From THE INSTITUTE OF ENVIRONMENTAL MEDICINE
DIVISION OF CARDIOVASCULAR EPIDEMIOLOGY
Karolinska Institutet, Stockholm, Sweden

CORONARY HEART DISEASE IN SWEDISH TWINS: QUANTITATIVE GENETIC STUDIES

Slobodan Zdravkovic

Stockholm 2006

To my beloved family

Eleonora, 2002

ABSTRACT

To this point in time, coronary heart disease (CHD) has been a major cause of morbidity and mortality in the industrialized part of the world. During the last decades a decrease in morbidity and mortality has been observed in the developed countries. The bases for this progress are mainly improvements in the health care system, medical interventions, and changes in people's lifestyle; habits related to an increased risk of the disease, like smoking, obesity and physical inactivity. In conclusion, this progress is mainly due to environmental changes. However, other factors, not possible to modify, like genetic factors, also contribute to the burden of CHD. Thus far, it has been shown that a positive family history of CHD increases risk. In addition, a large number of candidate genes have been suggested to be associated with CHD through related mechanisms such as lipid metabolism, blood pressure regulation, and insulin sensitivity. However, it has been difficult to quantify genetic influences by summarizing risks and effects of known genes, as the number of these genes is large and the effect of each gene small. The primary aim of this thesis was, therefore, to determine the overall impact of genetic and environmental factors on CHD-death and the CHD subphenotypes acute myocardial infarction (AMI) and angina pectoris (AP). The secondary aims were to study if genetic influences change with age and to what extent they are mediated through well known risk factors and AP. Twin pairs born between 1886 and 1958 from the Swedish Twin Registry were used to calculate concordances, hazard ratios and heritability. Heritability estimates were obtained by two approaches, the frailty and the liability approach. Analyses of concordances resulted in higher concordances for CHD in monozygotic (MZ) than dizygotic (DZ) twins as well as in higher estimates at younger ages. Heritability for CHD-death was estimated to 0.57 (95 % CI 0.45-0.69) among males and 0.38 (0.26-0.50) among females, and extension of the follow-up time resulted in a decrease in heritability with increasing length of follow-up (paper I). Including known risk factors (smoking, obesity, hypertension, diabetes, level of education, and marital status) into genetic analyses resulted in a slight decrease of genetic variance and thus a slight portion (among males and more so among females) of genetic variance was accounted for by the genetic variance for studied risk factors (paper II). AP was shown to be an important risk factor for CHD-death in both sexes, although more so in males. Heritability analyses resulted in moderate heritability estimates for AP in both sexes (0.39 (0.29-0.49) in males and 0.43 (0.08-0.51) in females). The correlation between AP and CHD was almost entirely explained by the influence of familial factors in both sexes (paper III). Heritability for AMI resulted also in moderate estimates, 0.35 (0.28-0.43) for males and 0.38 (0.29-0.46) for females (paper IV). In conclusion, genetic factors influence CHD-death to a moderate degree as well as the sub phenotypes AMI and AP. Genetic influences for death from CHD are slightly higher among males and more important at younger ages. Finally, genetic influences for CHD-death are slightly modified through risk factors and AP in males and more so in females.

LIST OF PUBLICATIONS

This thesis is based on the following papers and manuscripts:

I. Zdravkovic S, Wienke A, Pedersen NL, Marenberg ME, Yashin AI, De Faire U. Heritability of death from coronary heart disease: a 36-year follow-up of 20 966 Swedish twins. J Intern Med. 2002; 252(3):247-54.

II. Zdravkovic S, Wienke A, Pedersen NL, Marenberg ME, Yashin AI, de Faire U. Genetic Influences on CHD-Death and the Impact of Known Risk Factors: Comparison of Two Frailty Models. Behav Genet. 2004; 34(6):585-92.

III. Zdravkovic S, Wienke A, Pedersen NL, de Faire U. Genetic influences on angina pectoris and its impact on coronary heart disease. Submitted

IV. Zdravkovic S, Wienke A, Pedersen NL, de Faire U. Genetic susceptibility of myocardial infarction. Submitted

SUPERVISORS

Professor Ulf de Faire
Division of Cardiovascular Epidemiology
The Institute of Environmental Medicine
Karolinska Institutet
Stockholm, Sweden

Professor Nancy L. Pedersen
Department of Medical Epidemiology and Biostatistics
Karolinska Institutet
Stockholm, Sweden

Andreas Wienke PhD
Institute of Medical Epidemiology, Biostatistics, and Informatics
Faculty of Medicine
Martin-Luther-University Halle-Wittenberg
Halle, Germany

CONTENTS

LIST OF ABBREVIATIONS

AIC	Akaike information criterion
AMI	Acute myocardial infarction
AP	Angina pectoris
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence interval
DZ	Dizygotic
ICD	International classification of disease codes
IHD	Ischemic heart disease
MI	Myocardial infarction
MZ	Monozygotic
STR	The Swedish Twin Registry

1 INTRODUCTION

"They who are afflicted with it are seized while they are walking (more especially if it be uphill, and soon after eating) with a painful and most disagreeable sensation in the breast, which seems as if it would extinguish life, if it were to increase or continue; but the moment they stand still, all this uneasiness vanishes." This is the original description of angina pectoris (AP) written in the late eighteen century by William Heberden, a historian. AP, together with other heart diseases, belongs to the coronary family of heart diseases [1]. To this point, coronary heart disease (CHD) has been a major cause of morbidity and mortality in the industrialized part of the world [2]. In addition, CHD was reported to increase in developing countries during the 50s and 60s [3]. Up to recently CHD has been considered as a disease mainly affecting males. Nevertheless, the risk of CHD-death has been reported to be one in two for men and one in three in women at 40 years of age and one in three in men and one in four in women at 70 years of age [4]. There are, of course, substantial differences between the sexes but in general known risk factors seem to affect the risk for CHD similarly in both males and females. Hitherto, more than 200 risk factors [5] and a large number of genes related to cardiovascular diseases (CVD) [6] have been recognized. Smoking, diabetes, obesity, hypercholesterolemia and hypertension are only a few of all known risk factors with a large impact on the disease. Among novel risk factors that can be mentioned are various genes, C-reactive protein, homocysteine and lipoprotein (a) [7,8]. More recently the possible links between infection/inflammation and atherosclerosis have also been highlighted [9]. During the last decades, however, a decrease has been observed regarding mortality due to CHD in United States [10], a trend that has also been reported from various epidemiological surveys [11]. In Sweden, a decline in CHD-mortality has been observed for the last 20-25 years and predictions indicate that this trend will continue [12]. Incidence of acute myocardial infarction (AMI) has also been at a yearly decrease at 2% for men and 1.4% for women between 1984 and 1996 [13]. The decreased incidence of CHD has primarily been attributed to medical interventions and changes in health-related habits/lifestyles [14]. During recent years more and more attention has been directed towards exploring the genetic background to CHD. To that end a renewed interest has been noted for assessing the impact of familiality/hereditary factors by using appropriate study materials such as twins, family and adoption studies. It has for long time been known that a positive family history of CHD is associated with an increased risk of CHD events [15]. So far, though, it has been difficult to quantify the genetic influences by adding risks and effects of known genes, as the number of candidate genes is likely large and the effect of each gene small [16]. However, improvements in computer capacity and genetic epidemiological methods (molecular genetics) allow for more complex genetic analyses today than before, which will make it possible to delineate the importance of both genetic and environmental pieces included in the multifactorial puzzle of CHD.

2 BACKGROUND

2.1 CORONARY HEART DISEASE

CHD is the most common type of heart disease and is also known as coronary artery disease (CAD) and ischemic heart disease (IHD). It is usually caused by a narrowing of the blood vessels that supply blood and oxygen to the heart (coronary arteries). The underlying vascular disease, atherosclerosis, evolves gradually with a building up of fatty materials and plaque formation resulting in a smaller lumen which slows down or blocks the blood flow to the heart. The atherosclerotic process is normally a lifelong process that starts in early phases of life. It causes chest pain, shortness of breath, heart attacks or other symptoms/outcomes and may be pronounced, resulting in sudden severe arrhythmias causing death [17-19]. The following heart diseases are classified as CHD: AMI, other acute and sub-acute forms of IHD, chronic IHD, AP and asymptotic IHD.

2.1.1 Myocardial infarction

AMI usually results from a blood clot blocking the coronary artery and stopping the blood flow to the heart. Usually an atherosclerotic plaque has been ruptured, which triggers the formation of a blood clot. The outcome from an AMI is permanent damage or death of the affected area of the heart. Symptoms of an AMI are discomfort in the chest or upper body, shortness of breath, nausea etc.

2.1.2 Angina pectoris

AP or chest pain/discomfort occurs when the heart works harder due to not receiving enough blood and oxygen. It is a symptom of myocardial ischemia (insufficient blood supply to the heart) and occurs when at least one coronary artery is narrowed. Symptoms are pain/discomfort/squeezing in the chest, pain in the neck, jaw, shoulder and arm. AP consists of stable, unstable and variant angina.

2.1.2.1 *Stable angina*

Stable angina is the most common type of angina. This kind of angina occurs on a regular basis, after a heavy meal, exercise, stress etc. It usually lasts around 10 minutes and stops by rest or medicine.

2.1.2.2 *Unstable angina*

Unstable angina refers to an unstable situation with worsening of the angina symptoms. This kind of angina contains forms such as accelerating angina, new-onset angina, and progressive angina. It usually occurs during rest and little effort. The chest pain is stronger than the chest pain caused by stable angina and increases in severity during the attack. The pain is not relieved by rest or medicine. It may indicate that a heart attack is near to happen.

2.1.2.3 *Variant angina*

This is a rare type of angina which often occurs during the night (at rest). The pain is severe and is relieved by medicine. This type of angina affects women more often than men.

2.1.3 Genetic designs and risk factors

Multifactorial diseases such as CHD are caused by a large number of factors. In a review done in 1981 the number of risk factors exceeded 200 [5]. These factors are both environmental, genetic and in many case a combination of both. However, a digging into the background of this issue does not produce simple answers to whether a factor is purely environmental or genetic. Some factors seem at first to be purely environmental, which is not necessarily true as they can be a direct cause of an underlying state or disease totally or partly caused by genetic factors. It is extremely difficult to determine the origin of a risk factor. The fact that the individual risk factors interact with each other adds additional difficulties in understanding the aetiology of CHD (figure 1). Already in 1819, Samuel Black- an Irish physician - recognized a genetic contribution by noting, "We have seen that the disease appears to be connected with a plethoric state of the system and with obesity: that the great majority of the subjects of it have belonged to the better ranks of society, who were in the habit of sitting down everyday to a plentiful table, in the pleasures of which they may have indulged to a greater extent than was suitable to the tendency of their constitution" [20]. The traditional way of studying familiality (environment/behavioural patterns, and genetic effects) has been to calculate measures such as relative risks, hazard ratios, concordances and heritability for a given disease. In parallel with the genetic progress newer designs have also been applied. The following study designs are widely applied and have been of particular use in this research field.

2.1.3.1 *Adoption studies*

Adoption studies are very useful in order to evaluate the impact of environmental sources for the occurrence of a disease. In 1924, the first adoption study was performed investigating IQ. Since then this approach has been of great value for research on genetic and environmental influences on CHD and related states/diseases like smoking behaviour [21], body mass index (BMI), obesity [22, 23] and premature death [24]. This approach offers a unique possibility for studies of the pure impact of environmental factors. It also allows combined studies of environmental and genetic factors. In order to do so and address such questions it is, however, necessary to collect relevant information. Relevant information comes from information on adoptees and their adoptive parent's (unique questions on environmental importance) and information on biological parents (unique questions on genetic influences). The adoption design can also be based on twins reared apart as well as half-sibs reared apart.

2.1.3.2 *Family studies*

Studying disease occurrence in families has for a long time addressed questions on hereditary factors in terms of familiality (genetic and environmental). Such designs focus on information based on relatives of different genetic degrees. Usually relative risk of a disease is calculated given that a relative developed the disease. In order to determine the impact due to genetic factors different degrees of genetic relation can be compared such as parents and offspring, siblings etc. However, one difficulty when applying this approach is to depict the impact of familiality as the heritable factor which is either genetic, shared-environmental or both. In order to resolve such questions, information on risk factors is necessary. So far, this approach has, however, contributed significantly regarding the awareness of heritable risks, both genetic and environmental. Several studies have suggested that familial aggregation of CHD could

be due to a genetic predisposition for CHD as well as that family history of CHD increases the risk of the disease [15].

2.1.3.3 *Twin studies*

This particular design (the study design of this thesis) has been of great use for genetic studies, since twins are sampled from the same gene pool and thus share same genes, although to a different degree. Monozygotic or identical (MZ) twins share in principal all their genes in common, and dizygotic or fraternal (DZ) twins share on average half of their genes (like common siblings). The first twin study was performed in 1876, when Francis Galton studied developmental changes in twin similarity. The first study where MZ and DZ twins were compared occurred in 1924. The twin design covers the classical twin design, co-twin control, studies of twins and their relatives as well as genotyping at candidate loci/marker loci. The classical approach compares phenotypic resemblance of MZ and DZ twins in order to examine the contribution of environment (shared and non-shared) and genetic (additive and dominant) factors to the phenotypic variance. Twin studies have been very valuable regarding genetics of CHD (gender specific and age dependent) as well as lipid metabolism, and risk factors for CHD like blood pressure, haemostatic factor levels, and smoking [20].

2.1.3.4 *Association, linkage and linkage-disequilibrium studies*

Genetic association studies [25] address the question whether genetic polymorphisms are associated with a trait. This approach has greater power to detect small effects than linkage studies, but requires larger numbers of examined markers. The linkage design is used to detect regions of the genome that contain genes that are involved in disease occurrence, whereas linkage-disequilibrium aims at establishing associations between genetic polymorphisms and a disease. In linkage studies, the logarithm of the odds scores are calculated, where high positive scores are evidence of linkage and negative against [26]. It is also possible to apply this design to other approaches like the twin method [27]. These designs have so far been of great value for studying genetics of CHD [28].

More detailed information on these and other study designs as well as other related issues applied in genetic epidemiology are presented elsewhere [25-27, 29, 30].

2.1.3.5 *Reports on heredity/familiality/inheritance*

Familial aggregation of CHD has been reported from a number of studies and study designs. Early studies suggested that the familial aggregation of CHD could be due to a genetic predisposition for CHD [31-33]. From calculation of concordances on Swedish and Danish twins, similar conclusions were drawn [34-36]. The familial aggregation of CHD was not totally clustered by known risk factors (hypertension, diabetes, and hyperlipidemia) as it could be caused by undefined genetic factors, environmental factors common to family members and by gene x environment interaction [37]. At the same time another report suggested that the family history of myocardial infarction (MI) could be an important independent predictor of MI and that the familial aggregation for CHD was not reflected in measured levels of cholesterol, systolic blood pressure or smoking [38]. From another review it was suggested that a familial aggregation of CHD has to be considered as an independent predictor for CHD [15]. In addition, premature death in adults due to infections and vascular causes has also been

4

reported to have a strong genetic background [24]. Another study on Swedish twins showed by comparing relative hazards for MZ and DZ twins that genetic factors influence death from CHD, particularly at younger ages. In addition, adjusting for known risk factors such as hypertension, diabetes, smoking, high BMI, marital status and level of education did not change this conclusion [39]. Further studies in Danish twins also suggested moderate influences due to genetic factors [40]. Furthermore, following the significant progress in the field of genetic epidemiology both regarding molecular and population genetics, more advanced genetic studies have recently been performed. So far a large number of candidate genes have been suggested to be associated with CHD and related disorders [6, 7, 28]. Genes behind the regulation of lipoprotein (a) (Lp(a)), apolipoprotein B (ApoB), apolipoprotein E (APOE), lipoprotein lipase (LPL), low-density lipoprotein (LDL), adducin alpha (ADD1), angiotensinogen (AGT), fibrinogen α, β, and γ (FGA, FGB, and FGG respectively), insulin receptor subtrate-1 (IRS-1), interleukin 1-α and 6 (IL1A and IL6) are some of the genes so far suggested. These genes are involved in lipid metabolism, familial hypercholesterolemia, blood pressure regulation, insulin sensitivity, fibrinolysis, and inflammatory response. So far, genome-wide scans have been performed in European, Australian and Mauritian populations as well as a meta-analysis based on these studies [41]. The study on Finnish suggested a linkage for premature death from CHD on chromosome 2q21.1-22.and Xq23-26. In European families a significant linkage for MI was found on chromosome 14. In Mauritian families a suggested linkage for premature CHD was found on chromosomes 10q23, 16p13-pter and 3q27. In Australian families a suggested loci on chromosome 2q36-37 and two potential loci on 2q26-27 and 20q11-13 was found. However, the meta-analysis showed that genetic regions 3q26-27 and 2q34-37 could contain susceptibility genes for CHD [41]. It has also been suggested and reported that genetic and environmental factors interact and increase the risk of CHD as for example a gene-environment interaction between epsilon4, one of the three common alleles of APOE, which has been reported to interact with smoking, as well as an interaction between the alcohol dehydrogenase 3 gene variant and alcohol consumption, also increasing the CHD risk [42]. It is however important to note that replication of positive findings is a major problem when studying genetics. In order to address this issue, a meta-analysis consisting of 370 studies was conducted. By studying 36 genetic associations for various outcomes of disease it was shown that first studies often suggests stronger genetic effects as well as that the results correlate modestly with subsequent studies possibly due to bias and genuine population diversity [43].

2.1.3.6 *Environmental (behavioural) risk factors*

The most important known mainly environmental risk factors (high CHD-risk and easily modified) are smoking, diet, physical activity and psychosocial factors like stress. Smoking is mainly an environmental factor, where exposure on its own greatly increases the risk among smokers. This is not the only characteristic of tobacco use, as it increases the risk of other diseases, as well as interacts with various factors. One of the first studies on this matter was published in the beginning of the 1960's where it was shown that smoking increases the risk of heart disease in the Framingham population [44]. Furthermore, a dose-dependent association has been found where the risk of first AMI increases by 2-3 % for each gram of tobacco smoked daily [45]. In contrast, physical activity has been reported to have an inverse association with CHD as it decreases the risk. Inactivity, though, increases the risk of CHD at the same level as

hypertension, hypercholesterolemia and smoking [46]. A meta-analysis of this matter also found physical activity to have a dose-response decrease of CHD-risk in women [47]. Furthermore, dietary intake and in particular high fat intake has been considered as a major burden for CHD so far. It seems, however, that a reduction of the total fat intake is not as relevant for CHD as the type of fat intake. A diet high in saturated fat increases the risk of CHD whereas a replacement of the saturated fat intake with unsaturated tends to lower the risk of CHD to a higher degree than a reduction of total fat intake. A diet rich in n3-fatty acids (also known as omega-3 fatty acids), which exist in fish and plant sources, lowers the risk of CHD [48]. However, dietary intake may be considered as an environmental risk factor as a study on twins regarding habitual eating patterns (high in fat, salt and sugar as well as healthful eating patterns) showed that 60-85% of the variability in eating patterns was associated with environmental factors [49]. Another risk factor also considered as environmental is psychosocial stress, measured as work, home and financial stress, which increases the risk of AMI [50]. While keeping in mind that these factors are to some extent influenced by genetic factors [51-53], they are still considered as mainly environmental.

2.1.3.7 *Risk factors (genetically influenced)*

Major risk factors for CHD influenced by genetic factors are hypertension, diabetes, lipid metabolisms, total cholesterol levels, obesity and diet. Studying Finnish twins it has been reported that genetic factors tend to have a major role on Type 1 diabetes, and that for Type 2 diabetes, environmental factors also were involved to an important level [54]. In Danish twins insulin dependent diabetes (Type 1) was also reported to be influenced by genetic factors with a heritability estimate of 72% [55]. However, recent research has linked type 1 diabetes with environmental factors like infections [56]. Twin-studies have during the years contributed a great deal to our knowledge of the CHD-genetics. These studies have reported moderate heritability for resting blood pressure. Heritability for diastolic blood pressure was reported from 44% to 66% and for systolic blood pressure from 52% to 66% [20, 57]. Furthermore, heritability estimates of 50-70% of obesity in terms of BMI have been suggested as reasonable [52]. Age and gender specific heritability for BMI was also reported to be 46% for males aged 46-59 years and 61% for males aged 60-76 years. For females these estimates were higher in general, 77% for females aged 46-59 years and 75% for females aged 60-76 years [58]. Adoption studies have contributed a great deal regarding genetic influences on obesity [22, 23]. The importance of genetic factors has also been shown for eating behaviours like cognitive restraints (59% heritability) , emotional eating (60%) and uncontrolled eating (45%) in young Swedish male twins [51]. It has also been reported that serum or plasma levels of total cholesterol, low-density lipoprotein and high-density lipoprotein cholesterol, triglyceride, lipoprotein (a), insulin, plasminogen activator inhibitor-1 and fibrinogen are ranged from the maximum heritability of 32% for fibrinogen to 80% for lipoprotein (a) [59]. In addition, considerable heritability has also been reported for serum lipid levels of 28% to 78% as well as that the genetic effect decreased with age for some [60].

6

Figure 1. From risk to CHD, hypothetical illustration of a CHD-process.
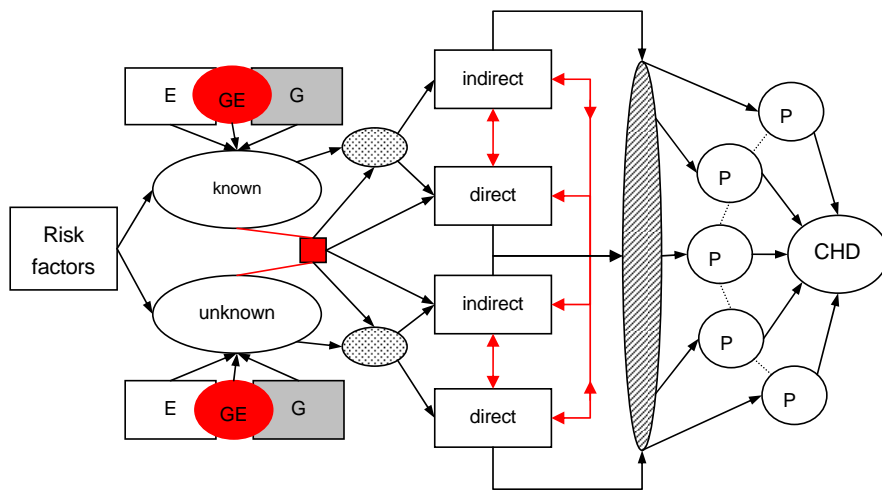
P = phenotype (coronary), E = environment factors, G = genetic factors,
GE=gene x environment.

2.1.4 Gender differences

Traditionally CHD has been considered to be a disease mainly affecting males. During the years this disease was, therefore, often unrecognized in women and many studies focused on men solely. However, calculating the lifetime risk of CHD resulted in a threefold greater risk of having a CHD than breast cancer being found in the Framingham population [61]. Therefore, several studies have been published in order to address gender differences [61-64]. At first, differences by gender in the treatment of CHD were observed. Men were twice as likely to undergo an invasive cardiac procedure as women [65] who when hospitalized for CHD undertook fewer major diagnostic and therapeutic procedures than men [66]. In decisions to refer patients for cardiac catheterization and coronary artery bypass surgery, the sex differential was not explained entirely by differences in the sensitivity of tests or the CAD-rates or either by differential benefits from surgery [67]. However, during the last decades it has been shown that one of the differences between the sexes is that CHD affects women in later phases of life. A study on Swedish AMI data showed that the incidence of MI is the same in women as in men 10 years younger [68]. However, this advantage seems to diminish after the occurrence of the first MI and women face a worse prognosis than men. Women below 50 years of age have worse prognosis than men and this excess mortality seems associated with diabetes [69]. Considering out-of hospital fatal MI, the impact of MI on survival in women increased as compared with men and MI had, thus, a greater impact on mortality in women [70].

There are sex differences between coronary phenotypes. The rare type of AP known as Variant angina is more common in women and AP in total is a more common clinical syndrome in women as compared to men, where MI is more prevalent [71]. In addition,

women face different symptoms of CHD such as jaw pain and nausea more frequently than men do [72]. There are also differences in diagnosing AP by the widely used Rose chest pain questionnaire. In Swedish twins, comparing the questionnaire diagnosis with clinical diagnosis (pathologic post exercise electrocardiogram reaction) where the examination was based on detailed medical history together with results from an exercise test, showed an association among males but not among females [73]. In addition, the reliability of reporting AP (standardized Rose Questionnaire) in The Lipid Research Clinics Prevalence Study was somewhat lower among women than men [74]. Another study on chest pain perception showed a significant difference between the sexes on the intensity of chest pain (more in women), the link of the pain to heart diseases (less in women) and a more atypical clinical picture of the pain in women [75]. Also, differences have been reported regarding symptom presentation in men and women hospitalized with AMI [76]. One of the main differences between the sexes regarding the risk of CHD is the effect of diabetes. Type 2 diabetes is a major risk factor for CHD, the impact of which has been shown to be larger in women than men. Females with diabetes have a risk from 3 to 7 as compared with 2 to 3 in males [62]. Other risk factors that increase the risk for CHD more in women than men are low high-density lipoprotein, high triglycerides and hormonal status/menopause, the last as the only factor known so far to affect only one gender [64]. It has also been reported that women experiencing menopause had three times greater risk of CHD [61]. In contrast to women and their risk factors, lipoprotein (a) seems to be a stronger risk factor in men though. There are also suggestions that family history, C-reactive proteins and smoking may have a larger influence in women the men [64]. Furthermore, high levels of plasma fibrinogen were independently associated with increased risk of MI in men but not in women [77]. Another risk factor affecting both sexes is depression, which has been shown to be more frequent in females (almost twofold) than in males [78].

3 MATERIAL

3.1 THE SWEDISH TWIN REGISTRY

The Swedish Twin Registry (STR) is today a valuable resource for research. It is a population based ongoing register containing information on twins born from the year 1886 onward [79, 80]. The registry was developed in late 1950's in order to study the effect that smoking and alcohol consumption have on health. Diseases of primary interest at that point of time were cardiovascular diseases and cancer. Since the start the registry has been mainly divided in three cohorts: twins born between 1886 and 1925, twins born between 1926 and 1958 and twins born from 1959 and 1990. Other interesting projects (completed and ongoing) within the register are briefly described below:

i) Screening Across the Lifespan Twin study (SALT)
Complete screening of all twins born 1958 or earlier for various things as contact with twin partner, birth weight, prescription and non-prescription medication use, occupation, hormone replacement therapy (in women), diseases and symptoms, occupation, alcohol consumption etc. Data collection was done by computer assisted telephone interviews.

ii) Swedish Adoption/Twin Study of Aging (SATSA)
This is a well defined subset of the STR. It contains twins reared apart and a matched sample of twins reared together with the aim to study successful aging. These studies are performed through five questionnaires mailed every third year and in-person measures of physical and psychological health and wellbeing, personality, cognitive status, family environment etc. Several important genetic studies on CHD-related factors like serum lipid levels, apolipoprotein and blood pressure have been obtained on SATSA [57, 60, 81].

iii) Origins of Variance in the Oldest Old (OCTO-Twin)
OCTO-twin focuses on studying the oldest old. This is a longitudinal study of aging in all like-sexed Swedish pairs 80 years of age or older. All like-sexed twin-pairs 80 years or older where both were alive at the start were recruited to the study. Data collection is based on questionnaires, health assessments and extensive cognitive testing in subject's homes.

Since this PhD thesis is based on data on twins born between 1886 and 1958 the two corresponding cohorts will be described more in detail. For completeness brief information on twins born between 1959 and 1990 is also presented. Detailed information on all projects included in this registry is presented elsewhere [79, 80].

3.1.1 Twins born between 1886-1925

All parishes in Sweden were contacted in 1959 in order to obtain information on multiple births between 1886 and 1925. In order to determine the status of all twin pairs they were followed until the status was established. In 1961 all complete same-sexed twin pairs (which means both twins alive) that lived in Sweden received a questionnaire with questions regarding twin similarity, urban/rural, marital and smoking status. This cohort of twins includes only twin pairs who were alive and responded to the

questionnaire sent in 1961, approximately 21,000 same-sexed twins. All twin pairs where at least one twin in a pair died before 1961 were never included in the registry. Four questionnaires were sent to the twins included in this cohort, the first in 1961, the second in 1963, the third in 1967. Due to non-response to the 1967 questionnaire some selected pairs received a questionnaire in 1970. The second questionnaire included questions regarding height, weight, education, dental status, family and list of diseases. The questionnaires sent out in 1967 and 1970 included questions regarding height, weight, cardiovascular, respiratory and headache symptoms, alcohol, smoking, diet, stress and occupation.

3.1.2 Twins born between 1926-1958

This cohort was established by use of nationalized birth registrations in 1970 including all twins born between 1926 and 1967. A birth register was established containing 50,000 twin births. In 1972/1973 a questionnaire was sent out to all same-sexed twin pairs born between 1926 and 1958 containing similar questions as the ones sent out to the members of the 1886-1925 cohort, as well as a questions regarding air pollution and noise. A short version of Eysenck Personality Inventory was developed and included.

3.1.3 Twins born between 1959-1990

Twins born between 1959 and 1968 were already identified in the birth register when the 1926-1958 cohort was compiled, but were not contacted. Twins born between 1969 and 1990 were included in 1993 by record linkage to the Medical Birth Register. Twins born 1959 to 1986 were recently contacted and participated in a web-based questionnaire.

3.1.4 Zygosity determination

In the first phases of the registry, twin similarity was determined by asking the twins a simple question: whether they and their twin partner were similar as two peas in a pod during childhood or not more than siblings in general. If both twins within a pair answered yes to this question (similar as two peas in a pod) they were classified as MZ twins. If both answered that they were more like ordinary siblings they were classified as DZ twins. Twin pairs where the twins answered differently to the question above were classified as not determined zygosity. This method has been proven to be adequate for determining similarity in twins. In total 95% of all similarity diagnoses have been proven to be correct. In order to determine similarity in twins for the SALT-project described briefly above as well as to further determine similarity for twins born between 1886 and 1958 for which zygosity could not be determined, blood samples and two questions regarding similarity during childhood were used. The questions used for twins born between 1886 and 1958 as well as an additional question regarding stranger difficulties in distinguishing between the twin partners during childhood were used. This way of determining zygosity improved the already good method described above and resulted in correct zygosity classification for 99% of the pairs (199 adult pairs) which was replicated (98%) in the Twin Moms study where 10 DNA micro satellite markers were used [79].

3.2 THE SWEDISH ACUTE MYOCARDIAL INFARCTION REGISTER

In 1978 Ahlbom developed a method by which hospital-care register kept by the Stockholm county council could be used in epidemiological studies [68, 82, 83]. This

method served as a base for establishing the Swedish Acute Myocardial Infarction Register in 1996. The register was established by record linkage of two already existing registers, the Hospital Discharge Register and the Cause of Death Register. It includes all persons (possessing a Swedish Civic Registration Number) having an AMI, both fatal and non-fatal, for the time period of 1987 to 2000 (at this point in time) reported to the Hospital Discharge Register or to the Cause of Death Register. The Hospital Discharge Register reports all patients discharged from public hospitals in the country. The Cause of Death Register includes all causes of death for people registered as Swedish residents, whether or not the death occurred in the country. All AMI events are identified by the date of admission or death and are separated in fatal and non-fatal. An event is classified as fatal if the patient dies from AMI outside the hospital or if the patient dies from AMI (as an underlying or a contributing cause of death) within 28 days from the admission. If a patient survives an episode of an AMI the event is classified as non-fatal. All AMI events that occur after 28 days from the admission are treated as new events.

3.3 CLASSIFICATION OF DISEASES

3.3.1 Coronary heart disease
The international classification of disease codes (ICD) for CHD are:

ICD7	420, 4221	between 1958 and 1968
ICD8	410-414	between 1969 and 1986
ICD9	410-414	between 1987 and 1996
ICD10	I20-I25	1997 +

3.3.2 Myocardial infarction
The Acute Myocardial Infarction Register started in 1987 and up to now it covers two revisions of the ICD. The codes for MI are:

ICD9	410	between 1987 and 1996
ICD10	I21, I22	1997 +

3.3.3 Angina pectoris
Information on AP was obtained from self-reports [73] and from the Swedish Hospital Discharge Register [84].

3.3.3.1 Rose's Questionnaire
The questionnaires included a set of slightly modified questions regarding chest pain elaborated by Rose [85] that the World Health Organization in 1963 recommended for use in epidemiological studies [86]. The following questions were used for AP:

1. Have you ever had any pain or discomfort in your chest?
   a. No
   b. Yes
2. If yes, when do you feel this pain or discomfort?
   a. When you are emotionally upset or excited
   b. When you walk fast or walk uphill
   c. When you walk at normal speed on level ground
   d. Under other circumstances

3. What do you do if you get this pain or discomfort while you are walking?
    a. Stop walking or walk more slowly
    b. Take medicine and continue walking at the same speed
    c. Continue walking at the same speed without taking medicine
4. If you stop walking, regardless of whether you take medicine or not, how is the pain or discomfort then?
    a. The pain usually passes within ten minutes
    b. The pain usually continues for more than ten minutes
5. Where are the pains or the discomfort located?
    a. In the middle of the chest
    b. In the left side of the chest
    c. In the left arm
    d. In some other place

A positive AP diagnosis was given if the respondent reported pain or discomfort in the chest (question 1, answer b), that was felt when emotionally upset or excited (2, a) and/or when walking fast or walked uphill (2, b) and/or when they walked at normal speed on level ground (2, c). Further, the respondent needed to state that they stopped walking or walked more slowly (3, a) and/or that they take medicine and continued walking at the same speed (3, b) and that the pain usually passes within ten minutes (4, a). Finally, the pain was localized either to the middle of the chest (5, a), to the left side of the chest (5, b), or to the left arm (5, c) [73].

3.3.3.2 *The Swedish Hospital Discharge Register*

Statistics of diseases and surgical treatments of patients has a long history in Sweden. Such data have been published for more than 100 years and have been available for the whole of the 20: th century. In the 1960's the National Board of Health and Welfare started to collect data on individual patients, who had been treated as in-patients at public hospitals. The register initially covered all patients treated in psychiatric care and around 16 per cent of patients in somatic care in six of the 26 counties in Sweden. The number of contributing county councils increased during the years and from 1987 this register covers all public, in-patient care in Sweden. More severe forms of AP diagnoses are covered on all in-patient care at Swedish hospitals from the period 1987 - 2003. The register includes 47 million discharges for the period 1964-2003 [84].

Two revisions of the ICD yields this period, the codes for AP are:

ICD9   413   between 1987-1996
ICD10   I200, I201, I208, I209   1997 +

4 METHODS

4.1 SURVIVAL ANALYSES

4.1.1 Proportional hazards model

The proportional hazards model or the Cox-model (1) has been considered as the standard model in basic survival analysis [87]. This model is useful when the aim is to determine the impact of risk factors on the time a disease occurs. The model is semi parametric as the baseline hazard in not specified or estimated.

(1) $$\lambda(t \mid X) = \lambda_0(t)e^{\beta X}$$

where $\lambda(t \mid X)$ is the hazard at time t with the covariate vector $X = (X_1,\ldots, X_j)$, and λ_0 the baseline hazard function.

4.1.2 Univariate frailty model

The univariate frailty model (2) was introduced in 1979 [88]. This model is an extension of the Cox-model, the main feature of which was to include an unobserved random variable for hidden (unobserved) heterogeneity.

(2) $$\lambda(t \mid X, Z) = Z\lambda_0(t)e^{\beta X}$$

where $\lambda(t \mid X, Z)$ is the hazard at time t with the covariate vector $X = (X_1,\ldots, X_j)$, Z an unobserved random variable (frailty), and λ_0 the baseline hazard function.

In standard models heterogeneity is included in the error term, which leads to an increase in variability of the response compared to the case. In survival analysis the increase of variability affects the hazard function. This, in turn, influences both the assumption of proportional hazards as well as the hazard ratios [89]. It is, therefore, not possible to interpret the estimates obtained by the univariate frailty model as relative hazard rates on a group level but rather on an individual level (conditional hazard rates). The key idea behind the concept of frailty is that individuals have different 'frailties' (susceptibility to the event under study), and that those who are most frail have an increased risk to die earlier than others. The frailty variable is a random effect and it is necessary to make an assumption about the distribution of this non-observable random variable. It is not possible that frailty takes a negative value, therefore, only distributions on the positive axis such as gamma are used.

4.2 QUANTITATIVE GENETIC ANALYSES OF TWINS

4.2.1 Quantitative genetics

Quantitative genetic analyses [90] examine the nature of individual differences as well as similarities of family members. In order to address the question regarding genetic and environmental impact on dissimilarities, the variance of a trait has to be studied. In order to do so it is necessary to perform studies on subjects with different degrees of genetic and environmental relationships. The twin-design is, therefore, of great use for genetic studies as twins are sampled from the same gene pool and they share same

genes, although to a different degree. MZ twins share in principal all their genes in common, and DZ twins shares on average, half of their genes (like ordinary siblings). The total phenotypic variance is, thus, depicted by components reflecting environmental and genetic factors. The phenotypic variance of a trait P is thus written as $Var(P) = \sigma^2 = \sigma^2_g + \sigma^2_o$, where σ^2_g is the genetic variance and σ^2_o is the environmental variance. The total variance due to genetic factors is written as $\sigma^2_g = \sigma^2_a + \sigma^2_d$, where σ^2_a is the variance due to additive genetic factors and σ^2_d is the variance due to dominant genetic factors. The total variance due to environmental factors is written as $\sigma^2_o = \sigma^2_c + \sigma^2_e$, where σ^2_c is the variance due to shared environmental factors and σ^2_e is the variance due to non-shared environmental factors. For this type of analyses equal environment for both types of twins, random mating, and no gene-environment interaction is assumed. DZ-twins do not necessary share their environment to the same degree as MZ twins do, and violations of this assumption can result in overestimation of genetic influences for a trait. The correlation coefficients ρ_{MZ} and ρ_{DZ} calculated for MZ and DZ twins provide information about genetic and environmental influences on a trait. Under the assumption of equal environments for both MZ and DZ twins, differences in the two correlation coefficients reflect the influence of genetic factors. Standard assumptions about the quantitative genetics yield the following relations: $\rho_{MZ} = a^2 + d^2 + c^2$, $\rho_{DZ} = 0.5a^2 + 0.25d^2 + c^2$ and $1 = a^2 + d^2 + c^2 + e^2$ (note that a^2 corresponds to the proportion of total variance associated with additive genetic effects (A), d^2 corresponds to the proportion of total variance associated with dominant genetic effects (D), c^2 corresponds to the proportion of total variance associated with shared environmental effects (C), and e^2 corresponds to the proportion of total variance associated with non-shared environmental effects (E)). The components are assumed to be independent. Theoretically up to six biometric models based on different assumptions about genetic structure can be estimated (ACE, ADE, AE, DE, CE, and E). From the estimation point of view no more than three components can be simultaneously represented in a model for MZ and DZ twins. Dominant genetic factors and shared environmental factors can not be estimated simultaneously because they are confounded in the classical study of twins reared together [91].

4.2.2 Heritability

Using variances explained above heritability can be calculated in order to determine the relative importance of genetic factors on a trait. This estimate is defined as the proportion of variation directly attributable to genetic differences among individuals to the total variation in a population [30]. Therefore, heritability can only take values between zero and one. Two types of heritability are estimated: the broad (ratio of variance attributed to all genetic differences among individuals to the total phenotypic variance) and the narrow sense heritability (ratio of variance contributed by the additive effects of alleles at one or more loci to the total phenotypic variance). In the simple case the genetic influence (additive and dominant) is calculated as $a^2 = 2(\rho_{MZ} - \rho_{DZ})$ and as $d^2 = 2\rho_{MZ} - 4\rho_{DZ}$. The proportion of the environmental influence (shared and non-shared) to the phenotypic variance is calculated as $c^2 = 2\rho_{DZ} - \rho_{MZ}$ and $e^2 = 1 - \rho_{MZ}$ [90]. Heritability takes a value of zero when phenotypic variation is attributable to non-genetic factors. Heritability estimates are also low when the studied population is genetically homogeneous. It is important to note that this is a population specific parameter used as a measure within the sample, and should, therefore, not directly be applied in other populations.

14

4.2.3 Probandwise concordances

Concordance rates [92] are calculated separately for MZ and DZ twins and if higher rates are observed among MZ than DZ twins, the difference is interpreted as caused by genetic factors. These rates are calculated as two times the number of concordant pairs (C) divided by the sum of two times the concordant and discordant pairs (D) [2C / (2C+D)]. Pairs considered as concordant are pairs where both twin partners experienced the event under study. Pairs considered as discordant are pairs where one twin partner experienced the event under study.

4.2.4 Genetic analyses of frailty

Genetic analyses of frailty are based on application of the correlated gamma-frailty model in order to estimate the relative influence of genetic factors (known as heritability). This model is particularly useful when dependent life times are analyzed [93, 94] and stems from the univariate frailty model [88]. Frailty is a latent (unobservable) variable and as mentioned above non-negative. In this model frailty is assumed to be gamma distributed. The reason why frailties are assumed to be gamma distributed is because of mathematical convenience; the gamma distribution is a relatively flexible family, but mathematically tractable. Frailty in twins is considered correlated as twins within a pair share a part of the frailty. Consider Z_1 to be the frailty of the first twin and Z_2 to be the frailty of the second twin. Furthermore, consider the decomposition of the frailties as $Z_1 = Y_0 + Y_1$ and $Z_2 = Y_0 + Y_2$ where Y_0, Y_1 and Y_2 are independent gamma distributed random variables with $Y_0 \sim G(k_0, \lambda)$, $Y_1 \sim G(k_1, \lambda)$ and $Y_2 \sim G(k_2, \lambda)$. Parameters k_0, k_1 and k_2 are positive. Frailty is obviously correlated, due to Y_0, which is a part of both Z_1 and Z_2. This bivariate lifetime model allows dealing with censored and truncated observations, which allows a combination of survival analysis and methods of quantitative genetics. Selection of the best fitting nested model is based on the likelihood ratio test, and selection of the best fitting non-nested model was based on the Akaike information criterion (AIC) [95].

4.2.5 Genetic analyses of liability

Liability (susceptibility to an event under study) is considered as an underlying unknown trait influenced by both genetic and environmental factors [96]. This approach utilizes threshold models, where the threshold is a point reflecting prevalence on a latent distribution of liability. Individuals above the threshold are assumed to develop the disease and individuals below this point are assumed not to develop the disease. Structural equation modelling techniques implemented in the software Mx [97] are adapted to calculate twin (intraclass) correlations as well as to evaluate heritability based on the liability threshold model. Twin correlation estimates the within-phenotype correlation in disease liability under the assumption of bivariate normal distribution as well as cross-phenotype and cross-twin cross-phenotype correlations. Calculation of twin correlations and heritability can be performed by use of contingency tables and by use of raw data. Heritability can be estimated in the univariate (single phenotype crude and adjusted) as well as in the bivariate case. The bivariate model includes two phenotypes and is used to analyze genetic contributions to the variation for both phenotypes as well as the genetic contributions to the correlation between the two phenotypes. In order to determine which full model is the most suitable, the correlation estimates, based on gender and zygosity, were compared. A model with additive

genetic variance is to be preferred when the correlation among MZ twins is around two times larger than the correlation among DZ twins. If the DZ correlation is less than half the MZ correlation, the dominant genetic model is to be preferred. If the DZ correlation is greater than half the MZ correlation, shared environmental influences are indicated. Likelihood ratio test was used in order to compare nested models by comparing 2log-likelihhod statistics for the full and the reduced models. If the difference (following a chi-square distribution with one degree of freedom) is observed to be significant the conclusion is that the full model fits the data better than the reduced. In order to determine the best fitting non-nested model the AIC [95] was used.

5 AIMS

The particular aims of this PhD-project were:

I. To determine the relative importance of genetic and environmental factors for CHD-death.

II. To evaluate if genetic influences for CHD-death differ at different stages in life and if they differ among sexes.

III. To evaluate to what extent the genetic impact for CHD-death is mediated through well-known risk factors.

IV. To determine the relative importance of genetic and environmental factors for AP as well as to determine the effect of AP on CHD-death.

V. To determine the relative importance of genetic and environmental factors for the first episode of AMI.

6 RESULTS

6.1 PAPER I

Aim

The aim of this particular study was to evaluate and distinguish between environmental and genetic effects for death from CHD as well as to determine whether the importance of genetic influences are changing with age, using information on twins born between 1886 and 1925.

Material

The total number of same-sexed twins included in the analyses was 20,966 of which 7,288 were MZ twins and 13,678 DZ twins. A total number of 4,007 CHD-deaths was observed during the follow-up period until 1996 (2,208 male and 1,799 female CHD-deaths).

Analysis of probandwise concordances

The proportion of concordant MZ pairs was greater than the corresponding proportion of DZ pairs in both sexes (presented in Table 1.1). The difference in concordance rates between MZ and DZ twins was statistically significant (p<0.05) in males, which indicates a possible role of genetic factors in death from CHD. In females the MZ concordance rate was greater than the corresponding rate for DZ female twins, although the difference was not statistically significant.

Table 1.1. The number of concordant and discordant twin pairs and proband-wise concordance rates for death due to CHD, by sex and zygosity.

CHD	Men		Women	
	MZ	DZ	MZ	DZ
Concordant pairs	153	248	97	155
Discordant pairs	451	955	424	871
Concordance rate	**40.4***	**34.2***	**31.4**	**26.2**

* Differences between concordances are statistically significant (P<0.05).

Age-specific concordance rates in males and females (presented in Figure 1.1) showed successively lower concordance rates with increasing age at death in males both among MZ and DZ-twins. As the difference in concordance rates between male MZ and DZ twins is almost the same for all age intervals, it seems as if genetic influences are fairly stable over the age-at-death intervals. In female twins, on the other hand, no decreasing trend was apparent, but the data suggested that genetic influences on CHD-mortality were more important among females whose twin partners died from CHD before the age of 65 years than those whose partners died after 65 years of age.

Figure 1.1. Probandwise concordance rates dependent on the age at death of the index twin, by sex and zygosity.
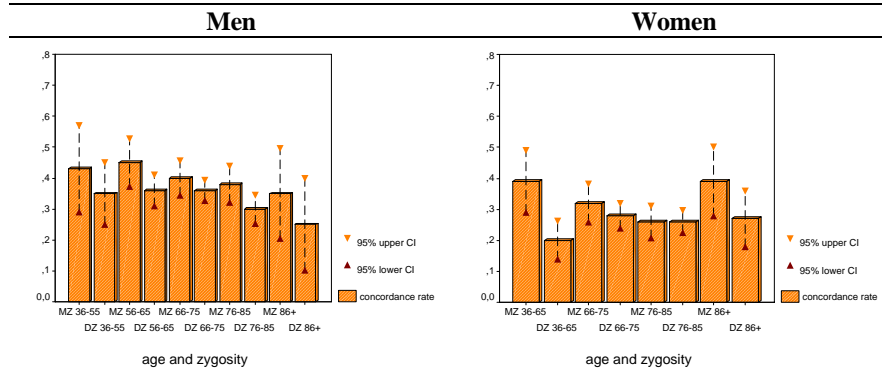
Genetic and environmental analysis of frailty

In the analyses of the correlated gamma-frailty model, the model with an additive genetic component and a non-shared environmental component gave the best fit to the data according to the AIC- for both male and female twins. The purely environmental CE-model gave the worst fit to data, for both males and females, suggesting that the susceptibility to die from CHD is not purely environmental. The heritability estimate for the best fitting model, AE, was approximately 0.38 (95%CI, 0.26-0.50) among female twins. Among male twins the heritability estimate was 0.57 (0.45-0.69), which is larger (borderline significant, p=0.06) than the corresponding estimate among females. Analyses based on the three follow-up times (presented in table 1.2) indicated that the highest heritability estimates were noted for follow-up through 1987 (i.e. the shortest period of follow-up time). The small but noticeable decrease in heritability over extended follow-up times confirms the hypothesis that the heritability decreases slightly over time, and that genetic effects seems to be stronger in early phases of life, although the decrease may not be considered as statistically significant.

Table 1.2. Heritability estimates obtained from the best fitting model, the AE-model with regard to different follow-up times, by sex.

	Heritability		
Follow-up	**1987**	**1992**	**1996**
Males	0.66	0.63	0.57
Females	0.44	0.38	0.38

6.2 PAPER II

Aim

The main focus of this study was to determine to what extent genetic influences for CHD-death are affected by genetic effects for cardiovascular risk factors. Two frailty models were compared regarding differences and similarities in results. A univariate gamma-frailty (the non-twin) model and a correlated gamma-frailty (twin) model were compared.

Material

A total number of 2,499 CHD-deaths (1,437 male and 1,062 female) was observed among twin pairs with complete information on related risk factors (a total number of 14,170 twins).

Non-twin model analyses

In general, estimates obtained by the twin model were larger, particularly for risk factors with a low number of cases such as diabetes and hypertension. Independently of gender, the health-related risk factors hypertension, diabetes, and high BMI, as well as life-style habits such as smoking, contributed significantly to an increase in the risk of dying from CHD. The relative hazard of CHD-death was significantly lower for twins with a level of education above grade school (among males) and among married compared to unmarried twins (among both males and females). The adjusted relative hazards (Figure 2.1) indicate an apparent difference in the influence of genetic factors on CHD-death. The relative hazard for MZ twins whose partner died from CHD was significantly larger than the relative hazard for DZ twins among both sexes.

Figure 2.1. Adjusted relative hazards of CHD-death for MZ and DZ twins whose partner died from CHD, by sex.
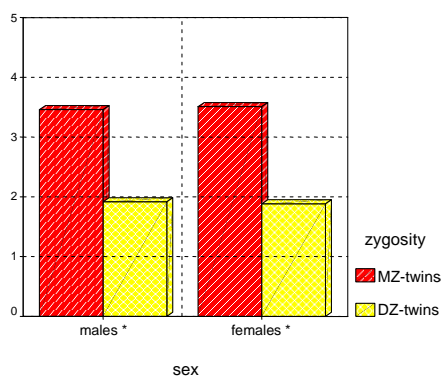
* Adjusted relative hazard for MZ twins is significantly different from the adjusted relative hazard for DZ twins. * p-value < 0.01.

Twin model analyses

The best fitting genetic model for both sexes was the AE-model, indicating that the nature of genetic factors influencing CHD-death is additive and the nature of environmental effects is non-shared or individual specific (presented in Table 2.1). Furthermore, the purely environmental model (CE) did not fit the data, and hence

20

CHD-death is not influenced solely by environmental factors. The heritability estimate obtained in the crude analysis (not considering risk factors) was 0.40 (0.26-0.54) among females and 0.59 (0.41-0.76) among males.

Table 2.1. Estimates of the components of variance in frailty to mortality from CHD obtained by the AE-model with and without risk factors.

Genetic model	Males		Females	
	crude	adjusted [a]	crude	adjusted [a]
AE	s^2	s^2	s^2	s^2
additive genetic factors	2.97	2.26	4.66	2.07
non-shared environmental factors	2.09	0.80	6.95	1.72

[a] Adjusted for smoking, BMI, hypertension, diabetes, marital status, level of education, and birth periods. s^2 is the variation due to additive genetic factors (A) or due to non-shared environmental factors (E).

When including risk factors in the analysis, a slight decrease of the genetic variance was observed, more apparent among females, and a substantial decrease of the non-shared environmental variance among both sexes. Thus, a very slight portion of the genetic variance among males, and a moderate portion of the genetic variance among females, is accounted for by the genetic variance for these risk factors. On the other hand, the risk factors do account for a substantial part of the individual specific environmental variance.

6.3 PAPER III

Aim

The primary aim of this study was to explore further the possible impact of genetic influences on AP. Secondly, we were also interested in assessing the impact of AP on CHD-death as well as the genetic contribution to the correlation between these two phenotypes.

Material

All same-sexed twins born between 1886 and 1958 served as a base for this study. As the main aim of this particular study was to focus on self-reported AP (in early 1960's and 1970's) all twins born after 1945 were excluded from the main analyses as the twins born after 1945 were too young to be considered for the diagnosis of AP when the questionnaires were sent out. The final sample included, therefore, 38,924 twins. In total we observed 2,225 cases of AP (835 among males and 1,390 among females).

Proportional hazards model

The impact of self-reported AP on CHD-death was significant both among males and females. Hazard ratios (HR) for CHD-death obtained by the proportional hazards model yielded ratios of 2.0 (95% CI, 1.8-2.3) among males, and 1.6 (1.4-1.8) among females. Analyzing males and females together resulted in a HR of 1.6 (1.5-1.8). Including sex in the model increased the AP estimate to HR 1.8 (1.6-2.0). Men had a significantly greater risk of CHD-death than women (HR 2.2 (2.0-2.3)). Including an interaction term for AP and sex indicated that the impact of AP on CHD-death was significantly higher among males as well.

Analysis of probandwise concordance and intraclass correlations

The probandwise concordances (presented in table 3.1) indicated that genetic factors influence both AP and CHD. Probandwise concordances were larger for MZ-twins as compared to DZ-twins both regarding AP and CHD. The concordance rates for AP were slightly larger in MZ females (0.26) than MZ males (0.19). Concordances for CHD-death were slightly larger in males than females in both MZ and DZ-pairs. Similarly, intraclass correlations (presented in table 3.1) for AP were higher in MZ-pairs (0.39 for males and 0.46 for females) than in DZ-pairs (0.27 for males and 0.15 for females) which indicates an impact of genetic factors in both sexes. The difference in intraclass correlation between MZ and DZ-twins was larger for female twins. The relatively large difference (the correlation in DZ-twins was less than half of the correlation in MZ-twins) between the two zygosity groups in females indicated presence of dominant genetic factors. In males the difference less than 2:1, indicating the presence of shared environmental influences. Intraclass correlations for CHD-death were large in both males and females. The correlation in MZ-pairs was 0.50 for males and 0.48 for females and in DZ-pairs 0.39 for females and 0.38 for males. The difference in correlation between MZ and DZ-pairs indicated the presence of shared environment in both sexes. Estimates of AP-CHD correlation, (presented in table 3.2) were higher in males as compared to females. The estimates in MZ males were 0.32. In DZ males the estimates were 0.28. In females the correlation estimates were 0.24 for MZ as well as 0.17 for DZ-twins.

Table 3.1. Concordant and discordant pairs, probandwise concordances and tetrachoric (intraclass) correlations, by sex and zygosity.

	Males		Females	
AP	**MZ**	**DZ**	**MZ**	**DZ**
Concordant pairs	30	32	63	49
Discordant pairs	249	420	362	750
concordances	**0.19**	**0.13**	**0.26**	**0.12**
Tetrachoric correlation	**0.39**	**0.27**	**0.46**	**0.15**
CHD	**MZ**	**DZ**	**MZ**	**DZ**
Concordant pairs	180	288	118	191
Discordant pairs	586	1188	529	1038
concordances	**0.38**	**0.33**	**0.31**	**0.27**
Tetrachoric correlation	**0.50**	**0.38**	**0.48**	**0.39**
AP and CHD	**MZ**	**DZ**	**MZ**	**DZ**
AP-CHD correlation	**0.32**	**0.28**	**0.24**	**0.17**

Genetic and environmental analysis of liability

The difference in correlations between MZ and DZ twins suggested that a full model with shared environment should be used instead of a model with dominant genetic effects among males. Comparing AE and the CE-model with the ACE model by the Likelihood ratio test (ACE v AE, difference of 0.95, 1_{df}) and (ACE v CE, 2.6, 1_{df}) indicated that both nested models give a better fit than the full model. Comparison of AIC-values indicated that the AE model was the better fitting model for AP in males. In females the large difference in correlations were better matched to a full model including dominant genetic effects instead of the ACE model. Using the Likelihood ratio test to compare AE with the ADE model resulted in a significant difference (5.7, 1_{df}). The dominant genetic component D was significant. The heritability for AP (presented in figure 3.1) in males was 0.39 (95% CI, 0.29-0.49) and in females 0.43 (0.08-0.51).

Figure 3.1. Heritability estimates and 95% CI for self-reported AP, by sex

Males	Females

White slices = heritability, Black slices = non-shared environment
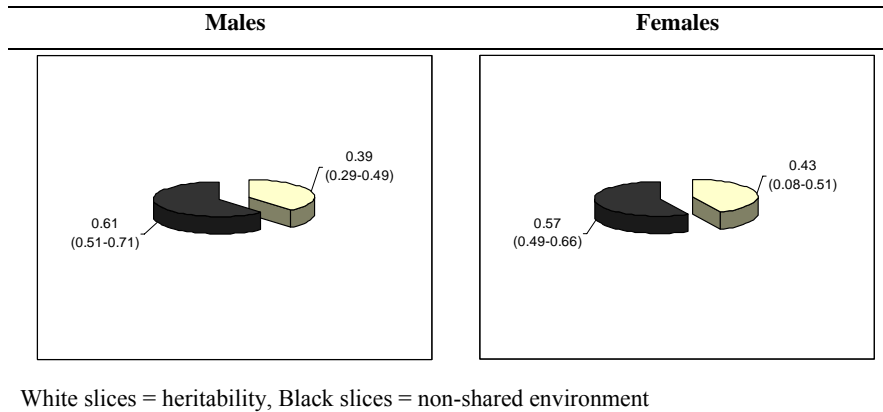
Including CHD in the analyses of heritability (presented in table 3.2) resulted in the ACE-model as the better fitting model for both sexes compared with the ADE-model. The phenotypic variation was thus explained by an additional factor C. The estimates were on the whole in line with the estimates obtained by the univariate model. Depicting the correlation between AP and CHD into components A, C and E resulted in moderate familial influence on the correlation among both sexes. Both genetic (A) and shared environmental (C) correlations were positive. The phenotypic correlation was almost completely explained by familial factors. The genetic proportion of the correlation was 0.44 among males and 0.55 among females. The contribution of the shared environmental part to the total phenotypic correlation was 0.67 for males and 0.39 for females. The proportion of the non-shared (unique) environmental factors to the correlation was smaller for both males and females with the difference that the estimate was negative for males.

Table 3.2. Heritability and proportions of AP-CHD correlation obtained by the bivariate model (ACE), by sex.

	a^2	c^2	e^2
Males			
AP	0.24	0.16	0.60
CHD	0.25	0.26	0.49
Proportion of correlation	0.44	0.67	-0.11
Females			
AP	0.38	0.03	0.59
CHD	0.16	0.32	0.53
Proportion of correlation	0.55	0.39	0.06

6.4 PAPER IV

Aim

The aim of this study was to determine the relative contribution of genetic factors for the first episode of AMI.

Material

Same-sexed twin pairs born between 1886 and 1958 where both twins were alive in 1987 were included in the analyses as the acute myocardial infarction register started in 1987. In total 41,492 twins were included in the analyses. 2,341 first episode cases of AMI were observed (1,326 among males and 1,015 among females). 824 first episode AMI cases died within 28 days whereof 786 died from AMI. Females experienced their first episode of AMI at older ages as compared to men (74.5 and 68.4, respectively).

Analysis of probandwise concordances

Probandwise concordances were in general larger in MZ than in DZ-pairs (presented in table 4.1). The estimates for MZ-pairs were similar for both sexes (0.26 for males and 0.27 for females). For DZ-pairs the estimates were 0.20 for males and 0.16 for females. The difference between the zygosity groups by gender indicates a genetic influence for both sexes, although higher in females, as inferred from a larger difference. The estimated tetrachoric correlation was also larger in MZ as compared to DZ-twins (presented in table 4.1). As for the concordances, the difference between the estimates by zygosity was larger for females. The estimates were 0.48 for MZ and 0.34 for DZ-males and 0.56 and 0.35 for female MZ and DZ-pairs, respectively. These patterns of correlations indicate both a genetic and shared environmental predisposition for the first occurrence of AMI.

Table 4.1. Number of concordant and discordant pairs, probandwise concordance rates and tetrachoric correlation for the first episode of AMI, by zygosity and sex.

	Males		Females	
AMI	**MZ**	**DZ**	**MZ**	**DZ**
Concordant pairs	63	83	50	50
Discordant pairs	352	682	276	539
concordances	**0.26**	**0.20**	**0.27**	**0.16**
Tetrachoric correlation	**0.48**	**0.34**	**0.56**	**0.35**

Genetic and environmental analysis of liability

Heritability estimates for AMI (presented in table 4.2) were moderately large for both sexes. The AE-model model fitted the data for males best. Twice the difference in log-likelihood for the full (ACE) and the reduced AE-model with one degree of freedom was not significant. The CE model fits the data significantly less well than the ACE model. The heritability estimate for males was thus 0.35 (95%CI, 0.28 - 0.43). For females the best fitting model was also the AE-model. The heritability estimate for females was thus 0.38 (0.29-0.46).

Table 4.2. Age adjusted heritability estimates and 95% CI for the first episode of AMI.

AMI	a^2	c^2	e^2	Difference in 2log-l [†]
Males				
ACE	0.30	0.04	0.66	
	(0.07 - 0.42)	(0.00 - 0.21)	(0.58 - 0.75)	
AE*	0.35		0.65	0.268
	(0.28 - 0.43)		(0.57 - 0.72)	
CE		0.25	0.75	6.390
		(0.19 – 0.31)	(0.69 – 0.81)	
Females				
ACE	0.38	0.00	0.62	
	(0.20 - 0.46)	(0.00 - 0.13)	(0.54 - 0.71)	
AE*	0.38		0.62	0.000
	(0.29 - 0.46)		(0.54 - 0.71)	
CE		0.25	0.75	12.465
		(0.19 - 0.32)	(0.68 - 0.81)	

* Best fitting model, † one degree of freedom

7 DISCUSSION

Data from this thesis suggest that genetic influences are of importance for death due to CHD in both sexes, although to a slightly higher degree in males. These influences tend to operate throughout the entire life span, while they seem to be particularly important for earlier ages at death. Furthermore, genetic influences for CHD-death are only partly mediated through known risk factors in males, but more so in females. AP as diagnosed by responses to a questionnaire is an important risk factor for CHD for both sexes with a slightly higher impact in males. The association between AP and CHD is moderately large and almost entirely explained by familial factors (shared environment and genetic factors). The influence of genetic factors plays a moderate role among both sexes for the occurrence of AP as well as for the first episode of AMI.

7.1 PREVIOUS REPORTS

These findings confirm and extend early findings regarding genetic influences on CHD and its sub-phenotypes. Familial aggregation for CHD has been reported in various studies and study designs [15, 24, 28, 39, 40]. Early studies on Swedish and Danish twins suggested a genetic predisposition for CHD, although the Danish study reported higher genetic influence in females [36, 40]. In a recent study on genetic influences for death from heart diseases it was noted in a subgroup analysis that heritability of death from CHD (based on the correlated gamma-frailty model as well) among twins in the Danish twin registry was 0.53 (0.31-0.75) among males and 0.58 (0.31-0.85) among females [40]. These data are in agreement with our findings regarding male twins. Heritability was, however, greater among Danish female twins than presented in paper I regarding Swedish female twins. A later study on Swedish twins showed that genetic factors influence death from CHD particularly at younger ages [39]. This is also in parallel with finding presented in paper I where a slight decrease in heritability depended on the length of follow-up time (and hence inclusion of higher ages at death). This decrease may partly reflect an age dependent expression of genes, and/or a change in the system's resilience to the more major effects of a genetic influence in early phases of life. A possible explanation may also be that the accumulation of environmental influences plays a larger role in older ages. In the context of evolutionary theories of ageing, genes, sex and ageing are often intricately intertwined [98-101]. CHD is a consequence of an age-related underlying disorder, coronary atherosclerosis. The pathogenesis of the atherosclerotic process is a result of an interaction between aging processes and disease-specific factors, both intrinsic and extrinsic. A large number of polymorphisms of genes such as APOE, the low-density lipoprotein receptor, apolipoprotein (a) and genes of relevance for homocysteine metabolism as well as various cardiovascular risk factors such as hypertension, diabetes, smoking, diet high in saturated fats, physical inactivity, psychological stress etc., are most likely involved. The evolutionary theories of aging and in that sense the role of chance at advanced ages are also in line with our findings on increased influence of non-shared environment with increasing age.

Furthermore, the findings presented in paper II showed that known risk factors for CHD-death are important and that these factors marginally account for genetic influences for CHD-death among males and even more so among females. In addition, previous genetic studies have suggested genetic influences for BMI [22, 23, 52, 58], smoking

[21, 53], educational attainment [102], marital status, personality and divorce [103], blood pressure [20, 57] and diabetes [54, 55]. Despite the role of genetic factors for these risk factors, their impact is not reflected to any great extent in the genetic variance for CHD-death. In addition, multivariate studies showed that genetic influences on body fat, insulin, and CVD differed among sexes, and that heritability estimates were higher for females in general. They suggested that there were shared genetic and environmental effects among all variables except CVD [104]. Another multivariate study examining several phenotypes and common genetic factors is the study by Herskind and colleagues who showed that only a small fraction of genetic influences on longevity is mediated by genetic factors in common to smoking and BMI [105]. Furthermore, a meta-analysis performed on four genome wide scan studies showed that genetic regions 3q26-27 and 2q34-37 could contain susceptibility genes for CHD, also linked to Type 2 diabetes and metabolic syndrome [41].

However, results presented in Paper III with longer follow-up times indicated similar heritability estimates for CHD-death as presented in paper I and II. These analyses showed that self-reported AP as a single factor has a significant impact on CHD-death in both sexes, although this is more apparent in males. In addition, follow-up studies of the Framingham population showed that CHD manifestations differ between sexes. MI in that study was more likely unrecognised in women, compared to men, and AP in women more often seemed uncomplicated. AP in men was also more often related to MI [71]. Therefore, merging of AP and MI into one endpoint (CHD) may be more motivated in males [106]. In addition, causes other than CAD for chest discomfort could be more common in women [74]. The risk of experiencing an MI is increased once there is a positive family history of CHD [107], a finding in parallel with the results presented in paper IV. Parental history of CHD, MI ($\leq$ 60 years) and AP has also been shown to significantly increase the risk in women [108]. In addition, a parental history of MI was showed to be positively associated with risk of CHD and is thus to be considered as an independent risk marker for CHD [109].

7.2 METHODOLOGICAL ASPECTS

7.2.1 Strengths
This PhD thesis is based on subjects drawn from one of the largest population based twin registries in the world with a large number of complete twin pairs. The twin design in itself is of great use for genetic studies, as twins are sampled from the same gene pool and they share same genes. In addition, the long length of follow up (from 1961 to 2001) regarding the information on CHD and thus the large number of cases is of particular importance. The main strengths of this thesis are, therefore, the good quality of the registers used as well as the long follow-up periods [68, 79, 80, 110].

7.2.2 Limitations

7.2.2.1 Selection
All analyses are based upon twins born in Sweden between 1886 and 1958. For those born between 1886 and 1925 only pairs alive in 1961 were collected. This selection has implied collection of more robust (less frail/liable) twins and, therefore, twin pairs where at least one twin died before 1961 never entered the analyses. A consequence of

this selection may be underestimation of the genetic impact, as genetic influences are suggested to be more important in earlier phases of life.

7.2.2.2 *Misclassification*

Delayed entry (although corrected for in the analyses) has resulted in underreporting of self-reported risk factors. Especially age-related risk factors as AP, hypertension, diabetes and for that matter also information on weight. This has implied that not all exposed twins are treated as exposed in the analyses due to short induction period. Differential misclassification on exposure has for this work not implied any bias as it was unrelated to the disease (cohort studies). On the other hand, non-differential misclassification has to be considered. It is likely that this bias may relate to hypertension, diabetes and AP. However, information on smoking may be considered quite valid as smoking was accepted at the time of data collection. This has been confirmed by comparing the questionnaire answers to personal interviews of 200 twin pairs [111]. It is also likely that information on marital status may be considered as correct. Level of education, though, may have resulted in more false reports as level of education as well as weight are parameters that may have been misreported. However, as these factors are not treated as the main exposure (except AP) in the analyses, it is unlikely that they could have affected conclusions regarding genetic influences. The main concern regarding misclassification is instead zygosity determination as this is the main exposure throughout this thesis. As shown, though, 95 % of all similarity diagnosis has been proven to be correct [79]. AP has to be considered in this matter as it was determined by self-reported answers from a slightly modified questionnaire developed in the early 1960's, a questionnaire that is more accurate for males than females [73, 74]. It is, thus, likely that a misclassification of the AP diagnoses may have some effect on the genetic findings regarding AP in women, as AP in women diagnosed by the questionnaire could be related to other causes of chest pain [74]. Another study on chest pain perception also reported that women experience chest pain more intensive than men did, and they also linked the pain less to heart diseases than men. They also reported a more atypical clinical picture of chest pain [75]. So the question is whether chest pain falsely classified as AP is genetically predisposed or not. If such pain was influenced by genetic factors it could have affected our conclusions on genetic influences for AP in women. In contrast, if such pain was not due to states influenced by genetic factors, then the results regarding the impact of AP on CHD in women should be considered as slightly underestimated, which is more likely. It is, however, important to note that results presented on self-reported AP (paper III) were partly confirmed by use of information from the Swedish Hospital Discharge Register, covering diagnoses on all in-patient care at Swedish hospitals in the period 1987 - 2003. Probandwise concordances for AP based on this information resulted in lower estimates (0.12 for MZ and 0.05 for DZ-twins) than the corresponding rates based on self-report but the patterns overall were quite similar. Misclassification regarding the main outcome, CHD-death, has most likely not contributed to significant biases as information on CHD-death was obtained from the Swedish Cause of Death Register for which the validity of cause-of-death certifications has been shown to be fairly good and at an acceptable level for epidemiological studies [110].

7.2.2.3 *Confounding*

Two confounding factors, age and sex, have been included throughout this thesis. However, these confounding factors have been taken care of in the analyses by stratification (sex) and multivariate analyses (age). The main problem of confounding regards, however, the genetic influences. In paper II and III genetic analyses included risk factors that are influenced by genetic components although to a different degree. It is likely, thus, that genetic factors covary with exposure (genetic and/or environmental part of the exposure) and consequently affects the risk of a disease. Approaches like those applied in this thesis where classical risks are not calculated implies additional difficulties as the degree of genetic influence is calculated theoretically. Focusing solely on heritability, however, does not resolve the questions on genetic confounding. Therefore, when dealing with confounding in these studies, it is essential to study the contribution of the adjustments to the depicted parts of the variance. It is, therefore, difficult to draw firm conclusions on the impact of genetic confounding.

7.2.2.4 *Methodological considerations*

7.2.2.4.1 Heritability

The main statistical tool for this PhD-project was genetic analyses of frailty and liability, two methods widely used in order to calculate heritability. Heritability is population specific and a result of analyses of phenotypic variance. In general, this estimate is defined as the proportion of variation directly attributable to genetic differences among individuals to the total variation in a population [30]. However, heritability estimates as obtained by the frailty and liability approach are not directly comparable as the models differ. Results presented in this thesis are, however, in parallel as both frailty and liability analyses resulted in similar heritabilities. Furthermore, two frailty models were applied (paper II) in order to contrast the types of interpretations that can be drawn. The important difference between these models is that the correlated frailty model (used for heritability calculation) estimates the impact of risk factors on a trait at the same time as the impact of genetic and environmental factors. This is, however, not possible by the univariate model which speaks in favour of the correlated gamma-frailty model.

7.2.2.4.2 Twin

One of the key assumptions (postnatal) in the classical twin design of genetic studies is the assumption of equal environments in both zygosity categories. However, DZ twins do not necessary share their environment to the same amount as MZ twins do, and violations of this assumption could result in overestimation of genetic influences for CHD. Another bias may relate differences between MZ and DZ twins regarding prenatal phases. MZ twins may differ regarding implantation patterns and intrauterine positions, as well as differences in delivery events, which could have a lowering effect on the concordance among MZ twins and thus result in lower genetic effects even when these factors are of importance [30, 90].

7.2.2.4.3 Quantitative genetics

In addition, all quantitative genetic analyses [90] performed in this PhD project did not include gene-environment interaction as necessary information regarding additional

relatives or molecular genetic data were unavailable. This kind of interaction occurs when environmental effects are conditioned on a particular genotype which is of considerable importance as gene-environment interactions may account for as much as 20% of the variance of a trait in a population. Not including such interaction in the analyses (as it is assumed and suggested for CHD) leads to biased estimates both regarding environmental and genetic components. If such interaction was due to gene by non-shared environment it would lead to an overestimation of the non-shared environmental component. In contrast, if such interaction was due to shared environment it would lead to an overestimation of the genetic impact and shared environmental component on the phenotype. In addition, not including assortative mating (positive) in the analyses leads to an underestimation of genetic estimates and overestimation of shared environmental influences. However, it is difficult to imagine the process by which mates select for a specific cause of death.

7.3   GENDER DIFFERENCES

CHD-studies focusing on gender as well as gender differences have so far suggested small differences between the sexes concerning CHD-risk [62, 64]. Findings presented in this thesis may add a piece to this puzzle with information on the impact of gender-specific genetic influence. Genetic factors tend to affect males more than females regarding mortality due to CHD, but no major differences were observed regarding AP and MI. However, genetic influences for CHD were modified through known risk factors to a slightly higher degree in females. The association between AP and CHD was genetically explained to a slightly higher degree in females than males.

7.4   CONCLUDING REMARKS

Findings presented in this thesis may contribute to better understanding of the genetic and environmental contribution for the occurrence and death by the coronary phenotype. These findings may also contribute positively for further strategies in this matter as more precise diagnostic tools are needed in order to resolve more complex genetic and gender-specific questions. However, the future seems bright regarding genetic studies due a rapid progress not only of molecular genetic techniques but also of advancements of statistical models and methods. Furthermore, the ongoing efforts to establish large population-based biobanks with frozen blood and DNA from twins like the STR as well as from non-twin samples will provide the necessary materials for application of novel genetic tools. It is therefore likely that the genetic influences outlined in this thesis will be identified down to its molecular genetic components.

8 CONCLUSIONS

- Genetic factors moderately influence the risk to die from CHD, particularly among males.
- These influences are in operation throughout the entire life span, although they tend to be particularly important for earlier ages of death.
- Genetic influences for CHD-death are only partly mediated through known risk factors in males and more so in females.
- AP is an important risk factor for CHD for both sexes, with a slightly higher impact for males as compared to females.
- The association between AP and CHD is moderately large and almost entirely explained by familial factors.
- Genetic factors play a moderate role for AP among both sexes.
- Genetic factors play a moderate role for the occurrence of the first episode of AMI in both sexes.

9 SAMMANFATTNING (SUMMARY IN SWEDISH)

Mortalitet orsakad av kranskärlssjukdom har under lång tid varit den mest frekventa dödsorsaken i utvecklade länder. Något som också börjar observeras i utvecklingsländer. Under senare delen dvs. slutet av 1900-talet observerades en neråtgående trend vad gäller denna sjukdom i västvärlden, ett betydande framsteg som mestadels har kopplats till omgivningsmässiga förändringar som förbättrad sjukvård, medicinska ingrepp samt ändring av CHD-relaterade tillstånd/vanor/beteenden såsom rökning, övervikt och fysisk inaktivitet. Trots att vi ej kan modifiera den genetiska risken har intresset för genetiska faktorer och deras betydelse ökat under senare år, inte minst pga. den snabba utvecklingen av olika molekylära tekniker. CHD-fall i familjen har visats öka risken att insjukna i samma sjukdom. Ett stort antal gener har också rapporterats vara relaterade till CHD och till olika riskfaktorer och deras mekanismer som blodtrycks reglering samt insulinkänslighet. Eftersom de genetiska effekterna förmedlas via en lång rad biologiska processer och steg har det varit svårt att kvantifiera och förstå det totala bidrag som utgörs av genetiska faktorer genom att summera risker från diverse genetiska effekter då antalet gener är stort men effekten av enskilda gener liten. Syftet med detta avhandlingsarbete har därför varit att studera den totala effekten av genetiska och omgivningsmässiga faktorer för CHD samt dess fenotyper såsom AMI och AP. Syftet har också varit att studera ifall genetiska influenser ändras med åren samt att studera om dessa medieras via kända risk faktorer. Avhandlingsarbetet har baserats på svenska tvillingar. Statistiska metoder/modeller har använts för att beräkna konkordanser, relativa risker samt heritabilitet.

Det genetiska bidraget för CHD-död visade sig vara måttligt för båda könen och något högre för män. Detta bidrag är av större betydelse vid lägre ålder men fortfarande betydelsefullt vid högre ålder (artikel I). Inkludering av kända risk faktorer som rökning, övervikt, diabetes, högt blodtryck, civilstånd och utbildningsnivå i dessa analyser resulterade i en liten minskning av den genetiska variansen bland män och en något större minskning bland kvinnor. Genetiska influenser för CHD-död är därför till högre grad medierade genom dessa faktorer bland kvinnor än män (artikel II). Resultat av genetiska analyser beträffande AP är i linje med slutsatser angående CHD resultaten. AP är en betydelsefull risk faktor för CHD oberoende av kön och är också måttligt influerad av genetiska faktorer. Sambandet mellan AP och CHD visades nästan helt vara en effekt av familjära faktorer bland båda könen (artikel III). Studier av AMI är också i linje med resultat beträffande CHD och AP med måttliga influenser av genetiska faktorer för kvinnor och män (artikel IV).

Sammanfattningsvis tycks genetiska faktorer ha en betydelsefull inverkan för att dö i CHD samt att drabbas av AP och AMI hos såväl män som kvinnor. Dessa influenser är något mer betydelsefulla för män avseende CHD-död. AP är också en viktig risk faktor för CHD med större inverkan på CHD-död bland män. Sambandet mellan AP och CHD tycks vara orsakade av familjära faktorer hos båda könen. Dessutom påverkar genetiska influenser CHD-död i något högre grad vid tidig ålder och är därtill medierade genom kända riskfaktorer och AP till en vis grad hos män och något mer hos kvinnor.

10 ACKNOWLEDGEMENTS

I would like to express my appreciation to all people somehow involved in this work.

Sincere appreciation goes to:

First of all, my head supervisor, professor Ulf de Faire, for giving me the opportunity to explore the enormously interesting field of cardiovascular epidemiology and in particular, the genetic epidemiology of cardiovascular diseases. Thank you Ulf for your excellent guidance, your encouragement, for always having time for my questions and for believing in me. It has been a pleasure working with you and being a member of your research team.

My supervisor, professor Nancy L. Pedersen, for patiently answering all my questions, and always having time. Thank you Nancy for your excellent advice and for guiding me through the field of genetic epidemiology in twins; it has been a pleasure working with you and being a member of your team.

My supervisor, Andreas Wienke PhD, for all the interesting discussions that we have had during these years, in Rostock, Halle, Szeged and Stockholm. Thank you Andreas for your rapid answers to all the e-mails I sent you and for being an excellent teacher of frailty models and related topics.

My family: my wife Vesna, my children Eleonora and Luka, my mother Divna, my father Vojislav, and my brother Dragan for their enormous support during these years. Also, my sister-in-law Mirjana for her support.

Lena Wärnlöf, division secretary, for being of great help, especially during the years I mainly worked from home.

My co-authors Marjorie E. Marenberg and Anatoli I. Yashin for valuable comments and discussions.

My friends and colleagues at IMM and MEB for interesting discussions regarding both topics related to this thesis and to other fields.

Professor Mads Melbye at Division of Epidemiology, Statens Serum Institut, Copenhagen, Denmark for his understanding and support during the final phase of my thesis as I worked in his department at the same time.

11 REFERENCES

1.   Hlatky MA, Mark DB, Califf RM, Pryor DB. Angina, myocardial ischemia and coronary disease: gold standards, operational definitions and correlations. J Clin Epidemiol 1989;42(5):381-4.
2.   Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 1997;349(9061):1269-76.
3.   Reddy KS, Yusuf S. Emerging epidemic of cardiovascular disease in developing countries. Circulation 1998;97(6):596-601.
4.   Lloyd-Jones DM, Larson MG, Beiser A, Levy D. Lifetime risk of developing coronary heart disease. Lancet 1999;353(9147):89-92.
5.   Hopkins PN, Williams RR. A survey of 246 suggested coronary risk factors. Atherosclerosis 1981;40(1):1-52.
6.   Tang Z, Tracy RP. Candidate genes and confirmed genetic polymorphisms associated with cardiovascular diseases: a tabular assessment. J Thromb Thrombolysis 2001;11(1):49-81.
7.   Nordlie MA, Wold LE, Kloner RA. Genetic contributors toward increased risk for ischemic heart disease. J Mol Cell Cardiol 2005;39(4):667-79.
8.   Mosca L. Novel cardiovascular risk factors: do they add value to your practice? Am Fam Physician 2003;67(2):264, 6.
9.   Leinonen M, Saikku P. Infections and atherosclerosis. Scand Cardiovasc J 2000;34(1):12-20.
10.   Rosamond WD, Chambless LE, Folsom AR, et al. Trends in the incidence of myocardial infarction and in mortality due to coronary heart disease, 1987 to 1994. N Engl J Med 1998;339(13):861-7.
11.   Tunstall-Pedoe H, Kuulasmaa K, Mahonen M, Tolonen H, Ruokokoski E, Amouyel P. Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. Lancet 1999;353(9164):1547-57.
12.   Peltonen M, Asplund K. Age-period-cohort effects on ischaemic heart disease mortality in Sweden from 1969 to 1993, and forecasts up to 2003. Eur Heart J 1997;18(8):1307-12.
13.   Linnersjo A, Hammar N, Gustavsson A, Reuterwall C. Recent time trends in acute myocardial infarction in Stockholm, Sweden. Int J Cardiol 2000;76(1):17-21.
14.   Rosen M, Alfredsson L, Hammar N, Kahan T, Spetz CL, Ysberg AS. Attack rate, mortality and case fatality for acute myocardial infarction in Sweden during 1987-95. Results from the national AMI register in Sweden. J Intern Med 2000;248(2):159-64.
15.   Friedlander Y. Familial clustering of coronary heart disease: a review of its significance and role as a risk factor for the disease. In: Goldbourt U, de Faire U, Berg K, eds. Genetic Factors in Coronary Heart Disease. Dordrecht ; Boston : Kluwer Academic 1994.
16.   Keavney B. Genetic epidemiological studies of coronary heart disease. Int J Epidemiol 2002;31(4):730-6.
17.   National Heart, Lung and Blood Institute. What Is Coronary Artery Disease? http://wwwnhlbinihgov/health/dci/Diseases/Cad/CAD_WhatIshtml 2006.
18.   American Heart Association. Angina pectoris. http://wwwamericanheartorg/presenterjhtml?identifier=4472 2006.
19.   MedlinePlus. Medical Encyclopedia. http://wwwnlmnihgov/medlineplus/encyclopediahtml 2006.
20.   Evans A, Van Baal GC, McCarron P, et al. The genetics of coronary heart disease: the contribution of twin studies. Twin Res 2003;6(5):432-41.
21.   Osler M, Holst C, Prescott E, Sørensen TI. Influence of genes and family environment on adult smoking behavior assessed in an adoption study. Genet Epidemiol 2001;21(3):193-200.

22.		Stunkard AJ, Sørensen TI, Hanis C, et al. An adoption study of human obesity. N Engl J Med 1986;314(4):193-8.

23.		Sørensen TI, Holst C, Stunkard AJ. Adoption study of environmental modifications of the genetic influences on obesity. Int J Obes Relat Metab Disord 1998;22(1):73-81.

24.		Sørensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. N Engl J Med 1988;318(12):727-32.

25.		Cordell HJ, Clayton DG. Genetic association studies. Lancet 2005;366(9491):1121-31.

26.		Dawn Teare M, Barrett JH. Genetic linkage studies. Lancet 2005;366(9490):1036-44.

27.		Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. Nat Rev Genet 2002;3(11):872-82.

28.		Scheuner MT. Genetic evaluation for coronary artery disease. Genet Med 2003;5(4):269-85.

29.		Plomin R, DeFries JC, McClearn GE, Rutter M. Behavioral Genetics. Third ed: New York : W. H. Freeman and Company; 1997.

30.		Khoury MJ, Beaty TH, Cohen BH. Fundamentals of Genetic Epidemiologi: New York : Oxford University Press; 1993.

31.		Gertler MM, White PD. Coronary heart disease in young adults: A multidisciplinary study. Cambridge, Massachusetts Harvard University Press; 1954.

32.		Thomas CB, Cohen BH. The familial occurrence of hypertension and coronary artery disease, with observations concerning obesity and diabetes. Ann Intern Med 1955;42(1):90-127.

33.		Rose G. Familial Patterns in Ischaemic Heart Disease. Br J Prev Soc Med 1964;18:75-80.

34.		Cederlöf R, Friberg L, Jonsson E. Hereditary factors and "angina pectoris". A study on 5,877 twin-pairs with the aid of mailed questionnaires. Arch Environ Health 1967;14(3):397-400.

35.		de Faire U, Friberg L, Lundman T. Concordance for mortality with special reference to ischaemic heart disease and cerebrovascular disease. A study on the Swedish Twin Registry. Prev Med 1975;4(4):509-17.

36.		Harvald B, Hauge M. Coronary occlusion in twins. Acta Genet Med Gemellol (Roma) 1970;19(1):248-50.

37.		ten Kate LP, Boman H, Daiger SP, Motulsky AG. Familial aggregation of coronary heart disease and its relation to known genetic risk factors. Am J Cardiol 1982;50(5):945-53.

38.		Snowden CB, McNamara PM, Garrison RJ, Feinleib M, Kannel WB, Epstein FH. Predicting coronary heart disease in siblings--a multivariate assessment: the Framingham Heart Study. Am J Epidemiol 1982;115(2):217-22.

39.		Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 1994;330(15):1041-6.

40.		Wienke A, Holm NV, Skytthe A, Yashin AI. The heritability of mortality due to heart diseases: a correlated frailty model applied to Danish twins. Twin Res 2001;4(4):266-74.

41.		Chiodini BD, Lewis CM. Meta-analysis of 4 coronary heart disease genome-wide linkage studies confirms a susceptibility locus on chromosome 3q. Arterioscler Thromb Vasc Biol 2003;23(10):1863-8.

42.		Talmud PJ. How to identify gene-environment interactions in a multifactorial disease: CHD as an example. Proc Nutr Soc 2004;63(1):5-10.

43.		Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nat Genet 2001;29(3):306-9.

44.		Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J, 3rd. Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med 1961;55:33-50.

45.		Nyboe J, Jensen G, Appleyard M, Schnohr P. Smoking and the risk of first acute myocardial infarction. Am Heart J 1991;122(2):438-47.

46.	Powell KE, Thompson PD, Caspersen CJ, Kendrick JS. Physical activity and the incidence of coronary heart disease. Annu Rev Public Health 1987;8:253-87.
47.	Oguma Y, Shinoda-Tagawa T. Physical activity decreases cardiovascular disease risk in women: review and meta-analysis. Am J Prev Med 2004;26(5):407-18.
48.	Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr 2001;20(1):5-19.
49.	van den Bree MB, Eaves LJ, Dwyer JT. Genetic and environmental influences on eating patterns of twins aged >/=50 y. Am J Clin Nutr 1999;70(4):456-65.
50.	Rosengren A, Hawken S, Ounpuu S, et al. Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. Lancet 2004;364(9438):953-62.
51.	Tholin S, Rasmussen F, Tynelius P, Karlsson J. Genetic and environmental influences on eating behavior: the Swedish Young Male Twins Study. Am J Clin Nutr 2005;81(3):564-9.
52.	Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K. The heritability of body mass index among an international sample of monozygotic twins reared apart. Int J Obes Relat Metab Disord 1996;20(6):501-6.
53.	Carmelli D, Swan GE, Robinette D, Fabsitz R. Genetic influence on smoking--a study of male twins. N Engl J Med 1992;327(12):829-33.
54.	Kaprio J, Tuomilehto J, Koskenvuo M, et al. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 1992;35(11):1060-7.
55.	Kyvik KO, Green A, Beck-Nielsen H. Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. Bmj 1995;311(7010):913-7.
56.	Feillet H, Bach JF. On the mechanisms of the protective effect of infections on type 1 diabetes. Clin Dev Immunol 2004;11(3-4):191-4.
57.	Hong Y, de Faire U, Heller DA, McClearn GE, Pedersen N. Genetic and environmental influences on blood pressure in elderly twins. Hypertension 1994;24(6):663-70.
58.	Herskind AM, McGue M, Sørensen TI, Harvald B. Sex and age specific assessment of genetic and environmental influences on body mass index in twins. Int J Obes Relat Metab Disord 1996;20(2):106-13.
59.	Higgins M. Epidemiology and prevention of coronary heart disease in families. Am J Med 2000;108(5):387-95.
60.	Heller DA, de Faire U, Pedersen NL, Dahlen G, McClearn GE. Genetic and environmental influences on serum lipid levels in twins. N Engl J Med 1993;328(16):1150-6.
61.	Kannel WB. The Framingham Study: historical insight on the impact of cardiovascular risk factors in men versus women. J Gend Specif Med 2002;5(2):27-37.
62.	Bello N, Mosca L. Epidemiology of coronary heart disease in women. Prog Cardiovasc Dis 2004;46(4):287-95.
63.	Schenck-Gustafsson K. Risk factors for cardiovascular disease in women: assessment and management. Eur Heart J 1996;17 Suppl D:2-8.
64.	Roeters van Lennep JE, Westerveld HT, Erkelens DW, van der Wall EE. Risk factors for coronary heart disease: implications of gender. Cardiovasc Res 2002;53(3):538-49.
65.	Steingart RM, Packer M, Hamm P, et al. Sex differences in the management of coronary artery disease. Survival and Ventricular Enlargement Investigators. N Engl J Med 1991;325(4):226-30.
66.	Ayanian JZ, Epstein AM. Differences in the use of procedures between women and men hospitalized for coronary heart disease. N Engl J Med 1991;325(4):221-5.
67.	Tobin JN, Wassertheil-Smoller S, Wexler JP, et al. Sex bias in considering coronary bypass surgery. Ann Intern Med 1987;107(1):19-25.
68.	Hammar N, Alfredsson L, Rosen M, Spetz CL, Kahan T, Ysberg AS. A national record linkage to study acute myocardial infarction incidence and case fatality in Sweden. Int J Epidemiol 2001;30 Suppl 1:S30-4.

69.	Rosengren A, Spetz CL, Koster M, Hammar N, Alfredsson L, Rosen M. Sex differences in survival after myocardial infarction in Sweden; data from the Swedish National Acute Myocardial Infarction Register. Eur Heart J 2001;22(4):314-22.

70.	Vaccarino V, Berkman LF, Krumholz HM. Long-term outcome of myocardial infarction in women and men: a population perspective. Am J Epidemiol 2000;152(10):965-73.

71.	Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am Heart J 1986;111(2):383-90.

72.	Milner KA, Funk M, Richards S, Wilmes RM, Vaccarino V, Krumholz HM. Gender differences in symptom presentation associated with coronary heart disease. Am J Cardiol 1999;84(4):396-9.

73.	Cederlöf R, Jonsson E, Lundman T. On the validity of mailed questionnaires in diagnosing "angina pectoris" and "bronchitis". Arch Environ Health 1966;13(6):738-42.

74.	Harris RB, Weissfeld LA. Gender differences in the reliability of reporting symptoms of angina pectoris. J Clin Epidemiol 1991;44(10):1071-8.

75.	Granot M, Goldstein-Ferber S, Azzam ZS. Gender differences in the perception of chest pain. J Pain Symptom Manage 2004;27(2):149-55.

76.	Goldberg RJ, O'Donnell C, Yarzebski J, Bigelow C, Savageau J, Gore JM. Sex differences in symptom presentation associated with acute myocardial infarction: a population-based perspective. Am Heart J 1998;136(2):189-95.

77.	Leander K, Wiman B, Hallqvist J, Falk G, De Faire U. The G-455A polymorphism of the fibrinogen Bbeta-gene relates to plasma fibrinogen in male cases, but does not interact with environmental factors in causing myocardial infarction in either men or women. J Intern Med 2002;252(4):332-41.

78.	Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB. Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. J Affect Disord 1993;29(2-3):85-96.

79.	Lichtenstein P, De Faire U, Floderus B, Svartengren M, Svedberg P, Pedersen NL. The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. J Intern Med 2002;252(3):184-205.

80.	Pedersen NL, Lichtenstein P, Svedberg P. The Swedish Twin Registry in the third millennium. Twin Res 2002;5(5):427-32.

81.	Heller DA, Pedersen NL, de Faire U, McClearn GE. Genetic and environmental correlations among serum lipids and apolipoproteins in elderly twins reared together and apart. Am J Hum Genet 1994;55(6):1255-67.

82.	Ahlbom A. Acute myocardial infarction in Stockholm--a medical information system as an epidemiological tool. Int J Epidemiol 1978;7(3):271-6.

83.	Hammar N, Nerbrand C, Ahlmark G, et al. Identification of cases of myocardial infarction: hospital discharge data and mortality data compared to myocardial infarction community registers. Int J Epidemiol 1991;20(1):114-20.

84.	Socialstyrelsen. The Swedish Hospital Discharge Register. http://wwwsocialstyrelsense/Statistik/statistik_amne/sluten_vard/patientregistret+engelskhtm 2006.

85.	Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull World Health Organ 1962;27:645-58.

86.	WHO. Arterial Hypertension and Iscemic Heart Disease. WHO Chron 1963;17(97).

87.	Cox D. Regression models and life-tables (with discussion). J R Stat Soc 1972;34:187-220

88.	Vaupel JW, Manton KG, Stallard E. The impact of heterogeneity in individual frailty on the dynamics of mortality. Demography 1979;16(3):439-54.

89.	Hougaard P. Analysis of multivariate survival data. New York: Springer-Verlag.; 2000.

90.	Posthuma D, Beem AL, de Geus EJ, et al. Theory and practice in quantitative genetics. Twin Res 2003;6(5):361-76.

91.	Heath AC, Neale MC, Hewitt JK, Eaves LJ, Fulker DW. Testing structural equation models for twin data using LISREL. Behav Genet 1989;19(1):9-35.

92.   McGue M. When assessing twin concordance, use the probandwise not the pairwise rate. Schizophr Bull 1992;18(2):171-6.
93.   Yashin AI, Iachine IA. Genetic analysis of durations: correlated frailty model applied to survival of Danish twins. Genet Epidemiol 1995;12(5):529-38.
94.   Yashin AI, Vaupel JW, Iachine IA. Correlated individual frailty: an advantageous approach to survival analysis of bivariate data. Math Popul Stud 1995;5(2):145-59, 83.
95.   Akaike H. Factor analysis and AIC. Psychometrica 1987;52:317-22.
96.   Falconer D. The inheritance of liability to certain diseases, estimated from the incidence among relatives. Ann Hum Genet 1965;29:51-76.
97.   Neale MC. Mx: Statistical modeling. Richmond, VA: Department of Psychiatry, 4th edition 1997.
98.   Kirkwood TB. Sex and ageing. Exp Gerontol 2001;36(3):413-8.
99.   Kirkwood TB. The origins of human ageing. Philos Trans R Soc Lond B Biol Sci 1997;352(1363):1765-72.
100.   Kirkwood TB, Austad SN. Why do we age? Nature 2000;408(6809):233-8.
101.   Finch CE, Tanzi RE. Genetics of aging. Science 1997;278(5337):407-11.
102.   Lichtenstein P, Pedersen NL, McClearn GE. The origins of individual differences in occupational status and educational level: A study of twins reared apart and together. Acta Sociologica 1992;35:13-31.
103.   Jockin V, McGue M, Lykken DT. Personality and divorce: a genetic analysis. J Pers Soc Psychol 1996;71(2):288-99.
104.   Nelson TL, Vogler GP, Pedersen NL, Hong Y, Miles TP. Genetic and environmental influences on body fat distribution, fasting insulin levels and CVD: are the influences shared? Twin Res 2000;3(1):43-50.
105.   Herskind AM, McGue M, Iachine IA, et al. Untangling genetic influences on smoking, body mass index and longevity: a multivariate study of 2464 Danish twins followed for 28 years. Hum Genet 1996;98(4):467-75.
106.   Wilcosky T, Harris R, Weissfeld L. The prevalence and correlates of Rose Questionnaire angina among women and men in the Lipid Research Clinics Program Prevalence Study population. Am J Epidemiol 1987;125(3):400-9.
107.   Leander K, Hallqvist J, Reuterwall C, Ahlbom A, de Faire U. Family history of coronary heart disease, a strong risk factor for myocardial infarction interacting with other cardiovascular risk factors: results from the Stockholm Heart Epidemiology Program (SHEEP). Epidemiology 2001;12(2):215-21.
108.   Colditz GA, Stampfer MJ, Willett WC, Rosner B, Speizer FE, Hennekens CH. A prospective study of parental history of myocardial infarction and coronary heart disease in women. Am J Epidemiol 1986;123(1):48-58.
109.   Friedlander Y, Kark JD, Stein Y. Family history of myocardial infarction as an independent risk factor for coronary heart disease. Br Heart J 1985;53(4):382-7.
110.   de Faire U, Friberg L, Lorich U, Lundman T. A validation of cause-of-death certification in 1,156 deaths. Acta Med Scand 1976;200(3):223-8.
111.   Cederlöf R, Jonsson E, Kaij L. Respiratory symptoms and "angina pectoris" in twins with reference to smoking habits. An epidemiological study with mailed questionnaire. Arch Environ Health 1966;13(6):726-37.