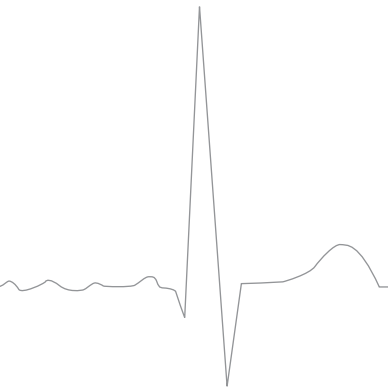
RISK FACTORS FOR ATRIAL Fibrillation

Bouwe Pieter Krijthe



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Hartstichting

Het verschijnen van dit proefschrift werd mede mogelijk gemaakt door de steun van de Nederlandse Hartstichting

CONTENTS

MANUSCRIPTS THAT FORM THE BASIS OF THIS THESIS

Chapter 2

Krijthe BP, Kunst A, Hofman A, Benjamin EJ, Lip GY, Franco OH, Hofman A, Hofman A, Stricker BH, Heeringa J. Projections on the number of patients with atrial fibrillation in the European Union. Submitted

Chapter 3.1

Krijthe BP, Leening MJ, Hofman A, Franco OH, Witteman JC, Stricker BH, Heeringa J. Unrecognized myocardial infarction and risk of atrial fibrillation: The Rotterdam Study. Int J Cardiol. 2013 Jan 16. pii: S0167-5273(12)01686-5. doi: 10.1016/j.ijcard.2012.12.057. [Epub ahead of print]

Chapter 3.2

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Chapter 3.3

Krijthe BP, Heeringa J, Kors J, Hofman A, Franco OH, Witteman JC, Stricker BH. Serum potassium levels and the risk of atrial fibrillation. Submitted

Chapter 3.4

Krijthe BP, Heeringa J, Hofman A, Franco OH, Witteman JC, Stricker BH. Non steroidal anti inflammatory drugs use and the risk of atrial fibrillation. In preparation

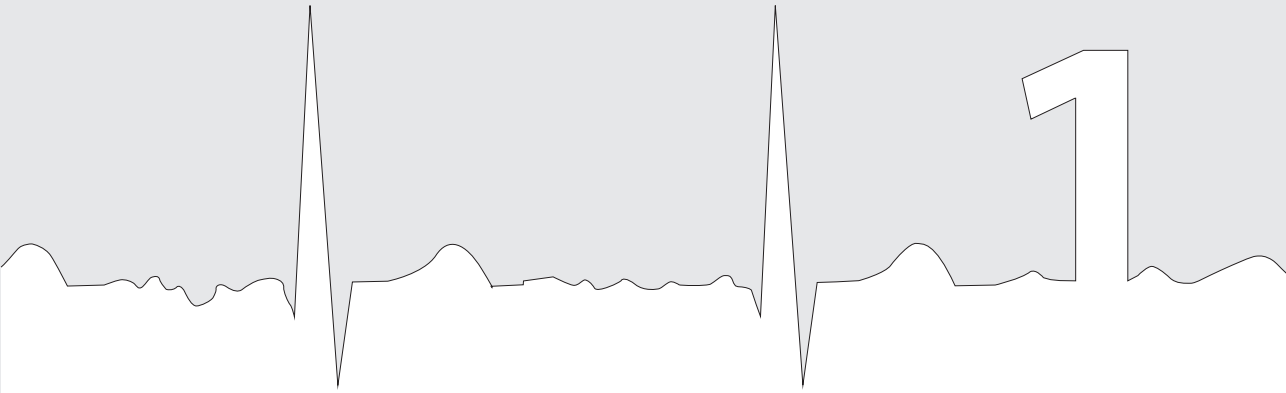
Chapter 3.5

Ellinor PT*, Lunetta KL*, Albert CM*, Glazer NL*, Ritchie MD*, Smith AV*, Arking DE*, Müller-Nurasyid M*, Krijthe BP*, Lubitz SA*, Bis JC*, Chung MK*, Dörr M*,Ozaki K*, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagoner DR, Magnani JW,Wakili R, Clauss S, Rotter JI, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Völker U, Völzke H, Milan DJ, Hofman A, Boerwinkle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N,Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann HE,Witteman JC, Kao WH, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjögren M, Newman AB, Liu Y, Gollob MH, Melander O*,Tanaka T*, Stricker BH*, Felix SB*, Alonso A*, Darbar D*, Barnard J*, Chasman DI*, Heckbert SR*, Benjamin EJ*, Gudnason V*, Kääb S*. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. Nat Genet. 2012 Apr 29;44(6):670-5. doi: 10.1038/ng.2261.

Chapter 4

Alonso A* , Krijthe BP*, Aspelund T*, Stepas KA*, Pencina MJ, Moser CB, Sinner MF, Sotoodehnia N, Fontes JD, Janssens AC, Kronmal RA, Magnani JW,Witteman JC, Chamberlain AM, Lubitz SA, Schnabel RB, Agarwal SK, McManus DD, Ellinor PT, Larson MG, Burke GL, Launer LJ, Hofman A, Levy D, Gottdiener JS, Kääb S, Couper D, Harris TB, Soliman EZ, Stricker BH, Gudnason V, Heckbert SR, Benjamin EJ. A simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: the CHARGE-AF consortium. J Am Heart Assoc. 2013 Mar 18;2(2):e000102. doi: 10.1161/JAHA.112.000102.

** These authors contributed equally to the manuscript*



CHAPTER 1

Introduction

INTRODUCTION

Atrial fibrillation is a common cardiac arrhythmia that is characterized by rapid disorganized atrial electrical activity resulting in absence of atrial contractions. It is diagnosed on the basis of typical findings on an electrocardiogram (ECG). The characteristic ECG findings are absence of P-waves, and an irregular heart rate. Symptoms of atrial fibrillation include palpitations, dyspnea, reduced exercise capacity, chest pain and dizziness, but it often goes without symptoms.[1] Although atrial fibrillation is often asymptomatic it has serious consequences for the health of affected individuals and is a substantial burden for the health care system.[2] Atrial fibrillation is associated with a higher risk of several serious complications. It is associated with a three to five fold higher risk of stroke.[3] Furthermore, it is associated with a higher risk of dementia,[4] heart failure,[5] and it is associated with increased mortality independent of age sex and other cardiovascular risk factors.[6] Also, it is associated with lower quality of life,[7] even patients without symptoms have a lower perceived general health and gobal life satisfaction than healthy subjects.[7]

The prevalence and incidence of atrial fibrillation increase with age. It is estimated that the lifetime risk for development of atrial fibrillation is one in every four adults over 40 years of age.[8, 9] As Western populations are projected to age in the coming decades it is likely that there will be an increase in the number of affected individuals with several types of chronic disease.[10-12] Several studies projected that the future number of adults with atrial fibrillation in the United States will have doubled by the year 2050.[13-15] Not much is known about the potential rise in the number of individuals with atrial fibrillation in the Netherlands and in the European Union but since these populations are projected to age, an increase in the number of patients can be expected.

The mechanisms behind the development and continuation of atrial fibrillation are complex. It is currently believed that ectopic foci along with electrical and structural remodelling play a role. Ectopic foci consisting of pulse generating areas other than the sinus node, are supposed to trigger fibrillation. Electrophysiological changes that occur during high atrial rate are responsible for a shortening of the refractory time of atrial myocytes, which facilitates atrial tachycardias.[16] Structural remodelling, being morphological changes in the atrial substrate, may facilitate both pulse re-entry and trigger ectopic foci.[17] Much research is focused on the role of structural remodelling as the pathogenic substrate of atrial fibrillation. The mechanisms leading to atrial structural remodelling and ultimately atrial fibrillation are largely unknown. It is suggested that multiple factors may have a role such as atrial ischemia, atrial stretching, volume and/or pressure overload and inflammatory processes.[16]

Several risk factors that are involved in these mechanisms have been studied. It is well established that the risk of atrial fibrillation is associated with age, sex, smoking and underlying cardiovascular diseases including heart failure, valvular disease, diabetes, hypertension, and

previous myocardial infarction.[18, 19] But based on these risk factors a substantial portion of the atrial fibrillation risk remains unexplained.[20] In recent years, several other risk factors for atrial fibrillation have been identified. Among those are conditions that do not attract direct clinical attention such as elevated systolic blood pressure within normotensive range, subclinical coronary artery disease, high normal thyroid function.[19, 21] Also several biomarkers involved in various physiological processes, have been associated with the risk of atrial fibrillation including, B-type Natriuretic Peptide levels, C-reactive protein, Interleukin-6, osteoprotogerin, troponin, endothelin, and plasminogen activator inhibitor-1.[20] Furthermore, lifestyle factors such as alcohol abuse, physical exercise, obesity, and also use of several drugs have been associated with the risk of atrial fibrillation.[18, 19, 22] Finally, it was suggested that the risk of atrial fibrillation includes a genetic component, as a Danish Twin Study estimated the heritability of atrial fibrillation attributable to additive genetic effects to be 62%[23] and several studies found multiple genetic loci associated with atrial fibrillation.[24-27] However, much of the underlying mechanisms leading to atrial fibrillation remain incompletely understood. Therefore, further risk factor identification is relevant.

Aim of this thesis

This thesis has several objectives. The first objective was to project the number of individuals with atrial fibrillation in the Netherlands and the European Union (chapter 2). The main objective of this thesis was to identify new risk factors for atrial fibrillation. Hereto, we used data from the Rotterdam Study, a large prospective population-based cohort study among elderly (>55 years old).[28] In chapter 3, we report on the results of a study on the association between clinically unrecognized myocardial infarction and the risk of atrial fibrillation. Furthermore, we studied whether levels of serum dehydroepiandrosterone sulphate (chapter 4), and serum potassium are associated with the risk of atrial fibrillation (chapter 5). In chapter 6, we studied in more detail the association of use of non–steroidal anti-inflammatory drugs with the risk of atrial fibrillation, an association which was investigated only in healthcare databases with relatively little clinical detail. In collaboration with several other population-based cohort studies, we performed a genome-wide association study of genetic loci and atrial fibrillation (chapter 7). Finally, in chapter 8 we investigate the predictability of atrial fibrillation using a prediction model that included several easy obtainable risk factors. In chapter 9, the main findings are discussed and suggestions for future research are given.

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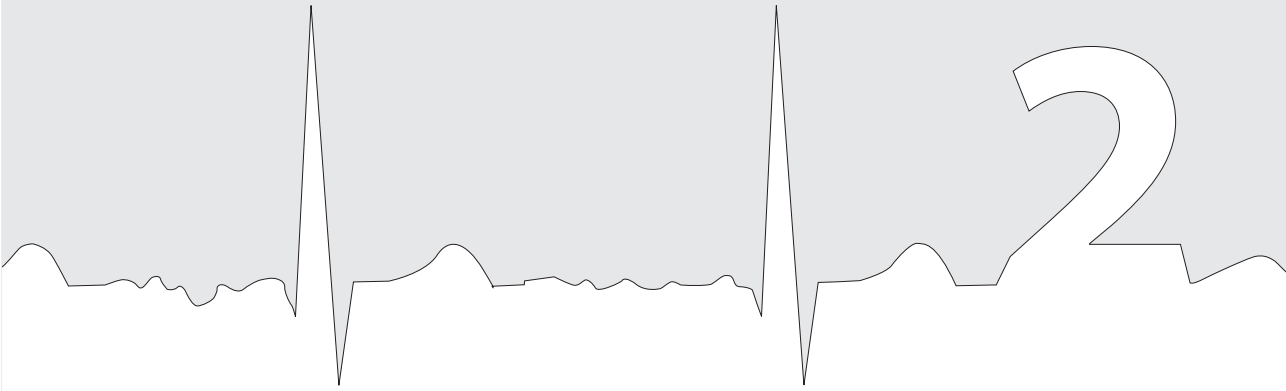

CHAPTER 2

Projections on the number of patients with atrial fibrillation

ABSTRACT

Background: Since atrial fibrillation is associated with increased risks of cardiovascular and cerebrovascular complications, estimations on the number of individuals with atrial fibrillation are relevant to healthcare planning. We aimed to project the number of individuals with atrial fibrillation in the Netherlands and in the European Union from 2000 to 2060.

Methods: Age- and sex-specific atrial fibrillation prevalence estimates were obtained from the prospective community-based Rotterdam Study. Population projections for the Netherlands and the European Union were obtained from the European Union's statistics office.

Results: In the age stratum of 55-59 years the prevalence of atrial fibrillation was 1.3% in men, and 1.7% in women. The prevalence of atrial fibrillation increased to 24.2% in men, and 16.1% in women, for those >85 years of age. We estimate that 259,600 adults over 55 years (95%-CI projections: 189,300 - 367,600) had atrial fibrillation in the Netherlands in 2010. We project that this number will double by 2060 to 547,700 (95%-CI projections: 416,000 - 729,100). Furthermore, we estimate that in the European Union, 8.8 million adults over 55 years had atrial fibrillation in 2010 (95%-CI projections: 6.5 million- 12.3 million), and project that this number will double by 2060 to 17.9 million (95%-CI projections: 13.6 million - 23.7 million).

Conclusion: We estimate that from 2010 to 2060, the number of adults 55 years and over with atrial fibrillation in the Netherlands and in the European Union will more than double. Our results support the importance of developing effective preventative strategies aiming to reduce the risk of atrial fibrillation.

INTRODUCTION

European populations are undergoing a substantial demographic shift in age. The European statistics' office 'Eurostat' estimates that in the European Union, 29.5% of the total population was at least 55 years of age in 2010, and this percentage is expected to rise to 41.0% by 2060.[1] The increased proportion of older adults has several public health consequences, including an increase in the number of older individuals surviving with chronic disease and disability, with a consequent increase in health service utilization.[2, 3]

Little is known about the potential rise in the number of individuals with atrial fibrillation in European populations. Atrial fibrillation is the most common sustained arrhythmia in the general population and it has been associated with an increased risk of stroke,[4] dementia,[5] and heart failure.[6] Moreover, atrial fibrillation is associated with an increased risk of cardiac and total mortality[7, 8] as well as substantial costs from diagnostics, interventions, treatments, and inpatient care.[9] Considering the risks of diseases, mortality, and costs associated with atrial fibrillation it is essential to have valid estimates and projections of the future population prevalence of atrial fibrillation.

The objective of our study was to calculate projections on the number of individuals with atrial fibrillation in the European Union from 2000 to 2060 using information collected in the community-based prospective cohort study: the Rotterdam Study.

METHODS

Study cohorts

The current investigation was performed within the Rotterdam Study, a community-based prospective cohort study.[10] Our study was designed to examine the onset of, and risk factors for disease in older adults. We used data from 2 independent cohorts –within the Rotterdam Study-, Rotterdam Study 1(RS1) and Rotterdam Study 2 (RS2).[10]

RS1 started with a baseline visit between 1990 and 1993. All inhabitants age 55 years and over in the Ommoord district of Rotterdam, The Netherlands were invited to participate (n=10,275). Of those who were invited 7,983 (78%) were enrolled. Between 2000 and 2001, the RS2 started with a baseline visit. All inhabitants of the Ommoord district who had become at least 55 years of age after the start of the Rotterdam Study or who had moved into the study district of Ommoord in the meantime were invited. Of those who were invited (4,472 inhabitants) 3,011 (67.3%) participated. At baseline, participants were interviewed at home and were examined at the research center. The examination included a 10 second, 12-lead resting electrocardiogram (ECG). Information on the presence of disease was available by collaboration with the general practitioners in the study area.[11]

The medical ethics committee of Erasmus Medical Center, Rotterdam, approved the study, and all participants gave written informed consent to participate in the study and to obtain medical information from treating physicians separately.

Atrial fibrillation assessment

Prevalent and incident atrial fibrillation were ascertained using three methods.[12] At baseline and during follow-up examinations 10 second, 12-lead ECGs were recorded, stored digitally, and analyzed by the Modular ECG Analysis System (MEANS).[13, 14] To verify the diagnosis of atrial fibrillation, all ECGs with a diagnosis of atrial fibrillation, atrial flutter, or any other rhythm disorder based on the algorithms of the MEANS software were recoded independently by 2 research physicians who were blinded to the MEANS diagnosis. The judgment of a cardiologist was sought and taken as decisive in those cases in which disagreement persisted between the coding physicians. Additionally, medical information was obtained from general practitioners, which included their own results as well as results from physicians practicing in hospitals and outpatient clinics. atrial fibrillation was considered present after diagnosis by a medical specialist or by a general practitioner if it was ascertained by an ECG. Finally, information was obtained from a national registry of all hospital discharge diagnoses. atrial fibrillation occurring during a serious disease resulting in death, during myocardial infarction or during cardiac operative procedures in which the individual recovered during the hospital admission was not included. We did not distinguish between atrial fibrillation and atrial flutter when we identified cases because both conditions are very similar with respect to risk factors and consequences.[15, 16]

Information on vital status was obtained on a regular basis from the central registry of the Municipality of Rotterdam, from collaborating general practitioners and by obtaining information during follow-up examinations. For those participants whose information on vital status remained missing, the Central Registry of Genealogy of the Netherlands was consulted. This national institute receives population registry records of all deceased inhabitants in the Netherlands.[11]

European estimates

Population projections were obtained from the statistics office of the European Union, Eurostat.[1] We used 1st January population projections from 2000 to 2060 by sex and 5-year age group, using a 5-year time interval. The projections were based on fertility rates, life expectancy at birth and net international migration. We used projections for the Netherlands, and the combination of the 27 European Union member states.

General baseline measurements

Weight and height were obtained at last preceding research center visit. Data on medications were obtained during the home interview by copying the labels of all medications used and further from a pharmacy database including drug use information from automated medical

records. A history of myocardial infarction was defined as self-reported myocardial infarction with hospital admission, the presence of a myocardial infarction on the ECG, or myocardial infarction as judged from the general practitioners records and coded independently by 2 research physicians.[17] Prevalent heart failure was assessed using a European Society of Cardiology validated score.[18, 19] Prevalent diabetes mellitus was defined as the use of hypoglycemic medication or a pre or post-load serum glucose level of >11.0 mmol/L.

Statistical analyses

Prevalence of atrial fibrillation was calculated as the proportion of those who had atrial fibrillation in the study population at the index date. For the main analysis, we used information from participants who were alive at January 1, 2002 and for whom information on atrial fibrillation was available. We chose January ,1 2002 as the index date in this analysis as this was the first date that all participants from RS1 and RS2 had a baseline ECG available and thereby the most recent date with accurate atrial fibrillation assessment in all age categories over age 55 years. We calculated prevalence by sex and 5 year age groups. Wilson's score method for a binomial proportion was used to calculate 95% confidence intervals. The estimates of atrial fibrillation prevalence were then used to extrapolate to the population projections from Eurostat to estimate the total number of cases in the Netherlands and in the European Union. These extrapolations were done per sex and 5-year age group, and then combined to estimate the total number of adults over 55 years of age with atrial fibrillation. In the main analyses we assumed the prevalence to remain stable from 2000 to 2060. We used the estimated age- and sex-specific confidence interval limits to estimate prevalence projections (95%-CI projections).

Several studies suggested that the prevalence of atrial fibrillation is rising over time.[20-23] To evaluate the change in prevalence of atrial fibrillation during the follow up time of the Rotterdam Study, we calculated prevalence figures after the completion of each research center visit.(January 1 of 1994, 1996, 2002 and 2006). We used generalized estimating equation methods to test for trends in prevalence of atrial fibrillation.[20, 21] This method adjusts for repeated measurements in the same individual. The year of the examination was used to test for trends in prevalence. Age, sex and calendar year were entered as variables in this model. Results from this analysis were then used in a sensitivity analysis in which we assumed a sex-specific yearly increase in atrial fibrillation prevalence, starting from January 1, 2002. Data were analyzed using the SPSS PASW statistical software, version 20.0 (IBM corporation, Armonk, New York, USA).

RESULTS

Prevalence of atrial fibrillation in the reference population

General characteristics of the reference population are described in Table 1. The study sample consisted of 533 individuals with prevalent atrial fibrillation, 242 men and 291 women, at January 1, 2002 (Table 1). Those with atrial fibrillation were older (mean age 78.9 years (SD:8.0)) than those without atrial fibrillation (mean age 72.4 years (SD:8.8)). Moreover, those with atrial fibrillation were more likely to be men, were taller, weigh more and were more likely to use blood pressure lowering medication, to have heart failure, a history of myocardial infarction, or to have diabetes (Table 1).

Table 1. Characteristics of Study Cohort, at index date (n=6,934)

Characteristic	No Atrial fibrillation (n=6,401)	Atrial fibrillation (533)	P-value[a]
Age (yrs)	72.4 (8.8)	78.9(8.0)	<0.01
Sex (women)	3,818(59.6)	291(54.6)	<0.01
Height (cm)	167.0(8.6)	168.0(8.2)	<0.01
Weight (kg)	75.4(12.0)	76.3(11.0)	<0.01
Use of blood pressure lowering medication	2281(35.6)	326(61.2)	<0.01
History of Myocardial Infarction	565(8.8)	91(17.1)	<0.01
Prevalent Heart failure	266(4.2)	153(28.7)	<0.01
Prevalent Diabetes Mellitus	888(13.9)	110(20.6)	<0.01

Denotes mean±SD for continuous variables, and n (%) for categorical variables
[a]From logistic or linear regression, age- and sex-adjusted

The prevalence of atrial fibrillation in men was 8.6% and in women 7.1% (Table 2.). In the age stratum of 55-59 years the prevalence was 1.3% in men, and 1.7% in women. The prevalence was higher with advancing age; for those >85 years of age 24.2% of men, and 16.1% of women had atrial fibrillation.

During follow-up of the Rotterdam Study, the age-adjusted risk of atrial fibrillation did not significantly change between 1994 and 2006 (age-adjusted per year of follow-up OR:1.004, 95%-CI:0.995 to 1.014), and was similar in men and women (age adjusted OR:1.004 (95%CI:0.989-1.018) in men, age-adjusted OR:1.004(95%CI:0.992-1.017), in women) (Supplemental table 1).

Estimates of atrial fibrillation in the Netherlands

Using the prevalence estimates from the study population, we estimate approximately 259,600 (95%-CI projections: 189,300 - 367,600) adults have atrial fibrillation in the Netherlands in 2010, reflecting 1.6% of the total Dutch population (Table 3 and Figure 1). Approximately half of them were men (n=128,100). If the prevalence estimates remain stable, the number of individuals with atrial fibrillation will more than double to a peak of about 553,700 (95%-CI projections:

Table 2. Age- and sex-specific atrial fibrillation prevalence.

	N	n	%	95% CI
		Men		
55-59	235	3	1.3	(0.4-3.6)
60-64	429	8	1.9	(0.9-3.6)
65-69	567	31	5.5	(4.0-7.8)
70-74	617	45	7.3	(5.7-9.6)
75-80	472	59	12.5	(9.8-15.8)
80-84	316	51	16.1	(12.4-20.4)
>85	186	45	24.2	(18.5-30.7)
Total	**2825**	**242**	**8.6**	**(7.6-9.7)**
		Women		
55-59	286	5	1.7	(0.7-4.0)
60-64	523	7	1.3	(0.6-2.7)
65-69	738	20	2.7	(1.8-4.2)
70-74	778	40	5.1	(3.8-6.9)
75-80	712	68	9.6	(7.6-11.9)
80-84	556	68	12.2	(9.7-15.1)
>85	516	83	16.1	(13.1-19.4)
Total	**4,109**	**291**	**7.1**	**(6.3-7.9)**
		All		
Total	**6,934**	**533**	**7.7**	**(7.0-8.3)**

Abbreviations: CI, Confidence interval; N, total number of participants; n, number of participants with atrial fibrillation

420,900 - 736,500) in the year 2050 and then decreases slightly to 547,700 (95%-CI projections: 416,000 - 729,100) in the year 2060. In 2060 the number of adults over 55 with atrial fibrillation would reflect 3.2% of the Dutch population. This increase is similar in men and women. Especially, the number of adults over age 75 with atrial fibrillation will increase from 156,500 in 2010 to 418,100 in 2060, whereas in adults younger than 75 years of age this number would only increase from 103,100 in 2010 to 129,600 in 2060. If we assume a yearly rise in the prevalence of 0.4%, we project that the total number of adults with atrial fibrillation could even increase to approximately 690,400 in the Netherlands, by 2060 (Supplemental table 2, Supplemental figure 1).

Estimates of atrial fibrillation in the European Union

We estimate that there were approximately 8.8 million adults with atrial fibrillation in the European Union (95%-CI projections: 6.5 - 12.3 million), in 2010 (Table 3 and Figure 2). If the prevalence estimates of atrial fibrillation remain stable, this number will more than double and could reach 17.9 million by the year 2060 (95%-CI projections: 13.6 million - 23.7 million). In

Table 3. Projected Number of Adults with Atrial Fibrillation in The Netherlands and the European Union between 2000 and 2060

	The Netherlands (in thousands)						European Union (in millions)					
Year	**Men**	**Women**	**Age <75**	**Age >75**	**Total**	**%***	**Men**	**Women**	**Age <75**	**Age >75**	**Total**	**%***
2000	98.2	112.9	83.9	127.3	211.1	1.3	3.4	3.9	2.9	4.3	7.2	1.5
2005	110.2	121.1	92.7	138.6	231.3	1.4	3.7	4.2	3.1	4.9	7.9	1.6
2010	128.1	131.5	103.1	156.5	259.6	1.6	4.2	4.6	3.2	5.6	8.8	1.8
2015	151.2	145.0	119.8	176.3	296.1	1.7	4.8	5.0	3.4	6.3	9.8	1.9
2020	175.9	162.2	135.6	202.5	338.0	2.0	5.3	5.4	3.8	6.9	10.7	2.1
2025	203.9	183.5	139.0	248.4	387.5	2.2	5.9	5.8	4.0	7.7	11.7	2.3
2030	231.3	205.7	145.5	291.5	436.9	2.5	6.6	6.3	4.2	8.6	12.9	2.5
2035	255.8	226.9	147.1	335.7	482.8	2.7	7.2	6.9	4.4	9.7	14.1	2.7
2040	272.9	244.3	140.8	376.3	517.1	2.9	7.9	7.4	4.4	10.9	15.3	2.9
2045	284.1	256.5	129.4	411.3	540.7	3.1	8.4	7.9	4.3	12.0	16.3	3.1
2050	291.0	262.7	122.3	431.4	553.7	3.2	8.9	8.2	4.2	12.9	17.1	3.3
2055	291.7	261.9	125.4	428.1	553.6	3.2	9.2	8.4	4.2	13.4	17.6	3.4
2060	289.7	258.0	129.6	418.1	547.7	3.2	9.4	8.5	4.1	13.8	17.9	3.5

* Percentage of total population

2010 the number of adults age 55 and over with atrial fibrillation would reflect 1.8% of the total population, and this number could rise to 3.5% by 2060. Especially the number of adults over age 75 with atrial fibrillation will increase from 5.6 million in 2010 to 13.8 million in 2060. If we assume a yearly rise of 0.4% in the prevalence of atrial fibrillation, we project that the number of adults over 55 years of age who will have atrial fibrillation would increase to approximately 22.6 million in the European Union by 2060 (Supplemental table 2 and Supplemental figure 2).

DISCUSSION

Due to the expected aging of the European population the number of individuals with atrial fibrillation is likely to increase. We project that from 2010 to 2060, the number of adults age 55 and over with atrial fibrillation will more than double in the Netherlands and in the European Union. Most of the projected increase will occur until 2050. Especially the number of adults older than 75 years with atrial fibrillation will increase substantively. As atrial fibrillation is associated with significant morbidities and mortality, this increasing number of individuals with atrial fibrillation may have major public health implications.

Several studies estimated the atrial fibrillation prevalence in European populations.[22-29] Our atrial fibrillation prevalence estimates are broadly similar to estimates from Iceland, Germany, the United Kingdom and Italy,[23-26] and our prevalence estimates are higher compared to those from other studies in the United Kingdom, Greece and Sweden.[22, 27-29] Several reasons for these

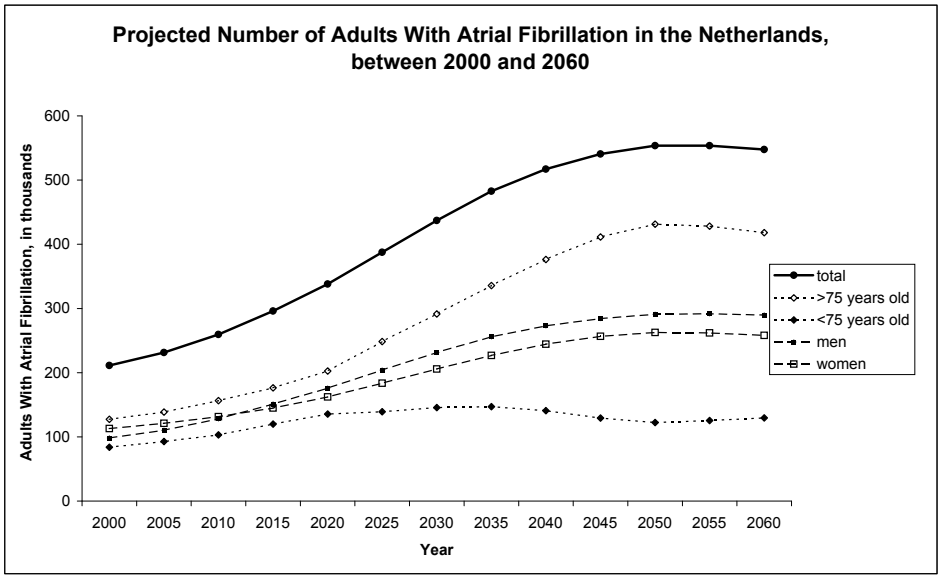

Figure 1. Projected number of adults with atrial fibrillation in the Netherlands between 2000 and 2060.

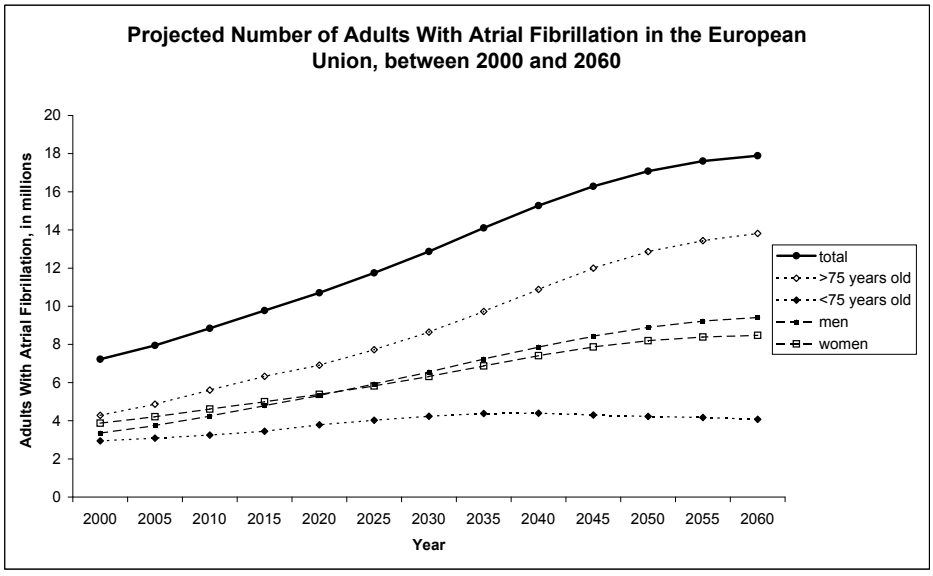

Figure 2. Projected number of adults with atrial fibrillation in the European Union between 2000 and 2060.

differences can be raised. First, differences in assessment may play a role. As atrial fibrillation often escapes clinical attention, prevalence estimates are easily underestimated. Furthermore, racial or cultural differences may partly explain the differences as it has been suggested that

whites have a higher risk of atrial fibrillation than non-whites.[30-32] Finally, as it was suggested that the prevalence of atrial fibrillation has increased, older studies may present lower prevalence estimates.[20-23]

Several studies have projected future numbers of adults with atrial fibrillation in the United States, all of them projecting a doubling of the number of atrial fibrillation patients by the year 2050.[30, 33, 34] Go et al. estimated that the prevalence of adults with atrial fibrillation in the US will rise from 2.66 million in 2010 to 5.61 million in 2050.[30] Miyasaka et al. estimated the United States prevalence of atrial fibrillation to be 6.1 million in 2010 and projected an increase to 12.1 million in 2050.[33] Finally, Nacarelli et al. estimated the number of adults in the US with atrial fibrillation to rise from 3,03 million in 2005 to 7,56 million in 2050.[34] Again, differences between these studies may be explained by differences in study setting, racial variation, and differences in atrial fibrillation assessment. Of note, projections for European populations are limited to Stefansdottir et al. who estimated that the number of Icelandic inhabitants with atrial fibrillation will more than double, rising from 4495 (2.0% of total population) in 2008 to 11 088 in 2050 (3.5% of total population).

The strength of our study is that our projections are based on prevalence estimates from a population-based cohort study, the Rotterdam Study, with extensive follow-up with examination at 3-4 yearly intervals, and a continuous registration of disease and mortality. First, we used screening ECGs at each follow-up examination. Second, we used all information available in the general practitioners' files of all participants. In the Dutch health care system, patients have one general practitioner, who serves a gatekeeper function, including a registration and filing of medical information from their own work as well as the results from other physicians practicing in hospitals and outpatient clinics. When individuals switch to another general practitioner, all medical information is transferred to the new practice. Finally we used a nation-wide registry of all hospital discharge diagnoses.

However, our study has some limitations. Our projections are based on some assumptions. Most importantly, our projections are based on a Dutch population of mainly north-western European descent. As whites have a higher risk of atrial fibrillation than non-whites,[30-32] our projections for the European Union might be overestimated because of ethnic variation within the European Union. Also other risk factors for atrial fibrillation such as, height, weight and cardiovascular disease may vary between European countries, and thereby may lead to differences in atrial fibrillation prevalence. The numbers of individuals should therefore be cautiously interpreted and only be regarded as estimations. Second, in the main analyses we assumed that the atrial fibrillation prevalence remains stable whereas several studies suggested that the prevalence is rising over time.[20-23] This rise in prevalence might be explained by more clinician monitoring and awareness of atrial fibrillation, improved survival of atrial fibrillation patients, or from increased prevalence of clinical conditions that are associated with a higher risk of atrial fibrillation.[35] Indeed, it may not be realistic to assume that this increase will continue for future decades. It is also the possible consequence of increased attention to cardiovascular risk factors

and subsequent treatment may reduce the risk of atrial fibrillation.[35] The rise in age-adjusted prevalence during the years of follow up in the Rotterdam Study did not reach statistical significance. Therefore, we assumed in our main results that the prevalence of atrial fibrillation is stable over time - if at all, this might have led to some underestimation in these results.

In the European Union, the number of adults with atrial fibrillation will substantially increase in the coming decades and may even double in the period from 2010 to 2060. As atrial fibrillation is associated with significant morbidities and mortality, this increasing population burden of atrial fibrillation will have major public health implications. Our results underly the importance of preventative strategies aiming to reduce the risk of atrial fibrillation within comprehensive programs aimed to improve the health of the ageing population.

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SUPPLEMENTARY MATERIAL

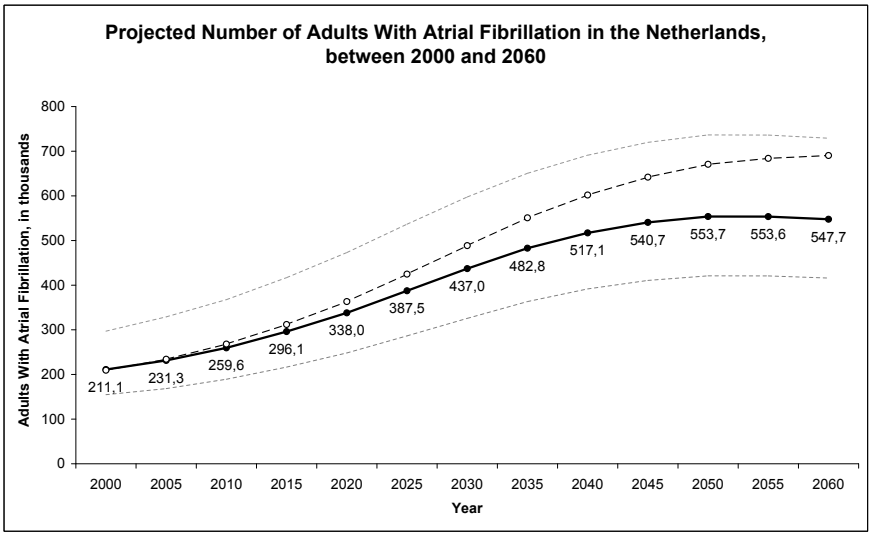
Supplemental table 1. Secular trends atrial fibrillation prevalence (%), at January 1 of each year

	1994	1996	2002	2006	Exp(B)*	(95%-CI)
Men	(183/2583) 7.1	(197/2423) 8.1	(242/2825) 8.6	(224/2338) 9.6	1.004	(0.989 to 1.018)
Women	(223/3849) 5.8	(224/3399) 6.2	(291/4109) 7.1	(279/3521) 7.9	1.004	(0.992 to 1.017)
Total	(406/6432) 6.3	(421/6046) 7.0	(533/6934) 7.7	(503/5859) 8.6	1.004	(0.995 to 1.014)

* Of annual change in atrial fibrillation prevalence. Age and sex adjusted

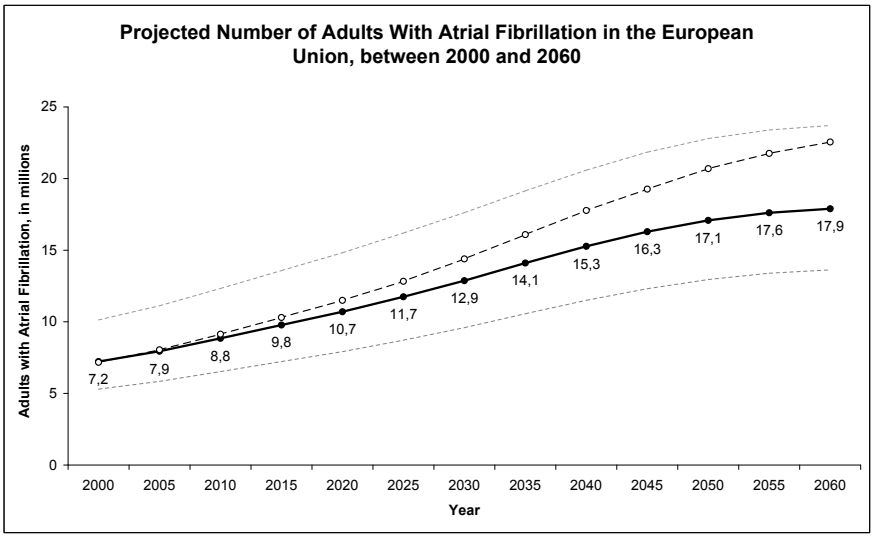
Supplemental table 2. Projected Number of Adults with Atrial Fibrillation in The Netherlands and the European Union between 2000 and 2060, assuming stable prevalence and assuming yearly increasing prevalence (0.4%), starting from January 1st 2002.

	The Netherlands (in thousands)		European Union (in millions)	
Year	Stable Prevalence	Increasing Prevalence	Stable Prevalence	Increasing Prevalence
2000	211.1	209.4	7.2	7.2
2005	231.3	234.1	7.9	8.0
2010	259.6	268.0	8.8	9.1
2015	296.1	311.9	9.8	10.3
2020	338.0	363.2	10.7	11.5
2025	387.5	424.7	11.7	12.9
2030	436.9	488.6	12.9	14.4
2035	482.8	550.8	14.1	16.1
2040	517.1	601.9	15.3	17.8
2045	540.7	641.9	16.3	19.3
2050	553.7	670.6	17.1	20.7
2055	553.6	684.0	17.6	21.8
2060	547.7	690.4	17.9	22.6



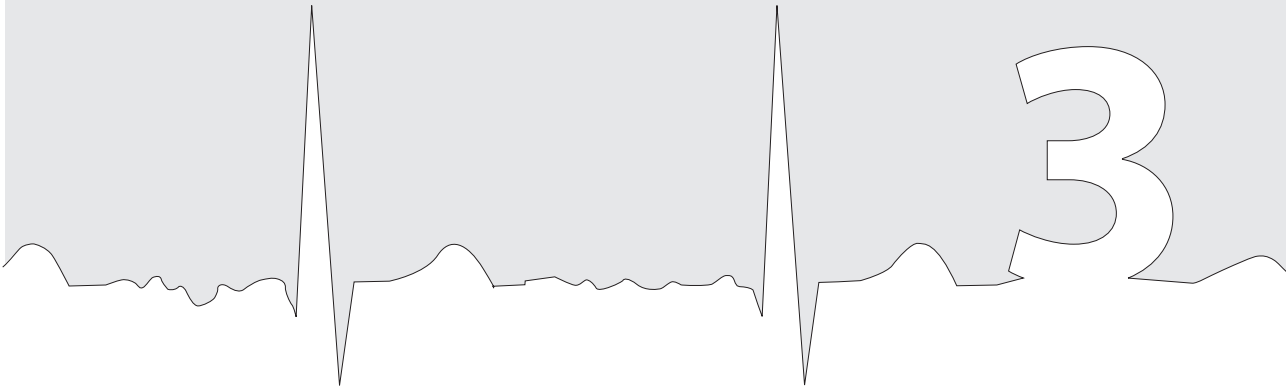
Supplemental figure 1. Projected number of adults with atrial fibrillation in the Netherlands between 2000 and 2060, assuming yearly rise in atrial fibrillation prevalence.

Bold solid line curve represent projections if atrial fibrillation prevalence to remain stable, dotted curves represent the projections of the 95% confidence intervals. Dashed curve assumes atrial fibrillation prevalence to yearly rise.



Supplemental figure 2. Projected number of adults with atrial fibrillation in the European Union between 2000 and 2060, assuming a yearly rise in atrial fibrillation prevalence.

Bold solid line curve represent projections if atrial fibrillation prevalence to remain stable, dotted curves represent the projections of the 95% confidence intervals. Dashed curve assumes atrial fibrillation prevalence to yearly rise.



CHAPTER 3

Risk factors for atrial fibrillation

CHAPTER 3.1

Unrecognized myocardial infarction

ABSTRACT

Background: Persons with a clinically recognized myocardial infarction are at increased risk for atrial fibrillation. However a large proportion of all myocardial infarctions remains clinically unrecognized. Whether subjects with electrocardiographic signs of an unrecognized myocardial infarction are also at an increased risk of developing atrial fibrillation is unknown. The objective of this study was to investigate whether unrecognized myocardial infarction was associated with an increased risk of atrial fibrillation in a prospective population-based cohort study.

Methods: The study is set within the prospective population-based Rotterdam Study. The study population comprised 2,505 men and 3,670 women without atrial fibrillation at baseline. Participants were classified based on electrocardiography, interview, and clinical data into those with recognized myocardial infarction , those with ECG based unrecognized myocardial infarction and those without myocardial infarction . Atrial fibrillation was ascertained from ECG assessments as well as medical records.

Results: During a mean follow-up of 11.7 years (SD 5.0), 329 men and 398 women developed atrial fibrillation. Unrecognized myocardial infarction was associated with a two-fold risk of developing atrial fibrillation in men (HR: 2.21, 95%CI:1.51 to 3.23) compared to men without a history of myocardial infarction, independent of age, and cardiovascular risk factors. In women, unrecognized myocardial infarction was not associated with atrial fibrillation (HR: 0.92, 95%CI:0.59 to 1.44).

Conclusion: The presence of an unrecognized myocardial infarction is associated with a twofold increased risk of atrial fibrillation in men, independent of known cardiovascular risk factors.

INTRODUCTION

Atrial fibrillation is the most common sustained arrhythmia in the older population. Its prevalence and incidence increase with age,[1-4] Atrial fibrillation has significant impact on prognosis and quality of life. It is a major cause of morbidities such as dementia,[5] stroke,[6] and heart failure[7] and it is also associated with increased cardiovascular and overall mortality.[8-10] Persons with a history of myocardial infarction (MI) are at increased risk of atrial fibrillation.[4, 11] However, it has previously been demonstrated that the overall detection of MIs is far from complete and that a large proportion of all MIs remains clinically unrecognized.[12-13] It was previously estimated that the proportion of unrecognized MIs from all MIs ranges from 21 to 33% in men and 26 to 54 % in women.[12-17] Furthermore, in approximately one to six percent of the general elderly population electrocardiographic characteristics can be detected of an unrecognized MI.[12, 14, 18] Whether subjects with electrocardiographic signs of an unrecognized MI are also at an increased risk of developing atrial fibrillation is unknown.

Therefore, the objective of this study was to investigate whether unrecognized MI was associated with an increased risk of atrial fibrillation in a prospective population of community-dwelling elderly.

METHODS

Study population

The current study was performed within the Rotterdam Study, a population-based prospective cohort study, designed to examine the onset of, and risk factors for disease in older adults, which started with a baseline visit between 1990 and 1993.[19] All participants aged 55 years and over in the Ommoord district of Rotterdam, The Netherlands were invited to participate (n=10,275). Of them, 7,983 (78%) participated in the study. At baseline, participants were interviewed at home and were examined at the research center, which included a 10 second, 12-lead resting electrocardiogram (ECG). From that visit onwards, participants were followed continuously and re-examined at three follow-up examination rounds (1993-1995, 1997-1999, and 2002-2004). Information on the presence and occurrence of disease at baseline and during follow-up is available by collaboration with the general practitioners in the study area. General practitioners in the Netherlands have a key position in the Dutch healthcare system. They register all diagnoses available from their own work and the work from physicians in the hospital and the out-patient clinic. The medical ethics committee of the Erasmus Medical Center, Rotterdam, approved the study, and all participants gave informed consent to participate in the study and to obtain information from treating physicians. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.[20]

Myocardial infarction assessment

ECGs were processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurement and interpretation.[21-23] To determine MI, MEANS uses an extensive set of criteria that is partially derived from the Minnesota code.[24] Subsequently, two research physicians blinded to other clinical information validated the ECGs selected by MEANS. A cardiologist, who specialized in ECG methodology, ascertained the final diagnosis of MI. The diagnosis of MI using MEANS is mainly driven by pathological Q waves and auxiliary criteria, such as QR ratio and R-wave progression. ST-T changes were not considered as criteria for MI by MEANS, but were taken into account by the cardiologist validating and ascertaining the diagnosis of MI based on the ECG. Assessment of recognized MI status was done by verification of the medical records after either self-reported MI or ECG abnormalities indicative of prior MI, as reported previously for the Rotterdam Study.[25] We classified participants at baseline as follows: a history of 'recognized MI' included people with self-reported MI and/or ECG characteristics matching an MI, confirmed by clinical data. A history of 'unrecognized MI' included all participants without documented or self-reported MI, but with ECG characteristics matching an MI. All other participants were classified as having 'no MI'.

Atrial fibrillation assessment

Prevalent and incident atrial fibrillation was ascertained using three methods.[1] At baseline and at each follow-up examination an ECG was recorded, stored digitally, and analyzed by MEANS.[21-23] Notably, MEANS is characterized by a high sensitivity (96.6%) and a high specificity (99.5%) in coding arrhythmias.[21] Additionally, information was obtained from the treating general practitioners, which included their own results as well as results from medical specialists practicing in hospitals and outpatient clinics. Finally, information was obtained from a nationwide medical registry of all hospital discharge diagnoses. To verify the diagnosis of atrial fibrillation, all ECGs with a diagnosis of atrial fibrillation, atrial flutter, or any other rhythm disorder were coded independently by two research physicians who were blinded to the MEANS diagnosis. The judgment of a cardiologist was taken as decisive in those cases in which disagreement persisted between the coding physicians. We did not distinguish between atrial fibrillation and atrial flutter when we identified cases because both conditions are very similar with respect to risk factors and consequences.[26-27] Also, we did not discriminate between paroxysmal atrial fibrillation and chronic atrial fibrillation. The date of incident atrial fibrillation was defined as the date of the first occurrence of symptoms with subsequent ECG verification. In a minority of the cases, when atrial fibrillation had been diagnosed at the research center only and when no further information was available on a more precise date of onset, we defined the date of onset as the midpoint of the time interval between examination at which atrial fibrillation was detected and the previous examination at the research center.

Vital status

Information on vital status of each participant was obtained on a weekly basis from the Central Population Register of the municipality of Rotterdam, from collaborating general practitioners, and by collecting information during follow-up examination rounds. If no information could be obtained from these sources, the Central Registry of Genealogy of the Netherlands was consulted. This national institute receives population registry records of all inhabitants of the Netherlands who have died.

Covariable assessment

Age at baseline was included in all analyses. Body mass index (BMI) was calculated by dividing weight in kilograms by height in squared meters (kg/m^2). Blood pressure was measured twice at the right upper arm with a random zero Hg sphygmomanometer in the sitting position. Systolic and diastolic blood pressures were calculated as the average of the two consecutive measurements. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol levels were measured with an automated enzymatic method. Data on medication use were obtained during the home interview by copying the labels of all the medication used. Information on smoking status was also acquired from the home interview. Heart failure was assessed using a validated score based on the definition of heart failure by the European Society of Cardiology.[28-29] Prevalent chronic obstructive pulmonary disease (COPD) was obtained from the medical records, and defined as the diagnosis of COPD by a medical specialist.[30] Prevalent diabetes mellitus was defined as the use of anti-diabetic medication or a pre- or post-load serum glucose level of >11.0 mmol/L.

Population for analysis

The study population comprised 7,983 persons. Persons who did not visit the research center at baseline and therefore did not have an ECG recorded were excluded (n=894). Also those with missing baseline ECG data, due to technical problems or lack of qualified personnel, were excluded (n=536). Furthermore 378 participants with prevalent atrial fibrillation at baseline were excluded from the analyses. This resulted in a population for analyses of 6,175 participants. All participants were followed from their baseline ECG assessment in the Rotterdam Study (1990-1993) until the date of diagnosis of atrial fibrillation, the date of death, loss to follow up, or end of the study period (January 1st, 2008).

Statistical analyses

All analyses were stratified by sex. Participants were categorized as those having unrecognized MI on baseline ECG, those with clinically recognized MI at baseline, and as those without a history of MI. Baseline characteristics of all participants were compared according to these categories. We then assessed the association of recognized and unrecognized MI with atrial fibrillation, using Cox proportional hazards regression analyses. First, we adjusted only for age. Age-adjusted hazard curves are presented comparing the cumulative incidence of atrial fibrillation between

the MI categories. Second, we additionally adjusted for the following cardiovascular risk factors at baseline: systolic and diastolic blood pressure, use of blood pressure lowering medication, BMI, total and HDL cholesterol, smoking status, diabetes mellitus, COPD, and heart failure. Finally, we adjusted for incident heart failure during follow-up (time-dependent). Approximately 5% of the participants had missing values for one or more covariables. These missing values were handled using the expectation maximization algorithm. All measures of association are presented with 95% CIs. Data were analysed using the SPSS PASW statistical package, version 17.0 (IBM corporation).

RESULTS

Baseline characteristics

Baseline characteristics of the study population are described in table 1. The population included 2,505 men of whom 142 were classified with an unrecognized MI and 270 with a

Table 1. Baseline characteristics of the study population.

Characteristics	Men			Women		
	No MI	Recognized MI	Unrecognized MI	No MI	Recognized MI	Unrecognized MI
N	2093	270	142	3365	112	193
Age (years)	67.2 (7.9)	68.8 (9.0)[a]	69.7 (8.6)[a]	68.8 (9.0)	74.6(8.6)[a]	74.0(9.3)[a]
Systolic Blood pressure (mmHg)	139 (22)	135 (20)[b]	144 (21)[b, c]	139 (23)	138(26)	147 (20)[b, c]
Diastolic blood pressure (mmHg)	75 (11)	72 (10)[b]	76 (13)[c]	73 (11)	70(14)[b]	75(11)[b,c]
Blood pressure lowering drugs	445(21.3)	185(68.5)[b]	36(25.4)[c]	1021(30.3)	91(81.3)[b]	69(35.8)[c]
Total cholesterol(mmol/L)	6.3(1.2)	6.5(1.2)	6.4(1.2)	6.9(1.2)	7.0(1.3)	6.9(1.3)
HDL cholesterol(mmol/L)	1.2(0.3)	1.1(0.3)	1.2(0.3)	1.5(0.4)	1.3(0.4)	1.4(0.3)
BMI (kg/m2)	25.6(2.9)	26.1(3.1)[b]	25.8(3.6)	26.7(3.9)	27.3(3.9)	27.8(5.0)[b]
Smoking:						
- Never	179(8.6)	14(5.2)[b]	11(7.7)	1794(53.3)	57(50.9)[b]	106(54.9)
- Current	626(29.9)	58(21.5)[b]	58(40.8)[b,c]	622(18.5)	19(17.0)	38(19.7)[b]
- Former	1288(61.5)	198(73.3)[b]	73(51.4)[b,c]	949(28.2)	36(32.1)[b]	49(25.4)
Diabetes	183(8.7)	42(15.6)[b]	25(17.6)[b]	322(9.6)	20(17.9)	27(14.0)
COPD	106(5.1)	13(4.8)	5(3.5)	88(2.6)	2(1.8)	4(2.1)
Heart failure	14(0.7)	29(10.7)[b]	8(5.6)[b, c]	55(1.6)	28(25.0)[b]	20(10.4)[b, c]
Q-wave MI on ECG	0(-)	145(53.7)	142(100.0)	0(-)	51(45.5)	193(100.0)

All values are means (standard deviations) or absolute numbers (%).

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; HDL, high-density lipoprotein; MI, myocardial infarction; N. number at risk

[a] *p*-value<0.05, compared with no MI

[b] *p*-value<0.05, compared with no MI, adjusted for age

[c] *p*-value<0.05, compared with recognized MI, adjusted for age

recognized MI. Of the 3,670 women, 193 were classified with an unrecognized MI and 112 with a recognized MI. Compared to those without a history of MI, participants with an unrecognized MI were older, had higher systolic and diastolic blood pressure, were more likely to smoke and to have heart failure independent of age. Men with unrecognized MI were also more likely to have diabetes than men without MI. Compared to participants with a recognized MI, participants with an unrecognized MI had a higher systolic blood pressure, were less likely to use blood-pressure lowering medication and were less likely to have been diagnosed with heart failure. Additionally, men with unrecognized MI were more likely to smoke than those with a recognized MI. During a mean follow-up of 11.7 years (SD 5.0), 727 participants, of whom 329 men, developed atrial fibrillation, and 2,301 died. During follow-up, 638 participants developed heart failure.

Unrecognized MI and risk of atrial fibrillation

Figure 1 displays the age-adjusted hazard curves for developing atrial fibrillation for men and women separately, based on the presence of recognized and unrecognized MI. Compared to men without MI, men with unrecognized MI had a higher risk of atrial fibrillation (age-adjusted HR of atrial fibrillation: 2.36 (95%CI: 1.62 to 3.43) (table 2). The age-adjusted HR of atrial fibrillation in men with recognized MI was 1.70 (95%CI: 1.26 to 2.31). Additional adjustment for the other covariables slightly attenuated the estimate to 2.21 (95%CI: 1.51 to 3.23) for unrecognized MI and remained unchanged for recognized MI. Finally, adjustment for heart failure during follow-up lowered the associations to 2.03 (95%CI: 1.38 to 2.97) for unrecognized MI and 1.48 (95%CI: 1.07 to 2.05) for recognized MI.

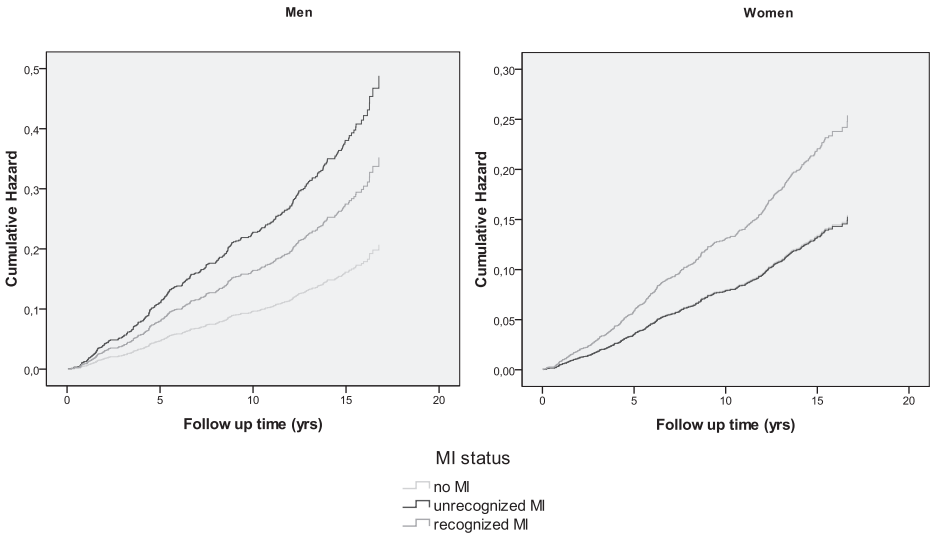


Figure 1. Age-adjusted hazard curves for the risk of atrial fibrillation for men and women, seperately.

In women, unrecognized MI was not associated with atrial fibrillation. Adjusted for age, the HR was 0.99 (95%CI: 0.64 to 1.54), and additional adjustment for the other risk factors only slightly changed this estimate (HR: 0.92, 95%CI: 0.59 to 1.44). Recognized MI was significantly associated with atrial fibrillation in women, when adjusted for age (HR: 1.64, 95%CI: 1.02 to 2.65). However, after adjustment for other risk factors this association was no longer statistically significant (HR: 1.36, 95%CI: 0.82 to 2.26).

Table 2. Hazard rates on the association of unrecognized MI with atrial fibrillation, stratified on sex

	N	n (%)	Model 1[a]	Model 2[b]
			HR (CI)	HR (CI)
Men				
No MI	2093	248 (11.8)	1.0 (Ref.)	1.0 (Ref.)
Recognized MI	270	50 (18.5)	1.70 (1.26-2.31)	1.66 (1.21-2.29)
Unrecognized MI	142	31(21.8)	2.36 (1.62-3.43)	2.21 (1.51-3.23)
Women				
No MI	3365	359 (10.7)	1.0 (Ref.)	1.0 (Ref.)
Recognized MI	112	18 (16.1)	1.64 (1.02-2.65)	1.36 (0.82-2.26)
Unrecognized MI	193	21 (10.9)	0.99 (0.64-1.54)	0.92 (0.59-1.44)

Abbreviations: HR, Hazard rate ratio; MI, myocardial infarction; N, number at risk; n, number of atrial fibrillation events (% of N)

[a]Adjusted for age.

[b]Additionally adjusted for systolic and diastolic blood pressure, use of bloodpressure lowering drugs, BMI, total cholesterol, HDL cholesterol, smoking status, diabetes mellitus, chronic obstructive pulmonary disease and/or heart failure at baseline.

DISCUSSION

Our results indicate that unrecognized MI in men is associated with a more than two-fold increased risk of developing atrial fibrillation, compared to men without a history of MI. This association was independent of cardiovascular risk factor. In women, unrecognized MI was not associated with an increased risk of atrial fibrillation.

Unrecognized MI has previously been associated with an increased risk of cardiovascular and total mortality comparable to recognized MI.[13, 15-17] Furthermore, unrecognized MI is reported to be associated with increased risks of morbidities, such as recurrent coronary heart disease,[31-32] stroke,[32] and heart failure.[32] These results were later replicated within the Rotterdam Study for stroke,[33] heart failure[34] and also dementia.[35] Like our current results, these studies showed that unrecognized MI was a strong risk factor for disease in men, but not in women.

Benjamin et al previously showed that the association of recognized MI with atrial fibrillation was different for men and women.[4] They found that clinically recognized MI was significantly

associated with atrial fibrillation in men, independent of cardiovascular risk factors. In women however, recognized MI was associated with atrial fibrillation after adjustment for age, but this association was no longer statistically significant after adjustment for other cardiovascular risk factors. Our results show a similar difference in prognosis between men and women for both recognized and unrecognized MI.

Several mechanisms may explain the association of MI with atrial fibrillation. Possibly atrial dysfunction or atrial stretching in response to infarction plays a role. This can lead to increased atrial pressures and a restrictive filling pattern, which has previously been related to development of atrial fibrillation.[36] Moreover, in reaction to atrial stretching catecholamines are produced, which have previously been associated with atrial fibrillation.[37] Also it has been suggested that atrial fibrillation follows secondary to left ventricular dysfunction and hemodynamic disturbances after MI.[38-39] Finally, atrial ischemia might create both atrial fibrillation triggers as well as a substrate for atrial fibrillation maintenance.[40] It is unclear why the association of MI with atrial fibrillation is different between men and women. In our study, recognized MI was associated with atrial fibrillation in men and women after adjustment for age. In women this association did not remain statistically significant after adjustment for cardiovascular risk factors. It might be possible this can be explained by a lack of power. However it has also been suggested that in response to acute coronary ischemia women are relatively protected from apoptosis and experience less adverse cardiac remodeling than men.[41] It remains to be elucidated to what extend this contributes to the development of atrial fibrillation.

While for recognized MI the risk estimate was lower in women than in men, the most obvious difference between men and women was seen for unrecognized MI. It has been suggested that the pathophysiology of unrecognized MI is similar to those that go clinically recognized.[18] This suggests that the most likely explanation for the absence of the association of unrecognized MI with atrial fibrillation in women is misclassification of this condition. Murabito et al. suggested that ECG abnormalities not caused by coronary artery disease but resulting from misplacement of ECG electrodes due to difficulties with lead placement owing to breast tissue, can be mistaken for MI.[42] Nevertheless, the reason behind the currently identified gender differences remain unexplained and deserve further evaluation.

Finally, our results suggest that the risk of atrial fibrillation is somewhat higher for those with unrecognized MI than for those with recognized MI. We also found that those with an unrecognized MI had higher systolic and diastolic blood pressure, were less likely to use antihypertensive drugs, and were more likely to smoke, compared to those with recognized MI. It is possible that lifestyle changes and treatment following the diagnosis of MI, lower the risk of atrial fibrillation and thereby explain why recognized MI is associated with a lower risk of atrial fibrillation than unrecognized MI.

Strengths of this study are the population-based design, with a long-term follow-up of 11.7 years. We were able to use data from a large population that included 6,175 participants of whom 335 had ECG characteristics matching an unrecognized MI at baseline and 692 who

developed atrial fibrillation during follow-up. Also, at baseline we did not inform participants or their treating physicians about the finding of an unrecognized MI on their ECG. The decision reflected the perception at that time that an unrecognized MI was less severe than a recognized MI, and was motivated by a lack of evidence that treatment could effectively reduce the risk of subsequent cardiovascular disease.[33] Our results therefore adequately assess the natural history of unrecognized MI with atrial fibrillation in the general population. Since the fourth research visit in 2002 we started to report findings of unrecognized MI to the participants and treating doctors.

By combining multiple sources of data on the occurrence of atrial fibrillation we limited the chance of misclassification. It is possible that the classification of unrecognized MI includes some misclassification, for instance in the case of non-Q-wave MIs or Q-waves that have disappeared over time. Finally, because we were unaware of the exact date of the occurrence of the unrecognized MI, we used the date of the ECG as the date of unrecognized MI diagnosis. This restricts our findings to the long-term risk of atrial fibrillation. Finally we were not able to adjust our analyses for valvular disease or thyroid function which are known risk factors for atrial fibrillation.

Unrecognized MI as identified by ECG is associated with a more than twice increased risk of atrial fibrillation in men, compared to those without an MI. Atrial fibrillation is an important health problem. Subjects with unrecognized MI are not treated for the disease, while detection of the MI followed by lifestyle changes and appropriate treatment may lower the risk of atrial fibrillation.

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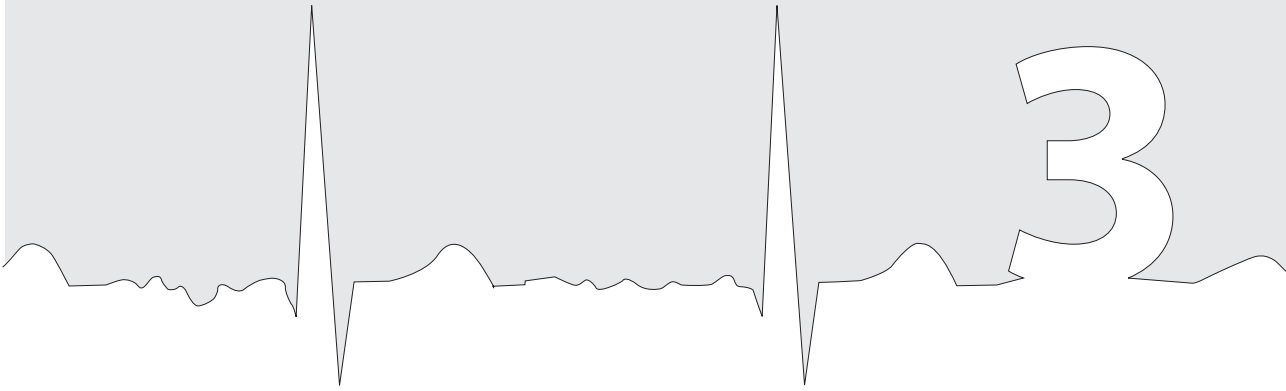

CHAPTER 3.2

Serum dehydroepiandrosterone sulfate levels

ABSTRACT

Background: High plasma dehydroepiandrosterone sulfate (DHEAS) levels have been associated with a reduced risk of cardiovascular disease and atherosclerosis. To our knowledge, no previous follow-up study investigated the association between DHEAS and development of atrial fibrillation. Our objective was to investigate the association between DHEAS levels and incident atrial fibrillation.

Methods: The study is set within a random sample of the prospective population-based Rotterdam Study. The study population comprised 1,180 participants without atrial fibrillation at baseline for whom baseline levels of DHEAS were measured in plasma. Atrial fibrillation was ascertained from centre visit ECG assessments as well as medical records.

Results: During a mean follow-up of 12.3 years, 129 participants developed atrial fibrillation. DHEAS levels were inversely associated with the risk of atrial fibrillation (HR per SD:0.74, 95%CI:0.58-0.94). Subjects in the highest DHEAS quartile had an almost three times lower risk of atrial fibrillation during follow-up, compared to those in the lowest DHEAS quartile (HR: 0.34, 95%-CI:0.18-0.64) adjusted for age, sex and cardiovascular risk factors.

Conclusions: DHEAS can be regarded as an important indicator of future atrial fibrillation in both men and women, independent of known cardiovascular risk factors.

INTRODUCTION

Dehydroepiandrosterone sulfate (DHEAS) is a precursor in the biosynthetic pathway of androgenic and estrogenic sex steroids. It is sulfated from dehydroepiandosterone (DHEA) by sulfotransferase in the adrenal glands, and in small part by the liver and small intestine. In blood, most of the DHEA is found as DHEAS which – due to its long plasma half-life - has much higher concentrations than any other sex steroid and shows little diurnal variation.[1] Previous studies indicated that higher DHEAS levels are associated with a lower risk of cardiovascular disease[2-3] and lower all-cause and cardiovascular mortality.[2, 4-7] These associations might in part be explained by results of later studies that suggested that high DHEAS levels are associated with a reduced risk of atherosclerosis.[8-10]

Both a history of cardiovascular disease as well as atherosclerosis are important independent risk factors for atrial fibrillation.[11-12] Atrial fibrillation is the most common sustained arrhythmia in the older population. Atrial fibrillation is associated with increased morbidity such as heart failure[13] and stroke[12] and also with increased total as well as cardiovascular mortality.[12, 14] Whether DHEAS levels are associated with the risk of atrial fibrillation is unknown.

The objective of this study was to investigate the association between DHEAS and incident atrial fibrillation, in a population-based setting. As sex specific effects may arise from DHEAS we also investigated the association in men and women separately.

METHODS

Study population

The current study was performed within The Rotterdam Study, a population based prospective cohort study designed to examine the onset of, and risk factors for disease in older adults, which started with a baseline visit between 1990 and 1993.[15] All participants aged 55 years and over in the Ommoord district of Rotterdam, The Netherlands were invited to participate (n=10,275). Of them 7,983 (78%) participated in the study. At baseline, participants were interviewed at home and were examined at the research centre, which included a 10 second, 12-lead electrocardiogram (ECG). Since then, participants are followed continuously and were re-examined during three follow-up examination rounds (1993-1995, 1997-1999, and 2002-2004). Medical information is available of all participants by collaboration with the general practitioners and with the pharmacies in the area of Ommoord. The medical ethics committee of Erasmus University, Rotterdam, approved the study, and all participants gave written informed consent.

DHEAS assessment

Levels were measured in plasma. Blood samples were drawn by venapuncture from non-fasting participants and collected in 5-ml tubes containing 0.5 ml sodium citrate solution. All tubes

were stored on ice before and after blood sampling. Plasma levels of DHEAS were estimated using coated tube radioimmunoassays purchased from Diagnostic Systems Laboratories, Inc. (Webster, Texas, USA).

Atrial fibrillation assessment

Prevalent and incident atrial fibrillation was ascertained using three methods.[16] At baseline and during follow-up examinations 12-lead ECGs were recorded, stored digitally, and analyzed by the Modular ECG Analysis System (MEANS).[17-18] To verify the diagnosis of atrial fibrillation, all ECGs with a diagnosis of atrial fibrillation, atrial flutter, or any other rhythm disorder were recoded independently by 2 research physicians who were blinded to the MEANS diagnosis. The judgment of a cardiologist was sought and taken as decisive in those cases in which disagreement persisted between the coding physicians. Additionally, medical information was obtained from general practitioners which included their own results as well as results from physicians practicing in hospitals and outpatient clinics. Patients were considered as a case of atrial fibrillation after diagnosis by a medical specialist or diagnosis by a general practitioner with ascertainment from an ECG. Finally, information was obtained from a national registration of all hospital discharge diagnoses. Atrial fibrillation occurring during a serious disease resulting in death, during myocardial infarction or during cardiac operative procedures who recovered during the hospital admission were not included as cases. We did not distinguish between atrial fibrillation and atrial flutter when we identified cases because both conditions are very similar with respect to risk factors and consequences.[19-20]

Vital status

Information on vital status was obtained on a regular basis from the Central Register of Population of the municipality of Rotterdam, from collaborating GPs and by collecting information during follow-up rounds. For the participants for whom information remained missing, the Central Registry of Genealogy of the Netherlands was consulted. This national institute receives population registry records of all inhabitants of the Netherlands who have died.

Covariable assessment

Body mass index was calculated from weight in kilograms and height in meters. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressure were calculated as the average of two consecutive measurements. Data on blood pressure lowering medication were obtained from questionnaires and from the participating pharmacies. Information on sex hormone therapy (ATC code: G03) was obtained in a similar way. Serum total cholesterol and HDL levels were measured with an automated enzymatic method. Information on smoking status and alcohol use was obtained during a home interview. Smoking status was coded as never, former or current smoker. Alcohol use was calculated in grams per day. A history of myocardial

infarction was defined as a self-reported myocardial infarction with hospital admission or the presence of a myocardial infarction on the ECG.[21] The presence of heart failure at baseline was assessed using a validated score based on the definition of heart failure of the European Society of Cardiology.[22] Information on cardiovascular events during follow-up, including myocardial infarction and heart failure, was obtained from the GPs, and was coded independently by 2 research physicians.[21-22] Prevalent diabetes mellitus was defined as the pre or post-load serum glucose level of > 11.1 mmol/L or the use of glucose-lowering medication. Carotid plaques were visualized by ultrasonography, as described in detail elsewhere.[11]

Population for analysis

Levels of DHEAS were assessed in a random sample of the Rotterdam Study participants. This random sample was drawn from participants who attended the baseline visit (1990-1993), and consisted of 1,284 participants. The sample included 68 participants with prevalent atrial fibrillation at baseline who were excluded from the analysis. Also 36 participants with missing baseline ECG data, due to technical problems or lack of qualified personnel, were excluded. This resulted in a study population of 1,180 participants. All participants were followed from the day of blood sampling in the Rotterdam Study (1990-1993) to the date of onset of atrial fibrillation, the date of death or loss to follow-up until January 1, 2008.

Statistical analysis

DHEAS levels were analyzed continuously (per SD increment), and categorized into quartiles. Baseline characteristics are presented for the total group and for DHEAS quartiles. Age and sex adjusted hazard curves for atrial fibrillation were computed using Cox proportional hazards analyses, comparing the DHEAS quartiles. Furthermore, to assess the association of DHEAS levels with atrial fibrillation risk, adjusted for several well-known risk factors, we used Cox proportional hazards analyses. The proportional hazards assumption was tested using time dependent interaction terms. We decided to use two models, first one adjusting for age and sex, and a second model additionally adjusting for other cardiovascular risk factors (systolic blood pressure, diastolic blood pressure, blood pressure lowering therapy, BMI, total and HDL cholesterol, smoking status, alcohol use, sex hormone therapy, prevalent myocardial infarction, heart failure and diabetes mellitus at baseline, and carotid plaque score). In secondary analyses, we checked whether the additional adjustment for incident heart failure and/or myocardial infarction (time dependent), added separately in one model, could modify our results. We included an interaction term of DHEAS levels*sex in the multivariate models. Second, we stratified analyses by sex, and used sex specific DHEAS quartiles. Finally, because DHEAS levels are associated with smoking we performed an analysis in non smokers (never and past smokers). Data were analysed using the SPSS PASW statistical software, version 17.0 (IBM corporation, Armonk, New York, USA).

RESULTS

Baseline Characteristics

Follow-up included 129 participants with incident atrial fibrillation during a mean follow-up of 12.3 years (SD=4.7). Table 1 shows the baseline characteristics of the study population in the full sample and stratified by DHEAS quartiles. Participants in the higher DHEAS quartiles were significantly younger, and were more likely to be male compared to those in the lower quartiles. Also participants in the higher DHEAS quartile were more likely to smoke or have a history of smoking compared to subjects in the lower quartiles, after adjustment for age and sex.

Table 1. Baseline characteristics of study population.

	Full sample	DHEAS quartiles			
Characteristic	**(n=1,180)**	**Q1 0.01 – 1.73 µmol/l (n=295)**	**Q2 1.74 – 2.95 µmol/l (n=295)**	**Q3 2.96 – 4.74 µmol/l (n=295)**	**Q4 4.75 – 23.08 µmol/l (n=295)**
Age (years)	69(8.4)	72(8.4)	70(8.3)[a]	68(8.0)[a]	66(7.1)[a]
Sex, male	547(46.3)	63(21.4)	124(42.0)[b]	154(52.0)[b]	206(69.8)[b]
Systolic blood pressure (mmHg)	138(21)	138(22)	138(21)	138(21)	136(21)
Diastolic blood pressure (mmHg)	73(11)	73(12)	73(11)	73(11)	74(10)
Use of antihypertensive medication	291(24.7)	81(27.5)	72(24.5)	75(25.3)	63(21.4)
BMI (kg/m^2)	26.1(3.4)	26.2(3.6)	26.2(3.4)	26.4(3.4)	25.7(3.2)
Total cholesterol (mmol/l)	6.7(1.2)	6.8(1.2)	6.7(1.2)	6.7(1.2)	6.6(1.2)
HDL cholesterol (mmol/l)	1.4(0.4)	1.4(0.4)	1.4(0.4)	1.3(0.3)	1.3(0.4)
Smoking status:					
- Current	279(23.6)	51(17.3)	50(16.9)	62(20.9)	116(39.3)[c]
- Past	490(41.5)	101(34.2)	127(43.1)	139(47.0)	123(41.7)[c]
Alcohol use (g/day)	11.9(15.5)	7.2(10.3)	10.5(14.3)	13.6(16.5)	16.3(18.2)
Myocardial infarction	148(12.5)	33(11.2)	34(11.5)	42(14.2)	39(13.2)
Heart failure	15(1.3)	4(1.4)	4(1.4)	4(1.4)	3(1.0)
Diabetes mellitus	84(7.1)	21(7.1)	27(9.2)	18(6.1)	18(6.1)
Carotid plaques:					
- No	465(39.5)	113(38.3)	106(36.1)	116(39.3)	130(44.1)
- Mild	143(12.1)	44(14.9)	29(9.9)	40(13.6)	30(10.2)
- Moderate	182(15.3)	43(14.6)	53(17.7)	43(14.6)	43(14.6)
- Severe	242(20.5)	58(19.7)	70(23.8)	57(19.3)	57(19.3)
- Missing	148(12.5)	37(12.5)	37(12.6)	39(13.2)	35(11.9)
Use of steroid hormone therapy	14(1.2)	6(2.0)	4(1.4)	3(1.0)	1(0.3)

Values are number of participants (%) or means (SD).

Abbreviations: DHEAS, Dehydroepiandrosterone sulfate; BMI, Body mass index; HDL, high-density lipoprotein

[a] $p<0.05$, compared to lowest DHEAS quartile, adjusted for sex.

[b] $p<0.05$, compared to lowest DHEAS quartile, adjusted for age.

[c] $p<0.05$, compared to lowest DHEAS quartile, adjusted for age and sex.

DHEAS levels and risk of atrial fibrillation

Figure 1 displays the age and sex adjusted hazard curves for risk of atrial fibrillation across DHEAS quartiles. The incidence rate of atrial fibrillation was lowest in the highest quartile of DHEAS. By 18 years of follow-up, the cumulative incidence in the highest quartile was 5.6% compared to 13.5% in the lowest quartile. After adjustment for age and sex, DHEAS levels were significantly associated with incident atrial fibrillation (HR per SD=0.76, 95%-CI:0.60-0.95; Table 2). Additional adjustment for cardiovascular risk factors only slightly changed this estimation (HR per SD =0.74, 95%-CI: 0.58-0.94). Participants in the highest quartile had a 66% lower risk to develop atrial fibrillation compared to the lowest quartile (HR on atrial fibrillation: 0.34, 95%-CI: 0.18-0.64). Of the 1,180 participants included in the analyses, 196 developed heart failure (n=137) and/or myocardial infarction (n=91) during follow-up of whom 21 later developed atrial fibrillation. However, the inclusion of a time dependent covariate for incident heart failure and/or myocardial infarction in the multivariable model adjusted for age, sex and cardiovascular risk factors, resulted in only a minor change of the risk estimate. DHEAS levels in the highest quartile were associated with a reduced risk of atrial fibrillation with an HR of 0.34 (95%-CI:0.19-0.64) for the participants in the highest quartile compared to those in the lowest quartile.

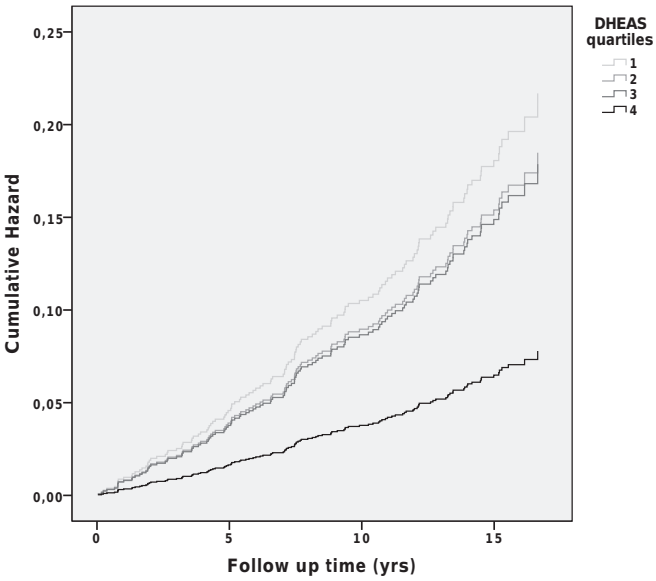

Figure 1. Age and sex adjusted hazard curves on risk for atrial fibrillation.

DHEAS levels and atrial fibrillation by gender

Mean DHEAS levels were 4.52 µmol/L (SD: 2.99) in men and 2.74 µmol/L (SD: 2.06) in women (*p*-value<0.01; Table 2). However, there was no statistically significant interaction between DHEAS levels and sex in the association with atrial fibrillation (*p*-for interaction=0.17 in age

Table 2. Hazard rates of the association of DHEAS levels with incident atrial fibrillation.

			Model 1[a]	Model 2[b]
	N	n	HR (95%-CI)	HR (95%-CI)
			All	
DHEAS per SD (µmol/L)	1180	129	0.76 (0.60-0.95)	0.74 (0.58-0.94)
DHEAS quartiles:				
- Q1. 0.01 – 1.73 µmol/l	295	39	Ref.	Ref.
- Q2. 1.74 – 2.95 µmol/l	295	37	0.85 (0.55-1.40)	0.79 (0.49-1.25)
- Q3. 2.96 – 4.74 µmol/l	295	36	0.82 (0.51-1.32)	0.74 (0.46-1.19)
- Q4. 4.75 – 23.08 µmol/l	295	17	0.36 (0.19-0.66)	0.34 (0.18-0.64)
			Men	
DHEAS per SD (µmol/L)	547	67	0.83 (0.61-1.11)	0.79 (0.58-1.08)
DHEAS quartiles:				
- Q1. 0.10 – 2.44 µmol/l	136	22	Ref.	Ref.
- Q2. 2.45 – 3.87 µmol/l	137	20	0.90 (0.48-1.69)	0.88 (0.46-1.70)
- Q3. 3.88 – 4.84 µmol/l	137	15	0.71 (0.36-1.40)	0.68 (0.34-1.39)
- Q4. 4.85 – 23.08 µmol/l	137	10	0.46 (0.21-1.00)	0.42 (0.19-0.96)
			Women	
DHEAS per SD (µmol/L)	633	62	0.81 (0.68-0.96)	0.80 (0.67-0.95)
DHEAS quartiles:				
- Q1. 0.01 – 1.29 µmol/l	158	22	Ref.	Ref.
- Q2. 1.30 – 2.19 µmol/l	159	18	0.79 (0.42-1.47)	0.74 (0.39-1.32)
- Q3. 2.20 – 3.81 µmol/l	158	15	0.74 (0.38-1.42)	0.67 (0.34-1.34)
- Q4. 3.82 – 13.56 µmol/l	158	7	0.34 (0.14-0.82)	0.33 (0.14-0.80)

Abbreviations: DHEAS, Dehydroepiandrosterone sulphate; HR, Hazard rate ratio; CI, Confidence interval; SD, Standard deviation; Ref, Reference

[a] Adjusted for age and sex

[b] Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, blood pressure lowering therapy, BMI, total and HDL cholesterol, smoking status, alcohol use, sex hormone therapy, prevalent myocardial infarction, heart failure and diabetes mellitus at baseline, and carotid plaque score.

adjusted model, *p*-for interaction=0.08 after additional adjustment for cardiovascular risk factors). The association of DHEAS levels with risk of atrial fibrillation was slightly more apparent in women then in men in stratified analyses. The HR on atrial fibrillation of participants in the highest quartile of DHEAS levels compared to the those in the lowest quartile was 0.42 (95%-CI:0.19-0.96) in men. In women, this association was even stronger with an HR of 0.33 (95%-CI:0.14-0.80).

DHEAS levels and atrial fibrillation in non-smokers

After exclusion of smokers, the study population included 901 participants of whom 113 developed atrial fibrillation during follow-up. In non-smokers, DHEAS levels were associated

with atrial fibrillation (HR per SD increment: 0.62, 95%-CI:0.47-0.84). Also in this subsample, participants in the highest quartile of DHEAS levels had a 68% lower risk of developing atrial fibrillation during follow-up (HR: 0.32, 95%-CI:0.16-0.64).

DISCUSSION

Our results suggest that high DHEAS levels are associated with a lower risk of atrial fibrillation, also after adjustment for age, sex, and known cardiovascular risk factors. Participants in the highest quartile of DHEAS levels were 3 times less likely to develop atrial fibrillation during follow-up when compared with participants in the lowest quartile. Although DHEAS levels varied significantly between men and women, the association of DHEAS levels and atrial fibrillation was not significantly different between men and women.

A previous cross-sectional study in 436 men of 65-94 years showed that subjects with atrial fibrillation had significantly lower DHEAS levels, even after adjustment for cardiovascular risk factors.[23] As far as we know, no previous population based follow-up study described the association of DHEAS levels with development of atrial fibrillation. Similar to the inverse association of DHEAS levels with risk of atrial fibrillation we report here, previous longitudinal studies suggested that higher DHEAS levels are associated with lower cardiovascular mortality.[2, 4-7] Trivedi et al[5] showed that male participants in the highest DHEAS quartile were at lower risk for cardiovascular mortality compared to those in the lowest quartile (multivariate adjusted HR:0.56, 95%-CI:0.32-0.97) in a community based study of 963 men and 1171 women aged 65 to 76 years old. In women this study found no significant association but the point estimate suggested a similar magnitude of association (HR: 0.63, 95%-CI:0.29-1.34). Similar results have been presented by Ohlsson et al,[7] in a multicentre study in 2644 men aged 69 to 81 years old, in which participants in the highest quartile had a lower risk of cardiovascular mortality (HR:0.60, 95%-CI 0.44-0.83). Shufelt et al[4] showed similar results in 270 postmenopausal women, in which participants in the lowest tertile of DHEAS levels had a higher cardiovascular mortality (HR: 2.43, 95%-CI:1.06-5.56) compared to those in the highest tertile, after adjustment for cardiovascular risk factors.

Our results show that DHEAS levels varied significantly between men and women. Where men receive a continuous albeit decreasing supply of sex steroids from the testes during their whole postpubertal life,[24] DHEA is the quantitatively most significant source of sex steroids in postmenopausal women.[24] However, our results did not suggest any significant difference in the strength of the association of DHEAS levels with risk of atrial fibrillation between men and women. Previous studies suggested DHEAS levels to be associated with several health outcomes in both men and women. Several studies described DHEAS levels to be associated with mortality,[2, 5-7] cardiovascular disease,[2-3] and atherosclerosis[10] in men and not in women, whereas other studies did suggest DHEAS levels to be associated with mortality,[4] and atherosclerosis[8, 25]

only in women. The reason for these differences in results is unclear, but is most likely due to differences in study populations and methodological approaches.[26]

Our data indicate that participants with high DHEAS levels were more likely to smoke. Previous studies also described smoking, which is a known risk factor for atrial fibrillation,[27] to be associated with higher DHEAS levels.[5, 28-29] It is possible that these higher DHEAS levels in smokers are just a consequence of smoking, but it is also possible that higher DHEAS levels play a protective role in the association of smoking with atrial fibrillation. To exclude the possible influence of smoking on the association of DHEAS levels with development of atrial fibrillation, we performed an additional analysis only in non smokers. This analysis showed similar results compared to the results in the full sample.

Several explanations for the association of DHEAS levels with atrial fibrillation can be raised. A previous study suggested that the zona reticularis of the adrenal gland, responsible for most DHEAS production, is highly susceptible to vascular damage.[30] It was therefore suggested that a low DHEAS level is only reflecting underlying vascular disease.[31] This might suggest that DHEAS levels are a non-etiological biomarker rather than a step in the causal pathway.

It has also been suggested that DHEAS could have tissue specific effects, either directly or indirectly by conversion to biologically active androgens and estrogens.[1] Li et al[9] recently suggested that DHEAS inhibits vascular remodeling by reducing neointima formation after arterial injury. The observation that DHEAS has an inhibitory effect on cell growth and proliferation was supported in several recent studies that suggested DHEAS to be inversely associated with atherosclerosis.[8-10] Moreover, several studies suggested that DHEAS may have also an anti-inflammatory role.[32-34] Recent studies indicated that inflammation is associated with atrial fibrillation and plays a role in its etiology.[35]

Strengths of this study are its population-based design, with follow-up of up to 18 years. The study included extensive information on clinical details and multiple covariables. This allowed proper adjustment and minimized the risk of false-positive misclassification, especially as diagnoses were unrelated to DHEAS levels and made prospectively and without knowledge of the research hypothesis. This study is limited in that we were not able to distinguish between paroxysmal and persistent atrial fibrillation. Also as atrial fibrillation may occur without symptoms, false-negative misclassification may have occurred. However we used three different methods for the case gathering and assessment, and included every clinically recognized case from two different sources of medical records. In addition, we included repeated screening ECG assessments of the study population at the research centre. Moreover, any false-negative misclassification is likely to be random and therefore will have led to an underestimation of the true risk estimate.

Since atrial fibrillation is highly prevalent in the elderly, and is associated with serious morbidity and mortality, it is a major public health problem.[36] Effective prevention strategies may arise from studies on biomarkers that are associated with the risk of atrial fibrillation. Our results show that DHEAS can be regarded as an important indicator for the risk of atrial fibrillation.

Although further causal evidence is required, future measures aimed to prevent the occurrence of atrial fibrillation and improve the detection and management of this condition, should take into account the potential role of DHEAS.

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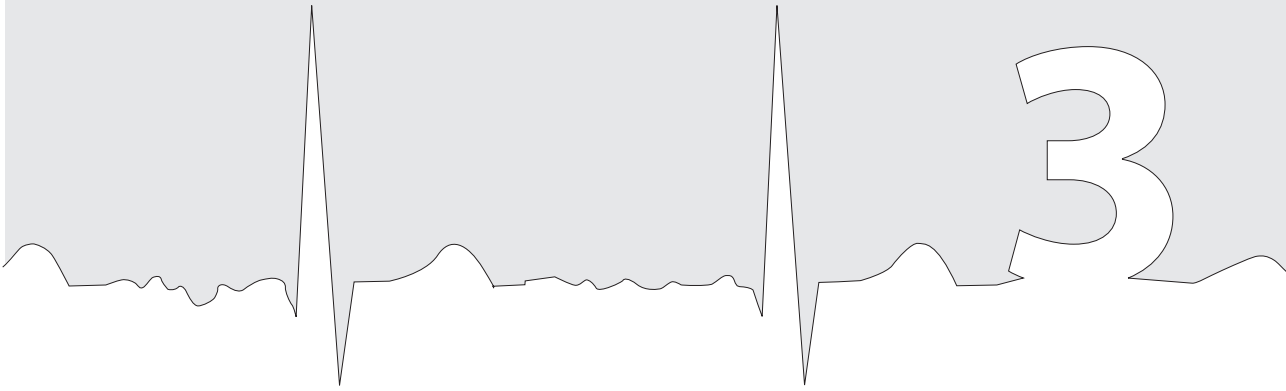
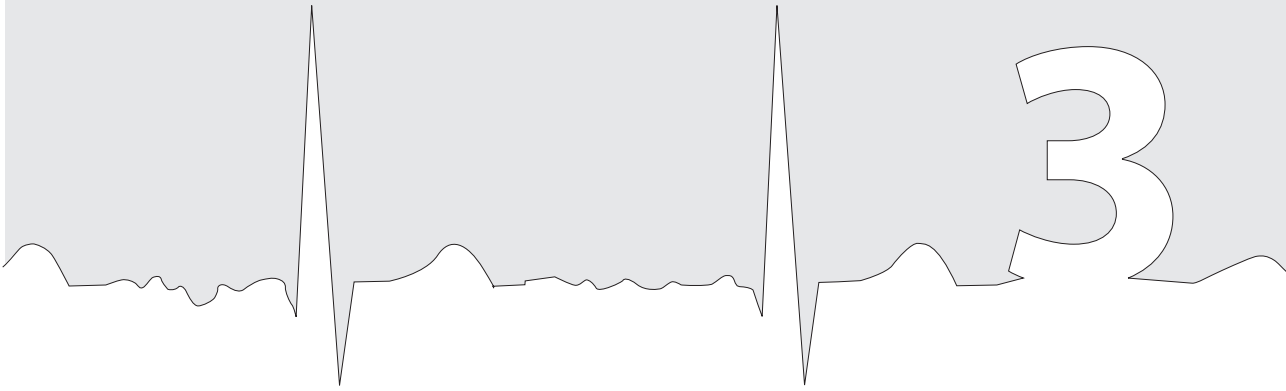
CHAPTER 3.3

Serum potassium levels



CHAPTER 3.4

Use of non-steroidal anti-inflammatory drugs



CHAPTER 3.5

Genome wide association study

ABSTRACT

Atrial fibrillation is a highly prevalent arrhythmia and major risk factor for stroke, heart failure, and death.[1] We conducted a genome-wide association study (GWAS) in individuals of European ancestry, including 6,707 with and 52,426 without atrial fibrillation. Six novel atrial fibrillation susceptibility regions were identified and replicated in an additional 5,381 European ancestry individuals with and 10,030 without atrial fibrillation. Four of the loci identified in Europeans were further replicated *in silico* in a GWAS of 843 Japanese individuals with and 3,350 without atrial fibrillation. The identified loci implicate transcription factors related to cardiopulmonary development, cardiac-expressed ion channels, and cell signaling molecules.

Genome-wide association studies (GWAS) in subjects of European descent have identified three genomic regions associated with atrial fibrillation at chromosomes 4q25/*PITX2*,[2-5] 16q22/*ZFHX3*[3, 5], and 1q21/*KCNN3*[2]. Studies of electrocardiographic traits have also identified a number of loci associated with atrial fibrillation.[6-7] However, despite these findings much of the heritability of atrial fibrillation remains unexplained, justifying the search for additional genetic variants underlying atrial fibrillation risk.[8] Large-scale meta-analysis of GWAS results is a powerful method to identify additional genetic variation underlying traits and conditions. We therefore conducted a meta-analysis of multiple, well-phenotyped GWAS samples of European ancestry to identify additional atrial fibrillation susceptibility loci.

Six prospective cohort and 10 prevalent study samples contributed to the discovery analysis, which was age- and sex-adjusted (Table 1 and Methods). Atrial fibrillation status was systematically ascertained in each sample (Methods). After application of quality control SNP exclusion criteria in each study (Supplementary Table 1), meta-analysis was performed applying genomic control to each study. The genomic control inflation factor for the meta-analysis was 1.042 for the full set of SNPs and 1.040 after omitting all SNPs within 500kb of the genome-wide significant hits. Supplemental Figure 1 displays the quantile-quantile plot of the expected versus observed *P* value distributions for associations of the 2,609,549 SNPs analyzed. We identified 10 loci that exceeded our pre-specified threshold for genome wide significance ($P<5x10^{-8}$) (Figure 1). The three loci most significantly associated with atrial fibrillation were at previously identified atrial fibrillation susceptibility loci on chromosomes 4q25/*PITX2* (rs6817105; $P=1.8x10^{-74}$),[4] 16q22/*ZFHX3* (rs2106261; $P=3.2x10^{-16}$),[3, 5] and 1q21/*KCNN3* (rs6666258; $P=2.0x10^{-14}$; Table 2).[2]

Seven novel genomic regions were associated with atrial fibrillation with $P<5x10^{-8}$ in the discovery stage (Table 2). The most significantly associated SNP in each of the seven novel regions was genotyped and tested for association with atrial fibrillation in an additional 3,132 to 5,289 independent individuals with atrial fibrillation and 8,159 to 11,148 referent individuals derived from six studies of European ancestry (Supplementary Table 2). Six of the genomic regions associated with atrial fibrillation in the discovery stage met our criteria for independent replication. Study-specific replication results are detailed in Supplementary Table 3. Meta-analysis of the discovery and replication results are displayed in Table 2 and regional plots are displayed in Figure 2. Recognizing that the genes in closest physical proximity to the associated SNPs are not always the causative genes, we report below the genetic associations in order of statistical significance along with the nearest gene.

The most significant novel association in the discovery stage was on chromosome 1q24 (rs3903239; overall $P=8.4x10^{-14}$) in *PRRX1*, a homeodomain transcription factor highly expressed in the developing heart, particularly in connective tissue. Biological interaction between *PRRX1* and a related homeobox transcription factor, *PRRX2*, results in abnormalities of great vessel development in a mouse knockout model.[9] In a separate *PRRX1* knockout, fetal pulmonary vasculature development was impaired.[10]

Table 1. Subject characteristics.

Cohort	Cohort type	Participants, n	AF, n	Males, n (%)	Age at DNA collection, mean ± SD	Age at DNA collection range	Age of atrial fibrillation onset, mean ± SD	Hypertension, n (%)	Body mass index, kg/m^2, mean ± SD	Diabetes, n (%)	Myocardial infarction, n (%)	Heart failure, n (%)
Incident AF		***N Overall***										
ARIC	Cohort	8,890	802	4,181 (47.0)	54.3 ± 5.7	44-66	67.8 ± 6.8	2,376 (26.7)	27.0 ± 4.8	763 (8.6)	354 (4.0)	325 (3.7)
AGES	Cohort	2,959	158	1,154 (39.0)	76.5 ± 5.5	66-95	75.0 ± 8.8	2,595 (87.7)	27.1 ± 4.4	319 (10.8)	189 (6.39)	55 (1.9)
CHS	Cohort	3,204	764	1,242 (38.8)	72.2 ± 5.3	65-98	81.2 ± 6.0	1,678 (52.4)	26.3 ± 4.4	377 (11.8)	0	0
FHS	Cohort	4,062	310	1,771 (43.6)	64.7 ± 12.6	31-101	77.8 ± 10.6	2,001 (49.3)	27.7 ± 5.2	328 (8.07)	231 (5.7)	55 (1.4)
RS-I	Cohort	5,665	542	2,282 (40.3)	69.1 ± 9.0	55 - 99	77.6 ± 7.7	1,866 (32.9)	26.3 ± 3.7	567 (10.0)	632 (11.2)	156 (2.8)
WGHS	Cohort	20,836	648	0	54.1 ± 7.0	43 - 89	68.0 ± 8.2	5,022 (24.1)	25.9 ± 4.9	503 (2.4)	0	17 (0.1)
Prevalent AF		***N per group***										
AFNET	Cases	468	–	236 (50.4)	51.8 ± 7.2	29-74	51.3 ± 7.6	252 (53.8)	28.0 ± 4.9	36 (7.7)	6 (1.8)	14 (4.8)
KORA	Referents	438	–	219 (50.0)	56.2 ± 7.1	45-69	–	185 (42.2)	27.7 ± 4.5	37 (8.4)	6 (1.3)	13 (2.9)
AGES[a]	Cases	241	–	88 (55.7)	78.5 ± 5.9	67-95	80.9 ± 6.2	143 (90.5)	27.7 ± 4.4	20(12.7)	8 (5.1)	5 (3.2)
	Referents	2,718	–	70 (36.1)	76.1 ± 5.4	66-94	80.4 ± 5.4	2,002 (78.2)	27.0 ± 4.5	269(10.5)	122 (4.8)	27 (1.1)
CC	Cases	496	–	375 (75.6)	58.8 ± 10.7	20-84	51.7 ± 12.0	269 (54.2)	30.2 ± 6.2	28 (5.6)	0 (0)	0 (0)
	Referents	2,971	–	1,124 (37.8)	28.5 ± 22.2	0-87	–	–	–	–	–	–
HVH	Cases	95	–	28 (29.5)	59.5 ± 6.5	40-68	57.4 ± 6.4	50 (52.6)	34.1 ± 9.9	14 (14.7)	0	0
	Referents	193	–	106 (54.9)	59.5 ± 6.0	40-69	–	153 (79.3)	31.4 ± 7.2	31 (16.1)	9 (4.7)	7 (3.6)
CHS*	Cases	67	–	38 (56.7)	76.3 ± 5.8	66-90	–	35 (52.2)	26.6 ± 4.3	14 (20.9)	0	0
	Referents	3,204	–	1,242 (38.8)	72.2 ± 5.3	65-98	–	1,678 (52.4)	26.3 ± 4.4	377 (11.8)	0	0
FHS*	Cases	253	–	151 (59.7)	76.9 ± 9.9	45-97	70.9 ± 10.8	180 (71.1)	27.4 ± 4.8	41 (16.21)	60 (23.7)	57 (0.23)
	Referents	4,151	–	1,807 (43.5)	64.7 ± 12.6	31-101	-	2,036 (49.1)	27.7 ± 5.2	329 (7.9)	235 (5.7)	55 (1.32)

Table 1. Subject characteristics. *(continued)*

Cohort	Cohort type	Participants, n	AF, n	Males, n (%)	Age at DNA collection, mean ± SD	Age at DNA collection range	Age of atrial fibrillation onset, mean ± SD	Hypertension, n (%)	Body mass index, kg/m^2, mean ± SD	Diabetes, n (%)	Myocardial infarction, n (%)	Heart failure, n (%)
MGH	Cases	366	–	295 (80.6)	53.4 ± 10.5	21-77	46.1 ± 11.7	85.8 (22.7)	27.8 ± 5.0	12 (3.2)	4 (1.1)	10 (2.8)
MIGEN	Referents	911	–	485 (53.2)	47.9 ± 8.8	18-83	–	–	–	–	–	–
RS-I*	Cases	309	–	145 (46.9)	76.2 ± 8.7	56–98	–	131 (42.4)	25.9 ± 3.6	64 (20.7)	69 (22.3)	54 (17.5)
	Referents	5,665	–	2,282 (40.3)	69.1 ± 9.0	55–99	–	1,866 (32.9)	26.3 ± 3.7	567 (10.0)	632 (11.2)	156 (2.8)
SHIP	Cases	107	–	69 (64.5)	65.1 ± 11.5	21-81	–	59 (55.1)	29.6 ± 5.1	23 (21.5)	14 (13.1)	44 (41.1)
	Referents	1,816	–	906 (49.9)	50.7 ± 14.9	21-81	–	437 (24.1)	27.2 ± 4.5	131 (7.2)	54 (3.0)	157 (8.6)
Vanderbilt	Cases	1,081	–	738 (68.3)	59.5 ± 12.6	16-87	51.5 ± 14.7	625 (57.8)	30.7 ± 6.9	197 (18.2)	95 (8.8)	191 (17.6)
	Referents	880	–	551 (62.6)	50.0 ± 17.4	18-91	–	463 (52.6)	28.0 ± 5.8	180 (20.5)	299 (34.0)	120 (13.6)

Abbreviations: SD, standard deviation. [a]Studies were community-based prospectively ascertained prevalent atrial fibrillation cases. The other prevalent atrial fibrillation studies were case-control design.

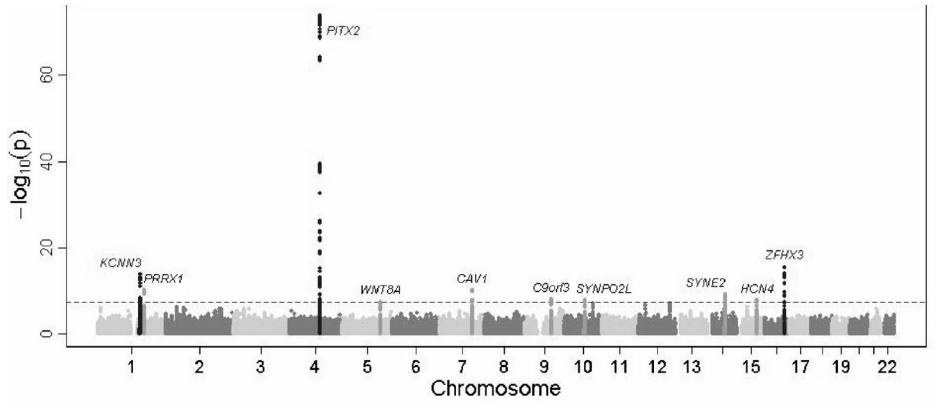


Figure 1. Manhattan plot of meta-analysis results for genome-wide association of atrial fibrillation.
The -$\log_{10}$(P value) is plotted against the physical positions of each SNP on each chromosome. The threshold for genome-wide significance, $P < 5 \times 10^{-8}$, is indicated by the dashed line. The three previously reported loci for atrial fibrillation are indicated in blue and the seven new loci that exceed the genome wide significance threshold are indicated in orange.

A second locus was located at chromosome 7q31 (rs3807989; overall $P=3.6 \times 10^{-12}$) in *CAV1*, which encodes caveolin-1, a cellular membrane protein involved in signal transduction. *CAV1* is selectively expressed in the atria,[11] and its knockout has been associated with dilated cardiomyopathy.[12] CAV1 co-localizes with and negatively regulates the activity of *KCNH2*,[13] a potassium channel involved in cardiac repolarization that has been associated with atrial fibrillation in a candidate gene association study, though not in our present analysis.[14] The top SNP at the CAV1 locus identified in the current study, rs3807989, was previously identified in a GWAS of the PR and QRS intervals and related to atrial fibrillation.[6-7] The relations between other previously reported PR loci and atrial fibrillation are reported in Supplementary Table 4. Interestingly, a significant association is observed with atrial fibrillation for SNPs related to the PR interval at *SOX5*, *TBX5*, *SCN5A*, and *SCN10A*.[6-7]

The third locus on chromosome 14q23 (rs1152591; overall $P=5.8 \times 10^{-13}$) is located in an intron of *SYNE2*, which encodes numerous nesprin-2 isoforms, some of which are highly expressed in the heart and skeletal muscle. Nesprin-2 is located throughout the sarcomere and is involved in maintaining nuclear structural integrity by anchoring the nucleus to the cytoskeleton. In a candidate gene approach, mutations in *SYNE2* have been found to segregate in some families with Emery-Dreifuss muscular dystrophy,[15] which is characterized by skeletal muscle atrophy, cardiomyopathy, and cardiac conduction defects.

The fourth locus on chromosome 9q22 (rs10821415; overall $P=4.2 \times 10^{11}$) is located in an open reading frame on chromosome 9. Genes at this locus include *FBP1* and *FBP2*, which are important for gluconeogenesis. Autosomal recessive FBP1 deficiency has been described, but cardiovascular features do not appear prominent.[16] Variants at 9q22 have been implicated in

Table 2. Summary of GWAS meta-analysis results with *P* value < 5×10^{-8}

SNP	Locus	Closest gene	SNP Location relative to closest gene	Minor / major allele	MAF (%)	Discovery			Replication		Overall	
						RR 95% CI	Meta *P* value	I^2(%) *P* value	RR 95% CI	Meta *P* value	RR 95% CI	Meta *P* value
rs6666258	1q21	*KCNN3/ PMVK*	Intronic	C/G	29.9	1.18 1.13-1.23	2.0×10^{-14}	42.3 0.04	-	-	-	-
rs3903239	1q24	*PRRX1*	46 kb upstream	G/A	44.7	1.14 1.10-1.18	9.1×10^{-11}	53.2 6.3×10^{-3}	1.13 1.06-1.20	2.0×10^{-4}	**1.14 1.10-1.17**	**8.4×10^{-14}**
rs6817105	4q25	*PITX2*	150 kb upstream	C/T	13.1	1.64 1.55-1.73	1.8×10^{-74}	80.8 1.4×10^{-10}	-	-	-	-
rs2040862	5q31	*WNT8A*	Intronic	T/C	17.8	1.15 1.09-1.21	3.2×10^{-8}	10 0.34	1.04 0.96-1.12	3.6×10^{-1}	1.12 1.07-1.17	2.5×10^{-7}
rs3807989	7q31	*CAV1*	Intronic	A/G	40.4	0.88 0.84-0.91	9.6×10^{-11}	10 0.34	0.93 0.88-0.97	2.7×10^{-3}	**0.90 0.87-0.92**	**3.6×10^{-12}**
rs10821415	9q22	*C9orf3*	Intronic	A/C	42.4	1.13 1.08-1.18	7.9×10^{-9}	49.5 0.015	1.09 1.04-1.15	7.2×10^{-4}	**1.11 1.08-1.15**	**4.2×10^{-11}**
rs10824026	10q22	*SYNPO2L*	5 kb upstream	G/A	15.8	0.85 0.81-0.9	1.7×10^{-8}	37.9 0.06	0.91 0.83-0.99	3.5×10^{-2}	**0.87 0.83-0.91**	**4.0×10^{-9}**
rs1152591	14q23	*SYNE2*	Intronic	A/G	47.6	1.13 1.09-1.18	6.2×10^{-10}	25.7 0.16	1.12 1.06-1.19	1.9×10^{-4}	**1.13 1.09-1.17**	**5.8×10^{-13}**
rs7164883	15q24	*HCN4*	Intronic	G/A	16.0	1.16 1.10-1.22	1.3×10^{-8}	0 0.85	1.24 1.16-1.32	1.3×10^{-10}	**1.19 1.14-1.24**	**2.8×10^{-17}**
rs2106261	16q22	*ZFHX3*	Intronic	T/C	17.6	1.24 1.17-1.3	3.2×10^{-16}	58.8 1.6×10^{-3}	-	-	-	-

Abbreviations: MAF, minor allele frequency; RR, relative risk; CI, confidence interval. I^2: Proportion of variability in the effect size due to between-study variability. We did not attempt replication of the previously published genetic loci associated with atrial fibrillation on chromosomes 1q21/*KCNN3*,[2] 4q25/*PITX2*,[4] and 16q22/*ZFHX3*.[3, 5]

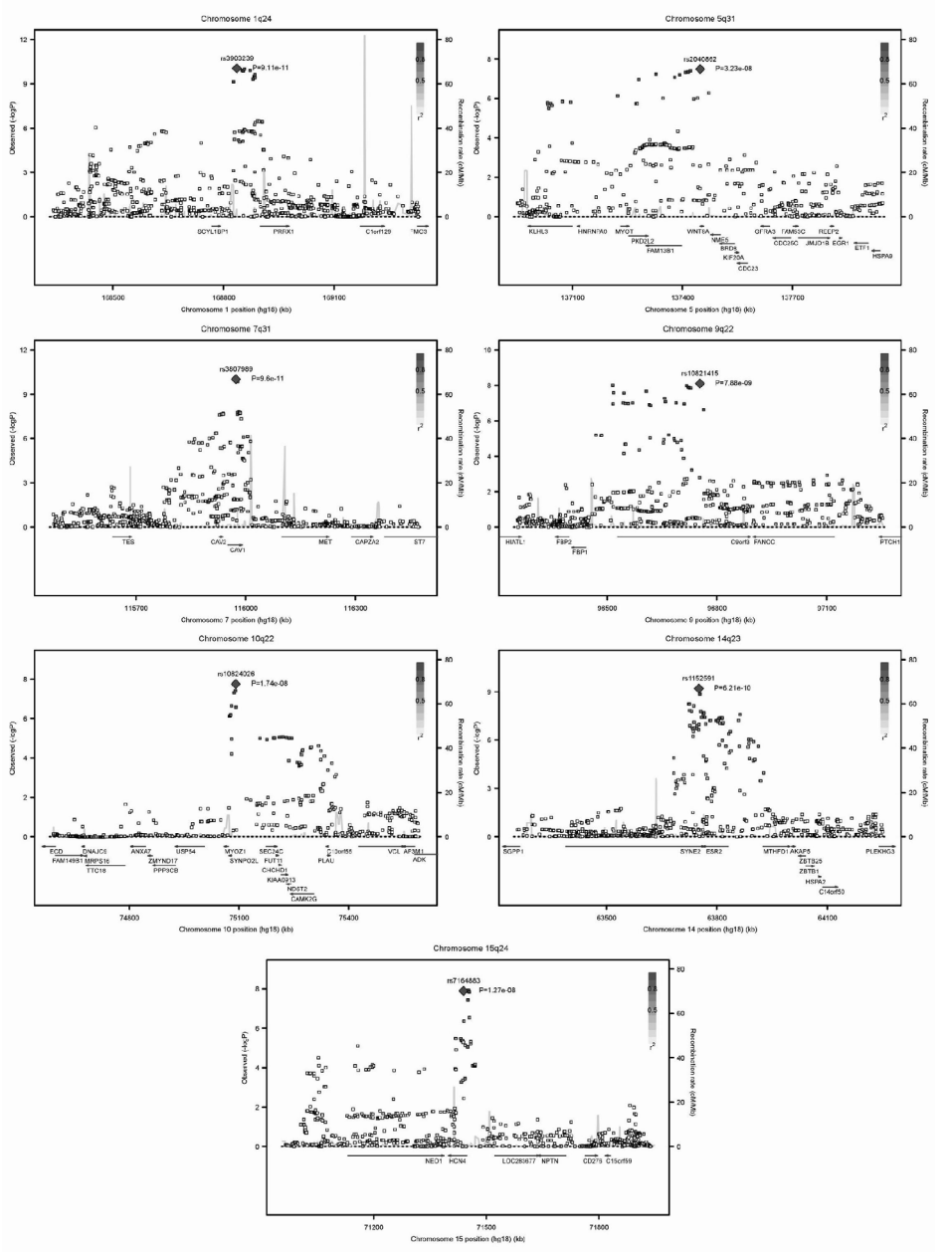


Figure 2. Regional plots for seven novel atrial fibrillation loci in discovery sample with $P<5\times10^{-8}$.
SNPs are plotted with the meta-analysis ***P*** value and genomic position (NCBI Build 36). The SNP of interest is labeled. The strength of the linkage disequilibrium (LD) is indicated by gradient of red. Estimated recombination rates are shown by the blue spikes and gene annotations are indicated by dark green arrows. LD and recombination rates are based on the CEU HapMap release 22. Figures prepared using SNAP.[26]

the regulation of height, pulmonary function, angiogenesis, and attention-deficit hyperactivity disorder, though rs10821415 is not in substantial LD with any of these SNPs (r^2<0.30).

A fifth signal is located at 15q24 (rs7164883; overall *P*=2.8x10^{-17}) in the first intron of *HCN4*. HCN4 is the predominant cardiac hyperpolarization-activated cyclic nucleotide-gated channel and is highly expressed in the sinoatrial node. HCN4 underlies the funny current (I_f) that governs cardiac pacemaking, and mutations in *HCN4* have been associated with various forms of sinus nodal dysfunction.[17-18]

A sixth locus on chromosome 10q22 (rs10824026, overall *P*=4.0x10^{-9}) is located 5 kb upstream of *SYNPO2L*, and 20 kb upstream of *MYOZ1*. *SYNPO2L* and *MYOZ1* are both expressed in skeletal and cardiac muscle, localize to the Z-disc, and interact with numerous other proteins. However, the precise role of either gene in cardiovascular physiology is unknown.[19-20] A mouse knockout of *MYOZ1* demonstrated increased calcineurin activity and cardiac hypertrophy in response to pressure overload. However, candidate gene approaches have not supported a prominent role for mutations in *MYOZ1* as a cause of familial dilated cardiomyopathy.[21] Interestingly, the *SYNPO2L* locus is located within a previously reported atrial fibrillation susceptibility locus identified in a family with autosomal dominant atrial fibrillation.[22]

One other locus was identified in the meta-analysis of atrial fibrillation at the gene *WNT8A* (rs2040862, *P*=3.2x10^{-8}); however, it failed to replicate in additional independent cohorts with atrial fibrillation (replication *P*=0.36, combined *P*=2.5x10^{-7}).

There was evidence of significant heterogeneity in the discovery meta-analysis at the previously published atrial fibrillation susceptibility signals on chromosome 4q25/*PITX2* and 16q22/*ZFHX3* (Table 2). Effect heterogeneity at the *PITX2* locus has already been observed.[4, 23-24]

We then sought to determine if the top SNPs or their proxies at each locus were associated with alterations in gene expression in the eQTL browser. The top SNP at the *SYNPO2L* locus, rs10824026, is in strong linkage disequilibrium with a SNP, rs12570126 (R^2=0.932), that was found to correlate with the expression of *MYOZ1* and *SYNPO2L*. Furthermore, the top SNP at the *SYNPO2L* locus is in linkage disequilibrium with a nonsynonymous SNP in *SYNPO2L*, rs3812629 (R^2=0.8; *SYNPO2L* P707L with respect to transcript Q9H987-1), that is predicted to be damaging by both the PolyPhen-2 and the SIFT algorithms. None of the other identified atrial fibrillation risk SNPs were associated with variations in gene expression in the eQTL browser.

We next examined the generalizability of our findings by examining our results in a separate GWAS of individuals of Japanese ancestry including 843 with and 3,350 without atrial fibrillation in the BioBank Japan study (Supplemental Figures 2A and 2B). Within the Japanese GWAS, only the chromosome 4q25/*PITX2* locus exceeded the preset threshold for genome-wide significance (rs2634073, OR 1.84, 95% CI 1.59-2.13, 3.7x10^{-17}; Supplemental Table 5 and Supplemental Figures 2A and 3). At the previously published locus on 16q22/*ZFHX3*,[3, 5] rs12932445 was associated with atrial fibrillation in participants of Japanese ancestry (*P*=6.8x10^{-4}). The relation between atrial fibrillation and variants at the *KCNN3*/*PMVK* locus on chromosome 1q21 failed to replicate (*P*=0.17); however, a regional plot of this locus revealed a distinct signal

at SNP rs7514452, approximately 375 kb away from rs6666258, the top SNP in the European ancestry sample, that was modestly associated with atrial fibrillation in the Japanese sample (P=4.9x10$^{-5,}$ R^2=0.047). At *PRRX1* and *CAV1*, the top SNPs in the European samples also were associated with atrial fibrillation in Japanese individuals. For the loci near *PRRX1* and *C9ORF3,* alternate SNPs in the Japanese cohort were more significantly associated with atrial fibrillation than the top SNP in Europeans (Supplemental Table 5 and Supplemental Figure 3).

Our study was subject to a number of limitations. To maximize both the power and the generalizability of our study, we included all available individuals with atrial fibrillation and thus some individuals had comorbidities such as systolic dysfunction and hypertension. However, none of the identified risk variants for atrial fibrillation were strongly associated with systolic dysfunction in the EchoGen Consortium,[25] a meta-analysis of echocardiographic data from 5 community-based cohorts consisting of over 12,000 individuals of European descent (P value < 10^{-5}). Further, when our replication results are adjusted for hypertension status, the identified variants remain significantly associated with atrial fibrillation (Supplemental Table 3). Ultimately, the development of a comprehensive risk score incorporating clinical, biochemical and genetic marker data will be necessary to clarify the incremental benefit of our findings in clinical care. Our eQTL analyses were limited to data available within the eQTL browser, future eQTL analyses derived from cardiac tissue may be helpful in identifying a relation between the atrial fibrillation risk SNPs and gene expression. Finally, we acknowledge that identified variants may not be causal, rather they may tag causal elements along the same or different molecular pathways; future statistical, bioinformatic, and biological analyses investigating potential genetic interactions are warranted. Fine mapping and deep resequencing will be necessary to uncover the genetic architecture accompanying the identified common atrial fibrillation susceptibility signals.

In summary, our GWAS meta-analysis for atrial fibrillation has identified six novel susceptibility loci in or near plausible candidate genes involved in pacemaking activity, signal transduction, and cardiopulmonary development. Our results demonstrate that atrial fibrillation has multiple genetic associations and identifies new targets for biological investigation.

METHODS

In each center the local institutional review board reviewed and approved all study procedures; written, informed consent was obtained from each participant, including consent to use DNA for genetic analyses of cardiovascular disease.

Detailed Description of Study Cohorts

The Age, Gene/Environment Susceptibility Study (AGES) Reykjavik study is comprised of 5,764 survivors drawn from the Reykjavik (Iceland) Study founded in 1967. AGES was designed to

examine the genetic susceptibility and risk factors for disease and disability in old age. Various vascular, neurocognitive, musculoskeletal and body composition related phenotypes have been ascertained. AGES examinations were conducted between 2002 and 2006. The AGES study ascertained atrial fibrillation based on AGES examination Minnesota coded electrocardiograms, and International Classification of Diseases, 9th revision (ICD-9) code 427.3 or ICD-10 code I48 from hospitalization records from 1997 through March 2008.

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

The Cleveland Clinic Lone atrial fibrillation GeneBank Study has enrolled patients with lone atrial fibrillation, defined as atrial fibrillation in the absence of significant structural heart disease. Participants were at least 18 years of age with a history of recurring or persistent lone atrial fibrillation, ≤50% coronary artery stenosis in the coronary arteries (if cardiac catheterization done) or with normal stress test results (documentation of normal cardiac catheterization or stress test required if age ≥50 years), and had normal left ventricular ejection fraction (LVEF) ≥ 50%. Subjects were excluded if they had heart failure, history of significant valvular disease (>2+ valvular regurgitation, any valvular stenosis), significant coronary artery disease (>50% coronary artery stenosis), prior myocardial infarction, prior percutaneous coronary intervention, or coronary artery bypass graft, or latest LVEF <50%. Referent subjects were drawn from the Illumina iControlDB online database. Referent subjects were included if they came from iControlDB Studies 64, 65, 66 or 67. All referent subjects in those studies were identified as Caucasian. Sex, age at DNA acquisition and race were the only available variables for the referent subjects.

Cardiovascular Health Study (CHS) is a prospective cohort study of cardiovascular disease in individuals 65 years or older recruited from four field centers (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA). Prevalent atrial fibrillation was that which was present on the baseline electrocardiogram. Incident atrial fibrillation was classified at the occasion of first atrial fibrillation identified during an annual CHS electrocardiogram or by ICD-9 code 427.3, 427.31, or 427.32 on a hospital discharge. Only subjects of self-reported European ancestry were included in this analysis.

Framingham Heart Study (FHS) is a community-based observational cohort initiated in 1948 to prospectively investigate CVD and its risk factors. The Original cohort (n=5,209) received biennial exams. The Original Cohort children (& spouses), termed the Offspring cohort (n=5,214), were recruited in 1971, and have been examined every four to eight years. In FHS, all cardiovascular hospital and outside records were routinely obtained and electrocardiograms were recorded at all FHS examinations; atrial fibrillation cases through 2007 were verified by two FHS cardiologists.

The German Competence Network for Atrial Fibrillation (AFNET) is a national registry of atrial fibrillation patients. In the context of the registry, additional DNA samples have been collected from patients with atrial fibrillation onset before age 60 years at the Medical Department I of the University Hospital Munich, Campus Grosshadern of the Ludwig-Maximilians University Munich in collaboration with the Institute of Epidemiology at the Helmholtz Zentrum Munich. Cases were selected if the diagnosis of atrial fibrillation was made on an electrocardiogram analyzed by a trained physician. Patients with signs of moderate to severe heart failure, moderate to severe valve disease or with hyperthyroidism were excluded from the study. Referent subjects were drawn from the KORA S4 study, with ages ranging from 25-74 years, and had no history of atrial fibrillation, myocardial infarction, heart failure or valve disease and had documented sinus rhythm at the time of blood draw. The KORA S4 study is a population-based epidemiological survey of persons living in or near the city of Augsburg, Southern Germany conducted between 1999 and 2001. The survey population consisted of German nationality residents born between July 1, 1925 and June 30, 1975 identified through the registration office. A sample of 6640 participants was drawn with ten strata of equal size according to sex and age, and 4261 individuals (66.8%) agreed to participate.

The Massachusetts General Hospital Atrial Fibrillation Study (MGH) enrolled serial patients with lone atrial fibrillation or atrial fibrillation and hypertension referred to the arrhythmia service between July 5, 2001 and February 19, 2008. Inclusion criteria were atrial fibrillation documented by electrocardiography, and age less than 66 years. Individuals with structural heart disease as assessed by echocardiography, hyperthyroidism, myocardial infarction, or heart failure were excluded. Each patient underwent a physical examination and standardized interview. All patients were evaluated by 12-lead electrocardiogram, echocardiogram, and laboratory studies. Referent subjects were selected from the control population of the MIGEN study, and were healthy patients from MGH without a history of MI or atrial fibrillation.

The Heart and Vascular Health Study (HVH) is a study of incident atrial fibrillation in the setting of Group Health Cooperative, a large integrated healthcare system in Washington State, USA. All plan members assigned a new ICD-9 code of 427.31 or 427.32 in the inpatient or outpatient setting between 1 October 2001 and 31 December 2004 were identified. Incident atrial fibrillation was verified by review of medical records with the requirement that the atrial fibrillation be documented by 12-lead electrocardiogram and clinically recognized by a physician, with no previous evidence of atrial fibrillation in the medical record. Control subjects were

identified from the Group Health membership, and had no history of atrial fibrillation. atrial fibrillation cases included in the discovery analysis had early-onset atrial fibrillation; they were less than 66 years of age at diagnosis, without a history of coronary artery disease, valvular disease, heart failure, poor left ventricular function, chronic obstructive pulmonary disease, active cancer or hyperthyroidism. Referent subjects were identified from the enrollment of Group Health Cooperative, were 40-69 years old, and had no history of atrial fibrillation.

The Rotterdam Study (RS-I) is a community-based study of elderly individuals from a suburb of Rotterdam with a focus on identifying determinants of health and cardiovascular, neurogeriatric, bone, and eye diseases. Participants age ≥55 years were examined up to 4 times every 3 years. Atrial fibrillation was diagnosed based on study visit electrocardiograms, review of hospital discharge information, and general practitioner diagnoses. Atrial fibrillation was verified by two physicians and disagreements settled by review of a cardiologist.

Study of Health in Pomerania (SHIP) is a longitudinal population-based cohort study in West Pomerania, a region in the northeast of Germany. SHIP was designed to assess prevalence and incidence of common risk factors, subclinical disorders and clinical diseases and to investigate complex associations among risk factors, subclinical disorders and clinical diseases. From the total population comprising 212,157 inhabitants in 1995, a two-stage stratified cluster sample of adults aged 20 to 79 years was drawn. From the net sample of 6,265 eligible subjects, 4,308 subjects (2,192 women) of European ancestry participated in the baseline examination, SHIP-0 (response 68.8%). During the baseline examination between 1997 and 2001 (SHIP-0) as well as during the 5-year follow-up examination between 2002 and 2006 (SHIP-1) resting electrocardiograms were digitally stored (Personal 120LD, Esaote, Genova, Italy) and processed by the MEANS ECG Interpretation and Measurement software (Welch Allyn, Skaneateles Falls, NY) according to the method described above for the RS. In addition, a Tele-ECG subproject was conducted in SHIP-1 to assess the prevalence of symptomatic and asymptomatic cardiac arrhythmias. Subjects were considered as having atrial fibrillation if it was present in at least one of these examinations.

The Vanderbilt Lone Atrial Fibrillation Registry consists of patients between 18 and 65 years of age with documented atrial fibrillation in the absence of hypertension, heart failure, coronary disease, or significant valve disease as assessed by echocardiography. Consecutive patients with atrial fibrillation were prospectively enrolled beginning in October 2002 from the Vanderbilt Cardiology and Arrhythmia Clinics, the emergency department, and in-patient services. Standardized medical and drug histories, and self-completed questionnaires detailing symptoms were administered to all participants. Referent subjects were free from atrial fibrillation as documented by electrocardiography.

The Women's Genome Health Study (WGHS) is a prospective cohort comprised of over 25,000 initially healthy female health professionals enrolled in the Women's Health Study, which began in 1993. All participants in WGHS provided baseline blood samples and extensive survey data. Women were asked to report diagnoses of atrial fibrillation at baseline, 48 months,

and then annually thereafter. Beginning on September 19, 2006, women enrolled in the continued observational follow-up who reported an incident atrial fibrillation event on at least one yearly questionnaire were sent an additional questionnaire to confirm the episode and to collect additional information. They were also asked for permission to review their medical records, particularly available ECGs, rhythm strips, 24-hour ECGs, and information on cardiac structure and function. For all deceased participants who reported atrial fibrillation during the trial and extended follow-up period, family members were contacted to obtain consent and additional relevant information. An end-point committee of physicians reviewed medical records for reported events according to predefined criteria. An incident atrial fibrillation event was confirmed if there was ECG evidence of atrial fibrillation or if a medical report clearly indicated a personal history of atrial fibrillation. The earliest date in the medical records when documentation was believed to have occurred was set as the date of onset of atrial fibrillation. Only confirmed events are included in this analysis.

Replication Cohorts

Independent subjects with atrial fibrillation were identified from the AFNET Study and controls without atrial fibrillation were obtained from the KORA S4 study for the replication phase of the current study. Independent subjects with atrial fibrillation and controls without atrial fibrillation were identified from the HVH study for replication; included in the replication sample were atrial fibrillation cases ≥66 years of age or with clinically recognized structural heart disease at atrial fibrillation diagnosis, and referent subjects without atrial fibrillation, frequency matched to atrial fibrillation cases on age, sex, hypertension, and year of identification. Independent subjects with early-onset atrial fibrillation were identified from the MGH atrial fibrillation Study; referent subjects without atrial fibrillation were drawn from the local hospital catchment.

The Health Aging and Body Composition (Health ABC) Study is a National Institute of Aging-sponsored ongoing cohort study of the factors that contribute to incident disability and the decline in function of healthier older persons, with a particular emphasis on changes in body composition in old age. Health ABC enrolled well-functioning, community-dwelling black (n=1,281) and white (n=1,794) men and women aged 70-79 years between April 1997 and June 1998. Participants were recruited from a random sample of white and all black Medicare eligible residents in the Pittsburgh, PA, and Memphis, TN, metropolitan areas. The key components of Health ABC include a baseline exam, annual follow-up clinical exams, and phone contacts every 6 months to identify major health events and document functional status between clinic visits.

The Malmö Study consists of cases with prevalent or incident atrial fibrillation from two population-based cohorts from Malmö, Sweden (Malmö Diet and Cancer and the reexamination of the Malmö Preventive Project identified from national registers as previously described) and matched 1:1 to controls from the same cohort by sex, age (±1 year), date of baseline exam (±1 year) and requiring a follow-up exceeding that for the corresponding case.

The Ottawa Heart atrial fibrillation study consists of patients with lone atrial fibrillation, or atrial fibrillation and hypertension, recruited from the Arrhythmia Clinic at the University of Ottawa Heart Institute (UOHI). Enrollment requires at least one episode of electrocardiographically documented atrial fibrillation characterized by erratic atrial activity without distinct P waves and irregularly irregular QRS intervals. Exclusion criteria consist of a history of coronary artery disease, left ventricular ejection fraction <50%, or significant valvular disease on echocardiography. Control subjects were drawn from the control arm of the Ottawa Heart Genomics Study, an ongoing case control study for coronary artery disease at the UOHI. Male control subjects were ≥ 65 years, while female controls were ≥ 70 years of age. Control subjects with a documented history of atrial fibrillation were excluded from this study. All cases and controls were of Western European ancestry.

Atrial fibrillation GWAS in Japanese

We used 843 atrial fibrillation cases who participated in the BioBank Japan project between 2003 and 2006. Control subjects consisted of 2,444 Japanese individuals registered in BioBank Japan as subjects with eleven diseases (hepatic cirrhosis, osteoporosis, colorectal cancer, breast cancer, prostate cancer, lung cancer, uterine myoma, amyotrophic lateral sclerosis, drug eruption, gallbladder and bile duct cancer, and pancreatic cancer) and 906 healthy volunteers recruited from the Osaka-Midosuji Rotary Club, Japan.

Illumina Human610-Quad and Illumina HumanHap550v3 Genotyping BeadChip were used for case and control groups, respectively. We applied quality control criteria as below (call rate of ≥0.99 in both cases and controls and Hardy-Weinberg equilibrium test $P \geq 1.0 \times 10^{-6}$ in control population); 430,963 SNPs on all chromosomes passed the quality control filters. All cluster plots were checked by visual inspection by trained personnel, to exclude SNPs with ambiguous calls.

Genotyping

Detailed information on the genotyping platforms and exclusions in each cohort for the GWAS meta-analysis are provided in Supplemental Table 1. Replication genotyping was performed using a TaqMan assay (Applied Biosystems, Inc., Foster City, CA) in the Ottawa sample, or Sequenom iPlex single base primer extension with MALDI-TOF mass spectrometry (Sequenom, San Diego, CA) for AFNET/KORA S4, HVH, Malmö, and MGH.

Statistical Analysis

For the meta-analysis, over 2.5 million HapMap SNPs were imputed within each study using the HapMap CEU population. Mach v1.0.1x was used by AFNET, AGES, Rotterdam, Vanderbilt, MGH, FHS, ARIC, Cleveland Clinic and WGHS; BIMBAM was used by HVH and CHS; IMPUTE v0.5 was used by SHIP. In studies for which population structure was associated with the atrial fibrillation phenotype (FHS, MGH, and Cleveland Clinic), analyses were adjusted for the principal components of genotype associated with phenotype,[27] The primary analysis in each center used

logistic or proportional hazards regression, as appropriate, adjusting for age at DNA draw and sex. ARIC and CHS also adjusted for study site. Each SNP was modeled using an additive genetic effect. The ratio of observed to expected variance in the imputed SNP genotype counts,[28] the MACH Rsq statistic, which is a variation on this metric, or a measure of the observed statistical information associated with the imputed genotype that was computed by IMPUTE, were used as a quality control metrics for imputed SNPs. All three metrics range from 0 to 1, with 1.0 indicating high imputation quality, and 0 no imputation information. For each SNP, all studies with quality scores greater than 0.10 were included in meta-analyses. For each SNP, a fixed effects model was used for meta-analysis of the genotype logistic regression parameters (log odds ratios), using inverse variance weights as implemented in the meta-analysis utility METAL. Prior to meta-analysis, genomic control was applied to each study having genomic control inflation factor (λ) >1.0 by multiplying the standard error of the SNP regression parameter by the square-root of the study-specific λ. A total of 2,609,549 SNPs with average minor allele frequencies $\geq$0.01 across participating studies were included in meta-analyses. We pre-specified a $P<5x10^{-8}$ corresponding to Bonferroni adjustment for 1 million independent tests as our criterion for genome-wide significance.[29] Our pre-specified criterion for replication was that the meta-analysis of the discovery + replication studies would have a smaller p-value than the discovery meta-analysis.

Prediction of SNP function and eQTL analyses

The proxy of each of the three previously published and seven novel top SNPs was obtained from SNAP Proxy Search. HapMap release 22 CEU population was used as the reference panel and r^2 threshold was 0.8. We limited the maximum physical distance to 500kb. The seven novel top SNPs, along with their proxies, were then used for SNP function and eQTL analysis. eQTL analysis was performed by searching against the eQTL Browser, which complied 13 datasets collected from multiple studies. Functional annotation of these SNPs was obtained from the dbSNP database. Nonsynonymous SNPs were selected and submitted to PolyPhen-2 and SIFT for functional effect prediction.

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SUPPLEMENTARY MATERIAL

(Starting on next page)

Supplemental Table 1. Details regarding study samples, genotyping and data cleaning.

	AFNET / KORA	AGES	ARIC	CHS	CCAF	FHS
Study	German AF Network	Age, Gene/ Environment Susceptibility Study	Atherosclerosis Risk in Communities Study	Cardio-vascular Health Study	Cleveland Clinic AF Study	Framingham Heart Study
Design papers		[1]	[2,3]	[4]		[5,6]
Array	Illumina HumanCNV370 And Illumina Human550K	Illumina HumanCNV370-Duo BeadChip	Affymetrix 6.0	Illumina 370 CNV	Illumina Hap550 v1 or v3 and Hap610 v1	Affymetrix Gene Chip® 500K Array Set & 50K Human Gene Focused Panel
Calling Algorithm	BeadStudio	BeadStudio	Birdseed	BeadStudio	BeadStudio	BRLMM
Per SNP Call rate	<98%	<97%	<95%	<97%	<95%	<97%
HWE p-value	$<10^{-5}$	$<10^{-6}$	$<10^{-6}$	$<10^{-5}$	FDR < 0.20	$<10^{-6}$
Mendelian errors	NA	NA	NA	≤2	NA	N>100
Excess heterozygosity	ND	NA	NA	ND	FDR < 0.01	subject hetero-zygosity >5 SD away from the mean
MAF	<5%	<1%	<1%	Excluded SNPs with 0 hetero-zygotes	<5%	<1%

HVH	**MGH**	**RS-I**	**SHIP**	**Vanderbilt**	**WGHS**	**BioBank Japan**
Heart and Vascular Health Study	Mass. General Hospital AF Study & MIGEN	Rotterdam Study	The Study of Health in Pomerania	Vanderbilt AF Registry	Women's Genome Health Study	BioBank Japan
7	8	9	10	11	12	13
Illumina 370 CNV	Affymetrix 6.0	Illumina Infinium HumanHap550-chip v3.0	Affymetrix 6.0	Illumina 610 quad	Illumina HumanHap300 Duo+	Illumina Human610-Quad and Illumina Human Hap550v3 BeadChip
BeadStudio	Birdseed	BeadStudio	BeadStudio v2	BeadStudio v3.1	BeadStudio	BeadStudio
<97%	<97%	<98%	ND	<99%	<90%	<99%
$<10^{-5}$	$<10^{-6}$	$<10^{-6}$	ND	$<10^{-4}$ flagged	$<10^{-6}$	$<10^{-6}$
≤2	NA	NA	NA	NA	NA	NA
ND	ND	>0.336; n=21	ND	ND	ND	ND
Excluded SNPs with 0 heterozygotes	<1%	<1%	ND	Excluded monomorphic SNPs	<1%	<1%

Supplemental Table 1. Details regarding study samples, genotyping and data cleaning. *(continued)*

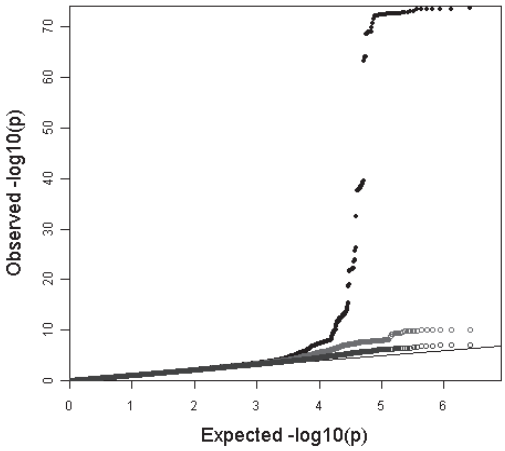
	AFNET / KORA	AGES	ARIC	CHS	CCAF	FHS
Selection criteria for PCs	P<0.05	P<0.05	Eigenstrat: anyone >8 SD from top 10 PCs was removed (225/9747 individuals)	PCs examined in relation to incident and prevalent AF. Bonferroni adjusted P value threshold to determine whether PCs were associated with AF.	P< 0.0005	All PCs unassociated, p>0.05
Number of PCs in the model	4	NA	NA	-	6	0
Number of SNPs used for imputation	315,972	308,340	602,642	306,655	460,569	385,958
Imputation software	Mach1 v 1.0.10[14]	Mach1 v 1.0.16[2]	Mach1 v 1.0.16[14]	BIMBAM	Mach1 v 1.0.16[14]	Mach1 v 1.0.15[14]
Imputation Backbone / NCBI Build	Build 35	Build 36	Build 36	Build 36	Build 36	Build 36
SNP position from NCBI build	Build 35	Build 36	Build 36	Build 36	Build 36	Build 36
GWAS Statistical Analysis	ProbABEL[16], R[17]	ProbABEL[16], R[17]	ProbABEL[16], PLINK[18], R[17]	R, version 2.7	ProbABEL[16], R[17]	R packages kinship, gee, coxpH [17]
Total number of SNPs used in the analysis (MAF>0.005)	2,521,723	2,408,991	2,512,759	I:2,319,581 P: 2,317,847	2,509,367	I: 2501666 P: 2501188
Inflation factor (λ)	1.02	I: 1.005 P:1.062	1.007	I: 1.045 P: 1.038	1.034	I:1.017 P:1.038

Abbreviations: AF, Atrial fibrillation; I, incident; NA, not available; ND, not done; P, prevalent;
BRLMM, denotes the Bayesian Robust Linear Modeling.
PLINK, http://pngu.mgh.harvard.edu/purcell/PLINK/
Eigenstrat, http://genepath.med.harvard.edu/~reich/Software.htm
MACH, http://www.sph.umich.edu/csg/abecasis/MaCH/index.html
BIMBAM, http://stephenslab.uchicago.edu/software.html

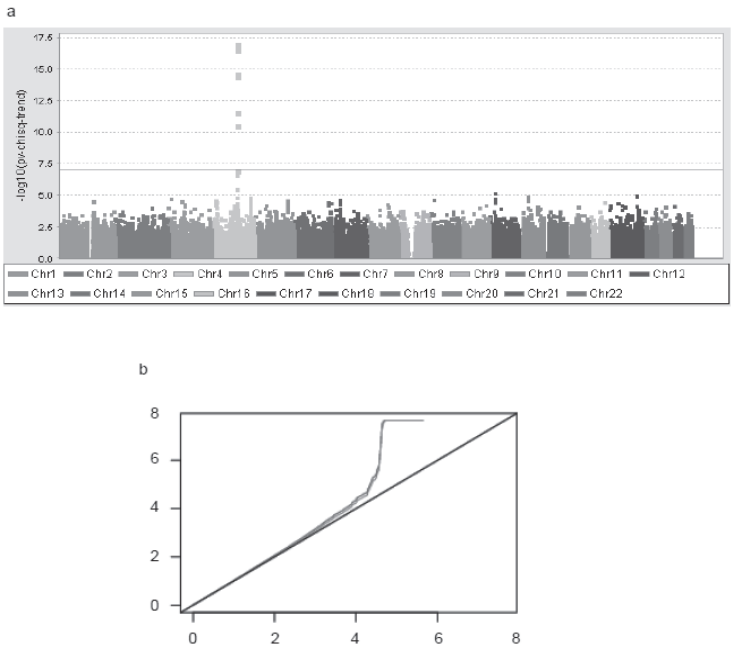
HVH	MGH	RS-I	SHIP	Vanderbilt	WGHS	BioBank Japan
ND	P<0.05	Outliers as identified by IBS clustering were excluded	AF, adjusted for sex+age, was associated with the PC (p<0.05), tested for first 10 PCs obtained from EIGENSTRAT	ND	Association with AF (p<0.05)	Within ± 0.02 from centroid for both components
NA	6	NA	0	NA	1 EV for incident AF, 3 EVs for prevalent AF	2
305,353	638,338	530,683	869,224	488,523	331959	ND
BIMBAM[15]	Mach1 v1.0.16[14]	Mach1 v 1.0.15[14]	IMPUTEv0.5.0 against HapMap II CEU v22	Mach v 1.0.16	Mach1 v. 1.0.16 HapMap II CEU r22	ND
Build 36	Build 36	Build 36	Build 36	Build 36	Build 36	ND
Build 36	Build 36	Build 36	Build 36	Build 36	Build 36	Build 36
R[17]	R[17]	Mach2QTL GenABEL + PLINK, R[17], GRIMP[19]	QUICKTEST v0.94	PLINK, PLATO	ProbABEL, R	R, version 2.10.0
2,316,203	2,508,401	I: 2,502,002 P: 2,501,903	2,598,639	2,543,887	2,608,508	430,963
1.09	1.022	I:1.035 P:1.024	0.998	1.023	1.017	1.03

Supplemental Table 2. Replication study samples.

Cohort	Affection Status	Ancestry	Participants, n	Males, n (%)	Age at DNA collection, mean ± SD	Age at DNA collection range	Age of AF onset, mean ± SD	Hypertension, n (%)	BMI, kg/m^2, mean ± SD	Reference
AFNET	Cases	European	1,489	1,119 (75.2)	62.9 ± 11.1	20-95	62.9 ± 11.2	934 (63)	27.7 ± 4.5	20
KORA	Controls	European	3,614	1,772 (49.0)	48.3 ± 14.3	25-74	-	1,293 (36)	27.1 ± 4.7	
Malmö	Cases	European	2,316	1,449 (63.0)	64.8 ± 7.4	45-85	69.8 ± 8.5	1,922 (83)	27.3 ± 4.4	21,22
	Controls		2,322	1,454 (63.0)	64.8 ± 7.4	45-85	-	1,672 (72)	26.3 ± 3.9	
MGH AF	Cases	European	181	136 (75.1)	59.7 ± 11.3	23-86	-	99 (55)	28.7 ± 5.8	23
	Controls		362	191 (52.8)	68.9 ± 11.6	24-94	-	221 (61)	27.0 ± 5.0	
Ottawa	Cases	European	301	240 (79.7)	-	-	47.2±11.4	65 (22)	-	24
	Controls		1,517	724 (47.7)	75.1 ± 5.4	65-100	-	652 (43)	26.4 ± 4.1	
Health ABC	Cases	European	434	264 (60.8)	74.1 ± 2.9	69-80	79.7 ± 4.3	286 (66)	27.2 ± 4.3	
	Controls		1,100	534 (48.6)	73.6 ± 2.8	69-80		659 (60)	26.4 ± 4.1	
HVH	Cases	European	660	255 (38.6)	74.0 ± 9.0	39 - 88	71.9 ± 8.9	505 (77)	30.3 ± 7.0	7
	Controls		1,115	406 (36.4)	72.7 ± 8.2	37 - 88	-	861 (77)	29.0 ± 5.8	
Biobank Japan	Cases	Japanese	843	579 (68.7)	67.3 ± 10.4	24-94	-	661 (78.4)	23.9 ± 3.6	
	Controls		3,350	1,821 (54.4)	52.4 ± 15.2	3-96	-	1117 (33.3)	22.5 ± 3.7	



Supplemental Figure 1. Quantile-Quantile plots for the discovery GWAS in Europeans.
Black points: GWAS p-values for N=2609549 SNPs with average minor allele frequency >0.01. Red points: GWAS p-values for N= 2607396 SNPs with minor allele frequency >0.01, excluding SNPs within 500kb of the three previously published loci. Blue points: GWAS p-values for the N= 2602637 SNPs SNPs with minor allele frequency >0.01, excluding SNPs within 500kb of the ten loci with $p<5\times10^{-8}$ in the discovery data set. Genomic control λ=1.04 for all 3 sets of p-values.



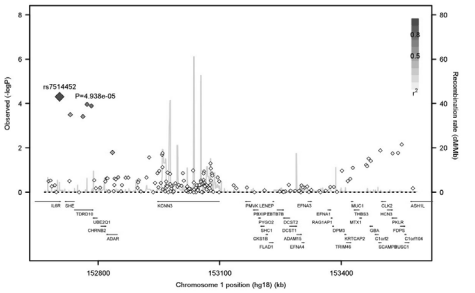
Supplemental Figure 2. Manhattan plot and quantile-quantile plot of genome-wide association results for atrial fibrillation in Japanese subjects.
A. The $-\log_{10}$(p value) is plotted against the physical positions of each SNP on each chromosome. B. Quantile-quantile plots of the observed against expected $-\log_{10}$ (p-value) distributions of SNPs related to atrial fibrillation.

Supplemental Table 3: Replication of novel genome-wide hits for atrial fibrillation.

					AFNET/KORA		HABC incident	
SNP	**Locus**	**Closest gene**	**Minor/ major allele**	**Model**	**RR 95% CI**	***P* value**	**RR 95% CI**	***P* value**
rs3903239	1q24	*PRRX1*	G/A	1	1.17 1.07-1.27	$6.0x10^{-4}$	0.88 0.77-1.00	0.07
				2	1.19 1.08-1.30	$1.3x10^{-4}$		
rs2040862	5q31	*WNT8A*	T/C	1	1.05 0.94-1.16	0.38	1.10 0.90-1.30	0.46
				2	1.02 0.92-1.14	0.35		
rs3807989	7q31	*CAV1*	A/G	1	0.83 0.76-0.90[2]	0.10	1.07 0.94-1.21	0.34
				2	0.92 0.83-1.02[2]	0.10		
rs10821415	9q22	*C9orf3*	A/C	1	1.14 1.05-1.24[3]		1.07 0.94-1.23	0.31
				2	1.16 1.05-1.28[3]	$3.8x10^{-3}$		
rs10824026	10q22	*SYNPO2L*	G/A	1	-	-	0.89 0.74-1.07	0.21
				2	-	-		
rs1152591	14q23	*SYNE2*	A/G	1	1.19 1.10-1.29	$3.2x10^{-5}$	1.03 0.90-1.18	0.62
				2	1.18 1.08-1.28	$6.2x10^{-5}$		
rs7164883	15q24	*HCN4*	G/A	1	1.27 1.13-1.42	$4.9x10^{-5}$	1.26 1.06-1.50	0.01
				2	1.32 1.16-1.50	$2.4x10^{-5}$		

Effect estimates correspond to the minor allele. [1]Proxy SNP rs11718898; [2]Proxy SNP rs11773845; [3]Proxy SNP rs4744411; [4]Proxy SNP rs6480708. No attempt was made to replicate the previously published loci for atrial fibrillation at ***PITX2***, ***KCNN3***/***PMVK***, or ***ZFHX3***. Model 1 adjusted for age and sex; model 2 adjusted for age, sex and HTN.
Please note that the HVH cohort matched subjects on hypertension status, so model 1 was not performed.

HABC prevalent		HVH		MGH AF		Malmö		Ottawa	
RR 95% CI	***P* value**	**RR 95% CI**	***P* value**	**RR 95% CI**	***P* value**	**RR 95% CI**	***P* value**	**RR 95% CI**	***P* value**
1.00 0.81-1.40	0.72			1.37 1.04-1.81	0.03	-	-	-	-
		0.99 0.86-1.10	0.93	1.37 1.03-1.81	0.03	-	-	-	-
0.94 0.66-1.3	0.73			1.02 0.71-1.48	0.90	-	-	-	-
		1.08 0.89-1.3	0.43	1.03 0.71-1.48	0.89	-	-	-	-
0.83 0.64-1.07	0.15			-	-	0.88 0.81-0.96	0.003	0.8 0.66-0.96	0.02
		1.04 0.9-1.19[2]	0.64	-	-	0.88 0.81-0.96	0.004	0.8 0.66-0.96	0.02
0.89 0.68-1.16	0.39			-	-	1.02 0.94-1.11	0.58	1.31 1.09-1.57	0.004
		1.12 0.98-1.29[3]	0.11	-	-	1.02 0.94-1.11	0.58	1.32 1.10-1.59	0.003
0.92 0.65-1.32	0.66	-	-	-	-	0.88 0.78-0.98	0.02	1.14 0.69-1.45	0.3
		-	-	-	-	0.87 0.78-0.98	0.02	1.12 0.87-1.43	0.37
1.10 0.85-1.42	0.46			1.05 0.80-1.40	0.71	-	-	-	-
		1.09 0.95-1.24	0.23	1.06 0.80-1.40	0.70	-	-	-	-
0.84 0.58-1.22	0.37			-	-	1.18 1.06-1.32	0.002	1.36 1.09-1.71	0.007
		1.25 1.04-1.50	0.02	-	-	1.20 1.08-1.34	0.001	1.38 1.10-1.73	0.006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P=4.938e-05
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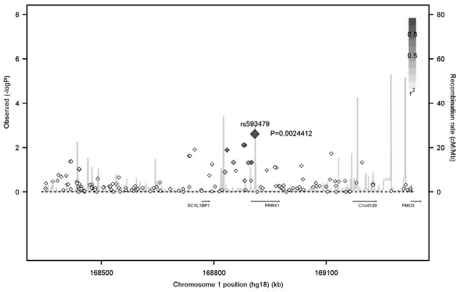
Recombination rate (cM/Mb)
Chromosome 1 position (hg18) (kb)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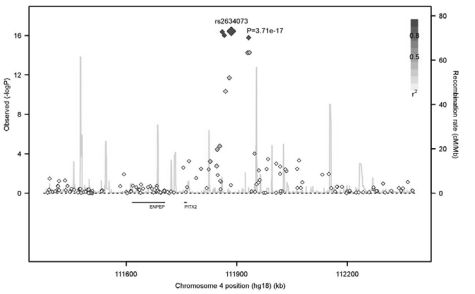
Recombination rate (cM/Mb)
Chromosome 1 position (hg18) (kb)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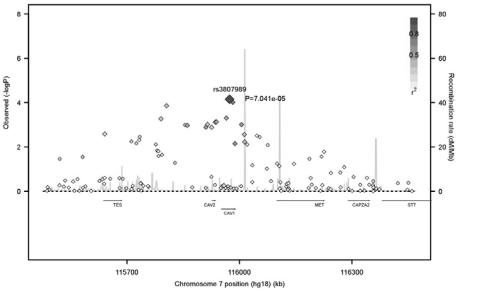
Observed (-logP)
Recombination rate (cM/Mb)
Chromosome 4 position (hg18) (kb)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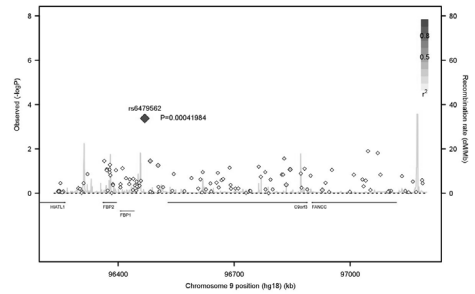
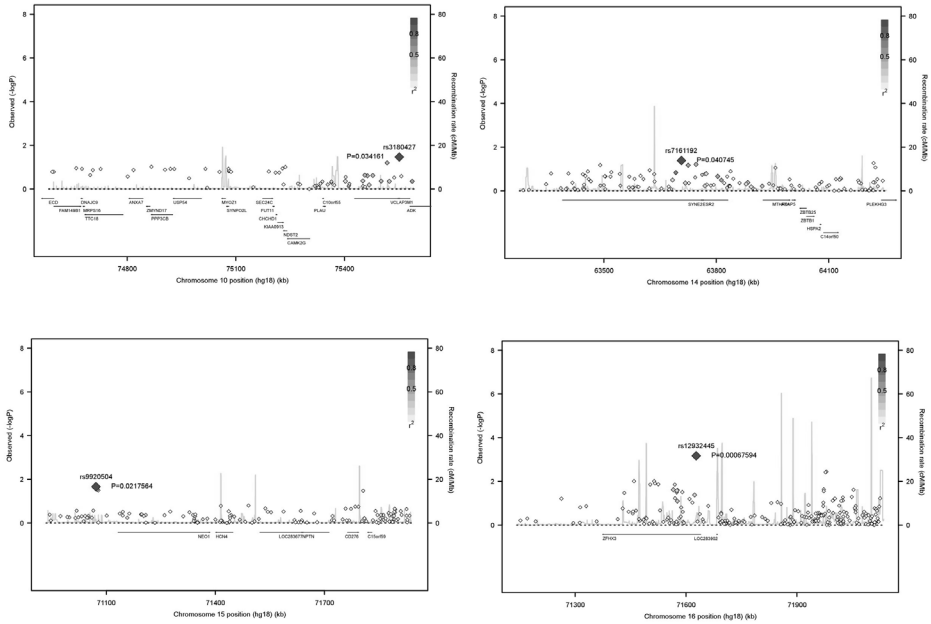
Observed (-logP)
Recombination rate (cM/Mb)
Chromosome 7 position (hg18) (kb)


rs6479562
P=0.00041984
Observed (-logP)
Recombination rate (cM/Mb)
Chromosome 9 position (hg18) (kb)



Supplemental Figure 3. Regional plots from Japanese GWAS data for 9 atrial fibrillation loci in Europeans. SNPs are plotted with the meta-analysis P value and genomic position (NCBI Build 36). The SNP of interest is labeled. The strength of the linkage disequilibrium (LD) is indicated by gradient of red. Estimated recombination rates are shown by the blue line and gene annotations are indicated by dark green arrows. LD and recombination rates are based on the CHB/JPT HapMap release 28. Figures prepared using SNAP.[27]

Supplemental Table 4: Relation between atrial fibrillation and previously reported PR GWAS loci.

Loci associated with the PR interval					Relation of PR SNPs to Atrial fibrillation			Reference
Locus	**SNP**	**Allele**	**Chromosome**	**Position**	**RR**	**95% CI**	***P* value**	
MEIS1	rs11897119	C	2	66625504	1.01	0.97-1.05	0.57	[25]
SCN5A	rs11708996	C	3	38608927	0.93	0.88-0.98	0.0084	[25]
SCN10A	rs6795970	A	3	38741679	0.92	0.88-0.94	$2.2x10^{-5}$	[26]
	rs6800541	C	3	38749836	0.91	0.87-0.95	$2.8x10^{-6}$	[25]
ARHGAP24	rs7692808	A	4	86860173	0.97	0.93-1.01	0.18	[25]
	rs7660702	T	4	86870488	0.97	0.93-1.01	0.19	[26]
NKX2-5	rs251253	C	5	172412942	1.04	1.00-1.09	0.04	[25]
CAV1	rs3807989	A	7	115973477	0.88	0.84-0.91	$9.6x10^{-11}$	[25,26]
WNT11	rs4944092	G	11	75587267	1	0.96-1.04	0.82	[25]
SOX5	rs11047543	A	12	24679606	1.16	1.10-1.23	$3.5x10^{-7}$	[25]
TBX5	rs3825214	G	12	113279826	0.88	0.83-0.92	$4.1x10^{-7}$	[26]
TBX5-TBX3	rs1896312	C	12	113830807	0.99	0.94-1.03	0.52	[25]
MYH6	rs365990	G	14	22931651	1.02	0.98-1.06	0.3	[26]

Supplemental Table 5: *In silico* replication of genome-wide hits for atrial fibrillation in Japanese.

		European GWAS			Japanese GWAS			
Locus	**Closest gene**	**Top SNP for AF**	**Minor major allele**	**MAF**	**Proxy for top SNP for AF**	**Minor major allele**	**MAF**	**R^2**
1q21	*KCNN3*	rs6666258	C/G	29.9	rs6426987	A/C	1.5	1.0
1q24	*PRRX1*	rs3903239	G/A	44.7	rs12755237	G/A	46.1	1.0
4q25	*PITX2*	rs6817105	C/T	13.1	rs2220427	T/C	47.3	1.0
5q31	*WNT8A*	rs2040862	T/C	17.8	-	-	-	-
7q31	*CAV1*	rs3807989	A/G	40.4	-	A/G	34.5	-
9q22	*C9orf3*	rs10821415	A/C	42.4	rs356131	A/G	31.2	0.731
10q22	*SYNPO2L*	rs10824026	G/A	15.8	-	-	-	-
14q23	*SYNE2*	rs1152591	A/G	47.6	rs1152592	A/G	40.7	0.74
15q24	*HCN4*	rs7164883	G/A	16.0	-	G/A	10.9	-
16q22	*ZFHX3*	rs2106261	T/C	17.6	rs12932445	C/T	37.0	0.816

OR 95% CI	P value	Top SNP at locus in Japanese AF GWAS	Minor major allele	MAF	R^2 to top SNP in European AF GWAS	OR 95% CI	P value
1.46 0.85-2.51	0.17	rs7514452	C/T	16.6	0	0.72 0.62-0.84	**$4.9x10^{-5}$**
1.17 1.03-1.32	**0.013**	rs593479	C/T	50.3	0.771	1.21 1.07-1.37	**$2.4 x10^{-3}$**
1.64 1.45-1.86	**$5.4x10^{-15}$**	rs2634073	C/T	31.9	0.363	1.84 1.59-2.13	**$3.7x10^{-17}$**
-	**-**	rs6878439	A/G	12.7	-	1.20 1.00-1.44	0.053
0.76 0.67-0.87	**$7.0x10^{-5}$**	rs3807989	A/G	34.5	-	0.76 0.67-0.87	**$7.0x10^{-5}$**
1.03 0.91-1.18	0.61	rs6479562	A/G	14.7	0	0.72 0.59-0.87	**$4.2x10^{-4}$**
-	-	rs3180427	T/G	7.9	0.003	1.16 1.01-1.33	0.034
1.13 0.99-1.28	0.061	rs7161192	C/A	48.4	0.443	0.88 0.78-0.99	0.041
0.98 0.81-1.19	0.86	rs9920504	C/A	2.8	0.051	0.68 0.50-0.94	0.022
0.80 0.71-0.91	**$6.8 x10^{-4}$**	rs12932445	C/T	37.0	0.816	0.80 0.71-0.91	**$6.8x10^{-4}$**

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CHAPTER 4

Prediction of atrial fibrillation

ABSTRACT

Background: Tools for the prediction of atrial fibrillation may identify high-risk individuals, more likely to benefit from preventive interventions, and serve as a benchmark to test novel putative risk factors.

Methods: Individual-level data from three large cohorts in the United States (Atherosclerosis Risk in Communities (ARIC) study, Cardiovascular Health Study (CHS), and Framingham Heart Study (FHS)), including 18556 men and women age 46-94 years (19% African Americans, 81% whites) was pooled to derive predictive models for atrial fibrillation using clinical variables. Validation of the derived models was performed in 7672 participants from the Age, Gene and Environment – Reykjavik study (AGES) and the Rotterdam Study (RS).

Result: The analysis included 1186 incident atrial fibrillation cases in the derivation cohorts and 585 in the validation cohorts. A simple 5-year predictive model including the variables age, race, height, weight, systolic and diastolic blood pressure, current smoking, use of anti-hypertensive medication, diabetes, and history of myocardial infarction and heart failure had good discrimination (C-statistic, 0.765; 95% CI, 0.748 to 0.781). Addition of variables from the electrocardiogram did not improve the overall model discrimination (C-statistic, 0.767; 95% CI, 0.750 to 0.783; categorical net reclassification improvement, -0.0032; 95% CI, -0.0178 to 0.0113). In the validation cohorts, discrimination was acceptable (AGES C-statistic, 0.664; 95% CI, 0.632 to 0.697 and RS C-statistic, 0.705; 95% CI, 0.664 to 0.747) and calibration was adequate.

*Conclusion***:** A risk model including variables readily available in primary care settings adequately predicted atrial fibrillation in diverse populations from the United States and Europe.

INTRODUCTION

Atrial fibrillation, a common cardiac arrhythmia, has emerged as a major public health problem as a result of wide prevalence,[1] close relation to stroke and mortality,[2] and associated costs.[3] Tools for the prediction of atrial fibrillation could help identify high-risk individuals, and serve as a benchmark to test potential novel risk factors. To this end, the Framingham Heart Study (FHS) developed a risk score for atrial fibrillation, which included a number of variables easily obtained during routine clinical examination.[4] This risk score was recently validated in two additional population-based cohorts, the Age Gene/Environment Susceptibility-Reykjavik (AGES) Study and the Cardiovascular Health Study (CHS), where it demonstrated reasonable performance.[5] An alternative score has been developed in the Atherosclerosis Risk in Communities (ARIC) Study, with similar predictive ability.[6] These studies included atrial flutter in their definition of atrial fibrillation. This inclusion is reasonable because, even though atrial flutter and atrial fibrillation are electrophysiologically distinct, most patients with atrial flutter have or will develop atrial fibrillation and the risk of stroke in atrial flutter is similar to that observed in atrial fibrillation.[7-8]

Previous risk models are limited as a result of being developed in single cohorts. Though the FHS risk score has predicted atrial fibrillation reasonably well in other populations,[5-6] it is unknown whether a risk model developed in a more geographically or racially diverse population would better predict atrial fibrillation. Previously developed models also require information from a 12-lead electrocardiogram, which might be unavailable in some primary care settings. Therefore, we developed and validated a new predictive score for atrial fibrillation (including atrial flutter) in five U.S. and European cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) atrial fibrillation consortium.[9]

METHODS

Study Cohorts

Participant-specific data from three community-based cohorts in the United States (ARIC, CHS, and FHS) were pooled to develop a risk score for the prediction of atrial fibrillation, and validation of this score was performed in two additional cohorts in Europe (AGES and the Rotterdam Study [RS]). A brief description of each participating cohort is provided below. For each cohort the choice of which examination to select as baseline was based on availability of potential predictors and adequate follow up for the development of atrial fibrillation. Participants were excluded from this analysis if they had atrial fibrillation at baseline, were younger than 46 or older than 94 years of age, had serum creatinine ≥2.0 mg/dL, identified themselves as other than white or African-American (n=30 ARIC, n=32 CHS, and n=62 RS participants), or had missing values for any of the variables of interest. After applying exclusion criteria, the derivation cohort included 18556 participants and the validation cohorts included a total of 7672

participants. The number of individuals excluded by cohort is provided in the supplemental table 1. Institutional Review Boards at the participating institutions approved the individual studies. Study participants provided written informed consent.

Atherosclerosis Risk in Communities Study

The ARIC study recruited 15792 men and women, aged 45-64 years, from 4 communities in the United States (Forsyth County, NC; Washington County, MD; Jackson, MS; and suburbs of Minneapolis, MN) in 1987-89.[10] Participants were mostly white in the Minnesota and Washington County field centers, white and African-American in Forsyth County, and exclusively African-American in the Jackson field center. After study inception, participants had 3 follow-up examinations, each approximately 3 years apart. For the present analysis, we included individuals attending the last follow-up examination (visit 4, conducted in 1996-98, n=11,656), with this examination used as baseline in all models. Of these, 10675 met inclusion criteria.

Cardiovascular Health Study

In 1989-90, CHS recruited 5201 men and women 65 years or older from 4 communities (Forsyth County, NC; Washington County, MD; Sacramento County, CA; and Pittsburgh, PA). Because of the different age inclusion criteria there was no overlap in ARIC and CHS participants. In 1992-93, 687 African Americans were recruited in 3 of the 4 communities to increase minority representation.[11] CHS participants had annual study exams through 1999; surveillance for cardiovascular events has been ongoing from baseline through the present. The 1989-90 examination was considered baseline for 3768 (approximately 65%) of the eligible CHS participants in this analysis, while 1992-93 was the baseline examination for the rest (n=1275).

Framingham Heart Study

The FHS Offspring cohort recruited 5124 predominantly white men and women, offspring (and their spouses) from the Original FHS cohort, in 1971-1975, with follow-up exams every 4-8 years.[12] The current analysis included participants of the FHS Offspring cohort free of atrial fibrillation attending the 6th examination cycle (1995-1998, n=3113); 2838 met inclusion criteria and were included in the analysis.

Age, Gene/Environment Susceptibility Reykjavik Study

The original Reykjavik Study, conducted between 1967 and 1996, included approximately 19000 men and women living in the greater Reykjavik area, born between 1907 and 1935.[13] Survivors of this study were invited to be part of AGES, which recruited 5764 men and women in 2002-2006. Of these, 5427 had a complete clinic exam, and 4469 met inclusion criteria and were considered for this analysis.

Rotterdam Study

The RS, a prospective population-based study aimed to assess the determinants of chronic conditions in the elderly, examined 7983 men and women, aged 55 years and older, living in the Rotterdam suburb of Ommoord in 1989-1993.[14] Since then, participants have been continuously followed and were reexamined in 1993-1994, 1997-1999, 2002-2004 and 2008-2010. The present analysis included 3203 study participants examined in 1997-1999 meeting inclusion criteria.

Ascertainment of incident atrial fibrillation

Incident atrial fibrillation cases in all five studies were ascertained from study electrocardiograms and hospitalization discharge diagnosis codes (ICD9-CM 427.3, 427.31 or 427.32, or ICD10 I48 in any position).[15-18] Individuals with atrial flutter were included as atrial fibrillation cases. Atrial fibrillation ascertainment in FHS required additional adjudication of cases by study cardiologists using electrocardiographic and clinical data from the FHS clinic, outside hospital or general practitioner records.[17] Cases included in the present analysis occurred between 2002-2011 in AGES, 1996-2005 in ARIC, 1989-2000 in CHS, 1995-2005 in FHS, and 1997-2005 in RS. Further details of atrial fibrillation ascertainment are available in supplementary material.

Other measurements

In all five study cohorts, examinations included a 12-lead electrocardiogram, standardized measurements of anthropometry, blood pressures, blood lipids, and fasting glucose, as well as assessment of prior cardiovascular disease and medication use.[10-13, 19] Details on measurement methods are provided in the supplement material. Protocols for variable ascertainment and definitions of cardiovascular risk factors were comparable across cohorts.

Statistical analysis

Derivation of the predictive model

Means and standard deviation, and frequency distribution of relevant covariates were calculated by cohort and race. We initially ran cohort- and race-specific Cox proportional hazard models to assess individual predictors of atrial fibrillation after age- and sex-adjustment in each cohort up to 7 years of follow-up. Variables considered included age, sex, height, weight, current smoking, systolic and diastolic blood pressure, use of antihypertensive medication, history of diabetes, fasting blood glucose, estimated glomerular filtration rate (eGFR)<60 ml/kg/m^2,[20] total blood cholesterol, HDL cholesterol, triglycerides, heart rate, electrocardiographic-derived left ventricular hypertrophy, PR interval, history of coronary artery bypass graft (CABG), history of heart failure, history of myocardial infarction, and history of stroke. We selected as candidate predictors for our pooled model any variable significantly associated with atrial fibrillation ($p<0.05$) in at least two of the three cohorts, and ran the final Cox proportional hazards model on our participant-specific pooled data using backward selection of variables ($p<0.05$ to remain in

the model). Age, sex and race interactions were tested, as was the assumption of proportional hazards. Model-based individual 5-year risk of atrial fibrillation was calculated. We evaluated model performance using the C-statistic,[21] discrimination slopes,[22] and Nam and D'Agostino's modified Hosmer-Lemeshow chi-square statistic for survival analysis.[23]

To facilitate the use of our score in the clinical settings with limited access to electrocardiograms or blood tests, we first developed a predictive model that did not require information from electrocardiogram and blood tests (which we labeled 'simple model'). We then developed a more complex model adding electrocardiographic variables and blood tests (labeled 'augmented model'). Variables were retained in the models if they were significantly associated with atrial fibrillation incidence ($p<0.05$). We calculated the added predicted value of the augmented model vs. the simple model with the increment in the C-statistic and the categorical net reclassification improvement (NRI) using the following risk categories: <2.5%, 2.5-5%, >5%.[22]

Validation analysis

The models developed in the derivation cohorts were applied in AGES and the RS to estimate the 5-year risk of developing atrial fibrillation. As in the derivation analysis, model performance was assessed using the C statistic, discrimination slopes, and Nam and D'Agostino's chi-square statistic metrics. To improve adjustment fit in the validation cohorts, we accounted for the baseline survival of the respective cohort and the corresponding risk factor means.[24]

Additional analyses

We compared the performance of the newly developed risk score with the previous FHS atrial fibrillation risk score.[4] To this end, we calculated model quality measures in the pooled data from ARIC, CHS, and FHS, and separately in AGES and RS after applying the atrial fibrillation risk function previously derived from FHS.[4] Because presence of cardiac murmur, one of the variables included in the FHS atrial fibrillation risk score, was not available in AGES and RS, and given its low prevalence (<3% in the FHS cohort),[4] we assumed it to be absent for all participants in whom it was not ascertained. Finally, we compared calibration and discrimination of the derived risk model and the model independently derived including those same variables in each validation cohort. SAS 9.1 was used for all analyses.

RESULTS

Baseline characteristics of eligible individuals by cohort and race (in ARIC and CHS) are presented in Table 1. Average age in years ranged from 60 in FHS to 76 in AGES; the proportion of women was between 55 and 66% across cohorts. African Americans comprised 19% of the derivation sample. The prevalence of cardiovascular risk factors was generally higher in African Americans than in

Table 1. Baseline characteristics in derivation cohorts and validation cohorts

	Discovery cohorts					Validation cohorts	
	FHS	**CHS whites**	**CHS AA**	**ARIC whites**	**ARIC AA**	**AGES**	**RS**
N	2838	4324	719	8305	2370	4469	3203
Atrial fibrillation cases	143	560	64	343	76	408	177
Age, years	60 (8)	73 (5)	73 (5)	63 (6)	62 (6)	76 (6)	72 (7)
Sex, % female	54.5	58.8	65.9	54.5	64.4	60.4	58.9
Height, cm							
Women	161 (6)	159 (6)	160 (6)	161 (6)	163 (6)	161 (6)	161(6)
Men	175 (7)	173 (7)	174 (7)	175 (7)	176 (7)	175 (6)	174(7)
Weight, kg	78.3 (17.1)	71.7 (14.3)	77.5 (15.6)	79.7 (16.9)	85.7 (18.2)	75.3 (14.5)	74.4 (12.3)
Current smoker, %	14.6	9.0	14.5	14.6	17.5	12.2	15.6
Systolic blood pressure, mmHg	130 (19)	136 (21)	142 (22)	126 (18)	134 (20)	142 (21)	143 (21)
Diastolic blood pressure, mmHg	76 (9)	70 (11)	76 (11)	70 (10)	76 (11)	74 (10)	75 (11)
Antihypertensive medication use, %	29.6	43.8	61.5	29.6	58.4	61.2	37.1
Diabetes, %	10.2	15.0	24.3	10.2	25.9	11.5	10.2
Fasting blood glucose, mg/dL	104 (28)	109 (33)	119 (55)	107 (31)	123 (56)	104 (21)	107 (27)
eGFR<60 ml/kg/m^2, %	9.1	21.1	25.2	9.1	14.8	28.8	13.4
Total cholesterol, mg/dL	207 (38)	212 (38)	210 (38)	202 (36)	200 (38)	219 (44)	225 (38)
HDL cholesterol, mg/dL	51 (16)	54 (16)	58 (15)	49 (16)	53 (17)	62 (17)	54 (15)
Triglycerides, mg/dL	140 (95)	146 (82)	116 (65)	151 (90)	115 (63)	106 (57)	135 (66)
Heart rate, bpm	64 (10)	65 (11)	67 (14)	62 (10)	65 (11)	66 (11)	68 (11)
Electrocardiogram-derived LVH, %	0.7	3.5	9.0	0.7	4.8	-	5.0
PR interval, ms	164 (24)	170 (31)	172 (34)	166 (27)	171 (27)	173 (30)	170 (26)
CABG history, %	1.6	4.4	2.8	1.6	1.7	5.9	4.0
Prevalent heart failure, %	0.6	3.6	4.7	3.9	7.2	1.7	3.5
Prevalent myocardial infarction, %	4.0	9.4	8.2	4.0	4.6	7.0	10.8
Stroke history, %	0.5	3.3	5.8	0.5	3.6	6.0	3.7

Values correspond to percent or mean (standard deviation). AA: African-Americans; CABG: coronary artery bypass graft surgery; eGFR: estimated glomerular filtration rate; LVH: left ventricular hypertrophy

Table 2. Hazard ratios (95% confidence intervals) of atrial fibrillation by selected variables, by cohort. Models with individuals risk factors adjusted for age and sex

	FHS	CHS whites	CHS AA	ARIC whites	ARIC AA	AGES	RS
Age, per 5 years	1.64 (1.49-1.82)	1.49 (1.39-1.60)	1.32 (1.09-1.63)	1.62 (1.46-1.78)	1.54 (1.27-1.88)	1.43 (1.31-1.56)	1.43(1.29-1.59)
Sex, male vs. female	1.95 (1.39-2.72)	1.87 (1.46-2.04)	1.28 (0.77-2.13)	1.38 (1.11-1.70)	1.21 (0.76-1.92)	1.53 (1.26-1.86)	1.68(1.25-2.27)
Height, per 10 cm	1.25 (0.96-1.62)	1.26 (1.11-1.44)	1.11 (0.76-1.64)	1.39 (1.17-1.65)	1.44 (1.08-1.91)	1.17 (0.98-1.39)	1.41(1.10-1.79)
Weight, per 15 kg	1.22 (1.03-1.44)	1.18 (1.07-1.31)	1.31 (1.04-1.65)	1.39 (1.26-1.53)	1.23 (1.03-1.46)	1.17 (1.04-1.31)	1.55(1.28-1.87)
Current smoker vs non smoker	1.57 (0.98-2.51)	1.35 (1.02-1.81)	0.66 (0.28-1.53)	1.33 (0.99-1.79)	1.27 (0.71-2.29)	1.38 (1.04-1.83)	0.96(0.62-1.50)
Systolic BP, per 20 mmHg	1.15 (0.97-1.37)	1.19 (1.10-1.28)	1.15 (0.92-1.42)	1.14 (1.02-1.28)	0.98 (0.78-1.24)	1.09 (1.00-1.19)	1.21(1.05-1.38)
Diastolic BP, per 10 mmHg	0.89 (0.75-1.06)	1.00 (0.93-1.08)	0.90 (0.72-1.13)	0.89 (0.80-0.99)	1.04 (0.84-1.29)	0.97 (0.88-1.08)	1.02(0.89-1.17)
Antihypertensive medication use	1.82 (1.31-2.55)	1.63 (1.38-1.92)	1.31 (0.77-2.22)	2.02 (1.63-2.51)	2.31 (1.34-3.98)	1.59 (1.28-1.97)	1.62(1.21-2.19)
Diabetes	1.63 (1.08-2.51)	1.51 (1.23-1.86)	1.17 (0.67-2.04)	1.83 (1.42-2.36)	1.64 (1.03-2.62)	1.21 (0.91-1.61)	1.23(0.80-1.90)
Blood glucose,[a] per 10 mg/dL	1.05 (1.00-1.10)	1.05 (1.02-1.07)	1.02 (0.98-1.06)	1.06 (1.03-1.08)	1.02 (0.98-1.05)	1.01 (0.96-1.06)	1.06(1.00-1.11)
eGFR<60 ml/min/m^2, vs ≥60	0.66 (0.39-1.12)	1.24 (1.02-1.50)	1.54 (0.90-2.63)	2.05 (1.52-2.76)	1.25 (0.71-2.19)	1.07 (0.87-1.33)	1.17(0.79-1.75)
Total cholesterol,[a] per 40 mg/dL	0.91 (0.76-1.10)	0.86 (0.78-0.94)	0.91 (0.70-1.18)	0.75 (0.66-0.85)	0.81 (0.63-1.04)	0.89 (0.81-0.98)	0.98(0.83-1.16)
HDL cholesterol,[a] per 15 mg/dL	0.86 (0.71-1.04)	0.99 (0.91-1.08)	1.10 (0.86-1.41)	0.82 (0.73-0.93)	0.80 (0.63-1.02)	0.98 (0.89-1.08)	0.86(0.72-1.02)
Triglycerides,[a] per 40 mg/dL	1.02 (0.96-1.08)	1.02 (0.98-1.07)	0.90 (0.74-1.10)	1.03 (0.98-1.07)	1.14 (1.04-1.26)	1.02 (0.95-1.09)	1.09(1.01-1.17)
Heart rate, per 10 bpm	0.99 (0.84-1.16)	1.05 (0.97-1.13)	0.99 (0.82-1.19)	1.12 (1.00-1.24)	0.99 (0.80-1.23)	0.97 (0.89-1.06)	0.92(0.80-1.06)
LVH by electrocardiogram	2.96 (1.08-8.08)	1.91 (1.36-2.67)	1.66 (0.82-3.37)	2.19 (1.26-3.82)	1.52 (0.61-3.76)	-	2.44(1.54-3.86)
PR interval, per 30 ms	1.03 (0.85-1.26)	1.13 (1.05-1.22)	1.22 (1.01-1.47)	1.05 (0.94-1.18)	1.06 (0.83-1.35)	1.18 (1.09-1.28)	1.04(0.99-1.10)
CABG history	0.95 (0.35-2.58)	1.84 (1.34-2.51)	0.62 (0.09-4.45)	2.35 (1.70-3.25)	1.32 (0.32-5.44)	1.66 (1.18-2.35)	1.40(0.75-2.60)
Heart failure history	2.08 (0.66-6.56)	3.27 (2.44-4.39)	3.20 (1.52-6.73)	2.57 (1.80-3.67)	3.94 (2.30-6.75)	2.52 (1.51-4.23)	1.45(0.76-2.79)
Myocardial infarction history	1.71 (0.99-2.96)	2.04 (1.63-2.55)	1.57 (0.71-3.45)	2.39 (1.72-3.32)	2.13 (0.98-4.65)	1.43 (1.03-1.98)	1.37(0.91-2.06)
Stroke history	0.71 (0.10-5.11)	2.38 (1.71-3.30)	1.91 (0.86-4.21)	1.96 (1.12-3.42)	1.64 (0.60-4.50)	1.01 (0.71-1.44)	2.24(1.31-3.83)

[a]Denotes fasting; AA: African-Americans; CABG: coronary artery bypass graft surgery; BP: blood pressure; eGFR: estimated glomerular filtration rate; LVH: left ventricular hypertrophy

whites. The analysis included 1186 incident atrial fibrillation cases among 18556 participants in the derivation cohorts, and 585 cases among the 7672 participants in the validation cohorts.

A number of sociodemographic variables and cardiovascular risk factors were consistently associated with age- and sex-adjusted atrial fibrillation incidence across cohorts (Table 2). We observed a higher risk of incident atrial fibrillation in men, older individuals, those with higher height, weight, blood pressure, blood glucose, individuals with lower total cholesterol, and those with electrocardiographic left ventricular hypertrophy, hypertension medication use, diabetes, current smokers, and a previous history of heart failure or myocardial infarction. Alcohol intake was not significantly associated with atrial fibrillation risk in any of the derivation cohorts (data not shown).

Derivation of the predictive model

Using a backward-selection algorithm in pooled data from ARIC, CHS and FHS, the following variables were included in the simple risk prediction score: age, race, height, weight, systolic blood pressure, diastolic blood pressure, current smoking, use of antihypertensive medication, diabetes,

Table 3. Final multivariable model for 5-year risk of atrial fibrillation derived in ARIC, CHS, and FHS[a]

	Simple model		Augmented model	
Variable	**Estimated β (SE)**	**HR (95% CI)**	**Estimated β (SE)**	**HR (95% CI)**
Age (5 years)	0.508 (0.022)	1.66 (1.59,1.74)	0.501 (0.022)	1.65 (1.58,1.72)
Race (white)	0.465 (0.093)	1.59 (1.33,1.91)	0.486 (0.094)	1.63 (1.35,1.95)
Height (10 cm)	0.248 (0.036)	1.28 (1.19,1.38)	0.243 (0.037)	1.28 (1.19,1.37)
Weight (15 kg)	0.115 (0.033)	1.12 (1.05,1.20)	0.121 (0.033)	1.13 (1.06,1.20)
Systolic BP (20 mmHg)	0.197 (0.033)	1.22 (1.14,1.30)	0.186 (0.033)	1.20 (1.13,1.29)
Diastolic BP (10 mmHg)	-0.101 (0.032)	0.90 (0.85,0.96)	-0.098 (0.032)	0.91 (0.85,0.97)
Smoking (current)	0.359 (0.091)	1.43 (1.20,1.71)	0.365 (0.091)	1.44 (1.20,1.72)
Antihypertensive medication use (Yes)	0.349 (0.063)	1.42 (1.25,1.60)	0.341 (0.063)	1.41 (1.24,1.59)
Diabetes (Yes)	0.237 (0.073)	1.27 (1.10,1.46)	0.242 (0.073)	1.27 (1.10,1.47)
Heart failure (Yes)	0.701 (0.106)	2.02 (1.64,2.48)	0.678 (0.107)	1.97 (1.60,2.43)
Myocardial infarction (Yes)	0.496 (0.089)	1.64 (1.38,1.96)	0.469 (0.090)	1.60 (1.34,1.91)
LVH by electrocardiogram (Yes)	-	-	0.401 (0.129)	1.49 (1.16,1.92)
PR Interval (< 120 v. 120-199)	-	-	0.645 (0.200)	1.91 (1.29,2.82)
PR Interval (> 199 v. 120-199)	-	-	0.118 (0.077)	1.13 (0.97,1.31)

BP: Blood pressure; LVH: Left ventricular hypertrophy

[a]All risk factors are classified at baseline examination. The 5-year risk for the simple model can be calculated as $1-0.9718412736^{\exp(\Sigma\beta X - 12.5815600)}$ where β is the regression coefficient and X is the level for each risk factor; the risk for the augmented model is given as $1-0.9719033184^{\exp(\Sigma\beta X - 12.4411305)}$. When calculating the 5-year risk, estimated β for age, height, weight, systolic and diastolic blood pressure must be divided by the number of presented units.

history of myocardial infarction, and history of heart failure. In addition to these variables, the PR interval and electrocardiogram-derived left ventricular hypertrophy were selected to be included in the augmented prediction score. The augmented score did not select variables requiring measurement of lipid levels, blood glucose, or creatinine. No significant interactions with age, sex or race were observed. Table 3 includes the beta coefficients, standard errors and hazard ratios with their 95% CIs corresponding to the final simple and augmented predictive models. The simple predictive model had good performance (C-statistic, 0.765; 95% CI, 0.748 to 0.781).

Addition of information from the electrocardiogram provided no gain in predictive ability (C-statistic, 0.767; 95% CI, 0.750 to 0.783). Inclusion of pulse pressure instead of systolic and diastolic blood pressure, or of body mass index or waist circumference instead of weight provided similar results (data not shown). Similarly, the categorical NRI showed that addition of electrocardiographic variables did not improve the predictive ability of the model (NRI, -0.0032; 95% CI, -0.0178 to 0.0113; Supplemental table 2).

The distribution of predicted 5-year risk of atrial fibrillation in the derivation cohorts is provided in Figure 1 and the observed cumulative risk of atrial fibrillation by predicted risk based on the simple model is presented in Supplemental figure 1, separately for whites and African Americans. An Excel spreadsheet available in the Framingham Heart Study website (available also as a supplemental file) allows calculation of atrial fibrillation risk using this predictive model.

Calibration of both models was adequate in the entire derivation sample (Table 4, Figure 2) and individually in each derivation cohort (Supplemental table 3). Discrimination using the previously developed Framingham atrial fibrillation risk score (C-statistic, 0.734; 95% CI, 0.717 to 0.750) was lower than with the CHARGE score.

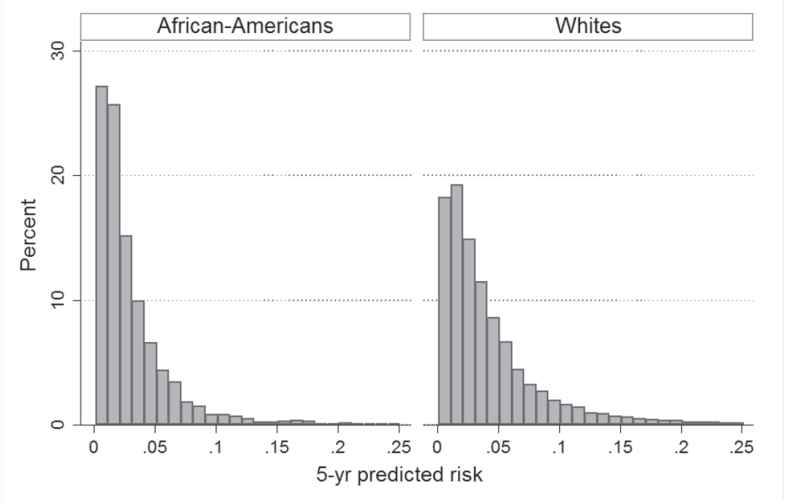

Figure 1. Distribution of predicted 5-year risk of atrial fibrillation in the derivation cohorts by race using the CHARGE-AF simple score.

Table 4. Model discrimination and calibration by cohort and risk score

	Pooled ARIC, CHS and FHS	AGES	RS
N	18,556	4469	3203
CHARGE atrial fibrillation simple score			
C-statistic (95% CI)	0.765 (0.748-0.781)	0.664 (0.632-0.697)	0.705 (0.663-0.747)
Calibration chi-square (p-value)	9.3 (0.41)	12.6 (0.18)	16.4 (0.06)
Discrimination slope	0.056	0.026	0.022
CHARGE atrial fibrillation augmented score			
C-statistic (95% CI)	0.767 (0.750-0.783)	0.665 (0.633-0.697)	0.716 (0.680-0.761)
Calibration chi-square (p-value)	5.4 (0.80)	16.7 (0.053)	10.1 (0.34)
Discrimination slope	0.059	0.027	0.023
FHS atrial fibrillation score[a]			
C-statistic (95% CI)	0.734 (0.717-0.750)	0.652 (0.621-0.684)	0.686 (0.642-0.729)
Calibration chi-square (p-value)	26.5 (0.002)	12.2 (0.20)	8.5 (0.49)
Discrimination slope	0.050	0.025	0.017
Cohort's own model			
C-statistic (95% CI)	-	0.668 (0.637-0.700)	0.733 (0.690-0.776)
Calibration chi-square (p-value)	-	11.8 (0.23)	10.9 (0.28)
Discrimination slope	-	0.025	0.026

[a] Discrimination and calibration of FHS atrial fibrillation score were obtained applying the published coefficients and calibrated using overall risk.

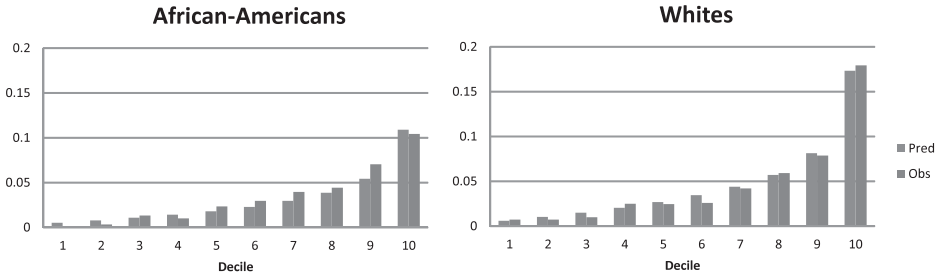

Figure 2. Calibration plots for the CHARGE-AF simple score model in the combined derivation cohorts, by race. The x-axis refers to deciles of predicted atrial fibrillation risk. Each bar in the graph represents the average observed and predicted atrial fibrillation risk.

Validation of the predictive model

The model developed in ARIC, CHS and FHS, was validated in two European cohorts, AGES and RS. Table 4 reports discrimination and calibration of the CHARGE-AF predictive models in the validation cohorts. C-statistic values were 0.664 in AGES and 0.705 in RS for the simple model, with similar results for the augmented model. Calibration of the predictive model after recalibration of the model using the average risk in each cohort was adequate in AGES and in RS (Table 4, Figure 2).

In RS, the new CHARGE score performed slightly better than the previous FHS risk score (C-statistic 0.705 for CHARGE simple score vs. 0.686 for FHS score), whereas in AGES the CHARGE and FHS scores had similar discrimination (C-statistic 0.664 for CHARGE simple score vs. 0.653 for FHS score).

Because of the relatively lower discrimination of the predictive model in AGES, we calculated the C-statistic of a model independently derived in the validation cohorts including the variables selected for the CHARGE risk model. Using this approach, the C-statistic in AGES was 0.668 (95% CI, 0.637 to 0.700) and in RS was 0.733 (95% CI, 0.690 to 0.776), not very different from values obtained using the CHARGE risk model (Table 4).

DISCUSSION

In our individual-level pooled analysis of 3 large community-based prospective studies in the United States, we found that a simple risk model including variables routinely collected in a primary care setting are useful to predict the future risk of atrial fibrillation. Discrimination ability of the model was comparable or superior to other risk stratification schemes developed for coronary heart disease or stroke.[24-26] The predictive model performed reasonably well in two additional cohorts in Europe when compared to the cohorts' own models. Including variables obtained from a 12-lead electrocardiogram provided no significant additional predictive ability.

Previous models for the prediction of atrial fibrillation have been reported already, but these were developed in single cohorts.[4, 6] Although the FHS atrial fibrillation risk score has shown acceptable discrimination in populations other than the cohort in which it was developed,[5-6] important improvements of the CHARGE-AF model were the availability of participant-specific data from several cohorts and larger sample size included in its development and validation. The CHARGE-AF model utilized more than 26000 individuals with over 1750 atrial fibrillation cases. The geographic and racial diversity of the participating cohorts provided increased generalizability over and above the FHS atrial fibrillation risk score alone. A further advantage of the CHARGE-AF predictive model is that it does not require extra diagnostic tests beyond what is usually available in primary care settings. We also found that the CHARGE-AF model performed better than the original FHS atrial fibrillation score in the derivation and validation cohorts. However, lack of information on cardiac murmur in ARIC, CHS, RS and AGES limits the value of

the FHS atrial fibrillation score in these cohorts. Similarly, we did not study discrimination of the ARIC risk score in the CHARGE cohorts since the ARIC score was derived in a middle-aged cohort (45-64 years old at baseline), whereas most individuals in the present analysis were older.

The CHARGE-AF predictive model shares some variables with previously developed risk scores for coronary heart disease,[24-25, 27] heart failure,[28-29] stroke,[26] or general cardiovascular risk.[30] However, the weight of individual risk factors in these other models differs from the CHARGE-AF model and their ability to accurately predict atrial fibrillation has been shown inadequate.[6]

The CHARGE-AF predictive model could have important research and clinical applications. The most immediate application might be to serve as a standard to evaluate the ability of putative novel clinical factors, biomarkers, subclinical measures, or 'omic (e.g. genomic, epigenomic, transcriptomic, proteomic, metabolomic) tests to reclassify an individual's risk of developing atrial fibrillation. In addition, the predictive model might be used to select high-risk individuals for trials of primary prevention of atrial fibrillation or intensive monitoring for atrial fibrillation detection. Our 5-year predictive model also may be useful once primary prevention strategies are developed, to facilitate identification of individuals more likely to benefit from them. Finally, given the association of some cardiovascular risk factors, such as hypertension, obesity, diabetes, or the metabolic syndrome,[31-35] with the risk of atrial fibrillation, the CHARGE-AF predictive model may, in the future, contribute to guidelines selecting candidates for more aggressive risk factor control. Future randomized trials and observational studies should determine if such approaches are useful and cost-effective.

In the proposed predictive model we found that higher systolic blood pressure was associated with higher atrial fibrillation risk, whereas diastolic blood pressure was inversely associated with atrial fibrillation incidence. This observation is consistent with a previous report from the FHS, in which pulse pressure was a better predictor of atrial fibrillation than systolic or diastolic blood pressure alone.[31] We chose to include systolic and diastolic blood pressure as separate variables in our model, instead of pulse pressure, because they are more commonly recorded in the clinical setting. Including pulse pressure provided similar results as those presented in the current analyses. Similarly, we included weight in the models even though waist circumference or body mass index, and not weight, may be the pathophysiologically relevant factors. In the derivation cohorts, however, models with waist circumference or body mass index offered similar discrimination ability. Which of these variables is more relevant from an etiopathogenic point of view needs to be addressed in future work.

Several variables included in the CHARGE-AF predictive model were part of both the published FHS and ARIC atrial fibrillation risk scores, including age, systolic blood pressure, use of antihypertensive medication, and history of heart failure (Supplemental table 4). Other variables in the CHARGE-AF model, however, were part of only one of the risk scores, such as race, smoking, height, diabetes, or myocardial infarction (in ARIC), and body mass index (in FHS). Similar to the ARIC model,[6] sex was not selected as a predictor in the CHARGE-AF model.

Even though atrial fibrillation incidence is higher in men than women, our model suggests that sex differences in the distribution of atrial fibrillation predictors may account for this disparity. In the initial analysis, we observed an unexpected inverse association between total cholesterol and atrial fibrillation risk. Upon further adjustment, cholesterol levels did not show a significant association with atrial fibrillation. Of note, an inverse association between total and LDL cholesterol was found in an analysis conducted in the ARIC study.[36]

We observed that the model had lower discrimination ability in AGES (C-statistic, 0.67). Discrimination only minimally improved in a model derived specifically in AGES using the CHARGE-AF variables (C-statistic, 0.68). In contrast, discrimination of the CHARGE-AF model was better in RS (C-statistic, 0.71). We can only speculate about the reasons to explain these differences. AGES participants were, on average, older than participants from other cohorts. Also, cohort differences in ascertainment of atrial fibrillation or in the impact of genetic risk factors may partly explain these results.

Our work has limitations that must be acknowledged. We restricted the age range of our risk score because very few individuals were younger than 46 or older than 94 years. The applicability of our risk model to individuals <46 or >94 years and to individuals not of African or European ancestry is uncertain. Our risk score will need to be validated outside the United States and Western Europe and in other ethnicities (e.g. Asians and Hispanics). Similarly, to be included, participants needed to attend a baseline cohort examination; the generalizability of the risk score to hospitalized patients or non-ambulatory settings is unknown. Most of the cohorts relied on periodic clinic examinations and hospitalization ICD codes leading to the potential for misclassification of atrial fibrillation, though validation studies in the ARIC study, CHS and other populations have shown adequate validity of this case definition.[15-16, 37] We also have shown previously that age- and race-specific incidence rates of atrial fibrillation in the derivation cohorts were similar in spite of the differences in atrial fibrillation ascertainment.[16] In addition, we note that atrial fibrillation is not infrequently asymptomatic or paroxysmal, being potentially missed in our cohorts. We included initial, paroxysmal, persistent and permanent atrial fibrillation, for which prediction may be heterogeneous. We acknowledge being unable to accurately comment on risk prediction for atrial fibrillation versus atrial flutter. We combined the two for several reasons including, they frequently complicate each other's course,[7] they are reported to have similar risk factors,[8] and because ICD codes may not accurately distinguish between the two.[38-39] Furthermore, we did not account for measurement error in risk factor ascertainment. We pooled participant-level data assuming a priori that the associations of risk factors with atrial fibrillation in the subjects representing three large United States cohort studies are sufficiently homogeneous. Strengths of our analysis include the large sample size, the number of atrial fibrillation cases included in the analysis, the inclusion of multiple cohort studies – enhancing generalizability, the availability of a large number of possible atrial fibrillation predictors, the racial diversity in the studied samples, and external replication.

In conclusion, we have developed a new risk model for the prediction of atrial fibrillation. The proposed model has the advantage of being simpler, using information readily available in a primary care setting, and having been developed in a larger population. Future research should determine whether biomarkers or genetic factors have value in the prediction of atrial fibrillation beyond that of clinical risk factors.

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SUPPLEMENTARY MATERIAL

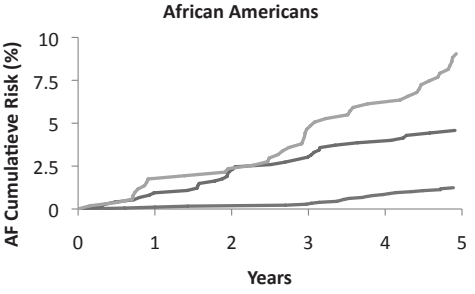
Supplemental table 1. Number of participants excluded by cohort applying exclusion criteria sequentially

	ARIC	CHS	FHS	AGES	RS
Initial sample	11656	5353	3113	5427[a]	4215
Prevalent atrial fibrillation	521	150	PE	527	282
Age <46 or >94, years	0	7	275	5	21
Creatinine ≥2 mg/dL	48	84	PE	55	8
Not white or African-American	30	32	0	0	62
Missing variables	382	37	PE	371	639
Eligible sample	10675	5043	2838	4469	3203

PE: Previously excluded from study sample

[a] There are 5764 participants in the AGES cohort, but only 5427 came to the study center. Other participants received a home-visit or provided blood samples only and, therefore, were excluded from this analysis.

Year	0	1	2	3	4	5
5-year predicted risk	Persons at risk by year					
<2.5%	6547	6516	6489	6440	6379	6305
2.5-5%	4297	4265	4201	4118	4041	3927
>5.0%	4623	4485	4303	4103	3867	3626



Year	0	1	2	3	4	5
5-year predicted risk	Persons at risk by year					
<2.5%	1817	1808	1798	1777	1751	1729
2.5-5%	748	732	707	691	675	651
>5.0%	524	501	484	454	433	396

Supplemental Figure 1. Observed cumulative risk of atrial fibrillation by categories of predicted risk according to CHARGE atrial fibrillation simple score in whites and African Americans. Green line: >5%, red line: 2.5-5%, blue line: <2.5%

Supplemental table 2. Reclassification among individuals who developed atrial fibrillation and who did not develop atrial fibrillation during a 5-year follow-up using the augmented score (which includes ECG measurements). Net reclassification improvement (NRI), -0.0032; 95% CI, -0.0178 to 0.0113.

Participants who developed atrial fibrillation				
	Augmented score			
Simple score	<2.5%	2.5-5%	>5%	Total
<2.5%	97	6	1	104
2.5-5%	4	161	5	171
>5%	0	16	517	532
Total	101	183	523	807
Participants who did not develop atrial fibrillation				
	Augmented score			
Simple score	<2.5%	2.5-5%	>5%	Total
<2.5%	8109	151	0	8260
2.5-5%	192	4499	184	4875
>5%	0	248	4366	4614
Total	8301	4898	4550	17749

Data are number of participants. Green cells correspond to desirable reclassification, while red cells correspond to undesirable reclassification.

Supplemental table 3. Model discrimination and calibration separately in each derivation cohort

	ARIC	**CHS**	**FHS**
N	10675	5042	2831
CHARGE atrial fibrillation simple score			
C-statistic (95% CI)	0.71 (0.68, 0.74)	0.70 (0.68, 0.73)	0.78 (0.74, 0.83)
Calibration chi-square (p-value)	6.6 (0.67)	17.3 (0.045)	14.2 (0.11)
Discrimination slope	0.017	0.054	0.057
CHARGE atrial fibrillation augmented score			
C-statistic (95% CI)	0.72 (0.68, 0.75)	0.71 (0.68, 0.73)	0.78 (0.74, 0.83)
Calibration chi-square (p-value)	5.1 (0.82)	11.1 (0.27)	8.0 (0.54)
Discrimination slope	0.018	0.058	0.060
FHS atrial fibrillation score			
C-statistic (95% CI)	0.67 (0.64, 0.71)	0.66 (0.64, 0.69)	0.78 (0.74, 0.82)
Calibration chi-square, p-value	32.2 (0.0002)	279.8 (<0.0001)	45.1 (<0.0001)
Discrimination slope	0.013	0.046	0.049

Supplemental table 4. Variables included in the CHARGE-AF simple model and previously published risk models for the prediction of atrial fibrillation

CHARGE-AF	FHS risk score[8]	ARIC risk score[9]
age	age	age
	sex	
race		race
height		height
weight	body mass index	
systolic BP	systolic BP	systolic BP
diastolic BP		
smoking		smoking
antihypertensive medication	antihypertensive medication	antihypertensive medication
diabetes		
myocardial infarction		coronary heart disease
heart failure	heart failure	heart failure
	cardiac murmur	cardiac murmur
	PR interval	
		ECG-derived LVH
		ECG-derived LAE

BP: blood pressure; ECG: electrocardiogram; LAE: left atrial enlargement; LVH: left ventricular hypertrophy

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ARIC: The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). This work was additionally funded by grants RC1HL099452 and RC1HL101056 from NHLBI and 09SDG2280087 from the American Heart Association.

CHS: The Cardiovascular Health Study was supported by NHLBI contracts N01-HC-85239, N01-HC-85079 through N01-HC-85086; N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133 and NHLBI grants HL080295 and R01HL088456, with additional contribution from NINDS. Additional support was provided through AG-023629, AG-15928, AG-20098, and AG-027058 from the NIA. See also http://www.chs-nhlbi.org/pi.htm.

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RS: The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

Atrial fibrillation ascertainment

AGES: Atrial fibrillation or atrial flutter was diagnosed from Minnesota-coded ECGs recorded at AGES study exams and from ICD-9 427.3 or ICD-10 I48 codes from hospital discharges in the National Hospital of Iceland database through April 2010.

ARIC: Atrial fibrillation in ARIC was identified from 3 sources: ECG conducted at each study visit, hospital discharge codes (ICD-9CM 427.31, atrial fibrillation, or 427.32, atrial flutter), and

death certificates (ICD-9 427.3 or ICD-10 I48). Atrial fibrillation cases occurring during the same hospitalization as open cardiac surgery were not included as events.[1, 2]

CHS: Incidence of atrial fibrillation and atrial flutter in CHS was identified from standard 12-lead electrocardiograms performed at each study visit, and from hospitalization discharge codes (ICD-9CM 427.31 or 427.32). Data from hospitalizations have been obtained through biannual contact with study participants and from information obtained from the Centers for Medicare and Medicaid Services.[3]

FHS: Participants were classified as having atrial fibrillation if atrial fibrillation or atrial flutter was present on an ECG derived from a Framingham Study clinic tracing, on an ECG during an encounter with an external clinician, or by Holter monitoring, or if it was noted in hospital records. All incident atrial fibrillation cases were reviewed and adjudicated by one of two Framingham cardiologists.[4]

RS: Atrial fibrillation cases were ascertained at baseline and during follow-up as described previously.[5] Briefly, ECGs were recorded and stored digitally, and analyzed by the Modular ECG Analysis System. Two research physicians and a cardiologist verified atrial fibrillation diagnoses. Additional information was obtained from general practitioner records, from outpatient clinics, and from a national database of hospitalizations, which records all hospitalization discharge diagnoses occurring in the Netherlands. Atrial fibrillation cases occurring during a serious disease resulting in death, or during myocardial infarction or cardiac operative procedures who recovered during the hospital admission were not included.

Assessment of covariates

AGES: Standard examination protocols and questionnaires were completed in the AGES study. Clinic visits included anthropometry, blood pressure measurement (defined as the mean value of two consecutive blood pressure measurements), electrocardiogram, and measures of different physical and cognitive function domains. Blood samples were drawn after overnight fasting. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose were analyzed on a Hitachi 912, using reagents from Roche Diagnostics and following the manufacturer's instructions. Low-density lipoprotein (LDL) was calculated using the Friedewald equation. Diabetes was defined from a self-report of a physician diagnosis, use of oral hypoglycemic agents or insulin, or a fasting blood glucose ≥126 mg/dL. Diagnoses of myocardial infarction and heart failure were based on hospital discharge records.

ARIC: Study participants were asked to fast for 12 hours before the clinic visit, during which a blood sample was obtained and a physical examination performed. Race, smoking status, and drinking status were determined by participant self-report. Blood collection and processing

techniques for the ARIC study have been previously described.[6] Enzymatic methods were used to measure total cholesterol and triglycerides. High-density lipoprotein cholesterol was measured enzymatically after dextran sulfate-Mg^{2+} precipitation of other lipoproteins. Low-density lipoprotein cholesterol levels were estimated using the Friedewald formula for subjects with triglycerides levels <400 mg/dl. Standing height and waist circumference (at the level of the umbilicus) were measured to the nearest centimeter in a scrub suit and without shoes. Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. Blood pressure was measured 3 times with the subject in the sitting position after 5 minutes of rest using a random-zero sphygmomanometer, and the last 2 measurements were averaged. Participants were asked to bring all medications with them to the clinic visits. A prescription bottle or self-report was used to determine cholesterol and blood pressure medication use. A 12-lead ECG at rest was used to define the PR interval and the presence of left ventricular hypertrophy (LVH). ECG-diagnosed LVH was considered present if the Cornell voltage was >28 mm in men or >22 mm in women. A participant was categorized as having diabetes if she or he had a fasting glucose of ≥126 mg/dl or nonfasting glucose level of ≥200 mg/dl, reported a physician diagnosis of diabetes, or was currently taking medication for diabetes. Prevalent coronary heart disease at baseline included a history of myocardial infarction, myocardial infarction adjudicated from the baseline ECG, or a history of coronary bypass or angioplasty. Prevalent heart failure was defined as a previous hospitalization for heart failure. Presence of cardiac murmur was assessed in the baseline examination but not in following visits.

CHS: Participants had annual examinations including assessment of cardiovascular risk factors, prior cardiovascular diseases, medications, height, weight, seated blood pressure, and a 12-lead electrocardiogram through 1999. Racial identity was provided by self-report. A history of heart failure at baseline was defined by signs, symptoms, clinical tests, physician diagnosis, and/or medical therapy. Prevalent coronary heart disease was defined by a history of myocardial infarction or angina pectoris confirmed by retrospective review of hospitalization medical records, or self-report of coronary angioplasty. Diabetes was defined as use of oral hypoglycemic agents or insulin, or a fasting blood glucose ≥126 mg/dl. ECG-LVH was defined according to Cornell voltage.

FHS: Cardiovascular disease risk factors were defined as follows: diabetes was diagnosed as fasting glucose ≥126 mg/dL, or use of hypoglycemic medications; the average of two Framingham Study physician systolic and diastolic blood pressure measurements in mm Hg with the participant seated. Medication, alcohol use and smoking were ascertained by self-report. Current smoking was defined as regular use of one or more cigarettes/day within the year prior to the Framingham clinic visit. Glucose and lipids were measured after an overnight fast in the Framingham Study laboratory. Cardiovascular events were adjudicated by a panel of

3 physicians, examining participant hospitalization and outpatient records. Heart failure was diagnosed based on major and minor clinical criteria that have been used for all heart failure cases of Framingham Heart Study participants. Myocardial infarction was diagnosed based on the presence of clinical history, electrocardiographic signs, and biomarkers. ECG-based left ventricular hypertrophy was considered present when a participant had voltage criteria for left ventricular hypertrophy with lateral repolarization changes.[7]

RS: Information on current health status, medical history, and smoking was obtained using a computerized questionnaire. Participants were classified as current or non-smokers. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressures were calculated as the average of the two consecutive measurements. A history of myocardial infarction was defined as a self-reported myocardial infarction with hospital admission or the presence of a myocardial infarction on the ECG. Left ventricular hypertrophy was diagnosed by the MEANS program using the Sokolow-Lyon cut-off of ≥3500 μV. Diabetes was defined as the use of antidiabetic medication or a random or post-load serum glucose level of 200 mg/dL or more. Diagnosis of heart failure was based on a score of heart failure symptoms, on medication prescribed with the indication of heart failure, on hospital discharge diagnoses, and on the information available in general practitioner files. Blood samples were drawn by venipuncture, and serum total cholesterol and HDL cholesterol were measured with an automated enzymatic method.

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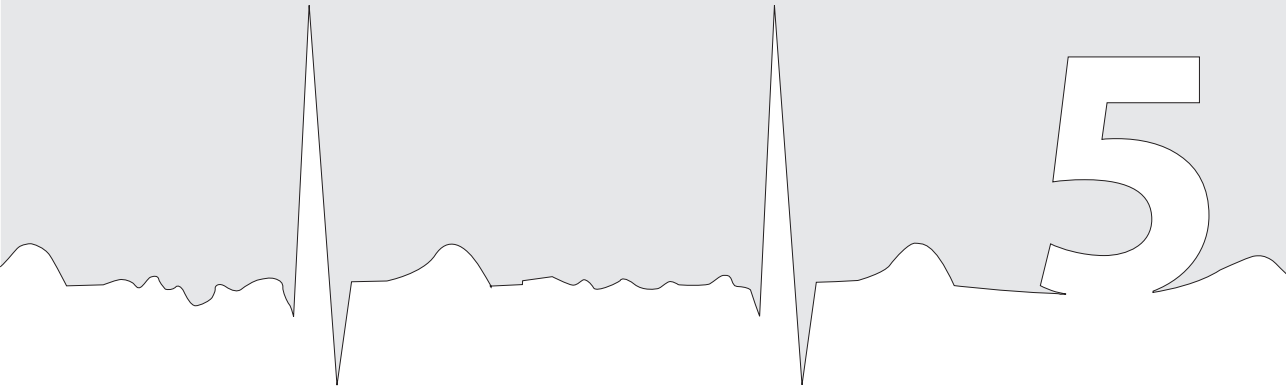

CHAPTER 5

General discussion

This thesis had several objectives. The first objective was to project the number of individuals with atrial fibrillation in the Netherlands and the European Union from 2000 to 2060 (chapter 2). Furthermore the objective of this thesis was to identify new risk factors for atrial fibrillation. Hereto, we reported on the association between clinically unrecognized myocardial infarction and the risk of atrial fibrillation (chapter 3.1). Also we found that levels of serum dehydroepiandrosterone sulphate (chapter 3.2), and serum potassium are associated with the risk of atrial fibrillation (chapter 3.3). In chapter 3.4, we studied the association of use of non–steroidal anti-inflammatory drugs with the risk of atrial fibrillation. Moreover, in collaboration with several other population-based cohort studies, we found several genetic loci to be associated with atrial fibrillation (chapter 3.5). Finally, in chapter 4 the objective was to develop and validate an atrial fibrillation risk prediction model that included several easily obtainable risk factors.

In this chapter, the main findings will be discussed and interpreted, and suggestions for future research are presented.

Main findings

Projections on the number of patients with atrial fibrillation

European populations are currently ageing and this is projected to proceed in coming decades. This will likely lead to an increase in the number of patients with atrial fibrillation since its risk is strongly associated with age. By combining data on atrial fibrillation from the population-based Rotterdam Study with population projections from the statistical bureau of the European Union, Eurostat,[1] we project that from 2010 to 2060, the number of adults aged 55 years and over with atrial fibrillation will more than double in the Netherlands. Also in the European Union the number of atrial fibrillation patients will likely double. The strength of our projections is that they are based on prevalence estimates from a cohort study that is derived from the general population and contains detailed information on the prevalence of atrial fibrillation. The projections have limitations and are based on some assumptions. Our reference population is a Dutch elderly population of mainly northwestern European descent, and this is probably not representative of the entire European Union. As Caucasians have a higher risk of atrial fibrillation compared to other ethnicities,[2-4] our projections for the European Union might be overestimated. However well validated atrial fibrillation prevalence estimates are unavailable for many countries in the European Union. Also, we assumed that the age adjusted prevalence will remain stable. However, some former studies found a rising atrial fibrillation age adjusted prevalence in previous years.[5-8] This rise in prevalence might be explained by more awareness and increased recognition by physicians, improved survival of atrial fibrillation patients, or from an increase of clinical conditions that are associated with a higher risk of atrial fibrillation.[9] However, it is also possible that increased attention for cardiovascular risk factors and thereby treatment and prevention may reduce the risk of atrial fibrillation.[9] In our study we were unable to find an increase in the age adjusted prevalence of atrial fibrillation in our study population.

In our main analysis, we therefore assumed the prevalence of atrial fibrillation to remain stable in future years which might have led to some underestimation in our projections. Our exact numbers of individuals should therefore be cautiously interpreted and only be regarded as estimations. Our projections indicate that due to aging of the European populations a substantial increase in the number of patients with atrial fibrillation can be expected. Since atrial fibrillation is associated with significant morbidities and mortality, this increasing number may have major public health implications.

Risk factors for atrial fibrillation

In this thesis, we studied the association of several factors with the risk of atrial fibrillation. It is well-known that cardiovascular disease leads to a higher risk of atrial fibrillation. Persons with a history of myocardial infarction are at increased risk of atrial fibrillation.[10, 11] However, it has previously been demonstrated that a large proportion of all myocardial infarctions remains clinically unrecognized.[12, 13] Whether these unrecognized myocardial infarctions are also associated with the risk of atrial fibrillation, was not previously investigated. We found that men with an unrecognized myocardial infarction have a more than two-fold increased risk of developing atrial fibrillation, compared to men without a history of myocardial infraction (chapter 3.1). This association of unrecognized myocardial infarction with atrial fibrillation was independent of classical cardiovascular risk factors. In women, unrecognized myocardial infarction was not associated with risk of atrial fibrillation. It is unclear why the association of myocardial infraction with atrial fibrillation is different between men and women. It may be explained by more misclassification in women as Murabito et al. suggested that ECG abnormalities due to difficulties with lead placement owing to breast tissue, can be mistaken for myocardial infarction.[14] These results support the hypothesis that undiagnosed cardiovascular disease may be an important risk factor for atrial fibrillation at least in men.

Previous studies indicated that higher dehydroepiandrosterone sulfate (DHEAS) levels are associated with a lower risk of cardiovascular disease[15, 16] and lower all-cause and cardiovascular mortality.[15, 17-20] DHEAS is a precursor in the biosynthetic pathway of androgenic and estrogenic sex steroids. In chapter 3.2, we show that high DHEAS levels are associated with a lower risk of atrial fibrillation, independent of age, sex, and known cardiovascular risk factors. Several explanations for the association of DHEAS levels with atrial fibrillation can be raised. A previous study suggested that the zona reticularis of the adrenal gland, responsible for most DHEAS production, is highly susceptible to vascular damage.[21] It is therefore possible that a low DHEAS level is only reflecting underlying vascular disease.[22] This suggests that DHEAS levels are a non-etiological biomarker rather than a step in the causal pathway. However, it has also been shown that DHEAS could have tissue specific effects, either directly or indirectly by conversion to biologically active androgens and estrogens.[23, 24] It is suggested that DHEAS inhibits vascular remodelling by reducing neo-intima formation after arterial injury.[25] Moreover, several studies

suggested that DHEAS may also have an anti-inflammatory role.[26-28] We were the first to show that high DHEAS levels are associated with a lower risk of atrial fibrillation. Further studies are needed to replicate these findings and understand the mechanism behind the association of DHEAS levels and the risk of atrial fibrillation.

In chapter 3.3 we studied the association of serum potassium levels with the risk of atrial fibrillation. It is known from previous studies that low serum potassium is associated with a higher risk of ventricular arrhythmias and cardiac arrest.[29] Similar results were found in clinical studies of patients undergoing cardiac surgery [30, 31] In our study, we showed that low serum potassium levels are also associated with a higher risk of atrial fibrillation in the general population. In addition, we found that low levels of serum potassium were associated with increased P-wave duration. This has also been shown in a study in haemodialysis patients.[32] P-wave duration increase is associated with a higher risk of atrial fibrillation.[33-37] This finding further supports the hypothesis that serum potassium is involved in atrial conduction and possibly in development of atrial fibrillation. Several mechanisms can explain the association of serum potassium with atrial fibrillation. First, it is possible that a low serum potassium level is a marker of underlying conditions. However it may also be possible low serum potassium itself leads to an increased risk of atrial fibrillation by the influence of potassium on the cellmembrane potential. It was suggested that a low serum potassium level might cause cellular hyperpolarity, increases resting potential and thereby hastens depolarization.[38] Further studies are needed to confirm these findings but these results suggest that monitoring potassium levels may also be useful to prevent atrial fibrillation.

In chapter 3.4, we showed that use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a higher risk of atrial fibrillation. Thereby, our results support most of these previously published results from database studies but has added value because of the detailed information from the Rotterdam Study.[39-41] Also our results suggest that the increased risk occurs shortly after starting treatment and may disappear over time. Several different mechanisms might explain the association of NSAIDs with the risk of atrial fibrillation. NSAIDs inhibit cyclo-oxygenases which are expressed in kidney tissue. Inhibition of these enzymes may lead to an increase in blood pressure due to expansion of plasma volume, increased peripheral resistance and thereby to atrial fibrillation.[40, 42] Also, cyclooxygenase inhibition may lead to fluctuation of serum potassium by decreased excretion in the distal nephron which in it self may cause atrial fibrillation. However it is also possible that NSAID use is an indicator of the presence of underlying inflammatory disease and these underlying inflammatory conditions might be associated with the risk of atrial fibrillation.[43] Therefore, the underlying mechanisms behind this association therefore deserve further attention.

Previous genome-wide association studies have identified three genomic regions associated with atrial fibrillation.[44-47] Genome wide association studies are commonly used to examine the association between variation in the genome of unrelated individuals with the phenotype of interest, free of any prior hypotheses. However, in these studies the ability to discover genetic

variants that are associated with the phenotype is largely dependent on sample size. By combining data from several studies we increased the power to detect additional genetic variants associated with atrial fibrillation. We meta-analyzed results from 6 prospective cohort and 11 case-control study samples in individuals of European ancestry, and found six novel atrial fibrillation susceptibility regions (chapter 3.5). The identified loci are close to genes that are related to cardiopulmonary development, cardiac-expressed ion channels, and cell signalling molecules. However it should be noted that these identified variants may not be causal, but also may tag causal elements along the same or different molecular pathways.

Prediction of atrial fibrillation

In chapter 4, we found that a simple risk model including variables routinely collected in a primary care setting is useful to predict the future risk of atrial fibrillation. For this we pooled data of three large community-based prospective studies in the United States on an individual-level. Also we found that the predictive model performed reasonably well in two additional cohorts in Europe. The model has several strengths. Where previously published prediction models were based in single cohorts,[48, 49] this model was based on combined information from three population based cohorts. A further advantage of this model compared to other prediction models is that it does not require additional tests other than what is usually available in clinical practice. The model could have various applications. The predictive model might be used to select high-risk individuals for trials of primary prevention of atrial fibrillation, or select candidates for more aggressive risk factor control. The most immediate application might be to serve as a standard to evaluate whether novel risk factors have value in the prediction of atrial fibrillation.

Future research and clinical applications

Our understanding on the pathophysiology of atrial fibrillation has advanced significantly in the past 10 to 15 years.[50] Understanding of the underlying mechanisms leading to atrial fibrillation may ultimately lead to treatment and preventive strategies. Preventive strategies are needed in Western populations as, with the current prevalence of atrial fibrillation, the number of patients with atrial fibrillation will likely double in future decades due to population aging. In this thesis, we studied several new risk factors for atrial fibrillation. However, it is clear that there is still much research needed and many recommendations for future research can be proposed. In general, studies in other populations are required to replicate findings and see if the results we found can be generalized from the study population to the population at large. Also, the underlying mechanism behind the studied risk factors in chapter 3 and atrial fibrillation are often not completely understood. Studies from various disciplines are relevant to study the underlying mechanisms in more detail. Moreover the genetic background of atrial fibrillation deserves further attention. Although genome wide association studies have become the primary approach for identifying common single nucleotide polymorphisms influencing complex

diseases, the common polymorphisms that are investigated in these studies are associated with small effects and thereby account for only a small fraction of disease heritability.[51] Many well-studied complex diseases are dealing with a large proportion of "missing heritability" and several explanations for this have been raised. It has been suggested that the total proportion of assumed heritability of complex diseases is overestimated.[52] This is mainly because the estimated heritability of a complex disease also includes the proportion that is due to gene-environment interactions. Some of the missing heritability therefore might be explained in gene-environment interactions, to study these interactions would require very large sample sizes.[52] Also, it has been argued that common diseases in the population are influenced by rare genetic variants with large effect on disease risk.[53] Next generation sequencing may be useful to identify these rare variants.[54]

In recent years, many novel risk factors for atrial fibrillation have been identified. However, so far the direct impact of this information on clinical practice has been limited. Studies are relevant to investigate whether interventions based on the identified risk factors may be useful for lowering the risk of atrial fibrillation. Based on the risk factors identified in this thesis, it may be useful to study whether interventions in those with an unrecognized myocardial infarction reduce the risk of atrial fibrillation. Also, it needs further elaboration whether supplementation of DHEAS or potassium may be useful for atrial fibrillation risk reduction.

Another use of the newly identified risk factors may be in predicting the risk of atrial fibrillation. An advantage of the model described in chapter 4 is its direct availability because it does not require additional tests other than what is usually available in clinical practice. However, it deserves further attention whether including novel risk factors improves the performance of the model and thereby more accurately identifies the risk of a person to develop atrial fibrillation. Accurate risk prediction models are useful for risk communication, patient motivation and clinical decision making.[55] This is important because, as with other forms of cardiovascular disease, more than half of the cases of atrial fibrillation may be preventable.[56]

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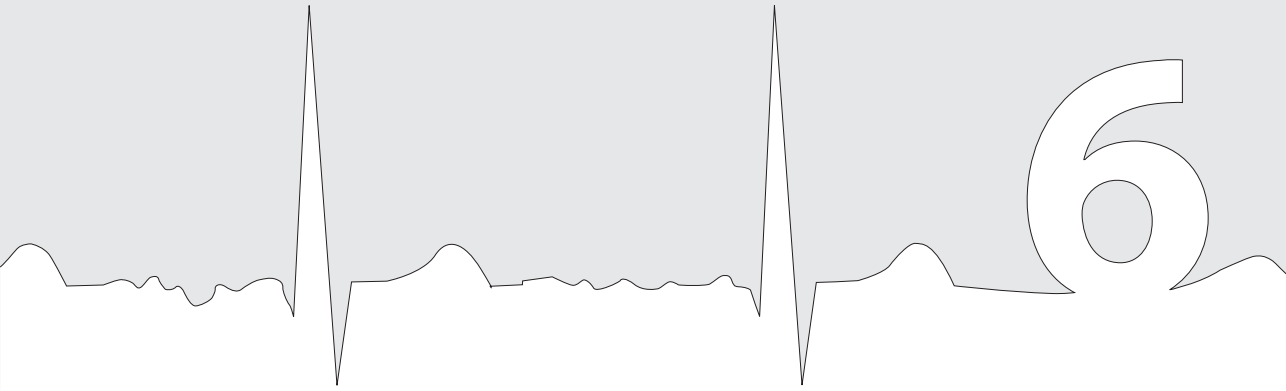

CHAPTER 6

Summary/Samenvatting

SUMMARY

Atrial fibrillation is a common cardiac arrhythmia in the elderly. It has serious consequences for the health of affected individuals and is a substantial burden for the health care system. It is associated with a three- to five-fold higher risk of stroke. Furthermore, it is associated with a higher risk of dementia, heart failure, and it is associated with increased mortality independent of age, sex and other cardiovascular risk factors. The mechanisms leading to atrial fibrillation need further investigation.

This thesis had several objectives. The first objective was to project the number of individuals with atrial fibrillation in the Netherlands and the European Union (chapter 2). European populations are currently ageing and this is estimated to proceed in coming decades. This will likely lead to an increase in the number of patients with atrial fibrillation since its risk is strongly associated with age. By combining data on atrial fibrillation from the population-based Rotterdam Study with population projections from the statistical bureau of the European Union, Eurostat, we project that from 2010 to 2060, the number of adults aged 55 years and over with atrial fibrillation will more than double in the Netherlands. Also in the European Union, the number of patients with atrial fibrillation will likely double. Since atrial fibrillation is associated with significant morbidities and mortality, this increasing number may have major public health implications.

In chapter 3, we aimed to identify novel risk factors for atrial fibrillation. In chapter 3.1, we report on the association between clinically unrecognized myocardial infarction and the risk of atrial fibrillation. Persons with a history of myocardial infarction are at increased risk of atrial fibrillation. However, it has previously been demonstrated that a large proportion of all myocardial infarctions remains clinically unrecognized. Whether these unrecognized myocardial infarctions are also associated with the risk of atrial fibrillation, was not previously investigated. We found that men with an unrecognized myocardial infarction have a more than two-fold increased risk of developing atrial fibrillation, compared to men without a history of myocardial infarction. This association of unrecognized myocardial infarction with atrial fibrillation was independent of several atrial fibrillation risk factors.

In chapter 3.2, we studied the association of levels of serum dehydroepiandrosterone sulphate (DHEAS) with atrial fibrillation. DHEAS is a precursor in the biosynthetic pathway of androgenic and estrogenic sex steroids. Previous studies indicated that higher dehydroepiandrosterone sulfate (DHEAS) levels are associated with a lower risk of cardiovascular disease and lower all-cause and cardiovascular mortality. We showed that high DHEAS levels are associated with a lower risk of atrial fibrillation, independent of age, sex, and known cardiovascular risk factors. Further studies are needed to replicate these findings and to understand the mechanism behind the association of DHEAS levels and the risk of atrial fibrillation.

In chapter 3.3, we studied the association of serum potassium levels with the risk of atrial fibrillation. Low serum potassium is found to be associated with a higher risk of ventricular

arrhythmias and cardiac arrest. We found in our study that low serum potassium levels are also associated with a higher risk of atrial fibrillation. In addition we found that low levels of serum potassium were associated with increased P-wave duration. P-wave duration increase is known to be associated with a higher risk of atrial fibrillation. Therefore these finding further supports the hypothesis that serum potassium is involved in atrial conduction and possibly development of atrial fibrillation.

In chapter 3.4, we found that use of non-steroidal anti-inflammatory drugs is associated with a higher risk of atrial fibrillation. Recent past use and current use were associated with a higher risk of atrial fibrillation, adjusted for age, sex and cardiovascular risk factors. Further studies are needed to investigate the underlying mechanism behind this association.

In chapter 3.5, we performed a genome wide association study on atrial fibrillation. Previous genome-wide association studies have identified three genomic regions associated with atrial fibrillation. By combining data from several studies, we increased the power to detect additional genetic variants associated with atrial fibrillation. We meta-analyzed results from 6 prospective cohort and 11 case-control study samples in individuals of European ancestry, and found six novel atrial fibrillation susceptibility regions. The identified loci are close to genes that are related to cardiopulmonary development, cardiac-expressed ion channels, and cell signalling molecules.

In chapter 4, we found that a simple risk model including variables routinely collected in a primary care setting is useful to predict the future risk of atrial fibrillation. Where previously published prediction models were based in single cohorts, this model was based on combined information from three population based cohorts. A further advantage of this model compared to other prediction models is that it does not require additional tests other than what is usually available in clinical practice.

In chapter 5, the main findings are discussed and interpreted, and suggestions for future research are discussed.

SAMENVATTING

Atriumfibrilleren is een veel voorkomende hartritmestoornis bij ouderen. Het heeft ernstige gevolgen voor de gezondheid van de aangedane individuen en is een aanzienlijke last voor de gezondheidszorg. Het is geassocieerd met een drie tot vijf maal hoger risico op een beroerte. Bovendien wordt het geassocieerd met een hoger risico op dementie, hartfalen, en met verhoogde mortaliteit onafhankelijk van leeftijd, geslacht en andere cardiovasculaire risicofactoren. Verder onderzoek naar de mechanismen die leiden tot atriumfibrillatie is daarom gewenst.

Dit proefschrift had verschillende doelstellingen. De eerste doelstelling was om het aantal personen met atriumfibrillatie in Nederland en in de Europese Unie in te schatten (hoofdstuk 2). Europese populaties zijn aan het verouderen en het wordt geschat dat dit zo door zal gaan in de komende decennia. Dit zal waarschijnlijk leiden tot een toename van het aantal patiënten met atriale fibrillatie, aangezien het risico sterk geassocieerd met de leeftijd. Door gegevens over de prevalentie van atriumfibrillatie in de bevolking vanuit de Rotterdam Studie, te combineren met bevolkingsprognoses van het statistisch bureau van de Europese Unie, Eurostat, schatten we dat van 2010-2060, het aantal volwassenen van 55 jaar en ouder met atriumfibrilleren meer dan zal verdubbelen in Nederland. Ook in de Europese Unie, zal het aantal atrium fibrillatie patiënten waarschijnlijk verdubbelen. Omdat atriumfibrilleren gerelateerd is aan een aanzienlijk hoger risico op morbiditeit en mortaliteit, kan dit belangrijke implicaties hebben voor de volksgezondheid.

In hoofdstuk 3 hebben we nieuwe risicofactoren voor atriumfibrilleren bestudeerd. In hoofdstuk 3.1 beschrijven we de relatie tussen klinisch niet herkende hartinfarcten en het risico op atriumfibrilleren. Het is bekend dat personen met een myocard infarct in hun voorgeschiedenis een verhoogd risico op atriumfibrillatie hebben. Echter een groot deel van alle myocardinfarcten wordt klinisch niet als dusdanig herkend. Of deze niet herkende myocardinfarcten ook geassocieerd aan het risico van atriumfibrilleren is niet eerder onderzocht. We vonden dat mannen met een niet-herkend myocardinfarct een meer dan twee-voudig verhoogd risico op het ontwikkelen van atriumfibrillatie hebben in vergelijking met mannen zonder een voorgeschiedenis van myocardinfarct. Deze relatie van niet herkende myocardinfarcten met atriumfibrilleren was onafhankelijk van de verschillende bekende risicofactoren voor atrium fibrillatie.

In hoofdstuk 3.2 hebben we de relatie tussen serum dehydroepiandrosterone sulfaat (DHEAS) concentraties en het risico op atriumfibrilleren bestudeerd. DHEAS is een precursor in de biosynthese van androgene en oestrogene geslachtshormonen. Eerdere studies lieten zien dat hogere dehydroepiandrosterone sulfate (DHEAS) niveaus geassocieerd zijn aan een lager risico op hart-en vaatziekten en lager risico op algemene sterfte en cardiovasculaire sterfte. We vonden dat hogere DHEAS concentraties ook geassocieerd zijn aan een lager risico op atriumfibrilleren, onafhankelijk van leeftijd, geslacht en cardiovasculaire risicofactoren. Verder onderzoek is nodig om het mechanisme achter deze associatie te onderzoeken.

In hoofdstuk 3.3 hebben we de relatie tussen serum kalium concentraties en het risico op atriumfibrilleren. Laag serum kalium is in verband gebracht met een verhoogd risico op ventriculaire aritmieën en hartstilstand. We vonden in ons onderzoek dat lage serum kalium spiegels ook geassocieerd zijn aan een hoger risico op atriumfibrillatie. Bovendien vonden we dat lage serum kalium geassocieerd waren aan een verlengde P-golf duur. Een toename van de P-golf duur is eerder geassocieerd met een hoger risico op atriumfibrillatie. Dit ondersteunt de hypothese dat serum kalium concentraties betrokken zijn in de atriale geleidingstijd en eventueel het ontwikkelen van atriumfibrillatie.

In hoofdstuk 3.4, vonden we dat het gebruik van niet-steroïdale anti-inflammatoire geneesmiddelen is geassocieerd met een hoger risico op atriumfibrillatie. Zowel huidig als recent gebruik was geassocieerd met een hoger risico op atriumfibrillatie, onafhankelijk van leeftijd, geslacht en cardiovasculaire risicofactoren. Verdere studies zijn nodig om het onderliggende mechanisme achter deze associatie te onderzoeken.

In hoofdstuk 3.5 hebben we een genoom-wijde associatie studie naar atriumfibrillatie gedaan. Vorige genoom-wijde associatie studies hebben drie genetische regio's gevonden die geassocieerd zijn aan het risico op atriumfibrilleren. Door het combineren van gegevens van verschillende studies wordt het vermogen om aanvullende genetische varianten te vinden vergroot. In een meta-analyse van 6 prospectieve cohort studies en 11 case-control studies vonden zes nieuwe genetische regio's die geassocieerd met het voor komen van atriumfibrillatie. De geïdentificeerde regio's liggen dichtbij genen die verband houden met cardiale-ontwikkeling, expressie van cardiale ionenkanalen, en cell verbindings moleculen.

In hoofdstuk 4 hebben we gevonden dat een voorspellingsmodel, met variabelen die routinematig verzameld worden in de eerste lijnzorg nuttig is om het risico op atriumfibrilleren te voorspellen. Waar eerder gepubliceerde voorspellingsmodellen waren gebaseerd op enkele cohorten, werd dit model gebaseerd op gecombineerde informatie van drie bevolkingsonderzoeken. Een verder voordeel van dit model vergeleken met andere is dat het niet nodig is aanvullende metingen te doen anders dan wat gewoonlijk beschikbaar is in de klinische praktijk.

In hoofdstuk 5 worden de belangrijkste bevindingen uit de voorgaande hoofdstukken besproken en geïnterpreteerd, en suggesties voor toekomstig onderzoek worden besproken.

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PHD PORTFOLIO

Name PhD student:	Bouwe Pieter Krijthe
Erasmus MC Department:	Epidemiology
Research School:	NIHES
PhD period:	2010-2013
Supervisors:	Prof.dr. B.H.Ch. Stricker and Prof.dr. J.C.M. Witteman

	Year
1. PhD training	
Research skills	
- Master of Science in Clinical Epidemiology, Netherlands Institute of Health Sciences, Rotterdam, The Netherlands	2007-2010
International conferences	
- CHARGE-meeting, Boston, MA, USA	2011
- CHARGE-meeting, Los Angeles, CA, USA	2011
- NCHA, Outreach meeting, Amersfoort, The Netherlands	2012
- WEON, Rotterdam, The Netherlands	2012
Seminars and workshops	
- Cardiovascular Pharmacology, COEUR, Erasmus MC, Rotterdam, The Netherlands	2010
- Research seminars, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands	2007-2013
2. Teaching activities	
Teaching assistant:	
- Pharmacoepidemiology, 4th year medical school students, Erasmus MC, Rotterdam, The Netherlands	2010
- Statistics, 4th year medical school students, Erasmus MC, Rotterdam, The Netherlands	2010
- "Data-analysis in Pharmaco-epidemiology", NIHES, Rotterdam, The Netherlands	2011-2013
- "Principles of Research in Medicine", NIHES, Rotterdam, The Netherlands	2011-2012
3. Other	
- Echocardiography, Post-HBO/Master programme, INHolland Academy Haarlem, The Netherlands	2010
- Conducting echocardiographic, ECG and carotid ultrasonic exams on participants of the Rotterdam Study	2010-2012

ABOUT THE AUTHOR

Bouwe Pieter Krijthe was born May 3rd 1986 in Wijk bij Duurstede, The Netherlands. He attended Revius Lyceum in Doorn and graduated in 2004. In the year 2000 he was selected for the Dutch national youth selection basketball. In the following years he combined school with basketball training.

In 2005 he started medical school at the Erasmus University Rotterdam. During his second year in medical school he started with the Master of Science programme in Clinical Epidemiology at the Netherlands Institute of Health Sciences. For this he attended Harvard University Summer School and, under supervision of Prof.dr. H. Tiemeier worked at he Department of Epidemiology at the Erasmus MC in Rotterdam. In 2010 he received both his "doctoraal" degree in Medicine and his Master of Science in Clinical Epidemiology. In 2010 he started his PhD research, under supervision of Prof.dr. B.H.Ch. Stricker, described in this thesis, at the department of Epidemiology, Erasmus MC, Rotterdam.