Determinants of heart failure

Drugs and gene effects in an epidemiological study

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Determinants of Heart Failure

Drugs and gene effects in an epidemiological study

Determinanten van hartfalen
Effecten van geneesmiddelen en genen in een epidemiologische studie

Proefschrift

ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam
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Contents

Manuscripts based on studies presented in this thesis

Chapter 2.1

Bleumink GS, Knetsch AM, Sturkenboom MCJM, Straus SMJM, Hofman A, Deckers JW, Witteman JCM, Stricker BHCh. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure. The Rotterdam Study. Eur Heart J (conditionally accepted).

Chapter 2.2

Bleumink GS, Rijnbeek PR, Kors JA, Van Herpen G, Hofman A, Witteman JCM, Sturkenboom MCJM, Stricker BHCh. Population-based reference values for electrocardiographic left ventricular hypertrophy in the elderly (submitted).

Chapter 3.1

Bleumink GS, Deinum J, Mosterd A, Witteman JCM, Hofman A, Stricker BHCh. Antihypertensive treatment is associated with improved left ventricular geometry. The Rotterdam Study. Pharmacoepidemiol Drug Saf 2004 (in press).

Chapter 3.2

Bleumink GS, Feenstra J, Sturkenboom MCJM, Stricker BHCh. Nonsteroidal anti-inflammatory drugs and heart failure. Drugs 2003;63(6):525-34.

Chapter 4.1

Bleumink GS, van Duijn CM, Kingma JH, Witteman JCM, Hofman A, Stricker BHCh. Apolipoprotein E $\in 4$ allele is associated with left ventricular systolic dysfunction. Am Heart J 2004;147:685-9.

Chapter 4.2

Bleumink GS, Schut AFC, Sturkenboom MCJM, Janssen JAMJL, Witteman JCM, van Duijn CM, Hofman A, Stricker BHCh. Absence of the 192-base pair allele in a promoter polymorphism of the insulin-like growth factor-I gene is associated with an increased risk of left ventricular hypertrophy. Heart (accepted for publication).

Chapter 5.1

Bleumink GS, Schut AFC, Sturkenboom MCJM, Deckers JW, van Duijn CM, Stricker BHCh. Genetic polymorphisms and heart failure. Gen Med (provisionally accepted).

Chapter 5.2

Bleumink GS, Rietveld I, Janssen JAMJL, Deckers JW, Hofman A, Witteman JCM, van Duijn CM, Stricker BHCh, Insulin-like growth factor-I gene polymorphism and risk of heart failure. The Rotterdam Study. Am J Cardiol (accepted for publication).

Chapter 5.3

Schut AFC, Bleumink GS, Stricker BHCh, Hofman A, Witteman JCM, Pols HAP, Deckers JW, Deinum J, van Duijn CM. Angiotensin converting enzyme Insertion/Deletion polymorphism and risk of heart failure in hypertensive subjects (submitted).

Chapter 6.1

Bleumink GS, Schut AFC, Sturkenboom MCJM, van Duijn CM, Deckers JW, Hofman A, Kingma JH, Witteman JCM, Stricker BHCh. Mortality in patients with hypertension on angiotensin-I converting enzyme (ACE)-inhibitor treatment is influenced by the ACE Insertion/Deletion polymorphism (submitted).

Chapter 1

Introduction

Heart failure is a progressive clinical syndrome and the final pathway of any structural or functional cardiac disorder that leads to an impairment of the ability of the heart to fill with or eject blood [1]. To preserve cardiac function after an injury to the myocardium, a complex cascade of interacting hemodynamic and neurohormonal-cytokine mechanisms is activated, which at a later stage may be detrimental for the progression of heart failure [2, 3]. Left ventricular hypertrophy and left ventricular systolic dysfunction are generally accepted to precede symptomatic heart failure [1].

Descriptions of heart failure already exist from ancient Egypt and Greece, and even the Romans were known to use foxglove (digitalis) as medicine [4]. However, it was not until the 1890s that investigations in heart failure improved after the discovery of X-rays by Röntgen and Einthoven's development of electrocardiography [4]. The definition of heart failure has undergone many changes over the past decades, together with advancing insight into its pathophysiology. Nevertheless, there is no satisfactory definition to date that encompasses all features of this complex syndrome. In the first half of the 20th century, circulatory pathophysiology was the focus of attention. This led in the 1950s to the examination of the role of cardiac hypertrophy and in the 1960s to studies on the role of the contractile process in the failing heart [5]. Studies in the 1960s, however, also demonstrated the presence of increased concentrations of noradrenaline in patients with heart failure. This finding led to a large number of studies on neurohormonal changes in heart failure. It became clear only in the 1980s that persistent activation of these neurohormonal systems is detrimental in chronic heart failure [5].

Heart failure constitutes a major public health problem in the western world. Prevalence and incidence of heart failure are substantial [2, 6]. In the Netherlands, for example, it is estimated that approximately 200,000 patients suffer from heart failure; i.e. 1-2% of the total Dutch population [7, 8]. In 1999, the care for these patients accounted for about 1% of the total costs of healthcare in the Netherlands, corresponding to € 299 million [8]. Since age is an important risk factor, the burden of this syndrome on health care systems in Europe and the United States will increase even further as these populations age [9, 10]. In addition, the growing incidence of heart failure is most likely explained by improved survival following cardiac diseases that lead to this condition, of which the most important is myocardial infarction, without effectively providing curative treatment. Hospitalization rates for heart failure have increased considerably in western societies, peaking in the early 1990s [11]. Readmissions for exacerbating heart failure occur frequently, and the proportion of patients with multiple hospital admissions is growing. Despite the tremendous advances in the treatment of heart failure that have taken place over the past decades, heart failure continues to be a highly fatal disease [2]. The true magnitude of the problem of heart failure in the community cannot be accurately described, since few large prospective population-based studies have been published that provide reliable estimates of its prevalence, incidence and prognosis. Moreover, nearly all of these studies have been performed in the United States. In

addition, comparison of studies is complicated, because they have used different definitions and methods to establish the presence of heart failure.

In Europe and the United States, the most common causes of heart failure are coronary artery disease, hypertension, valvular heart disease and idiopathic cardiomyopathy [1, 6, 12]. Many other less common causes exist, such as infectious diseases, endocrine diseases and the use of cardiotoxic drugs. There is also substantial evidence for a genetic contribution to the pathophysiology of heart failure [13-16]. Many studies have been published on the role of single gene mutations in cardiomyopathies [17, 18]. These rare mutations are important at an individual level and for the understanding of disease mechanisms. In addition, genetic association studies have investigated the role of several candidate genes in heart failure, albeit with contradictory results.

The diagnosis of heart failure is complex and relies on clinical judgment based on a history, physical examination and objective evidence of cardiac dysfunction at rest, evaluated e.g. by echocardiography [12]. Signs and symptoms are not specific, however, and a gold standard to establish the presence of this syndrome is lacking. Characteristically, patients present with symptoms of breathlessness or fatigue, which may limit exercise tolerance, and signs of fluid retention, which may lead to pulmonary crepitations or peripheral edema [1, 12]. Symptom-free periods are often alternated with periods of exacerbating symptoms. Although a clinical response to treatment directed at heart failure symptoms helps to make a diagnosis, it is not sufficient [12].

During the past centuries, the therapeutic approach to heart failure has also changed dramatically. Blood letting and leeches were used for centuries and the benefits of digitalis were published for the first time in 1785. Diuretics were not introduced until the 20th century. However, the early organo-mercurial agents, developed in the 1920s, were associated with substantial toxicity. The far less toxic thiazide diuretics were marketed in the 1950s [4]. Half a century ago, treatment of heart failure consisted of bed rest, a sodium restricted diet and the administration of digitalis and diuretics [5]. Present-day treatment is not only targeted towards symptomatic improvement, but also focuses on the prevention of the transition of asymptomatic structural heart disease to symptomatic heart failure, modification of the progressive nature of heart failure and reduction of mortality [1, 12]. Diuretics, angiotensin-I converting enzyme (ACE)-inhibitors and ß-blockers now form the cornerstone of heart failure treatment and physical activity is encouraged. Digitalis is currently only indicated as first-line therapy in heart failure complicated by atrial fibrillation in order to slow ventricular rate [12].

Outline of this thesis

This thesis comprises a number of epidemiological studies that are aimed at gaining insight into the effects of drugs and genetic determinants on the occurrence of heart failure in the general population. All quantitative studies were performed in the Rotterdam Study, a large-scale prospective population-based cohort study among 7983 inhabitants of Ommoord, a suburb of Rotterdam, who were 55 years of age and older [19]. This study provides an excellent setting for observational studies on heart failure, as this is primarily a disease of the elderly. The baseline examinations took place in 1990 to 1993, which allowed for a relatively long follow-up period in the prospective studies presented in this thesis.

In chapter 2, population-based estimates of the prevalence, incidence, lifetime risk and prognosis of heart failure are given to describe the magnitude of the problem of heart failure in the community. This chapter also presents age- and sex-specific reference values for the detection of left ventricular hypertrophy on the electrocardiogram in the elderly. These normal limits were established in an apparently healthy subgroup of participants of the Rotterdam Study and studied in relation to cardiovascular prognosis and the risk of heart failure. Chapter 3 presents two studies on drugs as determinants of heart failure. The association between current use of antihypertensive drugs and left ventricular geometry on the echocardiogram in an observational setting is described. Additionally, an extensive review of the medical literature is given in this chapter of recent findings on the association between the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and the occurrence of heart failure. Chapter 4 contains studies on the association between genetic polymorphisms and structural heart disorders that precede the development of heart failure (i.e. left ventricular hypertrophy and left ventricular systolic dysfunction) and chapter 5 contains studies on the association between genetic polymorphisms and heart failure. In chapter 6, a pharmacogenetic study is presented. The potential interaction between ACE-inhibitor therapy and the ACE Insertion/Deletion polymorphism in the prediction of incident heart failure and death is studied in a subgroup of subjects with hypertension. Finally in chapter 7, methodological limitations of pharmaco-epidemiologic and genetic association studies are discussed and a perspective is given on future directions and implications of pharmacogenetic research.

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Chapter 2

Heart failure

2.1

Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure. The Rotterdam Study

Abstract

Objective: To determine the prevalence, incidence rate, lifetime risk and prognosis of heart failure.

Design: Prospective population-based cohort study.

Setting and participants: 7,734 participants of the Rotterdam Study.

Main outcome measures: Heart failure was identified through general practitioner's medical records and hospital discharge letters. Presence of heart failure was determined according to criteria of the European Society of Cardiology. Information on vital status was obtained from municipal health authorities and general practitioners.

Results: Prevalence was higher in men than in women and increased with age from 0.9% in subjects aged 55-64 years to 17.4% in those aged ≥ 85 years. The incidence rate of heart failure was 14.4/1000 person-years (95% CI 13.4-15.5) and was higher in men (17.6/1000 man-years, 95% CI 15.8-19.5) than in women (12.5/1000 woman-years, 95% CI 11.3-13.8). The incidence rate increased with age from 1.4/1000 person-years in those aged 55-59 years to 47.4/1000 person-years in those aged ≥ 90 years. Lifetime risk was 33% for men and 29% for women at the age of 55. Survival after incident heart failure was 86% at 30 days, 63% at 1 year, 51% at 2 years and 35% at 5 years of follow-up and was similar for men and women.

Conclusions: The prevalence and incidence rate of heart failure are high. In individuals aged 55 years, almost 1 in 3 will develop heart failure during their remaining lifespan. Heart failure continues to be a fatal disease, despite advances in treatment, with only 35% surviving 5 years after the first diagnosis.

Introduction

Heart failure constitutes a major public health burden in the western world. Since incidence rates appear to remain stable over the years, at least in men [1], prevalence estimates of heart failure are bound to increase as the population ages. Hospitalisation rates for heart failure have increased considerably, peaking in the early 1990s [2]. The proportion of patients having multiple hospital admissions is rising. In addition, large observational studies have failed to show any substantial change in the prognosis of heart failure in the general population, despite advances in treatment [3]. Hospitalisation rates do not necessarily reflect the true incidence and prevalence of heart failure in the general population, as only the more serious stages of this syndrome require in-hospital evaluation and treatment. Although data regarding heart failure incidence, prevalence and prognosis in the community are vital, few large prospective population-based studies have been published that provide recent estimates, especially in European populations. Furthermore, most recent population-based estimates originate from relatively short-term studies [4-6], except for the Framingham Heart Study [1] and Cardiovascular Health Study [7], both performed in the United States. The diagnosis of heart failure is complex. Signs and symptoms are not specific and a gold standard to assess the presence of this disease is lacking. Previously published studies have used various criteria to assess the presence of heart failure. The European Society of Cardiology has therefore provided guidelines for the diagnosis of heart failure, for use in clinical practice and epidemiological surveys [8]. According to these guidelines, objective evidence of cardiac dysfunction has to be present to establish the presence of heart failure, in addition to typical symptoms (e.g. breathlessness) suggestive of the diagnosis.

This study was designed to calculate the prevalence, incidence, and lifetime risk of heart failure in participants of the Rotterdam Study, a large prospective population-based cohort study with more than ten years of follow-up. In addition, we studied the prognosis of cases of incident heart failure.

Methods

Setting and study population

The Rotterdam Study is a population-based prospective cohort study of cardiovascular, locomotor, neurologic and ophthalmologic diseases in the elderly [9]. All inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, who were 55 years of age or older were invited to participate. Of the 10,275 eligible subjects, 7,983 agreed to participate (78%). The Medical Ethics Committee of the Erasmus Medical Centre approved the study. The baseline examination was conducted between July 1989 and July 1993. Participants were visited at home for a standardized questionnaire and were subsequently examined at the

research centre. Since the start of the Rotterdam Study, cross-sectional surveys have been carried out periodically. In addition, participants are continuously monitored for major events that occur during follow-up, including heart failure, through automated linkage with files from general practitioners. Information on vital status is obtained regularly from municipal health authorities in Rotterdam and from the general practitioners working in the study district of Ommoord, and was complete for all participants until January 1, 2000. Furthermore, all drug prescriptions dispensed to participants by all pharmacies in the study area are routinely stored in the database.

To obtain recent estimates, the point prevalence of heart failure was determined at the 1st of January of 1997, 1998 and 1999. Calculations were performed in all participants of the Rotterdam Study who were alive and present at January 1 of each of these years. Four participants were excluded because of missing medical records. For estimation of incidence rates and lifetime risks, the study population comprised 7,734 subjects who were free from heart failure at baseline. Subjects were followed from baseline until the first of one of the following: a diagnosis of incident heart failure, death, loss to follow-up (<1%), date of last collection of information for determination of heart failure, or January 1, 2000. The date of last information on heart failure status preceded January 1, 2000 for 14.4% of participants. For the calculation of survival estimates, incident heart failure patients were followed from the date of incident heart failure until the earliest of death, removal from the study area, or January 1, 2000.

Heart failure assessment

Assessment of prevalent heart failure at the baseline examination in the Rotterdam Study has been described in detail earlier [10]. Briefly, a validated score was used, similar to the definition of heart failure of the European Society of Cardiology [8]. This score was based on the presence of at least two symptoms suggestive of heart failure (shortness of breath, ankle swelling and pulmonary crepitations) or use of medication for the indication of heart failure, in combination with objective evidence of cardiovascular disease. Questions on indication of cardiovascular medication and breathlessness were lacking at the start of the Rotterdam Study, but were subsequently added. Consequently, this information was obtained in only 5,540 participants. In addition, prevalent heart failure cases were obtained through a database containing hospital discharge diagnoses from all hospitals in the Rotterdam area as of January 1, 1991. Records from this database were linked to the Rotterdam Study database. For potential cases of heart failure identified in this way, copies of discharge letters were requested. Furthermore, all medical records were screened in retrospect for the occurrence of heart failure in the majority (97%) of participants of the Rotterdam Study. With these three methods, information on the presence of heart failure at baseline was available for all participants.

Cases of incident heart failure were obtained by continuously monitoring participants of the Rotterdam Study for the occurrence of heart failure during follow-up through automated linkage with files from general practitioners. All available data on these events, such as hospital discharge letters and notes from general practitioners, were copied from the medical records. Apart from this systematic follow-up procedure, we used verified hospital discharge diagnoses for case finding, gathered from all hospitals in the Rotterdam area as described above. The date of incident heart failure was defined as the day of the first occurrence of symptoms suggestive of heart failure, obtained from the medical records, or the day of receipt of a first prescription for a loop diuretic or an ACE-inhibitor, whichever came first.

The diagnosis of heart failure was classified as definite, probable, possible or unlikely. Definite heart failure was defined as a combination of heart failure diagnosed by a medical specialist and the presence of typical symptoms of heart failure, such as breathlessness at rest or during exertion, ankle oedema and pulmonary crepitations, confirmed by objective evidence of cardiac dysfunction (chest X-ray, echocardiography). This definition is in accordance with the criteria of the European Society of Cardiology [8]. Probable heart failure was defined as heart failure diagnosed by a general practitioner, with at least two typical symptoms suggestive of heart failure, and at least 1 of the following: history of cardiovascular disease (e.g. myocardial infarction, hypertension), response to treatment for heart failure, or objective evidence of cardiac dysfunction, while symptoms could not be attributed to another underlying disease, such as chronic obstructive pulmonary disease. Two research physicians independently classified all information on potential heart failure events. If there was disagreement, a consensus was reached in a separate session. Finally, a cardiologist verified all probable and possible cases, and all cases in which the two physicians could not reach consensus. If the cardiologist disagreed with the research physicians, the cardiologist's judgment was considered decisive. The research physicians and the cardiologist based their decisions on the same data. Only definite and probable cases were included in the analyses.

Statistical analysis

Prevalence of heart failure per calendar year was calculated by dividing the number of persons with prevalent heart failure by the number of subjects present in the study population at January 1 of each calendar year. 95% confidence intervals (CI) were calculated with Wilson's (score) method for a binomial proportion. Prevalence estimates were calculated for men and women separately and in 10-year age categories. The incidence rate of heart failure was determined by dividing the number of cases of incident heart failure by the total number of person-years accumulated in the study population without heart failure at baseline. The 95% CI around the estimates were calculated based on the Poisson distribution. Incidence rate estimates were calculated by gender and age (5-year categories).

To calculate the risk to develop heart failure over time, competing risk of death was taken into account. First, we calculated heart failure free survival at different ages with the Kaplan-Meier method, using incident heart failure and mortality data from the study cohort. Age at baseline was used as entry time variable and age at the end of follow-up, incident heart failure, or death, as failure time variable. Both death and incident heart failure were classified as failures. Second, the cumulative absolute risk of heart failure over a period was calculated as the integrated product of the age-specific heart failure incidence rates and heart failure free survival [11]. The risks of heart failure over time were calculated for the total population and for men and women separately at the ages of 55-, 65-, 75-, and 85 years.

The prognosis of heart failure was determined in 725 subjects with incident heart failure during follow-up. Survival after incident heart failure (30-day, 1-year, 2-year and 5-year) was calculated using the Kaplan-Meier method. Survival was also determined after exclusion of patients who died in the first 30 days, thereby excluding those with a first diagnosis of heart failure on the day of their death and the most severe cases of heart failure. We used Cox proportional hazards regression analysis to study gender differences in survival, adjusted for age.

Results

A total of 245 prevalent heart failure cases (88 men, 157 women) were identified at baseline in the Rotterdam Study. In the remaining study population (n=7,734), we identified 725 incident cases of heart failure (335 men, 390 women), of whom 673 were classified as definite-, and 52 as probable cases. The median follow-up time in this population was 7.1 years (interquartile range: 5.7-8.0) and we had in total 50,268 person-years of observation. The majority of our study population was female (61%) and mean age at baseline was 70.4 years (standard deviation 9.7 years). Mean age at the onset of heart failure was significantly higher in women than in men (82.5 years and 77.5 years respectively).

Prevalence

Point prevalence of heart failure was determined at January 1 of 1997, 1998 and 1999 and was 6.4% (95% CI 5.8-7.0), 6.7% (95% CI 6.1-7.4) and 7.0% (95% CI 6.4-7.7), respectively. Mean age of the study population increased from 73.3 years in 1997 to 74.5 years in 1999. Prevalence was higher in men than in women (e.g. 1998: men 8.0%, women 6.0%). There was a sharp rise of prevalence estimates with age. For example, in 1998 point prevalence increased from 0.9% (95% CI 0.5-1.6) in subjects aged 55-64 years, 4.0% (95% CI 3.3-4.8) in subjects aged 65-74 years, 9.7% (95% CI 8.4-11.1) in those aged 75-84 years to 17.4% (95% CI 14.8-20.4) in those aged 85 years or over.

Incidence rate

The overall incidence rate of heart failure was 14.4/1000 person-years (95% CI 13.4-15.5) and was significantly higher in men (17.6/1000 man-years, 95% CI 15.8-19.5) than in women (12.5/1000 woman-years, 95% CI 11.3-13.8). The incidence rate increased with age from 1.4/1000 person-years in those aged 55-59 years to 47.4/1000 person-years in those aged 90 years or older (table 1). This increase with age was evident for both genders (figures 1a and 1b). Incidence rates were on average approximately two times higher in men than in women in each age category, except for the youngest (55-59 years), in which no male cases occurred.

Table 1. Incidence rates for heart failure per 5-year age category.

Age category	Number of incident cases	Person-years	Incidence rate * (95% CI)
55-59 years	4	2888.6	1.4 (0.5-3.3)
60-64 years	27	8713.6	3.1 (2.1-4.4)
65-69 years	56	10392.1	5.4 (4.1-6.9)
70-74 years	113	9665.6	11.7 (9.7-14.0)
75-79 years	136	8012.8	17.0 (14.3-20.0)
80-84 years	166	5513.5	30.1 (25.8-35.0)
85-89 years	137	3269.0	41.9 (35.3-49.4)
≥ 90 years	86	1813.5	47.4 (38.6-58.2)

* per 1000 person-years

Period and lifetime risk

The period and lifetime risks for all subjects, and for men and women separately, at the ages of 55-, 65-, 75-, and 85 years are shown in table 2. All estimates account for the risk of competing causes of death. The lifetime risk of heart failure for a person aged 55 was 30.2%. For a man aged 55 years the lifetime risk was 33.0% and for a woman of the same age it was 28.5%. Lifetime risk of heart failure decreased with age in both sexes to approximately 23% in persons who reached 85 years of age without having heart failure. Stratification by gender showed that lifetime risks were higher in men than women at ages 55 to 75. In subjects aged 85 years, however, lifetime risks for developing heart failure were comparable and slightly higher in women (table 2 and figure 2). Cumulative risks in shorter time intervals (5-25 years) increased with age and were higher in men at all ages, reflecting the higher incidence rates in men.

Table 2. Cumulative risk of heart failure in different time periods for participants aged 55-, 65-, 75-, and 85 years; total and stratified by gender.

	Period risk* (years)							
Age	5	10	15	20	25	30	35	Lifetime
Total								
55	0.6	2.1	4.5	9.2	14.7	21.8	27.2	30.2
65	2.6	7.6	13.6	21.3	27.1			30.3
75	7.5	17.2	24.6					28.7
85	14.8							23.1
Men								
55	0	2.8	6.8	13.4	19.6	27.9	31.6	33.0
65	4.2	11.4	18.2	27.1	31.2			32.7
75	9.5	22.0	27.7					29.8
85	16.2							22.4
Women								
55	1.0	1.8	3.0	6.2	11.2	17.5	24.3	28.5
65	1.2	4.6	10.0	16.7	24.0			28.5
75	6.2	14.1	22.6					27.9
85	14.3							23.3

* numbers are percentages. Competing risk of death is taken into account.

Prognosis

Of the 725 persons with incident heart failure, 445 subjects died following the diagnosis (198 men and 247 women). Median survival was 2.1 years (range: 1 day- 9.0 years). Cumulative survival was 86% at 30 days after the onset of heart failure (95% CI 83%-88%), 63% at 1 year (95% CI 59%-66%), 51% at 2 years (95% CI 47%-55%) and 35% at 5 years (95% CI 31%-39%). There was no significant difference in cumulative survival after incident heart failure between men and women (figure 3, log rank test: p=0.15). Age-adjusted survival in Cox proportional hazards analysis was similar in men and women (hazard ratio female gender: 0.88, 95% CI 0.72-1.07). After exclusion of patients who died in the first 30 days, 1-, 2- and 5-year survival were 73%, 59% and 41% respectively. Age- and gender adjusted survival was significantly lower in subjects with incident heart failure than in the remainder of our study cohort (hazard ratio 4.3, 95% CI 3.8-4.8).

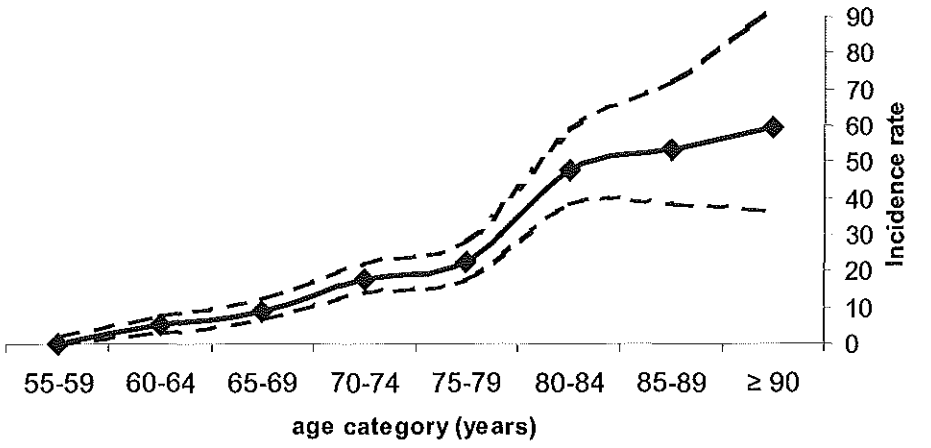

Figure 1a. Age-specific male incidence rates (/1000 man years) and 95% confidence band.

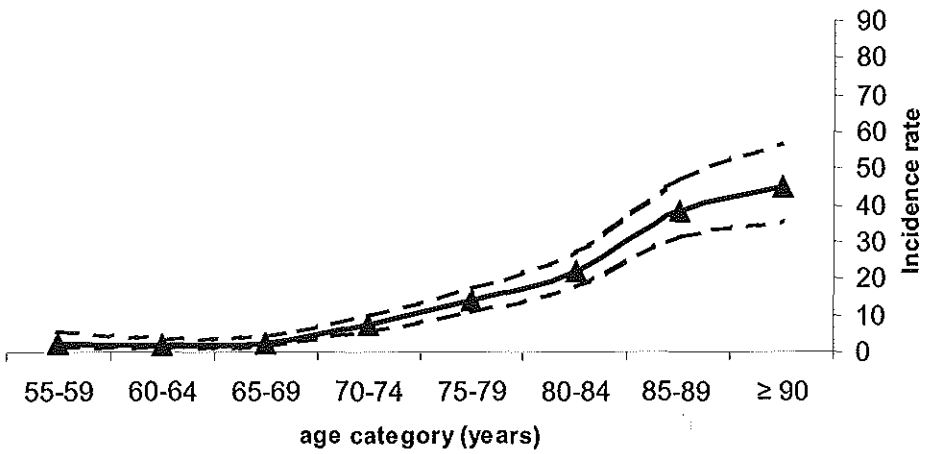

Figure 1b. Age-specific female incidence rates (/1000 woman-years) and 95% confidence band.

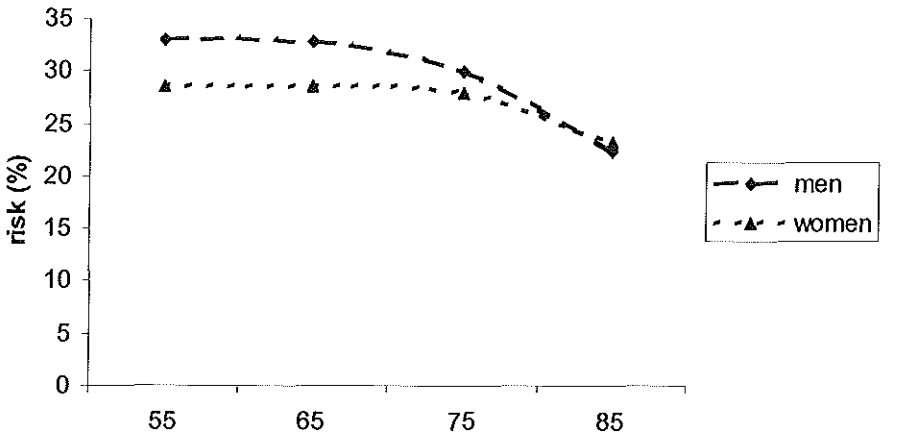

Figure 2. Age-specific lifetime risk of heart failure stratified by gender.

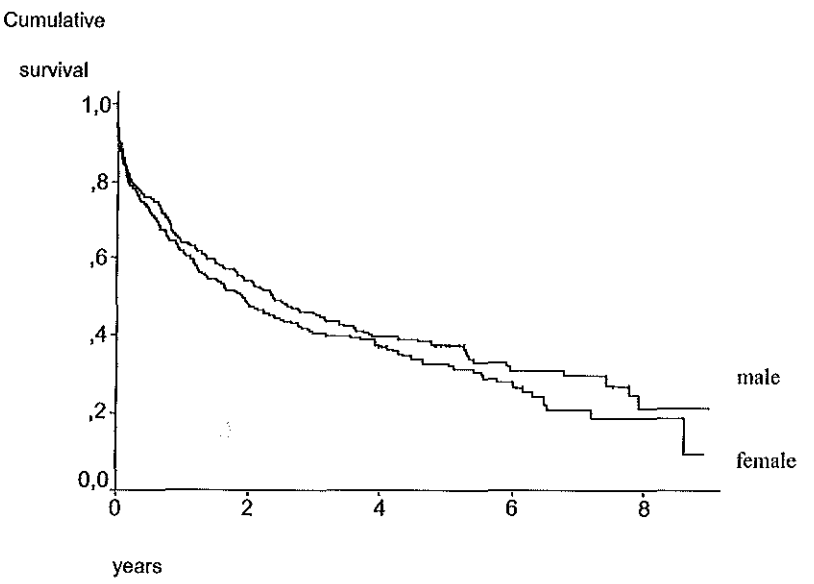

Figure 3. Kaplan - Meier survival curve for incident heart failure cases stratified by gender.

Discussion

In this long-term prospective population-based cohort study, we found that heart failure prevalence, incidence and risk are high. The incidence rate was significantly higher in men than in women and increased with age from 1.4/1000 person-years in subjects aged 55-59 years to 47.4/1000 person-years in those aged 90 years or older. Our study showed that the probability for an individual aged 55 years to develop heart failure during his or her remaining lifetime is 30.2%. As expected, lifetime risk decreased at older ages, probably because of depletion of susceptibles and a shorter remaining lifespan. In our study, lifetime risk of heart failure was higher in young men than in young women. In the older individuals, however, lifetime risks were practically the same in men and women. Heart failure remains a deadly disease for both genders, with a 5-year survival of only 35%.

Our age-specific incidence rate estimates are similar to the results from an investigation in a general practitioner's database in the United Kingdom [12], but differ somewhat from other recent population-based studies. Estimates in the Cardiovascular Health Study were higher in all age categories. Although this study also used clinical criteria for the assessment of heart failure, the investigators selected their participants through a Medicare eligibility list [7]. This may explain some of the difference with our study, which was performed in an unselected population. Besides differences in selection criteria and population characteristics, comparison between investigations is further complicated because studies have used different criteria to assess the presence of heart failure. For example, in the Framingham Heart Study, clinical criteria were used that do not include evidence of cardiac dysfunction on echocardiography, which is an important tool in heart failure diagnosis in clinical practice [1]. Therefore, in the Framingham Heart Study, the true incidence of heart failure may have been underestimated. In the Hillingdon heart failure study, potential cases were identified on the basis of referrals by general practitioners of patients with suspected heart failure [6]. Although similar criteria were used in this study, age-specific incidence was somewhat lower, possibly because not all potential cases were referred. Age-specific prevalence estimates of heart failure were also somewhat higher in the Cardiovascular Health Study, especially at younger ages [13]. Slightly lower prevalence estimates per age-category were found in a study in residents of Olmsted County, Minnesota and in the Framingham Heart Study [4, 14]. Both used Framingham criteria for case ascertainment. The Echocardiographic Heart of England Screening study used criteria based on the guidelines of the European Society of Cardiology and found age-specific prevalence estimates of heart failure that were similar to ours [5].

Only one other study, the Framingham Heart Study, calculated lifetime risks for heart failure [15]. Lifetime risk for the development of heart failure in this study was approximately 20% and was, in contrast to our findings, independent of age and gender. The investigators did not find a decrease in lifetime risk at older ages, which was attributed to an increasing

incidence with advancing age, outpacing the increasing mortality from competing causes. However, no age-limit was set for the calculation of cumulative risks in the Rotterdam Study, while in the Framingham Heart Study cumulative risks were calculated until the age of 94 years. Furthermore, lifetime risks in the Framingham Study were calculated from 1971 through 1996, while in the Rotterdam Study they were calculated from 1989 through 2000. Therefore, changes in mortality from competing causes over calendar time may explain some of the differences between the two studies. Furthermore, although questioned by some [16], improvements in myocardial infarction treatment over time might account for the higher incidence rates of heart failure that we found.

Heart failure is a fatal disease, despite advances in treatment over the past 15 years [3]. We found no differences between men and women in heart failure prognosis. Our survival estimates are very similar to those found in three other recent population-based studies [1, 12, 17]. However, compared to heart failure mortality in hospital-based studies [18-20], prognosis in our population-based study was better, probably as less severe cases of heart failure were also included. As the diagnosis of heart failure is difficult, some studies applied scores for the classification of heart failure, while other studies used clinical definitions or relied on hospital discharge codes. Therefore, a large part of the differences between studies may be explained by varying criteria. Besides a baseline screening in the majority of participants using a validated score, we applied clinical criteria for heart failure throughout the Rotterdam Study, based upon guidelines of the European Society of Cardiology. Apart from hospital discharge letters, medical records from general practitioners were available for assessment of cases. Consequently, also less severe cases were included in our study. However, some underestimation of the true prevalence and incidence may have been caused by the fact that old and diseased individuals were less likely to participate in the Rotterdam Study. Another limitation of our study is that we did not distinguish between underlying causes of heart failure. Among elderly patients, systolic hypertension and cardiac hypertrophy may be more important than ischemic heart disease [8].

In conclusion, heart failure prevalence and incidence are substantial. As age is an important risk factor for heart failure, the burden of this disease on health care systems in western societies increases as these populations age. In individuals aged 55 years, 30% will develop heart failure during their remaining lifespan; i.e. almost one out of three individuals. Heart failure continues to be a fatal disease, despite advances in treatment, with only 35% surviving 5 years after the first diagnosis. Prevention of the development of heart failure in high-risk patients is therefore fundamental.

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2.2

Population-based reference values for electrocardiographic left ventricular hypertrophy in the elderly

Abstract

Background: Left ventricular hypertrophy (LVH) is an independent predictor of cardiovascular disease. Although voltage amplitudes on the electrocardiogram (ECG) vary according to sex and age, reference values for LVH are lacking in the elderly. We established age- and sex-specific normal limits in elderly subjects for the Cornell, Sokolow-Lyon and 12-lead summed QRS voltage and voltage-duration products. Additionally, the prognostic value of all partition values for ECG-LVH was tested for heart failure and cardiovascular mortality.

Methods: The Rotterdam Study is a population-based cohort study in 7983 participants aged 55 years or older. 12-lead ECGs were digitally recorded at baseline for 6193 participants. Age- and sex-specific normal limits were calculated parametrically in 2915 apparently healthy participants. The 98th percentile was taken as the upper limit of normal. The prognostic value of partition values for ECG-LVH was assessed with Cox proportional hazards analysis.

Results: Newly assessed reference values for ECG-LVH were higher than traditional partition values, except for the Cornell voltage in men. 98th Percentiles were lower in women than in men up to the age of 75 years. In women, normal limits increased with age. In contrast, Sokolow-Lyon- and 12-lead summed voltage (-duration) criteria decreased with advancing age in men, whereas the Cornell voltage (-duration product) was not substantially influenced by age. ECG-LVH was significantly associated with heart failure and cardiovascular mortality for all partition values. The strongest association was seen for the Cornell voltage-duration product defined according to the new normal limits. Except for the Sokolow-Lyon voltage-duration product, all normal limits established in the present study showed stronger associations for ECG-LVH than the traditional partition values. Differences were more pronounced in women than in men.

Conclusion: We provide age- and sex-specific normal limits for three commonly used ECG-LVH voltage equations and their voltage-duration products in normal elderly individuals. ECG-LVH detected with these normal limits was associated with both heart failure and cardiovascular mortality for all voltage criteria.

Introduction

Over the past years, evidence has increased that left ventricular hypertrophy (LVH) is a strong and independent risk factor for cardiovascular morbidity and mortality [1-4]. Echocardiography is considered to be a gold standard for the detection of LVH. However, the greater availability, lower costs and higher reproducibility of the electrocardiogram (ECG) tend to favor its use in the assessment of LVH in large observational studies and clinical trials and support its wide application in clinical practice. In addition, due to technical problems, it is often difficult to obtain echocardiographic data of sufficient quality, particularly in the elderly [5, 6]. Both methods independently predict mortality, and represent, for some part, different manifestations of disease [7, 8].

Most available methods for the detection of LVH on the ECG utilize fixed voltage criteria, which are neither age- nor sex-specific. Evaluations of these criteria have largely been carried out manually and in clinical populations. Sources of normal limits for ECG measurements are scarce and reference values for common LVH-voltage criteria in healthy elderly individuals are lacking [9, 10]. Aging causes anatomic and functional changes in the cardiovascular system, which may affect ECG findings [11]. Furthermore, men have significantly longer QRS durations and greater ECG-voltages than women. All ECG criteria have a lower accuracy for the detection of LVH in women, even when differences in partition value selection are taken into account [12]. As both age and sex influence voltage amplitudes on the ECG, the traditional partition values used to detect LVH may not be valid in the elderly. Therefore, we performed a study in an apparently healthy subgroup of participants of the Rotterdam Study to establish age- and sex-specific reference values in the elderly for three commonly used voltage criteria - Cornell voltage, Sokolow-Lyon voltage and 12-lead sum of QRS voltage - and their duration products. In addition, the association between LVH classified according to these reference values and previously published partition values were studied in relation to cardiovascular mortality and the risk of heart failure.

Methods

Setting

The Rotterdam Study is a population-based prospective cohort study of cardiovascular, locomotor, neurologic, and ophthalmologic diseases in the elderly [13]. All inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, who were 55 years of age or older were invited and 7983 agreed to participate (78%). The baseline examination was conducted between 1990 and 1993. Participants were visited at home for a standardized questionnaire and were subsequently examined at the research center, where a 12-lead ECG was recorded. Information was collected on age, sex, present health status and medical history, including previous myocardial infarction,

coronary bypass surgery and coronary angioplasty. All reported myocardial infarctions were verified with the medical records. Hypertension was defined as use of antihypertensive medication for the indication high blood pressure, or as a systolic blood pressure of 160 mm Hg or over and/or a diastolic blood pressure of 100 mm Hg or over (i.e. moderate to severe hypertension). Diabetes mellitus was defined as the use of anti-diabetic medication, or as a random or post-load serum glucose level higher than 11.0 mmol/l. Information on medication use was assessed during the home interview. Participants subsequently showed all their currently used medication at the research center, where a physician determined the indication for each drug. Since the start of the Rotterdam Study, follow-up examinations have been carried out periodically. In addition, participants are continuously monitored for major events that occur during follow-up, including heart failure, through automated linkage with files from general practitioners. Information on vital status is obtained regularly from municipal health authorities in Rotterdam and from general practitioners working in the study district of Ommoord, and was complete for all participants.

ECG measurements

A 12-lead resting ECG was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally for 6193 (77.6%) participants. Missing ECGs were mainly due to technical and logistic problems. ECGs were processed by the Modular ECG Analysis System (MEANS) [14]. This system computes a representative averaged beat for each of the 12 leads from which ECG measurements are derived. QRS duration is determined from the first QRS onset in any of the 12 leads to the last QRS offset in any lead. MEANS has been evaluated extensively, showing good performance [14, 15].

Three widely used criteria for the detection of LVH in adult cardiology were examined: the Sokolow-Lyon voltage: sum of the amplitudes of the S wave in lead V1 and the R wave in lead V5 or V6 (whichever is longest) [16], the Cornell voltage: sum of the amplitudes of the R wave in lead aVL and the S wave in lead V3 [17] and the 12-lead summed QRS voltage: sum of peak-to-peak QRS amplitudes in all 12 leads [18]. In addition, voltage-duration products were calculated by multiplying the voltage with QRS duration. Voltage-duration products have been found to improve the accuracy of the identification of LVH on the ECG [19, 20].

Calculation of normal partition values for ECG-LVH

Study population

For the estimation of normal limits for ECG-LVH, a seemingly healthy subgroup of participants of the Rotterdam Study was selected without a known history of cardiovascular disease, and

for whom baseline data were complete. Participants with a history of myocardial infarction, heart failure, coronary bypass surgery or coronary angioplasty were excluded from our study population. Other exclusion criteria were the presence of hypertension, diabetes mellitus and the use of ACE-inhibitors, calcium antagonists, diuretics, β-blockers and other antihypertensive drugs. Plots of all ECGs displaying wave onsets and ends as found by MEANS were visually checked. ECGs with complete right- or left bundle-branch block, Wolff-Parkinson-White (WPW) syndrome, pacemaker rhythm or waveform recognition errors (mainly due to excessive noise) were also removed from the dataset, leaving a total study population of 2915 participants.

Normal limits estimation

Calculation of age- and sex-specific normal limits for ECG-LVH criteria was performed as described earlier [21]. The 98th percentile of the measurement distribution was a priori chosen as the upper limit of normal. Briefly, a linear regression analysis on age was performed in each age group. Percentiles were estimated parametrically on the residuals. Possible non-gaussianity of the residuals was removed using a transformation procedure, iteratively eliminating asymmetry (skewness) and peakedness (kurtosis) in two stages [22]. We used the Kolmogorov-Smirnov method to test the gaussianity of the transformed distribution. Estimated percentiles and their 95% confidence intervals (CI) were back-transformed to the original unit of measurement and presented according to sex and age groups.

In addition to this tabular presentation of normal limits according to sex and age groups, age-dependent curves were created for men and women separately, presenting the estimated normal limits in a continuous form. To this end, the two-stage transformation procedure was applied in windows of 200 measurements each, moving along the age-axis with a stepsize of one measurement. For each window position the 98th percentile and 95% CI were calculated and related to the median of the age values included in the window. To allow for estimates at young ages in our study population, the procedure started with a small initial window that was enlarged until 200 measurements were included. Polynomial curves were fitted through the point estimates of the 98th percentile values to obtain normal limits that smoothly change with age. The order of the polynomials was determined by visual inspection of the fit, selecting the lowest order that yielded curves remaining within the 95% CI of the 98th percentile values.

Prognosis of ECG-LVH for different partition values

We additionally tested the prognostic value of our newly estimated age- and sex-specific reference values and of previously published partition values in heart failure and cardiovascular mortality. Presence of ECG-LVH according to the different partition values

was assessed for all 6193 participants of the Rotterdam Study with a digitally stored ECG. The following 'traditional' partition values were used: Sokolow-Lyon voltage ≥ 3500 μV [16], Sokolow-Lyon voltage-duration product >322.4 μVs in women and >367.4 μVs in men [23], Cornell voltage >2000 μV in women and >2800 μV in men [17], Cornell voltage-duration product >244 μVs in men or (Cornell voltage + 600 μV) x QRS duration >244 μVs in women [19, 24], 12-lead sum >18499 μV in women and >19530 μV in men [23], 12-lead sum product >1683.8 μVs in women and >1957.9 μVs in men [23].

Heart failure

For the prognostic analyses with regard to heart failure, the study population comprised 5575 subjects out of the 6193 persons with an ECG, who were free from heart failure at baseline and had no bundle-branch block, WPW syndrome or pacemaker rhythm. Subjects were followed from baseline until the first of one of the following: a diagnosis of incident heart failure, death, loss to follow-up, date of last collection of information for determination of heart failure, or January 1, 2000. Cases of incident heart failure were obtained by continuously monitoring participants of the Rotterdam Study for the occurrence of heart failure during follow-up through automated linkage with files from general practitioners and through a hospital discharge diagnosis database. All available data on these events, such as hospital discharge letters and notes from general practitioners, were copied from the medical records. Heart failure was defined in accordance with the criteria of the European Society of Cardiology, which include the presence of typical symptoms of heart failure, such as pulmonary crepitations, and objective evidence of cardiac dysfunction [25].

Cardiovascular mortality

To study the predictive value of ECG-LVH according to the different partition values for cardiovascular mortality, we included 5740 of 6193 individuals with an ECG, without bundle-branch block, WPW syndrome or pacemaker rhythm. Subjects were followed from baseline until the earliest of death, loss to follow-up, or January 1, 2000. Information on vital status was obtained from municipal health authorities and from general practitioners working in the study area. Two research physicians independently coded potential cardiovascular deaths according to the 10th version of the International Classification of Diseases (ICD10). If the research physicians disagreed, a cardiologist reviewed the event and gave the definitive code. The following ICD10 codes were used in our analyses to define cardiovascular mortality: I20 (angina pectoris), I21 (acute myocardial infarction), I22 (subsequent myocardial infarction), I23 (complications following acute myocardial infarction), I24 (other acute ischemic heart diseases), I25 (chronic ischemic heart disease), I46 (cardiac arrest), I49 (other cardiac arrhythmias), I50 (heart failure), and R96 (other sudden death, cause unknown).

Statistical analysis

We used Cox proportional hazards regression analysis to calculate hazard ratios (HRs) and 95% CI for the estimation of relative risks of heart failure and cardiovascular mortality for LVH as determined by the different partition values. Analyses were adjusted for age and sex.

Results

Calculation of normal partition values for ECG-LVH

Table 1 presents general characteristics of the healthy subgroup of 2915 participants who were used to calculate normal limits. Mean age was 67 years and 59% of our study population was female. Body mass index and blood pressure measurements differed slightly between men and women. Mean voltages and voltage-duration products were significantly higher in men than in women for all criteria. There were marked differences between men and women in the association of age with voltage criteria. Mean voltages and voltage-duration products decreased with advancing age in men, except for the Cornell voltage and its duration product. In women, mean voltages increased significantly with age, except for the Sokolow-Lyon voltage-duration product (table 1).

Table 1. Baseline characteristics of an apparently healthy sub-population of the Rotterdam Study stratified by sex.

Variable	Men (n=1196) Mean (SD)	Women (n=1719) Mean (SD)
Age (years)*	66 (7.6)	67 (8.4)
Body mass index (kg/m²)*	25.5 (2.9)	25.9 (3.8)
Systolic blood pressure (mmHg)*	131 (15.5)	130 (16.6)
Diastolic blood pressure (mmHg)*	73 (9.8)	71 (9.5)
Sokolow-Lyon (μV)*	2406.6 (714.2)	2209.3 (638.3)
β age†	-12.1 (-17.3 - -6.8)	3.6 (0.06-7.2)
Sokolow-Lyon (μV) x QRS (s)*	244.4 (73.6)	207.3 (61.4)
β age†	-1.3 (-1.8 - -0.7)	0.3 (-0.01-0.7)
Cornell (μV)*	1420.1 (527.7)	1285.3 (487.8)
β age†	-0.32 (-4.2-3.6)	16.6 (14.0-19.2)
Cornell (μV) x QRS (s)*	145.7 (59.4)	122.2 (52.4)
β age†	-0.06 (-0.5-0.4)	1.6 (1.3-1.9)
12-lead summed (μV)*	14926.3 (3243.5)	13616.0 (2846.5)
β age†	-52.7 (-76.6 - -28.7)	50.6 (34.8-66.4)
12-lead summed (μV) x QRS (s)*	1521.2 (365.4)	1282.9 (301.2)
β age†	-5.7 (-8.4 - -3.0)	4.9 (3.2-6.5)

* mean difference between men and women: independent samples t-test: $p<0.05$
† linear regression model: dependent variable: microvoltage, independent variable: age; β (95%CI)

Table 2 presents the normal limits that we estimated for each voltage index for ECG-LVH, stratified by age and sex. In figures 1-3, estimated reference values for the three voltage criteria are also presented in age-dependent curves for men and women separately. In men, normal limits decreased with age for the Sokolow-Lyon voltage and 12-lead summed voltage and for their duration products (table 2, figures 1B and 3B). There was a slight increase in normal limits with age for the Cornell voltage-duration product, while the 98th percentile for the Cornell voltage remained relatively stable with age (table 2, figure 2B). Except for the Cornell voltage, normal limits for men found in the present study were higher than the traditional partition values at all ages. In women, normal limits increased with age for all ECG-LVH criteria. Normal partition values were greater than the traditional values for every voltage equation at all ages in women. Age-specific reference values were lower in women than in men, except in the oldest age group. In these participants, aged 75 years or older, 98th percentiles for the common voltage criteria in women were similar to or higher than in men.

Table 2. Estimated normal limits and 95% confidence interval for voltage equations of left ventricular hypertrophy in elderly men and women stratified by age group.

Voltage equation	55-64 years	65-74 years	75+ years
Men	N = 601	N = 422	N = 173
Sokolow-Lyon (μV)	4053 (3931-4178)	3977 (3837-4119)	3765 (3544-3996)
Sokolow-Lyon (μV) x QRS (s)	413 (401-426)	405 (391-420)	387 (364-407)
Cornell (μV)	2582 (2495-2669)	2609 (2487-2736)	2646 (2482-2815)
Cornell (μV) x QRS (s)	277 (267-288)	284 (269-301)	293 (270-318)
12-lead summed (μV)	22694 (22046-23372)	22482 (21682-23327)	21111 (20331-21881)
12-lead summed (μV) x QRS (s)	2349 (2283-2417)	2342 (2263-2424)	2300 (2178-2427)
Women	N = 858	N = 565	N = 296
Sokolow-Lyon (μV)	3528 (3442-3616)	3618 (3502-3736)	3823 (3678-3967)
Sokolow-Lyon (μV) x QRS (s)	333 (325-342)	349 (337-363)	385 (364-407)
Cornell (μV)	2153 (2091-2217)	2258 (2184-2334)	2946 (2735-3180)
Cornell (μV) x QRS (s)	216 (209-224)	235 (224-246)	316 (284-355)
12-lead summed (μV)	19037 (18667-19414)	20252 (19655-20876)	22096 (21196-23039)
12-lead summed (μV) x QRS (s)	1837 (1801-1874)	1964 (1904-2027)	2273 (2150-2409)

The 98th percentile of the measurement distribution was taken as the upper limit of normal.
In comparison: traditional values men (all ages): Sokolow-Lyon ≥ 3500, Sokolow-Lyon x QRS >367.4, Cornell >2800, Cornell x QRS >244, 12-lead summed >19530, 12-lead summed x QRS >1957.9. Traditional values women (all ages): Sokolow-Lyon ≥ 3500, Sokolow-Lyon x QRS >322.4, Cornell >2000, (Cornell+600mm) x QRS >244, 12-lead summed >18499, 12-lead summed x QRS >1683.8.

Table 3. Hazard ratios and 95% confidence intervals for heart failure and cardiovascular mortality by ECG-LVH partition value; overall and for men and women separately.

LVH partition value (number)	Heart failure			Cardiovascular mortality		
	*Overall** N° of cases: 432	*Men*** N° of cases: 206	*Women*** N° of cases: 226	*Overall** N° of cases: 275	*Men*** N° of cases: 135	*Women*** N° of cases: 140
Sokolow-Lyon traditional	1.9 (1.4-2.4)	1.7 (1.1-2.4)	2.1 (1.5-3.1)	1.9 (1.4-2.6)	1.4 (0.9-2.3)	2.4 (1.5-3.7)
Sokolow-Lyon new	**2.1 (1.5-2.9)**	**1.9 (1.1-3.1)**	**2.3 (1.5-3.6)**	**2.4 (1.6-3.4)**	**2.0 (1.1-3.7)**	**2.6 (1.6-4.3)**
Sokolow-Lyon x QRS traditional	2.6 (2.1-3.3)	2.3 (1.6-3.2)	2.9 (2.1-4.0)	2.6 (1.9-3.4)	2.3 (1.5-3.4)	2.9 (1.9-4.2)
Sokolow-Lyon x QRS new	**2.2 (1.6-3.0)**	**1.7 (1.1-2.8)**	**2.8 (1.8-4.3)**	**2.5 (1.7-3.5)**	**2.0 (1.2-3.4)**	**3.0 (1.8-4.9)**
Cornell traditional	1.9 (1.5-2.5)	2.3 (1.2-4.3)	1.9 (1.4-2.5)	1.8 (1.3-2.5)	0.9 (0.3-2.7)	1.9 (1.3-2.8)
Cornell new	**2.5 (1.8-3.5)**	**2.3 (1.4-3.6)**	**2.7 (1.7-4.3)**	**2.7 (1.8-3.9)**	**1.6 (0.8-3.0)**	**4.0 (2.5-6.4)**
Cornell x QRS traditional	2.0 (1.6-2.5)	2.1 (1.5-3.0)	2.0 (1.5-2.7)	2.2 (1.7-2.8)	2.0 (1.3-3.1)	2.3 (1.6-3.2)
Cornell x QRS new	**2.8 (2.0-3.9)**	**2.3 (1.4-3.7)**	**3.4 (2.2-5.2)**	**3.4 (2.4-4.8)**	**2.4 (1.4-4.1)**	**4.6 (2.9-7.3)**
12-lead summed traditional	1.4 (1.1-1.8)	1.0 (0.6-1.5)	1.8 (1.3-2.6)	1.7 (1.2-2.3)	1.1 (0.6-1.8)	2.3 (1.5-3.4)
12-lead summed new	**1.8 (1.2-2.7)**	**1.5 (0.8-2.7)**	**2.1 (1.2-3.6)**	**2.3 (1.5-3.5)**	**1.0 (0.4-2.4)**	**3.7 (2.2-6.2)**
12-lead summed x QRS traditional	1.8 (1.4-2.2)	1.7 (1.2-2.4)	1.9 (1.4-2.5)	2.0 (1.5-2.6)	1.8 (1.2-2.6)	2.2 (1.5-3.2)
12-lead summed x QRS new	**2.0 (1.4-2.9)**	**1.7 (0.9-2.9)**	**2.4 (1.5-3.8)**	**2.6 (1.8-3.8)**	**2.1 (1.2-3.8)**	**3.0 (1.8-5.0)**

New: age- and sex specific normal limits Rotterdam Study, * adjusted for age and sex, ** adjusted for age

Table 4. Age- and sex-adjusted hazard ratios for heart failure and cardiovascular mortality by ECG-LVH partition value, stratified by age.

	Heart failure			Cardiovascular mortality		
LVH partition value	55-64 years N° of cases: 52	65-74 years N° of cases: 167	75+ years N° of cases: 213	55-64 years N° of cases: 32	65-74 years N° of cases: 98	75+ years N° of cases: 145
Sokolow-Lyon traditional	1.9 (0.8-4.3)	2.1 (1.4-3.3)	1.7 (1.1-2.5)	2.7 (1.1-6.7)	1.4 (0.7-2.7)	2.0 (1.3-3.1)
Sokolow-Lyon new	**2.6 (1.0-7.3)**	**1.8 (1.0-3.3)**	**2.2 (1.4-3.4)**	**6.0 (2.3-15.7)**	**1.1 (0.4-3.0)**	**2.6 (1.6-4.1)**
Sokolow-Lyon x QRS traditional	2.5 (1.2-5.5)	2.6 (1.8-3.9)	2.5 (1.8-3.4)	4.5 (2.0-10.2)	2.1 (1.2-3.6)	2.6 (1.8-3.8)
Sokolow-Lyon x QRS new	**2.4 (0.9-6.8)**	**2.2 (1.3-3.8)**	**2.1 (1.4-3.2)**	**5.6 (2.2-14.7)**	**2.4 (1.2-4.7)**	**2.2 (1.4-3.5)**
Cornell traditional	1.8 (0.5-5.7)	2.2 (1.3-3.5)	1.9 (1.3-2.6)	2.1 (0.5-9.0)	2.6 (1.5-4.6)	1.5 (1.0-2.2)
Cornell new	**1.7 (0.5-5.4)**	**2.4 (1.5-4.0)**	**2.7 (1.6-4.3)**	**3.7 (1.3-10.5)**	**3.1 (1.7-5.4)**	**2.1 (1.2-3.8)**
Cornell x QRS traditional	2.3 (1.1-4.7)	2.3 (1.6-3.2)	1.9 (1.4-2.5)	3.6 (1.6-8.0)	2.7 (1.7-4.2)	1.8 (1.2-2.6)
Cornell x QRS new	**2.0 (0.6-6.3)**	**3.0 (1.8-4.8)**	**2.8 (1.7-4.4)**	**7.3 (3.0-17.7)**	**4.6 (2.8-7.8)**	**2.0 (1.1-3.7)**
12-lead summed traditional	1.8 (0.8-4.0)	1.5 (0.9-2.3)	1.3 (0.9-1.8)	3.1 (1.3-7.4)	1.4 (0.8-2.5)	1.7 (1.1-2.5)
12-lead summed new	**2.2 (0.7-6.9)**	**2.3 (1.2-4.2)**	**1.4 (0.8-2.6)**	**2.4 (0.6-10.1)**	**2.2 (1.03-4.8)**	**2.3 (1.3-4.1)**
12-lead summed x QRS traditional	2.1 (1.1-4.0)	2.1 (1.5-3.0)	1.6 (1.1-2.1)	6.6 (3.3-13.3)	2.1 (1.3-3.2)	1.5 (1.1-2.2)
12-lead sum x QRS new	**1.5 (0.4-6.3)**	**2.0 (1.1-3.5)**	**2.1 (1.3-3.4)**	**7.2 (2.8-18.8)**	**3.4 (1.9-6.0)**	**1.7 (1.0-3.1)**

Prognosis based on partition values for ECG-LVH

General characteristics of the 5740 subjects that were included to study the risk of cardiovascular mortality with ECG-LVH partition values are given below. Mean age of these subjects was 69 years and 60% was female. In total, 10% had diabetes mellitus, 6% had a history of myocardial infarction, 3% had a history of heart failure and 33% had hypertension. Coronary artery bypass surgery and coronary angioplasty had been performed in 2% and 1% of participants, respectively. The number of subjects with ECG-LVH varied greatly with the use of the traditional cut-off points: Sokolow-Lyon voltage: 452, Sokolow-Lyon voltage-duration product: 503, Cornell voltage: 511, Cornell voltage-duration product: 808, 12-lead summed voltage: 598, and 12-lead summed voltage-duration product: 865. Numbers for the age- and sex-specific normal limits were much lower and showed less variation: Sokolow-Lyon voltage: 250, Sokolow-Lyon voltage-duration product: 266, Cornell voltage: 259, Cornell voltage-duration product: 253, 12-lead sum voltage: 212 and 12-lead sum voltage-duration product: 247. A total of 275 subjects died of cardiovascular disease. In the population of 5575 subjects used to evaluate the association between ECG-LVH and heart failure, 432 cases of incident heart failure occurred during follow-up (mean follow-up 6.8 years, SD 2.1 years).

Hazard ratios of heart failure and cardiovascular mortality are presented in table 3 for the various ECG-LVH partition values. Overall, ECG-LVH was significantly associated with heart failure and cardiovascular mortality, regardless of the cut-off point used. Except for the Sokolow-Lyon voltage-duration product, for every voltage equation the presence of LVH defined with our normal limits yielded higher HRs of incident heart failure and cardiovascular mortality than LVH according to the traditional partition values. Differences were more pronounced for the Cornell voltage and its duration product and were larger in cardiovascular mortality than in heart failure. LVH according to the new normal limits for the Cornell voltage-duration product showed the strongest association with both outcomes (HR heart failure: 2.8, 95%CI 2.0-3.9; HR cardiovascular mortality: 3.4, 95%CI 2.4-4.8).

Stratification by sex showed that age-adjusted hazard ratios for LVH according to the new partition values compared to LVH defined by the traditional values were increased mainly in women and that differences in men were modest (table 3). In women, the association between ECG-LVH and heart failure or cardiovascular mortality was generally stronger than in men. Stratification by age demonstrated small differences in the magnitude of the hazard ratios for ECG-LVH between partition values per age group (table 4). However, numbers were small and hence confidence intervals wide, especially in the youngest age-category.

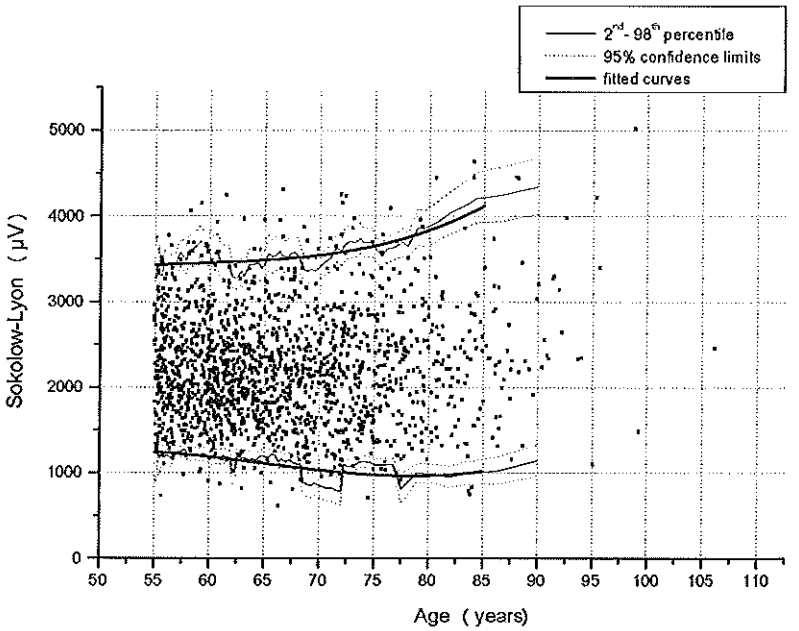

Figure 1a. Age-dependent curve of normal limits for Sokolow-Lyon voltage criteria in women. Graph represents 2nd and 98th percentile with 95% confidence interval and fitted curves.

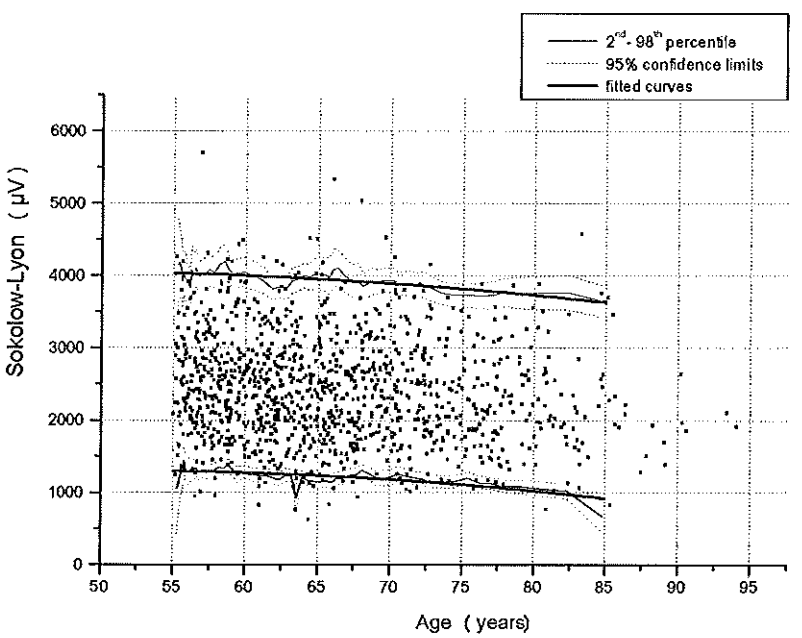

Figure 1b. Age-dependent curve of normal limits for Sokolow-Lyon voltage criteria in men. Graph represents 2nd and 98th percentile with 95% confidence interval and fitted curves.

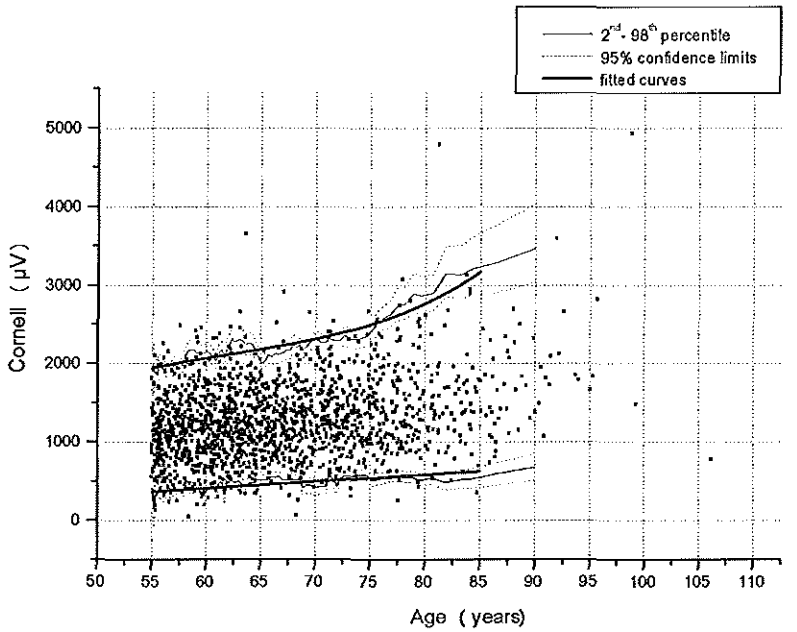

Figure 2a. Age-dependent curve of normal limits for Cornell voltage criteria in women. Graph represents 2nd and 98th percentile with 95% confidence interval and fitted curves.

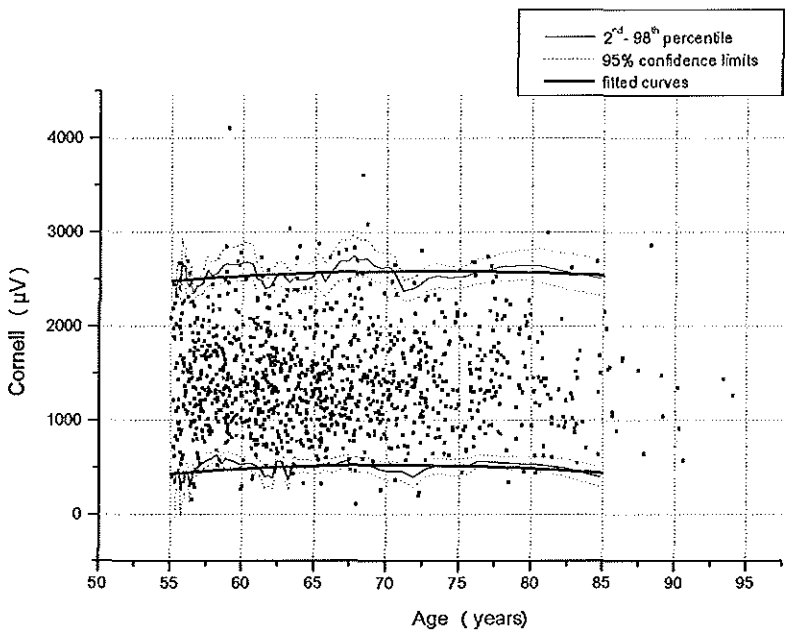

Figure 2b. Age-dependent curve of normal limits for Cornell voltage criteria in men. Graph represents 2nd and 98th percentile with 95% confidence interval and fitted curves.

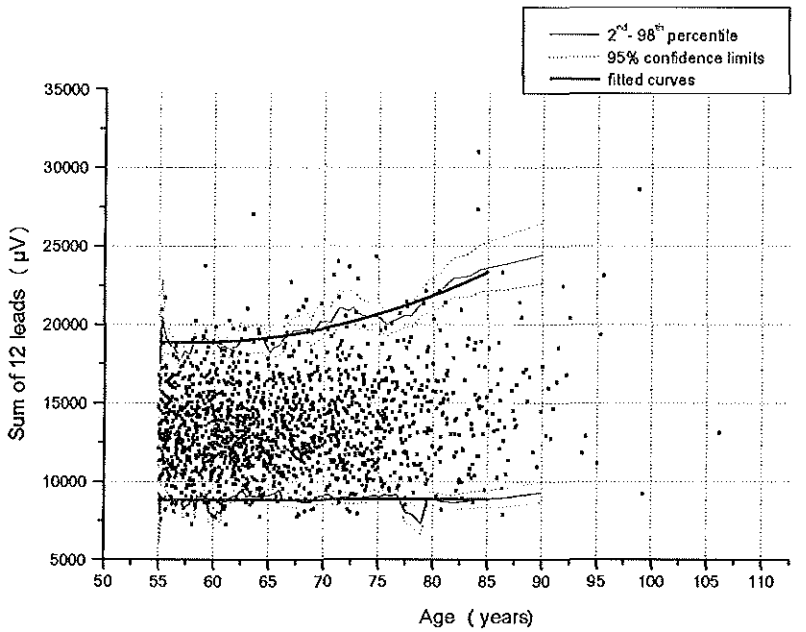

Figure 3a. Age-dependent curve of normal limits for sum of 12 leads voltage criteria in women. Graph represents 2nd and 98th percentile with 95% confidence interval and fitted curves.

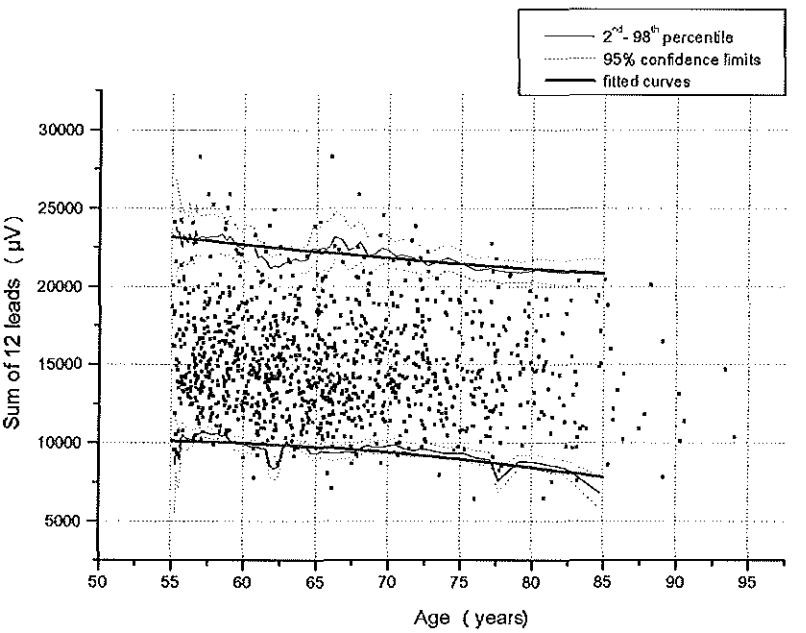

Figure 3b. Age-dependent curve of normal limits for sum of 12 leads voltage criteria in men. Graph represents 2nd and 98th percentile with 95% confidence interval and fitted curves.

Discussion

We established age- and sex-specific normal limits for three voltage equations that are frequently used to define ECG-LVH, and for their voltage-duration products, in a healthy elderly population. The new reference values for ECG-LVH were consistently higher than the traditional partition values, except for the Cornell voltage criteria in men. We found that 98th percentiles were lower in women than in men up to the age of 75 years. Age trends in amplitudes, and thus in normal limits, differed between sexes. In women, limits for every equation increased with age. In contrast, Sokolow-Lyon- and 12-lead summed voltage and voltage-duration products decreased with age in men, whereas the Cornell equations were not substantially influenced by age. We also studied associations between ECG-LVH, according to the different criteria and partition values, and incident heart failure and cardiovascular mortality. LVH as evidenced by ECG was significantly associated with both heart failure and cardiovascular mortality for all criteria and cut-off points. The strongest association was seen for the Cornell voltage-duration product defined according to the new normal limits. Except for the Sokolow-Lyon voltage-duration product, all age- and gender-specific normal limits established in the present study showed stronger associations between ECG-LVH and both cardiovascular outcomes than the traditional partition values. The differences in relative risks were more pronounced in women than in men.

As the aging process itself causes changes in ECG amplitudes and QRS duration, even in the absence of cardiovascular disease, a normal ECG in an elderly subject differs from that in a younger individual [9]. It has been demonstrated that aging increases the probability of a false-positive diagnosis of ECG-LVH [11]. Therefore, it is essential to define cut-off points for ECG-LVH that, besides sex, are also dependent on age. However, normal reference values for the detection of ECG-LVH in the elderly have not been determined so far. Although ECG amplitudes are generally expected to decrease with age, it has been shown previously that this does not apply to all voltages [10, 26, 27]. These studies described similar differences in age trends between men and women as we found in our study and their results also suggest that a partition value of 2800 μV for the Cornell voltage in men may be excessively high.

Besides the fact that most methods utilize fixed criteria to detect ECG-LVH, the three voltage criteria we assessed in this study have mainly been developed and evaluated in clinical populations. Also, the study populations that were used to develop these voltage criteria for ECG-LVH included, if any, only a limited number of elderly subjects, whereas our study cohort included a large number of elderly individuals. Moreover, the validity of ECG criteria for LVH has largely been tested against the echocardiogram as a gold standard. The correlation between left ventricular mass determined with the ECG and the echocardiogram is modest, however, and sensitivity of the ECG for detection of LVH on the echocardiogram is low [28]. A more useful method to validate ECG criteria for LVH might be to assess their prognostic ability. Therefore, we used heart failure incidence and cardiovascular

mortality as outcome measures in our study. Another advantage of our study is the use of a validated computer program (MEANS) to determine ECG amplitudes instead of manual ECG assessment. This reduces measurement error and intraobserver variability.

A priori, we chose the 98th percentile of ECG amplitudes as the upper limit of normal, which is a commonly used cut-off point for the determination of reference values in normal populations. However, other cut-off points are conceivable. Our choice for using the 98th percentile makes our age- and sex-specific normal limits more specific than, for example, 95th percentile cut-off points. Therefore, only the more serious cases of LVH are detected. Cardiovascular disease risks are, however, already elevated with the use of less specific limits for LVH, albeit probably at the expense of more false positive misclassification. Ultimately, the choice for a cut-off value for ECG-LVH will depend on the preference for the a priori magnitude of baseline risk.

Because of the uncertainty in LVH prevalence estimates, which occurs when traditional dichotomous ECG criteria are used, multivariate continuous ECG models for the estimation of echocardiographic left ventricular mass or the risk of cardiovascular disease have been introduced, which include factors such as age, sex, race and body mass index [29-31]. These algorithms perform better in estimating echocardiographic mass or predicting cardiovascular risk than voltage criteria alone. However, the application of these models is limited in a clinical setting as they are more complicated to use and more difficult to interpret than simple cut-off points for ECG-LVH. Moreover, a clinical diagnosis of LVH is not only dependent on criteria of high voltage, but also includes the morphology of the QRS complex, ST-T abnormalities, QRS-axis, left atrial hypertrophy and QRS duration [32].

Our study is limited by the predominantly Caucasian nature of our study population. ECG-amplitudes that are used in equations for LVH are known to differ significantly between human races [10, 27, 29]. Consequently, our results are not generalizable to non-white populations. Although we studied a large cohort of elderly individuals, the number of 'apparently healthy' subjects over 75 years old was still somewhat limited, especially in men, and therefore in this age group normal limits are less accurate. We presented age- and sex-specific normal limits in tables and in plots. Although the tabular presentation is easier for use in clinical practice, one should be aware that the tabulated values are estimates for the median age in age groups and that an age effect within these groups may still be present. Therefore, the continuous age-dependent curves are preferred for computerized determination of ECG-LVH.

In summary, ECG amplitudes are significantly associated with age and sex. Also, age-trends in amplitudes differ between men and women. Therefore, the traditional partition values for ECG-LVH voltage criteria may not adequately distinguish between physiology and disease in elderly individuals. Despite its low sensitivity for the detection of LVH, the ECG is widely used in clinical practice and in large clinical and observational studies because of its low costs, simplicity, greater availability and high reproducibility. Therefore, age- and

sex-specific cut-off points for ECG-LVH may be of great clinical importance. We provide age- and sex-specific normal limits for three commonly used voltage equations and their voltage-duration products to overcome the lack of proper reference values for ECG-LVH in normal elderly individuals. ECG-LVH detected with these normal limits was associated with both heart failure and cardiovascular mortality for all voltage criteria. Associations were strongest for ECG-LVH according to the new normal limits for the Cornell voltage-duration product.

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Chapter 3

Pharmacoepidemiology

3.1

Antihypertensive treatment is associated with improved left ventricular geometry. The Rotterdam Study

Abstract

Purpose: Left ventricular hypertrophy (LVH) increases the risk of cardiovascular disease. We evaluated the association between antihypertensive therapy and echocardiographically determined LVH.

Methods and results: The Rotterdam Study is a population-based prospective cohort study among 7,983 participants aged 55 years or over. Echocardiography was performed in 2,823 participants. The study population consisted of 740 participants with grade 1 hypertension or antihypertensive monotherapy, without heart failure. Of these, 646 had an adequate echocardiogram for analysis of relative wall thickness and 642 for left ventricular mass index. Participants were followed from January 1, 1991 until the date of echocardiography, between September 1992 and June 1993. Outcome measures were defined as being in the highest gender-specific quintile of left ventricular mass index and as having a relative wall thickness higher than 0.43. A Cox regression model with duration of use of antihypertensives defined as time-dependent covariates was used for data-analysis. Antihypertensive treatment lowered the risk of increased left ventricular mass index (RR 0.6, 95%CI 0.4-0.9). ACE-inhibitors, diuretics, and β-blockers all showed a risk reduction. Use of antihypertensives was also associated, although non-significantly, with a decrease of high relative wall thickness (RR 0.8, 95%CI 0.6-1.0). ACE-inhibitors, β-blockers, and calcium antagonists showed similar risk reductions, while diuretics seemed to increase the risk, possibly by reducing left ventricular end diastolic diameter.

Conclusions: The use of antihypertensive drugs is associated with a decreased risk of echocardiographically determined LVH in a population-based setting.

Introduction

Left ventricular hypertrophy (LVH) is associated with an increased risk of cardiovascular disease, even after adjustment for major risk factors, such as hypertension [1, 2]. Depending on the method of indexation of left ventricular mass (LV mass) and the partition values used, echocardiographically determined LVH is present in 42%-77% of patients with hypertension [3]. Several factors influence LV mass, of which blood pressure, age, gender, body mass index, diabetes mellitus, and pre-existing cardiovascular disease are most important [4-6]. Cardiovascular morbidity and mortality increase as the geometric pattern of the left ventricle changes from normal to concentric remodelling (increased LV wall thickness), eccentric LVH (increased LV mass) and concentric LVH (increased LV mass and increased LV wall thickness) [7].

Regression of LVH is associated with decreased morbidity and mortality in essential hypertension. Findings indicate that patients who fail to achieve reduction in LV mass after antihypertensive treatment are at higher risk for subsequent cardiovascular events [8, 9]. Although there have been a number of clinical trials reporting regression of LVH after antihypertensive treatment [10], its effect under everyday circumstances remains unclear. The relative efficacy of the different classes of antihypertensive drugs also remains a matter of controversy [11]. In cross-sectional studies, chronic antihypertensive therapy was not associated with normalisation of LV geometry, nor were there any differences between classes of antihypertensive drugs [12, 13].

The objective of this study was to evaluate the association between antihypertensives and echocardiographically determined LVH in a population of community-dwelling elderly.

Methods

Setting

The Rotterdam Study is a population-based prospective cohort study. An outline of the study has been published previously [14]. The first survey started in 1990 and was completed in 1993. All inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, aged 55 years or over were invited to participate. Of the 10,275 eligible subjects, 7,983 gave informed consent (78%). Participants were visited at home for a standardised questionnaire and were subsequently examined at the research centre, where echocardiography was performed in a sample of 2,823 participants. The performance of cardiac ultrasound depended on the availability of ultrasound technicians. Other than a small subset of participants living in nursing homes, no participants were a priori excluded from receiving an ultrasound. In 19.7% (n=556), the echocardiographic registrations were considered inadequate for a reliable measurement of LV dimensions. These participants were more likely to be older,

had a higher body mass index, and more often used medication for chronic obstructive pulmonary disease [15]. In addition to medication history from computerised pharmacy records, information on the current use of medication and type of medication were also assessed during the home interview. The participants subsequently showed all their currently used medication at the research center, where a physician determined the indication for each drug. In case of blood pressure levels below the cutoff points, originally at baseline defined as a systolic blood pressure of 160 mm Hg or over, and/or a diastolic blood pressure of 95 mm Hg or over, and inconsistencies or missing values regarding indication, additional information was used to classify subjects as hypertensive. This additional information was obtained, first from the response to the question, "Have you ever been told by a doctor that you have hypertension?" and, second, from the response to the question, "Were you ever treated with drugs because of high blood pressure?" [16].

Study population

To be eligible for this study, at least 1 year of medication history before the date of echocardiography had to be available. Computerised pharmacy records were available of all participants of the Rotterdam Study as of January 1, 1991. Therefore, those individuals who underwent echocardiography before January 1, 1992 were excluded from this study (n=345).

Furthermore, the study population was restricted to participants who had at least grade 1 hypertension, and participants who used antihypertensive medication for the treatment of high blood pressure (remaining population: n=1015). Grade 1 hypertension was defined as a systolic blood pressure of 140-159 mm Hg, and/or a diastolic blood pressure of 90-99 mm Hg, according to World Health Organization guidelines [17]. Furthermore, subjects who had used multiple antihypertensive agents during the study period, either concomitantly or consecutively, were excluded (n=220). Patients with prevalent congestive heart failure at the time of the baseline interview or incident congestive heart failure during follow-up in the Rotterdam Study were also excluded (n=55). Of the remaining 740 echocardiograms, 646 (87.3%) were technically adequate for determination of relative wall thickness, and 642 (86.8%) for determination of LV mass index. Participants were followed as of January 1, 1991 until the date of echocardiography, which varied between September 1992 and June 1993.

Exposure definition

All prescriptions dispensed to participants by seven automated pharmacies in the study area were routinely stored in the database. We included as antihypertensive agents: ACE-inhibitors, diuretics, ß-blockers, calcium antagonists, and other antihypertensives (such as centrally acting antihypertensives). Participants were considered exposed if the date of the

echocardiography fell within the legend duration (prescription length) of an antihypertensive agent. The legend duration was calculated by dividing the total number of filled tablets/capsules of consecutive prescriptions of a drug by the prescribed daily number. We added a carry-over period of 3 months as we assumed that any protective effect on LV mass index and relative wall thickness would not immediately subside after discontinuation of an antihypertensive drug. A protective effect on both aspects of LV geometry was considered to require a duration of use of at least two months [18]. The duration of exposure on the date of echocardiography was divided into three mutually exclusive periods: 1) non-use; 2) duration of use of 2 months or less (induction period); 3) duration of use of more than 2 months.

Outcome definition

Two manifestations of LVH were studied. The first was defined as being in the highest gender-specific quintile of LV mass index, the second as having an increased relative wall thickness. Echocardiography was carried out with the participant in the partial left decubitus position using a 2.75-MHz transducer (Toshiba SSH-60A Nasuworks, Otawara, Japan). Two-dimensional imaging using parasternal long-axis views was performed to aid M-mode studies. Measurements of the left ventricle were performed at end-diastole, as defined by the onset of the QRS complex, according to the American Society of Echocardiography (ASE) recommendations [19]. LV mass was determined using the Devereux-modified ASE cube formula [20]: LV mass (grams) = $0.8x(1.04x[(LVED+IVS+LVPW)^3-(LVED)^3])+0.6$, where LVED = LV end diastolic diameter, IVS = interventricular septum thickness, and LVPW = LV posterior wall thickness. LV mass was indexed to body surface area and divided into gender-specific quintiles, which has been shown to provide good cardiovascular risk estimates [21]. Relative wall thickness (RWT) was calculated as RWT = 2 x LVPW/LVED [22]. Increased relative wall thickness was present when the ratio exceeded 0.43, according to previously published criteria [3].

Other variables

Information was gathered on several potential confounders such as age, gender, history of myocardial infarction, congestive heart failure, smoking (current/former/never), and body mass index. Diabetes mellitus was defined as the use of anti-diabetic medication, or as a random or post-load serum glucose level higher than 11.0 mmol/l [23].

Analysis

A Cox regression model with the duration of use of antihypertensive drugs defined as time-dependent covariates was used for data analysis. Non-use of the antihypertensive drug of interest served as reference. The associations were expressed as relative risks (RR) with 95% confidence limits. In the multivariate analyses we adjusted for age, gender, smoking, body mass index, diabetes mellitus, and history of myocardial infarction. We repeated the multivariate analyses after restricting the population to those subjects with hypertension defined as use of antihypertensive medication for the treatment of high blood pressure, or as having a systolic blood pressure of 160 mm Hg or over, or a diastolic blood pressure of 95 mm Hg or over (n=317) to see if we created more or less similar groups [16]. Additional analyses were performed comparing treated antihypertensive patients who had well-controlled hypertension (systolic blood pressure lower than 140 mm Hg and diastolic blood pressure lower than 90 mm Hg) with non-treated hypertensive patients, and comparing antihypertensive therapy in patients with refractory hypertension with non-treated hypertensive patients. All statistical analyses were carried out with the statistical software packages SPSS 9.0 and SAS 6.12.

Results

General characteristics

In table 1 general characteristics of participants who met the inclusion criteria are presented. The mean age of eligible participants was 66 years. There was a small majority of women (54%). The mean follow-up was 2.2 years. Mean systolic blood pressure was 154 mm Hg and mean diastolic blood pressure was 80 mm Hg. Mean LV mass index was 99 g/m^2 and mean relative wall thickness was 0.42. A history of myocardial infarction was present in 7% of participants, and 8% had diabetes mellitus. Of all participants 19% were current smokers, whereas 46% were former smokers. Antihypertensive drugs were used by 31% of participants; ß-blockers (14%) and diuretics (8%) were most frequently prescribed.

Left ventricular mass index

Table 2 represents the estimates of the association between the use of antihypertensive drugs and LV mass index. Since sample size was too small to render estimates in most of the drug exposure categories in the induction period, the relative risk for exposure in the first two months is only shown for the use of antihypertensives in general. The use of antihypertensive drugs decreased the risk of high LV mass index (RR 0.6, 95%CI: 0.4-0.9) after adjustment for confounding variables. Of the individual antihypertensives, only diuretics showed a significant reduction in the risk of elevated LV mass index after

the induction period. Restriction of the analyses to subjects with more stringent criteria for hypertension (systolic blood pressure ≥ 160 mm Hg, and/or diastolic blood pressure ≥ 95 mm Hg, and/or antihypertensive drug use for the indication hypertension) revealed similar relative risk estimates (third column, table 2). Antihypertensive drug use in patients with well-controlled treated hypertension revealed a RR of 0.3 (95%CI 0.2-0.7) of being in the highest quintile of LV mass index as compared to non-treatment, while treatment in refractory hypertension revealed a RR of 0.8 (95%CI 0.5-1.2). Point estimates for LV mass index and relative wall thickness were similar in analyses restricted to participants without a prior history of myocardial infarction.

Table 1. General characteristics.

	Population (n=646)
Age (years)	66 (7.3)
Female	347 (54%)
Follow-up (years)	2.2 (0.2)
Body mass index (kg/m²)	26 (3.3)
Body surface area (m²)	1.9 (0.2)
Systolic blood pressure (mm Hg)	154 (16.6)
Diastolic blood pressure (mm Hg)	80 (10.5)
Left ventricular mass index (g/m²)	99 (25.7)
Relative wall thickness	0.42 (0.09)
Fractional shortening (%)	39 (7.5)
History of myocardial infarction *	47 (7%)
Diabetes mellitus †	49 (8%)
Smoking	
Current	121 (19%)
Former	295 (46%)
Never	230 (35%)
Use of antihypertensive drugs during study period:	198 (31%)
Diuretics	52 (8%)
ß-blockers	93 (14%)
Calcium antagonists	20 (3%)
ACE-inhibitors	29 (5%)
Other antihypertensives	4 (1%)

Values are means (SD) or numbers (%), * myocardial infarction according to general practitioner, cardiologist or ECG, † the use of anti-diabetic medication, or a random or post-load serum glucose level higher than 11.0 mmol/l.

Table 2. Association between LV mass index and use of antihypertensive agents.

	RR (95%CI) * Total population	RR (95%CI) * Sub-population †
All antihypertensives ‡		
≤ 2 months	0.2 (0.03-1.7)	0.2 (0.03-1.6)
>2 months	0.6 (0.4-0.9)	0.5 (0.3-0.8)
ACE-inhibitors §		
>2 months	0.6 (0.2-1.3)	0.5 (0.2-1.2)
Diuretics §		
>2 months	0.3 (0.1-0.8)	0.3 (0.1-1.0)
ß-blockers §		
>2 months	0.7 (0.4-1.2)	0.7 (0.4-1.2)
Calcium antagonists §		
>2 months	1.5 (0.7-3.2)	1.2 (0.5-2.7)

* Adjusted for age, gender, diabetes mellitus, smoking, history of myocardial infarction, and body mass index, † Subjects with hypertension defined as use of antihypertensive medication for the treatment of high blood pressure, or as having a systolic blood pressure of 160 mm Hg or over, or a diastolic blood pressure of 95 mm Hg or over, ‡ The use of any of the described antihypertensive drugs as monotherapy, § Because of low numbers for the individual antihypertensives in the induction period (≤ 2 months), only relative risks are given for use of more than 2 months.

Relative wall thickness

Table 3 represents the estimates of the association between the use of antihypertensive drugs and increased relative wall thickness. Treatment with antihypertensives in general was associated with a decrease in the risk of elevated relative wall thickness (RR 0.8; 95%CI 0.6-1.0), although this failed to reach statistical significance. ACE-inhibitors, β-blockers, and calcium antagonists showed similar reductions in risk of increased relative wall thickness, of which β-blockers were the only drug class with a significant association. The use of diuretics was associated with a higher risk of increased relative wall thickness, which was significant when restricting the analyses to the stricter criteria for the presence of hypertension. Mean LV end diastolic diameter tended to be significantly lower and mean LV posterior wall thickness tended to be similar with the use of diuretics, as compared to non-treatment (independent samples t-test: p=0.01, respectively p=0.68). Again, restriction to more stringent criteria for hypertension revealed similar relative risk estimates for the association between elevated relative wall thickness and antihypertensive therapy (table 3). Antihypertensive drug use in patients with well-controlled treated hypertension revealed a RR of 0.5 (95%CI 0.3-0.8) of increased relative wall thickness as compared to non-treatment, while treatment in refractory hypertension yielded a RR of 1.0 (95%CI 0.7-1.3).

Table 3. Association between relative wall thickness and use of antihypertensive agents.

	RR (95%CI)* Total population	RR (95%CI)* Sub-population †
All antihypertensives ‡		
≤ 2 months	0.7 (0.3-1.8)	0.6 (0.2-1.8)
>2 months	0.8 (0.6-1.0)	0.8 (0.6-1.1)
ACE-inhibitors §		
>2 months	0.7 (0.4-1.2)	0.7 (0.4-1.3)
Diuretics §		
>2 months	1.6 (1.0-2.5)	1.8 (1.1-3.1)
ß-blockers §		
>2 months	0.6 (0.4-1.0)	0.6 (0.4-1.0)
Calcium antagonists §		
>2 months	0.7 (0.3-1.6)	0.8 (0.4-1.9)

* Adjusted for age, gender, diabetes mellitus, smoking, history of myocardial infarction, and body mass index, † Subjects with hypertension defined as use of antihypertensive medication for the treatment of high blood pressure, or as having a systolic blood pressure of 160 mm Hg or over, or a diastolic blood pressure of 95 mm Hg or over, ‡ The use of any of the described antihypertensive drugs as monotherapy, § Because of low numbers for the individual antihypertensives in the induction period (≤ 2 months), only relative risks are given for use of more than 2 months.

Discussion

The main findings of this study are that the use of antihypertensive drugs decreases the risk of echocardiographically determined LVH. Of all antihypertensive drugs, diuretics seemed to be most strongly associated with decreased LV mass index. A decrease in the risk of high relative wall thickness was observed with the use of ACE-inhibitors, β-blockers, and calcium antagonists, although with low statistical power. The use of diuretics seemed to increase the risk of elevated relative wall thickness. This may have been due to a tendency for LV end diastolic diameter to be lower and LV posterior wall thickness to be similar with the use of diuretics, as compared to no treatment. However, as this was not part of our prior hypothesis and numbers were small, these results should be interpreted with caution.

Since all data on exposure and outcome were recorded similarly for all subjects without prior knowledge of our study hypotheses, selection bias and information bias are not likely to have influenced the results of this study. Non-differential misclassification could have occurred due to inaccurate assumptions about carry-over effects. Little is known about the continued effect of antihypertensive agents on LVH. Washout periods lasting more than 2-6 months have been suggested before measurable redevelopment of hypertrophy occurs [24]. As only one echocardiography was carried out, the association between antihypertensive drugs and alterations of LV geometry over time could not be evaluated. An important problem in observational studies on drug effects is confounding by indication, due to the difficulty of ensuring comparability of prognosis across different treatment groups. In theory, the effect of confounding by indication can be completely removed by adjustment for all patient

characteristics that form the basis of the indications for treatment [25]. Statistical adjustment will be incomplete, however, if not all relevant determinants of the indication for treatment are known. Potential confounding by indication in this study was dealt with by including only patients with hypertension, making treatment groups more similar in terms of prognosis. In addition, patients with heart failure and users of multiple antihypertensive drugs were excluded, because of the possibility of substantial residual confounding in these groups. In the analysis, we adjusted for several potential confounders that are known to be associated with both exposure and LVH. Although blood pressure on the date of echocardiography was known, we considered this as a potential intermediate for which we should not adjust. Moreover, adjustment for systolic blood pressure did not change our results. Also, analyses in a population with more severe hypertension were performed, which showed similar risk estimates. Any residual confounding by indication would have led to higher relative risk estimates, and not to a protective effect as seen in this study. Residual confounding may have resulted from the prescription of antihypertensive drugs for alternate indications. Since we only selected participants with hypertension, and verified the indication of antihypertensive treatment, however, it seems fair to assume that the antihypertensive drugs were indeed prescribed for this indication. Also, specific patient or physician characteristics may have influenced the decision to treat patients for increased blood pressure. This may have led to a negative association if patients with less severe hypertension or patients who were more easy to control were preferentially treated. In this population, however, a large proportion of participants was unaware of having hypertension and their elevated blood pressure was discovered at the baseline examination in the Rotterdam Study [16]. Also, in the time of the baseline examination, different criteria for hypertension were used, and therefore patients with mild hypertension according to the new WHO criteria were probably not treated. These characteristics would have led to higher relative risk estimates, and do not explain the negative association seen in this study.

In several clinical trials regression of echocardiographically determined LVH has been reported following treatment with a wide range of antihypertensive agents, including ACE-inhibitors, diuretics, β-blockers, and calcium antagonists [10]. Many of these trials were of small size and short follow-up. More recent large randomized clinical trials have suggested that diuretics and ACE-inhibitors are most effective in reducing LV mass [26-29]. An advantage of our study is that it enabled us to determine the effectiveness of antihypertensive drugs in a general population of community dwelling elderly. So far as we are aware, population-based studies on antihypertensives and echocardiographically determined LVH are rare. There have been some cross-sectional studies, in which chronic antihypertensive therapy was not associated with normalisation of LV geometry [12, 13].

Reduction of cardiac afterload as a result of blood pressure lowering reduces myocardial wall stress and leads to a decrease in muscle mass. However, blood pressure reduction alone may not automatically lead to a decrease in LV mass [30]. Chronically increased

blood pressure results in concentric remodeling, while chronic volume overload on the left ventricle produces eccentric hypertrophy [31]. Both forms of hypertrophy are accompanied by complex changes in gene programming, including genes encoding components of hormonal pathways (e.g. renin-angiotensin system). In addition, variable expression occurs in genes that modify intracellular ion homeostasis, and key parasympathetic and sympathetic receptors are downregulated [32]. Circulating and tissue noradrenaline as well as increased production of angiotensin II participate not only in the production and maintenance of the increased pressure load on the ventricle, but also have a direct effect on myocyte hypertrophy, and hypertrophy and hyperplasia of nonmyocyte cells [31]. Blockade of the sympathetic system with a ß-blocker or blockade of formation of angiotensin II with an ACE-inhibitor may therefore contribute to the reversal of LVH. In this study, both agents were associated with a reduction in risk of LVH.

Despite activation of neurohormonal pathways by diuretics their hemodynamic effects seem to be more important for regression of LVH. The observed relative increase in wall thickness, presumably reflecting a decrease in transverse dimensions of the heart has been reported earlier with the use of diuretics, and may result from adaptive changes of cardiac muscle tissue around a smaller left ventricle [33]. With sustained therapy, short-acting calcium antagonists and long-acting dihydropyridines may promote sympathetic activation [34]. This might explain the lack of an association between calcium antagonists and LVH as observed in this study, besides low statistical power. Later introduced long-acting preparations do not have this characteristic and may have more beneficial effects on LV geometry [35].

In conclusion, the use of antihypertensive drugs is associated with a decreased risk of echocardiographically determined LVH in a population-based setting.

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3.2

Nonsteroidal anti-inflammatory drugs and heart failure

Abstract

Heart failure constitutes an increasing public health problem, because of its growing incidence and prevalence, poor prognosis, and high hospital (re)admission rates. Myocardial infarction is the underlying cause in the majority of cases, followed by hypertension, valvular heart disease and idiopathic cardiomyopathy. Nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the enzymes cyclo-oxygenase (COX) 1 and 2, have been associated with the occurrence of symptoms of heart failure in several case reports and quantitative studies, mainly in patients with a history of cardiovascular disease or left ventricular impairment. NSAIDs may impair renal function in patients with a decreased effective circulating volume by inhibiting prostaglandin synthesis. Consequently, water and sodium retention, and decreases in renal blood flow and glomerular filtration rate may occur, affecting the unstable cardiovascular homeostasis in these patients. In patients with pre-existing heart failure, this may lead to cardiac decompensation. Putative renal-sparing NSAIDs, such as COX-2 selective inhibitors have similar effects on renal function as the traditional NSAIDs, and can likewise be expected to increase the risk of heart failure in susceptible patients. NSAIDs are frequently prescribed to elderly patients, who are particularly at risk for the renal adverse effects. If treatment with NSAIDs in high risk patients cannot be avoided, intensive monitoring and patient education is important.

Introduction

Heart failure constitutes a major public health problem. The prevalence in the general population is estimated to range from 0.3% to 2.0%, and increases considerably with age [1]. It approximately doubles with every additional decade of life [2]. Estimates of the cumulative incidence per year follow a similar pattern and vary from 0.1% to 0.2% in middle aged men and women [1]. In the last two decades, hospital admission rates for heart failure have increased steadily [3]. Readmissions for exacerbating heart failure occur frequently, especially in the elderly. The incidence of heart failure increases over calendar time, which is most likely explained by improved survival following myocardial infarction, and increased longevity in industrialised countries. Prognosis remains poor, with a cumulative 5-year mortality of over 40% [4]. Because of the increasing number of patients with heart failure, and its high morbidity and mortality, it is important to identify potentially preventable risk factors for the occurrence of heart failure.

Heart failure can result from any structural or functional disorder that impairs the ability of the left ventricle to fill with or eject blood [5]. Diagnosis is complex and relies on clinical judgement based on history, physical examination and imaging procedures, such as echocardiography. Characteristically, patients present with signs of breathlessness or fatigue, either at rest or during exertion, pulmonary crepitations, or peripheral edema [6]. Symptom-free periods are often alternated with periods of exacerbating symptoms. Heart failure develops when compensatory hemodynamic and neurohormonal mechanisms of the injured heart are exhausted or overwhelmed [7]. As coronary heart disease is the underlying cause in the majority of cases, most patients have evidence of left ventricular systolic dysfunction. However, nearly all patients also exhibit diastolic impairment at rest [6]. This report will focus mainly on heart failure due to systolic dysfunction. The most frequent non-ischemic causes of heart failure are hypertension, valvular disease and idiopathic dilated cardiomyopathy [5].

Several drugs have demonstrated to be able to induce or exacerbate heart failure [8]. In a number of reports, the occurrence of heart failure was attributed to the use of nonsteroidal anti-inflammatory drugs (NSAIDs) [9-13]. NSAIDs are used extensively in the general population for rheumatologic conditions, especially in elderly patients. Nonrheumatic indications include acute and chronic pain, biliary and ureteric colic, and dysmenorrhoea [14]. In the United States, 70 million NSAID prescriptions are dispensed each year [15]. Worldwide these agents account for approximately 2.5% of all prescription dollars, corresponding with 6.8 billion US dollars spent annually [16]. Most patients who use therapeutic dosages for a short period of time tolerate NSAIDs well [17]. However, a considerable subset of individuals develops adverse effects involving the gastrointestinal, renal and cardiovascular systems.

In this report, we will discuss recent findings on the association between the use of NSAIDs and the occurrence of heart failure. Consecutively, we will focus on current knowledge of

the pathophysiology of heart failure, the mechanism of action and renal effects of NSAIDs, potential "renal-sparing" NSAIDs, cyclo-oxygenase-selectivity, and quantitative studies on the association between NSAIDs and heart failure.

Pathophysiology of heart failure

Left ventricular dysfunction begins with some form of injury to the myocardium, for example an acute myocardial infarction, which results in loss of functioning myocardial cells. In response, hemodynamic and neurohormonal mechanisms are activated to preserve cardiac function [18]. The decreased capacity of the left ventricle to empty during systole increases diastolic wall tension in the non-injured parts of the heart. The left ventricle responds by enhancing its contraction, following the Frank-Starling curve. Additionally, the sympathetic nervous system is activated, resulting in increased force and frequency of contraction. Both compensatory mechanisms also lead to a remarkable increase in internal wall stress during diastole. In response, synthesis of myofibrillar proteins is stimulated, resulting in increased wall thickness and a subsequent reduction of ventricular wall stress and dilatation, which reduces energy expenditure [7]. Moreover, an increase in diastolic wall stress in the atria suppresses the sympathetic nervous system [19] and leads to the release of atrial (A-type) natriuretic peptide. Also, B-type natriuretic peptide and C-type natriuretic peptide are released, respectively by the ventricular myocardium in response to elevations of end-diastolic pressure and volume, and by endothelial cells in response to shear stress [20]. The natriuretic peptides improve the loading conditions on the heart through their diuretic, natriuretic and vasodilator properties. In this way, a delicate hemodynamic balance is achieved, which restores cardiac function [7]. Long-term activation of these mechanisms, however, diminishes their favourable physiologic effects and results in progressive deterioration of ventricular function. As cardiac output declines, systemic perfusion is maintained by peripheral vasoconstriction and sodium retention [21]. Catecholamines, angiotensin II and vasopressin act to increase systemic blood pressure and expand intravascular volume, while prostaglandins and natriuretic peptides limit the pressor, antinatriuretic and antidiuretic effects of these vasoconstrictor systems. Water and salt retention result mainly from direct and indirect effects of the renin-angiotensin system on glomerular and tubular function.

Nonsteroidal anti-inflammatory drugs

Mechanism of action

NSAIDs are a structurally diverse group of drugs containing the salicylates, pyrazoles, oxicams, fenamates, arylacetic acids and arylpropionic acids [22]. They exert analgesic, antipyretic, anti-inflammatory and platelet-inhibitory actions. Inhibition of prostaglandin synthesis, through inhibition of cyclo-oxygenase (COX), is the major mechanism of action [23]. This enzyme metabolises arachidonic acid to prostaglandin G_2. This is reduced to prostaglandin H_2, the precursor of various other prostaglandins, prostacyclins and thromboxanes. COX consists of at least two different isoenzymes. COX-1 is constitutively expressed and regulates functions such as gastric cytoprotection and vascular homeostasis [24]. COX-2 is predominantly present throughout all stages of the inflammatory response, but is also constitutively expressed in parts of the kidney [16]. Most NSAIDs inhibit both COX-1 and COX-2, but in recent years selective COX-2 inhibitors, such as celecoxib and rofecoxib, have been developed.

Effects on renal function

At present, it is believed that the central mechanism by which NSAIDs influence cardiovascular homeostasis follows from their effect on renal function. Potential prostaglandin-mediated renal adverse effects of NSAID therapy are summarized in figure 1. Prostaglandins are known to exert their effects at the location at which they are synthesised and are therefore referred to as autocoids. The major sites of COX in the kidney comprise the arterial tree, including the afferent and efferent arterioles, the glomerulus, and the collecting tubule. Furthermore, interstitial cells adjacent to the thick ascending limb of Henle's loop and in the medulla are rich in COX [25]. Under normal, euvolemic conditions, prostaglandins do not play a major role in the maintenance of renal and glomerular circulation [26]. However, in settings of decreased effective circulating volume, such as heart failure, hepatic cirrhosis, chronic renal insufficiency, and dehydration, prostaglandin production is enhanced to preserve renal perfusion [27]. Whenever renal blood flow is compromised, the kidneys respond by releasing two types of hormones, angiotensin II and prostaglandins [21]. Angiotensin II decreases renal blood flow and leads to sodium and water retention, in part by stimulating the release of vasopressin. The principal renal prostaglandins, prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2), increase renal blood flow and enhance the excretion of sodium and water, partly by opposing the actions of vasopressin. Activation of one hormonal system immediately triggers the release of counterregulatory factors in the kidney. Although these two systems exert opposite effects, both act to preserve glomerular filtration rate [21]. Angiotensin II exerts a vasoconstrictor effect on the intrarenal efferent arterioles, while a vasodilator effect is exerted by prostaglandins on the afferent arterioles. Under these circumstances, inhibition of prostaglandin synthesis by an NSAID will lead to excessive vasoconstriction, with a

subsequent decline in renal blood flow and glomerular filtration rate [17]. Consequently, acute renal failure may occur.

Edema and sodium retention are the most common NSAID-associated adverse effects involving the kidney [27]. Although both have negligible consequences in healthy individuals, they may affect the unstable cardiovascular homeostasis in patients with heart failure. In case of renal hypoperfusion, a decrease in glomerular filtration rate results in increased water and electrolyte reabsorption in the proximal tubule. PGE_2 decreases sodium reabsorption at the thick ascending limb of the loop of Henle by inhibiting chloride transport. Inhibition of prostaglandin synthesis under these circumstances results in increased sodium, chloride and water reabsorption in the proximal convoluted tubule, and increased sodium and chloride absorption in the ascending limb of the loop of Henle. PGE_2 also antagonises the effect

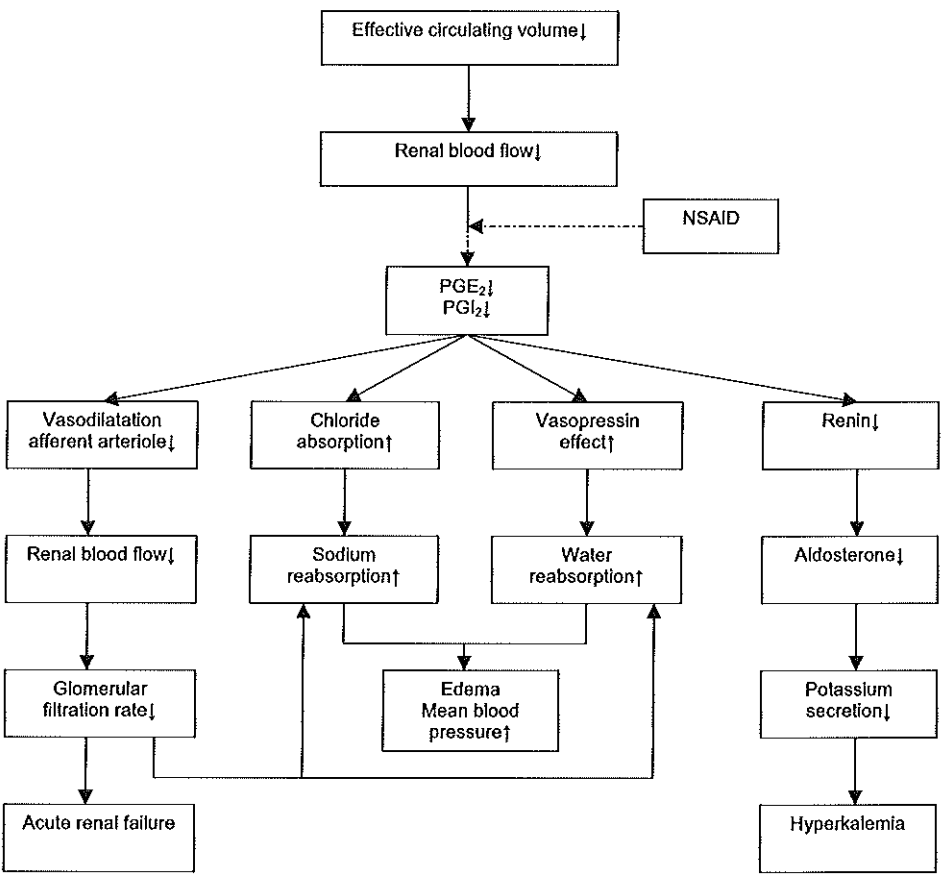

Figure 1. Prostaglandin-mediated renal adverse effects of NSAIDs with a potential effect on cardiovascular homeostasis. Effects of angiotensin II and natriuretic peptides A, B, and C, which are released in response to a decrease, respectively an increase in effective circulating volume, are not shown in this figure.

of vasopressin on the collecting tubule, hence reducing water reabsorption. Therefore, by blocking PGE_2, NSAIDs cause an increase in water absorption, a reduction in urinary volume and consequently, systemic fluid retention [26]. Water retention which is disproportionate to sodium retention may lead to the development of hyponatremia in some patients [28].

The antinatriuretic and vasoconstrictor effects of NSAIDs may also have important clinical implications with regard to blood pressure regulation. Several trials have studied the effect of NSAIDs on blood pressure. Results of a meta-analysis suggest that mean blood pressure is elevated by approximately 5 mm Hg in patients using these drugs [29]. The hypertensive effect in this analysis was most marked in hypertensive patients who were taking medication for their blood pressure. It has been shown that the use of NSAIDs decreases the antihypertensive effects of thiazides, loop diuretics, α-adrenergic blockers, β-adrenergic blockers, and angiotensin converting enzyme (ACE)-inhibitors [29, 30]. Moreover, in patients with severe volume depletion, NSAIDs may blunt the actions of diuretics and lead to severe fluid retention [31]. Renal effects of the combination of NSAIDs with ACE-inhibitors depend on pre-existing renal function [32]. Under baseline conditions, this combination has no overall effect on the kidneys. However, under conditions of renal hypoperfusion or renal impairment, the two drugs will interfere with the physiologic mechanisms that serve to protect glomerular filtration rate. Hence clinicians should avoid coprescribing an NSAID and an ACE-inhibitor under these circumstances.

Suppression of prostaglandin-mediated renin release by NSAIDs may lead to a state of hyporeninemic hypoaldosteronism, resulting in hyperkalemia and type IV renal tubular acidosis. The degree of hyperkalemia is usually mild, but cardiac arrest and even death may occur [33]. NSAID-induced hyperkalemia seldom occurs in the absence of other defects of potassium homeostasis. Patients at risk are, for example, users of potassium-sparing diuretics, β-blockers, and aldosterone antagonists. Especially insulin-dependent diabetics with renal dysfunction and patients with renal failure are at high risk [17].

Apart from the prostaglandin-mediated renal function abnormalities, NSAIDs are also associated with acute renal syndromes, such as nephrotic syndrome, acute interstitial nephritis, acute tubular necrosis, papillary necrosis, and acute glomerulonephritis [18, 21]. This is, however, relatively rare and outside the scope of this review.

Renal-sparing nonsteroidal anti-inflammatory drugs

Conventional renal-sparing nonsteroidal anti-inflammatory drugs

Efforts have been made to develop NSAIDs, which do not influence renal prostaglandin synthesis and thereby lack prostaglandin-mediated adverse effects on renal and cardiovascular homeostasis. Compared with other conventional NSAIDs, sulindac was reported to have a lower propensity for inhibiting renal prostaglandin synthesis and impairing renal function

[34-37]. Because the active sulphide metabolite of sulindac is inactivated by renal oxidative enzymes, this was thought to minimise the effect of the drug on local renal prostaglandin synthesis [34]. However, clinical studies have demonstrated that this drug is capable of inducing the same renal toxicity as other NSAIDs [38, 39]. Similarly, nabumetone has been postulated to have renal sparing properties [34-36, 40]. Like sulindac, nabumetone is a pro-drug, which is rapidly transformed in the liver to its active metabolite 6-methoxy-2-naphtylacetic acid (6-MNA). Before being excreted in the urine, 6-MNA is metabolized to inactive metabolites which are weak inhibitors of COX [34]. However, renal failure has been reported as a consequence of nabumetone use [41]. Overall, studies assessing whether nabumetone has renal-sparing properties do not provide a definitive answer to this question [33]. Current data strongly suggest that nabumetone and sulindac share the risks of adverse renal effects with other conventional NSAIDs. Therefore, use of these agents requires the same precautions in patients at risk of adverse renal effects during use of non-renal-sparing NSAIDs. Whether these effects may be less is currently unknown. Since most studies were small and carried out in patients with normal renal function, further studies are needed before conclusions can be drawn.

Cyclo-oxygenase-2 selective inhibitors

The COX-2 selective inhibitors, celecoxib and rofecoxib, were originally developed to reduce the incidence of gastrointestinal adverse effects associated with the use of conventional NSAIDs. Initially, it was assumed that these agents would also reduce nephrotoxicity, by inhibiting only COX-2, the inducible form of cyclo-oxygenase. However, renal impairment and heart failure due to treatment with COX-2 selective inhibitors have been suggested in several publications [42-45].

Although COX-1 is expressed constitutively and is involved in homeostasis while COX-2 is mainly induced during pathophysiologic processes, there seems to be a significant overlap between expression patterns and functions of these two isoforms [46]. Both are present in constitutive and inducible forms in the kidney [47-52]. The physiological roles of COX-1 and COX-2 are not fully understood. In the human renal cortex, COX-2 is predominantly expressed intraglomerularly in podocytes, suggesting a role in the regulation of glomerular hemodynamics through contraction of podocytes [53]. COX-2 expression decreases with salt depletion and increases with high-salt diet and dehydration [54-56]. Additionally, COX-2 expression is upregulated after treatment with angiotensin II inhibitors [57]. Based on the expression of COX-2 in the kidney, and the regulation of COX-2 by sodium intake and angiotensin II, it may be anticipated that COX-2 selective inhibitors exert similar effects on renal function as conventional NSAIDs. It has been suggested that COX-2 selective inhibitors spare glomerular filtration rate, which may be mediated primarily by COX-1, while effects on sodium excretion are similar to conventional NSAIDs [58, 59]. However, this hypothesis was

based on clinical studies in healthy individuals on a sodium-replete diet. Additional studies in healthy subjects on a sodium restricted diet have demonstrated significant decreases in glomerular filtration rate with the use of COX-2 selective inhibitors [60, 61]. Therefore, it appears that COX-2 selective NSAIDs have similar effects on renal function as conventional NSAIDs.

Data from clinical trials also suggest that rofecoxib and celecoxib have similar effects on blood pressure as the conventional NSAIDs [62]. With the exception of one study, which compared a half-maximal dose of celecoxib with a maximal dose of rofecoxib [63], trials comparing these two agents did not show significant differences in effects on blood pressure [62]. The incidence of edema with the use of COX-2 selective inhibitors corresponds with that of conventional NSAIDs [62, 64]. Currently, data are lacking on the risk of heart failure associated with the use of COX-2 selective inhibitors. Most clinical trials have been carried out in healthy subjects. Comparative studies of these agents in patients who are at risk for NSAID-induced heart failure are needed to evaluate their safety in susceptible individuals. Since the renal effects of COX-2 selective inhibitors appear to be similar to the effects of conventional NSAIDs, administration of these drugs in patients susceptible to heart failure should be carried out with caution.

Recently, it was suggested that the use of COX-2 selective inhibitors may be associated with an increased risk of cardiovascular events, one of the major risk factors for heart failure. In an analysis of clinical trials, Mukherjee et al. concluded that the use of rofecoxib increased the risk of cardiovascular events [65]. Their conclusion was mainly based on the results of the Vioxx Gastrointestinal Outcomes Research (VIGOR) trial, in which the gastrointestinal safety of rofecoxib was compared with naproxen [66]. In contrast, data from the Celecoxib Long-term Arthritis Safety Study (CLASS), comparing celecoxib with ibuprofen and diclofenac, did not support this association [64, 67]. Important differences in patient characteristics, concomitant use of aspirin, and conventional NSAID comparators may partially account for the different findings between the two large clinical trials [46]. A pooled analysis by Konstam et al of 23 phase IIb through V trials found no excess in the number of cardiovascular events with the use of rofecoxib as compared to placebo or NSAIDs other than naproxen [68]. The data did, however, indicate that use of naproxen was associated with a decreased risk of cardiovascular events relative to rofecoxib. Unlike conventional NSAIDs, COX-2 selective inhibitors have no effect on platelet derived thromboxane A_2 (TXA_2) production [59]. This thromboxane causes platelet aggregration and is a potent vasoconstrictor. However, COX-2 selective inhibitors do inhibit the production of vasodilatory and antiaggregatory PGI_2. It was therefore hypothesized that the increased risk of cardiovascular events with the use of rofecoxib in the VIGOR trial may be explained by tipping the balance in favour of prothrombotic eicosanoids [65]. However, it could not be excluded that a potential antithrombotic effect of naproxen could also explain the difference between rofecoxib and naproxen. Also, by inhibiting the inflammation processes, COX-2 selective inhibitors may

Table I. Observational studies on the association between current use of NSAIDs and heart failure.

First author, year of publication	Study design	Population characteristics, sample size	Outcome definition	RR/OR (95% CI)
Heerdink, 1998[74]	Cohort study	Elderly recipients of diuretics and NSAIDs, identified in community pharmacies, n=10519	First hospitalisation with a primary or secondary diagnosis of heart failure	1.8 (1.4-2.4)
Page, 2000[75]	Case control study	Patients admitted to the emergency department of a hospital, n=1023	Hospitalisation with a primary diagnosis of heart failure	2.1 (1.2-3.3)
Feenstra, 2000[76]	Cohort study	Elderly recipients of NSAIDs, with one hospitalisation for heart failure, identified in community pharmacies, n=559	Rehospitalisation with a primary diagnosis of heart failure	2.2 (1.4-3.4)
Merlo, 2001[77]	Ecological study	National patient register Sweden	Hospitalisation with a primary diagnosis of heart failure	1.08 (1.04-1.12)[a]
Feenstra, 2002[78]	Cohort study	Elderly recipients of NSAIDs in population: 1) without heart failure, n=5062 2) with incident heart failure, n=85	 1) First occurrence of heart failure 2) Hospitalisation for relapse heart failure	 1) 1.2 (0.8-1.8) 2) 9.9 (1.7-57.0)

a: Per increase of one standard deviation of NSAID utilisation (5.8 defined daily doses/1000 inhabitants/day)
RR = relative risk, OR = odds ratio

actually exert anti-atherogenic effects [69]. Several observational studies have recently been published that examined the relationship between the use of naproxen and incidence of thrombotic events. Ray et al found no evidence for a protective effect of naproxen [70]. Three other studies, however, demonstrated a lower risk of cardiovascular events with the use of naproxen as compared to non-use or use of other NSAIDs [71-73]. Therefore, the weight of evidence is in favour of a cardioprotective effect of naproxen. However, findings of these studies do not clarify the role of rofecoxib. More research is necessary to assess the true risk of prothrombotic cardiovascular events associated with the use of COX-2 selective inhibitors.

Quantitative studies

As mentioned above, several case reports have been published in which the occurrence or exacerbation of heart failure was attributed to the use of NSAIDs [9-13]. Most of these patients had pre-existing heart disease. In view of the role of prostaglandins in the pathophysiology of heart failure, and the adverse renal effects of NSAIDs, it seems plausible that NSAIDs exert adverse effects in patients at risk for developing heart failure. So far, only few observational studies on the association between NSAID treatment and the onset of heart failure have been published (table I).

The first analytic study that was published was performed in a cohort of users of diuretics, aged 55 years and older [74]. In this study, the concomitant use of NSAIDs and diuretics as compared with the use of diuretics alone was associated with a two-foldly increased risk of first hospitalisation for heart failure. Most hospitalisations occurred within one month after initiation of combined therapy, with the highest risk occurring within the first days of NSAID use. As this study was performed in a cohort of users of diuretics, and the relative risk was higher in individuals with a history of heavy diuretic use, it seems likely that a number of these patients had symptomatic cardiac dysfunction preceding the date of first hospitalisation for heart failure. Page et al. conducted a matched case-control study in two public hospitals [75]. They found a doubling of the risk for hospital admission with heart failure in patients using NSAIDs in the preceding week. The estimated odds ratio was higher for a first admission with heart failure, and increased with high-dose and long plasma drug half-life. A much stronger association was found in patients with a history of heart disease. The findings of this study are consistent with an important effect of NSAIDs in patients with left ventricular impairment. In agreement with the above mentioned studies, are the results of a cohort study conducted in patients aged 50 years or older with a previous hospitalisation for heart failure [76]. Among patients who had received at least one NSAID prescription during the follow up period, current use of NSAIDs was associated with a two-foldly increased risk of rehospitalisation for heart failure. In the total cohort, risk of relapsing heart failure associated

with the use of NSAIDs was non-significantly increased by 41%. To study the impact of NSAID utilisation on hospitalisations for heart failure in Sweden, Merlo et al. performed a nationwide ecological study [77]. The relative risk of hospitalisation because of heart failure was significantly increased by 8% with every increase of one standard deviation of NSAID utilisation. However, a pitfall of the ecological design is that individual events are not linked to individual exposure or covariate data. Secondary diagnoses of heart failure were not used in this study and primary diagnoses were not validated. In a recently published, large population-based cohort study in the Netherlands, among community dwelling elderly, current use of NSAIDs was not associated with an increased risk of incident heart failure [78]. Incident heart failure was defined as the first occurrence of heart failure, irrespective of whether this event led to a hospital admission. This definition is more specific than in the previous described studies, in which only hospital admissions were considered. The risk of hospital admission for relapsing heart failure was, however, significantly increased in current users of NSAIDs among persons who had filled at least one NSAID prescription at any time since first diagnosis of heart failure (adjusted relative risk 9.9).

Overall, the observational studies that have been published strongly suggest that the risk of developing symptoms of heart failure is elevated during the use of NSAIDs by patients who are susceptible to the development of myocardial decompensation. No significant association was found between incident heart failure and NSAID treatment.

Conclusion

Considering current knowledge of the effects of NSAIDs on renal function and water and salt homeostasis, the important role of prostaglandins in the pathophysiology of heart failure, and evidence from observational studies, it is very likely that NSAIDs may increase the risk of developing symptoms of heart failure in susceptible patients. Patients with a history of cardiovascular disease, such as pre-existing heart failure, are particularly at risk. NSAIDs should therefore be prescribed to such patients as little as possible. If a prescription is justified, monitoring of renal function, adequate patient education and increased attention for signs and symptoms of heart failure is mandatory. It is unlikely that these drugs can also induce heart failure in otherwise healthy individuals.

The actual risk of NSAID-induced heart failure is currently unknown. Few quantitative studies have been performed. These studies consistently found that the risk is at least doubled in patients with left ventricular impairment. NSAIDs are utilised widely, mainly among elderly individuals, who are particularly susceptible to the adverse renal effects of these agents. At the same time, heart failure is an increasingly prevalent disease. Further studies are needed to quantify the risk of NSAID-induced heart failure in patients with asymptomatic cardiac dysfunction and to assess the risks of individual NSAIDs. Differences

between individual NSAIDs in the ability to induce heart failure have not yet been demonstrated, but may exist. Although several agents have been claimed to have renal sparing properties, including sulindac and nabumetone, insufficient evidence has so far been provided to substantiate this. Overall, it appears that COX-2 selective inhibitors have similar effects on renal function as the conventional NSAIDs. Since the central mechanism by which NSAIDs influence cardiovascular homeostasis is their effect on renal function, it seems plausible to assume that COX-2 selective inhibitors may also induce heart failure in susceptible patients.

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Chapter 4

Genetics and left ventricular abnormality

4.1

Apolipoprotein E ∈4 allele is associated with left ventricular systolic dysfunction

Abstract

Background: Apolipoprotein (APOE) ∈4 allele has been associated with cardiac dysfunction in Alzheimer's disease and β-thalassemia. We investigated the association between APOE genotypes and left ventricular dysfunction in a population of community-dwelling elderly.
Methods: This study was performed in the Rotterdam Study, a population-based prospective cohort study among elderly. For 2,206 participants, a baseline echocardiogram and blood specimens for APOE typing were available. Cardiac dysfunction was considered present if fractional shortening was 25% or less. Multivariate logistic regression was used to calculate odds ratios. The ∈3/∈3 genotype served as a reference category.
Results: In participants homozygous for the ∈4 allele, the odds of cardiac dysfunction was increased three-fold (OR 3.1, 95%CI 1.2-8.1), while the odds of persons with APOE ∈3/∈4 was non-significantly increased (OR 1.5, 95%CI 0.9-2.5). There was a significant allele-effect relationship for the ∈4 allele (p-trend<0.05). These elevated odds remained after adjustment for cholesterol levels and atherosclerosis parameters. Risks associated with APOE ∈4/∈4 and APOE ∈3/∈4 were more pronounced in participants aged 65 years or older.
Conclusion: The APOE ∈4 allele is an independent risk factor for cardiac dysfunction in the elderly. Besides well-known effects on atherosclerosis and cholesterol levels, there may be other mechanisms, such as apoptosis, through which this allele exerts negative effects on myocardial performance.

Introduction

It is well established that the ∈4 allele of the apolipoprotein E (APOE) gene is a major risk factor for Alzheimer's disease [1], and that it is associated with an increased risk of coronary heart disease, in particular at early age [2]. The APOE gene is located on chromosome 19 and has three common alleles (∈2, ∈3, and ∈4), resulting in six APOE genotypes. The APOE polymorphism involves the coding region of the APOE gene and results in changes in the gene product, leading to an altered metabolism and transport of lipoprotein particles [3].

Besides being a risk factor for coronary heart disease in most populations, there are indications that the APOE ∈4 allele is associated with an increased risk of (a)symptomatic left ventricular dysfunction in patients with Alzheimer's disease [4] and in patients with β-thalassemia [5]. Asymptomatic left ventricular systolic dysfunction is generally accepted to be a precursor of heart failure. A reduced ejection fraction increases the risk of cardiovascular mortality and hospital admissions for heart failure [6]. Most common causes are coronary artery disease, hypertension, valvular disease and cardiomyopathy [7]. Early detection and treatment of impaired left ventricular function might lead to a considerable reduction in morbidity and mortality in the elderly.

So far, the role of APOE in cardiac dysfunction has not been investigated in the general population. We examined the relationship between APOE genotypes and left ventricular systolic dysfunction in a population of elderly.

Methods

Setting and study population

The Rotterdam Study is a population-based prospective cohort study of cardiovascular-, locomotor-, neurologic- and ophthalmologic diseases in the elderly [8]. Participants were recruited from inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, who were 55 years of age and older. Of the 10,275 eligible subjects, 7,983 gave informed consent (78%). Participants were visited at home for a standardised questionnaire and were subsequently examined at the research centre, where echocardiography was performed in a sample of 2,823 subjects. In 19.7% (n=556), echocardiographic registrations were considered inadequate for reliable measurement of left ventricular dimensions [9]. This percentage is comparable to other population-based studies [10]. The analyses were restricted to persons for whom blood specimens were available for APOE typing (n=2,206).

Left ventricular systolic dysfunction

For assessment of left ventricular systolic function echocardiography was carried out with the participant in the partial left decubitus position using a 2.75-MHz transducer (Toshiba SSH-60A Nasuworks, Otawara, Japan). Two-dimensional imaging using parasternal long-axis views was performed to aid M-mode studies. Measurements were made according to guidelines of the American Society of Echocardiography. Left ventricular internal dimension was measured at end-diastole (LVIDed), as defined by the onset of the QRS complex, and at end-systole (LVIDes), as determined at the nadir of septal motion. The percentage fractional shortening was calculated as 100 * ((LVIDed-LVIDes)/LVIDed). This percentage reflects left ventricular systolic function in the absence of major wall motion abnormalities [11]. Left ventricular systolic dysfunction was considered present if fractional shortening was less than or equal to 25%, corresponding to a left ventricular ejection fraction of 42.5% [9].

APOE genotype

Genotyping for APOE was performed on coded DNA specimens, as described in detail elsewhere [12]. Briefly, a polymerase chain reaction was performed and the amplification products were digested with HhaI. The resulting restriction fragments were separated on precast gels (ExcelGel, Pharmacia Biotech, Uppsala, Sweden) by electrophoresis (MultiPhorII, Pharmacia Biotech) and visualized by silver staining. Three persons read the results independently, and were blinded to the participant's assessment of left ventricular systolic function. In case of discrepancies, APOE genotyping was repeated.

Other variables

Information was gathered during the interview and at the research centre on several potential confounders such as age, gender, pre-existing cardiovascular disease (myocardial infarction, hypertension, history of coronary artery bypass graft (CABG), history of percutaneous transluminal coronary angioplasty (PTCA)), smoking (classified as never/former/current), alcohol intake, and body mass index. Hypertension was defined as use of antihypertensive medication for the indication high blood pressure, or as a systolic blood pressure of 160 mm Hg or over, or a diastolic blood pressure of 95 mm Hg or over. Diabetes mellitus was defined as use of anti-diabetic medication, or a random or post-load serum glucose level higher than 11.0 mmol/l. Serum total and high density lipoprotein (HDL)-cholesterol concentrations were determined by using an automated enzymatic procedure. For determination of ankle-arm index, the ratio of systolic blood pressure at the ankle and the systolic blood pressure at the right arm was calculated for each leg. Peripheral arterial disease was considered present when the ankle-arm index was lower than 0.9 on at least one side. Aortic atherosclerosis was diagnosed by radiographic detection of calcific deposits in the abdominal aorta. Lateral

abdominal X-rays were made from a fixed distance with the subject seated. Calcifications were judged to be present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). In the analyses, aorta calcifications were classified as absent or present. To measure carotid intima-media thickness, ultrasonography of the left and right common carotid artery was performed with a 7.5-MHz linear-array transducer (ATL Ultra-Mark IV). On a longitudinal, two-dimensional ultrasound image of the carotid artery, the anterior (near) and posterior (far) walls of the carotid artery are displayed as two bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright line of the far wall and the leading edge of the second bright line indicates the intima-media thickness. The actual measurements of intima-media thickness were performed off-line. The procedure has been described in detail previously [13, 14]. The average of the mean anterior and posterior intima-media thickness of both the left and right common carotid artery was used for the current analyses. When one or more of the intima-media thickness measurements could not be obtained, the average of the remaining measurements was used for analyses.

Analysis

Continuous variables were expressed as mean ± SD and categorical variables as proportions. Comparisons were evaluated with t-tests and χ^2-tests as appropriate. Agreement of the genotype frequencies with the Hardy-Weinberg equilibrium expectations was tested using a χ^2-test. For the estimation of relative risks of left ventricular systolic dysfunction by genotype, a case-control analysis was performed, in which a multiple logistic regression model was used to calculate odds ratios for the different genotypes plus 95% confidence intervals (CI). The ∈3/∈3 genotype served as a reference category. Age (years), gender, hypertension, smoking, diabetes mellitus, history of myocardial infarction, history of CABG, history of PTCA, alcohol intake (0, <30 g/day, ≥30 g/day), peripheral arterial disease, aorta calcifications, body mass index (kg/m^2), total cholesterol (mmol/l), HDL-cholesterol (mmol/l), and mean carotid intima-media thickness (divided in quartiles) were added to the models to adjust for potential confounding. None of these factors changed the point estimate of the association between APOE genotype and left ventricular systolic dysfunction by more than 10% when added to the age- and gender corrected model. Some of these covariates are not considered as confounders but as intermediates in the association between APOE genotype and impaired systolic function. They were added to the model to evaluate if other mechanisms besides atherosclerosis and coronary heart disease can be responsible for an association between APOE genotype and left ventricular dysfunction. The same models were used to test for an allele-effect relationship (trend test) of the ∈4 allele. For categorical covariates with missing values we incorporated missing indicator variables in the model. On all potential confounders more than 98% of information was available, except on peripheral

arterial disease (94%), aorta calcifications (89%), alcohol intake (83%), and mean carotid intima-media thickness (79%). To study potential effect modification by age, the population was divided into two strata: younger than 65 years, and 65 years of age or older. Interaction terms for these two age-groups, hypertension and diabetes mellitus were added to the multivariate model.

Results

General characteristics of the study population are presented in table 1. Participants with left ventricular systolic dysfunction were on average older, more often male, and had a higher mean carotid intima-media thickness compared with controls with normal cardiac function. Aorta calcifications, history of myocardial infarction, CABG, or PTCA were also more frequent in cases than in controls, and the percentage of former smokers was higher. The distribution of APOE genotypes was in Hardy-Weinberg equilibrium (χ^2 = 1.4, df=3, 2p=0.7).

As shown in table 2, the odds ratio (OR) for systolic dysfunction associated with APOE ∈4/∈4 was 3.1 (95%CI 1.2-8.1), after adjustment for confounding variables. Participants with APOE ∈3/∈4 had a 1.5-foldly increased odds (95%CI 0.9-2.5), although this did not reach statistical significance. However, there was a significant allele-effect relationship (p-trend <0.05). According to the model, the adjusted odds of cardiac dysfunction is multiplied by 1.57 per APOE ∈4 allele present (including ∈2/∈4-, ∈3/∈4-, and ∈4/∈4 genotypes). The APOE ∈2 allele was not significantly associated with impaired systolic function, but numbers were small. Age, gender, history of CABG, and history of myocardial infarction were the only covariates significantly associated with decreased percentage fractional shortening in the multivariate model. Restriction to participants without a history of myocardial infarction revealed an OR of 2.9 (95%CI 0.9-8.8) for the association between APOE ∈4/∈4 and left ventricular dysfunction, and an OR of 1.4 (95%CI 0.8-2.6) for APOE ∈3/∈4.

Stratification of the study population by age showed that the increase in odds associated with APOE ∈4/∈4 was only present in participants of 65 years of age or older (OR 3.8, 95%CI 1.1-13.1). In this age-group, APOE ∈3/∈4 was also associated with a significantly increased odds of impaired systolic function (OR 2.0, 95%CI 1.0-4.0). Analyses in the younger age group yielded only 2 cases with APOE ∈4/∈4 (OR 2.7, 95%CI 0.6-13.2), and 6 cases with APOE ∈3/∈4 (OR 1.0, 95%CI 0.4-2.7). In a multivariate model interaction terms of APOE genotypes and the two age-groups, hypertension and diabetes mellitus were non-significant, indicating that there was no significant effect modification by these variables in this population.

Table 1. General characteristics.

	Cases (n=82)	Controls (n=2,124)
Age		
< 65 years	27 (33%)	1,171 (55%)
65-75 years	40 (49%)*	693 (33%)
≥ 75 years	15 (18%)*	260 (12%)
Female	26 (32%)*	1,170 (55%)
Body mass index (kg/m²)	26 (±2.9)	26 (±3.3)
Total cholesterol (mmol/l)	6.4 (±1.1)	6.6 (±1.2)
HDL-cholesterol (mmol/l)	1.3 (±0.4)	1.3 (±0.4)
Fractional shortening (%)	11 (±13.1)*	40 (±6.7)
Peripheral arterial disease	13 (16%)	197 (9%)
Aorta calcifications	57 (70%)*	1,122 (53%)
Mean carotid intima-media thickness (mm)	0.84 (±0.18)*	0.78 (±0.15)
Hypertension †	30 (37%)	648 (31%)
History of myocardial infarction ‡	24 (29%)*	181 (8%)
Diabetes mellitus §	9 (11%)	149 (7%)
Smoking:		
Current	14 (17%)	466 (22%)
Former	52 (63%)*	940 (44%)
Never	16 (20%)	703 (33%)
Alcohol intake (g/day)	10.9 (±13.1)	10.4 (±14.3)
History of CABG	9 (11%)*	36 (2%)
History of PTCA	3 (4%)*	18 (1%)

Values are means (±SD) or numbers (%)
* comparison cases and controls: $p<0.05$
† use of antihypertensive medication for treatment of high blood pressure, or systolic blood pressure of 160 mm Hg or over, or diastolic blood pressure of 95 mm Hg or over.
‡ myocardial infarction according to general practitioner, cardiologist or ECG.
§ use of anti-diabetic medication, or as a random or post-load serum glucose level higher than 11.0 mmol/l.

Table 2. Association between left ventricular systolic dysfunction and APOE genotypes.

APOE genotype:	Cases	Controls	OR adjusted for age and sex	Adjusted OR*
∈3/∈3†	41 (50.0%)	1,212 (57.0%)	1.0 (reference)	1.0 (reference)
∈2/∈2	1 (1.2%)	15 (0.7%)	1.5 (0.2-12.2)	2.4 (0.3-21.0)
∈2/∈3	9 (11.0%)	263 (12.4%)	1.1 (0.5-2.5)	1.1 (0.5-2.3)
∈2/∈4	1 (1.2%)	63 (3.0%)	0.5 (0.1-3.4)‡	0.5 (0.1-3.9)‡
∈3/∈4	24 (29.3%)	510 (24%)	1.4 (0.8-2.4)‡	1.5 (0.9-2.5)‡
∈4/∈4	6 (7.3%)	61 (2.9%)	2.8 (1.1-7.0)‡	3.1 (1.2-8.1)‡

number (%); odds ratio (OR) with 95% confidence interval.
* Adjusted for APOE genotype, age (years), gender, body mass index (kg/m^2), total cholesterol (mmol/l), HDL-cholesterol (mmol/l), peripheral arterial disease, aorta calcifications, mean carotid intima-media thickness (quartiles), hypertension, history of myocardial infarction, diabetes mellitus, smoking (never, former, current), alcohol intake (0, < 30 gr/day, ≥30 gr/day), history of CABG, and history of PTCA. † Reference group, ‡ ∈4 allele-effect relationship: p-trend <0.05.

Discussion

The results of this study indicate that the APOE ∈4 allele is associated with left ventricular systolic dysfunction in an elderly population. Participants homozygous for the ∈4 allele carried a three-foldly increased odds of impaired systolic function, while the odds of persons with APOE ∈3/∈4 was non-significantly increased by 50%. There was a significant allele-effect relationship. The elevated risk remained after adjustment for total- and HDL-cholesterol levels, atherosclerosis parameters, and coronary heart disease, which were considered as intermediates in the potential association between APOE genotype and cardiac dysfunction. Point estimates were similar in analyses restricted to participants without a prior history of myocardial infarction. The odds associated with APOE ∈4/∈4 and APOE ∈3/∈4 were more pronounced in participants of 65 years of age or older. However, numbers were small, and since there was no significant effect modification by age, these results should be interpreted with caution. Although no significant association was found for the APOE ∈2 allele, sample size was too small to conclude that this allele is not associated with cardiac dysfunction.

Two other studies have found an association between cardiac dysfunction and the APOE ∈4 allele. The first demonstrated that, in patients with Alzheimer's disease, APOE ∈4/∈4 carriers have a 7.2-foldly increased risk of ECG-abnormalities indicative of left ventricular dysfunction [4]. The second study compared the ∈4 allele frequency in patients with β-thalassemia major, stratified by cardiac status, with the frequency in a control sample of healthy blood donors [5]. The ∈4 allele frequency in patients with left ventricular failure was doubled as compared to the frequency in healthy controls.

An advantage of the present investigation over the previous studies is that it enabled us to determine relative risk estimates in the general population, rather than in specific subgroups. The selection of cases and controls in this study was independent of APOE

genotype, which minimized the risk of selection bias. However, because the APOE ∈4 allele has been associated with increased mortality in patients with ischemic heart disease [15], and as this study was performed in an elderly population, survival bias may have played a role. Moreover, old and diseased individuals were less likely to participate, resulting in a healthier study population. It seems likely, however, that this may lead to an underestimation rather than to an overestimation of the risks. Information bias is not likely, since all data on APOE genotype and left ventricular function were recorded similarly for all participants without prior knowledge of our study hypotheses. The time of onset of cardiac dysfunction was unknown. Symptomatic dysfunction prior to the date of echocardiography may have led to alterations in life style or medical interventions, thereby altering the risk factor profile of the cases, making them more similar to the controls. This might explain the modest effect of some well-known confounders in this study.

The actual mechanism by which the APOE ∈4 allele increases the risk of impaired left ventricular function is unknown. In this study, the potential influence of APOE genotype on serum cholesterol and atherosclerosis could not explain the relationship. Earlier, we did report an effect of APOE ∈2 on atherosclerosis [16]. There are several studies in which the association between APOE genotype and coronary artery disease or Alzheimer's disease was independent of atherosclerosis and plasma cholesterol concentrations [17-19]. We hypothesize that cardiomyocyte apoptosis is one of the mechanisms through which the APOE ∈4 allele exerts negative effects on left ventricular function. Investigations in Alzheimer's disease have shown that APOE is involved in apoptosis and that the ∈4 allele exerts isoform-specific neurotoxicity through various pathways, one of which is Fas-mediated apoptosis [20, 21]. Fas-mediated apoptosis has also been shown to occur in cardiomyocytes [22]. Studies in failing human- and animal hearts have led to the hypothesis that progressive left ventricular dysfunction may result, in part, from ongoing loss of cardiomyocytes [23]. Adult cardiac muscle cells are thought to have a very limited capacity for self-renewal. Death of a significant number of adult cardiomyocytes, either by necrosis or apoptosis, could therefore permanently diminish cardiac performance. Apoptosis has been detected in the failing heart, regardless of predisposing factors, and generally affects scattered individual cells [24]. Cardiac dysfunction increases as a consequence of elevated reactive oxygen species formation, which in turn promotes myocyte apoptosis [25]. APOE possesses anti-oxidant activity [5]. The effect seems to be isoform-specific, with ∈4 expressing the least anti-oxidant activity [26]. The aging process occurring in the heart is characterised by a significant loss of cardiomyocytes and reactive hypertrophy of the remaining cells [27]. The susceptibility of myocytes to cell death, including apoptosis, increases with age. It is estimated that the aging process itself contributes to loss of nearly 30% of all left ventricular cardiomyocytes [23]. This ongoing cell loss may be partly responsible for the increased risk of cardiac dysfunction seen in the elderly.

In conclusion, the APOE ∈4 allele is an independent risk factor for left ventricular systolic dysfunction. Besides well-known effects on atherosclerosis and cholesterol levels, there may be other mechanisms, such as apoptosis, through which the APOE ∈4 allele exerts negative effects on myocardial performance.

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4.2

Absence of the 192-base pair allele in a promoter polymorphism of the insulin-like growth factor-I gene is associated with an increased risk of left ventricular hypertrophy

Abstract

Objective: Altered serum levels of insulin-like growth factor I (IGF-I) have been associated with adverse cardiac remodeling. A promoter polymorphism in the IGF-I gene may alter serum IGF-I levels. We investigated the association between this polymorphism and left ventricular hypertrophy.

Methods and results: This study was performed in the Rotterdam Study, a population-based prospective cohort study among elderly. Analyses were performed with baseline measurements in subjects aged between 55 and 75 years, without a history of myocardial infarction (n=1,678). Left ventricular hypertrophy was defined as a left ventricular mass index ≥ 104 g/m² in women and ≥ 116 g/m² in men. Non-carriers of the 192-base pair allele of a cytosine-adenosine repeat in the IGF-I gene had a 1.5 foldly-increased risk of left ventricular hypertrophy as compared to subjects homozygous for the wild type allele. Although we found no clearly increased association in heterozygotes, there was a significant allele-effect relationship (p-trend<0.05).

Conclusion: Non-carriers of a 192-base pair polymorphism in the IGF-I gene are more susceptible to the development of left ventricular hypertrophy than individuals homozygous for this allele.

Introduction

Left ventricular hypertrophy is a strong predictor for cardiovascular morbidity and mortality [1]. The structure of the left ventricle is influenced by several factors such as blood pressure, age, gender, body mass index, diabetes mellitus, and pre-existing cardiovascular disease [2, 3]. These factors, however, do not completely explain the variability in left ventricular mass. Of the other factors involved, insulin-like growth factor I (IGF-I) may explain part of this variability [4]. IGF-I is a polypeptide growth factor that is expressed in many organs. It is the product of the IGF-I gene, which has been mapped to chromosome 12 [5]. Evidence has accumulated that, in addition to its growth-promoting and metabolic effects, IGF-I has cardioprotective effects by reducing myocardial apoptosis and injury in response to ischemia [6]. Moreover, findings suggest that lowered free IGF-I levels are associated with a higher prevalence of cardiovascular disease [7].

There are several limitations of studies conducted on serum IGF-I levels in relation to cardiovascular disease. Most of these studies have been performed cross-sectionally, making it difficult to distinguish whether altered serum IGF-I levels are a cause or a consequence of the underlying disease. In addition, serum levels of IGF-I are profoundly influenced by various factors such as growth hormone, insulin, age, diet, physical activity and genetic factors [8, 9]. A genetic polymorphism in the IGF-I promoter region has been identified which influences IGF-I production [10, 11]. Recently, we observed lower circulating total IGF-I levels in non-carriers of the wild type allele of this polymorphism than in homozygous carriers [12, 13]. Studying the effect of this polymorphism in relation to pathology may better reflect the effects of long-term IGF-I exposure than studies on circulating serum IGF-I levels, which may fluctuate considerably. Furthermore, studies of genetic determinants of IGF-I levels may suffer less from the confounding influence of other factors.

This study aims to investigate the association between a promoter polymorphism of the IGF-I gene and the occurrence of left ventricular hypertrophy on the echocardiogram.

Methods

Setting and study population

The Rotterdam Study is a population-based prospective cohort study of cardiovascular-, locomotor-, neurologic- and ophthalmologic diseases in the elderly [14]. All inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, who were 55 years of age or older were invited to participate. Of the 10,275 eligible subjects, 7,983 agreed to participate (78%). The baseline examination was conducted between 1990 and 1993. Participants were visited at home for a standardized questionnaire and were subsequently examined at the research center, where echocardiography was performed. Due to costs and logistic

problems, cardiac ultrasound was carried out in a random subpopulation of the Rotterdam Study consisting of 2,823 subjects, who were not living in nursing homes. In 19.7% (n=556), echocardiographic registrations were considered inadequate for reliable measurement of left ventricular dimensions. This percentage is comparable to other population-based studies [15]. The present study was performed with baseline measurements in subjects between 55 and 75 years of age. Participants with a history of myocardial infarction were excluded. The analyses were restricted to persons for whom blood specimens were available for IGF-I typing, and for whom all measurements were available to determine left ventricular mass index (n=1,678).

Left ventricular hypertrophy

For assessment of the presence of left ventricular hypertrophy, echocardiography was carried out with the participant in the partial left decubitus position using a 2.75-MHz transducer (Toshiba SSH-60A Nasuworks, Otawara, Japan). Measurements were made by experienced staff, trained at the echo lab of the Thorax Center in the Erasmus MC in Rotterdam, according to a protocol based on the recommendations of the American Society of Echocardiography (ASE) [16]. Two-dimensional imaging using parasternal long-axis views was performed to aid M-mode studies. Measurements of the left ventricle were performed at end-diastole, as defined by the onset of the QRS complex, according to ASE recommendations. Left ventricular mass was determined using the Devereux-modified ASE cube formula: left ventricular mass (grams) = $0.8x(1.04x[(LVED+IVS+LVPW)^3-(LVED)^3])+0.6$, where LVED = left ventricular end diastolic diameter, IVS = interventricular septum thickness, and LVPW = left ventricular posterior wall thickness [17]. Left ventricular mass was indexed to body surface area.

Cases with left ventricular hypertrophy were defined as having a left ventricular mass index equal to or greater than 104 g/m^2 in women and 116 g/m^2 in men [18, 19]. Since there is no consensus concerning left ventricular hypertrophy thresholds in the medical literature, left ventricular mass index was also divided into gender specific quintiles, which has been demonstrated to provide a good prediction of cardiovascular risk estimates [20]. For this second analysis, cases were defined as being in the highest gender specific quintile, and controls as being in the remaining quintiles.

To study differences in left ventricular remodeling patterns according to IGF-I genotype, relative wall thickness (RWT) was also determined and calculated as RWT = 2 x LVPW / LVED [21]. Increased relative wall thickness was considered present when this ratio exceeded 0.43, according to previously published criteria [22]. Three mutually exclusive patterns were identified: normal left ventricular geometry (normal relative wall thickness and normal left ventricular mass index), concentric remodeling (increased relative wall thickness but normal left ventricular mass index), and left ventricular hypertrophy (as defined above).

IGF-I genotype

The polymorphism under study was a cytosine-adenosine repeat in the promoter region, 1 kilobase upstream from the transcription site of the IGF-I gene. Genotyping for the IGF-I polymorphism was performed as described elsewhere [12]. Earlier, we identified ten different alleles in the promoter region of the IGF-I gene in a sample of 900 subjects of the Rotterdam Study [12]. Of these participants, 88.4% carried at least one 192-base pair (bp) allele, which suggests that this is the wild type allele from which all other alleles originated. The frequency of the other 9 alleles was low. Based on this observation, using the wild type allele, our study population was divided into three genotypes: individuals homozygous for the 192-bp allele (43.6%), individuals heterozygous for the 192-bp allele (45.6%), and non-carriers of the 192-bp allele polymorphism (10.7%).

Other variables

Information on several risk factors, such as age, gender, history of myocardial infarction (confirmed by a general practitioner, cardiologist, or the electrocardiogram), smoking (classified as never/former/current), hypertension, diabetes mellitus, and body mass index (kg/m^2) was obtained at baseline. Information on the use of medication and type of medication was assessed during the home interview. Participants subsequently showed all currently used medication at the research center, where a physician determined the indication for each drug. Systolic and diastolic blood pressures from the right upper arm were measured with a random-zero sphygmomanometer twice with the patient in a sitting position. The mean of the two readings was used to determine blood pressure levels. Hypertension was defined as use of antihypertensive medication for the indication of high blood pressure, or as a systolic blood pressure of 140 mm Hg or over, or a diastolic blood pressure of 90 mm Hg or over [23]. Diabetes mellitus was defined as use of anti-diabetic medication, or a random or post-load serum glucose level higher than 11.0 mmol/l.

Analysis

Univariate comparisons were evaluated with a logistic regression model. Agreement of the genotype frequencies with the Hardy-Weinberg equilibrium expectations was tested using a χ^2-test. To investigate the association between left ventricular hypertrophy and IGF-I genotypes, we used multivariate logistic regression to calculate odds ratios plus 95% confidence intervals (CI). An allele-effect model was assumed to explain differences between genotype groups. Participants homozygous for the 192-bp allele served as the reference category for all analyses. Men and women were pooled in the analyses, because there was no effect modification by gender. Age (years), gender, hypertension, diabetes mellitus, and body mass index (kg/m^2) were added to the model to adjust for potential

confounding. The decision to keep potential confounders in the final model was based on biological plausibility. None of these factors changed the point estimate of the association between IGF-I genotype and left ventricular hypertrophy by more than 5% when added to the univariate model. On each potential confounder more than 97% of data was available. For categorical covariates with missing values we incorporated missing indicator variables in the model. We performed age- and gender adjusted logistic regression analysis to test for an allele (dose)-effect relationship (trend test). To evaluate the distribution of left ventricular geometry patterns according to IGF-I genotype, percentages per genotype were calculated. To test differences between genotypes, we used an ordinal regression model adjusted for age, with the different patterns of left ventricular geometry as outcome variables.

Results

In total, 358 cases were identified with left ventricular hypertrophy. Genotype and allele distributions were in Hardy-Weinberg equilibrium (total population: p-value = 0.33). Table 1 presents the baseline characteristics of the study population. Participants with left ventricular hypertrophy were on average two years older than patients with normal left ventricular mass index (mean age 65- and 63 years respectively, t-test $p<0.001$). In addition, cases had a higher mean body mass index, systolic- and diastolic blood pressure, and had more co-morbidity such as diabetes mellitus and hypertension. The number of smokers did not differ significantly between participants with or without left ventricular hypertrophy. The difference in gender distribution was not statistically significant.

In table 2, point estimates are presented for the association between IGF-I genotypes and left ventricular hypertrophy on the echocardiogram. Non-carriers of the 192-bp allele had a 1.50 foldly-increased odds of echocardiographically determined left ventricular hypertrophy as compared to individuals homozygous for the 192-bp allele. This association remained significant after adjustment for potential confounding factors (OR 1.49; 95%CI 1.01-2.20). The frequency of left ventricular hypertrophy was slightly but non-significantly increased in participants heterozygous for the 192-bp allele. However, there was a significant allele (dose)-effect relationship (p-trend <0.05). According to the model, the age- and gender adjusted odds of left ventricular hypertrophy is multiplied by 1.20 per risk allele present (non-wild type). Findings were similar for the association between IGF-I genotypes and the highest versus other gender specific quintiles of left ventricular mass index (age-adjusted OR non-carriers 192-bp allele 1.48, 95%CI 1.001-2.18). However, after additional adjustment for body mass index, hypertension, and diabetes mellitus this association was not statistically significant (OR 1.46, 95%CI 0.99-2.17).

Table 1. General characteristics.

	Cases* (n=358) [LVH +]	Controls (n=1320) [LVH -]	OR (95% CI)
Female	192 (54%)	754 (57%)	0.87 (0.69-1.10)
Age (years)	65 (5.3)	63 (5.3)	1.07 (1.05-1.09)
Diabetes mellitus	30 (8%)	62 (5%)	1.85 (1.18-2.92)
Smoking			
Never	101 (28%)	435 (33%)	1.00 (reference)
Former	171 (48%)	584 (44%)	1.26 (0.96-1.66)
Current	84 (24%)	296 (23%)	1.22 (0.88-1.69)
Hypertension†	223 (64%)	578 (45%)	2.16 (1.69-2.75)
Body mass index (kg/m^2)	27 (3.1)	26 (3.3)	1.09 (1.05-1.13)
Systolic blood pressure (mm Hg)	144 (21.7)	134 (21.1)	1.02 (1.02-1.03)
Diastolic blood pressure (mm Hg)	76 (12.0)	74 (11.0)	1.02 (1.01-1.03)

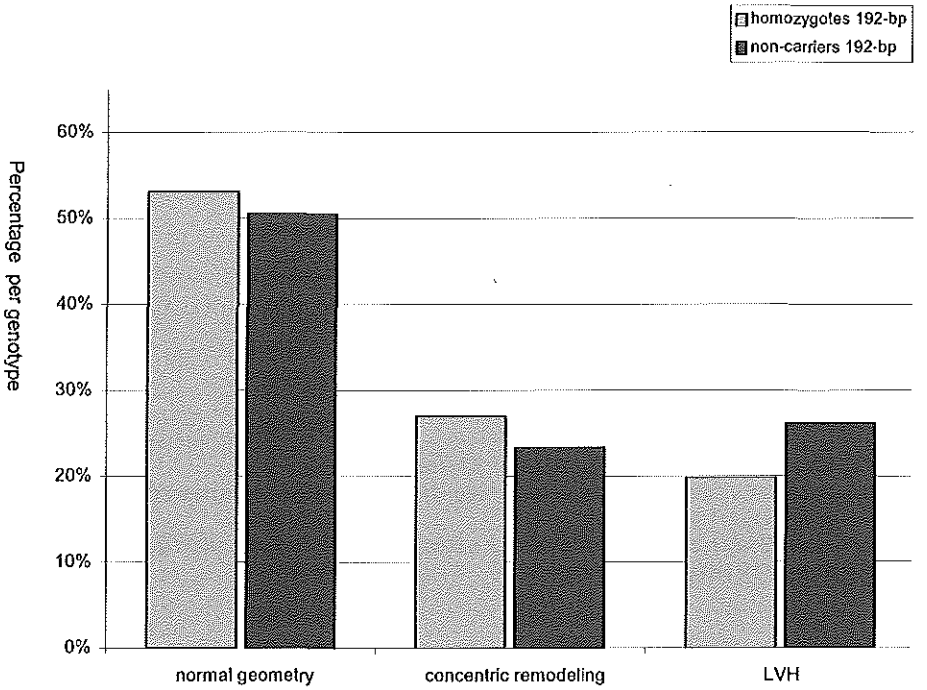
Values for characteristics of cases and controls are means (±SD) or numbers (%)
* Case definition: left ventricular hypertrophy (LVH) was considered present when left ventricular mass index was equal or greater than 104 g/m^2 in women or 116 g/m^2 in men.
† Defined as systolic blood pressure of 140 mm Hg or higher or diastolic blood pressure of 90 mm Hg or higher or use of antihypertensive drugs with the indication hypertension.

Table 2. Association between echocardiographically determined left ventricular hypertrophy and IGF-I genotypes.

IGF-I genotype	Cases* (n=358)	Controls (n=1320)	OR model 1†	OR model 2‡
Homozygous 192-bp allele	145 (41%)	587 (44%)	1.00 (reference)	1.00 (reference)
Heterozygous 192-bp allele	166 (46%)	600 (46%)	1.13 (0.88-1.46)	1.11 (0.86-1.43)
Non-carrier 192-bp allele	47 (13%)	133 (10%)	1.50 (1.02-2.21)	1.49 (1.01-2.20)

Number (% among cases or controls); odds ratio (OR) with 95% confidence interval.
* Case definition: left ventricular hypertrophy was considered present when left ventricular mass index was equal or greater than 104 g/m^2 in women and 116 g/m^2 in men.
† Adjusted for age (years) and gender. Allele-effect relationship: p-trend <0.05.
‡ Adjusted for age (years), gender, body mass index (kg/m^2), hypertension, and diabetes mellitus.

Figure 1 shows the percentages per IGF-I genotype of geometric patterns of the left ventricle for non-carriers and participants homozygous for the 192-bp allele. Although ordinal regression analysis estimates were not statistically significant (p=0.17), this graph shows a tendency of percentages for non-carriers to be higher in the most unfavorable pattern of left ventricular geometry, while concentric remodeling was more frequent in individuals homozygous for the wild type allele.



LVH= left ventricular hypertrophy. Bars add up to 100% per genotype. Numbers in bars are the absolute number per genotype. Ordinal regression: p=0.17.
OR[age and sex adjusted] LVH versus concentric remodelling: 1.6 (1.0-2.6), OR[age and sex adjusted] LVH versus normal geometry: 1.5 (1.0-2.2).

Figure 1. Left ventricular remodeling and IGF-I genotypes.

Discussion

In this study, non-carriers of the 192-bp allele of a cytosine-adenosine repeat in the promoter region of the IGF-I gene were more susceptible to the development of left ventricular hypertrophy than participants homozygous for the wild type allele. To our knowledge, this is the first population-based study on the association between this IGF-I polymorphism and left ventricular hypertrophy. The risk of elevated left ventricular mass index detected by echocardiography in these subjects was significantly increased by 50%. Although there was no clearly increased difference in risk for participants heterozygous for the wild type allele, there was a significant allele-effect relationship in this population. In addition, there was a tendency for non-carriers to have a higher frequency of adverse cardiac remodeling with cardiac enlargement than participants homozygous for the 192-bp allele. In contrast, concentric remodeling, which is a more appropriate response, was more frequent in

individuals homozygous for the wild type allele. Cardiovascular morbidity and mortality are known to increase as the geometric pattern of the left ventricle changes from normal to concentric remodeling and finally to left ventricular hypertrophy [24]. In vitro and in vivo studies suggest that IGF-I may help maintain appropriate myocardial remodeling in reaction to an injury to the heart [25]. This may provide an explanation for our findings.

Most studies on the association between IGF-I serum levels and left ventricular mass have been performed cross-sectionally, making it difficult to distinguish whether altered serum IGF-I levels are a cause or a consequence of left ventricular hypertrophy. In addition, serum levels of IGF-I are influenced by several factors, which vary over time, including growth hormone, insulin, nutrition and physical activity. Therefore, residual confounding can be an issue in these studies. Studying the association with the IGF-I polymorphism may circumvent these difficulties, since this approach will probably better approximate long-term exposure to circulating IGF-I. Moreover, disturbances by factors that regulate IGF-I serum levels will not influence the genetic background. Accordingly, potential confounding factors did not have an effect on the point estimates in this study.

It cannot be excluded that the IGF-I polymorphism itself is not functional but just serves as a marker for a nearby genetic variant functionally involved in IGF-I expression. However, the polymorphism investigated in the present study has been associated with serum IGF-I concentrations in several studies, albeit with opposite directions [11, 12]. Recently, we found in a sample of the Rotterdam Study that non-carriers of the 192-bp allele had lower circulating total IGF-I levels and lower body height than carriers of this polymorphism. The absence of this allele was also significantly associated with an increased risk of type-2 diabetes mellitus and myocardial infarction [12]. Moreover, we observed that the normal gradual decline in circulating serum IGF-I levels during aging was highly influenced by the presence of two 192-bp alleles in the IGF-I gene [13]. These findings in subgroups of our study population make a functional relationship between this polymorphism and left ventricular remodeling plausible.

In recent years, several studies provided support to the hypothesis that low serum IGF-I is a risk factor for ischemic heart disease and atherosclerosis [7, 26-28]. In addition to its favorable effects on glycaemic control and the lipid profile [29]. IGF-I has beneficial effects on cardiac remodeling by reducing myocyte apoptosis in response to ischemia [25, 30], and by improving myocardial contractility and stroke volume [31]. Furthermore, IGF-I promotes cardiac hypertrophy of a physiologic phenotype [32, 33] and causes systemic vascular vasodilatation [34]. Moreover, basal IGF-I levels are reduced in patients with dilated cardiomyopathy [35], and a recent study demonstrated an inverse association between the severity of heart failure, by both clinical assessment and left ventricular performance, and IGF-I levels [36]. This may imply that progression of disease from compensated to decompensated heart failure may be partly influenced by the ability to generate IGF-I for cardiac remodeling. Hence, evidence suggests that IGF-I deficiency may lead to diminished

cardiac performance and adverse remodeling in reaction to an injury to the heart. On the other hand, IGF-I directly stimulates growth of cardiac myocytes through induction of cardiac protein synthesis. In patients with acromegaly, IGF-I hypersecretion leads to ventricular hypertrophy with interstitial fibrosis [37]. Also, in a cross-sectional study of patients with untreated essential hypertension and normal glucose tolerance, IGF-I was a powerful independent determinant of left ventricular mass [4]. Therefore, both low and high levels of IGF-I seem to have adverse effects on cardiac function and structure.

This observational study is potentially limited by selection- and information bias and confounding. Old and diseased individuals were less likely to participate, resulting in a healthier study population. Random misclassification of the outcome may have occurred due to measurement error, but this will only lead to conservative risk estimates. Potential confounding factors were dealt with in the analyses. None of these factors had a major influence on the association studied.

In conclusion, non-carriers of a 192-bp allele polymorphism in the promoter region of the IGF-I gene are more susceptible to the development of left ventricular hypertrophy than participants homozygous for the wild type allele. This may be the consequence of a relative IGF-I deficiency, leading to a faulty remodeling in response to myocardial injury.

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Chapter 5

Genetics and heart failure

5.1

Genetic polymorphisms and heart failure

Abstract

Heart failure is a complex clinical syndrome. There is evidence for a genetic contribution to the pathophysiology of heart failure. Considering the fundamental role of neurohormonal factors in the pathophysiology and progression of cardiac dysfunction and hypertrophy, variants of genes involved in this system are logical candidate genes in heart failure.

In this report, genetic polymorphisms of the major neurohormonal systems in heart failure will be discussed. Studies on polymorphisms of the renin-angiotensin-aldosterone system (RAAS), adrenergic receptor polymorphisms, endothelin (receptor) polymorphisms and a group of miscellaneous polymorphisms that may be involved in the development or phenotypic expression of heart failure will be reviewed. Research on left ventricular hypertrophy is also included.

The majority of genetic association studies focused on the ACE I/D polymorphism. Initial genetic associations have often been difficult to replicate, mainly due to problems in study design and lack of power. Promising results have been obtained with genetic polymorphisms of the RAAS and sympathetic system. Considering the evidence so far, a modifying role for these polymorphisms seems more likely than a role of these variants as susceptibility genes. Besides the need for larger studies to examine the effects of single nucleotide polymorphisms and haplotypes, future studies also need to focus on the complexity of these systems and study gene-gene interactions and gene-environment interactions.

Introduction

Heart failure is a complex clinical syndrome with high morbidity and mortality [1, 2]. Impairment of cardiac function activates compensatory neurohormonal mechanisms, which at a later stage may be detrimental for progression of heart failure [3]. Coronary heart disease and hypertension are major underlying causes of heart failure. Other frequent underlying conditions include valvular heart disease and idiopathic dilated cardiomyopathy. It is difficult to predict who will develop heart failure in response to myocardial injury. Racial differences in occurrence and outcome of heart failure [4, 5] and heritability estimates of variability in left ventricular mass of 28% to 65% [6, 7] suggest a genetic contribution to the pathophysiology of left ventricular remodeling and heart failure.

Many studies have been published on the role of single gene mutations in (familial) cardiomyopathies [8, 9]. These Mendelian traits are by nature rare, and although important at an individual level and for the understanding of disease mechanisms, of limited significance in terms of prediction of heart failure occurrence in the population [10]. Moreover, in patients with identical gene mutations clinical manifestations of cardiomyopathy may differ. This suggests that probably also environmental- and/or other genetic factors play a role.

In this report, genetic polymorphisms of major neurohormonal systems involved in the pathophysiology of heart failure will be discussed, including the renin-angiotensin-aldosterone system (RAAS), adrenergic receptor polymorphisms, endothelin (receptor) polymorphisms and a group of miscellaneous polymorphisms. Research on left ventricular hypertrophy is also included, because this condition often precedes heart failure. Studies on the association between heart failure and human leucocyte antigen complex alleles and pharmacogenetic studies are outside the scope of this review.

Methods

Literature for this report was systematically identified by searching PubMed for all English-language articles published up to July 2003 related to heart failure and genetic polymorphisms. Bibliographies in articles provided further references. A two-step approach was used. First, genetic polymorphisms were identified in a search with the keywords 'heart failure' and 'polymorphism'. Second, polymorphisms that were identified with this search, were used as keywords with the addition of one of the following keywords 'heart failure', 'left ventricular hypertrophy', 'left ventricular mass', and 'cardiomyopathy'. We focused on genetic association studies.

Heart failure pathophysiology

Left ventricular dysfunction begins with an injury to the myocardium, e.g. myocardial infarction, which results in loss of functional cells. In response, a complex cascade of interacting hemodynamic and neurohormonal mechanisms is activated to preserve cardiac function [3]. These factors alter the shape and function of the ventricle through a process called left ventricular remodeling, in which fibrosis and myocyte damage appear to be decisive morphological alterations [11, 12]. The most important regulators of this process are components of the RAAS, growth factors and endocrine hormones such as norepinephrine.

A decreased capacity of the left ventricle to empty during systole increases diastolic wall tension. The ventricle responds by enhanced contraction, following the Frank-Starling curve [13]. Additionally, the sympathetic nervous system is activated, which provides inotropic support and maintains cardiac output [3]. Both compensatory mechanisms also lead to increased internal wall stress during diastole. In response, synthesis of myofibrillar proteins is stimulated, resulting in increased wall thickness and a reduction in ventricular wall stress and dilatation [13]. Sustained sympathetic stimulation, however, activates the RAAS and other neurohormones. The main effector of the RAAS is angiotensin II, a potent vasoconstrictor and stimulator of renal sodium reabsorption and aldosterone and vasopressin release [14]. Hypoxia, shear stress, and vasoactive hormones also stimulate the generation of endothelin, the most potent endogenous vasoconstrictor [15]. Endothelin has positive inotropic and chronotropic effects, influences salt and water homeostasis, stimulates the RAAS and sympathetic system and appears to play a role in cardiac remodeling [16]. With worsening fibrosis and cardiac myocyte degeneration, left ventricular end-diastolic pressure increases and later ejection fraction decreases [12].

To counterbalance the pressor and volume expanding effects of vasoactive neurohormones, natriuretic peptides are generated, which exert diuretic, natriuretic and vasodilator properties [17]. Also, the kallikrein-kinin system forms bradykinin, which results in natriuresis and vasodilatation, and stimulates the production of prostaglandins [3]. In this way, a delicate hemodynamic balance is achieved, which restores cardiac function temporarily. Long-term activation of these mechanisms, however, results in progressive deterioration of ventricular function.

Genetic polymorphisms

In common multi-factorial diseases, such as heart failure, the candidate gene approach is widely used to study genetic polymorphisms [18]. This allows for the identification of gene defects directly involved in the pathophysiology of heart failure (susceptibility genes), or

variants involved in modification of its phenotypic expression (modifier genes). The case-control study is the most frequently used design. Candidate genes are selected based upon their biological plausibility. Polymorphisms located in coding or promoter regions of a gene may alter the function or expression of proteins encoded by the gene. Even if these polymorphisms are not functional, they are more likely to be in linkage disequilibrium with causative alleles [10].

Considering the fundamental role of neurohormonal factors in the pathophysiology and progression of cardiac dysfunction and hypertrophy, variants of neurohormonal genes are logical candidate genes in heart failure. Successively, we will describe the potential role of these variants as susceptibility genes and as modifier genes in heart failure due to ischemic and/or dilated cardiomyopathy and in hypertrophic cardiomyopathy. Also, studies on left ventricular dimensions will be discussed. Table 1 shows genetic polymorphisms that have been associated with one or more of these disease manifestations in at least one study.

Renin-angiotensin-aldosterone system

The RAAS is one of the major systems involved in the pathophysiology of heart failure. Since it is a complex system, effects of polymorphisms influencing the expression of one of its components may be counterbalanced by compensatory changes in other components. Therefore, for individual mutations, associations are probably modest.

Angiotensin converting enzyme I/D polymorphism

ACE enzymatically transforms angiotensin I to angiotensin II. The human ACE gene is located on chromosome 17q23. A genetic polymorphism in intron 16 of this gene is strongly associated with serum levels of ACE [19]. It is characterized by an insertion (I) or a deletion (D) of a 287 non-coding base pair (bp) sequence within an Alu repeat. Its functional role has been debated. Probably, this polymorphism is in strong linkage disequilibrium with another functional mutation within the gene [20].

Raynolds and colleagues published an association between the ACE I/D polymorphism and heart failure susceptibility in Caucasians [21]. They observed an excess of DD genotype both in subjects with ischemic- and idiopathic dilated cardiomyopathy compared to organ donors. However, since their control group was not in Hardy-Weinberg equilibrium, results may have been biased. Subsequently, many conflicting studies followed. A study in 70 Chinese patients appeared to confirm the presence of an association [22] but was also not in Hardy-Weinberg equilibrium. Most studies did not find an association between the ACE polymorphism and heart failure secondary to ischemic and/or dilated cardiomyopathy in 1506 Caucasian- [23-26], 287 Chinese- [27], 281 Japanese- [28] and 724 black South African

subjects [29, 30]. Hence, it seems unlikely that susceptibility to ischemic or idiopathic dilated cardiomyopathy in the general population is associated with the ACE I/D polymorphism.

In contrast, studies have reported more promising results on the potential role of the ACE polymorphism as a modifier gene in heart failure, at least in Caucasian populations. Andersson found an association between poor survival and DD genotype in 194 Swedish patients with heart failure [31]. A study in 57 white American patients demonstrated that DD genotype was associated with impaired exercise tolerance [32]. Others found that the D allele was associated with an increased risk of death or heart transplantation in 328 American patients [33]. Two small studies in 90 Czech- and 84 Turkish subjects could, however, not detect an association between heart failure phenotype and ACE genotype [24, 26]. Studies in Japanese [28, 34] and Chinese patients [35] failed to associate this polymorphism with heart failure phenotype, while studies in blacks were contradictory. Candy et al. found an association between DD genotype and reduced cardiac function and increased cavity size in South African patients with cardiomyopathy [30], whereas others did not [29].

The first study on the ACE polymorphism and hypertrophic cardiomyopathy found an excess of DD genotype in Caucasian patients, especially in families with a history of sudden cardiac death [36]. Other studies have since then confirmed the higher frequency of DD genotype in hypertrophic cardiomyopathy in white and Japanese patients [37-39]. Yamada et al. did not detect an association between ACE genotypes and hypertrophic cardiomyopathy in Japanese. In addition, no association was found between ACE genotypes and echocardiographic measurements [28]. Also, a study in 104 Dutch patients did not support a role for the I/D polymorphism as a modifier in this disorder [40]. An association of ACE genotype with phenotypic expression of hypertrophic cardiomyopathy was, however, observed in Caucasians by others [41]. A study conducted in a family affected by a single mutation in the myosin binding protein-C (MyBP-C) gene confirmed these results [42]. It seems that the influence of ACE genotype in patients with hypertrophic cardiomyopathy depends on the specific disease-causing mutation, which may offer an explanation for the conflicting results [43]. Similar to ischemic and idiopathic dilated cardiomyopathy, it is more likely that the ACE I/D polymorphism acts as a modifier in hypertrophic cardiomyopathy than as a susceptibility gene.

Many studies have investigated the influence of the ACE polymorphism on left ventricular hypertrophy. There is considerable controversy on this topic. Schunkert and colleagues described an association between DD genotype and electrocardiographic left ventricular hypertrophy in 1428 Europeans [44]. Another large-scale investigation was carried out in the Framingham Heart Study in 2439 subjects [45]. No association was found between ACE genotypes and echocardiographic left ventricular hypertrophy. Kuznetsova et al. have summarized the majority of studies (n=28, overall sample size 6638) in a meta-analysis [46]. There was significant heterogeneity among studies and the definition of left ventricular hypertrophy varied widely. Using data from 12 case-control studies, the pooled odds

ratio (OR) of left ventricular hypertrophy with presence of the D allele was 1.09 (95% confidence interval (CI) 0.98-1.21). Subgroup analyses, however, showed that in untreated hypertensives, the risk of cardiac hypertrophy for the DD genotype compared to the II genotype was significantly increased (OR 2.92; 95% CI 1.50-5.70). Also, left ventricular mass as a continuous trait was only associated with ACE genotype in never-treated hypertensives. Therefore, findings suggest that the ACE polymorphism may only have an impact on cardiac dimensions in hypertensives. From a clinical perspective one might speculate that this effect can be neutralized by the use of antihypertensive drugs. Although there are many studies on the ACE I/D polymorphism, study populations are often not in Hardy-Weinberg equilibrium. There may be various explanations, including differential mortality, but also genotyping error cannot be excluded. This calls for a different approach in which multiple markers are tested that are more robust than the original ACE I/D variant.

Angiotensinogen M235T polymorphism

Angiotensinogen is converted to angiotensin I. The human angiotensinogen gene is located on chromosome 1q42-43 [47]. Three polymorphisms have been studied: two mutations in exon 2 resulting in a methionine to threonine exchange at position 235 (M235T) and a threonine to methionine substitution at position 174 (T174M), and a G-6A variant, which represents a guanine to adenine substitution 6 bp upstream from the initiation site of transcription in the promoter region [48]. The G-6A variant is in close linkage disequilibrium with the M235T polymorphism and will not be discussed separately. As the T174M variant has been studied only once with respect to cardiac dimensions [49], we focus on the M235T polymorphism.

The 235T allele is associated with a stepwise increase in angiotensinogen levels and with a moderate increase in risk of hypertension [50]. Nevertheless, most studies did not find an association between the M235T polymorphism and development or progression of ischemic and dilated cardiomyopathy [25, 28, 29, 35]. Therefore, it is unlikely that this polymorphism plays a role in these disorders. An increased risk of heart failure in subjects carrying both 235M/T and –6G/G genotypes has been reported, however, suggesting the presence of a disease related haplotype [51].

In hypertrophic cardiomyopathy, conflicting findings have been published. In Japanese subjects, one study revealed a higher frequency of the 235T allele in hypertrophic cardiomyopathy [38]. This could not be confirmed by others [28]. A study by Ortlepp and colleagues in 26 family members carrying a single mutation in the MyBP-C gene revealed a significant association between the angiotensinogen polymorphism and cardiac hypertrophy [42]. In contrast, the extent of hypertrophy in 108 unrelated Canadian patients was not influenced by angiotensinogen genotype [52].

Most studies on left ventricular hypertrophy have been performed in Caucasians [48, 49, 53-58]. The majority did not find an association [49, 54-58]. In contrast, studies in Chinese dialysis patients [59], South Korean patients with chest discomfort or hypertension [60] and Japanese outpatients of a cardiovascular clinic [61] found an association between TT genotype and cardiac hypertrophy. This suggests a more important role for the M235T polymorphism in Asian populations, corresponding with a higher frequency of the T allele in these populations [35]. A study in 103 Japanese patients, however, did not show an association between this polymorphism and left ventricular remodeling after myocardial infarction [62].

Angiotensin II receptor polymorphisms

The gene for human angiotensin II type 1 receptor (AT1R) is located on chromosome 3. Stimulation of the AT1R results in vasoconstriction, increased atherogenecity, inflammation, growth, proliferation or coagulation, depending on local conditions [63]. In chronic heart failure, the AT1R is down regulated in the heart. Various polymorphisms have been detected in this receptor, of which an adenine/cytosine (A/C) substitution located at position 1166 has been associated with heart failure and cardiac dimensions. This variant is probably not functional [40]. The C allele is considered to be the risk allele.

A potential interaction between ACE DD and AT1R AC/CC genotypes was described as a predictor of survival in Swedish patients with heart failure [31]. A large study in a French population (CARDIGENE study) failed to detect an association between the AT1R polymorphism and idiopathic dilated cardiomyopathy [25]. This study, however, used unconditional analyses despite matching of cases and controls on gender and age-distribution. Sanderson and colleagues did find an association between clinical course of heart failure and the C allele in 82 Chinese patients [35].

One study has been performed on hypertrophic cardiomyopathy in Japanese subjects. Although there was a significant difference in allele frequencies between patients and relatives, no difference in genotype frequencies was found between patients and healthy controls [64]. However, controls were not in Hardy-Weinberg equilibrium, which may have biased the results, and statistical analysis was performed unconditional, despite matching on age and gender. Two studies in Caucasian populations also found no association between the AT1R polymorphism and occurrence of hypertrophic cardiomyopathy [40, 52]. Some studies showed that the 1166C allele might adversely affect the phenotypic expression of hypertrophic cardiomyopathy [40, 42]. Therefore, A1166C polymorphism does not seem to increase heart failure susceptibility, but may play a modifying role. As only one out of six studies found an association between this variant and cardiac hypertrophy, a role for the A1166C polymorphism in left ventricular remodeling seems unlikely, at least in Caucasians.

Moreover, this positive study was small and, unexpectedly, the A allele was more frequent in cases [53].

The angiotensin II type 2 receptor (AT2R) gene is located on the X chromosome. The AT2R probably counteracts the effects of AT1R [63]. Deinum investigated the association between a polymorphism in exon 3 (A3123C) of the AT2R gene and extent of hypertrophy in 103 Dutch patients with hypertrophic cardiomyopathy. The extent of echocardiographic hypertrophy decreased with the number of C alleles in women [65]. A potential interaction between AT1R A1166C and AT2R A3123C polymorphisms was detected in men only.

Erdmann and colleagues found no significant differences between allele frequencies of a G1675A polymorphism in intron 1 of the AT2R in 107 patients with hypertrophic cardiomyopathy, 95 patients with dilated cardiomyopathy and 160 normal controls [66]. In 120 young white males, however, an association between the 1675A allele and structural cardiac changes was found in mildly hypertensive persons [67]. Two independent studies on left ventricular hypertrophy in Glasgow residents showed inconsistent results [68]. As the G1675A polymorphism is located in a gene region that is involved in the transcriptional control of the AT2R gene, this variant is potentially functional [67, 68]. Therefore, more studies are needed to determine the role of this polymorphism in heart failure.

Aldosterone synthase C-344T polymorphism

Aldosterone excess is a well-documented cause of hypertension and there is convincing evidence that mineralocorticoids have adverse effects in heart failure [69]. The key enzyme in aldosterone synthesis is aldosterone synthase. The corresponding gene CYP11B2 is located on chromosome 8 [70]. A cytosine/thymidine (C/T) substitution in the 5' promoter region at location –344 of the CYP11B2 gene has been identified. The functionality of this variant is unclear [70]. Few studies have described the association between heart failure and C-344T polymorphism. Studies in Caucasian and Japanese patients did not find an association between this variant and idiopathic dilated cardiomyopathy [25, 71]. However, the C allele was associated with increased left ventricular volume in the Japanese patients [71]. This finding could not be confirmed in black South Africans with heart failure [29]. Studies in hypertrophic cardiomyopathy also yielded conflicting results [42, 72]. Consequently, there is no firm evidence for a role of this polymorphism in heart failure.

An association between the C-344T variant and left ventricular hypertrophy was detected in young healthy Finns [73]. Although one small study confirmed this association [74], other larger studies failed to detect a significant effect of this polymorphism on left ventricular structure [75, 76]. Mayosi et al. investigated the contribution of several markers in the CYP11B2 gene, including the C-344T polymorphism, as individual variants and haplotypes [77]. Polymorphisms and haplotypes at the CYP11B2 locus were associated with a small but significant effect on variation in septal wall thickness and left ventricular cavity size

(variants G5937C and A4450C respectively). No significant association was detected with cardiac mass.

Sympathetic system

Adrenergic receptors are divided into α- and β-adrenergic receptors. α_1-Adrenergic receptors are mediators of cardiomyocyte hypertrophy, while α_2-adrenergic receptors are pre-synaptic inhibitors of norepinephrine release [78]. β_1- and β_2-adrenergic receptors increase cardiac inotropy and chronotropy, the β_1-receptor being the dominant subtype [79]. In heart failure, chronic sympathetic activation leads to selective down-regulation of β_1-adrenergic receptors and uncoupling of β_1- and β_2-adrenergic receptors, markedly blunting both signaling pathways [78]. Therefore, one may hypothesize that genetic variants of adrenergic receptors play a role in heart failure. In addition, β-adrenergic receptor blockers have been found to improve symptoms and mortality in heart failure, albeit with substantial interindividual variation [79].

α_2-adrenergic receptor Del322-325 polymorphism

The Del322-325 polymorphism in the gene for the α_{2c}-adrenergic receptor, located on chromosome 4, causes a substantial loss of agonist-mediated receptor function in vitro [80]. So far, only one study addressed the potential role of this polymorphism in heart failure susceptibility. Small et al. found that black subjects who were homozygous for the α_{2c} Del322-325 variant were more than 5 times as likely to have heart failure as those who were not. A two-locus analysis indicated a significant interaction between the α_{2c}Del322-325 polymorphism and the 389Arg variant of the β_1-adrenergic receptor [81]. Although not significant, associations for white subjects were similar in magnitude and direction. The α_{2c}Del322-325 variant was more than 10 times more common in black- than in white controls. As this polymorphism potentially changes the functionality of the α_{2c}-adrenergic receptor, it is an important candidate for further research on genetic factors in heart failure.

β_1-adrenergic receptor polymorphisms

Two major polymorphic loci have been identified in the gene coding for the β_1-adrenergic receptor located on chromosome 10. A guanine/cytosine substitution at nucleotide 1165 produces a change in the amino acid sequence, substituting a glycine (Gly) for an arginine (Arg) residue at position 389. This residue lies within the intracellular carboxy-terminus that is critical for G-protein coupling. Data indicate that this polymorphism has functional consequences for intracellular signaling [82]. The second variant, an adenine/guanine

substitution at nucleotide position 145, results in a serine (Ser) or glycine (Gly) at amino acid 49 [83]. Genetic variation in this residue has been shown in vitro to affect receptor desensitization and agonist-dependent down-regulation [84]. So, both polymorphic loci may lead to functional changes of the β_1-adrenergic receptor and hence both may play a role in heart failure. Genetic association studies, however, have been contradictory.

In the CARDIGENE study, no association was found between the Arg389Gly polymorphism and idiopathic dilated cardiomyopathy and disease severity [85]. Others have confirmed the lack of an association with heart failure occurrence [34, 81, 86]. Small and colleagues did find a significant interaction between the α_{2c}Del322-325 variant and the β_1 Arg389 allele in heart failure susceptibility [81]. An impaired exercise performance was demonstrated in patients with ischemic or idiopathic dilated cardiomyopathy, who were homozygous for Gly389 compared to Arg389 homozygotes, with an intermediate level of performance in heterozygotes [87]. This may suggest a role for the Arg389Gly polymorphism as a disease modifier. Also, a study in patients with renal disease found higher cardiac mass in Gly389 homozygotes [88]. In contrast, a study in Japanese cardiomyopathy patients showed a protective effect of the Gly389 allele in susceptibility to ventricular tachycardia [34].

Few studies have been published on the Ser49Gly polymorphism. A study in dilated cardiomyopathy demonstrated that 5-year transplant-free survival was worse in Ser49 homozygotes than in patients with the Ser49Gly variant [83]. Allele frequencies in patients did not differ from those in normal controls. Another study found impaired exercise capacity in patients with ischemic or dilated cardiomyopathy homozygous for the Ser49 allele compared to Gly49 carriers [87]. In contrast, Podlowski and colleagues only found the Gly49 mutation in patients with idiopathic dilated cardiomyopathy and not in healthy volunteers [86].

One other polymorphic locus in the gene for the β_1-adrenergic receptor, T-2146C, has been associated with idiopathic dilated cardiomyopathy [89]. Thus far, there is not enough evidence to draw firm conclusions about the role of β_1-adrenergic receptor polymorphisms in heart failure.

β_2-adrenergic receptor polymorphisms

An intronless gene on chromosome 5 encodes the β_2-adrenergic receptor. Attention has focused on three polymorphisms that display altered receptor function in experimental studies [79, 90]. The most functionally altered receptor is due to a threonine (Thr) to isoleucine (Ile) switch at amino acid 164. This rare variant receptor exhibits abnormal coupling to stimulatory G-protein [91]. The other two polymorphic loci occur on amino acid positions 16 (arginine (Arg)/glycine (Gly) substitution) and 27 (glutamine (Gln)/glutamic acid (Glu) substitution) [90]. There is marked linkage disequilibrium between these two polymorphisms [92]. The Gly16 receptor displays enhanced agonist-promoted down regulation, while the Glu27 form is resistant to down regulation [90, 93].

Two studies have investigated the effect of β_2-adrenergic polymorphisms in heart failure. Especially the Thr164Ile variant seems to be important in its phenotypic expression. The first study found no difference in frequency of the three polymorphisms between heart failure patients and normal subjects [90]. However, patients who were heterozygous for the Ile164 mutation had significantly worse survival than homozygous Thr164 patients. Trends for Arg16Gly and Gln27Glu did not reach statistical significance. The second study demonstrated that patients with heart failure carrying the Ile164 allele had a lower exercise capacity than patients homozygous for Thr164 [94]. Patients homozygous for the Gly16 allele also demonstrated lower exercise capacity than Arg16 homozygotes. As the Thr164Ile variant is rare, both studies did not include homozygous Ile164 subjects. A study in normotensive twins indicated that all three polymorphisms might be associated with ventricular wall thickness [92]. However, other studies have failed to detect any association between the Arg16Gly and Gln27Glu polymorphisms and cardiac dimensions [95-97]. These studies did not investigate the role of the Thr164Ile variant.

Endothelin

There is considerable evidence to support a role for the endothelin system in heart failure [98]. The endothelin A receptor mainly mediates the vasoconstrictor effects of endothelin-1, the predominant endothelin isoform. The endothelin B receptor has similar affinity for all isoforms and mediates vasodilatation in endothelial cells and vasoconstriction in smooth muscle cells [16]. Few genetic association studies have been performed.

Studies on dilated cardiomyopathy did not detect a role of genetic polymorphisms in the endothelin-1 gene [99, 100]. Brugada and colleagues showed that a G8002A polymorphism located in the 4th intron of the endothelin-1 gene on chromosome 6 might act as a modifier gene in hypertrophic cardiomyopathy [52]. Two variants of the endothelin A receptor have been studied with promising results. In the CARDIGENE study, a cytosine/thymidine (C/T) substitution in exon 8 at nucleotide position 1363 was associated with idiopathic dilated cardiomyopathy [99]. Individuals homozygous for the T allele were at significantly increased risk for this disease. A C/T substitution in exon 6 at position 69, which does not alter the amino acid sequence of the receptor, has been associated with survival in patients with non-ischemic dilated cardiomyopathy [100]. Carriers of the T allele had a more than 5-fold increased risk of death within 2 years after the diagnosis. A study in 528 never-treated hypertensives demonstrated that variants in the genes encoding endothelin-1 and the endothelin A receptor are not significant determinants of cardiac morphometric parameters [101]. Although several studies also investigated the role of endothelin B receptor polymorphisms, no significant associations were found [99-101].

Table 1. Genetic polymorphisms that have been associated with heart failure occurrence (susceptibility gene), - phenotype (modifier gene) and/or left ventricular structure in genetic association studies.

Genetic polymorphism	Risk allele*	Heart failure occurrence‡	Heart failure phenotype ‡	DCM / DCM phenotype	HCM / HCM phenotype	LVH / LV dimensions §
Renin-angiotensin-aldosterone						
ACE I/D	**D**	+	+	+	+	+
AGT M235T	**T**	+	-	-	+	+
AGT -G6A	**G**	+	-	-	-	+
AGT T174M	**M**	-	-	-	-	+
AT1R A1166C	**C**	-	+	-	+	+ (A)
AT2R A3123C	**A**	-	-	-	+	-
AT2R G1675A	**A**	-	-	-	-	+
CYP11B2 C-344T	**C**	-	-	+	+	+
Sympathetic system						
A2AR α_{2c}Wt/Del322-325	**Del**	+	-	-	-	-
B1AR Ser49Gly	**Ser**	-	+	+	-	-
B1AR Arg389Gly	**†**	-	+	+	-	+
B1AR T-2146C	**C**	-	-	+	-	-
B2AR Arg16Gly	**Gly**	-	+	-	-	+ (Arg)
B2AR Gln27Glu	**Glu**	-	-	-	-	+
B2AR Thr164Ile	**Ile**	-	+	-	-	+
Endothelin						
END1 G8002A	**A**	-	-	-	+	-
ETAR C1363T	**T**	-	-	+	-	-
ETAR C69T	**T**	-	-	+	-	-
Miscellaneous						
Bradykinin receptor [106]	**+9**	-	-	-	-	+
CMA A-1903G [37,42,107]	**A**	-	-	-	+	+
TNF G-308A [72,108,109]	**A**	-	-	+	+	-
TGFB1 Leu10Pro [110]	**heterozy**	+	-	+	-	-
SOD2 Ala16Val [111]	**Val**	-	-	+	-	-
PAF G994T [112,113]	**T**	-	-	+	+	-
CCR2 Val64Ile [114]	**Ile**	+	-	-	-	-
NOS3 Glu298Asp [115]	**Asp**	-	+	-	-	-
APOE ε2/ ε3/ ε4 [116-118]	**ε4**	+	-	-	-	+
α-adducin Gly460Trp [58]	**Trp**	-	-	-	-	+
GNB3 C826T [119,120]	**T**	-	-	-	-	+
AMPD1 +/- [121]	**+**	-	+	-	-	-

* in majority studies, † indecisive, ‡ heart failure with multiple causes, not exclusive in DCM or HCM, § in patients without heart failure; +: at least one positive study, -: no positive association studies, DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, LVH: left ventricular hypertrophy, ACE: angiotensin converting enzyme, AGT: angiotensinogen, AT1R: angiotensin II type 1 receptor, AT2R: angiotensin II type 2 receptor, CYP11B2: alodosterone synthase gene, α_2-AR: α_2-adrenergic receptor, B1AR: β1-adrenergic receptor, B2AR: β2-adrenergic receptor, END1: endothelin-1, ETAR: endothelin type A receptor, CMA: cardiac chymase, TNF: tumor necrosis factor α, TGFB1: transforming growth factor β-1, SOD: superoxide dismutase, PAF: platelet-activating factor, CCR: chemokine receptor, NOS: nitric oxide synthase, APOE: apolipoprotein E, GNB3: G-protein β_3 subunit, AMPD1: adenosine monophsophate deaminase 1.

Miscellaneous genetic polymorphisms

Multiple other polymorphisms have been investigated in heart failure, with variable success. These are mainly genetic variants of factors that play a role in cardiac remodeling, inflammation,

signal transduction or protection from oxidative damage. Genetic polymorphisms that have been associated with heart failure, heart failure phenotype and/or left ventricular structure are presented in table 1. However, few studies have been published for most of these variants and their exact role in heart failure susceptibility and modification needs to be elucidated further.

Comment

There is substantial evidence that genes play a role in the pathophysiology of heart failure. Numerous studies have investigated the association of heart failure with polymorphisms in candidate genes. Even more studies examined the role of genetic variants in left ventricular hypertrophy. Most studies focused on the ACE I/D polymorphism. In addition, genetic polymorphisms have been studied of other RAAS components, adrenergic receptors, endothelin-1 and endothelin receptors and of factors that play a role in cardiac remodeling, inflammation, signal transduction or protection of cells from oxidative damage. Still, many genes in heart failure remain to be discovered. So far, genetic association studies in heart failure and cardiac remodeling have been highly inconsistent. As heart failure is a complex trait, there are probably several genetic variants that together result in the expression of its pathologic phenotype [79]. Therefore, for individual polymorphisms, associations are likely to be modest. The same holds for variants associated with cardiac hypertrophy. The ultimate evidence of these genetic polymorphisms being more than just risk markers depends on the characterization of intermediate phenotypes that can be linked to the disease [102]. Since most studies on heart failure susceptibility did not find an association, a role for neurohormonal polymorphisms as modifier genes seems more likely.

Genetic associations that were found in initial studies have often been difficult to reproduce. There are several potential explanations for this. The major problem has been lack of power to detect the typically small effects in genetic association studies of multifactorial traits [103]. Besides increasing the sample size of a study population, this problem may be resolved by studying the combination of several genetic markers into haplotypes [77]. Small studies with statistically significant associations are more readily published. This may overestimate the true effect. Several studies used unconditional statistical methods for data analysis, despite the fact that they matched their cases and controls on population characteristics [e.g. 25, 31, 34, 37, 64, 71, 85]. This may underestimate the effect of the polymorphisms studied, as it will bias the results towards the null hypothesis.

An association between a genetic polymorphism and a disease (phenotype) may merely be caused by its linkage disequilibrium with a mutation of a nearby gene that is the actual functional gene. Patterns of linkage disequilibrium can vary significantly within and between populations due to several factors, including population admixture and

age of the mutation [18]. If a polymorphism is not functional, varying degrees of linkage disequilibrium may explain variations between populations. Much emphasis has been put on the role of population stratification in the generation of false positive results. When the population under study consists of a mixture of subpopulations that have different allele frequencies and disease risks, genetic associations can be confounded by population stratification [103]. The most important confounder in this respect is ethnicity. Frequency and outcome of heart failure differ significantly between races [4, 5], as do allele frequencies of genetic polymorphisms [28, 35, 38, 80]. Nearly all populations are confounded by genetic admixture at some level [104]. Lack of replicability of an association in different ethnic groups does not rule out the possibility of a causal association, because of potentially different background risks, allele frequencies and environmental factors. Genotyping error may also affect the results of genetic association studies and is the most common cause of deviations from the Hardy-Weinberg equilibrium. An important example of this type of exposure misclassification is the underestimation of ACE I/D heterozygotes that may occur with the conventional genotyping method [105]. Most studies on heart failure used this non-I-allele-specific method for ACE genotyping may have underestimated the effect of the DD genotype. In addition, large heterogeneity in outcome measures between studies may account for contradictory findings.

In summary, genetic association studies on heart failure and cardiac remodeling have focused on polymorphisms that may influence neurohormonal factors. Initial genetic associations have often been difficult to replicate, mainly due to faulty study designs and lack of power. Most promising results have been obtained with polymorphisms of RAAS and sympathetic system. A role for these polymorphisms as modifier rather than susceptibility genes seems more likely considering the evidence so far. Heart failure is probably caused by many genetic factors that are all components of larger complex systems and interact with environmental factors. Besides the need for larger studies to examine the effects of single genetic variants and haplotypes, future studies also need to focus on the complexity of these systems and study gene-gene interactions and gene-environment interactions.

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5.2

Insulin-like growth factor-I gene polymorphism and risk of heart failure. The Rotterdam Study

Abstract

Objective: Low serum insulin-like growth factor-I (IGF-I) levels have been associated with heart failure. A polymorphism in the IGF-I promoter region may influence the expression of IGF-I. We studied the association between this polymorphism and incident heart failure.
Methods and results: We used data from 4963 participants of the population-based Rotterdam Study without heart failure at baseline aged 55 to 75 years. Incident heart failure was determined according to established criteria. IGF-I genotypes were classified based upon presence or absence of the 192-bp allele and upon presence or absence of the following combination of genotypes: 192-bp/192-bp, 192-bp/194-bp and 194-bp/194-bp. Persons with any other genotype than 192-bp/192-bp, 192-bp/194-bp and 194-bp/194-bp had an increased risk of heart failure (RR 1.39, 95% CI 1.06-1.80).
Conclusions: Our study confirms recent findings and suggests that genetically determined chronic exposure to low IGF-I levels is associated with an increased risk for heart failure in elderly individuals.

Introduction

Heart failure is a complex clinical syndrome with high morbidity and mortality [1, 2]. Over the years, evidence for the cardioprotective effects of serum insulin-like growth factor-I (IGF-I) has accumulated [3, 4]. In a recent prospective study, performed in elderly without a myocardial infarction participating in the Framingham Heart Study, a low serum IGF-I level was associated with an increased risk of heart failure [5].

Twin studies have shown that at least 40% of circulating IGF-I levels is genetically determined [6]. A polymorphism in the IGF-I gene promoter region, comprising a variable length of a cytosine-adenosine (CA)-repeat sequence, may influence IGF-I production [7-9]. Earlier, we observed lower circulating total IGF-I levels in non-carriers of the wild type allele of this polymorphism than in carriers [8, 9]. We also found an association between this genetic variant and diabetes mellitus, body height and myocardial infarction [8] and carotid intima-media thickness in hypertensive individuals [10]. Because serum IGF-I levels may fluctuate, this polymorphism might be a better indicator for chronic IGF-I exposure in the body, both locally and systemically, than an incidental measurement of the actual serum IGF-I concentrations.

We investigated the association between this IGF-I polymorphism and the incidence of heart failure in a prospective population-based cohort study.

Methods

Setting and study population

The Rotterdam Study is a population-based cohort study in 7983 subjects of 55 years or older [11]. The baseline examination was conducted in 1990-1993, during which information was obtained on age, gender, smoking, hypertension, diabetes mellitus, body mass index (BMI), verified history of myocardial infarction, coronary artery bypass graft (CABG) and percutaneous transluminal coronary angioplasty (PTCA). Hypertension was defined as use of antihypertensives, or as a systolic blood pressure of 160 mm Hg or higher, or a diastolic blood pressure of 100 mm Hg or higher. Participants are continuously monitored through automated linkage with files from general practitioners for major events that occur during follow-up, including incidence of heart failure, myocardial infarction, CABG, PTCA and death. Information on vital status is also obtained regularly from municipal health authorities in Rotterdam. Furthermore, all drug prescriptions dispensed to participants by automated pharmacies are routinely stored in the database.

As serum IGF-I levels show an age-dependent decline, which we found to be genotype dependent and may be associated with longevity, we excluded all subjects aged 75 years or older to avoid any bias in our results because of selective mortality [9, 10, 12]. Hence,

the study population consisted of 4963 persons younger than 75 years, for whom blood specimens were available for IGF-I genotyping and who were free from heart failure at baseline. Participants were followed from baseline until the earliest of incident heart failure, death, loss to follow-up, date of last data collection, or January 1, 2000.

IGF-I genotype

IGF-I genotyping was performed as described elsewhere [8]. Two a priori defined classifications for IGF-I genotypes were examined. First, we studied genotypes based upon the presence of the 192-bp allele (corresponding to 19 CA-repeats), as described in previous studies [7-9], resulting in participants homozygous (reference group) or heterozygous for the 192-bp allele and non-carriers of this allele. Second, we studied genotypes based upon the presence (reference group) or absence of one of the following genotypes: 192-bp/192-bp, 194-bp/194-bp or 192-bp/194-bp. This second categorization was investigated because we found earlier that circulating serum IGF-I levels were highest for persons with 192-bp and 194-bp alleles, while both alleles shorter than 192-bp and longer than 194-bp seemed to have lower serum IGF-I levels, suggesting a broader optimum for IGF-I gene regulated transcriptional activity [Rietveld I. Functional aspects of a $(CA)_n$ polymorphism in the promoter region of the IGF-I gene. Presented at the Endocrine Society's 85th annual meeting, 19-22 June 2003, Philadelphia].

Incident heart failure

Incident heart failure events were obtained by continuously monitoring participants of the Rotterdam Study for the occurrence of heart failure during follow-up through automated linkage with files from general practitioners. All available data on these events, such as hospital discharge letters and notes from general practitioners, were copied from the medical records. Apart from this systematic follow-up procedure, we used verified hospital discharge diagnoses for case finding, gathered from all hospitals in the Rotterdam area. The definition of heart failure was based on the criteria of the European Society of Cardiology, which include the presence of typical symptoms of heart failure, such as pulmonary crepitations, and objective evidence of cardiac dysfunction [1].

Analysis

We used Cox proportional hazards regression analysis to estimate relative risks and 95% confidence intervals (95% CI). Age in days was used as time-axis of the model instead of follow-up for optimal age-adjustment. Analyses were initially adjusted for gender and follow-up. In the second model, we additionally adjusted for the following baseline factors:

smoking status, BMI, diabetes mellitus, hypertension, atrial fibrillation, the ratio of serum total cholesterol to high-density lipoprotein (HDL) cholesterol, history of myocardial infarction, CABG and PTCA. Furthermore, this model was adjusted for the incidence of an ischemic heart disease event, consisting of either a myocardial infarction, CABG or PTCA. This model was also adjusted for the occurrence of a first automated filled prescription of any anti-diabetic drug during follow-up, as a proxy for incident diabetes mellitus, and of any antihypertensive drug. Men and women were pooled in the analyses, as there was no significant effect-modification by gender. Analyses were repeated in a sub-population without myocardial infarction.

Results

During a mean follow-up of 7.2 years (SD 1.7), 280 cases of incident heart failure were identified. Mean age of the 4963 participants was 65 years (SD 5.5), 57% were women and mean BMI was 26.3 kg/m^2 (SD 3.6). Diabetes mellitus was present at baseline in 8%, history of myocardial infarction in 5%, and hypertension in 31% of participants. Based upon the 'traditional' IGF-I genotype classification, 2170 (44%) participants were homozygous and 2196 (44%) were heterozygous for the 192-bp allele, while 597 (12%) were classified as non-carriers. Having either an IGF-I 192-bp/192-bp, 192-bp/194-bp or 194-bp/194-bp genotype was present in 3586 (72%) and absent in 1377 (28%) participants. IGF-I genotype distributions were in Hardy-Weinberg equilibrium.

Table 1 shows that absence of the 192-bp allele was not significantly associated with incident heart failure. Relative risk of heart failure was slightly, but non-significantly increased in heterozygotes and non-carriers of the 192-bp allele. Absence of 192-bp/192-bp, 192-bp/194-bp and 194-bp/194-bp genotypes significantly increased the risk of incident heart failure (RR 1.39, 95% CI 1.06-1.80, table 1). Results for both genotype categorizations did not change after restriction of the population to subjects without a prevalent myocardial infarction. Relative risk of heart failure for the absence of 192-bp/192-bp, 192-bp/194-bp and 194-bp/194-bp genotypes in this population was 1.41 (model 2, 95% CI 1.05-1.89).

Table 1. Relative risks of incident heart failure and IGF-I genotypes.

IGF-I genotype	model 1†	model 2‡
192-bp/194-bp present*	1.00 (reference)	1.00 (reference)
192-bp/194-bp absent*	1.38 (1.07-1.76)	1.39 (1.06-1.80)
Homozygous 192-bp allele	1.00 (reference)	1.00 (reference)
Heterozygous 192-bp allele	1.21 (0.94-1.56)	1.27 (0.98-1.65)
Non-carrier 192-bp allele	1.26 (0.87-1.83)	1.16 (0.78-1.74)
Heterozygous and non-carrier combined	1.22 (0.96-1.55)	1.25 (0.97-1.60)

Relative risk (95% confidence interval)
* present: presence of one of the following combination of genotypes: 192-bp/192-bp, 194-bp/194-bp and 192-bp/194-bp; 192-bp/194-bp, absent: all other IGF-I genotypes
† adjustment for age, gender and follow-up
‡ adjustment for age, gender, follow-up, smoking status, body mass index, diabetes mellitus, hypertension, atrial fibrillation, ratio serum total cholesterol to high-density lipoprotein cholesterol, myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, incident ischemic heart diseases, first prescription anti-diabetic drug and first prescription antihypertensive drug

Discussion

In this large prospective population-based study, absence of IGF-I 192-bp/192-bp, 192-bp/194-bp and 194-bp/194-bp genotypes was associated with an increased risk of incident heart failure in subjects aged between 55 and 75 years. No significant association was found for the traditional division in genotypes based upon the presence of the 192-bp allele. This might be explained by the existence of another genetically determined optimum in IGF-I serum levels than previously hypothesized.

There is substantial evidence that IGF-I plays a pivotal role in the pathophysiology of cardiac remodeling and heart failure [3, 4]. Recently, Vasan and colleagues found an association between low serum IGF-I levels and increased risk of incident heart failure in elderly without a history of myocardial infarction participating in the Framingham Heart Study [5]. Our study seems to confirm their findings, since the lowest serum IGF-I levels were found in non-carriers of the 192-bp and 194-bp IGF-I gene alleles and these subjects had a higher risk of incident heart failure. In addition, our study suggests that to some extent the risk of incident heart failure is genetically determined.

Several studies have investigated the potential functionality of the IGF-I polymorphism with conflicting results. This CA-repeat polymorphism has been associated with serum IGF-I levels, albeit with opposite directions [7-9, 13, 14]. Differences in population background (environmental- and genetic factors), age and small sample sizes may all account for contradictory results. Also, all studies used the conventional division of genotypes based

upon presence of the 192-bp allele, while we found earlier that there may be a broader optimum for IGF-I gene regulated transcriptional activity.

Confounding bias may provide an alternative explanation for our results. However, as we studied a homogeneous Caucasian population and potential confounding factors did not greatly influence our results, this explanation seems less likely.

In conclusion, we found an association between a promoter polymorphism in the IGF-I gene and incident heart failure in the Rotterdam Study population. Our study confirms recent findings in the Framingham Heart Study that low serum IGF-I levels increase the risk of heart failure in normal elderly individuals and suggests that to some extent the risk of incident heart failure is genetically determined.

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5.3

Angiotensin converting enzyme Insertion/ Deletion polymorphism and risk of heart failure in hypertensive subjects

Abstract

Aims: Cardiac angiotensin-I converting enzyme (ACE) activity is influenced by the ACE I/D polymorphism. Evidence suggests that the DD-genotype may be a risk factor for cardiac hypertrophy and heart failure, especially in hypertensive subjects. We assessed the relation between the ACE I/D polymorphism and the risk of incident heart failure in normotensive and hypertensive subjects.

Methods and Results: We investigated 4264 normotensive and 2174 hypertensive participants of the Rotterdam Study; a population based prospective cohort study. All subjects were available for follow-up from 1990 until 2000. Incidence rates (IR) of heart failure in normotensive subjects were the same over all genotype strata (10 per 1000 person-years). In hypertensive subjects, the IR significantly increased with the number of D-alleles present (II: IR=13, ID: IR=18 and DD: IR=20 per 1000 person-years). Hypertensive subjects carrying the II-genotype did not have an increased risk of heart failure compared to normotensive subjects. However, hypertensive subjects carrying one or two copies of the D-allele did have a significantly increased risk of heart failure (ID: RR: 1.4 (1.1-1.7) and DD: RR: 1.5 (1.2-2.0)).

Conclusion: Our findings suggest that the ACE I/D polymorphism may play a modifying role in the development of heart failure in hypertensive subjects.

Introduction

Heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs adequate ventricular filling or ejecting of blood. Coronary artery disease and hypertension are among the most common risk factors. Regardless of the initial cause of cardiac damage, the heart will respond with a set of adaptive mechanisms in order to maintain its pumping function. Both clinical and experimental data suggest that activation of local renin angiotensin system (RAS) in the heart plays an important role in this compensatory mechanism to maintain adequate hemodynamic function [1]. Recent studies have shown that cardiac expression of angiotensin-I converting enzyme (ACE) and angiotensinogen is increased in experimental heart failure [2, 3]. In patients with chronic heart failure, cardiac expression of ACE was found to be increased up to threefold compared to the hearts of subjects without heart failure [4].

An Insertion/Deletion (I/D) polymorphism, characterized by the presence or absence of a 287-base pair Alu repeat sequence in intron 16 of the ACE gene, has been reported to be responsible for about 50% of the interindividual variability in serum ACE levels [5, 6]. Both serum ACE levels and cardiac ACE activity were highest in subjects carrying two copies of the D-allele [5-7]. The DD-genotype has been put forward as a risk factor for left ventricular remodelling in hypertensive subjects [8, 9]. Raynolds et al. observed an increased frequency of the DD-genotype in patients with both ischemic and idiopathic dilated cardiomyopathy [10].

We examined the role of the ACE I/D polymorphism in the development of heart failure in a population-based cohort study. Since several studies reported an effect of the D-allele on cardiac disease in hypertensive subjects only, we analysed normotensive and hypertensive subjects separately.

Methods

Study Population

The study was conducted within the Rotterdam Study, a single-centre prospective follow-up study in which all residents aged 55 years and over of the Rotterdam suburb of Ommoord were invited to take part. The baseline examination of the Rotterdam Study was conducted between 1990 and 1993. The Medical Ethics Committee of Erasmus Medical Centre Rotterdam approved the study. Written informed consent was obtained from all participants. The design of the study has been described previously [11]. 7983 participants were examined (response 78%). In 6869 subjects, the ACE I/D polymorphism was genotyped successfully (86%). In the remaining 1114 subjects, no genotypes were available. We excluded 211 subjects because no information on blood pressure levels was available.

At baseline, information concerning medical history, medication use and smoking behavior was obtained with a computerized questionnaire [11]. Blood pressure was measured, after a minimum of 5 minutes rest, in the sitting position at the right upper arm using a random zero sphygmomanometer. Participants were asked to abstain from smoking and drinking alcoholic or caffeine-containing beverages at least two hours before blood pressure measurements were taken. The average of two measurements was used for analysis. Hypertension was defined as a diastolic blood pressure (DBP) of 100 mmHg or higher and/or a systolic blood pressure (SBP) of 160 mmHg or higher and/or use of anti-hypertensive medication indicated for treatment of hypertension (grade 2 and 3 of the 1999 WHO/ISH criteria) [12, 13].

Heart failure assessment

Assessment of prevalent heart failure at the baseline examination in the Rotterdam Study has been described in detail earlier [14]. We excluded subjects with prevalent heart failure from our study (n=220). All participants of the Rotterdam Study were continuously monitored for the occurrence of heart failure during follow-up using automated linkage with files from general practitioners. All available medical data, such as hospital discharge letters and notes from general practitioners, were obtained from the medical records in case of possible heart failure. Apart from this systematic follow-up procedure, we used verified hospital discharge diagnoses for case finding, gathered from all hospitals in the Rotterdam area as described above. The date of incident heart failure was defined as the day of the first occurrence of symptoms suggestive of heart failure or the date of the first prescription of a loop diuretic or an ACE-inhibitor.

The diagnosis of heart failure was classified as definite, probable, possible or unlikely. Definite heart failure was defined as a combination of heart failure diagnosed by a medical specialist and the presence of typical symptoms of heart failure, such as breathlessness at rest or during exertion, ankle edema and pulmonary crepitations, confirmed by objective evidence of cardiac dysfunction (chest X-ray, echocardiography). This definition is in accordance with the criteria of the European Society of Cardiology [15]. Probable heart failure was defined as heart failure diagnosed by a general practitioner, with at least two typical symptoms suggestive of heart failure, and at least 1 of the following: history of cardiovascular disease (e.g. myocardial infarction, hypertension), response to treatment for heart failure, or objective evidence of cardiac dysfunction, while symptoms could not be attributed to another underlying disease.

Two research physicians independently classified all information on potential heart failure events. If there was disagreement, a consensus was reached in a separate session. Finally, a cardiologist verified all probable and possible cases, and all cases in which the two physicians could not reach consensus. If the cardiologist disagreed with the research

physicians, the cardiologist's judgment was considered decisive. The research physicians and the cardiologist based their decisions on the same data. Only definite and probable cases were included in the analyses.

Genotyping

The II, ID and DD genotypes were detected using the polymerase chain reaction technique (PCR) according to the method of Lindpaintner et al with some modifications [16]. In order to avoid misclassification of ID genotypes into DD genotypes, a second PCR was performed using an I-specific primer.

Statistical Analysis

Overall general characteristics of normotensive and hypertensive subjects and those stratified by ACE genotype, were compared using univariate analysis of variance for continuous variables and chi-square statistics for dichotomous variables. Incidence rates were calculated as number of cases per 1000 person-years using the exact Poisson formula and presented with 95% confidence intervals (CI). Relative risks of incident heart failure were assessed using Cox proportional hazard regression analysis. All risk estimates are presented with 95% CI. Normotensive subjects were used as the reference group in these analyses. We adjusted for age and sex in all analyses. To assess the effect of the ACE I/D polymorphism independent of possible confounding or mediating factors, analyses were repeated adding body mass index (BMI), diabetes mellitus, smoking, myocardial infarction, total and HDL-cholesterol to the model. Furthermore, we tested for statistical interaction between the ACE I/D polymorphism and hypertension by adding an interaction term to the regression model: hypertension (dichotomous) x ACE-genotype (categorical). We performed all analyses with SPSS version 11.0.

Results

A total of 6438 subjects were available for follow-up until January 1, 2000. Baseline descriptives of the total study population are presented in table 1. We included 4264 normotensive subjects and 2174 hypertensive subjects in our study. Both groups followed Hardy-Weinberg Equilibrium proportions for the ACE I/D polymorphism. Mean follow-up was 6.9 ± 2.1 years for normotensive subjects and 6.4 ± 2.4 years for hypertensive subjects. In hypertensive subjects, mean follow-up was significantly shorter for subjects carrying two copies of the D-allele than for subjects carrying two copies of the I-allele. Hypertensive subjects were significantly older and less often male than normotensive subjects. This difference was

Table 1. Baseline descriptives normotensive and hypertensive subjects: overall and stratified by ACE genotype.

	Normotensive subjects				Hypertensive subjects			
ACE genotype	Overall	II	ID	DD	Overall	II	ID	DD
Number (%)	4264 (66.0)	962 (22.6)	2106 (49.4)	1196 (28.0)	2174 (34.0)	441(20.3)	1116 (51.3)	617 (28.4)
Mean follow-up (yrs)	6.9 ± 2.1	6.9 ± 2.0	6.9 ± 2.1	6.9 ± 2.1	6.4 ± 2.4	6.6 ± 2.4	6.5 ± 2.3	6.3 ±2.4
Age (yrs)	68.0 ± 8.9	67.6 ± 8.8	68.1 ± 8.8	68.4 ± 9.2	71.0 ± 8.8	70.6 ± 9.0	71.1 ± 8.6	71.2 ± 9.1
Sex (male)	42.9	43.2	43.0	42.4	35.3	34.9	36.1	34.0
SBP (mmHg)	130.7 ± 16.1	130.6 ± 16.3	130.2 ± 16.4	131.4 ± 15.8	156.8 ± 22.4	156.3 ± 22.7	156.5 ± 22.2	157.6 ± 22.5
DBP (mmHg)	70.9 ± 10.0	70.8 ± 10.1	70.6 ± 9.9	71.4 ± 9.9	80.0 ± 12.0	80.4 ± 12.5	80.0 ± 12.0	79.4 ± 11.7
Diabetes mellitus	7.1	6.5	8.0	6.0	15.0	15.9	15.8	13.0
Myocardial Infarction	11.3	10.2	11.8	11.3	15.6	14.6	16.0	15.5
BMI (kg/m^2)	25.8 ± 3.5	25.8 ± 3.7	25.7 ± 3.5	25.9 ± 3.5	27.2 ± 3.9	27.5 ± 3.8	27.2 ± 4.0	27.1 ± 3.7
Total cholesterol	6.6 ± 1.2	6.5 ± 1.3	6.6 ± 1.2	6.6 ± 1.2	6.7 ± 1.2	6.8 ± 1.3	6.7 ± 1.3	6.7 ± 1.2
HDL-cholesterol	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.4	1.3 ± 0.4
Smoking (current)	24.7	25.9	25.5	22.4	18.4	16.6	19.3	17.9

All values are presented as percentage or mean ± standard deviation

the same over all genotype strata. Within the normotensive group, DBP was significantly higher in subjects carrying the DD-genotype compared to subjects carrying the ID-genotype. Prevalence of diabetes mellitus and myocardial infarction, mean BMI and total cholesterol levels were significantly higher in hypertensive subjects compared to normotensive subjects. In the hypertensive group, BMI was significantly higher in subjects carrying the II-genotype compared to subjects carrying the DD-genotype. HDL-cholesterol and percentage current smokers were significantly lower in hypertensive subjects than in normotensive subjects. This difference was the same over all genotype strata.

Table 2 shows number of cases, person-years and incidence rates (IR) of heart failure observed in normotensive and hypertensive subjects stratified by ACE genotype. During 44,883.1 person-years of follow-up 543 participants developed heart failure. In normotensive subjects, the IR of heart failure was about 10 per 1000 person-years, independent of genotype status. In hypertensive subjects, the IR of heart failure significantly increased with the number of D-alleles present. In subjects carrying the II-genotype the IR of heart failure was 13 per 1000 person-years (95%CI: 9-17). In subjects carrying one or two copies of the D-allele the IR of heart failure increased up to 18 (15-21) and 20 (16-24) per 1000 person-years, respectively (p for trend<0.05).

Table 2. Number of cases and incidence rates of heart failure stratified by ACE genotype in normotensive and hypertensive subjects.

	Normotension			Hypertension		
ACE genotype	Number of cases	Person years	IR (95%CI)	Number of cases	Person years	IR (95% CI)
II	67	6823.4	10 (8-12)	39	3020.2	13 (9-17)
ID	131	14970.7	9 (7-10)	138	7616.4	18 (15-21)
DD	88	8377.2	11 (8-13)	80	4075.2	20 (16-24)

Incidence rate (IR) presented as number of cases per 1000 person-years with 95% confidence interval.

Table 3. Risk of heart failure in hypertensive subjects compared to normotensive subjects: overall and stratified by ACE genotype.

	Model 1	Model 2
Normotensive subjects: Overall risk of heart failure	1.0 (ref.)	1.0 (ref.)
Hypertensive subjects: Overall risk of heart failure	1.6 (1.4-1.9)	1.4 (1.2-1.7)
Risk of heart failure stratified by ACE genotype		
II-subjects	1.2 (0.8-1.6)	1.2 (0.8-1.6)
ID-subjects	1.6 (1.3-1.9)	1.4 (1.1-1.7)
DD-subjects	1.7 (1.3-2.2)	1.5 (1.2-2.0)

All risk estimates are compared to all normotensive subjects (reference group). Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, body mass index, smoking, diabetes mellitus, myocardial infarction, total and HDL-cholesterol.

In table 3, the relative risks (RR) of heart failure for hypertensive subjects, overall and stratified by ACE genotype, compared to normotensive subjects are presented. Overall, hypertensive subjects had a significantly increased risk of heart failure of 1.4 (1.2-1.7) compared to normotensive subjects (model 2). Hypertensive subjects carrying two copies of the I-allele did not have an increased risk of heart failure compared to normotensive subjects (RR: 1.2 (0.8-1.6) (model 2). However, hypertensive subjects carrying one or two copies of the D-allele had a significantly increased risk of heart failure compared to normotensive subjects: ID: RR: 1.4 (1.1-1.7) and DD: RR: 1.5 (1.2-2.0) (model 2). Statistical interaction between the D-allele of the ACE-genotype and hypertension was borderline significant, (p=0.059).

Discussion

We observed an increased risk of heart failure in hypertensive subjects compared to normotensive subjects that was dependent on the presence of the D-allele of the ACE I/D polymorphism. Hypertensive subjects did not have a significantly increased risk of heart failure compared to normotensive subjects, unless they carried one or two copies of the D-allele. The incidence rate of heart failure in hypertensive subjects significantly increased with the number of D-alleles present. As the incidence of heart failure marks the end of the follow-up period, this may also explain the shorter follow-up period observed in hypertensive DD subjects compared to II subjects.

Hypertension is the most common condition antedating heart failure in the general population [17, 18]. Especially in the elderly, heart failure is often preceded by long standing high blood pressure and LVH [19, 20]. However, the extent of cardiac remodelling does not always seem to correlate with the extent of cardiac damage. In fact, mild hypertension may lead to severe heart failure whereas severe hypertension may be without any perceivable effects on cardiac function. As a consequence, it has been hypothesized that genetic factors may modulate the manifestation or progression of cardiac remodeling [21].

The ACE I/D polymorphism is by far the most frequently studied candidate gene in the development of left ventricular hypertrophy and heart failure. Homozygosity for the D-allele has been associated with higher prevalence of LVH and increased heart weight in (untreated) hypertensive subjects [8, 9, 22-24]. Raynolds et al. were the first to report an association between the ACE I/D polymorphism and heart failure. They observed an increased frequency of the DD-genotype in subjects with ischemic and dilated cardiomyopathy [10]. Since local formation of angiotensin II within the myocardium is thought to be involved in the cardiac remodelling process, elevated cardiac angiotensin II levels in subjects carrying the D-allele, may partly explain the association between the DD-genotype and various cardiac disorders [7].

Nevertheless, findings remain controversial and so far positive and negative results seem to outweigh each other [25-27]. Many of the conflicting findings on the ACE I/D polymorphism and cardiac disease are most likely due to small sample sizes and large heterogeneity of the populations that were studied. Another reason for the inconsistent findings may be that the ACE I/D polymorphism by itself does not have enough biological significance to exert an effect on cardiac tissue, especially since the RAS is normally under strict negative feedback inhibition. This has led to the hypothesis that an effect of the ACE I/D polymorphism on cardiac function may only become clinically relevant under specific conditions in which the cardiac growth machinery is already activated [28]. In line with this hypothesis, Montgomery et al. observed increased left ventricular mass after rigorous exercise only in those participants who carried a copy of the D-allele [29]. Another study observed increased adverse cardiac remodelling in subjects with the ACE ID- and DD-genotype after they had experienced a myocardial infarction [30].

We believe our findings provide additional evidence for a modifying effect of the ACE I/D polymorphism in the development of cardiac disease. In our study, the D-allele was associated with an increased risk of heart failure in hypertensive subjects only, which may suggest that the D-allele has an effect on the heart merely when local RAS is already activated because of increased heamodynamic load. Since asymptomatic cardiac remodelling usually precedes the development of clinically overt heart failure in hypertensive subjects, we believe our findings are in accordance with the observation that, especially in subjects with hypertension, the D-allele of the ACE I/D polymorphism is associated with increased levels of various echocardiography measures of cardiac hypertrophy [8, 9, 31, 32].

Until now, our study is the largest population based study that assessed the role of the ACE I/D polymorphism in heart failure in a relatively homogenous population, as 98% of the participants in our study are Caucasians and they all live in the same area of Rotterdam. In contrast to case-control studies on heart failure that have been conducted so far, the prospective nature of our study makes our results less prone to survival bias. Still several issues need to be addressed. In our study, we were not able to discern the different etiologies of heart failure (idiopathic, ischemic or other). However, we think that the ACE I/D polymorphism may be more important as a modulator in the way the myocardium responds to cardiac damage ("remodelling") than in the events leading to cardiac damage. The ACE I/D polymorphism may increase the risk of heart failure through an effect on blood pressure or an increased risk of myocardial infarction; however, our results do not support this. On the contrary, our findings suggest that hypertension by itself is not a real strong predictor of heart failure, unless one or two copies of the D-allele are present. Furthermore, correction for baseline and incident MI in our analyses did not change the association between heart failure and the ACE I/D polymorphism. In addition, the prevalence of MI did not differ significantly between the genotype groups in hypertensive subjects.

In conclusion, our findings suggest that the ACE I/D polymorphism may play a modifying role in the development of heart failure in hypertensive subjects, regardless of the initial cause of cardiac damage. We believe these findings may provide an additional genetic clue as to whether some hypertensive subjects do develop cardiac hypertrophy resulting in heart failure, whereas others do not.

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Chapter 6

Pharmacogenetics

6.1

Mortality in patients with hypertension on angiotensin-I converting enzyme (ACE)-inhibitor treatment is influenced by the ACE Insertion/ Deletion polymorphism

Abstract

Background: The response to angiotensin-I converting enzyme (ACE)-inhibitor therapy is highly variable. Residual ACE activity during treatment, potentially modified by the ACE Insertion/Deletion (I/D) polymorphism, may explain part of this variability. We studied the possible interaction between ACE-inhibitor therapy in patients with hypertension and the ACE I/D polymorphism in incident heart failure and death.

Methods: We studied 3365 hypertensive participants of the population-based Rotterdam Study, without heart failure at baseline for whom ACE-genotyping was successful. Incident heart failure was defined according to established criteria. In addition, total and cardiovascular mortality were studied as endpoints. A Cox regression model with use of ACE-inhibitors defined as time-dependent covariates was used for data-analysis. Interaction was tested in this model assuming an allele-effect relationship.

Results: Although we could not demonstrate a beneficial effect of ACE-inhibitors, there was significant interaction between the ACE I/D polymorphism (II-ID-DD) and ACE-inhibitor use in the prediction of total and cardiovascular mortality. Mortality risk associated with treatment increased with the number of D alleles present; e.g. for total mortality in the II genotype group: RR=0.95 (95% CI 0.63-1.45), in the ID genotype group: RR=1.08 (95% CI 0.84-1.38) and in the DD genotype group: RR=1.61 (95% CI 1.18-2.18). No statistically significant interaction was found for incident heart failure.

Conclusion: The results of our study suggest a relative resistance to ACE-inhibitor therapy in subjects with hypertension and the DD genotype compared to the II genotype, with the ID genotype in an intermediate position.

Introduction

Heart failure is a complex clinical syndrome resulting in substantial morbidity and mortality [1, 2]. Especially in elderly subjects, heart failure is often preceded by hypertension [1]. It is, however, difficult to predict who will develop heart failure or other end-organ damage in response to a high blood pressure. The renin-angiotensin-aldosterone system (RAAS) plays a major role in cardiovascular pathophysiology. An Insertion/Deletion (I/D) polymorphism located in intron 16 of the angiotensin-I converting enzyme (ACE) gene is responsible for approximately 50% of inter-individual variability in circulating ACE levels, which are highest in subjects with the DD genotype [3]. The ACE I/D polymorphism has also been demonstrated to influence ACE expression in cardiac tissue [4]. Therefore, many genetic association studies have investigated the ACE I/D polymorphism as a risk factor for cardiovascular disease with, in general, contradictory results [5-8]. However, promising results have been reported in studies examining the ACE polymorphism as a modifier gene in heart failure and hypertension; hence, a role for this variant in the development of cardiovascular disease may only become apparent under specific stressful conditions [5, 6].

ACE-inhibitors are widely used in the treatment of hypertension and heart failure, because of their blood pressure lowering effects and their positive effects on cardiovascular morbidity and mortality [9,10]. However, the individual response to ACE-inhibitor therapy is highly variable and the RAAS is not uniformly suppressed during treatment with ACE-inhibitors [11]. This diversity in response is partially explained by environmental factors, such as salt intake [12]. In addition, residual levels of enzyme activity during ACE-inhibition, potentially modified by ACE I/D genotype, may be important [13, 14].

Studies on the interaction between ACE I/D genotype and the use of an ACE-inhibitor in subjects with hypertension have focused on the efficacy of ACE-inhibitors in blood pressure reduction and regression of left ventricular hypertrophy [5, 13-17]. Results have been inconclusive so far. None of these studies included major cardiovascular endpoints. Therefore, we studied the possible interaction between ACE-inhibitor therapy and the ACE I/D polymorphism in the prediction of incident heart failure and death in a large cohort of Caucasian elderly subjects with hypertension.

Methods

Setting and study population

The Rotterdam Study is a population-based prospective cohort study in 7983 subjects of 55 years and older and has been approved by the Medical Ethics Committee of the Erasmus Medical Center [18]. The baseline examination was conducted between 1990 and 1993. Participants were visited at home for a standardised questionnaire and were subsequently

examined at the research center. At baseline, information was obtained on several characteristics, including age, gender, smoking, blood pressure, diabetes mellitus, body mass index (BMI), medication use, verified history of myocardial infarction, heart failure, coronary artery bypass graft (CABG), and percutaneous transluminal coronary angioplasty (PTCA). A research physician determined the indication for each drug that was currently used by the participant. Hypertension was defined as use of antihypertensive drugs for the indication of high blood pressure, or as a systolic blood pressure of 140 mm Hg or higher, or a diastolic blood pressure of 90 mm Hg or higher. Hypertension was present in 3678 participants. For the present study we included 3365 participants with hypertension, without prevalent heart failure (n=135), for whom ACE genotyping was successful.

In the Rotterdam Study, participants are continuously monitored for major events that occur during follow-up, including heart failure, myocardial infarction, CABG and PTCA. This occurs through automated linkage with files from general practitioners. All available information on these events is copied from the medical records for verification of the diagnosis. Furthermore, all drug prescriptions dispensed to participants by all pharmacies in the study area are routinely stored in the database since January 1, 1991. Information on vital status is obtained regularly from municipal health authorities in Rotterdam and from the general practitioners in the study district, and was complete for all participants until January 1, 2000.

ACE genotyping

The ACE II, ID and DD genotypes were detected using a polymerase chain reaction (PCR) technique according to the method of Lindpaintner et al. with some modifications [19]. In order to avoid misclassification of ID-genotypes as DD-genotypes, a second independent PCR was performed with a primer pair that recognises an insertion specific sequence (5'TGG GAC CAC AGC GCC CGC CAC TAC3' and 5'TCG CCA GCC CTC CCA TGC CCA TAA3'). To optimise the second PCR, 10% dimethyl sulfoxide (DMSO), 0.35 units AmpliTaq Gold DNA polymerase and GeneAmp PCR Gold buffer (Applied Biosystems) were used in the PCR mix. This reaction yielded a 335-bp amplicon only if the I-allele was present. Two independent investigators read pictures from each gel and were not aware of the objective of the present study. All ambiguous samples were analysed a second time.

Exposure to ACE-inhibitors

Complete information on all prescriptions for ACE-inhibitors dispensed to participants was available in automated form since January 1, 1991 and was used to create time-dependent variables of use and non-use of ACE-inhibitors. Participants were considered to be exposed if a prescription for an ACE-inhibitor was filled during follow-up, in the period before the index date. Hereto, for every prescription, the length was calculated by dividing the number

of dispensed pills by the prescribed daily number. In this way the follow-up period for every participant was distinguished into periods of use and non-use. The index date was defined as the date on which an endpoint occurred for a participant of this study.

Heart failure assessment

Assessment of prevalent heart failure at baseline has been described in detail earlier [20]. Briefly, a validated score was used, similar to the definition of the European Society of Cardiology [1]. This score was based on the presence of at least two symptoms suggestive of heart failure or treatment for heart failure, in combination with objective evidence of cardiovascular disease. This score was, however, not implemented from the first beginning of the Rotterdam Study, but was subsequently added. Consequently, this information was obtained in only 5540 participants. In addition, prevalent heart failure cases were obtained through a database containing hospital discharge diagnoses from all hospitals in the Rotterdam area. Furthermore, all medical records were screened in retrospect for the occurrence of heart failure in the majority (97%) of participants. With these three methods, information on prevalent heart failure was available for all participants.

Cases of incident heart failure were obtained by continuously monitoring participants of the Rotterdam Study for the occurrence of heart failure during follow-up through automated linkage with files from general practitioners. All available data on these events, such as hospital discharge letters and notes from general practitioners, were copied from the medical records. Apart from this systematic follow-up procedure, we used verified hospital discharge diagnoses for case finding, gathered from all hospitals in the Rotterdam area as described above. The diagnosis of heart failure was classified as definite, probable, possible or unlikely. Definite heart failure was defined as a combination of heart failure diagnosed by a medical specialist and the presence of typical symptoms of heart failure, such as breathlessness at rest or during exertion, ankle edema and pulmonary crepitations, confirmed by objective evidence of cardiac dysfunction (chest X-ray, echocardiography). This definition is in accordance with the criteria of the European Society of Cardiology [1]. Probable heart failure was defined as heart failure diagnosed by a general practitioner, with at least two typical symptoms suggestive of heart failure, and at least 1 of the following: history of cardiovascular disease (e.g. myocardial infarction, hypertension), response to treatment for heart failure, or objective evidence of cardiac dysfunction, while symptoms could not be attributed to another underlying disease, such as chronic obstructive pulmonary disease. Two research physicians independently classified all information on potential heart failure events. If there was disagreement, a consensus was reached in a separate session. Finally, a cardiologist verified all probable and possible cases, and all cases in which the two physicians could not reach consensus. If the cardiologist disagreed with the research physicians, the cardiologist's judgment was considered decisive. Only definite and probable cases were included in the analyses.

Analysis

We used Cox proportional hazards regression analysis to estimate relative risks (RR) and 95% confidence intervals (95% CI) for exposure to an ACE-inhibitor in relation to the incidence of three predefined endpoints: heart failure, death and the combined endpoint of heart failure or death (whichever came first). Participants were followed from January 1, 1991 until the earliest of incident heart failure, death, loss to follow-up, date of last data collection, or January 1, 2000. Exposure to an ACE-inhibitor was represented in the model by a time-dependent covariate to compare any use with non-use at different points in time. To ensure optimal adjustment for age, age in days was used as the time-axis of the model instead of follow-up. This resulted in a model that compares the exposure status of each case with the status of all other participants who are alive and free of heart failure at exactly the age of the case at the index date. Analyses were performed stratified by ACE I/D genotype.

Analyses were initially adjusted for gender and calendar time. In the second model, we additionally adjusted for the following baseline factors: smoking status (current/former/never), BMI, diabetes mellitus, systolic blood pressure and history of ischemic heart disease, consisting of either a myocardial infarction, CABG or PTCA. Furthermore, this model was adjusted for incident ischemic heart disease events. We also adjusted this model in a time varying manner for the occurrence of a first automated filled prescription of any anti-diabetic drug during the study period, as a proxy for incident diabetes mellitus, and of any other antihypertensive drug, including β-blockers, diuretics, calcium antagonists and other antihypertensives. All incident cofactors were defined in the time-varying model using age in days as the time-axis.

We tested for interaction between the ACE I/D polymorphism and the use of ACE-inhibitors by adding an interaction term to the regression model: ACE-genotype (0-1-2: DD-ID-II) x ACE-inhibitor use (0-1), assuming an allele-effect relationship. A two-sided p-value < 0.05 was considered statistically significant. Additional analyses were performed with the endpoint cardiovascular mortality (ICD10 codes I20-I25, I46, I49, I50 and R96) and after exclusion of participants, who were exposed to an ACE-inhibitor in the first 100 days of follow-up, used as a proxy for prevalent exposure.

Results

A total of 3365 individuals with hypertension were included in our study population. Approximately 45% of these subjects used antihypertensive therapy according to their baseline interview. Table 1 presents baseline characteristics according to ACE genotype and the use of an ACE-inhibitor at any time during the study period. Genotype and allele distributions were in Hardy-Weinberg equilibrium for both groups and for the total study population (p=0.5). During a mean follow-up of 7.8 years (SD 2.1), 354 cases of incident heart

Table 1. Baseline characteristics according to the use of an angiotensin converting enzyme (ACE)-inhibitor during the study period: overall and stratified by ACE genotype.

	ACE-inhibitor (-)				ACE-inhibitor (+)			
ACE genotype	Overall	II	ID	DD	Overall	II	ID	DD
Number	2410	500 (21%)	1211 (50%)	699 (29%)	955	206 (22%)	481 (50%)	268 (28%)
Mean follow-up (years)	7.7 (2.1)	7.7 (2.2)	7.7 (2.2)	7.8 (2.1)	8.0 (1.8)*	8.2 (1.7)**	8.1 (1.8)**	7.8 (2.0)**
Age (years)	69.6 (9.0)	69.4 (9.1)	69.7 (8.9)	69.5 (9.2)	68.4 (8.0)*	67.9 (8.1)	68.6 (7.9)	68.5 (8.1)
Female	1500 (62%)	322 (64%)	740 (61%)	438 (63%)	563 (59%)	119 (58%)	285 (59%)	159 (59%)
SBP (mm Hg)	153 (17)	153 (17)	152 (17)	153 (17)	155 (21)*	153 (21)	156 (21)	156 (20)
DBP (mm Hg)	78 (11)	78 (11)	78 (11)	78 (10)	80 (12)*	80 (12)	80 (12)	80 (12)
Diabetes mellitus	287 (12%)	62 (12%)	156 (13%)	69 (10%)	143 (15%)*	27 (13%)	81 (17%)	35 (13%)
Myocardial infarction	117 (5%)	25 (5%)	63 (5%)	29 (4%)	83 (9%)*	13 (6%)	50 (10%)	20 (7%)
Body mass index (kg/m2)	26.7 (3.8)	26.7 (3.8)	26.7 (3.8)	26.6 (3.7)	27.4 (3.9)*	27.7 (4.4)	27.3 (3.9)	27.4 (3.7)
CABG	48 (2%)	6 (1%)**	22 (2%)**	20 (3%)**	37 (4%)*	7 (3%)	23 (5%)	7 (3%)
PTCA	18 (1%)	2 (0.4%)	15 (1%)	1 (0.1%)	8 (1%)	1 (0.5%)	7 (2%)	8 (3%)
Smoking (current)	472 (20%)	94 (19%)	253 (21%)	125 (18%)	182 (19%)	31 (15%)	98 (20%)	53 (20%)

All values are presented as number (percentage) or mean ± standard deviation. * Significantly different from non-users, $p<0.05$. **Significant trend over genotypes in users or non-users, $p<0.05$. Overall no significant trend over genotypes, $p>0.05$. SBP: systolic blood pressure, DBP: diastolic blood pressure, CABG: coronary artery bypass graft, PTCA: percutaneous transluminal coronary angioplasty. SBP and DBP: participants who were treated with antihypertensive drugs at baseline were included

failure were identified and 821 subjects died. Throughout the study period, 955 participants accumulated person-time in the exposed category. These subjects were on average younger at baseline and more likely to have diabetes mellitus, a history of myocardial infarction and a history of CABG (table 1). Also, blood pressure levels and BMI were on average higher in these subjects. Mean follow-up was 0.3 years longer for participants who received an ACE-inhibitor than for those who did not. The ACE I/D genotype distribution did not differ between the two exposure groups. Exposure to the other antihypertensive drugs during the study period was also not associated with the ACE I/D polymorphism. Overall, baseline characteristics were not significantly different between ACE I/D genotypes (p-value trend>0.05). In subjects who did not receive an ACE-inhibitor during the study period, a history of CABG was more frequent in the DD genotype group than in the II genotype group, with ID heterozygotes in an intermediate position. In persons who were exposed to an ACE-inhibitor during the study period, mean follow-up increased with the number of I alleles present.

Table 2 presents relative risk estimates for the associations between the use of an ACE-inhibitor and incident heart failure, death and the combined endpoint of heart failure or death, stratified by ACE I/D genotype. Despite the adjustment for a large number of cardiovascular risk factors, a beneficial effect of ACE-inhibitor therapy could not be demonstrated in our study population. ACE I/D genotype did not modify the association between the use of an ACE-inhibitor and incident heart failure. There was, however, a significant interaction between the ACE I/D polymorphism and ACE-inhibitor use in the prediction of total mortality. The risk of death of any cause was elevated in users of ACE-inhibitors compared to non-users in the DD group, while this was not observed in both ID and II genotype groups. The same trend over the three ACE genotypes was observed for the association between ACE-inhibitor therapy and the combined outcome of heart failure or death (table 2).

When we restricted our analyses to cases of cardiovascular mortality (ICD10 codes I20-I25, I46, I49, I50 and R96; number of cases: 206), a similar worsening trend over ACE genotypes was detected: the relative risk for cardiovascular mortality with the use of an ACE-inhibitor was 1.15 (95% CI 0.53-2.49) in the II genotype group, 1.58 (95% CI 1.01-2.47) in the ID genotype group, and 2.97 (95% CI 1.61-5.48) in the DD genotype group; p-value for interaction = 0.05. To investigate the effect of exposure misclassification due to prevalent use of ACE-inhibitors at baseline, we excluded all participants who were exposed to an ACE-inhibitor in the first 100 days of follow-up. This yielded the same results: a significant interaction between ACE genotype and ACE-inhibitor use for death and the compound endpoint of heart failure or death, but not for incident heart failure (results not shown).

Table 2. Relative risks for the associations between the use of an angiotensin converting enzyme (ACE)-inhibitor and heart failure, death and the combined endpoint heart failure or death, stratified by ACE genotype.

	Heart failure (n=354)		Death* (n=821)		Heart failure or death *† (n=989)	
Genotype stratum	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**
II	1.30 (0.75-2.25)	1.01 (0.55-1.85)	1.06 (0.72-1.56)	0.95 (0.63-1.45)	1.05 (0.74-1.49)	0.93 (0.64-1.36)
ID	1.29 (0.91-1.83)	1.14 (0.79-1.65)	1.22 (0.97-1.54)	1.08 (0.84-1.38)	1.22 (0.99-1.51)	1.09 (0.87-1.36)
DD	1.58 (1.01-2.46)	1.20 (0.74-1.92)	1.93 (1.45-2.57)	1.61 (1.18-2.18)	1.68 (1.29-2.19)	1.36 (1.02-1.80)

Model 1: adjusted for age, gender and calendar time; **Model 2**: adjusted for age, gender, calendar time, body mass index, smoking status (current/former/never), prevalent and incident diabetes mellitus, systolic blood pressure, prevalent and incident ischemic heart disease, consisting of myocardial infarction, coronary artery bypass graft and percutaneous transluminal coronary angioplasty, and a first prescription during the study period of a diuretic, a -blocker, a calcium antagonist or an other antihypertensive drug

* p-value < 0.05 for the interaction term in multivariate models: ACE I/D genotype (0-1-2: II-ID-DD) x ACE-inhibitor (0-1), † whichever came first

Discussion

In this large prospective population-based study, we found a relative resistance to ACE-inhibitor therapy in subjects with hypertension and the DD genotype compared to the II genotype, with the ID genotype in an intermediate position. Mortality risk associated with treatment increased with the number of D alleles present. This trend was observed for both total and cardiovascular mortality. The risk of incident heart failure associated with ACE-inhibitor therapy was, however, not significantly different between strata of ACE genotypes. A potential explanation for this might be that our study lacked sufficient power to identify small differences between genotype strata in the relative risk of heart failure with the use of ACE-inhibitors. Another explanation might be that subjects with the DD genotype could have died before they developed heart failure, and that these subjects would have developed heart failure if they had lived longer. We could not demonstrate a beneficial effect of ACE-inhibitor therapy in this observational setting.

Clinical investigations on the presence of an interaction between ACE genotype and response to ACE-inhibitor therapy have focused on its hemodynamic effects in the treatment of hypertension and on the efficacy of ACE-inhibitors in the reduction of proteinuria [5, 12, 14-17, 21, 22]. Also, ACE-inhibition has been reported to influence the association between the ACE I/D polymorphism and left ventricular remodelling after myocardial infarction [23]. However, studies on hard cardiovascular endpoints are lacking. So far, findings have been highly inconsistent, mainly due to lack of statistical power and heterogeneity of study populations. Although there have been a few studies that tested for an interaction between ACE I/D genotype and response to ACE-inhibitor therapy in patients with heart failure [11, 13], our study is the first that tested for an interaction of this polymorphism with the effects of treatment on the risk of incident heart failure and death in subjects with hypertension. Contrary to our findings on mortality in hypertensive subjects, post hoc analyses in a large trial in patients with stroke or transient ischemic attack did not demonstrate modification of the effects of ACE-inhibitor therapy on mortality by ACE I/D genotype [24].

A major limitation of our study is its observational nature, which inevitably has led to confounding by indication of the effects of ACE-inhibitor use. Despite the fact that large clinical trials have established the efficacy of ACE-inhibitors in the treatment of hypertension and heart failure [9, 10], we could not demonstrate a beneficial effect of these drugs in our population-based study. As physicians were free to choose antihypertensive treatment strategies in this study, specific patient characteristics may have influenced treatment decisions. As was shown in table 1, baseline characteristics of persons who received an ACE-inhibitor during the study period were significantly different from those who did not. Although we adjusted for these variables in the analyses, immeasurable residual confounding prevented the detection of a protective effect, probably because ACE-inhibitor treatment is not considered first line therapy for hypertension and not all relevant characteristics

that lead to the indication for ACE-inhibitor therapy were identified. Moreover, optimal adjustment for the confounding effects of systolic blood pressure, as part of the indication for treatment, was not possible, since we included subjects who used antihypertensive drugs at baseline in our analysis and we did not have blood pressure measurements shortly before the start of antihypertensive treatment. Besides incomplete adjustment for the 'confounding by indication' effects of blood pressure, the fact that our unexposed group consisted of both otherwise treated and untreated individuals may have confounded our results. Adjustment for the use of other antihypertensive drugs during follow-up did affect the magnitude of the point estimates, but was not sufficient to show a protective effect of ACE-inhibitor therapy over the reference group.

However, even though residual confounding by indication probably affected our risk estimates for the effects of ACE-inhibitor treatment, we could still study the interaction between the use of ACE-inhibitors and the ACE I/D polymorphism, because it is unlikely that residual confounding by indication was non-randomly distributed over ACE genotypes. Since genotyping is not part of routine clinical practice, the prescribing physicians were unaware of the individual's genotype. We did not find a difference in genotype distribution between users and non-users of ACE-inhibitors or other antihypertensive drugs, suggesting that the ACE polymorphism indeed did not affect the choice of antihypertensive treatment, as has been reported earlier by others [25]. Moreover, baseline characteristics were not significantly different between ACE I/D genotypes. Therefore, it seems unlikely that confounding by indication explains the interaction between ACE genotype and ACE-inhibitor therapy in our study. To ensure maximum power, we did not distinguish between different ACE-inhibitor types and there was heterogeneity in cumulative dose and duration of the agents that were used. However, the affinity for tissue ACE varies among the different agents and drug dosage might be able to overcome genetically determined ACE overactivity [12]. Therefore, our results may not be conclusive.

Since the ACE I/D polymorphism is located in an intron, it is probably not functional but in strong linkage disequilibrium with a functional mutation [5]. However, there is substantial evidence that the DD genotype is associated with serum ACE levels. ACE activity in the heart may also be associated with the ACE I/D polymorphism [4]. Todd and colleagues have demonstrated that subjects with the DD genotype, starting from greater initial values, had a significantly larger absolute decrease of serum ACE activity after enalapril administration than the other genotypes [14]. Nevertheless, residual enzyme activity remained higher in the DD genotype group. Therefore, individuals with the II genotype might benefit more from ACE-inhibitor therapy due to a more complete blockade of the RAAS. One could on the other hand also argue that DD individuals have greater benefit, because of the greater decrease in ACE activity after ACE-inhibitor administration [26]. However, in the study by Todd et al., the hypotensive response did not differ between genotypes. Therefore, it is important to consider the question whether ACE is rate-limiting for the production of angiotensin II [12]. Although

under physiological conditions, renin mainly determines the production of angiotensin II, it is not unlikely that in pathophysiological states ACE activity becomes the rate-limiting step. Tissue ACE appears to be more important in the determination of RAAS activity than serum ACE levels and these are not necessarily correlated [12]. Besides a differential effect on ACE activity, a relative resistance to the hemodynamic effects of ACE-inhibitors in the DD genotype group might increase their risk of heart failure or death. However, pharmacogenetic studies in hypertension have not consistently supported this theory. Another potential mechanism is provided by a study of Cicoira and coworkers [11]. They found that the DD genotype was associated with elevated plasma levels of aldosterone despite long-term ACE-inhibitor administration. This aldosterone escape may be an important factor in the development of ACE-inhibitor resistance and has been reported in up to 20% of patients with heart failure during long-term ACE inhibition [11, 27]. Aldosterone has deleterious cardiovascular effects under pathophysiological conditions, including myocardial hypertrophy, and may serve as an important prognostic marker [27].

In conclusion, the results of our study suggest a relative resistance to ACE-inhibitor therapy in subjects with hypertension and the DD genotype compared to the II genotype, with the ID genotype in an intermediate position. Although a statistically significant interaction in the prediction of incident heart failure could not be demonstrated, there were significant differences in relative risks of total and cardiovascular mortality over genotype strata for the use of an ACE-inhibitor. As this study was performed in an observational setting, confirmation and quantification of our results is necessary in an adequately powered randomised clinical trial, which is specifically designed to study drug-genotype interactions.

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Chapter 7

General discussion

In this chapter, the main methodological limitations of pharmaco-epidemiological studies and genetic association studies are discussed to facilitate a proper interpretation of the study results that are described in this thesis. Moreover, a perspective is given on the potential future directions and implications of pharmacogenetic research, a developing field with its own specific pitfalls.

Pharmaco-epidemiology

Pharmaco-epidemiology may be defined as the study of the beneficial and adverse effects of drugs in populations, by using the techniques of chronic disease epidemiology [1]. In addition to the main internal validity issues in classical epidemiology, such as the role of chance, selection bias, information bias and confounding, pharmaco-epidemiological studies have their own specific validity issues. These problems will be addressed in the following section. This part of the discussion will focus on drug effectiveness studies, because the quantitative studies that are presented in this thesis investigate intended drug effects.

Observational data are considered to be very useful to verify drug safety issues and therefore, pharmaco-epidemiologists usually study the unintended (adverse) effects of drugs, where confounding by indication is usually not an issue [2]. Observational studies on the intended effects of drugs, such as drug efficacy, are far less frequently performed. The gold standard in research on intended drug effects is the randomised controlled clinical trial. The most important advantage of this design is that baseline prognoses of patient groups are made comparable by randomisation, to obtain unbiased estimates of the effects of a particular intervention [3]. However, there are some important drawbacks of controlled clinical trials that may justify the use of other designs to study intended drug effects. Clinical trials are usually very expensive to carry out and will not generally be performed by the pharmaceutical industry for total drug classes and for drugs for which the patent has expired. For example, thiazide diuretics have widely been used for many years in the treatment of hypertension and heart failure. Thiazides reduce urinary calcium excretion and therefore may also have beneficial effects on bone. Consequently, the use of thiazide diuretics may reduce fracture incidence. Since this potential benefit of thiazides is probably a class effect and patents have expired, this topic will not be of special interest to individual pharmaceutical companies and hence will not be studied in a clinical trial setting. Therefore, academic researchers recently studied the association between the use of thiazide diuretics and the risk of hip fracture in the Rotterdam Study [4]. For interventions with very small postulated effects and for the investigation of rare but important outcomes, performance of adequately powered randomised trials may even be unfeasible, given the extreme requirements of sample size and follow-up [5]. In addition, randomisation may lead to ethical problems. For example, for drugs that have already shown large beneficial effects, randomisation of these

drugs and the use of a placebo as a comparator may be questionable. Finally, an important drawback of the controlled clinical trial is that it typically involves subjects who are fit to enter and are likely to finish the study [6]. Certain types of patients, such as patients with severe disease, children, or elderly patients, are mostly excluded. Compliance to the study medication during these trials is maximized and studies are performed in a highly controlled clinical setting, which may not mimic the setting in which the drug will ultimately be used. This compromises the generalizability of the results of a clinical trial to patients that are not represented in the study, and may result in a lack of effectiveness of an efficacious drug after it is introduced on the market [1, 2, 6]. This might explain why heart failure remains a highly fatal disease, despite advances in treatments that have been proven to be efficacious in clinical trials. Therefore, additional information may be needed on whether, in the real world of daily medical practice, a drug that has been evidenced to be efficacious in a clinical trial setting, actually achieves its intended effects, i.e. is effective.

A major threat to the internal validity of pharmaco-epidemiological studies on drug effects is confounding by indication, due to the difficulty of ensuring comparability of prognosis across different treatment groups [3, 7]. In non-experimental studies, the allocation of treatment is by definition not random. Therefore, if we assume that prescribing is rational, the prognosis of treated patients will obviously differ from the prognosis of untreated patients, because the untreated patients generally will not have an indication for the treatment under study. So, treated patients will have a higher rate of any disease that the drug is intended to treat, which will manifest itself by making the drug appear ineffective or even harmful [2, 3, 7]. This phenomenon is known as confounding by indication and makes the performance of pharmaco-epidemiological studies on intended drug effects, at best, extremely difficult. The direction of confounding may be hard to predict, especially if two drugs from the same class are compared or when drugs are administrated for purposes of secondary prevention of disease [7]. A special form of confounding by indication is confounding by severity, in which the severity of the disease that forms the indication for treatment, rather than the mere presence of the disease itself, acts as a confounder. This bias is particularly important to acknowledge in studies that compare subjects with the same indication but different treatments. Another bias in pharmaco-epidemiology is protopathic bias, which occurs when the first symptoms of the disease outcome under study form the indication for treatment [8].

There are several methods that can be used in the design and analysis of a study to reduce the problems of confounding. In theory, the effect of confounding by indication can be completely removed by adjustment for all patient (and physician) characteristics that form the basis of the indication for treatment [3]. This is often not feasible, as many factors are not measurable, and residual confounding will result. In practice, there is no conclusive test to prove that there is no residual confounding. It may sometimes be useful, however, to examine the potential effect of residual confounding by means of sensitivity analyses that study the robustness of a result to the pressure of unknown covariates. This

can be done by imagining a certain confounder with different prevalence values and varying magnitudes of association with both exposure and outcome [7]. It may also be useful to investigate the determinants of prescribing itself, before doing a pharmaco-epidemiological study on intended drug effects, because we have to be satisfied that we were able to create treatment groups that are similar with regard to important prognostic factors and that any dissimilarities that arise due to prescribing are known and can be properly adjusted for [3, 7]. Another approach to deal with confounding by indication is to apply restriction criteria and include only those subjects who are similar for all prognostic factors except treatment [3]. Also, exposed and unexposed subjects can be matched on risk factors, which removes the confounding effect of these variables. One method that has been developed to reduce the problem of confounding by indication, and that appears to be especially useful as a matching criterion, is the propensity score [7, 9, 10]. The idea of this score is to model all (known) determinants of exposure, usually by logistic regression, and to predict the probability of receiving treatment for each person given his covariate pattern. This predicted probability could then be used as a variable on which subjects can be matched or could be used as a cofactor in the analysis to adjust for confounding. However, a major disadvantage of the propensity score is that the researcher loses insight into which variables are responsible for the adjustments that are made to the model, while this score does not reduce the problem of unmeasured residual confounding. Moreover, matching on propensity score, or any other variable, has the disadvantage that it decreases statistical power in case control studies. If confounding by indication cannot be adequately controlled for with these methods, it is not possible to interpret drug-disease associations. However, an association in the opposite direction than explained by confounding by indication may still be meaningful, because it endorses a genuine protective effect. It must be emphasized, however, that such results should be interpreted with caution.

Other major issues in pharmaco-epidemiological studies pertain to the definition of drug exposure. Accurate and complete information on drug exposure is essential to pharmaco-epidemiological studies [11]. To avoid information bias due to exposure misclassification, the drug exposure under study should be well defined in terms of dose, timing and duration. Different dosages and potencies of the same drug class are likely to have different effects, and this should be taken into account when two compounds are compared. Reducing information into a dichotomous expression, that is exposed versus non-exposed, may increase the rate of exposure misclassification, which will bias the results [1]. Timing of the outcome of interest in relation to the start of the drug exposure is crucial for causal inference. Obviously, the onset of an event must occur after start of the exposure. A sequence of acute effects for every prescription can be defined in a hazard function for each patient [12]. In case of adverse effects, there is usually a period of no effect, the induction period, followed by a period of high risk, then a period of moderate risk, and finally there is a return to the background incidence rate [13]. For beneficial effects, an opposite hazard curve may be

expected. For many chronic treatments, the rate at which treatment-related outcomes occur varies with time since the start of therapy and with cumulative exposure [14]. This apparent time dependence of risk can result from the early depletion of patients who are susceptible to the adverse events of a drug, from drugs that have beneficial and adverse effects with different induction periods, or from the physiologic adaptation that occurs during prolonged periods of treatment [14]. Also, bias could be introduced due to differing confounding effects of recent and distant past diagnoses, especially if, with evolving knowledge, patterns of indications and contra-indications change. Hence, hazard ratios are usually not constant over time, especially when new and chronic users are mixed [2].

In general, a pharmaco-epidemiological study preferably includes new users of the drug of interest, which is called an inception cohort. Any previous experiences with a drug will influence decisions with regard to future exposure and, if associated with the outcome of interest, will bias the study results [2]. Moreover, when two treatments are compared, the inclusion of prevalent users can lead to exposure groups with different durations of therapy, which can introduce bias if hazard ratios are not constant over time, as described above [14]. Also, the inclusion of prevalent users complicates the control for potential confounders, since these factors can be influenced by the drug itself, as well as form an indication for treatment. However, the exclusion of prevalent users will reduce sample size and thus limit the power of a study. This can potentially be addressed by assessing the magnitude of the potential biases that are introduced when prevalent users are included in the analyses. If no evidence of material presence of these biases is found, prevalent users could be included in the analyses [14]. Also, large computerised databases containing detailed information on drug prescriptions and medical records for hundreds of thousands of subjects, such as the Integrated Primary Care Information (IPCI) project in the Netherlands, are becoming increasingly available for pharmaco-epidemiological research, making the new-user design more practicable.

In many prospective fixed cohort studies, limited information is available on drug exposure, which is often obtained by interview, and sometimes only acquired at the beginning of follow-up [11]. Baseline information on drug use is not sufficient to study drug effects, because the exposure of interest, the drug, and its effects naturally change over time. If sufficient information is available, standard time-varying approaches can be used to adequately analyse the data in cohort studies. Case control studies, in which information on previous drug exposure is typically obtained by interview after the outcome has occurred, may suffer from recall bias if patients remember their drug exposure history better than healthy controls. The use of pharmacy records bypasses the potential for recall bias, as these data are gathered before disease onset. Pharmacy records form an abundant, continuous and reliable source of information on drug exposure, providing that data are complete [15]. Pharmacy records supply data on a day-to-day basis, allowing for time-varying analysis of drug exposure. However, even with the use of pharmacy records, misclassification of

exposure may still be an issue when the definition of the risk window is flawed. Moreover, non-compliance may seriously threaten the results of a study that uses pharmacy records. This will almost always be random and lead to an underestimation of the true effect [15]. A final drawback of pharmacy data is the lack of information on the use of over the counter drugs and non-reimbursed drugs.

Two quantitative studies that are presented in this thesis investigated the effectiveness of antihypertensive treatment in an observational setting and may have suffered from the problems described above. We dealt with possible confounding by indication in these studies by restricting our study populations to patients with hypertension, making the treatment groups more similar in terms of prognosis. Prevalent heart failure cases were also excluded in both studies. In addition, we adjusted for several potential confounders that are known to be associated with the exposure to antihypertensive drugs, left ventricular hypertrophy and heart failure. In the Rotterdam Study, extensive information is available on many of these variables. Our study on the association between antihypertensive drugs and left ventricular geometry may have suffered from residual confounding by indication, if antihypertensive drugs were prescribed for alternate indications than a high blood pressure. However, since we only selected participants with hypertension, and verified the indication of antihypertensive treatment, it seems fair to assume that the antihypertensive drugs were indeed prescribed for this indication. Nevertheless, as physicians were free to choose antihypertensive treatment strategies in the Rotterdam Study, specific patient or physician characteristics may have influenced the decision to treat patients for an increased blood pressure. However, these characteristics would have led to higher relative risk estimates, and do not explain the negative association that we found in this study. This endorses the presence of a genuine beneficial effect of antihypertensive drugs on left ventricular geometry, corresponding to the results of large randomised clinical trials. Our study on the interaction between angiotensin-converting enzyme (ACE)-inhibitors and the ACE Insertion/Deletion (I/D) polymorphism suffered from immeasurable residual confounding by indication, despite our efforts to diminish this problem. Therefore, we could not demonstrate a beneficial effect of ACE-inhibitors in this study. However, it was still possible to study the possible interaction between the use of ACE-inhibitors and the ACE I/D polymorphism, because it was unlikely that this residual confounding was non-randomly distributed over the ACE genotypes.

In the Rotterdam Study, all drug prescriptions dispensed to participants by all pharmacies in the study area are routinely stored in the database. This means that complete information is available on dispensed drug prescriptions, including the product name, the number of tablets or other vehicles in the filled prescription, the date of delivery, the prescribed daily number of tablets to be taken and the drug dosage. With these data it is possible to create time-dependent variables for drug use on a day-to-day basis. We used these data in our studies to calculate prescription lengths, by dividing the total number of filled tablets of consecutive prescriptions of a drug by the prescribed daily number. In this way the

follow-up period could be divided into periods of use and non-use of the drugs under study. Potential misclassification of drug exposure may have occurred, because we did not distinguish between drug types of the same class, and because there was heterogeneity in dosage and duration. This could have biased the results of our studies towards the null hypothesis. To enhance the power of our analyses, we did include prevalent users in our studies, because leaving them out did not materially change our conclusions.

In conclusion, despite the fact that non-experimental studies on intended drug effects are very difficult to perform, mainly due to confounding by indication and difficulties in drug exposure definition, there is definitely a place for pharmaco-epidemiological studies besides the gold standard, the randomised controlled clinical trial. Nevertheless, conclusions of these studies should not be accepted without criticism, especially when prognostic factors are very different for treated and untreated patients.

Genetic epidemiology

Genetic epidemiology focuses on the role of inherited factors in disease aetiology [16]. Traditionally, the family-based study design has been used to investigate genetic components of disease, in particular in monogenic disorders. With respect to genetic factors in heart failure, the role that heritable gene mutations have in (familial) cardiomyopathies is increasingly well understood [17, 18]. However, these rare single gene mutations are not the only potential causes of heart failure and, although they are important for the understanding of disease mechanisms, they are of limited significance at the level of populations [19]. Many genes and environmental factors probably play a role in the pathophysiology of the complex syndrome of heart failure. To study genetic components of multi-factorial disorders such as heart failure, the most commonly applied strategy is the genetic association study. In this approach, candidate genes are selected based upon their biological plausibility, which implies prior knowledge of the involvement of their products - i.e. proteins - in the pathogenesis of the disease. This design allows for the identification of gene polymorphisms directly involved in the aetiology of heart failure (susceptibility genes), or sequence variants involved in the modification of its phenotypic expression (modifier genes). Because the studies that are described in this thesis deal with this alternative approach to study genetic determinants of heart failure and cardiac abnormalities, the discussion in the next section will concentrate on the main limitations of genetic association studies.

In general, results of genetic association studies have been highly inconsistent and their use has therefore been widely debated [16, 19-22]. Initial positive genetic associations have often been difficult to replicate. There are several explanations for this. A major problem in candidate-gene studies is the possibility of generating false-positive results [16, 21, 23]. These studies generally evaluate one genetic polymorphism at a time. For most common

complex diseases, however, hundreds of known genes are potential candidates, and in most genes, dozens of polymorphisms can be identified [21]. Even if none of the genotypes is actually associated with the outcome, one can expect many significant associations to occur by chance alone, when all these variants are tested and treated independently [21, 23]. Particularly studies with ill-defined candidate markers suffer from this problem, since a low prior probability is more likely to lead to false positive results [19]. How to handle this issue of multiple testing in genetic association studies is still a matter of debate. The formation of haplotypes, combinations of multiple markers in a gene that are located closely together and that tend to be inherited together, may provide a solution, because it decreases the number of genetic markers that are tested. Further overestimation of the true effect of a genetic marker might occur due to publication bias, as negative results are generally less likely to be submitted for publication.

Probably the most important problem so far in genetic association studies has been the lack of power to detect the typically small effects in genetic association studies of multi-factorial disorders [21]. Common, complex disorders are under the control of many (interacting) genes of minor individual effect, interacting with a number of environmental factors [20]. Therefore, the effect of individual genetic polymorphisms will be small. Hence, large populations are needed in genetic association studies to avoid generation of false-negative results. Besides increasing the sample size of a study population, statistical power may be increased by the formation of haplotypes over multiple markers in a candidate gene, as opposed to the examination of single genetic markers [23]. Another cause of false negative results is that several genetic association studies have used unconditional statistical methods for data analysis, despite the fact that they matched their cases and controls on population characteristics. This could have biased the results of these studies towards the null hypothesis.

As in classical epidemiology, misclassification of the outcome may weaken associations in candidate-gene studies and heterogeneity in outcome measures between studies may account for many of the contradictory findings. Misclassification of exposure, that is genotyping error, may also affect the results of genetic association studies, and is the most common cause of deviations from the Hardy-Weinberg equilibrium [24]. Departure from Hardy-Weinberg equilibrium, a fundamental rule of population genetics, increases the chance of finding a spurious association, but can also lead to false-negative results. Although random misclassification can be caused by DNA contamination from plates, primers and other environmental components of a laboratory, the possibility of systematic errors due to un-blinded genotyping is particularly troubling [24]. Another well-known example of exposure misclassification due to genotyping error that is relevant to this thesis is the underestimation of ACE I/D heterozygotes that may occur with the conventional genotyping method [25]. Most genetic association studies have used this non-I-allele-specific method for ACE genotyping, which may have led to an underestimation of the true effect

of the DD genotype. In the Rotterdam Study, mistyping of ACE I/D genotypes was avoided by applying modifications to the conventional procedure, including the performance of a second polymerase chain reaction (PCR) with an Insertion-specific primer pair.

An important issue in genetic epidemiology is the phenomenon of population stratification. When the study population comprises a mixture of subgroups that have different allele frequencies and disease risks, genetic associations can be confounded by population stratification [21]. This distortion is only present if the differences in disease prevalence between subgroups are not caused by the allele under study. The most important example of population stratification is a difference in genetic make-up between subpopulations with different ethnicity [19, 20, 26]. Allele frequencies at random marker loci are known to vary between ethnic groups [26]. It is possible to detect population stratification by typing additional unlinked genetic markers, which should also show associations with the disease under study if this bias is present [26]. One solution to the problem of population stratification is to match study subjects on ethnicity or genetic background [19, 20]. Another way to minimize the possibility of stratification is the use of genetically homogeneous populations, such as genetically isolated populations, which originate from a limited number of ancestors (founder populations, e.g. Iceland). Complex traits are expected to be more homogeneous in these populations. However, a drawback of studies in founder populations is that isolation may have caused these populations to have a more or less private make-up of the genome. This may limit the generalizability of these studies. Therefore, disease-related mutations or polymorphisms detected in isolated populations also need to be studied in other populations [16]. Finally, family-based designs are generally accepted to definitively control for confounding by population stratification [27]. However, these studies also have some important drawbacks. For example, a major limitation of using parents or siblings as controls is loss of power because of overmatching [19, 27]. Also, relatives may be unavailable, especially if late-onset diseases are studied. Nevertheless, there is now substantial evidence that well-designed and appropriately interpreted population-based studies with unrelated controls are largely robust to confounding from population stratification [20].

An association between a genetic polymorphism and a disease phenotype may merely be caused by its linkage disequilibrium with a mutation of a nearby gene that is the actual functional gene. Patterns of linkage disequilibrium over chromosomal regions can vary significantly between populations due to several factors, including population admixture and the age of a mutation [28]. Additionally, the genetic structure of a population can lead to linkage disequilibrium between unlinked loci, causing false-positive signals [29]. If a polymorphism is not functional, but simply acts as a genetic marker, varying degrees of linkage disequilibrium may explain variations in association between populations. Polymorphisms located in coding or promoter regions of a gene may alter the function or expression level of proteins encoded by the gene and are a priori more likely to be of functional significance. Even if these genetic variants are not functional, they are more likely

to be in linkage disequilibrium with the true causative alleles [19]. However, the ultimate evidence of these genetic polymorphisms being more than just risk markers depends on the characterization of intermediate phenotypes that can be linked to the disease [30].

Although replication of a genetic association in different (ethnic) populations provides support for a causal association, its absence does not necessarily exclude it [19]. True variation in the presence or size of an association between a polymorphism and a disease in different populations could result in non-replicable associations. This heterogeneity can result from effect modification by other genetic or environmental factors, if these vary between study settings, and from differences in background risks [21]. However, in populations of similar genetic background and with similar disease rates and life styles, it is unlikely that gene-gene or gene-environment interactions play an important role in explaining heterogeneity of effect size between studies [21].

The genetic association studies described in this thesis are potentially limited by the methodological problems presented in the previous section. Although our work represents some of the largest candidate-gene studies in the general population performed until now, we cannot entirely rule out the possibility that chance may provide an alternative explanation for our positive results; especially, since we studied one genetic polymorphism at a time. Also, the mechanisms by which the polymorphisms that were investigated in this thesis may exert their effects on the pathophysiology of heart failure or left ventricular abnormalities are largely unknown. The ACE I/D polymorphism, for example, is located in an intron and therefore is probably not functional itself, but rather in strong linkage disequilibrium with another functional mutation [31]. Also, the functionality of the insulin-like growth factor-I (IGF-I) promoter polymorphism has been debated, since studies so far have provided conflicting results [32-35]. However, findings in the Dutch population have been highly consistent, strongly suggesting that the IGF-I polymorphism can be used as a genetic marker, at least in Dutch subjects [33, 34, 36]. Replication of our findings is therefore needed in other populations. As already mentioned ACE I/D mistyping was dealt with in the Rotterdam Study by the use of I-specific primers. Confounding by population stratification was not an important issue in the studies presented in this thesis, because we used data from a relatively homogeneous population living in the same area in Rotterdam, in which approximately 98% is Caucasian. Also, we adjusted our analyses for factors that were potentially associated with the polymorphisms under study and heart failure or left ventricular abnormality. Finally, in all studies, genotype and allele distributions were in Hardy-Weinberg equilibrium, suggesting no effect of migration or selection pressure in our study populations.

In 1995, the departments of genetic epidemiology and clinical genetics started the research program "Genetic Research in Isolated Populations" (GRIP) in a genetically isolated population in the Southwest of the Netherlands, to study several complex genetic disorders, including hypertension. In 2002, the ERF-study (Erasmus Rucphen Familieonderzoek) was

started, which includes three-generation families selected from the same population as the GRIP study. The combination of the detection of disease-related mutations or polymorphisms in these two studies with the possibility of replicating these results in the population-based Rotterdam Study provides a unique opportunity to study genetic causes in complex diseases.

In conclusion, genetic association studies offer a potentially powerful approach to identify genetic factors that influence the susceptibility to common multi-factorial diseases, such as heart failure, or modify their phenotypic expression. However, lack of replicability of the results has caused the use of this design to be widely debated. False-negative, underpowered studies have been a major problem. Other important issues include false-positive results due to multiple testing, genotyping error, population stratification, population-specific linkage disequilibrium, gene-gene/gene-environment interactions, and insufficient knowledge about the functionality of genetic polymorphisms. However, if all these issues are properly addressed, there is clearly an important role for this study design in the genetic epidemiology of heart failure.

Pharmacogenetics

Heterogeneity in the individual response to drugs is a major problem in clinical practice and drug development [37, 38]. When several patients are prescribed the same recommended daily dosage of a drug, the drug can be efficacious in most, have little or no effect in others, and/or result in adverse drug reactions - occasionally fatal - in a small group of patients [39]. Besides the importance of clinical factors that determine variability in drug response, including age, organ function, concomitant therapy and patient compliance, it is now clear that inherited factors can have an even greater influence on the efficacy and toxicity of drugs [37, 40]. Unlike environmental factors, inherited determinants generally remain stable throughout a person's lifetime [41]. Two main strategies are currently being used to identify genes that cause individual variations in drug response. Pharmacogenetics studies the variability in drug responses attributed to hereditary factors, such as genetic polymorphisms, in different populations [38, 42]. Pharmacogenomics encompasses a genome-wide search for genes relevant for the application of drugs in humans and involves analysis of the genome and its products (RNA and proteins) as they relate to drug response [42-44]. Although both approaches represent different tactics, they share technologies and the terms are commonly used interchangeably. Research in these fields is developing into two directions. First, genetic variants are investigated, which may affect the individual response to drugs that are currently marketed. Second, studies are performed to identify specific genes and their products that may provide targets for the development of new drugs [37].

Pharmacogenetics studies both efficacy and drug safety. Essentially, there are three mechanisms by which genes can influence the response to pharmacotherapy: inherited variability in pharmacokinetics, variability in pharmacodynamics, and gene-drug interactions in the causal pathway of disease [43]. Pharmacokinetic variability in pharmacogenetics refers to inherited differences in drug metabolising enzymes, such as the cytochrome P450 enzyme family, and in drug transport molecules that mediate drug uptake into, and efflux from, intracellular sites [45]. Inherited differences in drug metabolising enzymes have been extensively studied and generally present as monogenic traits. Genetic polymorphisms have now been identified in over 20 of these enzymes in humans, several with substantial ethnic differences in frequencies, many of which translate into functional changes in the encoded proteins [37, 41]. Inherited differences in drug metabolism can affect the response to a drug by influencing the concentration of the active compound. This can result in profound toxicity for medications with a narrow therapeutic index that are not adequately inactivated by a polymorphic enzyme, or reduced efficacy of medications that are rapidly metabolised into inactive compounds [38, 40]. Reduced efficacy can also result from inactivity of a polymorphic enzyme, when drugs require activation to an active metabolite for their effect. For many of these polymorphisms, there is no evident phenotype in the absence of pharmacotherapy. An example of a drug-metabolising enzyme that may have implications in the response to cardiovascular pharmacotherapy is the cytochrome P450 enzyme CYP2D6 [37, 38]. This enzyme is highly polymorphic and is inactive in about 6% of Caucasians. CYP2D6 is responsible for the metabolism of many drugs, including antiarrhythmics and certain ß-blockers.

Information on the effect of genetic polymorphisms in transporter proteins on drug response is scarce. Studies of polymorphisms in the MDR1 gene, which codes for the ATP-dependent transmembrane efflux pump P-glycoprotein, suggest an impact of this gene on requirements of dose adjustments of digoxin and verapamil [38]. Pharmacodynamic variability refers to differences in drug effects at the molecular site of action [45]. Most drugs interact with specific target proteins to exert their effects, including receptors and proteins involved in signal transduction. Many genes encoding these targets exhibit genetic polymorphisms, which may alter their sensitivity to specific drugs and hence affect drug efficacy and toxicity [40]. Examples include polymorphisms in $ß_2$-adrenergic receptors and their effect on sensitivity to ß-agonists in asthmatics [46], and the ACE I/D polymorphism affecting the renoprotective effect of ACE-inhibitors [47]. Finally, genetic polymorphisms that underlie disease pathogenesis and are neither direct targets of drugs nor involved in their disposition, can also be determinants of drug response; e.g. the apolipoprotein E polymorphism may affect the efficacy of lipid lowering drugs [40].

Because pharmacogenetics combines the techniques of pharmaco-epidemiology and genetic epidemiology, it faces, in principle, the methodological issues of both fields, which are described in the previous sections of this discussion. The problem of confounding by

indication in pharmacogenetic efficacy studies will probably be less important, since a physician will usually not be aware of the genetic profile of a patient before prescribing a drug. However, there are some additional critical issues that must be considered when studies are conducted on inherited determinants of drug effects. First, most common disorders have a polygenetic origin and therefore different pathways may contribute to the same clinical phenotype. These genetic differences, together with gene-environment interactions, may also lead to different responses to pharmacotherapy [43]. This might explain, for example, the numerous inconsistent studies on the potential interaction between the ACE I/D polymorphism and ACE-inhibitor therapy [48]. Second, most drug effects are determined by the interplay of several gene products and environmental factors that influence the pharmacokinetics (metabolism and disposition) and pharmacodynamics (targets) of drugs [41]. Very large study populations may be needed to detect a significant pharmacogenetic effect. Another complication of pharmacogenetic strategies is that knowledge of the pharmacokinetics and mechanisms of action of many drugs is incomplete [41]. This may lead, together with the enormous quantity of genetic polymorphisms that have been identified in the human genome, to a large number of false positive findings. Besides polymorphisms in coding regions, variants in regulatory regions, including promoters, 3' untranslated regions and splice sites, are particularly likely to influence drug response by controlling the level and site of expression of drug targets [49]. Biomedical research is now putting great efforts in defining molecular mechanisms of pharmacological effects and identifying genetic determinants of disease pathogenesis and pharmacokinetics, facilitating the performance of future candidate-gene studies in pharmacogenetics [40].

Substantial investments are now being made by pharmaceutical and biotechnology industries to use the approach of pharmacogenomics in the discovery of novel therapeutic compounds [40, 44]. Single nucleotide polymorphism (SNP) mapping technology enables the entire human genome to be searched to identify SNP profiles that are associated with responses to pharmacotherapy [44, 50]. Genes that are identified by genomic strategies generally require functional validation and replication in different racial or ethnic groups [41, 44]. By incorporating whole genome SNP linkage disequilibrium mapping to patients during phase II clinical trials, it may be possible to select small regions of SNP linkage disequilibrium that are associated with efficacy and common adverse event phenotypes. These abbreviated SNP profiles could then form the basis for selection criteria of patients in phase III clinical trials. This would limit unnecessary exposure of non-responsive patients in phase III studies, who would only experience the adverse effects of the new chemical entities under study. Moreover, phase III clinical trials could then be performed faster, with fewer patients and higher cost-efficiency [44, 50]. Also, information from genetic profiles of non-responders will identify new targets for drug research. There is a risk that new medicines will only be developed for the most common, and therefore commercially attractive, genotypes. Also, pharmaceutical companies might worry that they are limiting their markets, by segmenting

the patient groups for which a drug is indicated. However, focusing the development of new drugs on subgroups of patients who are selected by specific disease phenotypes or medicine response profiles will provide additional opportunities to develop more drugs for a larger proportion of patients with common multi-factorial disorders than currently available [44]. As drug efficacy and toxicity become more predictable, this will maximize the value of each prescribed medicine and reduce total health care expenditure. Another frequently heard argument against the application of pharmacogenomics in drug development is that, in clinical practice, newly marketed drugs may be prescribed to patients who do not meet the pharmacogenetic criteria that were used to select patient subgroups in phase III trials, and thus without proper knowledge of the potential benefit-risk ratio of these agents [44]. The assessment of pharmacogenetic profiles before prescribing a particular drug to a patient may be able to prevent this. It is, however, difficult to predict to what extent pharmaceutical industries are willing to incorporate mandatory pharmacogenetic testing into future prescribing schedules. This will to some extent be influenced by the attitude of regulatory authorities [37]. Therefore, regulatory authorities and pharmaceutical industries will need to work together to develop guidelines for the application of pharmacogenomics in drug development and post-marketing strategies. The abbreviated SNP profiles that are developed during the pre-marketing phase can potentially be extended during post marketing surveillance, including phase IV trials, to encompass further efficacy phenotypes and adverse event profiles [44].

In the far future, pharmacogenomics and pharmacogenetics are expected to yield standardised screens of genetic variants that will become routine tools in the selection of medications and drug dosages for each individual patient [41, 44]. A patient's genotype will need to be determined only once for any given gene, because it will not change, except for some rare somatic mutations [41]. The key factor in determining the success of these strategies, besides economical reasons, will include access to data on patient genotypes and other relevant molecular markers [43]. However, we are still a long way from having a personal pharmacogenetic DNA chip that can be used by physicians to predict individual drug responses. Also, before any genetic-based prediction is accepted in clinical practice, its clinical utility needs to be obviated [50]. Potentially, pharmacogenetics can streamline the treatment of complex disorders such as heart failure, in which treatment is largely on a trial-and-error basis and polypharmacy is a common problem, which often leads to decreased compliance. If genetic information can be used to identify the combination of heart failure drugs that provides the maximum benefit-risk ratio for each patient separately, drug regimens can be simplified, costs will be reduced and patient compliance may improve, potentially leading to improvement of patient outcomes [48]. The term 'genetic profile' or 'genetic test' suggests that, in the future, it will be possible to include all relevant genetic information into a single test, and then precisely predict the response to pharmacotherapy for any individual patient. However, this view may be too optimistic and it is important to bear in mind that,

even in the case of fairly obvious bimodality in responses, individual patients will still fall into a distribution pattern of responses, and all predictions will be of a probabilistic nature [51]. In addition, based upon our current understanding of the heterogeneous nature of complex disorders, we will only be able to exclude those gene variants that do not appear to contribute to the disease, and therefore rule out certain treatments. So, physician experience will continue to play an important role in future pharmacotherapy [51].

Pharmacogenetics presents future challenges with respect to physician's ethical and professional responsibilities. There is a clear need for guidelines and regulatory policies to avoid the misuse of genetic information in ways that could harm people; for example by denying them access to health insurance, employment, or loans [52, 53]. This fear for the implications of breaching confidentiality is further fuelled by the notion that limiting access to medical records to the patient and the treating physician is not possible and often undesirable. People tend to think of genetic information as more definitive and predictive than other types of data. However, physicians usually have a limited ability to predict when, how, and even whether a person with a genetic predisposition to a certain disorder will actually become diseased [53]. Also, it is important to distinguish between disease susceptibility genes and genes that only influence the response to certain drugs. The former can help diagnose the disease, determine the carrier status of patients and their relatives and/or predict the occurrence of the disease at an early stage. Genetic tests to determine disease susceptibility may therefore have greater ethical and social implications on the patient and his or her family than genetic profiles that predict an individual's response to pharmacotherapy. However, insurance companies may also misuse information on inherited differences in drug response, if this information will determine the extent of future health care costs [44]. It is not unthinkable that in the future, it may even be unethical not to carry out pharmacogenetic tests on a routine basis, to avoid the exposure of patients to potentially harmful and/or inefficacious drugs [37].

In conclusion, heterogeneity in individual drug response is a major problem in clinical practice and drug development. Pharmacogenetics and pharmacogenomics offer great opportunities to remove much of the uncertainty in current drug prescribing, and to improve the discovery and development of new drug compounds [50]. Such developments will enable individual patients to be classified according to their likely response to a drug and hence, maximize the utility of each medicine. Although most of the developments in pharmacogenetics and pharmacogenomics lie far ahead, there are already some examples of their application in clinical practice today, mostly restricted to academic centres. The most widely accepted application of pharmacogenetic testing is the use of CYP2D6 genotyping to appropriately dose psychiatric drugs [37]. The potential of predictable efficacy, limited adverse events, lower complications due to targeted delivery, and increased cost-effectiveness of medicines will likely improve future health-care delivery [44].

In the near future, pharmacogenetic research in heart failure in the Rotterdam Study may focus on several genetic polymorphisms that have been associated with heart failure and may have functional consequences for the (intended) response to pharmacotherapy. Future pharmacogenetic studies could, for instance, investigate the interaction between the use of β-blockers and β-adrenergic receptor polymorphisms in heart failure. Also, the potential modification of the effects of lipid lowering therapy in heart failure by the apolipoprotein E polymorphism could be studied. However, several other combinations of drugs that are used in the treatment of heart failure and genetic polymorphisms are conceivable. Also, inherited differences in drug metabolising enzymes may be studied in relation to drug-induced heart failure, or in relation to the prevention of sudden cardiac death in heart failure patients. Finally, the interaction between genetic polymorphisms in the MDR1 gene and digoxin use may be studied as determinants of increased mortality in heart failure patients.

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Chapter 8

Summary/Samenvatting

Summary

Heart failure, a complex clinical syndrome, constitutes a major public health problem in the western world. Prevalence and incidencé of heart failure are substantial and will continue to grow as populations age, and because the survival of cardiovascular diseases that lead to this disorder improves. The most common causes of heart failure are coronary artery disease, including myocardial infarction, hypertension, valvular heart disease and idiopathic cardiomyopathy. There is also substantial evidence for a genetic contribution to the pathophysiology of heart failure. Despite the advances in its treatment, heart failure is still a highly fatal disease. Heterogeneity in the individual response to drugs and polypharmacy are major problems in clinical practice. This thesis comprises a number of epidemiological studies that were aimed at gaining insight into the effects of drugs and genetic determinants on the occurrence of heart failure in the general population. All quantitative studies were performed in the Rotterdam Study, a large-scale prospective population-based cohort study among 7983 individuals aged 55 years and older.

After a general introduction in **chapter 1**, **chapter 2** focuses on the magnitude of the problem of heart failure in the community. This chapter also presents age- and sex-specific reference values for the detection of left ventricular hypertrophy on the electrocardiogram (ECG) in the elderly. Chapter **2.1** provides population-based estimates of the prevalence, incidence, lifetime risk and prognosis of heart failure. In the Rotterdam Study, the incidence of heart failure is identified through general practitioner's medical records and hospital discharge letters on a continuous basis. Presence of heart failure is determined according to criteria of the European Society of Cardiology. Information on vital status is obtained from municipal health authorities and general practitioners. We observed a higher prevalence of heart failure in men than in women. Prevalence increased with age from 0.9% in subjects aged 55-64 years to 17.4% in those aged ≥ 85 years. The incidence rate of heart failure was substantial: 14.4/1000 person-years (95% confidence interval (CI) 13.4-15.5) and was higher in men (17.6/1000 man-years, 95% CI 15.8-19.5) than in women (12.5/1000 woman-years, 95% CI 11.3-13.8). The incidence rate increased with age from 1.4/1000 person-years in those aged 55-59 years to 47.4/1000 person-years in those aged ≥ 90 years. Lifetime risk was 33% for men and 29% for women at the age of 55. This means that in individuals aged 55 years, almost 1 in 3 will develop heart failure during their remaining lifespan. Heart failure was highly fatal, with survival after incident heart failure being 86% at 30 days, 63% at 1 year, 51% at 2 years and 35% at 5 years of follow-up. Chapter **2.2** provides age- and sex-specific normal limits in elderly subjects for the Cornell, Sokolow-Lyon and 12-lead summed QRS voltage and voltage-duration products, which are used to detect left ventricular hypertrophy on the ECG. Voltage amplitudes on the ECG vary according to age and sex. However, reference values for left ventricular hypertrophy are lacking in the elderly. At

baseline, 12-lead ECGs were digitally recorded for 6193 participants. Age- and sex-specific normal limits were calculated parametrically in 2915 apparently healthy participants. The 98th percentile was taken as the upper limit of normal. Additionally, the prognostic value of all partition values for left ventricular hypertrophy on the ECG, the traditional values and those established in this study, was tested for heart failure and cardiovascular mortality. Newly assessed reference values for left ventricular hypertrophy on the ECG were higher than traditional partition values, except for the Cornell voltage in men. 98th Percentiles were lower in women than in men up to the age of 75 years. In women, normal limits and voltage (-duration) criteria increased with age. In contrast, Sokolow-Lyon- and 12-lead summed voltage and voltage-duration criteria decreased with advancing age in men, whereas the Cornell voltage and voltage-duration product was not substantially influenced by age. Left ventricular hypertrophy on the ECG was significantly associated with heart failure and cardiovascular mortality for all partition values. Except for the Sokolow-Lyon voltage-duration product, all normal limits established in the present study showed stronger associations for left ventricular hypertrophy on the ECG than the traditional partition values. Differences were more pronounced in women than in men.

Chapter 3 presents two studies on drugs as determinants of heart failure. In chapter **3.1**, an observational study is presented on the association between current use of antihypertensive drugs and left ventricular geometry on the echocardiogram. In the Rotterdam Study, echocardiography was performed in 2823 participants. We studied 740 participants with at least mild hypertension or on antihypertensive monotherapy, without heart failure. Of these, 646 had an adequate echocardiogram for analysis of relative wall thickness and 642 for left ventricular mass index. Participants were followed from January 1, 1991 until the date of echocardiography, between September 1992 and June 1993. Outcome measures were defined as being in the highest gender-specific quintile of left ventricular mass index and as having a relative wall thickness higher than 0.43. A Cox regression model with duration of use of antihypertensive drugs defined as time-dependent covariates was used for data-analysis. Antihypertensive treatment lowered the risk of increased left ventricular mass index (relative risk (RR) 0.6, 95% CI 0.4-0.9) and also, although non-significantly, decreased the risk of high relative wall thickness (RR 0.8, 95% CI 0.6-1.0). In chapter **3.2**, an extensive review of the medical literature is given of recent findings on the association between the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and the occurrence of heart failure. NSAIDs may impair renal function in patients with a decreased effective circulating volume by inhibiting prostaglandin synthesis. Consequently, water and sodium retention, and decreases in renal blood flow and glomerular filtration rate may occur, affecting the unstable cardiovascular homeostasis in these patients. In patients with pre-existing heart failure, this may lead to cardiac decompensation. Putative renal-sparing NSAIDs, such as cyclo-oxygenase-2 selective inhibitors have similar effects on renal function as the traditional

NSAIDs, and can likewise be expected to increase the risk of heart failure in susceptible patients.

Chapter 4 consists of studies on the association between genetic polymorphisms and structural heart disorders that often precede the development of heart failure. The apolipoprotein E (APOE) ∈4 allele has been associated with cardiac dysfunction in Alzheimer's disease and β-thalassemia. We studied the association between APOE genotypes and left ventricular systolic dysfunction, assessed by echocardiography, in the general population. This study is described in chapter **4.1**. A baseline echocardiogram and blood specimens for APOE genotyping were available for 2206 participants. Cardiac dysfunction was considered present if fractional shortening was 25% or less. We found that the APOE ∈4 allele is an independent risk factor for cardiac dysfunction in the elderly. Our study results also suggest that, besides well-known effects on atherosclerosis and cholesterol levels, there may be other mechanisms through which this allele exerts negative effects on myocardial performance. The association between a cytosine-adenosine (CA) repeat polymorphism in the promoter region of the insulin-like growth factor I (IGF-I) gene and left ventricular hypertrophy on the echocardiogram is described in chapter **4.2**. Altered serum levels of IGF-I have been associated with adverse cardiac remodelling, and the IGF-I promoter polymorphism may influence circulating IGF-I levels. Analyses were performed with baseline measurements in 1678 subjects aged between 55 and 75 years, without a history of myocardial infarction. Left ventricular hypertrophy was defined as a left ventricular mass index ≥ 104 g/m² in women and ≥ 116 g/m² in men. We found that non-carriers of a 192-base pair (bp, 19 CA repeats) polymorphism in the IGF-I gene are more susceptible to the development of left ventricular hypertrophy than individuals homozygous for this allele.

Studies on the association between genetic polymorphisms and heart failure are presented in **chapter 5**. A comprehensive review of the medical literature on the role of genetic polymorphisms of the major neurohormonal systems in heart failure is given in chapter **5.1**. The majority of genetic association studies have focused on the angiotensin-I converting enzyme Insertion/Deletion (ACE I/D) polymorphism. Initial genetic associations have often been difficult to replicate, mainly due to problems in study design and lack of power. Promising results, however, have been obtained with genetic polymorphisms of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic system. Considering the evidence so far, a modifying role for these polymorphisms seems more likely than a role of these variants as susceptibility genes in the general population. Chapter **5.2** contains a study on the association between the CA repeat polymorphism in the promoter region of the IGF-I gene and incident heart failure. Data were used from 4963 participants of the Rotterdam Study without heart failure at baseline aged 55 to 75 years. IGF-I genotypes were classified based upon presence or absence of the 192-bp allele (19 CA repeats, traditional classification)

and upon presence or absence of the following combination of genotypes: 192-bp/192-bp, 192-bp/194-bp and 194-bp/194-bp. This second categorization was investigated because earlier it was found, in subjects from the Rotterdam Study, that circulating serum IGF-I levels were highest for persons with 192-bp and 194-bp alleles, while both alleles shorter than 192-bp and longer than 194-bp seemed to have lower serum IGF-I levels. This suggests that there is a broader optimum for IGF-I gene regulated transcriptional activity. Persons with any other genotype than 192-bp/192-bp, 192-bp/194-bp and 194-bp/194-bp had an increased risk of heart failure (RR 1.39, 95% CI 1.06-1.80). Therefore, our findings suggest that genetically determined chronic exposure to low IGF-I levels is associated with an increased risk for heart failure in elderly individuals. In chapter **5.3**, the relation between the ACE I/D polymorphism and the risk of incident heart failure in 4264 normotensive and 2174 hypertensive subjects (at least moderate hypertension) is described. Incidence rates of heart failure in normotensive subjects were the same over all genotype strata. In hypertensive subjects, the incidence rate increased significantly with the number of D-alleles present. We observed that hypertensive subjects carrying one or two copies of the D-allele had a significantly increased risk of heart failure compared to normotensive subjects (relative risk ID 1.4, 95% CI 1.1-1.7 and DD 1.5, 95% CI 1.2-2.0), while hypertensive subjects carrying the II-genotype did not have an increased risk of heart failure compared to individuals with a normal blood pressure. These findings suggest that the ACE I/D polymorphism plays a modifying role in the development of heart failure in hypertensive subjects.

In **chapter 6**, a pharmacogenetic study is presented. The potential interaction between ACE-inhibitor therapy and the ACE I/D polymorphism in the prediction of incident heart failure and death was studied in a subgroup of subjects with hypertension. We used data from 3365 patients with mild to severe hypertension, without heart failure at baseline for whom ACE-genotyping was successful. Incident heart failure, total mortality and cardiovascular mortality were studied as endpoints. A Cox regression model with any use of ACE-inhibitors defined as time-dependent covariates was used for data-analysis. Interaction was tested in this model assuming an allele-effect relationship. Although we could not demonstrate a beneficial effect of ACE-inhibitor use in this observational setting, possible pharmacogenetic interaction could still be investigated. The results of this study suggest that there is a relative resistance to ACE-inhibitor therapy in subjects with hypertension and the DD genotype compared to the II genotype, with the ID genotype in an intermediate position. Mortality risk associated with treatment increased with the number of D-alleles present; e.g. for total mortality in the II genotype group: RR=0.95 (95% CI 0.63-1.45), in the ID genotype group: RR=1.08 (95% CI 0.84-1.38) and in the DD genotype group: RR=1.61 (95% CI 1.18-2.18). No statistically significant interaction was found for incident heart failure, probably due to low statistical power.

In the general discussion in **chapter 7**, methodological limitations of pharmaco-epidemiological and genetic association studies are discussed to facilitate a proper interpretation of the study results that are described in this thesis. Moreover, a perspective is given in this chapter on the potential future directions and implications of pharmacogenetic research, a developing field with its own specific pitfalls.

Samenvatting

Hartfalen is een complex klinisch syndroom en vormt een belangrijk probleem voor de volksgezondheid in the westerse wereld. De prevalentie en incidentie van hartfalen zijn aanzienlijk en zullen verder stijgen als een gevolg van de vergrijzing van populaties en een verbeterde overleving van de cardiovasculaire ziekten, die deze aandoening kunnen veroorzaken. De meest voorkomende oorzaken van hartfalen zijn coronaire hartziekte, met name het myocard infarct, hypertensie, kleplijden en idiopathische cardiomyopathie. Er zijn tevens sterke aanwijzingen voor een belangrijke bijdrage van genetische factoren aan de pathofysiologie van hartfalen. Ondanks de enorme vooruitgang in de behandeling van hartfalen, blijft de prognose van deze aandoening slecht. In de klinische praktijk vormt de vaak sterk wisselende respons van individuen op geneesmiddelen tegen hartfalen een groot probleem. Dit proefschrift bevat een aantal epidemiologische studies, die tot doel hadden om inzicht te verkrijgen in de effecten van geneesmiddelen en genetische determinanten op het optreden van hartfalen in de algemene bevolking. Alle kwantitatieve studies werden uitgevoerd met behulp van data afkomstig van het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek, een grootschalig bevolkingsonderzoek van 7983 personen van 55 jaar en ouder.

Na een algemene inleiding in **hoofdstuk 1**, wordt in **hoofdstuk 2** een schatting gegeven van het optreden van hartfalen in de algemene bevolking. In dit hoofdstuk worden ook leeftijds- en geslachtsspecifieke referentiewaarden gegeven, die gebruikt kunnen worden voor de detectie van linker ventrikel hypertrofie op het electrocardiogram (ECG) in een oudere populatie. Hoofdstuk **2.1** verschaft schattingen van de prevalentie en incidentie van hartfalen, het cumulatieve risico op hartfalen gedurende het leven en de prognose van hartfalen in de algemene bevolking. Het medisch dossier van personen die deelnemen aan het ERGO onderzoek wordt continu onderzocht met betrekking tot het optreden van hartfalen. Dit dossier bevat onder andere aantekeningen van de huisarts en ontslagbrieven van ziekenhuisopnames. Of een deelnemer van het ERGO onderzoek werkelijk hartfalen heeft, wordt bepaald aan de hand van criteria die zijn opgesteld door de "European Society of Cardiology". Informatie over overlijden wordt verkregen via de gemeentelijke autoriteiten en de huisartsen van de deelnemers. Wij vonden een hogere prevalentie van hartfalen bij

mannen dan bij vrouwen. De prevalentie steeg van 0.9% in personen van 55-64 jaar oud naar 17.4% in personen van 85 jaar en ouder. De incidentie van hartfalen was aanzienlijk: 14.4 per 1000 persoonsjaren (95% betrouwbaarheidsinterval (BI) 13.4-15.5) en was hoger bij mannen (17.6/1000 man-jaren, 95% BI 15.8-19.5) dan bij vrouwen (12.5/1000 vrouw-jaren, 95% BI 11.3-13.8). De incidentie steeg met de leeftijd van 1.4 per 1000 persoonsjaren bij individuen van 55-59 jaar oud naar 47.4 per 1000 persoonsjaren bij individuen van 90 jaar en ouder. Het cumulatieve risico op hartfalen gedurende het leven bedroeg 33% voor mannen en 29% voor vrouwen van 55 jaar. Dit betekent dat ongeveer 1 op de 3 personen van 55 jaar hartfalen zal ontwikkelen gedurende zijn of haar resterende leven. Hartfalen had een zeer slechte prognose in onze studie. De overleving bedroeg 86% na 30 dagen, 63% na 1 jaar, 51% na 2 jaar en 35% na 5 jaar volgend op een eerste diagnose van hartfalen. Hoofdstuk **2.2** geeft leeftijds- en geslachtsspecifieke normaalwaarden voor de detectie van linker ventrikel hypertrofie op het ECG in ouderen voor de Cornell, Sokolow-Lyon en de "12-lead summed" QRS voltage en voltage*duur produkten. Amplitudes van voltages op het ECG variëren met leeftijd en geslacht. Referentiewaarden voor linker ventrikel hypertrofie op het ECG ontbreken echter bij ouderen. Tijdens de eerste ronde van het ERGO onderzoek zijn van 6193 participanten digitale ECGs opgeslagen. Leeftijds- en geslachtsspecifieke normaalwaarden voor linker ventrikel hypertrofie op het ECG werden bepaald met behulp van een parametrische methode bij 2915 gezonde deelnemers van ERGO. Het 98^{e} percentiel gold als de bovengrens van normaal. Tevens werden de traditionele en de door ons bepaalde afkappunten voor linker ventrikel hypertrofie op het ECG getest op hun relatie met het optreden van hartfalen en cardiovasculaire mortaliteit. De door ons bepaalde referentiewaarden voor linker ventrikel hypertrofie op het ECG waren hoger dan de traditioneel gebruikte afkappunten, behalve voor het Cornell voltage bij mannen. Vrouwen hadden lagere waarden voor de 98^{e} percentielen dan mannen tot aan de leeftijd van 75 jaar. Normaalwaarden en voltage(*duur) criteria stegen met de leeftijd bij vrouwen. Echter, bij mannen daalden de Sokolow-Lyon en "12-lead summed" QRS voltage en voltage*duur criteria met het toenemen der leeftijd, terwijl het Cornell voltage en het voltage*duur product bij mannen niet noemenswaardig werd beïnvloed door de leeftijd. Linker ventrikel hypertrofie op het ECG was significant geassocieerd met het optreden van hartfalen en met cardiovasculaire mortaliteit voor alle afkappunten. Associaties waren sterker voor de normaalwaarden die waren bepaald in onze studie dan voor de traditioneel gebruikte afkappunten, behalve voor het Sokolow-Lyon voltage*duur product. Verschillen waren meer uitgesproken bij vrouwen dan bij mannen.

In **hoofdstuk 3** worden twee onderzoeken gepresenteerd, waarin onderzocht werd in hoeverre geneesmiddelen determinanten vormen van hartfalen. Hoofdstuk **3.1** bevat een observationele studie naar de associatie tussen het huidige gebruik van antihypertensiva en linker ventrikel geometrie op het echocardiogram. Een echocardiogram was vervaardigd

bij 2823 deelnemers in de eerste ronde van ERGO. Wij bestudeerden 740 deelnemers zonder hartfalen met tenminste milde hypertensie en/of het gebruik van maximaal één antihypertensivum. Van deze participanten hadden 646 personen een deugdelijk echocardiogram voor de analyse van relatieve wanddikte en 642 voor de analyse van linker ventrikel massa index. Deelnemers werden gevolgd vanaf 1 januari 1991 tot de datum van echocardiografie, tussen september 1992 en juni 1993. Uitkomstmaten werden op de volgende manier gedefinieerd: het zich bevinden in het hoogste geslachtsspecifieke quintiel van de linker ventrikel massa index en het hebben van een relatieve wanddikte groter dan 0.43. Een Cox regressie model met de duur van het gebruik van antihypertensiva gedefinieerd als tijdsafhankelijke variabele werd gebruikt voor analyse van de data. Het gebruik van antihypertensiva verlaagde het risico op het hebben van een verhoogde linker ventrikel massa index (relatief risico (RR) 0.6; 95% BI 0.4-0.9) en, hoewel niet statistisch significant, verlaagde het ook het risico op een hoge relatieve wanddikte (RR 0.8; 95% BI 0.6-1.0). In hoofdstuk **3.2** wordt een uitgebreide beschouwing gegeven van de recente medische literatuur over de associatie tussen het gebruik van niet steroïde anti-inflammatoire geneesmiddelen (NSAID's) en het optreden van hartfalen. NSAID's kunnen de nierfunctie schaden bij patiënten met een verminderd effectief circulerend volume door een blokkade van de synthese van prostaglandines. Als gevolg hiervan kan retentie van water en natrium optreden en een vermindering in de doorbloeding van de nier en van de glomerulaire filtratie. Hierdoor kan de instabiele cardiovasculaire homeostase bij deze patiënten verder negatief beïnvloed worden. Bij patiënten met hartfalen kan dit leiden tot verschijnselen van decompensatie. NSAID's met een veronderstelde nier-sparende werking, zoals de cyclo-oxygenase-2 selectieve remmers, hebben gelijkwaardige effecten op de nierfunctie als de traditionele NSAID's en zullen derhalve een verhoogd risico op hartfalen geven bij daarvoor gevoelige patiënten.

Hoofdstuk 4 bevat enkele onderzoeken waarin associaties worden bestudeerd tussen genetische polymorfismen en structurele aandoeningen van het hart welke vaak voorafgaan aan hartfalen. Het apolipoproteïne E (APOE) ∈4 allel is in eerdere studies geassocieerd met cardiale disfunctie in patiënten met de ziekte van Alzheimer en in patiënten met β-thalassemie. Wij bestudeerden de associatie tussen APOE genotypes en systolische disfunctie van de linker ventrikel op het echocardiogram in de algemene bevolking. Deze studie wordt beschreven in hoofdstuk **4.1**. Een echocardiogram en bloedmonsters waren beschikbaar voor 2206 deelnemers. Cardiale disfunctie was gedefinieerd als een "fractional shortening" van 25% of minder. Wij vonden in onze studie dat het APOE ∈4 allel een onafhankelijke risicofactor is voor cardiale disfunctie in een oudere populatie. Onze bevindingen suggereren tevens dat er naast de algemeen bekende effecten van dit polymorfisme op atherosclerose en cholesterol andere mechanismen zijn, die de negatieve effecten van dit allel op de prestaties van het myocard kunnen verklaren. De associatie tussen een cytosine-adenosine

(CA) repeat polymorfisme in de promoter regio van het insuline-achtige groeifactor (IGF)-I gen en linker ventrikel hypertrofie op het echocardiogram wordt beschreven in hoofdstuk **4.2**. Veranderingen in IGF-I bloedspiegels zijn geassocieerd met nadelige remodeling van het hart. Het IGF-I promoter polymorfisme zou de IGF-I bloedspiegels kunnen beïnvloeden. Analyses werden uitgevoerd met de metingen van de eerste ERGO ronde van 1678 deelnemers van 55 tot 75 jaar oud, zonder een voorgeschiedenis van myocard infarct. Linker ventrikel hypertrofie was gedefinieerd als een linker ventrikel massa index ≥ 104 g/m^2 in vrouwen en ≥ 116 g/m^2 in mannen. Wij vonden in deze studie dat non-carriers van het 192-base paar allel (bp, 19 CA repeats) polymorfisme in het IGF-I gen vatbaarder waren voor het ontwikkelen van linker ventrikel hypertrofie dan personen die homozygoot waren voor dit allel.

In **hoofdstuk 5** worden onderzoeken gepresenteerd naar de associatie tussen genetische polymorfismen en hartfalen. Een uitgebreid overzicht van de medische literatuur over de rol van genetische polymorfismen van de belangrijkste neurohormonale systemen in hartfalen wordt gegeven in hoofdstuk **5.1**. Een meerderheid van de genetische associatie studies in de medische literatuur is gericht op het angiotensin-I converting enzyme Insertion/Deletion (ACE I/D) polymorfisme. Genetische associaties, die aanvankelijk werden gevonden, bleken vaak moeilijk om te reproduceren in latere studies, voornamelijk vanwege problemen in het ontwerp van de betreffende onderzoeken en een gebrek aan onderscheidingsvermogen ("power"). Veelbelovende resultaten zijn echter geboekt met genetische polymorfismen van het renine-angiotensine-aldosteron systeem (RAAS) en het sympathische systeem. Uitgaande van de huidige kennis lijkt een modificerende rol van deze polymorfismen op hartfalen in de algemene bevolking waarschijnlijker dan een rol van deze genetische varianten als ontvankelijkheids genen. Hoofdstuk **5.2** bevat een onderzoek naar de associatie tussen het CA-repeat polymorfisme in de promoter regio van het IGF-I gen en incident hartfalen. Voor deze studie werden data gebruikt van 4963 deelnemers aan ERGO van 55 tot 75 jaar oud, zonder hartfalen bij het begin van de studie. IGF-I genotypes werden geclassificeerd op basis van de aan- of afwezigheid van het 192-bp allel (19 CA repeats, traditionele classificatie) en op basis van de aan- of afwezigheid van de volgende combinatie genotypes: 192-bp/192-bp, 192-bp/194-bp en 194-bp/194-bp. Deze tweede categorisatie werd onderzocht in deze studie omdat er in een eerdere studie in de ERGO-populatie was gevonden dat IGF-I bloedspiegels het hoogst waren voor deelnemers met 192-bp en 194-bp allelen, terwijl deelnemers met zowel kortere allelen dan het 192-bp allel als deelnemers met allelen langer dan het 194-bp allel lagere IGF-I bloedspiegels hadden. Deze bevinding suggereert dat er een breder optimum is voor de regulatie van de transcriptionele activiteit van het IGF-I gen. Personen met elk ander genotype dan 192-bp/192-bp, 192-bp/194-bp en 194-bp/194-bp hadden een verhoogd risico op hartfalen (RR 1.39; 95% BI 1.06-1.80). Onze resultaten suggereren derhalve dat een genetisch bepaalde chronische blootstelling aan lage IGF-I spiegels het risico op

hartfalen verhoogt bij oudere individuen. In hoofdstuk **5.3** wordt de relatie beschreven tussen het ACE I/D polymorfisme en het risico op incident hartfalen bij 4264 participanten van het ERGO onderzoek met een normale bloeddruk en 2174 deelnemers met hypertensie (ten minste matige hypertensie). De incidentiecijfers van hartfalen waren gelijk voor alle genotypes bij mensen met een normale bloeddruk. Bij deelnemers met hypertensie steeg het incidentiecijfer echter significant met het aantal D-allelen. Wij vonden dat personen met hypertensie en één of twee D-allelen een significant verhoogd risico hadden op hartfalen ten opzichte van deelnemers met een normale bloeddruk (relatief risico ID 1.4.95% BI 1.1-1.7 en DD 1.5, 95% BI 1.2-2.0). Hypertensieven met het II genotype hadden geen verhoogd risico op hartfalen ten opzichte van personen met een normale bloeddruk. Onze bevindingen suggereren dat het ACE I/D polymorfisme een modificerende rol speelt bij de ontwikkeling van hartfalen bij patiënten met hypertensie.

In **hoofdstuk 6** wordt een farmacogenetische studie gepresenteerd. Het bestaan van een mogelijke interactie tussen behandeling met ACE-remmers en het ACE I/D polymorfisme bij het optreden van hartfalen en overlijden werd bestudeerd bij een groep ERGO deelnemers met hypertensie. Hiertoe maakten wij gebruik van de data van 3365 patiënten met milde tot ernstige hypertensie, zonder hartfalen bij het begin van de studie, voor wie de genotypering van het ACE polymorfisme succesvol was verricht. Als eindpunten werden incident hartfalen, totale mortaliteit en cardiovasculaire mortaliteit bestudeerd. Een Cox regressie model met het gebruik van ACE-remmers gedefinieerd als tijdsafhankelijke variabele werd toegepast voor analyse van de data. Hoewel wij geen gunstig effect van het gebruik van ACE-remmers konden aantonen in deze observationele studie, kon het bestaan van een mogelijke farmacogenetische interactie wel worden bestudeerd. Onze bevindingen suggereren dat er een relatieve resistentie is tegen de effecten van ACE-remmers bij personen met hypertensie en het DD genotype ten opzichte van het II genotype. Het ID genotype neemt hier een middenpositie in. Het overlijdensrisico geassocieerd met de behandeling steeg met het aantal D-allelen; bijvoorbeeld voor totale mortaliteit (alle doodsoorzaken) in de II genotype groep: RR=0.95 (95% BI 0.63-1.45), in de ID genotype groep: RR=1.08 (95% BI 0.84-1.38) en in de DD genotype groep: RR=1.61 (95% BI 1.18-2.18). Er werd geen statistisch significante interactie gevonden voor incident hartfalen, waarschijnlijk vanwege een te laag onderscheidingsvermogen van onze studie.

In de algemene discussie in **hoofdstuk 7** worden methodologische beperkingen van farmaco-epidemiologische en genetische associatie studies besproken, teneinde een juiste interpretatie mogelijk te maken van de onderzoeken die in dit proefschrift worden beschreven. Daarnaast wordt in dit hoofdstuk een perspectief gegeven van de potentiële toekomstige richting en consequenties van farmacogenetisch onderzoek.

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