as prospective population-based cohort studies, some studies as population-based case-control studies. Each of the individual studies has its specific strengths and limitations. The Rotterdam Study contains a wide range of clinical characteristics and individual drug exposure for a large number of participants. However, the exposure of some individual drugs may be limited. In the studies assessing drug use at the time of an ECG, multiple follow-up ECGs per participant were available (**chapter 2**). This allowed us to assess the effect of a drug within

a person over time, which facilitates more detailed analyses. In the studies assessing the influence of endocrine factors on the QTc interval, only a single measurement was available (**chapters 4.1 and 5**). Due to this cross-sectional design, we cannot exclude that QTc or RR interval variation was already present in some participants before the change in thyroid hormone, testosterone, glucose or insulin levels. In the study assessing the effect of antithyroid drugs on sudden cardiac death, we took changes in exposure status into account by using a Cox-regression model with individual drug use as time-dependent variables, since the pharmacy data provided us information about drug use at each point in time during follow-up (**chapter 4.2**). The IPCI database contains all relevant medical information and prescription information from the GP for a large number of patients. In the studies assessing the relationship between drug use and ventricular arrhythmias or sudden cardiac death, we were able to assess the drug use at the day of sudden cardiac death, since the prescription data provided us information about drug use at each point in time (**chapters 3 and 4.2**).

Bias and confounding

In observational studies, selection bias, information bias and/ or confounding may influence the validity. The population-based design of the studies probably limited the chances of selection bias. In the Rotterdam Study and SHIP, there is a chance that non-participants to the study or to (part of) the examinations at the research center had more - or more serious - co-morbidity. Since we also have information from GPs, pharmacies and specialists from the participants that are not able to visit the research center, the chance of selection bias is limited. In the studies performed within the IPCI database, selection bias is unlikely, since both cases and controls were originating from the same IPCI population, and the controls were randomly selected from the source population.

Since all information in the three study bases was gathered prospectively and irrespective of disease status, without knowledge of drug-use, genotype or endocrine measurements, the chance of information bias is unlikely. However, misclassification of exposure may however have occurred for various reasons. In the studies investigating drug use as exposure, the legend duration for a calculated prescription may not reflect actual use. Furthermore, in the Rotterdam Study we used pharmacy data and in none of the sources we had information whether the drugs were actually taken. However, this will probably lead to non-differential misclassification. Over the counter drug use may cause misclassification since this will not be registered in the pharmacy or GP databases. This was relevant for the study on domperidone, where we observed strong heterogeneity in the effect of domperidone in publicly and privately insured persons, which generally have different patterns of OTC drug use. To limit differential misclassification we stratified by insurance. In the studies investigating endocrine factors as exposure,

misclassification may have occurred, since we used a single measurement. However, if exposure misclassification occurred in one of the studies it is probably non-differential.

We cannot exclude that some misclassification of outcome occurred. However, this is unlikely in the studies with the QTc interval as outcome, since the use of digital ECG recordings, all measured using the MEANS system (in both the Rotterdam Study as well as in SHIP), likely reduced intra- and interobserver variability in the assessment of the QTc interval. In the studies with sudden cardiac death as outcome, we may have missed some deaths, although this will be minimal, since death is consistently registered by GPs. Second, since an autopsy was not available in all acute deaths, we cannot be certain that all acute deaths have been of cardiac origin. We determined sudden cardiac death, however, on the basis of the full medical records and most circumstances surrounding death were available. Recently, an evaluation comparing different methods to determine the incidence of sudden cardiac death suggested that this method provides a very reliable way of determining cases of sudden cardiac death.[78]

Confounding is an important issue in epidemiological studies. Although we adjusted for all known confounders, residual confounding may exist. In the study on domperidone and the risk of ventricular arrhythmias, we considered GP visits to be a confounder, since prodromal symptoms of sudden cardiac death, such as nausea and vomiting, can be mistakenly diagnosed as symptoms due to gastric pathology. Many potential confounders are well recorded in the three studies, such as use of concomitant drugs, comorbidities, and lifestyle factors. However, some factors potentially associated with sudden cardiac death, such as smoking, alcohol abuse or body weight, are not complete in the IPCI database.

Confounding by indication is an important factor to consider in pharmaco-epidemiological studies.[79] As shown in the study on use of antithyroid drugs and the risk of sudden cardiac death, it can be difficult to determine whether the drugs or the underlying indication for use are associated with an increased risk of sudden cardiac death.

Clinical implications

In the studies described in this thesis, we were able to identify several risk factors for QTc prolongation and/ or sudden cardiac death. Prescribers should be aware which drugs are associated with an increased risk of QTc prolongation and sudden cardiac death, in order to be able to make a proper benefit-risk assessment. Preferably, a physician should not prescribe two or more QTc prolonging drugs concomitantly. Nonetheless, when this is required, it is advised to record an ECG to examine the length of the QTc interval. However, in a recent study it was shown that doctors who overrule high-level drug-drug interaction alerts on QTc prolongation rarely record an ECG as a safety measure. If ECGs

were recorded before and after QTc overrides, clinically relevant QTc prolongation was found in one-third of the cases with an average change in QTc interval of 31 ms.[80] In an ideal situation, QTc prolonging drugs should not be prescribed to patients with an increased risk of sudden cardiac death, unless for life-threatening indications.

Genotyping variants in the *NOS1AP* gene might reduce the risk of QTc prolongation due to use of verapamil and potentially other QTc prolonging drugs. However, a disadvantage would be that genotype information may not only inform physicians and patients about the risk of adverse events, but also about the risk of future non-drug-related diseases which are associated with such variants. This may raise ethical issues.

Also, from a regulator's point of view, it is important to know which drugs have the potential to prolong the QTc interval. For example, we confirmed that thioridazine was associated with a significant increase in the QTc interval. This drug has been withdrawn in 2005 due to QTc prolongation, cardiac arrhythmias and sudden death.[12] The over-the-counter availability of domperidone has been the subject of discussion due to the increased risk of sudden cardiac death. In line with regulatory recommendations, most new drugs are tested for their ability to block *hERG*-encoded potassium channels.[81] We have shown that the margin between binding capacity and free plasma concentration of potentially *hERG*-encoded potassium channels blocking drugs might be an important indicator of the risk of sudden cardiac death. Regulators could use this margin to decide whether a drug is safe enough to be registered or whether QTc prolongation or sudden cardiac death should be labelled in the safety section of the product information.

Furthermore, physicians should be aware of the fact that certain endocrine factors are associated with an increased risk of QTc prolongation. First, our findings suggest that in patients with increased thyroid hormone levels euthyroid values should be reached as soon as possible to decrease the risk of sudden cardiac death. Second, patients with several risk factors for sudden cardiac death, including increased thyroid hormone levels or an impaired fasting serum glucose, can be identified to be able to take preventive measures, such as avoiding use of QTc prolonging drugs if possible.

Future research

A number of studies described in this thesis show results based on a limited number of exposed individuals. In two different studies, we presented data from two independent populations to confirm our findings. However, there is still a need for replication in other populations for the remaining study results.

To gain better insight into the risk of individual drugs in addition to class effects, data from several populations should be pooled, which will increase the number of exposed individuals. In Europe, there are initiatives to combine population databases for the early

detection of adverse events.[82] This will increase the power to detect an adverse event during the use of an individual drug, including the effect of duration of use and dosage. Also, with a larger population the role of different drugs can be studied within subpopulations with risk-increasing co-morbidity such as diabetes mellitus, thyroid disorders, and other cardiovascular and endocrinological diseases. Furthermore, it is hypothesized that adverse events will be detected earlier than in spontaneous reporting systems, which depend on voluntary reporting by physicians, pharmacists and patients.

Pharmacogenetic studies are currently conducted to identify genetic high risk groups for adverse events. However, most studies are conducted in populations with limited exposure of genetic variants in combination with use of specific drugs. Nevertheless, the combination of a rare genetic trait and a rarely used drug may remain difficult to investigate. Collaborations of several studies in Europe and the United States of America, e.g. the CHARGE Consortium, have the intention to pool data to increase the power to identify genetic high risk groups for adverse events.[83]

Finally, since thyroid hormone levels were only available in a subcohort of the Rotterdam Study, we could not directly study the association between thyroid hormone and sudden cardiac death. Future research studying the direct association and the underlying mechanism will be necessary.

Main conclusions

In the past decade, one of the most frequent causes of withdrawal or restriction of marketed drugs was QTc interval prolongation. Nevertheless, in a clinical setting it was recently shown that automatically generated warnings about QTc prolonging drugs interaction rarely result in the preferred approach to subsequently record an ECG.[80] Identification of QTc prolonging drugs in order to restrict the use or to withdraw a drug from the market when it causes cardiac arrhythmia, may therefore improve the safe use of drugs.

The studies on drugs and repolarization disturbances confirmed the important role of antidepressants and antipsychotics as potential contributors to QTc-prolongation. Especially tricyclic antidepressant drugs appear to prolong the QTc interval, and this seems to be a class effect. In addition, we demonstrated that the minor alleles of both *NOS1AP* SNPs significantly potentiate the QTc prolonging effect of verapamil.

The studies on drugs and ventricular arrhythmia showed that current use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs in the general population (both over-the-counter as well as prescription only drugs) was associated with an increased risk of sudden cardiac death. We demonstrated that *hERG*-encoded potassium

channels inhibiting capacity tended to be associated with an increased risk of sudden cardiac death. Furthermore, we demonstrated that use of anticonvulsants with sodium channel-blocking activity is associated with an increased risk of sudden cardiac death.

The studies on thyroid disturbances and repolarization disturbances and fatal ventricular arrhythmias demonstrated that high levels of free T4 are associated with substantial QTc prolongation in men. Subsequently, we showed in two independent populations that use of antithyroid drugs was associated with a threefold increased risk of sudden cardiac death. Although this might be directly caused by antithyroid drug use, it might be more readily explained by underlying poorly controlled hyperthyroidism, since treated patients who developed sudden cardiac death still had low TSH levels shortly before death.

The studies on other endocrine factors and repolarization disturbances showed that serum testosterone levels were associated with both the QTc as well as the RR interval. However, we could not demonstrate an association with the QT interval. Lower QTc intervals in men with higher serum testosterone levels were probably due to the association of serum testosterone with prolongation of the RR interval. Furthermore, we demonstrated that impaired fasting glucose and hyperinsulinemia are associated with a significantly increased QTc interval and shortening of the RR interval. We demonstrated in the same population that both the QTc/QT as well as the RR interval are associated with an increased risk of sudden cardiac death.

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Chapter 7.1

Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation

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Abstract

Background

A recent genome-wide association study identified a haplotype block on chromosome 4q25 associated with atrial fibrillation (AF). We sought to replicate this association in four independent cohorts.

Methods

The Framingham Heart Study and Rotterdam Study are community-based longitudinal studies. The Vanderbilt AF Registry and German AF Network (AFNet) are case-control studies.

Results

Participants with AF ($n=3508$) were more likely to be male and were older than referent participants ($n=12\,173$). Single nucleotide polymorphism (SNP) rs2200733 was associated with AF in all four cohorts, with odds ratios (ORs) ranging from 1.37 in Rotterdam [95% confidence interval (CI) 1.18–1.59; $P=3.1 \times 10^{-5}$] to 2.52 in AFNet (95% CI 2.22–2.84; $P=1.8 \times 10^{-49}$). There also was a significant association between AF and rs10033464 in Framingham (OR 1.34 (1.03–1.75); $P=0.031$) and AFNet (OR 1.30 (1.13–1.51); $P=0.0002$), but not Vanderbilt (OR 1.16 (0.86–1.56); $P=0.33$). A meta-analysis of the current and prior AF studies revealed an OR of 1.90 (1.60–2.26; $P=3.3 \times 10^{-13}$) for rs2200733 and of 1.36 (1.26–1.47; $P=6.7 \times 10^{-15}$) for rs10033464.

Conclusions

The non-coding SNPs rs2200733 and rs10033464 are strongly associated with AF in four cohorts of European descent. These results confirm the significant relations between AF and intergenic variants on chromosome 4.

Introduction

Atrial fibrillation (AF) is the most commonly sustained arrhythmia affecting over two million Americans, a number that is expected to increase to between 6 and 12 millions by the year 2050.[1, 2] AF is a major source of morbidity and mortality. It is associated with a five-fold increased risk of stroke [3], a doubling in risk of dementia [4], and an increased risk of heart failure (HF).[5, 6]

Multiple risk factors for AF exist, including hypertension (HTN), valvular heart disease [7], HF, and a family history of the arrhythmia.[8-10] In the past 5 years, data have emerged to support a genetic contribution to AF. Several genetic loci for AF have been identified [11-13], although the genes responsible for AF at these loci remain unknown. Mutations in the cardiac sodium channel [14, 15], potassium channel complexes [16-19], and gap junction proteins [20] have been reported to cause AF; however, these ion channel variants appear to account for only a small fraction of AF cases.[21] Finally, with the exception of the association between a common variant in *KCNH2* and AF [22], most case-control association studies to date have been underpowered and have not been replicated.

Recently, a genome-wide association study in Icelanders identified a haplotype block on chromosome 4q25 containing variants that predispose to AF.[23] In this block, two single nucleotide polymorphisms (SNPs), rs2200733 and rs10033464, are in strong linkage disequilibrium and define three haplotypes. The haplotype identified by rs2200733 was found to confer a relatively higher risk [odds ratio (OR) = 1.72] of AF or atrial flutter, whereas the haplotype identified by rs10033464 conferred a more modest risk (OR = 1.39) compared with the common sequence. The association of these SNPs with AF was replicated in two small cohorts of Northern Europeans and in a study of people of Asian descent. The 4q25 haplotype block is located in a 'genomic desert' of approximately 1.5 million base pairs without any known genes. The closest gene, *PITX2*, is more than 50 000 bp away from these variants.

PITX2 is a transcription factor critical for determining left-right asymmetry and for the differentiation of the left atrium.[24] Furthermore, it is necessary for the development of the pulmonary myocardium [25], the source of ectopic, electrically active foci associated with paroxysmal AF in many individuals. The pulmonary myocardium is the therapeutic target of pulmonary vein ablation procedures that have become increasingly frequent in the management of AF.[26]

In our study, we sought to determine whether the association between the 4q25 haplotype block and AF replicated in two community-based and two case-control studies with large numbers of AF participants. In an exploratory analysis, we studied whether there was effect modification by age.

Methods

Description of study cohorts

The Framingham Heart Study (FHS) is a longitudinal observational, community-based cohort initiated in 1948 to prospectively investigate cardiovascular disease and its risk factors, as previously described.[27] Participants were diagnosed with AF, if AF or flutter was present on an electrocardiogram obtained from the hospital or physician records or from routine Framingham clinic examination (every 2 years in the Original Cohort and every 4–8 years in the Offspring Cohort). AF cases were available through 21 April 2007. All protocols were approved by the Boston University Medical Center Institutional Review Board, and participants provided written informed consent.

The Rotterdam Study (RS) is a community-based study founded in 1990. The Medical Ethics Committee of Erasmus University Rotterdam approved the study, and participants signed consent. Inhabitants of a suburb of Rotterdam ($n = 10\,275$) aged 55 years and older were invited, and 7983 participants (78%) were examined. The participants were interviewed at their home and were examined during two visits at the research centre for baseline data collection. The participants were re-examined twice during three follow-up rounds. The first round was performed between July 1993 and 31 December 1994. The second round started in April 1997 and ended 31 December 1999. The third round started in January 2002 and ended 31 July 2004. Three methods were used to assess cases of AF or atrial flutter, as described previously [28]: (i) at baseline and during follow-up examinations, 10 s 12-lead ECGs were recorded at the research centre with an ACTA Gnosis IV ECG recorder (EsaOte, Florence, Italy), stored digitally, and analysed with the Modular ECG Analysis System (MEANS).[29] To verify the diagnosis of AF, all ECGs with a diagnosis of AF or flutter or any other rhythm disorder were re-coded independently by two physicians who were blinded to the MEANS diagnosis. The judgement of a cardiologist was considered decisive in the case of persistent disagreement; (ii) general practitioners information; and (iii) hospital discharge diagnoses were also obtained from the Landelijke Medische Registratie system.

The Vanderbilt AF Registry consists of consecutive patients with documented AF age >18 years, who were prospectively enrolled since October 2002 from the Vanderbilt Cardiology and Arrhythmia Clinics, the emergency department, and in-patient services. At enrolment, participants were asked a detailed medical and drug history and were asked to complete a symptom questionnaire.[30] Participants were excluded if AF was diagnosed in the setting of recent cardiac surgery or were unable to give informed consent or report for follow-up. An echocardiogram was obtained on all patients at the time of registry enrolment. The study protocol was approved by the Vanderbilt University Institutional Review Board, and participants were enrolled following informed written consent. Participants with AF ($n=556$) were enrolled and age- and sex-matched

to referent participants (n=598). The controls were participants who underwent cardiac surgery with no personal or family history of AF and had no AF documented after surgery.

The German Competence Network for Atrial Fibrillation (AFNet) is a national registry of AF patients comprising 10 000 probands. DNA samples are currently collected from patients with AF onset before age 60 years. In this analysis, all samples available by 1 October 2007 (n=906) from the nation-wide German Competence Network for Atrial Fibrillation were combined with AF patients collected at the Medical Department I of the University Hospital Munich, Campus Grosshadern of the Ludwig-Maximilians University Munich, the Medical Department I of the Technical University Munich Hospital, and the Deutsches Herzzentrum Munchen (in total, n=1715). Cases were selected if the diagnosis of AF was made on an electrocardiogram analysed by a trained physician. Patients with signs of moderate-to-severe HF, moderate-to-severe valve disease, or with hyperthyroidism were excluded from the study. Control probands were from a population-based epidemiological survey of persons living in or near the city of Augsburg, Southern Germany (KORA S4), conducted between 1999 and 2001.[31] The survey population consisted of German nationality residents born between 1 July 1925 and 30 June 1975 identified through the registration office. A sample of 6640 participants was drawn with 10 strata of equal size according to sex and age and 4261 individuals (66.8%) agreed to participate. Exclusion criteria for control probands were reported history of AF, signs or symptoms of AF on physical examination, or absence of sinus rhythm upon 12-lead resting ECG that all probands received. All studies involving humans were performed according to the declarations of Helsinki and Somerset West, were approved by local medical Ethics Committees, and participants signed informed consent.

Genotyping

SNP genotyping in the FHS and RS was performed using an ABI TaqMan assay.[32] Genotyping of samples from Vanderbilt University and AFNet was performed using PCR, iPLEX single base primer extension, and matrix assisted laser desorption/ionization–time of flight mass spectrometry in a 384-well format (Sequenom, San Diego, CA, USA), as described previously.[33] Subjects were considered to have failed genotyping if we were unable to detect a PCR product or to distinguish between the alleles in at least two separate experiments.

Statistical analysis

Exact Hardy–Weinberg equilibrium (HWE) tests were applied to all SNPs in the referent participants. In the Framingham sample, a subset of unrelated participants was used to test for deviation from HWE. SNP-age interactions were assessed using age as a continuous variable. All calculations were performed using R (FHS), SPSS (RS), SAS (Vanderbilt

AF Registry), and STATA SE 8.0 statistical package (AFNet). Logistic regression was used to adjust for participants' age and sex. In the community-based studies, a multi-variable analysis was performed using logistic regression to adjust for participants' age, sex, body mass index, and history of HTN, HF, myocardial infarction, and diabetes mellitus. A two-sided P-value of less than 0.05 was considered significant. Only subjects who failed genotyping were excluded from the analysis. Meta-analyses of the relations between AF and rs2200733 and rs10033464 were performed. Given the differences in study design, the community-based samples (FHS and RS) and case-control studies (AFNet and Vanderbilt AF Registry) were analysed separately. A meta-analysis was performed to determine the association between AF and rs2200733 or rs10033464 using all available case-control studies for AF.[23]

Results

A total of 3508 participants with AF and 12 173 referent participants were available from four cohorts of European descent (Table 1). Participants with AF were older ($P \leq 0.001$) and were more likely to be male in the FHS, RS, and AFNet ($P \leq 0.001$). By design, there was a similar number of men among those with and without AF in the Vanderbilt AF Registry (67.8 vs. 66.6%, $P=NS$). Genotype call rates and HWE were similar in subjects without AF (Table 2). Minor allele frequencies for rs2200733 are illustrated in Figure 1.

Table 1 Characteristics of the study cohorts

	Framingham Heart Study		Rotterdam Study		Vanderbilt AF Registry		German AF network	
	AF	Non-AF	AF	Non-AF	AF	Non-AF	AF	Non-AF
Number of participants	327	2006	910	5496	556	598	1715	4073
Age (years) mean± SD	82.2 ±9.9	71.4±12.8*	73.4±8.4	68.8±9.1*	54.2±13.7	56.6±14.2#	61.7±12.1	49.2±13.9*
Men, number (%)	189 (57.8)	833 (41.5)*	415 (45.6)	2178 (39.6)#	377 (67.8)	398 (66.6)	1328 (72.2)	2005 (49.2)*

For comparisons between participants with and without AF: * $P<0.0001$ and # $P=0.0001$

In an analysis adjusted for age and sex, rs2200733 was strongly associated with AF (Table 3), with ORs ranging from 1.37 (95% CI 1.18–1.59; $P = 3.1 \times 10^{-5}$) in the RS to 2.52 (95% CI 2.22–2.84; $P=1.8 \times 10^{-49}$) in AFNet. A multi-variable analysis adjusting for age, sex, HTN, HF, diabetes, and body mass index was performed in the population-based studies. The association between rs2200733 and AF remained significant with an OR of 1.47 (95% CI 1.11–1.94; $P = 0.0067$) in the FHS and 1.36 (95% CI 1.17–1.59; $P = 9.3 \times 10^{-5}$) in the RS. Exclusion of subjects with missing data for at least one co-variate

Table 2 Distribution of genotypes by cohort for single nucleotide polymorphisms rs2200733 or rs10033464

	rs2200733				rs10033464					
	CC	CT	TT	Call rate (%)	HWE	GG	GT	TT	Call rate (%)	HWE
Framingham Heart Study										
AF	229	83	12	99.1	0.15	253	70	2	99.4	0.40
Non-AF	1527	418	29	98.2	0.82	1676	305	24	99.9	0.08
Rotterdam Study										
AF	676	215	19	97.4	0.68	674	162	12	99.6	0.48
Non-AF	4392	1030	74	97.5	0.14	4194	855	52	99.6	0.25
Vanderbilt AF Registry										
AF	398	130	24	98.8	0.004	439	106	7	98.1	0.83
Non-AF	453	99	6	98.4	0.81	452	99	4	98.8	0.81
German AF network										
AF	1015	598	93	99.5	0.69	1328	351	22	99.1	0.83
Non-AF	3116	853	45	98.6	0.11	3315	675	29	98.7	0.40

AF=atrial fibrillation; HWE=P-value for exact Hardy-Weinberg equilibrium test

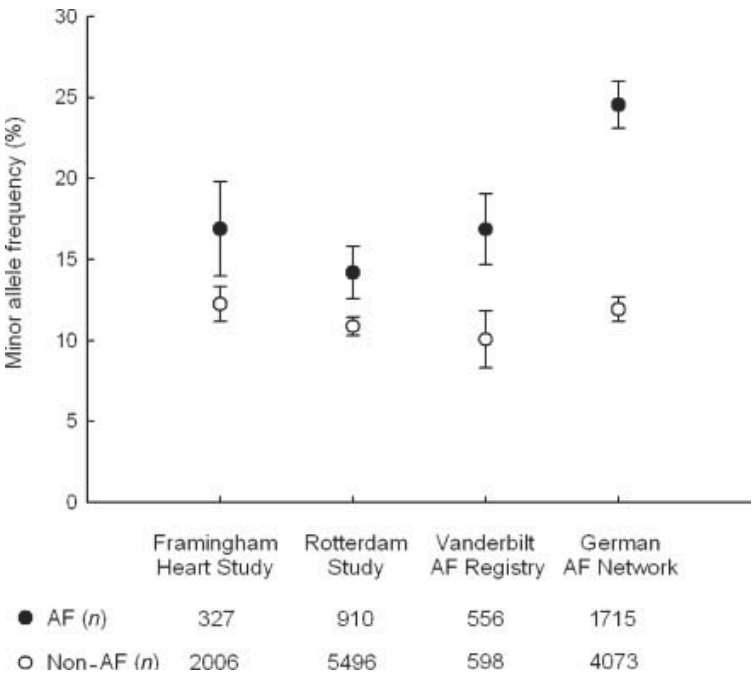


Figure 1 Comparison of the minor allele frequency for single nucleotide polymorphisms rs2200733 in participants either with (filled circle) or without (open circle) AF. 95% confidence intervals are indicated by the error bars.

did not alter our findings (FHS $n=28$, OR 1.47, 95% CI 1.11–1.94; RS $n=468$, OR 1.37, 95% CI 1.18–1.60). In a meta-analysis of the relations between rs2200733 and AF in the community-based studies (Framingham and Rotterdam), the OR was 1.38 (95% CI 1.21–1.57; $P=1.41 \times 10^{-6}$), compared with 2.09 (95% CI, 1.41–3.10; $P=2.4 \times 10^{-4}$) in the case–control studies (Vanderbilt AF Registry and AFNet). A meta-analysis of the case–control studies from both the current and prior [23] reports reveals an OR of 1.90 (95% CI 1.60–2.26; $P=3.3 \times 10^{-13}$). The association between AF and SNP rs10033464 was weaker (Table 3) and did not achieve statistical significance in the Vanderbilt AF Registry cohort. In a multi-variable analysis, rs10033464 was not significantly associated with AF in the FHS, with an OR of 1.22 (95% CI 0.92–1.62; $P=0.18$) or the RS

Table 3 Association between the single nucleotide polymorphisms rs2200733 or rs10033464 with atrial fibrillation

	Adjusted for age and gender				Adjusted for age, gender, HTN, HF, MI, BMI, DM			
	rs2200733		rs10033464		rs2200733		rs10033464	
	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value
Framingham Heart Study	1.40 (1.07 – 1.82)	0.01	1.34 (1.03 – 1.75)	0.031	1.47 (1.11 – 1.94)	0.007	1.22 (0.92 – 1.62)	0.18
Rotterdam Study	1.37 (1.18 – 1.59)	3.1×10^{-5}	1.17 (0.99 – 1.38)	0.07	1.36 (1.17 – 1.59)	9.3×10^{-5}	1.17 (0.98 – 1.39)	0.09
Vanderbilt AF Registry	1.68 (1.29 – 2.18)	0.0001	1.16 (0.86 – 1.56)	0.33	-	-	-	-
German AF network	2.52 (2.22 – 2.84)	1.8×10^{-49}	1.30 (1.13 – 1.51)	0.0002	-	-	-	-

HTN=hypertension; HF=heart failure; MI=myocardial infarction; DM=diabetes mellitus

1.17 (95% CI 0.98–1.39; $P=0.093$). A meta-analysis of the association between AF and rs10033464 in the case–control studies, with inclusion of the current and previous reports [23] reveals an OR of 1.36 (95% CI 1.26–1.47; $P=6.7 \times 10^{-15}$). In an exploratory analysis, we observed a significant interaction between rs2200733 genotype and age in the FHS ($P=0.0065$), but not in the RS ($P=0.62$). There was no significant interaction between rs10033464 genotype and age in the FHS ($P=0.24$) or in the RS ($P=0.47$). In order to compare with prior reports, we have presented these data dichotomized at 60 years of age in Table 4. A higher OR was noted between SNP rs2200733 and AF in participants <60 years in the FHS and AFNet, whereas the opposite was true in the Vanderbilt AF Registry with a higher OR in participants over 60 years.

In the RS, there were 575 cases of incident AF. After adjustment for age and sex, the association between AF and rs2200733 was significant (OR 1.39, 95% CI 1.17–1.65; $P=2.1 \times 10^{-4}$). Among the 2006 participants available in the FHS, there were 184 incident cases of AF. Acknowledging low power to detect an association in Framingham we did not observe a significant odds of incident AF with SNPs rs10033464 or rs2200733 (OR, 95% CI, P-value: 1.11, 0.81–1.52, 0.52; and 1.21, 0.89–1.64, 0.23, respectively).

Table 4 Relations between age of participants and risk of atrial fibrillation for single nucleotide polymorphisms rs2200733 or rs10033464

	rs2200733		rs10033464	
	Age<60, Odds Ratio (95% CI), AF/non-AF	Age≥60, Odds Ratio (95% CI), AF/non-AF	Age<60, Odds Ratio (95% CI), AF/non-AF	Age≥60, Odds Ratio (95% CI), AF/non-AF
Framingham Heart Study	2.13 (1.13 – 4.00), 29/1973	1.29 (0.98 – 1.72), 295/1973	1.48 (0.68 – 3.23), 30/2005	1.34 (1.01 – 1.78), 295/2005
Rotterdam Study	1.34 (0.75 – 2.39), 52/1007	1.33 (1.14 – 1.55), 858/4489	1.60 (0.90 – 2.86), 49/942	1.16 (0.97 – 1.38), 802/4181
Vanderbilt AF Registry	1.44 (1.02 – 2.01), 264/304	2.03 (1.34 – 3.07), 168/254	1.12 (0.76 – 1.64), 264/302	1.17 (0.73 – 1.86), 168/253
German AF network	2.71 (2.34 – 3.15), 697/2868	1.99 (1.68 – 2.36), 1009/1146	1.35 (1.12 – 1.63), 691/2872	1.31 (1.07 – 1.60), 1010/1147

Discussion

In the last 2 years, an increasing number of genome-wide association studies have emerged in the literature identifying genetic variants associated with macular degeneration [35], coronary disease [36], QT interval [37] and recently AF.[23] Although the prior studies provide an opportunity to identify variants associated with apparently complex traits in a population, upon the completion of these studies, two primary issues remain. First, given the inherent limits of multiple hypothesis testing in genome-wide association studies, can the findings from any one study be broadly replicated? Secondly, how do we go from association to mechanism or how precisely do these chromosomal variants lead to AF? In our current report, we address the reproducibility of the findings in the original study.

We found that two SNPs—rs2200733 and rs10033464—were significantly associated with AF in four cohorts of Northern European descent after accounting for age and sex of the participants. Meta-analyses of the relations between AF and both SNPs in all available case–control studies provided a convincing OR of 1.90 ($P = 3.3 \times 10^{-13}$) for rs2200733 or 1.36 ($P = 6.7 \times 10^{-15}$) for rs10033464.

Although the potential mechanism of action of the genetic locus identified by these two non-coding SNPs is unknown and may be mediated through effects of distant genes, it is interesting to note that the closest gene, located ~ 50 000 bp centromeric, is the transcription factor, *PITX2*. Mouse knockouts of this gene have demonstrated a critical role for one isoform, PITX2c, in left–right asymmetry[24] and specifically the development of the left atrium.[38, 39] The loss of PITX2c leads to right atrial isomerisation and a failure to suppress a default pathway for sinus node formation in the left atrium of the embryo.[40] Finally, in a recent elegant study, PITX2c has been demonstrated to be necessary for the development of the pulmonary myocardium or the sleeve of cardiomyocytes extending from the left atrium to the initial portion of the pulmonary vein.[25] Clinical and animal studies have demonstrated that ectopic foci of electrical activity arising from within the pulmonary veins and posterior left atrium play a substantial role

in initiating and maintaining fibrillatory activity.[26, 41] Furthermore, electrical isolation of the pulmonary veins and left atrial region is the goal of catheter ablation procedures that increasingly have been used to treat AF in the last decade.

The association between genetic variants on chromosome 4q25 and AF that we have observed implicates a novel pathway in the genesis of arrhythmia. AF has been reported to be associated with mutations in ion channel proteins, alterations in ion channel flux, and action potential shortening.[42] It is interesting to hypothesize that these variants may dysregulate *PITX2* during cardiogenesis or beyond, thus perturbing the normal structure or function of the left atrium and pulmonary veins and ultimately predisposing to AF.[43, 44] It is important to note that such reasoning is currently speculative and at present, no direct mechanistic relationship between these variants and *PITX2* has yet been demonstrated.

Although there was a strong association between rs2200733 and incident AF in the RS, the association was not statistically significant in the FHS. Although the failure to replicate the Rotterdam findings may simply be due to the limited number of incident cases ($n = 184$) in the Framingham cohort, the relatively low risk associated with rs2200733, even in the case–control studies, suggests that the broad use of such a SNP in predictive testing is of limited clinical value. Similarly, the variability noted in the ORs for the association between SNP rs2200733 and AF in younger vs. older subjects may be due to the limited number of younger subjects available in all studies but the German AF Network. Future studies to determine whether this SNP is associated with the age of onset of AF or other outcomes from AF such as the risk of HF, stroke, mortality, responses to drugs, or catheter ablation procedures will be helpful.

As each of the four studies we sampled consists of participants of European descent, the generalizability of our results to other races and ethnicities is uncertain. In addition, we had low statistical power to test for gene–environment or gene–gene interactions.

In conclusion, we report that two variants on chromosome 4q25 are strongly associated with AF. Although variation at this locus does not appear to be suitable for clinical testing, it does provide a starting point for exploration of a novel pathway for this morbid arrhythmia.

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Chapter 7.2

Variants in *ZFHX3* are associated with atrial fibrillation in individuals of European ancestry

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Abstract

We conducted meta-analyses of genome-wide association studies for atrial fibrillation (AF) in participants from five community-based cohorts. Meta-analyses of 896 prevalent (15,768 referents) and 2,517 incident (21,337 referents) AF cases identified a new locus for AF (*ZFHX3*, rs2106261, risk ratio $RR = 1.19$; $P = 2.3 \times 10^{-7}$). We replicated this association in an independent cohort from the German AF Network (odds ratio = 1.44; $P = 1.6 \times 10^{-11}$; combined $RR = 1.25$; combined $P = 1.8 \times 10^{-15}$).

Introduction

With increasing longevity of individuals in developed countries, late-onset chronic cardiovascular diseases such as AF have become important public health problems. AF is an electrical disorder of the heart's upper chambers characterized by an irregular heart rhythm. The overall lifetime risk of AF is almost 25% in the US and Europe.[1, 2] Furthermore, the incidence of AF is increasing over time; in the US it is projected that up to 15.9 million individuals may be affected by 2050.[3] The growing number of individuals with AF is of concern because of its association with significantly increased risks of stroke, heart failure and death.[4]

AF is a complex disease with many etiologies, including cardiovascular disease and its risk factors. Recently it was reported that, even for typical forms of AF, individuals with an affected relative are at higher risk of AF.[5] Moreover, a genome-wide association study (GWAS) identified SNPs in the chromosome 4q25 region that are associated with increased AF risk.[6] We hypothesized that additional common genetic variation contributes to the development of AF.

Methods

We conducted and combined meta-analyses of prevalent AF and incident AF, using existing GWAS data from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) AF Consortium. CHARGE included the following five community-based cohorts[7]: Age, Gene/Environment Susceptibility Reykjavik Study (AGES); Atherosclerosis Risk in Communities (ARIC); Cardiovascular Health Study; Framingham Heart Study; and Rotterdam Study. Genotyping inclusion criteria were unbiased toward AF, as genotyping was performed as a core effort for many phenotypes in each cohort.

The CHARGE AF Consortium included analyses from five community-based cohorts that collected AF cases systematically in longitudinal follow-up and had GWAS data. All participants included in this analysis were of European descent. In CHARGE cohorts (and AFNET and KORAS4) written informed consent was obtained from each subject, including consent to use DNA for genetic analyses of cardiovascular disease. Consent precluded participant-specific meta-analysis. African American participants from ARIC and CHS studies were not analyzed for the present study.

AGES represents the later follow-up of the midlife Reykjavik (Iceland) Study founded in 1967.[8] AGES was designed to examine the genetic epidemiology of four phenotypes known to alter with advancing age: vascular, neurocognitive, musculoskeletal and body composition. The AGES examinations were conducted between 2002 and 2006 on 5,764 survivors of the Reykjavik Study.

The ARIC study was initiated in 1987 to examine atherosclerosis in middle-aged adults and completed enrollment of 15,792 participants. ARIC was conducted in four US communities (Forsyth County, NC; Jackson, MS; Minneapolis suburbs, MN; and Washington County, MD). Participants were examined about every three years four times and followed for events.[9] Only white ARIC participants were included in the analyses.

CHS is a prospective population-based cohort study of CVD in adults 65 years and older. The four Field Centers (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA) completed enrollment of 5888 participants in 1989-1990 and 1992-1993.[10]

FHS is a community-based observational, cohort initiated in 1948 to prospectively investigate CVD and its risk factors. The Original cohort (n=5209) received biennial exams.[11] The Original Cohort children (& spouses), termed the Offspring cohort (n=5214), were recruited in 1971, and were examined every four to eight years.[12]

The community-based RS was founded in 1990 to examine the determinants of disease and health in the elderly with a focus on CVD, neurogeriatric, bone and eye diseases. Inhabitants of a Rotterdam suburb (n=10,275) age ≥55 years were invited and 7,983 participants (78%) were examined. The participants were examined up to four times approximately every three years.[13]

AF Ascertainment

Studies included initial, paroxysmal, persistent, and permanent atrial fibrillation and atrial flutter (ARIC did not include atrial flutter). Prevalent AF was considered present if AF was observed on baseline electrocardiograms (AGES, CHS, RS), or prior to DNA collection (FHS). Incident AF was defined as AF that first occurred after the collection of DNA (FHS, ARIC), or after the baseline examination (other cohorts). There was no overlap in AF cases between prevalent and incident AF analyses.

The AGES study ascertained AF based on AGES examination Minnesota coded electrocardiograms, and ICD-10 I48 recorded from hospitalizations from 1997 through March, 2008.

The ARIC study determined AF from three sources: electrocardiograms at study visits, hospital discharge records and death certificates[14] (first reviewed and confirmed by a cardiologist, latter two reviewed by trained abstractor, ICD-9 code 427.31 or 427.3; ICD-10 I48). Incidence of AF was identified through 2004 as the first occurrence of AF by any of the sources. ARIC excluded 37 atrial flutter without subsequent AF cases. In CHS, prevalent AF was identified by 12-lead ECG at baseline. Incident AF in up to 16 years follow-up (median 13 years) was identified by the first occurrence of AF on annual CHS study electrocardiograms or the first occurrence of a hospital discharge ICD-9 code for AF. CHS participant hospital discharge diagnosis codes for AF were found to have a sensitivity of 71% for AF.[15]

In FHS, all cardiovascular hospital and outside records were routinely obtained and electrocardiograms were recorded at all FHS examinations; AF cases through 2007 were verified by 2 FHS cardiologists.[16]

For the RS the ascertainment of AF included review of hospital discharge information, general practitioner diagnoses and RS electrocardiograms. AF was verified by 2 physicians and verified by electrocardiogram review by a cardiologist in the case of a disagreement.[2]

AF Replication sample

The replication German Competence Network for Atrial Fibrillation (AFNET) is a national registry of patients with prevalent AF onset before 60 years ($n=2,145$) without structural heart disease. Referent subjects without AF or structural heart disease ($n=4,073$) were drawn from the population-based KORA S4 study.[17] Association testing was performed using logistic regression with an additive genetic model adjusting for age at DNA draw, sex and hypertension status.

Genotyping and Imputation

Briefly, the five studies utilized a variety of high-density Illumina (Human CNV370, AGES and CHS; Infinium 550, RS) and Affymetrix (6.0, ARIC; 500K +50K human gene focused, FHS) platforms. Approximately 2.5 million autosomal genotypes were imputed within each study using the Phase II CEU HapMap reference panel (<http://hapmap.org>) and BIMBAM (CHS; <http://stephenslab.uchicago.edu/software.html>) or MACH v1.0.15/16 (all others; <http://www.sph.umich.edu/csg/abecasis/MaCH/index.html>) software.

Statistical methods

Primary GWAS were performed within each cohort separately for prevalent and incident AF using an additive genetic model adjusting for age, sex, and, if relevant, cohort (FHS Original versus Offspring) or site (ARIC, CHS). Prevalent AF was examined with logistic regression; controls for prevalent analyses included all eligible participants without prevalent AF at the time of DNA collection. Incident AF was examined with proportional-hazards regression, censoring at death, loss to follow-up or date of last contact. The incident AF analyses included eligible participants without prevalent AF at the time of DNA collection. To account for its pedigree structure data, FHS used generalized estimating equations for logistic analyses and robust variance estimates for proportional-hazards analyses.

Results and discussion

Our community-based participants were middle-aged to elderly, with mean ages at DNA collection from 57 (ARIC) to 76 (AGES) years (table 1). The Manhattan plot of $-\log_{10}$ P values for combined prevalent and incident AF analyses is displayed in figures 1. We prespecified genome-wide significance as $P < 5 \times 10^{-8}$, corresponding to significance at 5% adjusting for approximately one million independent tests as estimated in HapMap samples of European ancestry. To prioritize follow-up genotyping, we required that SNPs have $P < 4 \times 10^{-7}$ (corresponding to one expected false positive per GWAS) and that at least six of nine analyses (out of four prevalent and five incident AF analyses) contribute results for the SNP, to reduce possible false-positives due to poor imputation. We replicated the association with a previously reported chromosome 4 locus 7 (rs17042171, $P = 6.0 \times 10^{-27}$; table 2), which was approximately 150 kb telomeric to the transcription factor gene *PITX2*.

Table 1 Characteristics of the study cohorts

	Prevalent (n=16,664)				Incident (n=23,854)				
	AGES	CHS	FHS	Rotterdam	AGES	ARIC	CHS	FHS	Rotterdam
Age, years	≥66	≥65	≥30	≥55	≥66	≥44	≥65	≥30	≥55
Participants before exclusion	3219	3372	5282	5974	3219	11459	3372	5282	5974
Participants after exclusion	2959	3267	4464	5974	2718	8086	3201	4184	5665

SNP rs2106261 on chromosome 16q22, located in an intronic region of transcription factor *ZFHX3* (previously known as *ATBF1*), showed suggestive evidence of association (table 2, combined prevalent-incident $P = 2.3 \times 10^{-7}$, Fig. 1). Results were consistent in the separate prevalent ($P = 9.0 \times 10^{-6}$) and incident ($P = 7.9 \times 10^{-4}$) AF analyses.

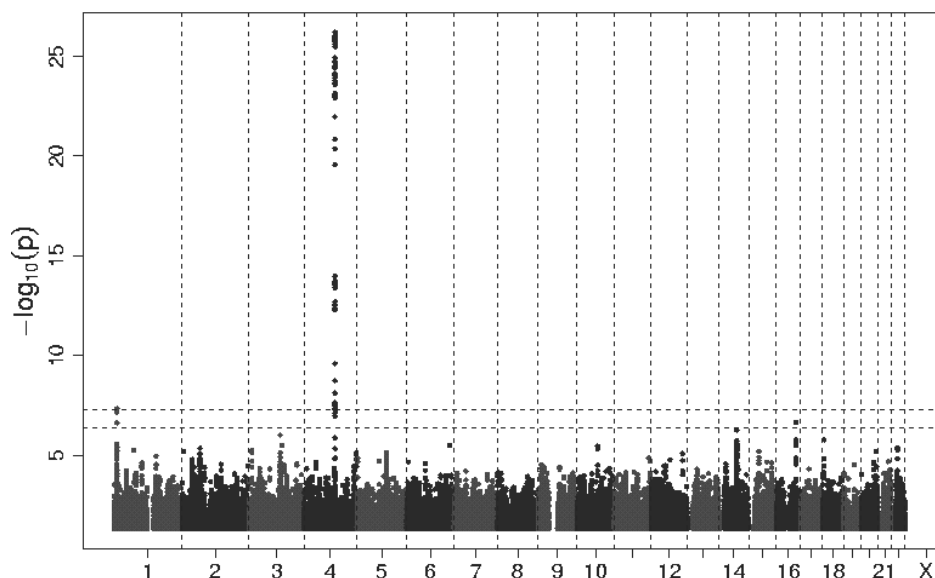


Figure 1 Manhattan plot of combined prevalent and incident analyses

We replicated the association between SNP rs2106261 and AF in a large independent cohort, the German AF Network (AFNET), consisting of 2,145 cases and 4,073 controls (odds ratio = 1.44, $P = 1.6 \times 10^{-11}$; table 2). In a meta-analysis of the results from the discovery (CHARGE community AF) and replication (German AFNET) studies, rs2106261 was significantly associated with AF (RR 1.25, $P = 1.8 \times 10^{-15}$; table 1). *ZFH3* appears to regulate myogenic [18] and neuronal differentiation.[19] *ZFH3* has been reported to be a tumor suppressor gene in several cancers [20], and recently SNPs in *ZFH3* have been associated with susceptibility to Kawasaki disease.[21] Although the function of *ZFH3* in cardiac tissue is unknown, it is expressed in mouse hearts.

Another significant association signal was on chromosome 1p36 within *MTHFR* (rs17375901, $P = 4.6 \times 10^{-8}$), which encodes 5,10-methylenetetrahydrofolate reductase. The association with the *MTHFR* locus was not confirmed in independent subjects from the AFNET cohort (table 2). The initial *MTHFR* finding may be a false positive result. However, the region may merit further investigation because *MTHFR* is in linkage disequilibrium with *NPPA*, the atrial natriuretic peptide gene; a *NPPA* frameshift mutation has been described in a family with AF.[22]

We acknowledge several study limitations. Although our findings were generally consistent, we observed some between-analysis heterogeneity in effect sizes ($P = 0.01$), possibly arising from variation in cohort participant characteristics, duration and etiology of AF, low study-specific precision, subtle locus-specific population stratification and population differences in underlying haplotype structure. We note that for the previously

Table 2 CHARGE AF GWAS meta-analysis with $P \leq 4 \times 10^{-7}$ and German AFNET replication

Locus		Chr	Minor/ major allele	MAF CHARGE AFNET	Combined analysis prevalent and incident AF		German AF net		Meta-analysis CHARGE and German AFNET	
SNP/ nearby gene					Relative risk	Meta P-value	Odds Ratio	P-value	Relative risk	P-value
rs17042171 PITX2		4	A/C	0.122 0.156	1.45	6.0×10^{-27}	2.46	6.9×10^{-51}	1.65	3.9×10^{-63}
rs2106261 ZFHX3		16	T/C	0.174 0.192	1.19	2.3×10^{-7}	1.44	1.6×10^{-11}	1.25	1.8×10^{-15}
rs17375901 MTHFR		1	T/C	0.053 0.058	1.34	4.6×10^{-8}	1.04	0.68	1.26	5.9×10^{-7}

MAF Minor allele frequency

validated *PITX2* locus we observed between-study heterogeneity. Thus, heterogeneity appears to be a general feature of even the strongest genome-wide findings for AF, and it remains to be addressed in follow-up studies. In addition, our findings may not be generalizable to other populations. It also was not possible to perform a pooled analysis using participant-specific data given the restrictions imposed by the Institutional Review Boards at some study sites. Furthermore, there is a potential for survival bias in the prevalent AF analysis if the variant is associated with both AF onset and lethality; in this situation, individuals who die shortly after AF onset might not survive until DNA collection. Nonetheless, a moderate association was present in prevalent, incident, and combined AF meta-analyses for both the validated chromosome 4q25 and the new chromosome 16q22 loci. Another limitation is that, beyond single SNPs, our study did not analyze patterns of haplotypes, and thus this it may not have captured complex haplotype associations. However, our use of imputation to the HapMap does take advantage of available linkage disequilibrium information. Finally, we recognize that we likely have identified variants in linkage disequilibrium with causal variants rather than the specific functional variants; the pathophysiology by which locus variation contributes to AF risk remains unknown. The strengths of our approach include the use of five large community-based cohorts, whose participants were not selected for phenotypic characteristics, thereby enhancing the generalizability of our findings. The robustness of the chromosome 16q22 result is strengthened by its documentation in samples ascertained with different study designs, including case-control and cohort studies. In summary, by examining GWAS data for AF in five community-based cohorts, we replicated the previously reported association with chromosome 4q25 variants and we identified a new locus on chromosome 16 in a gene encoding the transcription

factor *ZFHX3*. We provided confirmatory support for the *ZFHX3* finding by replicating our findings in a large independent study of AF. Further studies are needed to elucidate functional variants and mechanisms by which the 16q22 locus predisposes to AF.

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Chapter 7.3

Genome-wide association study of PR interval and relation to risk of atrial fibrillation

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Abstract

The electrocardiographic PR interval reflects atrial and atrioventricular nodal conduction, disturbances of which increase risk of atrial fibrillation (AF). To identify underlying common genetic variation, we meta-analyzed genome-wide association results for PR interval from seven community-based studies of European-ancestry individuals: AGES, ARIC, CHS, FHS, KORA, Rotterdam Study and SardinIA (n=28,517). Associated loci were tested for association with AF (n=5,741 AF cases). Significant association with PR interval was identified at nine loci ($P < 5 \times 10^{-8}$). We identified 9 independent signals. Five of the nine loci were also associated with AF ($P < 0.0056$). Common genetic variation contributes significantly to atrial and atrioventricular conduction and to AF risk.

Introduction

In myocardial excitation, the delay between the excitation of the atria and ventricles is determined by the sum of atrial and atrioventricular nodal conduction. This delay, measured in milliseconds, is reflected on the standard 12-lead electrocardiogram (ECG) by the PR interval or PQ interval. The PR interval has a substantial heritable component, with heritability estimates ranging between 30 and 50%. [1-4]

Atrial fibrillation (AF) is the most common sustained arrhythmia and is independently associated with increased risk of stroke, heart failure, dementia, and death. [5] AF prevalence increases markedly with age, to nearly 9% in those 80-89 years of age, and is estimated to triple by the year 2050. [6] Common genetic risk factors for AF [7] include variants on chromosome 4q25 near the *PITX2* gene [8], in 16q22.3 near the *ZFHX3* (*ATBF1*) gene [9] and the K897T variant in the *KCNH2* gene on 7q36.1. [10]

The PR interval is an intermediate phenotype for AF, as alterations in atrial action potential duration and in atrioventricular conduction influence both PR interval and AF risk. [11] Longitudinal data from the Framingham Heart Study (FHS) and the Atherosclerosis Risk in Communities Study (ARIC) demonstrate that PR interval prolongation is a predictor of increased AF risk. [12,13] In addition, PR interval prolongation has been shown in FHS to be an independent predictor in a multifactorial risk score for atrial fibrillation predisposition. [14]

We undertook a meta-analysis of GWAS to investigate the genetic determinants of the PR interval and their relationship to AF risk. Our goal was to identify genes that can provide insights into atrial disease and lead to novel opportunities for AF prevention and therapy.

Methods

We studied individuals of European descent from seven community based studies: the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES) [15], ARIC [16], the Cardiovascular Health Study (CHS) [17], FHS [18], the Kooperative Gesundheitsforschung in der Region Augsburg Study (KORA) [19], the Rotterdam Study (RS) [20], and the SardiNIA study [3]. Phenotypic data including resting 12-lead electrocardiography, height, weight, systolic blood pressure, and medication use were collected using standardized protocols in all studies.

Study participants were genotyped using a variety of genome-wide SNP arrays. To facilitate comparison of results across studies, we imputed to the 2.5 million HapMap SNPs. A recent review supports the validity of combining results across statistical and genotyping platforms.[21]

After exclusions, 28,517 individuals were available for study (table 1). The association of each SNP with the PR interval was adjusted for age, sex, RR interval, height, body mass index (BMI), systolic blood pressure, and study site in studies with multiple recruitment sites. Studies adjusted for or excluded individuals using drugs known to alter the PR interval including beta-blockers, diuretics and non-dihydropyridine calcium antagonists. Due to restrictions imposed by Institutional Review Boards at several of the study sites on the sharing of individual genetic data, it was not possible to perform analyses based on combined individual-level data. Therefore, we conducted a meta-analysis of the beta estimates from linear regression of PR interval. The coefficients, generated for each SNP, estimate the difference in PR interval per additional copy of the minor allele, adjusted for the covariates in the model. The genome-wide significance threshold was 5×10^{-8} .

Table 1 Characteristics of the study cohorts

	AGES	ARIC	CHS	FHS	KORA F3	KORA S4	Rotterdam	SardiNIA
Participants before exclusion	3219	11478	2084	12174	1644	1100	5271	4305
Participants after exclusion	2471	6486	1769	7636	1427	927	3710	4091
Gender, men (%)	922 (37.3)	2977 (45.9)	773 (43.7)	3511 (46.0)	728 (51.0)	447 (48.2)	1541 (39.8)	1782 (43.5)
Mean age, years \pm SD	76.1 \pm 5.4	53.9 \pm 5.7	72.9 \pm 5.4	39.9 \pm 10.3	61.6 \pm 9.9	56.2 \pm 7.1	67.8 \pm 8.5	42.5 \pm 17.1
Mean PR interval, ms \pm SD	171.4 \pm 27.7	162.7 \pm 23.7	167.9 \pm 28.5	152.1 \pm 21.9	163.3 \pm 23.3	164.6 \pm 21.2	165.9 \pm 24.1	154.2 \pm 26.7

To determine if there was an association between the PR-associated loci and AF risk, we meta-analyzed results from 4 studies of AF in subjects of European descent. The first was a meta-analytic study of 896 prevalent AF cases and 15,768 referents from the CHARGE cohorts. The second was a meta-analytic study of 2,517 incident AF cases and 21,337 referents from the CHARGE cohorts. The third and fourth were

independent case-control studies of prevalent AF: the German Competence Network on Atrial Fibrillation (AFNET, 2,145 cases and 4,073 controls); and the Cleveland Clinic AF study (CCAF, 183 cases and 164 controls). We performed a meta-analysis of the logistic-regression results from the prevalent AF studies and the proportional hazards result from the incident AF study. The Bonferroni adjusted significance threshold was $P = 0.05/9 = 0.0056$.

The study was performed in accordance with the Helsinki declarations and was approved by the local medical ethics and institutional review boards. All participants gave signed informed consent to use their DNA for genetic analyses.

Results and discussion

Overall, nine loci showed independent association signals with $P < 5 \times 10^{-8}$ (table 2). In one region we detected two association signals, ($P = 2.1 \times 10^{-74}$) and ($P = 6.0 \times 10^{-26}$). These variants are in low LD ($r^2 = 0.031$). In a meta-analysis of linear regression results from models including both SNPs, these SNPs remained independently associated with PR interval ($P = 9.7 \times 10^{-82}$; $P = 1.1 \times 10^{-33}$), suggesting they represent independent association signals. In addition, six PR interval associations were identified in or near genes involved in human cardiac development.

Table 2 Genome-wide significant association findings for PR interval

SNP	Minor/ major allele	beta (ms)	SE (ms)	P value
1	C/T	1.3624	0.207	$4.62 * 10^{-11}$
2	C/G	3.0403	0.2886	$6.00 * 10^{-26}$
3	C/T	3.7687	0.2065	$2.10 * 10^{-74}$
4	A/G	-2.0146	0.2203	$5.99 * 10^{-20}$
5	C/T	-1.4924	0.2091	$9.45 * 10^{-13}$
6	A/G	2.2959	0.2086	$3.66 * 10^{-28}$
7	G/A	-1.1916	0.2155	$3.22 * 10^{-8}$
8	A/G	-2.0907	0.2872	$3.34 * 10^{-13}$
9	C/T	1.9505	0.2311	$3.13 * 10^{-17}$

Genome-wide significant threshold $P < 5 * 10^{-8}$

Of the nine identified PR loci, five were associated with AF risk ($p < 0.0056$) (table 3). In all instances the minor alleles were associated with a decrease in AF risk, irrespective of the direction of their association with PR interval (table 3). Protective ratios against AF were between 0.93 and 0.88 for any minor allele. The observation that the sign of effect on PR interval was not predictive of the risk decreasing or increasing effect on AF may initially appear counterintuitive. The possibility that PR intervals at high and low extremes may both be associated with increase in AF risk may provide a possible

Table 3 Meta-analytic results for the association of the nine genome wide significant PR SNPs with atrial fibrillation

SNP	PR prolonging allele	Frequency of PR prolonging allele	OR (95% CI) for AF PR prolonging allele	P-value (unadjusted)	P-value (adjusted)	Effect of PR prolonging allele towards AF risk
1	C	0.39	1.01 (0.97 – 1.06)	0.65	-	-
2	C	0.15	0.90 (0.84 – 0.96)	$7.0 * 10^{-4}$	$6.30 * 10^{-3}$	Decreased
3	C	0.40	0.92 (0.88 – 0.96)	$1.5 * 10^{-3}$	$1.35 * 10^{-3}$	Decreased
4	G	0.69	1.01 (0.97 – 1.06)	0.56	-	-
5	T	0.61	1.07 (1.03 – 1.12)	$2.3 * 10^{-3}$	$2.07 * 10^{-2}$	Increased
6	A	0.40	0.91 (0.87 – 0.95)	$2.2 * 10^{-5}$	$1.98 * 10^{-4}$	Decreased
7	A	0.67	0.94 (0.90 – 0.99)	0.01	-	-
8	G	0.85	1.13 (1.06 – 1.20)	$2.1 * 10^{-4}$	$1.89 * 10^{-3}$	Increased
9	C	0.30	0.99 (0.95 – 1.04)	0.72	-	-

The Bonferroni adjusted significance threshold is $P = 0.05/9 = 5.6 * 10^{-3}$

explanation. Existing data from humans and animal models suggest that the effects of genetic variants on atrial repolarization and action potential duration, and their relationship with atrial arrhythmias, are complex. As long and short QT intervals are both associated with increased VT risk, also for AF linear association models with PR interval and with underlying genetic variants may not capture the entire complexity of these relationships.

Our study was subject to a number of potential limitations. False positive associations from multiple testing is a limitation of any GWAS, so we used a well-accepted genome-wide association significance threshold equivalent to a Bonferroni correction for 1 million independent tests to reduce false positive findings.[22] Population stratification is also a concern, so we only included study subjects of European descent. Our study also did not examine patterns of haplotype association. Thus complex haplotype associations may not have been captured. However, fully genome-wide meta-analyses of haplotypes are not currently feasible, and, in common with other GWAS, our use of imputation to the HapMap leverages available linkage disequilibrium information.

The biological mechanisms by which the identified variants influence PR interval and AF remain speculative, and detailed functional investigation will be required to determine the potential contribution of each genomic region.

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Chapter 8

Summary

Samenvatting

Summary

Sudden death is among the most common causes of death in developed countries. Sudden death from cardiac causes accounts for approximately 50% of all deaths from cardiovascular diseases and 20% of all deaths. The majority of sudden cardiac deaths are caused by acute ventricular arrhythmia. An important potential cause of ventricular arrhythmia is prolongation of ventricular repolarization. Prolongation of ventricular repolarization may result in early after depolarizations, which in turn may induce re-entry and thereby provoke Torsade de Pointes and fatal ventricular arrhythmia. This thesis focused on the effect of certain drugs and endocrine factors on QTc prolongation and sudden cardiac death.

Chapter 1 gives an overview of QTc prolongation and sudden cardiac death and its main risk factors, etiology and possibilities for prevention.

In **chapter 2.1** we studied whether listed putative QTc prolonging psychotropic drugs indeed prolong the QTc interval under everyday circumstances, and evaluated whether this is a class effect or an individual drug effect. We were able to confirm the importance of antidepressants (in particular amitriptyline, imipramine, maprotiline and nortriptyline) and antipsychotics (in particular lithium, olanzapine and thioridazine) as potential contributors to QTc prolongation. Especially starting of tricyclic antidepressant drugs is associated with a significant intra-individual increase in the QTc interval (approximately 10 milliseconds) in comparison to the change in non-users. The tricyclic antidepressants appear to prolong the QTc interval as a class effect.

Since common variation in the *NOS1AP* gene has been associated with QTc interval variation and NOS1 is presumed to influence intracellular calcium, we aimed to study in **chapter 2.2** whether *NOS1AP* single nucleotide polymorphisms modify the QTc prolonging effect of calcium channel blockers. We demonstrated that the minor alleles of both *NOS1AP* SNPs significantly potentiate the QTc prolonging effect of verapamil. Because QTc prolongation is associated with an increased risk of sudden cardiac death, this gene-drug interaction may be of clinical importance.

Virtually all QTc prolonging drugs act by blocking the *hERG* (human ether a-go-go related gene) encoded potassium channels, whereas not all QTc prolonging drugs are associated with an increased risk of serious cardiac arrhythmias. To investigate whether binding capacity to *hERG*-encoded potassium channels can actually predict hard clinical endpoints, we studied in **chapter 3.1** whether non-cardiovascular drugs, which are known to block the *hERG*-encoded potassium channels, are associated with an increased risk of sudden cardiac death and whether the ratio between therapeutic

plasma concentrations and the concentration which inhibits 50% of the potassium channels is an indicator of the risk of sudden cardiac death. We confirmed that current use of non-cardiovascular *hERG*-encoded potassium channels inhibiting drugs in the general population is associated with an increased risk of sudden cardiac death. In addition, we demonstrated that drugs with a high *hERG*-encoded potassium channels inhibiting capacity had a higher risk of sudden cardiac death than patients using drugs with a low potassium channels inhibiting capacity. In **chapter 3.2** we evaluated the association between use of domperidone, which is also known to block the *hERG*-encoded potassium channels, and serious non-fatal ventricular arrhythmias and sudden cardiac death in the general population. We confirmed that current use of domperidone and mainly high doses are associated with an increased risk of sudden cardiac death. We could not demonstrate an effect of domperidone on non-fatal ventricular arrhythmias due to absence of exposed cases.

Since sudden cardiac death is frequently reported in epilepsy and the indications of anticonvulsant drug prescriptions now include chronic pain syndromes, we studied whether use of anticonvulsants is associated with an increased risk of sudden cardiac death. In **chapter 3.3** we found that use of anticonvulsive drugs is associated with an increased risk of sudden cardiac death in the general population, even in persons who use anticonvulsive drugs for other indications than epilepsy. The risk of sudden cardiac death seems to be higher among users of carbamazepine and other anticonvulsants with sodium channel blocking properties.

Since the literature on the effect of excess thyroid hormone on ventricular repolarization is controversial, we aimed to examine whether free T4 and TSH are associated with QTc prolongation. In **chapter 4.1** we showed that high levels of free T4 are associated with QTc prolongation in men, which can be explained by an increased activity of cardiac Na⁺/K⁺ ATPase in thyroid hormone excess, leading to increased intracellular K⁺ with subsequent membrane hyperpolarization and an increase in QTc duration. Although a QTc interval prolongation of 10 milliseconds in one individual usually remains without clinical consequences, an average shift of 10 milliseconds in a Gaussian distribution on a population level will inevitably push more individuals into the upper percentiles of the QTc interval with its increased risk of Torsade de Pointes and sudden cardiac death. In **chapter 4.2** we investigated whether use of antithyroid drugs (as a direct cause or as an indicator of poorly controlled hyperthyroidism) is associated with an increased risk of sudden cardiac death. We demonstrated in two independent studies that use of antithyroid drugs seems to be associated with a threefold increased risk of sudden cardiac death. Although this might be due to antithyroid drug use itself, it could be more readily explained by underlying hyperthyroidism, since increased free T4 levels are associated with QTc prolongation and treated patients who developed sudden cardiac

death still had low TSH levels shortly before death. This suggests that hyperthyroidism may be a risk factor for sudden cardiac death.

It is assumed that testosterone is an important regulator of gender-related differences in ventricular repolarization. Therefore, we studied in **chapter 5.1** whether serum levels of testosterone are associated with QTc, QT and RR interval variation. We demonstrated in two population-based studies that serum testosterone is associated with a decreased QTc interval and increased RR interval, however, not with a difference in the QT interval. The decreased QTc interval in men with higher levels is probably due to the association of serum testosterone with prolongation of the RR interval.

Diabetes mellitus is a common disease in the elderly which adversely affects cardiac repolarization. Since hyperglycemia itself is a risk factor for cardiovascular disease, we studied whether non-diabetic persons with impaired fasting serum glucose and hyperinsulinemia have QTc/QT-interval prolongation and RR-interval shortening, and whether these were associated with an increased risk of sudden cardiac death. In **chapter 5.2** we showed that impaired fasting glucose and hyperinsulinemia are associated with a significantly increased QTc-interval and shortening of the RR interval. We demonstrated in the same population that both prolongation of the QTc/ QT as well as shortening of the RR interval are associated with an increased risk of sudden cardiac death.

In **chapter 6** we discussed the main findings of the studies presented in this thesis and the methodological considerations of these studies. In addition, we presented potential clinical implications and suggestions for future research.

In addition, we presented recently discovered genes involved in atrial fibrillation and the PR interval during studies. In **chapter 7.1** we have replicated variants on chromosome 4 associated with atrial fibrillation. The SNPs rs2200733 and rs10033464 are strongly associated with AF in four independent cohorts of European descent. The primary aim of genome-wide association (GWA) studies is to identify novel genetic loci associated with interindividual variation in the levels of risk factors and the degree of clinical disease. In **chapter 7.2** we conducted meta-analyses of genome-wide association studies for atrial fibrillation in collaboration with the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium and identified the new locus *ZFHX3* for atrial fibrillation. The electrocardiographic PR interval reflects atrial and atrioventricular nodal conduction, disturbances of which increase risk of atrial fibrillation. In **chapter 7.3** we conducted meta-analyses of GWA studies for the PR interval in 7 community-based studies and identified 9 loci. Five of these 9 loci were also associated with atrial fibrillation.

Samenvatting

Acute hartdood is één van de meest voorkomende doodsoorzaken in de westerse wereld. Acute hartdood is verantwoordelijk voor ongeveer 50% van alle cardiovasculaire doden en 20% van alle doden. De meerderheid van alle acute hartdoden wordt veroorzaakt door acute ventriculaire aritmieën. Een belangrijke oorzaak van ventriculaire aritmieën is verlenging van de ventriculaire repolarisatie (zichtbaar als verlenging van het QTc-interval op het ECG) resulterend in “early after depolarizations”, die op hun beurt re-entry kunnen induceren en Torsade de Pointes kunnen uitlokken, mogelijk leidend tot fatale ventriculaire aritmieën. Dit proefschrift bevat een aantal onderzoeken naar het effect van bepaalde geneesmiddelen en endocriene factoren op QTc verlenging en acute hartdood.

In **hoofdstuk 1** wordt een overzicht gegeven van QTc verlenging en acute hartdood, waarbij de belangrijkste risicofactoren, de etiologie en de mogelijkheden voor preventie zijn beschreven.

In **hoofdstuk 2.1** hebben we bestudeerd of psychotrope geneesmiddelen, waarvan wordt aangenomen dat ze het QTc interval verlengen, inderdaad het QTc interval verlengen. Bovendien hebben we geëvalueerd of dit een klasse-effect is of een effect van het individuele geneesmiddel. We hebben het belang van antidepressiva (met name amitriptyline, imipramine, maprotiline en nortriptyline) en antipsychotica (met name lithium, olanzapine en thioridazine) als mogelijke bijdrage aan QTc verlenging kunnen bevestigen. Vooral het starten van tricyclische antidepressiva is geassocieerd met een significante toename van het QTc interval (ongeveer 10 milliseconden) duidend op een klasse-effect.

Variatie binnen het *NOS1AP* gen is geassocieerd met variatie in de lengte van het QTc interval, waarbij NOS1 van invloed is op intracellulair calcium. In **hoofdstuk 2.2** hebben we bestudeerd of *NOS1AP* “single nucleotide polymorphisms” (SNPs) het QTc verlengende effect van calciumantagonisten veranderen. We hebben aangetoond dat de variant allelen van beide *NOS1AP* SNPs het QTc verlengende effect van verapamil significant potentiëren. Omdat QTc verlenging geassocieerd is met een verhoogd risico op acute hartdood, kan deze gen-geneesmiddel interactie van klinisch belang zijn.

Vrijwel alle QTc verlengende geneesmiddelen verlengen het QTc interval door blokkade van kaliumkanalen, gecodeerd door *hERG* (human ether a go-go related gene). Niet alle QTc verlengende geneesmiddelen zijn echter geassocieerd met een verhoogd risico op cardiale aritmieën. Om te onderzoeken of de bindingscapaciteit aan door *hERG* gecodeerde kaliumkanalen harde klinische eindpunten kan voorspellen, hebben we in

hoofdstuk 3.1 bestudeerd of het gebruik van niet-cardiovasculaire geneesmiddelen, waarvan bekend is dat ze deze kaliumkanalen kunnen blokkeren, geassocieerd is met een verhoogd risico op acute hartdood. Daarnaast hebben we onderzocht of de ratio tussen de therapeutische plasmaconcentratie en de concentratie die nodig is om 50% van de kaliumkanalen te remmen, een indicator is voor het risico op acute hartdood. We hebben bevestigd dat gebruik van niet-cardiovasculaire geneesmiddelen die deze kaliumkanalen blokkeren, in de algemene populatie geassocieerd is met een verhoogd risico op acute hartdood. Bovendien hebben we aangetoond dat gebruik van geneesmiddelen met een hoge capaciteit om deze kaliumkanalen te blokkeren, geassocieerd is met een hoger risico op acute hartdood dan het gebruik van geneesmiddelen met een lage capaciteit. Domperidon kan tevens door *hERG* gecodeerde kaliumkanalen blokkeren. In **hoofdstuk 3.2** hebben we de associatie tussen het gebruik van domperidon en ernstige non-fatale ventriculaire aritmieën en acute hartdood in de algemene populatie geëvalueerd. We hebben aangetoond dat gebruik van domperidon, voornamelijk in hoge doseringen, geassocieerd is met een verhoogd risico op acute hartdood. Doordat geen enkele patiënt - die een non-fatale ventriculaire aritmie ontwikkelde - domperidon gebruikte, konden we geen effect van domperidon op ventriculaire aritmieën vaststellen.

Aangezien acute hartdood vaak gerapporteerd wordt bij epilepsie en de indicaties van anti-epileptica uitgebreid zijn met het chronische pijn syndroom, hebben we bestudeerd of het gebruik van anti-epileptica geassocieerd is met een verhoogd risico op acute hartdood. In **hoofdstuk 3.3** hebben we gevonden dat gebruik van anti-epileptica geassocieerd is met een verhoogd risico op acute hartdood in de algemene populatie, zelfs bij patiënten die anti-epileptica gebruiken voor een andere indicatie dan epilepsie. Het risico op acute hartdood blijkt hoger bij gebruikers van carbamazepine en andere anti-epileptica met natriumkanal blokkerende eigenschappen.

Aangezien de literatuur controversieel is over het effect van schildklierhormoon op de ventriculaire repolarisatie, hebben we onderzocht of vrij T4 en TSH geassocieerd zijn met QTc verlenging. In **hoofdstuk 4.1** hebben we aangetoond dat hoge vrij T4 waarden geassocieerd zijn met QTc verlenging bij mannen. Dit effect kan verklaard worden door een verhoogde cardiale Na^+/K^+ ATPase activiteit bij verhoogde serumwaarden van het schildklierhormoon, mogelijk geassocieerd met een verhoogd intracellulair kalium en een toename van de lengte van het QTc interval. Alhoewel een verlenging van het QTc interval van 10 milliseconden in één persoon normaliter zonder klinische consequenties blijft, zal een gemiddelde toename van 10 milliseconden in een normale verdeling op populatie-niveau meer personen naar de hoogste percentielen van het QTc interval verschuiven, geassocieerd met een verhoogd risico op Torsade de Pointes en acute hartdood. In **hoofdstuk 4.2** hebben we bestudeerd of gebruik van thyreostatica (als een directe oorzaak of als indicator van matig ingestelde hyperthyreoïdie) geassocieerd is

met een verhoogd risico op acute hartdood. We hebben in twee onafhankelijke studies aangetoond dat gebruik van thyreostatica geassocieerd is met een drievoudig verhoogd risico op acute hartdood. De thyreostatica zouden dit zelf kunnen veroorzaken maar het is waarschijnlijker dat de acute hartdood veroorzaakt wordt door de onderliggende hyperthyreoïdie. Ten eerste omdat patiënten die waren behandeld met thyreostatica en een acute hartdood ontwikkelden verlaagde TSH spiegels hadden kort voor het overlijden en ten tweede omdat hoge waarden van vrij T4 geassocieerd zijn met QTc verlenging. Dit maakt aannemelijk dat hyperthyreoïdie zelf een risicofactor voor acute hartdood is. Het risico lijkt vooral verhoogd te zijn in het begin van de behandeling met thyreostatica.

Het wordt verondersteld dat testosteron een belangrijke regulator is van geslachtsgerelateerde verschillen in ventriculaire repolarisatie. Daarom hebben we in **hoofdstuk 5.1** bestudeerd of testosteronspiegels geassocieerd zijn met variatie in het QTc, QT en RR interval. We hebben in twee afzonderlijke studies aangetoond dat testosteron geassocieerd is met een verkort QTc interval en een verlengd RR interval, echter niet of in mindere mate met het QT interval. Het kortere QTc interval bij mannen met hoge testosteronspiegels kan waarschijnlijk worden verklaard door de associatie met het verlengde RR interval bij mannen. In de formule van Bazett voor het QTc-interval, hangen RR-interval en QTc-interval rechtstreeks samen.

Diabetes mellitus heeft een negatief effect op de cardiale repolarisatie. Aangezien hyperglycemie reeds een risicofactor voor cardiovasculaire ziekte is, hebben we onderzocht of personen zonder diabetes mellitus met een verhoogde nuchtere glucose spiegel en hyperinsulinemie, ook QTc/ QT-verlenging en RR-verkorting ontwikkelen. Daarnaast hebben we onderzocht of een verlengd QTc/ QT interval en een verkort RR interval geassocieerd is met een verhoogd risico op acute hartdood. In **hoofdstuk 5.2** hebben we aangetoond dat een verhoogde nuchtere glucose spiegel en hyperinsulinemie geassocieerd zijn met een significant verlengd QTc interval en een verkort RR interval. We hebben in dezelfde populatie aangetoond dat zowel verlenging van het QTc/ QT interval als een verkorting van het RR interval geassocieerd zijn met een verhoogd risico op acute hartdood.

In **hoofdstuk 6** hebben we de belangrijkste bevindingen van de studies in dit proefschrift en de methodologische overwegingen van deze studies besproken. Bovendien hebben we de klinische implicaties en suggesties voor toekomstig onderzoek besproken.

Daarnaast hebben we recent ontdekte genen besproken, die geassocieerd zijn met atriumfibrilleren en het PR interval. In **hoofdstuk 7.1** hebben we 2 SNPs op chromosoom 4 met betrekking tot atriumfibrilleren gerepliceerd. De SNPs rs2200733 and rs10033464

zijn sterk geassocieerd met atriumfibrilleren in 4 onafhankelijke cohorten van Europese afkomst. Het doel van genoom-brede associatie (GWA) onderzoeken is om nieuwe genetische loci te identificeren die geassocieerd zijn met interindividuele variatie van de ziekte. In **hoofdstuk 7.2** hebben we een meta-analyse uitgevoerd van GWA onderzoeken voor atriumfibrilleren in samenwerking met het CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium. We hebben een nieuw gen (*ZFHX3*) geïdentificeerd, dat geassocieerd is met een verhoogd risico op atriumfibrilleren. Het PR interval op het ECG is een maat voor de atriale en atrioventriculaire geleiding. In **hoofdstuk 7.3** hebben we een meta-analyse uitgevoerd van GWA onderzoeken voor het PR interval in 7 studies. We hebben 9 onafhankelijke loci geïdentificeerd. Vijf van deze 9 loci waren tevens geassocieerd met atriumfibrilleren.

Chapter 9

Dankwoord

Phd Portfolio

List of publications

About the author

Dankwoord

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Charlotte

PhD Portfolio

Name	Charlotte van Noord
Erasmus MC Department	Epidemiology
Research School	Netherlands Institute for Health Sciences
PhD period	2006-2009
Supervisors	Prof.dr. B.H.Ch. Stricker, Prof.dr. J.C.M. Witteman, Prof.dr. M.C.J.M. Sturkenboom and Dr. S.M.J.M. Straus

1. PhD Training

Research skills

2006 – 2008	Master of Science in Clinical Epidemiology, Netherlands Institute for Health Sciences, Erasmus University, Rotterdam, the Netherlands
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Presentations

Oral presentations

2009	Population-based studies of antithyroid drugs and sudden cardiac death, 25 th International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, United States of America
2009	Quantitative signal detection, Pharmacovigilance training, European Medicines Agency in London, United Kingdom
2008	Signal detection methodologies, Informa Life Science's annual Signal Detection & Risk Management in Amsterdam, the Netherlands
2008	Ventricular Arrhythmia and Sudden Unexpected Death and Domperidone, 8 th Annual Meeting of the International Society of Pharmacovigilance in Buenos Aires, Argentina
2008	Association of Cardiovascular Drugs and <i>NOS1AP</i> with QTc Prolongation, 8 th Annual Meeting of the International Society of Pharmacovigilance in Buenos Aires, Argentina
2008	Association of Cardiovascular Drugs and <i>NOS1AP</i> with QTc Prolongation and Sudden Cardiac Death, 24 th International Conference on Pharmacoepidemiology and Therapeutic Risk in Copenhagen, Denmark

Poster presentations

- 2009 Recent use of bisphosphonates associated with atrial fibrillation, 25th International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, United States of America
- 2009 Ventricular Arrhythmia and Sudden Unexpected Death and Domperidone, 25th International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, United States of America
- 2007 Psychotropic Drugs Associated with QTc Prolongation, 7th Conference of European Association for Clinical Pharmacology and Therapeutics in Amsterdam, the Netherlands
- 2007 Psychotropic Drugs Associated with QTc Prolongation, 23rd International Conference on Pharmacoepidemiology and Therapeutic Risk in Montreal, Canada

International Conferences

- 2009 4th CHARGE Meeting in Washington, United States of America
- 2009 25th International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, United States of America
- 2009 3rd CHARGE Meeting in Rotterdam, the Netherlands
- 2008 WHO Uppsala Conference 2008, Impacting patient safety: Adverse drug reaction signal detection in Uppsala, Sweden
- 2008 8th Annual Meeting of the International Society of Pharmacovigilance in Buenos Aires, Argentina
- 2008 24th International Conference on Pharmacoepidemiology and Therapeutic Risk in Copenhagen, Denmark
- 2007 7th Conference of European Association for Clinical Pharmacology and Therapeutics in Amsterdam, the Netherlands

Seminars and workshops

- 2009 "Advanced topics in Pharmacoepidemiological methods", special skills workshops, 25th International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, United States of America
- 2009 "Arrhythmia Research Methodology", COEUR, Erasmus MC, Rotterdam, the Netherlands
- 2008 "Advanced topics in Pharmacoepidemiological methods", special skills workshops, 24th International Conference on Pharmacoepidemiology and Therapeutic Risk in Copenhagen, Denmark
- 2008 EudraVigilance Training, EMEA in London, United Kingdom

- 2007 "SNPs and Human Diseases", Molecular Medicine, Erasmus MC, Rotterdam, the Netherlands
- 2007 Mid-Year Meeting of the International Society for Pharmacoepidemiology in Amsterdam, the Netherlands
- 2006 – 2009 Research seminars, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands

2. Teaching activities

Supervising practicals

- 2008 Principles of Research in Medicine, NIHES, Rotterdam, the Netherlands
- 2008 – 2009 Data-analysis in pharmacoepidemiology, NIHES, Rotterdam, the Netherlands
- 2007 – 2008 Pharmacoepidemiology, 4th year medical students, Erasmus MC, Rotterdam, the Netherlands
- 2006 – 2008 Statistics, 4th year medical students, Erasmus MC, Rotterdam, the Netherlands
- 2006 – 2007 Clinical Epidemiology, 4th year medical students, Erasmus MC, Rotterdam, the Netherlands

Supervising Master's thesis

- 2007 – 2008 Karen de Groot, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands

List of publications

Manuscripts based on the studies described in this thesis

Chapter 2.1

van Noord C, Straus SMJM, Sturkenboom MCJM, Hofman A, Aarnoudse ALHJ, Bag-nardi V, Kors JA, Newton-Cheh C, Wittteman JCM, Stricker BHC. Psychotropic Drugs Associated With Corrected QT Interval Prolongation. *Journal of Clinical Psychopharma-cology* 2009; 29 (1):9-15.

Chapter 2.2

van Noord C, Aarnoudse ALHJ, Eijgelsheim M, Sturkenboom MCJM, Straus SMJM, Hofman A, Kors JA, Newton-Cheh C, Wittteman JCM, Stricker BHC. Calcium channel blockers, *NOS1AP* and QTc prolongation. *Pharmacogenetics and Genomics*, 2009; 19 (4):260-6.

Chapter 3.1

van Noord C, Sturkenboom MCJM, Straus SMJM, Wittteman JCM, Stricker BHCh. Non-cardiovascular drugs which inhibit *hERG*-encoded potassium channels and risk of sudden cardiac death. *Submitted*.

Chapter 3.2

van Noord C, Dieleman JP, van Herpen G, Verhamme KM, Sturkenboom MCJM. Dom-peridone and ventricular arrhythmia or sudden cardiac death. *Submitted*.

Chapter 3.3

Bardai A, **van Noord C**, Blom MT, van Soest EM, Verhamme KM, Sturkenboom MCJM, Tan HL. Anticonvulsants and risk of sudden cardiac death. *Submitted*.

Chapter 4.1

van Noord C, van der Deure WM, Sturkenboom MCJM, Straus SMJM, Hofman A, Visser TJ, Kors JA, Wittteman JCM, Stricker BHC. High free thyroxine levels are associated with QTc prolongation in males. *Journal of Endocrinology* 2008; 198:253–60.

Chapter 4.2

van Noord C, Sturkenboom MCJM, Straus SMJM, Hofman A, Wittteman JCM, Stricker BHC. Population-based studies of antithyroid drugs and sudden cardiac death. *British Journal of Clinical Pharmacology* 2009; 68 (3):447-54.

Chapter 5.1

van Noord C, Dörr M, Sturkenboom MCJM, Straus SMJM, Reffellmann T, Felix SB, Hofman A, Kors JA, Haring R, de Jong FH, Nauck M, Uitterlinden AG, Wallaschofski H, Witteman JCM, Völzke H, Stricker BHCh. The association of serum testosterone levels and ventricular repolarization. *Submitted*.

Chapter 5.2

van Noord C, Sturkenboom MCJM, Straus SMJM, Hofman A, Kors JA, Witteman JCM, Stricker BHCh. Serum glucose and insulin are associated with QTc and RR intervals in non-diabetic elderly. *Submitted*.

Chapter 7.1

Kaab S*, Darbar D*, **van Noord C***, Dupuis J*, Pfeufer A*, Newton-Cheh C, Schnabel R, Makino S, Sinner MF, Kannankeril PJ, Beckmann BM, Choudry S, Donahue BS, Heeringa J, Perz S, Lunetta KL, Larson MG, Levy D, MacRae CA, Ruskin JN, Wacker A, Schömig A, Wichmann HE, Steinbeck G, Meitinger T, Uitterlinden AG, Witteman JCM, Roden DM, Benjamin EJ, Ellinor PT. Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. *European Heart Journal* 2009;30 (7):813-9. (*** equal contribution**)

Chapter 7.2

Benjamin EJ*, Rice KM*, Arking DE*, Pfeufer A*, **van Noord C***, Smith AV*, Schnabel RB, Bis JC, Boerwinkle E, Sinner MF, Dehghan A, Lubitz SA, D'Agostino Sr RB, Lumley T, Ehret GB, Heeringa J, Aspelund T, Newton-Cheh C, Larson MG, Marciante KD, Soliman EZ, Rivadeneira F, Wang TJ, Eiriksdottir G, Levy D, Psaty BM, Li M, Chamberlain AM, Hofman A, Vasan RS, Harris TB, Rotter JI, Kao WHL, Agarwal SK, Stricker BHC, Wang K, Launer LJ, Smith NL, Chakravarti A, Uitterlinden AG, Wolf PA, Sotoodehnia N, Köttgen A, van Duijn CM, Meitinger T, Mueller M, Perz S, Steinbeck G, Wichmann HE, Lunetta KL, Heckbert SR, Gudnason V, Alonso A, Kaab S, Ellinor PT, Witteman JCM. Variants in ZFX3 are associated with atrial fibrillation in individuals of European ancestry. *Nature Genetics* 2009; 41(8):879-81. (*** equal contribution**)

Chapter 7.3

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Najjar SS, Lakatta E, Schlessinger D, Uda M, Abecasis GR, Müller-Myhsok B, Ehret GB, Boerwinkle E, Chakravarti A, Soliman EZ, Lunetta KL, Perz S, Wichmann HE, Meitinger T, Levy D, Gudnason V, Ellinor PT, Sanna S, Kääb S, Witteman JCM, Alonso A, Benjamin EJ, Heckbert SR. Genome-wide association study of PR interval and relation to risk of atrial fibrillation. *Nature Genetics* 2009. *In press*. (*** equal contribution**)

Other manuscripts

Eijgelsheim M, Newton-Cheh C, Aarnoudse AL, **van Noord C**, Witteman JC, Hofman A, Uitterlinden AG, Stricker BHC. Genetic variation in NOS1AP is associated with sudden cardiac death: evidence from the Rotterdam Study. *Human Molecular Genetics* 2009. *In press*.

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van Noord C, Hooijkaas H, Dufour-van den Goorbergh BCM, van Hagen PM, van Daele PLA, van de Merwe JP. The diagnostic value of anti-cyclic citrullinated peptide antibodies in patients with Sjögren's syndrome in detecting rheumatoid arthritis. *Annals of the Rheumatic Diseases* 2005; 64: 160-2.

About the author

Charlotte van Noord was born on April 22nd, 1981 in Rotterdam, the Netherlands. In 1999, she graduated from the “Erasmiaans Gymnasium” in Rotterdam and started medical school at the Erasmus University, Rotterdam. In 2003, she participated in research on the diagnostic value of anti-cyclic citrullinated peptide antibodies to detect rheumatoid arthritis in patients with Sjögren’s syndrome at the department of Immunology of the Erasmus Medical Center in Rotterdam (supervisors: Dr.J.P. van de Merwe and Prof.dr.H. Hooijkaas). After obtaining her medical degree in 2005, she worked for one year as a resident in Internal Medicine at the “Medisch Centrum Rijnmond-Zuid” in Rotterdam (head Dr.A. Berghout).

In September 2006, she started the work described in this thesis at the pharmacoepidemiology unit (head: Prof.dr.B.H.Ch. Stricker) and the cardiovascular epidemiology unit (head: Prof.dr.J.C.M. Witteman) of the department of Epidemiology (head: Prof.dr.A. Hofman) of the Erasmus Medical Center in Rotterdam. During this period, she also worked at the Pharmacovigilance department (head: Dr.S.M.J.M. Straus) of the Dutch Medicines Evaluation Board in The Hague.

In August 2008, she obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES). In 2009, she received the Stanley Edlavitch Award for the best student abstract from the International Society for Pharmacoepidemiology. In January 2010, she will start her specialist training in Internal Medicine at the Maasstad Hospital in Rotterdam (head: Dr.M.A. van den Dorpel).