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RISK FACTORS FOR SUDDEN CARDIAC DEATH FROM AN ACUTE ISCHEMIC EVENT IN GENERAL POPULATION

A CASE-CONTROL STUDY

FACULTY OF MEDICINE,
DEPARTMENT OF INTERNAL MEDICINE,
UNIVERSITY OF OULU

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Abstract

Specific prevention of sudden cardiac death (SCD) caused by an acute coronary event in the general population has remained a challenge for clinicians since the recognizable risk factors for this fatal outcome of an underlying coronary artery disease (CAD) may be partly the same as those of a non-fatal coronary event.

This case-control study was designed to compare genetic and several other factors between consecutive series of survivors (n = 644) and victims of SCD (n = 425) from an acute coronary event. Only subjects with an acute coronary event verified at medico-legal autopsy were included in the SCD group. As controls, 809 subjects without any history of CAD, acute myocardial infarction or aborted cardiac arrest were examined. Subjects to the sub-studies were drawn from these study populations. The increased risk for SCD in the general population was associated with family history of SCD, male gender, smoking, cardiac hypertrophy and the severity of CAD. In the present study, 100% mortality was observed when all these risk factors were present at the time of an acute coronary event. In the subjects with a family history of SCD, the increased risk of SCD was correlated with the severity of CAD without any clustering of coronary risk factors, suggesting that genetic factors affecting the accelerated progression of CAD may have an important role in familial SCD. However, polymorphisms of genes affecting thrombosis, which are believed to have effects on plaque progression and the consequences of plaque complications, were not associated with an increased risk for SCD.

The present results show that the risk of SCD at the time of an acute coronary event can be assessed by generally available methods. If a subject is a male smoker and has a family history of SCD, the risk of SCD is substantially increased. In our study sample the currently known polymorphisms affecting thrombosis did not have a major impact in risk stratification of genetic susceptibility for SCD. Simple association studies have clear shortcomings when they attempt to reveal genetic associations with complex outcomes and thus new research strategies are needed to elucidate the genetic background of SCD.

Keywords: coronary disease, death, epidemiology, family history, genetics, myocardial infarction, risk factors, sudden

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Abbreviations

AMI	Acute myocardial infarction
BMI	Body mass index
BSA	Body surface area
CAD	Coronary artery disease
CI	Confidence interval
ECG	Electrocardiogra/m, -phic, -phy
EMD	Electromechanical dissociation
FDR	First degree relative
ICD	Implantable cardioverter defibrillator
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
OR	Odds ratio
SCD	Sudden cardiac death
SD	Standard deviation
THW	Total heart weight
VF	Ventricular fibrillation
VT	Ventricular tachycardia

List of original publications

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Kaikkonen KS, Kakko S, Kortelainen ML, Tapanainen JM, Savolainen MJ, Kesäniemi YA, Huikuri HV & Savolainen ER (2005). The –1C to T polymorphism in the Annexin A5 gene is not associated with the risk of acute myocardial infarction or sudden cardiac death in middle-aged Finnish males. Scand J Clin Lab Invest 65: 133–40.

II Kaikkonen KS, Kortelainen ML, Linna E & Huikuri HV (2006). Family history and the risk of sudden cardiac death as a manifestation of an acute coronary event. Circulation 114: 1462–7.

III Kaikkonen KS, Kortelainen ML & Huikuri HV (2008). Comparison of risk profiles between survivors and victims of sudden cardiac death from an acute coronary event. Ann Med 41: 120–7.

IV Kaikkonen KS, Kortelainen ML & Huikuri HV. Comparison of coronary risk factors and autopsy findings of subjects with and without a family history of sudden cardiac death caused by an acute coronary event. Manuscript.

Addendum to publication I

A Kaikkonen KS, Kortelainen ML, Kesäniemi YA, Huikuri HV. Polymorphisms of genes affecting thrombosis and risk of myocardial infarction and sudden cardiac death from an acute coronary event. Manuscript.

Contents

1 Introduction

Sudden cardiac death (SCD) due to coronary artery disease (CAD) is the single most prevalent cause of death in Western societies. Despite the fact that a number of studies on SCD have been conducted during the past few decades, prevention of SCD in the general population has remained a challenge (Huikuri et al. 2001, Huikuri et al. 2003). A large number of CAD patients die suddenly without exhibiting any prior symptoms and before preventive efforts can be instituted (Myerburg et al. 1997). Another problem is the overlap between the risk factors for coronary events, acute myocardial infarction (AMI), and SCD, which complicates the specific identification of patients vulnerable to SCD (Kannel & Thomas 1982, Suhonen et al. 1988, Escobedo & Zack 1996, Wannamethee et al. 1995).

At the end of 1990s, two population based studies observed familial clustering of SCD indicative of a genetic background. The first of these studies was published in 1998 by Friedlander *et al.* showing that a family history of AMI or SCD was independently associated with an increased risk to SCD. One year later, results from the Paris Prospective Study confirmed this finding but also indicated that even though a family history of SCD increased the risk for SCD it did not increase the risk for AMI. In opposite, for AMI, family history of AMI was an independent risk factor. (Jouven et al. 1999). This suggests that SCD is not just an incidental phenomenon occurring in the presence of acute ischemia but may partly be determined by the predisposing genetic risk factors. These early findings have inspired a number of studies to elucidate possible genetic risk factors for SCD that could enable early identification of subjects at risk and prevention of SCD in the general population.

One shortcoming of these two previous population based studies was the lack of comprehensive autopsy material as evidence to define the specific mode of sudden death. Therefore, it has not been fully established whether there is a familial background for the occurrence of SCD as a manifestation of an acute ischemic event, which would support further research efforts on specific genetic screening for vulnerability to ischemia-induced fatal arrhythmias.

The aim of this thesis was to study risk factors for SCD from an acute coronary event in a general northern Finnish population. For this purpose, the Finnish Genetic Study of Arrhythmic Events (FinGesture) was designed and implemented. In this case-control study, demographic variables, presence of familial clustering and possible genetic risk factors for ischemic SCD were

evaluated in a consecutive series of autopsy verified SCD victims and survivors of AMI. Subjects were collected from the Oulu University Hospital District in northern Finland.

2 Review of the literature

2.1 Sudden cardiac death

2.1.1 Definition

The present definition of SCD proposed in the task force report of the European Society of Cardiology is defined as follows: *'Natural death due to cardiac causes, heralded by abrupt loss of conciousness within one hour of the onset of acute symptoms; pre-existing heart disease may have been known to be present, but the time and mode of death are unexpected.'* (Priori *et al.* 2001).

The definition of SCD is not clear-cut and covers a vast variety of different underlying cardiac abnormalities. The definition does attempt to restrict SCDs to deaths caused by ventricular arrhythmias. The presumption is that these arrhythmias leading to SCD may be treated and prevented with specified therapy. Engdahl *et al.* (Engdahl *et al.* 2002) suggested that SCD could be replaced by the term 'presumed ventricular fibrillation', since from a treatment perspective these are the cases of primary interest.

In the current definition the 1 h time frame has been used, since this period seems to best describe the patients with arrhythmic SCD (Hinkle & Thaler 1982). In practice, estimation of duration of preceding symptoms is difficult as many SCDs occur at home and are unwitnessed (de Vreede-Swagemakers *et al.* 1997). In the literature, the time frame used to define SCD has thus varied between instantaneous death to 24 hours if an individual has been seen in a stable condition prior to death during this time-period (Farb *et al.* 1995).

The use of temporal criteria creates also another pitfall as many non-cardiac causes such massive pulmonary embolism, cerebral infarction and rupture of an aortic aneurysm etc. may mimic SCD. In the study of Pratt *et al*. (1996) including subjects with an implantable cardioverter-defibrillator (ICD), the autopsy information contradicted and changed the clinical perception of a SCD based solely on temporal criteria in 7 out of 24 SCD cases by revealing a non-cardiac cause of sudden death. In that study fourfold, differences in SCD rates were derived from the identical clinical database when different classifications were used.

2.1.2 Epidemiology

SCD due to CAD is the single most important cause of death in the adult population of the industrialized world. When the definition is restricted to death within less than 2 h from the onset of symptoms, 12 percent of all natural deaths are sudden, and 88 percent of these are due to cardiac disease. The yearly incidence of approximately 1/1000/year in the general population has been confirmed in several studies (de Vreede-Swagemakers *et al.* 1997, Myerburg *et al.* 1992). In Finland, of all deaths due to CAD (~12000 / year) 67% in males and 56% in females occur out-of-hospital or within one hour of the onset of symptoms, giving a similar yearly incidence of SCD as in other Western populations. Prior to death, CAD has been diagnosed in 62% of males and 57% of females suffering SCD. (Salomaa *et al.* 2002).

In both men and women, the incidence of SCD increases with age (Kannel & Thomas 1982). However, especially in younger age groups, the incidence in women is lower, being about half of that in men and it lags behind by almost 20 years. Thus the incidence of SCD in males between 55 to 64 years of age is about 2/1000 but women only reach the same incidence of SCD at about 75 years of age. (Kannel *et al.* 1998) Although the incidence of SCD increases with age, the proportion of deaths that are sudden decreases with increasing age (Kannel & Thomas 1982, Albert *et al.* 2003). The changes in incidence of SCD with age and the observed sex differences mainly reflect the epidemiology of CAD that is present in up to 80% of individuals suffering SCD.

A circadian variation of SCD has been documented in several studies. There is an increased incidence of SCD during the late morning, usually during the initial 3 hours after awakening (Muller *et al.* 1987, Willich *et al.* 1987, Willich *et al.* 1993). The morning peak of SCD incidence is thought to be related with an increased sympathetic stimulation predisposing to fatal arrhythmias. Also emotional stress may provoke fatal arrhythmias in subjects prone to SCD. Immediately after earthquakes an over fourfold increase in SCD incidence has been observed and it has been estimated that about 40% of SCDs may be precipitated by emotional stress (Leor *et al.* 1996, Gold *et al.* 2007).

In the population, specific subgroups of patients with a high incidence of SCD can be recognized (Myerburg *et al.* 1998). These include individuals with previous myocardial infarction, low ejection fraction, and ventricular tachycardia (VT) and those resuscitated from out-of-hospital cardiac arrest. However, only a small proportion of all SCDs occur among these high-risk subjects (Myerburg *et*

al. 1998). It is an epidemiological paradox that we lack the information about specific markers of an increased risk of death caused by arrhythmia both in the general population and among those with non-specific and intermediate risk profiles, i.e. the individuals who account for the largest absolute number of events (Huikuri *et al.* 2001). (Figure 1)

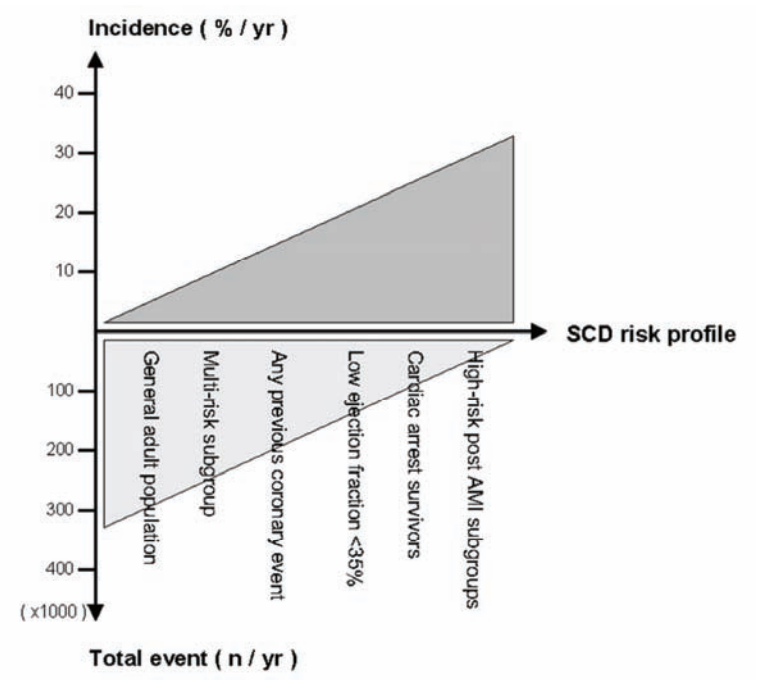

Fig. 1. Epidemiological paradox of sudden cardiac death (Modified from Myerburg *et al.* 1998).

2.1.3 Mechanism

VT degenerating to ventricular fibrillation (VF) and later to asystole appears to be the most common pathophysiological cascade involved in fatal arrhythmias. The next common cause of SCD is bradyarrhythmia or electromechanical dissociation, especially in patients with advanced heart disease. In patients without underlying ischemic heart disease or cardiomyopathy, inherited or acquired arrhythmias such torsades de pointes and idiopathic VF may evoke the SCD. (Huikuri *et al.* 2001) The assessment of initial arrhythmia at the time of abrupt loss of consciousness is

difficult as most reports are based on electrocardiography (ECG) recordings made by the ambulance personnel some time after the initial event (Engdahl *et al.* 2002).

The occurrence of SCD can be considered as an electrical accident proveked by the simultaneous interplay of an anatomic or functional *substrate,* a modulating *transient event* and *a triggering* arrhythmia mechanism (Zipes & Wellens 1998) (Figure 2).

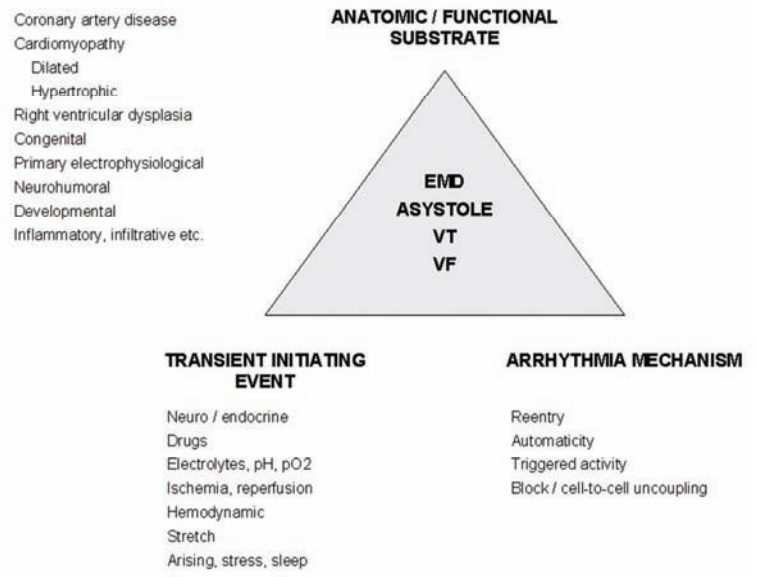

Fig. 2. Mechanisms of sudden cardiac death (Modified from Zipes & Wellens 1998).

In the majority of SCD victims, an underlying cardiac abnormality providing the substrate for arrhythmias can be found and only in about 5% of SCD victims does death occur without any evidence of heart disease (Zipes & Wellens 1998). The most frequent causes of out-of-hospital SCD are *ischemic cardiac disease, non-atherosclerotic disease of coronary arteries, cardiomyopathies, valvular heart disease, infiltrative and inflammatory myocardial disease, congenital heart disease, and primary electrical abnormalities* (Engdahl *et al.* 2002). In most cases underlying cardiac abnormality setting a substrate for fatal arrhythmias is structural such as old infarct scar enabling re-entry pathways but it may also be functional. Arrhythmias associated with structural substrates, manifests most commonly by monomorphic VT with or without degeneration into VF.

The most common factor triggering fatal arrhythmias is *acute myocardial ischemia*. Acute ischemia may cause VF that is at times preceded by polymorphic VT, especially in subjects without a previous history of heart disease. Acute phase ischemia affect the resting membrane potential and the inward and outward ionic fluxes during the action potential leading to alterations in conduction, refractoriness, and automaticity in cardiac muscle cells (Janse & Wit 1989). A non-homogeneous alteration of action potential around ischemic myocardium enables re-entry arrhythmias to occur (Boineau & Cox 1973, Kaplinsky *et al.* 1979, Bigger *et al.* 1977). Also triggered activity, secondary to afterdepolarizations and abnormal automaticity have been shown to precipitate ventricular arrhythmias during the acute phase of an acute ischemic event. In addition to ischemia, several other triggering mechanisms have been recognized, including *systemic, metabolic* and *hemodynamic alterations, neurochemical* and *neurophysiological factors,* and *exogenous toxic* or *pharmacologic effects* (Huikuri *et al.* 2001). Many of these factors increase the incidence of abnormal automaticity and triggered afterdepolarizations enabling the initiation of fatal ventricular arrhythmias.

2.1.4 Autopsy findings

In order to define probable cause of sudden death, autopsy is the only reliable method to confirm or exclude antemortem suspicion of the possible underlying etiology (Pratt *et al.* 1996, Lahti *et al.* 1998). In Finland, the cause-of-death diagnostics is regulated by an act-of-law in cases of sudden death. In contrast to many European countries and the United States, a medico-legal autopsy is mandatory in Finland, whenever sudden death is not due to a known disease and the deceased has not been treated by a physician during his/her last illness, or death has been otherwise unexpected (Act on the Inquest into the Cause of Death, 459/1973, 7th paragraph: Finnish law). In addition, the definitive death certificate containing causes of death and information on its circumstances is issued only after the autopsy with all its accessory examination. (Lahti 2005) In Finland, uniformly and unexceptionally performed autopsies ensure the high quality and reliability of death certificates providing a good basis for the SCD studies.

The autopsy findings in victims of SCD have been shown to vary according to age in many previous studies. Steinberger *et al.* (1996) have reported their observations in 20 subjects who died suddenly within less than one year of age. In 13 deceased individuals (65%), a cardiac abnormality was observed in autopsy, of

which 10 were coronary arterial anomalies. Another common cause for sudden death in newborns is sudden infant death syndrome of unknown reason. In adolescents and young adults, myocarditis, coronary artery anomalies and cardiomyopathies including right ventricular dysplasia and hypertensive and idiopathic cardiac hypertrophy, are the most frequent findings in autopsy (Virmani *et al.* 2001). Maron *et al.* (1986) studied the reasons for sudden death occurring in young competitive athletes (less than 35 years of age). In this special patient group, hypertrophic cardiomyopathy appeared to be the most common cause of SCD accounting for about half of the cases of sudden death. Other important causes of sudden death in young athletes were idiopathic left ventricular hypertrophy (LVH), coronary anomalies, CAD and ruptured aorta.

After 30 to 35 years of age, atherosclerosis is by far the most common autopsy finding in victims of SCD (>60%) followed by hypertensive and idiopathic LVH, and dilated and hypertrophic cardiomyopathies (Virmani *et al.* 2001, Burke *et al.* 1991).

While studying the largest group of SCD victims with underlying CAD, the autopsy findings can be divided into acute and chronic lesions of the coronary arteries and acute and chronic lesions of the myocardium (Myerburg *et al.* 1992). This division forms a reasonable basis to help to understand the interplay between the underlying anatomic substrate and an acute ischemic event triggering fatal arrhythmia.

Chronic atherosclerotic lesions are observed in the coronary arteries of the majority of SCD victims. As an indicator for critical coronary stenosis that may result in acute myocardial ischemia, greater or equal to 75% cross-sectional luminal narrowing has been used (Davies 1992). Critical stenosis is quite often an incidental finding in autopsy. In a study investigating men aged 50–69 years with traumatic or natural non-coronary deaths, the incidence of severe one-vessel disease was 10%, two-vessel-disease 3%, and three-vessel disease 1% (Davies 1992). However, in victims of SCD the proportion of hearts with severe stenosis (>75%) was much higher varying from 15–44% with one-vessel disease, 15–33% with two-vessel disease, and 22–47% with three-vessel disease depending on the population being studied (Farb *et al.* 1995, Virmani *et al.* 2001, Perper *et al.* 1975, Warnes & Roberts 1984, Thomas *et al.* 1988). In the previous autopsy studies, no pattern of specific vessel involvement has been noted (Farb *et al.* 1995, Perper *et al.* 1975, Taylor *et al.* 2000).

In atherosclerotic hearts, an acute coronary event leading to SCD is often associated with an occlusive or subocclusive thrombus (Naghavi *et al.* 2003). The

frequency of detected coronary thrombosis in autopsy has varied from 20% up to 70%, depending on the studied population (Farb *et al.* 1995, Scott & Briggs 1972, Davies & Thomas 1984). The time interval between the onset of symptoms and death, the presence of AMI and the type of prodromal symptoms, all affect the incidence of detected thrombi in SCD (Virmani *et al.* 2001). Also the presence of hypertension and cigarette smoking has been associated with thrombosis in SCD victims (Burke *et al.* 1996, Burke *et al.* 1997). An occlusive thrombus is most often associated with underlying plaque rupture (60%). Without rupture, a thrombus has been found in association with plaque erosion (35%) especially in cases with non-occlusive thrombus and in younger individuals whereas a calcified nodule is an infrequent cause of thrombosis (5%). (Virmani *et al.* 2000)

In approximately 40% of cases, neither thrombi nor plaque disruption could be identified even in careful autopsy (Virmani *et al.* 2000). Naghavi *et al.* (2003) hypothesized that in SCD victims with stable plaque terminal arrhythmic episode may be due to coronary spasm, emboli in the distal intramural vasculature, or myocardial damage related to previous injury. Myocardial pathology increasing the vulnerability towards cardiac arrhythmias and SCD has been associated with previous myocardial infarction (found approximately in 40% to 80% in autopsies) and cardiomegaly (Warnes & Roberts 1984, Scott & Briggs 1972, Davies *et al.* 1989). These two findings also seem to interact since an increased heart weight has been observed in SCD victims with healed infarct in autopsy (Burke *et al.* 1996). In addition, in normotensive hearts with severe stenosis, a stepwise increase in heart weight with one-, two-, and three-vessel disease has been detected (Burke *et al.* 1996).

Naturally, in autopsy exact arrhythmia mechanism cannot be determined and the diagnosis of SCD is set based on the combined information on the circumstances of death and the autopsy findings after exclusion of non-cardiac causes of death.

2.2 Risk factors for sudden cardiac death in the general population

As up to 80% of individuals suffering SCD have underlying CAD, the recognized risk factors for SCD mainly parallel those for CAD (Zipes & Wellens 1998). The risk for SCD has varied significantly in CAD subgroups, with post-MI patients with low ejection fraction being in the highest risk and the subjects with stable CAD without specific risk markers placed as the other end of the spectrum. Myerburg has proposed a three-step cascade for modelling individual-specific

risk of SCD within the general population: 1) Identify risk for CAD (conventional risk factors), 2) Susceptibility to acute coronary syndromes (vulnerable plaques, vulnerable blood), 3) Subgroup at specific risk for SCD (arrhythmia markers, familial clustering, genetic determinants). (Myerburg 2001)

2.2.1 Conventional risk factors

Several models have been developed to assess the risk for CAD in asymptomatic subjects. The European Society of Cardiology has recommended a model for estimation of ten-year risk of fatal CAD based on the SCORE (Systematic Coronary Risk Evaluation) system (De Backer *et al.* 2003). In the SCORE system, the following risk factors are integrated: gender, age, smoking, systolic blood pressure and either total cholesterol or the cholesterol/HDL ratio. The disadvantage of the SCORE-system is that it is better at predicting the evolution of the underlying CAD and total mortality whereas its sensitivity as an indicator for sudden arrhythmic death is very low (Myerburg 2001).

Smoking

Smoking is a major risk factor for CAD, AMI and SCD. In the Framingham study, the risk of SCD increased almost three-fold in cigarette-smokers compared with non-smokers (Kannel & Thomas 1982). It is also one of the few risk factors that has been shown to have an independent association with SCD. In the National Mortality Followback Survey, which included a random sample of about 1% of deaths of US residents, current smoking was the only risk factor differentiating the SCD from the non-sudden coronary deaths (Escobedo & Zack 1996). Current smoking may modify the outcome of an acute coronary event by increasing the propensity towards arrhythmias and by affecting the acute thrombosis (Escobedo & Zack 1996, Burke *et al.* 1997, Hallstrom *et al.* 1986).

Hypertension and left ventricular hypertrophy

Hypertension is a well-known risk factor for CAD but its role in SCD has also been well established (Kannel *et al.* 1988, Weijenberg *et al.* 1996, Cupples *et al.* 1992). Hypertension predispose to LVH setting the myocardium in proarrhythmic state (McLenachan *et al.* 1987). Ghali *et al.* (1991) showed that LVH is associated with an increase in the frequency and complexity of ventricular arrhythmias. The

study was conducted in patients without CAD and the relationship between LVH to arrhythmias was both graded and continuous. An independent role of LVH to ventricular arrhythmias has been confirmed also in other studies (Szlachcic *et al.* 1992). In animal models, LVH has been associated with longer action potential durations, increased dispersion of repolarization and increased vulnerability to arrhythmia induction (Kowey *et al.* 1991, Biagetti & Quinteiro 2006).

In addition to hypertension, the severity of CAD may have an effect on cardiac hypertrophy. In a subgroup analysis of hypertensive and normotensive victims of SCD, the stepwise increase in total heart weight (THW) with one-, two-, and three-vessel disease was shown in normotensive SCD victims in contrast to hypertensive subjects with constantly greater heart weight (Burke *et al.* 1996).

Lipids

Elevated blood cholesterol and triglyceride levels are well-documented risk factors for CAD and all its manifestations including SCD (Wannamethee *et al.* 1995, Jouven *et al.* 1999). In secondary prevention trials, lipid lowering drugs have been able to reduce total number of coronary deaths (Pedersen *et al.* 2000, Simes *et al.* 2002). However, these studies have not analyzed SCD separately from other causes of coronary death. One can conclude that 30–40% relative risk reduction in SCD could occur if an assumption of parallel reduction in risk with CAD death and non-fatal myocardial infarction is made (Priori *et al.* 2001).

Indirect evidence the effect of lipids on SCD emerged from a study in patients with CAD and life-threatening ventricular arrhythmias treated with ICD implantation. De Sutter *et al.* first showed that lipid-lowering drugs were associated with a reduction of recurrences of ventricular arrhythmias in these patients with ICD. One possible explanation for this finding was that lipid-lowering drugs had reduced the number of ischemic episodes that could have acted as a trigger on the myocardial substrate to initiate recurrences of ventricular arrhythmias. (De Sutter *et al.* 2000) The role of increased blood lipid levels for provoking SCD is thus more probably mediated by the progression of underlying CAD rather than by any direct effect on arrhythmogenesis.

In the study of Dekker *et al*. (2006), hypercholesterolemia was actually significantly lower in patients who developed VF during their first ST-elevation AMI than in those who did not have such arrhythmia. A similar result was found in the Nurses' Health Study , which reported that a history of

hypercholesterolemia was not a significant predictor of SCD (Albert *et al.* 2003). In both of these studies, the presence of hypercholesterolemia was based on the patient history and not on measured cholesterol-levels. It is possible that some of the subjects had undiagnosed hypercholesterolemia but the authors from the Nurses' Health Study considered that unlikely in their cohort of health professionals (Albert *et al.* 2003).

Impaired glucose metabolism

In subjects with impaired glucose tolerance or diabetes many factors, such as severe CAD, disturbed autonomic balance, and decreased fibrinolytic function, may contribute to the unfavorable outcome during an acute coronary event (Jacoby & Nesto 1992). An independent association of diabetes to SCD was detected in the Paris Prospective Study (Balkau *et al.* 1999). In the French men included in the study, the risk of suffering SCD increased progressively in conjunction with the severity of the disease from borderline diabetes to diabetes without microvascular, and diabetes with microvascular changes and this correlation was maintained even after adjustment for potential confounders. The importance of blood glucose control was also noted, as higher glucose levels were associated with the increased risk of SCD both in the absence and in the presence of diabetic complications. (Jouven *et al.* 2005) The association with diabetes and SCD has been confirmed in other studies (Kannel *et al.* 1998, Curb *et al.* 1995).

Since moderately impaired glucose metabolism does not cause any marked symptoms, it may remain undetected in many subjects with increased blood glucose levels. In the previous studies, a high prevalence (~30%) of unrecognised impaired glucose tolerance and diabetes was observed in patients with AMI (Norhammar *et al.* 2002, Hu *et al.* 2006, Andersen *et al.* 2006). It can be assumed that the proportion of subjects with unrecognised impaired glucose tolerance and diabetes is similar in victims of SCD.

ECG changes

ECG is a straightforward non-invasive method to evaluate electric disturbances in heart during a routine clinical examination. In the Framingham study, occurrence of signs of LVH (ECG-LVH) and silent myocardial infarction (ECG-AMI) in ECG has been associated with an increased risk for SCD in asymptomatic subjects free of clinically evident CAD (Kannel & Abbott 1986). In men, ten-year,

age-adjusted incidence (rate/100) of SCD was 8.2 for ECG-LVH and 6.3 for ECG-AMI being significantly higher than in general Framingham sample (2.7). In women the figures were similar.

Both of these ECG findings can be interpreted as signs of the underlying heart disease. In Framingham study, these unrecognised, ECG-MI, infarctions were as serious prognostic risk factors as the more common symptomatic infarctions (Kannel 1986). In these subjects, similar methods should be used to evaluate future SCD risk as applied in symptomatic post-AMI patients. The association of ECG-LVH with underlying cardiac hypertrophy is not so clear-cut. It has been suggested that the voltage criteria for LVH reflects the severity and duration of the hypertension whereas accompanying repolarization abnormalities signal the onset of a compromised coronary circulation and ischemic myocardial involvement (Kannel & Abbott 1986). In fact, observed repolarization abnormalities as well as increased QRS duration in ECG independently increased the risk for cardiovascular mortality in a study including hypertensive patients with ECG documented LVH (Oikarinen *et al.* 2004).

As noted earlier, silent myocardial ischemia and LVH have been independently associated with ventricular arrhythmias in asymptomatic patients with mild to moderate hypertension (Szlachcic *et al.* 1992). In the hypertensive patients without CAD, a higher frequency and complexity of ventricular arrhythmias have been found in association with the presence of ECG-LVH (Ghali *et al.* 1991, Coste *et al.* 1988).

2.2.2 Lessons from the previous follow-up studies

In some studies, specific risk factors for SCD have been evaluated as opposed to other manifestations of CAD (Escobedo & Zack 1996, Wannamethee *et al.* 1995). Previous follow-up studies have confirmed several risk factors for SCD including, LVH, age, serum cholesterol, smoking, relative weight, elevated blood pressure, hematocrit, vital capacity, diabetic status, heavy drinking, ECG changes, and a parental history of SCD (Escobedo & Zack 1996, Wannamethee *et al.* 1995, Jouven *et al.* 1999, Schatzkin *et al.* 1984, Cuddy & Tate 2006). In most of these studies, victims of SCD have been compared to non-sudden coronary disease death and only a few risk factors have shown an independent association with SCD. In the Paris Prospective Study, body mass index (BMI), diabetes status and parental history of SCD associated with SCD but not with fatal myocardial infarction (Jouven *et al.* 1999) and in the study of Escobedo *et al*. (1996), current

smoking was a specific risk factor for SCD. Only Wannamethee *et al.* (1995) included cases of nonfatal AMI. They found specific or particular associations of elevated heart rate, heavy drinking and arrhythmia to SCD examined in relation to non-sudden coronary disease death and nonfatal AMI. The role of risk factors has been shown to operate differently in subjects with and without previous coronary heart disease (Wannamethee *et al.* 1995, Schatzkin *et al.* 1984). In the Framingham study, only LVH and intraventricular block were positive predictors of SCD in men with prior CAD whereas hematocrit was a risk factor in women with prior CAD (Schatzkin *et al.* 1984). The low incidence of SCD in women has lead to the exclusion of women from most prospective studies. However, in the Framingham study, women seemed to be subject to the same risk factors as men (Kannel *et al.* 1998, Albert *et al.* 2003).

2.2.3 Family history

The role of family history as a risk factor for CAD has been well documented in the previous studies during the last decades (Sholtz *et al.* 1975, Rissanen 1979, Snowden *et al.* 1982). In a Swedish study over 21 000 twins were followed for 26 years and the risk of death from coronary heart disease in pairs of monozygotic and dizygotic twins was assessed. In both women and men, death from CAD at an early age in one's twin was a strong predictor of the risk of death from this disorder. The risk was greater in monozygotic twins than in dizygotic twins and was largely independent of other personal risk factors for CAD. Based on the results from this fundamental study, a genetic background for death from CAD was proposed. It was also noted that the genetic effect decreased at older ages. (Marenberg *et al.* 1994)

In 1998 Friedlander *et al.* reported that a family history of myocardial infarction or SCD was positively associated with the risk of SCD. This association was independent of familial aggregation of other common risk factors. (Friedlander *et al.* 1998) One year later, the results from the Paris Prospective Study suggested that parental history of sudden death was an independent risk factor for SCD in middle-aged men. In that study it was also noted that parental history of SCD was not associated with fatal myocardial infarction for which parental history of myocardial infarction was an independent risk factor. (Jouven *et al.* 1999) The result was interpreted as evidence of a familial risk of sudden death distinct from the familial risk pattern of myocardial infarction. Due to the frequent occurrence of CAD in sudden death patients over 40 years of age, the

authors hypothesized that the expression and mechanisms of CAD may be under partial familial control, which may cause sudden death rather than fatal myocardial infarction for some subjects (Jouven *et al.* 1999).

Dekker *et al.* (2006) provided further evidence of an independent role of family history of SCD as a specific risk factor for SCD. They performed a case-control study including a large sample of subjects with a first ST-elevation myocardial infarct to identify independent risk factors for primary VF. Primary VF before the thrombolysis had occurred significantly more often in subjects with positive family history of SCD. It was concluded that at present, family history, which can be viewed as an integrator of complex interactions of multiple genes and environmental factors, is a key predictor of SCD in AMI patients. (Dekker *et al.* 2006)

2.2.4 Genetic determinants

After the two population-based studies had proposed that there was a genetic background for SCD in general heterogenous populations, a report from the National Heart, Lung, and Blood Institute Workshop dealing with SCD, genes, and arrhythmogenesis concluded that genetic population studies directed at discovering common proximal sources of inherited molecular risk most directly linked to arrhythmia initiation and propagation would appear to have considerable potential in helping reduce cardiovascular mortality (Jouven *et al.* 1999, Friedlander *et al.* 1998, Spooner *et al.* 2001). Three broad pathways by which genetic variation could increase the susceptibility for arrhythmias and SCD were postulated: 1) atherothrombosis, 2) electrogenesis, and 3) neural regulation and control (Spooner *et al.* 2001).

During the last two decades several inherited arrhythmogenic disorders with an increased risk for SCD have been identified such as long QT-syndrome, familial hypertrophic cardiomyopathy, arrhythmogenic right ventricular dysplasia, dilated cardiomyopathy and Brugada syndrome etc. In some cases, the genetic locus and associated gene have been identified as in different forms of long QT-syndrome. (Priori *et al.* 1999) However, these disorders where a single genetic mutation is namely responsible for the clinical outcome of the patient constitute only a small part of the clinical conditions associated with cardiac arrhythmias. In contrast to these rare single-gene diseases, the development of CAD is a complex and multifactorial process and therefore in the general population there are

several genes that can modify the risk of an ischemic event and its consequences (AMI or SCD) (Sing *et al.* 1992).

Even though the literature about above-mentioned rare monogenic diseases have been rapidly increased, little is known about genetic vulnerability to SCD in the general population (Prutkin & Sotoodehnia 2008). In order to reveal association of SCD and common genetic variation present in genes responsible for electrogenesis and neural regulation, the candidate gene approach has been used in many previous studies. Genes where genetic mutations responsible for monogenic diseases have been observed, have gained special interest in this respect. For example, a common variant in the SCN5A gene, S1102Y, have been associated with an increased risk of arrhythmias and SCD in the presence of predisposing factors like hypokalemia and cardiac hypertrophy (Splawski *et al.* 2002, Burke *et al.* 2005). Rare mutations in the SCN5A gene are responsible for inherited monogenic diseases like long-QT and Brugada syndromres (Wang *et al.* 1995, Antzelevitch 2006). Many other common variants in genes with assumed effect on electrogenesis have been studied with less notable findings (Prutkin & Sotoodehnia 2008). In the studies concerning genetic variation affecting neural regulation, association of SCD and a common variant in a gene coding β2-adrenergic receptor, Q27E, have been replicated in a few populations (Lanfear *et al.* 2005, Sotoodehnia *et al.* 2006).

As the most common underlying pathology of SCD is CAD, the factors accelerating the progression of CAD and predisposing to acute ischemia are of special interest. Thrombosis can affect both plaque progression and the consequences of plaque complications leading to AMI and SCD (Davies 1996b). Genetic risk for susceptibility of SCD may be mediated through variation in genes affecting plaque development and stability, platelet aggregation, clotting proteins, inflammatory mediators and endothelial interactions (Spooner *et al.* 2001). Several polymorphisms and mutations in the genes coding proteins involved in the atherothrombotic process have been examined with some of them showing associations between the genotype and the risk of CAD. These genes with possible CAD-associated polymorphisms include fibrinogen, platelet glycoproteins Ia, Ib, IIIa, and VI, coagulation Factor V, Factor VII, Factor XII, Factor XIII, and Annexin A5 (Lane & Grant 2000, Endler & Mannhalter 2003, Ollikainen *et al.* 2004, Mikkelsson *et al.* 2005, Gonzalez-Conejero *et al.* 2002).

Even though the literature of the polymorphisms associated with CAD and its consequences have rapidly expanded, the results of gene-disease associations have often been contradictory (Lane & Grant 2000). These inconsistencies have

been explained for example by inadequate sample sizes unable to detect minor effects, publication, recruitment and survival biases and variation of the genetic polymorphisms between racial groups (Lane & Grant 2000, Colhoun *et al.* 2003). In most studies on the association between the genetic polymorphism and acute coronary events, survivors of AMI have been over-represented since as many as one out of every three patients with an acute ischemic event will suffer SCD before admission to the hospital. The genetic risk factors leading more likely to SCD than to non-fatal AMI as a manifestation of CAD may have been missed or falsely considered as positive protective factors against non-fatal AMI. Thus, the role of thrombotic polymorphisms in SCD from an acute coronary event has not been fully established.

2.3 Summary

In summary, the majority of SCDs occurs in a low risk general population. As the most common trigger for fatal arrhythmias is acute myocardial ischemia, the key to the specific prevention of SCD lies in achieving a better distinction of risk profiles between survivors and victims of SCD from an acute coronary event. A familial background of sudden death has been observed in previous studies. However, previous studies have not included comprehensive autopsy findings as criteria for defining the specific mode of sudden death. Therefore, it has not been unequivocally established whether there is a familial background for the occurrence of SCD as a manifestation of an acute coronary event, which would support further research efforts on genetic screening for vulnerability to ischemia-induced fatal arrhythmias.

3 Purpose of the present study

The overall aim of the present study was to compare risk factors in consecutive series of autopsy verified SCD victims and survivors of an AMI, in order to find specific risk profiles for SCD from an acute coronary event. The specific aims of this study were to investigate:

1. Whether a combination of traditional coronary risk factors, cardiac hypertrophy and the severity of coronary artery disease is associated with an increased risk for SCD from an acute coronary event
2. Whether SCD has a familial background indicating genetic predisposition for sudden death from an acute coronary event
3. Whether a family history of SCD has any effect on traditional coronary risk factors, cardiac hypertrophy or the severity of CAD
4. Whether genetic polymorphisms associated with thrombosis increase the risk for SCD from an acute coronary event

4 Subjects and methods

4.1 FinGesture- and other study populations

Finnish Genetic Study for Arrhythmic Events (FinGesture) started in 2000, after which all victims of sudden death autopsied at the Department of Forensic Medicine, University of Oulu, Oulu, Finland, were included in the study. At the end of April 2006, this study had records on 646 consecutive victims of out-of-hospital sudden death from a defined geographical area, i.e. Oulu University Hospital District in Northern Finland.

In contrast to many European countries and the United States, a medico-legal autopsy is mandatory in Finland whenever sudden death is not due to a known disease and the deceased has not been treated by a physician during his/her last illness, or death has been otherwise unexpected (Act on the Inquest into the Cause of Death, 459/1973, 7th paragraph: Finnish law) (Lahti RA, 2005). Therefore, the study includes practically all unexpected out-of-hospital sudden deaths that occurred in the Oulu University Hospital District during the study period.

The out-of-hospital SCD victims consisted of those with *(i)* a witnessed sudden death within 6 hours of the onset of the symptoms or within 24 hours of the time that the victim was last seen alive in a normal state of health and *(ii)* evidence of a coronary complication, defined as a fresh intracoronary thrombus, plaque rupture or erosion, intraplaque hemorrhage or critical coronary stenosis (>75%) in the main coronary artery were included in the SCD group (Virmani *et al.* 2001, Taylor *et al.* 2000). Figure 3. shows an example of culprit lesions found in autopsy. Victims with evidence of a non-cardiac cause and victims with mechanical causes of sudden death, such as a rupture of the myocardium and/or tamponade, extensive myocardial necrosis (>50%), rupture of entire papillary muscle, pulmonary edema, or any cause of death considered to be due to some reason other than ischemia-induced SCD were excluded. An end-point committee consisting of a forensic pathologist, an experienced cardiologist and the primary investigator defined the mode of deaths in each case. After the exclusions, 425 out of 646 victims of sudden death were defined to be due to an acute coronary event.

As controls (in papers I-III, A) we used consecutive AMI survivors (n=644) that were collected during the same time period in the Division of Cardiology, University of Oulu. These patients comprised all AMIs in the same geographic area from which the SCD victims had been collected. Survivors of AMI were

recruited to participate during the first seven days after the diagnosis of AMI, which was confirmed by using the contemporary guidelines at the beginning of the study. Subjects resuscitated from VT or VF during the acute ischemic event was excluded from the AMI population. (Tapanainen *et al.* 2001)

In papers I, II and A, 40–62 year old subjects without a history of coronary heart disease, AMI or aborted cardiac arrest were also included. These controls were the subjects from the Opera (Oulu Project Elucidating Risk of Atherosclerosis) study on randomly selected subjects from the social insurance register covering the whole population of the City of Oulu (Rantala *et al.* 1999). These subjects have been followed-up for 12 years and the subjects who died for any reason or experienced AMI during the follow-up before the end of 2004 were excluded from the study. In total, 809 controls were included.

Subjects to the original articles were drawn from the whole FinGesture-population using study specific criteria in each paper. Due to the small number of women among the consecutive series of AMI survivors and SCD victims, papers I and A included only males to the study groups. The age limit for these studies was set based on the previous selection criteria in the AMI survivor and in the control groups. In total, 98 victims of SCD, 212 AMI survivors and, 243 controls with complete information of the analysed polymorphisms were included in paper I. In paper A, number of subjects in each study group was 219, 349, and 197, respectively. In papers II and IV subjects without a complete family history of AMI and SCD were excluded, leaving 138 victims of SCD, 254 AMI survivors and 470 controls to the study population in paper II and 246 victims of SCD in paper IV. In paper III, no additional exclusions were performed.

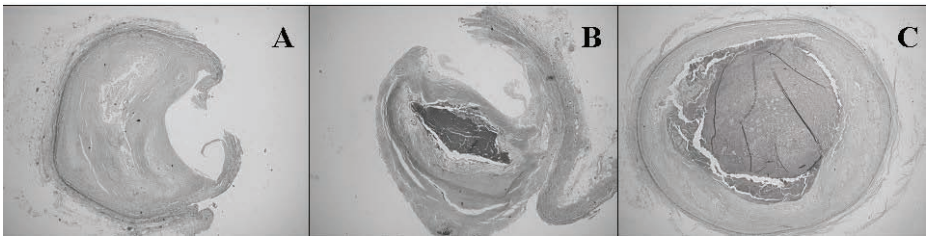

Fig. 3. An example of culprit lesions found in autopsy. A) Stable plaque (>75% stenosis), B) Intraplaque haemorrhage, C) Plaque rupture with a thrombus. Verhoeff-Masson trichrome staining (O'Connor & Valle 1982).

4.2 Demographic variables and coronary risk factors

In victims of SCD, information about coronary risk factors was obtained from the medico-legal autopsy records and from a questionnaire sent to the closest relative of the SCD victim. The postal inquiry included questions about previously detected hypertension, hypercholesterolemia, impaired glucose metabolism, angina pectoris, myocardial infarction and history of smoking. The presence of hypertension, hypercholesterolemia, and impaired glucose metabolism was based solely on patient history or the ongoing medication at the time of SCD, reported either in autopsy records or in the postal inquiry. In controls, a history of same coronary risk factors was assessed prior to clinical examination indicating the awareness of the risk factors before the event.

The severity of CAD was defined as the number of critically stenosed (>75%) main coronary arteries (left main, left anterior descending, left circumflex or right coronary artery) obtained in autopsy or in coronary angiography (III–IV, A). When coronary angiography had been performed within 3 months in relation to the AMI (225 patients, 35%), the results were included in the comparisons.

For AMI survivors, cardiac hypertrophy was defined by echocardiography using M-mode recordings under 2-D guidance according to the recommendations of the American Society of Echocardiography (ASE) (Sahn *et al.* 1978). Left ventricular mass (LVM) was calculated from the ASE measurements according to the corrected equation and cardiac hypertrophy was defined when the ratio between the LVM and body surface area (BSA) was over 134g/m^2 for men and over 110g/m^2 for women (Kauma *et al.* 1998, Devereux *et al.* 1986, Devereux *et al.* 1984, Du Bois & Du Bois 1916).

At autopsy, the THW was measured. THW was indexed by dividing it by the BSA. These values were compared to normal values drawn from the population-based table for normal THW separately for women and men in a previous Mayo Clinic study (Kitzman *et al.* 1988). In this study, the mean THW ± 2SD plotted against BSA for 392 normal women and 373 normal men was reported. We used this data to identify the normal heart weight. The cut-off points for hypertrophy were estimated by line fitting using the least square method for the given THW values exceeding +2SD plotted against BSA separately for males and females (III, IV).

Notably, even though different method was used to determine the presence of cardiac hypertrophy in AMI survivors and in victims of SCD, a close correlation

(r=0.92, SD = 43g) between these two methods in assessing LVM has been observed in a previous study (Devereux *et al.* 1986).

4.3 Family history

The family history of SCD and AMI in the first-degree relatives (FDR) (each biological parent, brother, sister or child) was ascertained by a mailed questionnaire or a telephone call from the control subjects, the survivors of AMI and the closest relatives of the SCD victims by using a standardized inquiry. In cases of a reported sudden death of a FDR, a description of the event was elicited to confirm the mode of death. Death certificates from the Central Statistical Office in Finland and the Causes of Death Register were used to confirm the mode of death in the FDR. The quality of these registers has been previously validated (Mahonen *et al.* 1999, Rapola *et al.* 1997, Pajunen *et al.* 2005). The end-point committee reviewed all cardiac deaths and AMI of the FDR blinded to the class of the cases, and used both predefined clinical criteria and the death certificates in the interpretation of sudden deaths and AMI. The same definitions for SCD regarding the time after onset of symptoms, witnessed vs. unwitnessed, were used for the relatives.

4.4 DNA analysis

Genomic DNA was extracted from peripheral blood nuclear cells with a salting-out method (Miller *et al.* 1988). Studying this thesis, we selected ten polymorphisms that have been associated with acute coronary events in some previous studies: Factor V R506Q (FV Leiden), Factor VII R353Q, Factor XII C46T, FXIII V34L, Glycoprotein (Gp) Ia C807T, GpIbalpha T[-5]C, GpIIIa $P1^{A1/A2}$, GpVI T13254C, Fibrinogen –455G/A and Annexin A5 –1C/T (Lane & Grant 2000, Endler & Mannhalter 2003, Ollikainen *et al.* 2004, Grant 2003, Simmonds *et al.* 2001). Previously described methods were used for polymerase chain reaction amplification and digestion procedures (Table 1) (Ollikainen *et al.* 2004, Bertina *et al.* 1994, Green *et al.* 1991, Zito *et al.* 2000, Kakko *et al.* 2002, Unkelbach *et al.* 1995, Thomas *et al.* 1991). After restriction enzyme digestion, the products were electrophoresed through a 3 % agarose gel, stained with GelStar (FMC BioProducts, Rockland, Me., USA), and visualized under ultraviolet light.

Table 1. Methods used for DNA analysis.

Polymorphism	Primers	Restriction enzyme	Fragment lengths	Reference
FV-Leiden	5'-TGCCCAGTGCTTAACAAGACCA-3'	MnlI	WT 37, 67, 163 bp	(Bertina *et al.* 1994)
	5'-TGTTATCACACTGGTGCTAA-3'		HO 67, 200 bp	
FVII (R353Q)	5'-GGGAGACTCCCCAAATATCAC-3'	MspI	WT 205bp	(Green *et al.* 1991)
	5'-ACGCAGCCTTGGCTTTCTCTC-3'		HO 272, 205bp	
FXII (C46T)	5'-GATAGGCAGCTGGACCAACG-3'	BsaHI	WT 116, 26 bp	(Zito *et al.* 2000)
	5'-TGATAGCGACCCCCCAGAAC-3'		HO 142 bp	
FXIII (V34L)	5'-ACCTGCCCACAGTGGACCTTCAGAGC-3'	AluI	WT 87 bp	(Kakko *et al.* 2002)
	5'-CCAGAGTGGTGGGGAAGGGGGGTATG-3'		HO 25, 62 bp	
GpIa (C807T)	5'-GTGTTTAACTGAACACATATAAAACC-3'	TaqI	WT 118 bp	(Kakko *et al.* 2002)
	5'-CTTACCTTGCATATTGAATTGCTTC-3'		HO 26, 92 bp	
GPIb T[-5]C	5'-CCTTCAACCGGCTGACCTCGCTGCC-3'	BsaHI	WT 242, 111bp	(Unkelbach *et al.* 1995)
	5'-TTCAGCATTGTCCTGCAGCCAGC-3'		HO 353bp	
GP IIIa $P1^{A1/A2}$	5'-CTGCAGGAGGTAGAGAGTCGCCATAG-3'	NciI	WT 247, 170, 77bp	(Unkelbach *et al.* 1995)
	5'-GTGCAATCCTCTGGGGACTGACTTG-3'		HO 170,77bp	
GpVI (T13254C)	5'-ACATCCACAACAGTCCAGTG-3'	MspI	WT 120, 112, 47 bp	(Ollikainen *et al.* 2004)
	5'-ATCGAGAAGTCTAGGCAGAG-3'		HO 112, 95, 47, 25 bp	
Fibrinogen –455G/A	5'-AAGAATTTGGGAATGCAATCTCTGCTACCT-'3	HaeIII	WT 383, 575 bp	(Thomas *et al.* 1991)
	5'-CTCCTCATTGTCGTTGACACCTTGGGAC-3'		HO 958 bp	
Annexin A5 –1C/T	5'-GGGCACGAGTTGCAAATGGCG-3'	NcoI	WT 87bp, 67bp	(Gonzalez-Conejero *et al.* 2002)
	5'-GTCGCAGCATACAAAGTTGTG-3'		HO 153bp	

4.5 Statistical analysis

All analyses were performed with the Statistical Package for Social Studies versions 10.1 (I) and 12.0 (II–IV, A) (SPSS Inc, Chicago, Ill., USA). The statistical data is presented as mean ± standard deviation (SD) for continuous variables and as percentages for categorical variables. The statistical significance of the differences between the study groups was analyzed with Chi-square test for categorical variables and two-sided t-test for continuous variables with a normal distribution. Odds ratios (OR) with 95% confidence intervals (CI) were reported. In addition sensitivity, specificity and predictive accuracy of each risk variable and the combination of the variables in differentiating the patients who had died suddenly from those who had survived an acute coronary event were reported (III). In paper II, logistic regression analysis was used to assess the significance of a family history of SCD after adjustment for other variables and in paper IV to analyze the role of coronary risk factors explaining the presence of three-vessel coronary disease in victims of SCD (IV). In the addendum to publication I (A), Sample Power version 2.0 (SPSS Inc., Chicago, Ill., USA) was used for power calculations. All tests were two sided and statistical significance was set at $p<0.05$.

5 Results

5.1 Characteristics of FinGesture-population

At the end of April 2006, the FinGesture-study consisted of 646 consecutive victims of sudden death of which 425 fullfilled the predefined criteria for SCD. Table 2 shows the characteristics of FinGesture-population.

Table 2. Characteristics of FinGesture-population.

Variables	Male (n = 356)	Female (n = 69)
Age (years)	62.7 (±10.7)	71.8 (±11.6)
Youngest – oldest (years)	35–92	40–90
BMI (kg/m^2)	27.1 (±4.3)	27.5 (±5.8)
History of		
Hypercholesterolemia	92/350 (26.3)	20/68 (29.4)
Diabetes type I or II	54/351 (15.4)	20/68 (29.4)
Hypertension	169/349 (48.4)	39/68 (57.4)
Angina pectoris	177/346 (51.2)	32/67 (47.8)
Previous myocardial infarction	49/351 (14.0)	11/68 (16.2)
Smoking		
Never	65/252 (25.8)	27/39 (69.2)
Ex	63/252 (25.0)	2/39 (5.1)
Current	124/252 (49.2)	10/39 (25.6)
Recent alcohol consumption	69/355 (19.4)	6/69 (8.7)
SCD event		
Estimated time of death (0–24h)	11.7 (±6.3)	12.1 (±6.2)
Place of SCD		
At home	196/333 (58.9)	51/61 (83.6)
Outside	137/333 (41.1)	10/61 (16.4)
Witnessed	145/350 (41.4)	33/67 (49.3)
Resuscitated	183/353 (51.8)	31/67 (46.3)

Variables	Male (n = 356)	Female (n = 69)
Autopsy		
Total heart weight	496.9 (±97.4)	403.1 (±75.5)
Critical stenosis (>75%)		
No critical stenosis	40/355 (11.3)	5/68 (7.4)
One coronary artery	71/355 (20.0)	13/68 (19.1)
Two coronary arteries	77/355 (21.7)	16/68 (23.5)
Three coronary arteries	167/355 (47.0)	34/68 (50.0)
Culprit lesion		
No acute lesion or severe stenosis	10/356 (2.8)	0/69 (0.0)
Stable plaque	171/356 (48.0)	37/69 (53.6)
Intraplaque haemorrhage	93/356 (26.1)	22/69 (31.9)
Plaque erosion	24/356 (6.7)	5/69 (7.2)
With thrombus	16/24 (66.7)	3/5 (60.0)
Plaque rupture	58/356 (16.3)	5/69 (7.2)
With thrombus	34/58 (58.6)	3/5 (60.0)
Location of culprit lesion		
Left main artery	8/351 (2.3)	2/68 (2.9)
Left anterior descending	88/351 (25.1)	18/68 (26.5)
Left circumflex	23/351 (6.6)	2/68 (2.9)
Right coronary artery	94/351 (26.8)	16/68 (23.5)
Uncertain	138/351 (39.3)	30/68 (44.1)
Acute infarction	243/351 (69.2)	40/68 (58.8)
Healed infarction	223/355 (62.8)	32/68 (47.1)

AMI = acute myocardial infarction, BMI = body mass index, SCD = sudden cardiac death.

Values are expressed as mean (±SD) or number of subjects (%), unless otherwise stated.

In the present study, 57% of the sudden deaths had occurred unwitnessed and thus met the 24h inclusion criteria when the subject was seen alive. The exact information about the onset of symptoms is known for 99 victims of SCD (23% of the total population). Of these subjects 45 suffered SCD within one-hour from the onset of symptoms and in 54 cases the death was instantaneous without any preceding symptoms. In the majority of the cases it is not possible to determine the exact time from the onset of symptoms as many SCDs occurred during the night or there was no reliable witness to the event. Cardiopulmonary resuscitation had been attempted in 51% of the SCD cases indicating that there had been a short time interval between the cardiac arrest and the provision of cardiovascular care.

5.2 Family history of sudden cardiac death (II)

The role of family history as a determinant for the final outcome of an acute coronary event is shown in tables 3 and 4. The incidence of coronary events, either SCD or AMI, among FDRs of SCD victims and AMI survivors was significantly higher than among FDRs of the control subjects. However, the incidence of coronary events among FDRs in general did not differentiate SCD victims from AMI survivors (Table 3). The same phenomenon was seen when the incidence of AMI among FDRs was analyzed separately. The incidence was significantly higher among FDRs of both the AMI and SCD subjects than of the control subjects but did not differ between the AMI and SCD groups. (Table 3).

SCD among FDRs of victims of SCD (5.2%) was significantly more common than SCD among the relatives of AMI survivors (3.3%) or controls (2.3%). The OR of having had SCD among FDRs was 1.6 (95% CI 1.2 to 2.2, p< 0.01) in SCD victims compared with AMI survivors and 2.2 (95% CI 1.6 to 3.0, p<0.001) compared with control subjects. The proportion of SCD in the context of an acute coronary event (AMI or SCD) was also higher among FDRs of SCD victims than among FDRs of the AMI or control subjects. (Table 3)

Table 3. History of sudden cardiac death and myocardial infarction among first-degree relatives.

Variables	FDR of control (n = 3748)	FDR of AMI (n = 2326)	FDR of SCD (n = 1223)
SCD or AMI among FDR	276/3748 (7.4)	262/2326 (11.3)*	148/1223 (12.1)*
SCD among FDR	87/3748 (2.3)	76/2326 (3.3)**	64/1223 (5.2)*, ***
AMI among FDR	189/3748 (5.0)	186/2326 (8.0)*	84/1223 (6.9)**
SCD proportion	87/276 (31.5)	76/262 (29.0)	64/148 (43.2)**, ***

The figures are number of subjects (%). Probability values refer to χ^2 analyses between the groups.
AMI = acute myocardial infarction, FDR = first-degree relatives, SCD = sudden cardiac death.
* p<0.001 vs. control, ** p<0.05 vs. control, *** p<0.01 vs. AMI.

When victims of SCD were compared with AMI survivors, the history of SCD in one family member did not differ significantly between the groups (OR 1.4, 95% CI 0.9 to 2.2, p<0.09), but it was higher in victims of SCD than among controls (OR 3.4, 95% CI 1.8 to 6.5, p<0.001). There were a greater number of subjects with SCD in two or more FDR in the SCD group than in the AMI group (OR 3.3. 95% CI 1.4 to 7.8, p<0.01) or in the control group (OR 11.3, 95% CI 4.0 to 31.8, p<0.001). (Table 4)

Table 4. Family history of sudden cardiac death and acute myocardial infarction.

Variables	Control (n = 470)	AMI (n = 254)	SCD (n = 138)
SCD among FDR			
No	388 (82.6)	189 (74.4)	93 (67.4)
1	77 (16.4)	56 (22.0)	30 (21.7)*
>1	5 (1.1)	9 (3.5)*	15 (10.9)*†
AMI among FDR			
No	304 (64.7)	128 (50.4)	79 (57.2)
1	144 (30.6)	78 (30.7)	39 (28.2)
>1	22 (4.7)	48 (18.9)*	20 (14.5)*

The figures are number of subjects (%). Probability values refer to χ^2 analyses between the groups.

AMI = acute myocardial infarction, FDR = first-degree relatives, SCD = sudden cardiac death.

* $p<0.001$ compared to control, † $p<0.01$ compared to AMI.

5.3 Comparison of risk profiles between survivors and victims of sudden cardiac death from an acute coronary event (III)

In comparison to AMI survivors the following risk factors were associated to the victims of SCD with an increased risk for SCD from an acute coronary event beside the family history of SCD: male gender (OR 1.8, 95% CI 1.3 to 2.4, $p<0.001$), current smoking (OR 2.0, 95% CI 1.5 to 2.6, $p<0.001$), cardiac hypertrophy (OR 3.0, 95% CI 2.3 to 3.9, $p<0.001$) and three-vessel CAD (OR 5.4, 95% CI 3.6 to 8.2, $p<0.001$). There was a cumulative increase in the risk of being a SCD victim vs. AMI survivor when more than one risk factor was present, the odds ratio being 44.3 (95% CI from 8.0 to 246.7, $p<0.001$) in a current male smoker with a family history of SCD and cardiac hypertrophy (Figure 4).

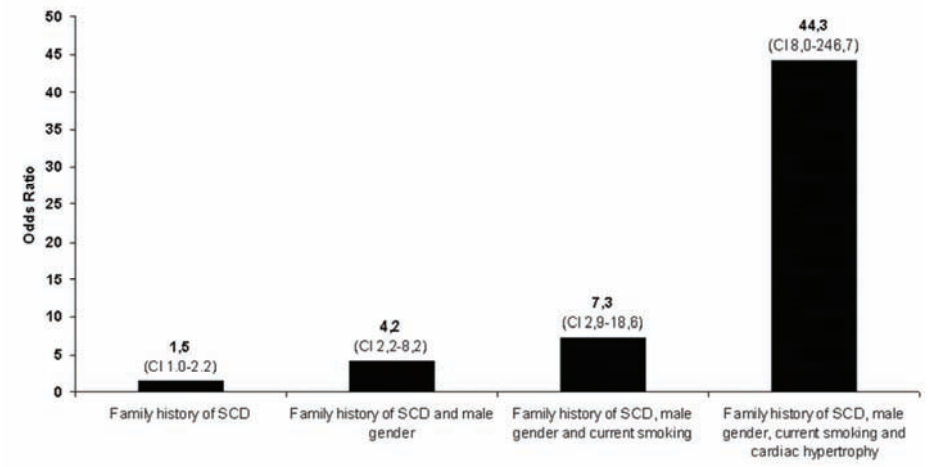

Fig. 4. Odds ratios for the combinations of risk factors showing the risk of sudden cardiac death from an acute coronary event. SCD = sudden cardiac death. CI = 95% confidence interval.

Of the other coronary risk factors, a history of hypercholesterolemia was less common in victims of SCD than in survivors of AMI (OR 0.18, 95% CI 0.14–0.24, p<0.001). Similarly, a history of diabetes (OR 0.71, 95% CI 0.52–0.97, p=0.03) and previous myocardial infarction (OR 0.61, 95% CI 0.44–0.86, p=0.04) tended to be less common among the SCD victims. No differences in age, BMI, family history of AMI, history of hypertension or angina pectoris, were observed. At autopsy, no significant differences in the location of the stenosis were observed in subjects with one-vessel CAD. In the comparison of victims of SCD to AMI subjects, it was noted that the affected artery was the left main artery in 4% vs. 1% of cases (p=0.31), the left anterior descending in 57% vs. 47% of cases (p=0.11), the left circumflex in 8% vs. 16% of cases (p=0.13), and the right coronary artery in 31% vs. 37% of cases (p=0.54).

Table 5 shows the sensitivity, specificity and predictive accuracy of each significant variable and the combination of the variables in differentiating the patients who had died suddenly from those who had survived an acute coronary event. The formulation of the risk combinations was based on the clinical practice beginning with the patient history, followed by the factors requiring non-invasive and invasive testing, respectively.

Table 5. Sensitivity, specificity and predictive accuracy of the risk factors.

Variables	Sensitivity	Specificity	PPV	NPV
Family history of SCD	32 (26–38)	76 (72–81)	50 (42–58)	60 (56–65)
Male gender	84 (80–87)	25 (22–29)	43 (39–46)	70 (66–76)
Current smoking	46 (41–52)	70 (66–73)	42 (36–47)	74 (70–77)
Cardiac hypertrophy	70 (66–75)	56 (52–60)	52 (48–56)	73 (69–77)
Three-vessel CAD	47 (43–52)	86 (81–90)	86 (82–91)	46 (42–51)
Combination 1.	80 (71–89)	51 (42–61)	55 (46–64)	77 (68–87)
Combination 2.	72 (68–87)	74 (62–85)	63 (49–78)	81 (70–92)
Combination 3.	88 (74–100)	86 (72–100)	88 (74–100)	86 (72–100)
Combination 4.	100 (95–100)	100 (95–100)	100 (95–100)	100 (95–100)

PPV = positive predictive value, NPV = negative predictive value, SCD = sudden cardiac death, CAD = coronary artery disease.

Values are expressed as % (95% confidence interval).

Combination 1. = Family history of SCD and male gender

Combination 2. = Family history of SCD, male gender and current smoking

Combination 3. = Family history of SCD, male gender, current smoking and cardiac hypertrophy

Combination 4. = Family history of SCD, male gender, current smoking, cardiac hypertrophy and three-vessel CAD

Combination 1, 2, 3, and 4 represents 26% (n=64/246), 11% (n=26/230), 9% (n=21/229) and 7% (n=15/228) of the SCD population for whom there was complete information about each variable in the combination, respectively

5.4 Comparison of coronary risk factors and autopsy findings of subjects with and without a family history of sudden cardiac death (IV)

Several factors through which a family history of SCD may modify the risk for SCD due to an acute ischemic event were evaluated in a subgroup of the FinGesture-population with the complete family history of SCD (n=246). The presence of three-vessel CAD was more common among the victims of SCD with a family history of SCD (58.2%) compared to those without any such history (41%) (OR 2.0, 95% CI 1.2 to 3.5, p=0.014) (Table 6). In logistic regression analysis, including age, gender, family history of SCD, and the history of hypertension, hypercholesterolemia, diabetes and current smoking in the analysis, positive family history of SCD remained as an independent predictor of the presence of three vessel disease at autopsy (OR 2.0, 95% CI 1.1 to 3.7, p=0.016) in addition to age (OR 1.0, 95% CI 1.0–1.1, p=0.01).

No statistically significant differences were observed in demographic variables and traditional coronary risk factors between subjects with and without a family history of SCD. However, the frequency of females, in whom the risk for CAD and SCD is generally low, tended to be higher among those subjects with a family history of SCD (19%) than those without (9%, p=0.06). A statistically non-significant trend toward a lower incidence of coronary risk factors including hypercholesterolemia, diabetes and current smoking, was also observed in victims with a family history of SCD compared to those without such a history.

A family history of SCD had no effects on autopsy verified THW or culprit lesion morphology responsible for an acute coronary event leading to SCD. (Table 6).

Table 6. Family history of sudden cardiac death and the severity of coronary artery disease.

Variables	Family history - N = 167	Family history + N = 79	P
Demographic variables and coronary risk factors			
Age (years)	63.3 (±11.1)	65.0 (±9.7)	0.25
Sex (male)	151/167 (90.4)	64/79 (81.0)	0.06
BMI (kg/m^2	27.4 (±4.1)	26.8 (±4.2)	0.31
History of			
Hypertension	88/166 (53.0)	46/78 (59.0)	0.41
Hypercholesterolemia	60/166 (36.1)	26/79 (32.9)	0.67
Diabetes type I or II	30/167 (18.0)	10/79 (12.7)	0.36
Smoking			0.39
Never	47/156 (30.1)	29/74 (39.2)	
Ex	37/156 (23.7)	15/74 (20.3)	
Current	72/156 (46.2)	30/74 (40.5)	
Angina pectoris	89/166 (53.6)	45/77 (58.4)	0.49
Previous myocardial infarction	28/167 (16.8)	10/79 (12.7)	0.46

Variables	Family history - N = 167	Family history + N = 79	P
Autopsy findings			
Total heart weight	489 (±93.4)	474 (±100.7)	0.26
Critical stenosis (>75%)			0.04
No critical stenosis	23/166 (13.9)	4/79 (5.1)	
One coronary artery	34/166 (20.5)	11/79 (13.9)	
Two coronary arteries	41/166 (24.7)	18/79 (22.8)	
Three coronary arteries	68/166 (41.0)	46/79 (58.2)	
Plaque complication			0.50
No acute lesion	7/167 (4.2)	1/79 (1.3)	
Stable plaque (>75%)	79/167 (47.3)	35/79 (44.3)	
Intraplaque haemorrhage	39/167 (23.4)	23/79 (29.1)	
Thrombus	25/167 (15.0)	13/79 (16.5)	
Plaque erosion	15/167 (9.0)	5/79 (6.3)	
With thrombus	9/15 (60.0)	4/5 (80.0)	
Plaque rupture	27/167 (16.2)	15/79 (19.0)	
With thrombus	16/27 (59.3)	9/15 (60.0)	
Acute infarction	119/164 (72.6)	48/77 (62.3)	0.13
Healed infarction	92/165 (55.8)	47/79 (59.5)	0.68

BMI, body mass index; CAD, coronary artery disease; SCD, sudden cardiac death.

Values are expressed as mean (±SD) or number of subjects (%). Probability values refer to two- sided *t*- test and χ^2 analyses comparing subjects with and without a family history of SCD

5.5 Genetic polymorphisms associated with thrombosis and the risk for sudden cardiac death from an acute coronary event (I,A)

Allele and genotype frequencies of ten previously described polymorphisms (Factor V R506Q, Factor VII R353Q, Factor XII C46T, FXIII V34L, Glycoprotein (Gp) Ia C807T, GPIbalpha T[-5]C, GP IIIa $P1^{A1/A2}$, GpVI T13254C, Fibrinogen –455G/A and Annexin A5) with possible effects on AMI and SCD risk were determined in each study group. With respect to the genetic studies only males were included due to the small number of women among the consecutive series of AMI survivors and SCD victims. As shown in table 7, none of the studied polymorphisms displayed any significant association with an increased risk of SCD when victims of SCD were compared to the AMI survivors or to the random sample of controls from the same geographical area in Northern Finland sharing a homogenous genetic background. The distribution of the genotypes was in Hardy-Weinberg equilibrium.

The role of polymorphisms affecting thrombosis as risk factors for acute coronary event in general was determined by comparing controls to the combined AMI and SCD groups. A borderline effect was seen for FXII (C46T) (Odds ratio (OR) 1.35; 95% confidence interval (CI) 0.97–1.88, p=0.08) and Fibrinogen – 455G/A polymorphisms (OR 1.36; 95% CI 0.96–1.91, p=0.09). However, the difference in genotype distribution was not statistically significant and no association with acute coronary event and genotype with any of the other studied polymorphisms was observed.

Table 7. Genotype and allele frequencies of the atherothrombotic polymorphisms.

Gene	Genotype / allele	Controls	AMI survivors	Victims of SCD
		n = 197	n = 349	n = 219
FV-Leiden	WT	190 (96.4)	333 (95.4)	202 (92.2)
	HE	6 (3.0)	16 (4.6)	16 (7.3)
	HO	1 (0.5)	0 (0.0)	1 (0.5)
	1	386 (98.0)	682 (97.7)	420 (95.9)
	2	8 (2.0)	16 (2.3)	18 (4.1)
FVII (R353Q)	WT	171 (86.8)	306 (87.7)	189 (86.3)
	HE	26 (13.2)	42 12.0)	27 (12.3)
	HO	0 (0.0)	1 (0.3)	3 (1.4)
	1	368 (93.4)	654 (93.7)	405 (92.5)
	2	26 (6.6)	44 (6.3)	33 (7.5)
FXII (C46T)	WT	119 (60.4)	178 (51.0)	123 (56.2)
	HE	68 (34.5)	151 (43.3)	84 (38.4)
	HO	10 (5.1)	20 (5.7)	12 (5.5)
	1	306 (77.7)	507 (72.6)	330 (75.3)
	2	88 (22.3)	191 (27.4)	108 (24.7)
FXIII (V34L)	WT	131 (66.5)	244 (69.9)	155 (70.8)
	HE	58 (29.4)	97 (27.8)	56 (25.6)
	HO	8 (4.1)	8 (2.3)	8 (3.7)
	1	320 (81.2)	585 (83.8)	366 (83.6)
	2	74 (18.8)	113 (16.2)	72 (16.4)
GpIa (C807T)	WT	74 (37.6)	118 (33.8)	75 (34.2)
	HE	95 (48.2)	166 (47.6)	99 (45.2)
	HO	28 (14.2)	65 (18.6)	45 (20.5)
	1	243 (61.7)	402 (57.6)	249 (56.8)
	2	151 (38.3)	296 (42.4)	189 (43.2)

Gene	Genotype / allele	Controls n = 197	AMI survivors n = 349	Victims of SCD n = 219
GPIb T[-5]C	WT	168 (85.3)	290 (83.1)	188 (85.8)
	HE	27 (13.7)	56 16.0)	29 (13.2)
	HO	2 (1.0)	3 (0.9)	2 (0.9)
	1	363 (92.1)	636 (91.1)	405 (92.5)
	2	31 (7.9)	62 (8.9)	33 (7.5)
GP IIIa $P1^{A1/A2}$	WT	158 (80.2)	264 (75.6)	157 (71.7)
	HE	35 (17.8)	79 (22.6)	55 (25.1)
	HO	4 (2.0)	6 (1.7)	7 (3.2)
	1	351 (89.1)	607 (87.0)	369 (84.2)
	2	43 (10.9)	91 (13.0)	69 (15.8)
GpVI (T13254C)	WT	154 (78.2)	274 (78.5)	170 (77.6)
	HE	40 (20.3)	71 (20.3)	47 (21.5)
	HO	3 (1.5)	4 (1.1)	2 (0.9)
	1	348 (88.3)	619 (88.7)	387 (88.4)
	2	46 (11.7)	79 (11.3)	51 (11.6)
Fibrinogen –455G/A	WT	135 (68.5)	215 (61.6)	135 (61.6)
	HE	59 (29.9)	118 (33.8)	74 (33.8)
	HO	3 (1.5)	16 (4.6)	10 (4.6)
	1	329 (83.5)	548 (78.5)	344 (78.5)
	2	65 (16.5)	150 (21.5)	94 (21.5)
Annexin A5 –1C/T	WT	202/243 (83.1)	169/212 (79.7)	82/98 (83.7)
	HE	37/243 (15.2)	43/212 (20.3)	16/98 (16.3)
	HO	4/243 (1.6)	0/212 (0.0)	0/98 (0.0)
	1	441 (90.7)	381 (89.9)	180 (91.8)
	2	45 (9.3)	43 (10.1)	16 (8.2)

Values are number of subjects and alleles (%). For Annexin A5 different n values were used.

No statistically significant differences in genotype or allele frequencies were observed between the study groups.

AMI = acute myocardial infarction, SCD = sudden cardiac death, WT = wild-type, HE = heterozygous for polymorphism, HO = homozygous for polymorphism.

Since the genetic predisposition for acute coronary events may be enhanced in younger subjects and in subjects with a positive family history, we further analysed the results based on the presence of family history of SCD and AMI and separately in males younger than 50 years old. However, when subjects with a family history of SCD were compared to subjects without such a family history, no statistical differences in genotype or allele frequencies were observed between the groups in any of the studied polymorphisms (p-values between 0.18 and 1.00). The results were similar with respect to the family history of AMI (p-values

between 0.13 and 1.00) and did not change also when the analysis was performed only in males less than 50 years of age (p-values 0.09 and 0.88). Due to the assumption that current smoking could intensify the possible prothrombotic effects of these polymorphisms, the results were evaluated separately for current smokers and non-smokers. However, no differences in genotype distribution were observed either in current smokers or non-smokers in the comparison of controls to combined AMI+SCD group (p-values between 0.09 and 1.00) or to SCD group separately (p-values between 0.11 and 1.00).

A subgroup analysis was conducted by dividing the victims of SCD into three groups based on the observed culprit lesions in autopsy: 1) stable plaque (n=102), 2) intraplaque haemorrhage (n=65), and 3) plaque rupture or erosion (n=47) but again no statistical differences were observed in the genotype and allele frequencies of the studied polymorphisms. A fresh intraluminal thrombus was observed in 27 victims of SCD in association with plaque rupture or erosion. No associations with the specific genotype of the studied polymorphisms and the presence of fresh thrombus in autopsy were observed (p-values between 0.14 and 1.00).

6 Discussion

To the best of our knowledge, this is the largest reported case-control study comparing the risk profiles between those individuals who die suddenly and those who survive during an acute coronary event. The challenge in the overall global problem of SCD is to identify the subjects at increased risk of SCD in the general population. The present results show that the risk of SCD at the time of an acute coronary event can be assessed by generally available methods.

6.1 FinGesture-population

The strength of the Finnish Genetic Study for Arrhythmic Events (FinGesture) is the large, consecutive, autopsy verified, population of out-of-hospital SCD victims from an acute coronary event. Since a medico-legal autopsy is mandatory in Finland for victims of sudden death, this study should include virtually all SCD victims autopsied in the department of forensic medicine of the Oulu University since the beginning of the trial.

In cases of sudden unexpected death, the medico-legal autopsy data, including the information on the circumstances of death, is the best guarantee of reliable cause-of-death diagnosis (Lahti *et al.* 1998). Many previous studies into the conventional and genetic risk factors of SCD in other western societies may have suffered significant selection bias when SCD has been verified only by death certificates, without a detailed clinical history and autopsy data (Jouven *et al.* 1999, de Vreede-Swagemakers *et al.* 1997, Pratt *et al.* 1996, Friedlander *et al.* 1998, Fox *et al.* 2005). For example, in the Framingham Heart Study the number of out-of-hospital SCD determined by death certificates, was shown to overestimate the rate of SCD by 47%, while comparing the incidence adjudicated by a physician panel (Fox *et al.* 2005).

In the FinGesture-study, one out of every three (34%) sudden death defined by clinical criteria could not be attributed to an acute ischemic event on the basis of the autopsy results. This highlights the importance of comprehensive autopsy in the studies of SCD. The proportion of the excluded cases in the present study was similar to that reported in the study of Pratt *et al.* (1996) in which autopsy contradicted the diagnosis of SCD based on the clinical criteria in 29% of subjects.

The complete autopsy that was performed on each SCD victim enables the exclusion of non-cardiac causes of sudden death and the use of 24-h inclusion criteria for non-witnessed out-of-hospital sudden death cases with reliable

restriction to the victims of SCD with presumable arrhythmic SCD triggered by an acute coronary event. A similar definition of SCD as death occurring within 6 hours of the onset symptoms (witnessed SCD) or within 24 hours of the time that the victim was last seen alive in normal state of health has been used in the other large autopsy series of SCD making the data of the present study comparable with these reports (Taylor *et al.* 2000). The 24-h definition has also been used in a population based study in the Maastricht area evaluating the true incidence of SCD including also the unwitnessed cases of SCD that would have been excluded by using the more strict 1-h criteria (de Vreede-Swagemakers *et al.* 1997).

For the genetic studies, in which population stratification is essential, FinGesture-population is ideal, as it does not suffer from the biases present in genetically mixed populations. The subjects in FinGesture-study represent the general population of a distinct geographical area in Northern Finland that has remained isolated for a long time and thus shares a homogenous genetic background in comparison with that of most Western societies (Peltonen *et al.* 1999).

Characteristics of the FinGesture-population

Demographic findings of the FinGesture-population were in good accordance with the previous reports about SCD. The circadian variation in the occurrence of SCD was confirmed with the highest incidence of SCD being around noon (Muller *et al.* 1987, Willich *et al.* 1987, Willich *et al.* 1993). The mean age of SCD victims was 63 years in men and 72 years in women as in the earlier reports (Zipes & Wellens 1998). In the present study, over four out of every five SCD victims was male. The changes in the incidence of SCD with age and sex differences have thought to reflect the epidemiology of CAD (Zipes & Wellens 1998). In accordance with the results from the Maastricht area, most out-of-hospital SCDs occurred at home and were unwitnessed (de Vreede-Swagemakers *et al.* 1997). Resuscitation had been attempted in about the half of the cases evidence of a rather short time interval between the cardiac arrest and provision of cardiovascular care.

Autopsy findings

Davies *et al.* (1992) have reported the incidence of severe one-vessel disease as being 10%, two-vessel-disease at 3%, with three-vessel disease being 1% in men

suffering either traumatic or natural noncoronary death. In the present study, severe coronary stenosis was much more common finding in autopsy, with a critical stenosis being present in at least one major coronary artery in about 90% of SCD victims, in agreement with previous studies (Farb *et al.* 1995, Virmani *et al.* 2001, Perper *et al.* 1975, Warnes & Roberts 1984, Thomas *et al.* 1988).

In the study of Taylor *et al.* (2000) the culprit plaque was defined as the plaque with an acute thrombus, or, in the absence of an acute thrombus, the arterial segment with the greatest narrowing. In the present study, a deep haemorrhage into the plaque without any plaque disruption was considered to be the culprit lesion in the absence of simultaneous plaque rupture or an intraluminal thrombus, in addition to the criteria provided by Taylor *et al.* (2000). We found the presence of intraplaque haemorrhage to be significantly higher (26–32% of subjects) than in the previous studies, which have reported an incidence of about 7% for deep plaque haemorrhage (Farb *et al.* 1995, Davies *et al.* 1989). We consider the sudden rush of blood into plaque with the subsequent intense vasoconstriction as a likely cause of an acute ischemia triggering fatal arrhythmia in the heart without protective preconditioning (Kloner & Jennings 2001a, Kloner & Jennings 2001b, Pasceri *et al.* 1996).

The presence of healed infarction in autopsy (about 60%) was similar as previously reported (Warnes & Roberts 1984, Scott & Briggs 1972, Davies & Thomas 1984). In particular, the prevalence was much higher than expected from the patient history, emphasizing the important role of "silent" infarctions in SCD (Kannel & Abbott 1986, Kannel 1986). The incidence of an acute infarct was relatively high (>60%) compared to the corresponding value in the other out-of-hospital SCD series where acute infarct has been present in less than 25% of cases (Farb *et al.* 1995, Virmani *et al.* 2001, Warnes & Roberts 1984, Scott & Briggs 1972). The difference is possibly explained by the differences in study populations and in the definition of SCD.

In a postmortem study on Finnish cases of death from external causes without significant heart diseases, the mean heart weight did not exceed 400g in any age group of either sex (Lehti 1971). In the present study, the mean THW was 497g for men and 403g for women strengthening the association between cardiomegaly and vulnerability towards cardiac arrhythmias and SCD observed in previous studies (Warnes & Roberts 1984, Scott & Briggs 1972, Davies & Thomas 1984). Similarly to the results of Burke *et al.*, increased heart weight was associated with healed infarct in autopsy and the severity of CAD in normotensive victims of SCD (Burke *et al.* 1996).

While evaluating the autopsy findings in victims of sudden death some limitations have to be recognized. Even though the exclusion of non-cardiac causes of death in autopsy justify the SCD diagnosis, especially when the information on the circumstances of death and evidence of a coronary complication support the diagnosis, nothing can be said about the exact arrhythmia mechanism causing the sudden death. The assumption of arrhythmic death in these cases is based on the evidence obtained from the epidemiologic studies that have established VT and VF as typical sequences of electrical events leading to SCD (Huikuri *et al.* 2001, Hinkle & Thaler 1982). In addition to VT and VF, bradyarrhythmias and electromechanical dissociation (EMD) may cause the SCD. Also acute pump failure as a cause of sudden death cannot be definitely ruled out even when the extensive autopsy findings are used.

6.2 Family history of sudden cardiac death (II, IV)

The main finding of the present study was that a family history of SCD increases the risk of experiencing a cardiac arrest during an acute coronary event. The risk of SCD appears to be high if two or more FDR have succumbed to SCD. On the basis of the present study, obtaining a detailed family history with regard to SCD may be one way to identify subjects prone to ischemic SCD. In these individuals, special attention should be paid to the prevention of their first acute coronary event. These results may be of major assistance in reducing the incidence of SCD in the general population and especially in individuals with non-specific and intermediate risk profiles, who together account for the largest absolute number of SCD events (Huikuri *et al.* 2001).

The present study confirms the previous observations of the importance of family history as a risk factor for SCD and primary VF during an acute coronary event (Jouven *et al.* 1999, Friedlander *et al.* 1998, Dekker *et al.* 2006). In contrast to the previous studies, we performed a comprehensive autopsy for each subject ensuring that we examined only victims of SCD with an ischemic background to the event. In agreement with the results of Dekker *et al.* who studied subjects with the first ST-elevation myocardial infarct our findings suggest that fatal arrhythmia during an acute coronary event is not just an incidental phenomenon but is partly determined by the predisposing genetic factors. Dekker *et al.* concluded that at present, family history, which acts as an integrator of complex interactions of multiple genes and environmental factors, is the key predictor of SCD in AMI patients. (Dekker *et al.* 2006)

The results from this present and the previous studies indicate that a familial risk of SCD which is distinct from the familial risk of myocardial infarction. Jouven *et al.* (1999) hypothesized that the expression and mechanism of CAD may be under partial familial control, which may evoke SCD rather than AMI in some subjects. In study IV, victims of SCD with a family history of SCD exhibited more commonly a three-vessel CAD at autopsy even though they displayed a similar or a trend toward a lower prevalence of conventional coronary risk factors when compared to those SCD victims who had no family history of SCD. The present results suggest that it is those factors, which accelerate the progression of CAD independently from previously known common coronary risk factors that partly explain the clustering of SCD into certain families. Genetic factors are the most probable candidates in this respect, although some still unrecognized environmental factors might also play a role. A family history of SCD had no effects on autopsy verified THW or culprit lesion morphology responsible for an acute coronary event leading to SCD.

Since the family history of SCD differentiated victims of SCD from the AMI survivors but also AMI survivors from the controls, it is presumable that in addition to the possible genetic factors accelerating the progression of CAD there are also other factors having direct effects on electrogenesis and neural regulation making the heart vulnerable to ischemia-induced arrhythmias (Spooner *et al.* 2001).

One possible bias in assessing the family history of SCD is the possibility that the relatives of SCD victims may report selectively more SCD (true or supposed) among their FDRs than the controls. We attempted to control for this recall bias by asking for a short description of the reported event to confirm that the death was truly sudden according to our criteria. In addition, death certificates from the Central Statistical Office in Finland were used to confirm the SCD of the relatives. In these cases, the majority of the victims also had undergone a medicolegal autopsy.

A relatively large proportion of the initial study populations had to be excluded by the end-point committee because of missing or inaccurate information about the exact mode of death. These strict criteria were used deliberately to avoid selection bias which has affected many previous studies, in which SCD was verified only by death certificates, without a detailed clinical history and autopsy data (de Vreede-Swagemakers *et al.* 1997, Fox *et al.* 2005). Furthermore, the proportion of accepted cases with accurate definition of the

mode of death did not differ between the relatives of SCD victims and relatives of AMI survivors (II).

6.3 Coronary risk factors, cardiac hypertrophy and the severity of coronary artery disease (III)

If one strives to target preventive therapy to those subjects prone to fatal arrhythmias, in order to prevent sudden deaths from an acute coronary event in a general population, then one would need a better identification of these subjects from those who will survive this traumatic event. Several risk factors for SCD have been recognized in the previous studies (Escobedo & Zack 1996, Wannamethee *et al.* 1995, Jouven *et al.* 1999, Schatzkin *et al.* 1984, Cuddy & Tate 2006). However, these risk factors mainly parallel those for a non-fatal coronary event and thus the prevention of SCD evoked by an acute coronary event has remained a challenge for clinicians. Currently used methods in general population are mainly predictors of the evolution of an underlying CAD and total mortality whereas their sensitivity to estimate the risk for sudden arrhythmic death is very low (Myerburg 2001, De Backer *et al.* 2003). In most of the previous studies victims of SCD have been compared to non-sudden coronary disease death and only a few risk factors have shown an independent association with SCD. Only Wannamethee *et al.* (1995) included cases of nonfatal AMI and examined the specific or particular association between elevated heart rate, heavy drinking and arrhythmia on the risk of SCD.

To the best of our knowledge, there have been no carefully designed case-controlled studies, including autopsy verified victims of SCD, that have evaluated risk profiles, severity of CAD and cardiac hypertrophy comparing victims of SCD with survivors of an acute coronary event. In the present study, in addition to the family history of SCD, male gender, current smoking, cardiac hypertrophy and three-vessel CAD represented the most significant differences between the SCD victims and survivors of an acute coronary event. A combination of various risk factors yielded high odds ratio and a high positive predictive value in differentiating the SCD victims from AMI survivors. In this Northern Finnish population, the presence of all these risk factors indicated 100% mortality from an acute coronary event.

While interpreting these results, some limitations need to be addressed. Coronary angiography was performed only in one out of every three patients with AMI within a 3-month time frame, making the comparison of the severity of

CAD less reliable. Obviously, symptomatic patients with evidence of active ischemia underwent coronary angiography more often because of their clinical symptoms. Therefore, the present results may in fact underestimate the importance of the severity of underlying CAD as a risk factor of SCD. Due to the low frequency of coronary angiograms (35%) among the AMI survivors the finding has to be considered as a preliminary result for the further studies. Cardiac hypertrophy was defined in different ways between the study groups. However, a previous study has confirmed the relatively good concordance of diagnosing cardiac hypertrophy from autopsy and echocardiography, respectively (Devereux *et al.* 1986). Therefore, it is unlikely that the higher prevalence of cardiac hypertrophy among the victims of SCD is simply due to these methodological aspects.

In the present study many conventional coronary risk factors (age, BMI, hypertension, angina pectoris, family history of AMI) had no effect to the risk of SCD or were actually less common in victims of SCD than the survivors of AMI (hypercholesterolemia, diabetes). It is presumable that even though these factors increase the risk for CAD they have no direct effects on arrhythmogenesis. Since the detection of the conventional risk factors in the present study was based on patient history, it is possible that in some subjects the presence of hypertension, hypercholesterolemia or diabetes had not been diagnosed. This interpretation is supported by the high prevalence (~30%) of unrecognised impaired glucose tolerance and diabetes in patients with AMI described in several previous studies (Norhammar *et al.* 2002, Hu *et al.* 2006, Andersen *et al.* 2006). A similar unawareness may explain the strikingly lower prevalence of hypercholesterolemia in victims of SCD compared to AMI survivors in the present study. However, in the Nurses' Health Study a reported history of hypercholesterolemia was not a significant predictor of SCD and in another study including patients who developed VF during their first ST-elevation myocardial infarction, a lower prevalence of hypercholesterolemia was reported than in those who did not suffer such arrhythmia (Albert *et al.* 2003, Dekker *et al.* 2006). The authors of the Nurses' Health Study considered that undiagnosed hypercholesterolemia is an unlikely explanation for this finding in a cohort of health professionals (Albert *et al.* 2003). To minimize this bias caused by inaccuracy and missing data about the previous medical history, we used a standardized and comprehensive questionnaire to collate all possible existing information regarding the patient's history.

The present results show that the risk of SCD at the time of an acute coronary event can be assessed by generally available methods in a general population. Information about the family history of SCD, sex, and smoking status are easily gathered during a routine clinical examination. The ECG that is generally available in the most clinical practices can be used with some limitations to estimate presence of cardiac hypertrophy. In previous studies, ECG-LVH has been associated with increased risk of SCD and ventricular arrhythmias (Szlachcic *et al.* 1992, Ghali *et al.* 1991, Coste *et al.* 1988). ECG may also reveal a "silent" infarct scar in the myocardium , a common finding in SCD victims and one associated with an increased risk for SCD (Warnes & Roberts 1984, Scott & Briggs 1972, Davies & Thomas 1984, Kannel & Abbott 1986, Kannel 1986).

Echocardiography and coronary angiography should perhaps be performed with more liberal indications in these high-risk subjects to diagnose cardiac hypertrophy and severe CAD, in attempts to target more actively therapy to achieve a regression of hypertrophy and adequate treatment of CAD.

However, the case-control nature of FinGesture-study limits the generalization of these results. Future prospective studies are needed to evaluate whether this strategy may help us to reduce incidence of SCD in the general population. Work to establish novel risk factors for SCD continues, for even though the accumulation of the risk factors presented in the thesis enables us to identify new high risk subgroups in the general population, the majority of SCD victims still cannot be distinguished from AMI survivors with currently known methods.

6.4 Genetic polymorphisms associated with thrombosis (I, A)

The clustering of SCD in certain families points to a genetic background of SCD from an acute coronary event. In the present study, an association between the family history of SCD and the severity of CAD was observed. Thrombosis has been suggested to be involved both in the development of atherosclerotic plaques and in the consequences of plaque complications leading to AMI and SCD and this has placed the genes affecting thrombosis as special candidates in assessments of the genetic risk for AMI and SCD from an acute coronary event (Davies 1996a, Davies 1996b).

In the present study, the role of polymorphisms that have been associated with the risk of CAD and AMI in the previous studies was evaluated in our setting where we had a large number of autopsy verified victims of SCD. The study

should thus minimize the survival bias, which has been present in most of the previous studies. The subjects for this case-control study were collected from the same geographical area and share the homogenous genetic background. The autopsy findings from the victims of SCD also enabled us to evaluate association between the polymorphisms and the different culprit lesion morphologies even though the number of subjects in subgroups was rather small.

In this population, none of the studied polymorphisms was associated with an increased risk of SCD or acute coronary event in general. No significant differences in allele or genotype frequencies were observed either in a subgroup of men less than 50 years of age or in subjects with a family history of SCD or AMI in whom the genetic risk for SCD would be anticipated to be more pronounced. No significant differences in genotype frequencies were detected when smokers and non-smokers were analysed separately. In addition, none of the polymorphisms showed any association with the more restricted phenotype defined as autopsy verified culprit lesion morphology, or the presence of an acute intraluminal thrombus.

The present study elucidates several problems encountered in simple association studies of gene-disease interactions of complex diseases such as SCD from an acute coronary event (Sing *et al.* 1992, Colhoun *et al.* 2003). It is notable that even though the familial aggregation of SCD has been confirmed in this Finnish population pointing to a genetic background for SCD from an acute coronary event, the influence of genetic variation to the SCD risk is likely to be mediated through several pathways and also to involve interactions with environmental factors. In addition to atherothrombosis, it is claimed that genetic variation in the genes affecting electrogenesis and neural regulation partly determine the observed clinical outcome (Spooner *et al.* 2001). Simple association studies are based on the assumption that common variants (i.e. minor allele frequency >5%) account for much of the susceptibility towards a certain disease, since they ignore etiological heterogeneity of complex diseases as well as gene-gene and gene-environment interactions (Kullo & Ding 2007). However, the minor gene effects reported in some studies of genetic risk factors for arterial thrombosis have not always been responsible in other studies and thus they have not really helped in overall risk stratification for CAD and its serious manifestations such AMI and SCD (Simmonds *et al.* 2001).

While studying the haemostatic factors, we have to keep in mind that in order to be clinically relevant, the studied polymorphism has to have an effect either on circulating protein levels (or the density of expression in the case of receptors) or

on the function of a protein and furthermore that the protein levels or function have to be associated with an increased risk of a particular clinical outcome (Lane & Grant 2000). Recently, Naran et al. observed a positive association between platelet GpIIIa PlA1/A2 polymorphism and platelet function. These workers concluded that the GpIIIa PlA2 allele may be a genetic factor that contributes to the risk of sudden death from myocardial infarction (Naran *et al.* 2008). They justified this conclusion by citing the earlier association between the platelet hyperreactivity and increased platelet aggregation with the risk for recurrent myocardial infarction (Trip *et al.* 1990). Even though there is a logical continuation from the genotype to the measurable phenotype (platelet function) and thus from the phenotype to the disease (AMI), the estimation of the impact of the genotype and the disease (CAD / AMI) have been contradictory in previous publications (Weiss *et al.* 1996, Aleksic *et al.* 2000, Ridker *et al.* 1997, Carter *et al.* 1997, Herrmann *et al.* 1997) and in the present study no association between the platelet GpIIIa PlA1/A2 polymorphism and AMI or SCD was observed. As concluded by Simmonds et al., if platelet GpIIIa PlA1/A2 polymorphism is associated with any increased risk of ischemic heart disease, the risk appears to be very minor and may arise only in some poorly characterized circumstances (Simmonds *et al.* 2001).

In many cases, positive correlations between genotype and disease phenotype have not been established, even though the appropriate laboratory parameters correlate consistently with disease and polymorphisms in certain genes correlate with plasma levels of their respective proteins. In their review, Lane and Grant discussed this issue using fibrinogen as an example (Lane & Grant 2000). Atherothrombotic disorders have been associated with an elevated level of circulating fibrinogen, of which about 50% is explained by genetic variance. Since several polymorphisms can affect the plasma level of fibrinogen, the estimation of the contribution of an individual polymorphism to the variance noted in the plasma level is only approximately 5%. From this point of view, it is not surprising that simple association studies have given contradictory results with respect to polymorphisms and the risk of CAD and its manifestations such as AMI and SCD. If these were to be observed in the general population, such small variations in genetic risk would require enormous sample sizes that are difficult to achieve at reasonable cost. Based on the results from the present study, group sizes close to 1000 subjects per group would have been required in order to reject the null hypothesis with the observed effect size of fibrinogen –455G/A polymorphism.

It is notable that the results from the present study contradict the previous, mainly positive, associations between the studied polymorphisms and fatal myocardial infarction from the Helsinki Sudden Death Study (HSDS) including Finnish men suffering out-of-hospital death (Ollikainen *et al.* 2004, Mikkelsson & Karhunen 2002, Mikkelsson *et al.* 2000, Mikkelsson *et al.* 1999, Mikkelsson *et al.* 2002, Mikkelsson *et al.* 2001, Mikkelsson *et al.* 2000). Even though the subjects in both FinGesture and HSDS studies are representatives of the homogenous Finnish population, we were not able to replicate the previous results. One explanation for this could be false positive results obtained by chance in the initial positive studies or the inadequate sample size in the present study to detect an association with a low impact on SCD phenotype. For example, in the study of Mikkelsson et al., platelet GpIIIa PlA1/A2 polymorphism was not directly associated with AMI but the prevalence of the $P1^{A2}$ allele was significantly higher in men with AMI caused by a coronary thrombosis than in men with AMI without thrombosis (Mikkelsson *et al.* 1999). In the present study, no such association was observed. In both studies, the association between polymorphism and thrombosis was performed in a patient subgroup with a low number of subjects. It is possible that the association between platelet GpIIIa PlA1/A2 polymorphism and thrombosis is a true positive and needs to be confirmed in a larger study including subjects with a more specific phenotype. On the other hand, many associations in patient subgroups represent false positive results simply due to multiple testing and chance (Colhoun *et al.* 2003). Publication bias i.e. the failure to publish possible negative results in other populations, may also falsely emphasize the importance of previously reported positive associations (Colhoun *et al.* 2003).

Due to the study setting, it was not feasible to evaluate the interactions between environmental risk factors and the studied polymorphisms as the awareness of the risk profile of the SCD victims prior to their death was based only on interview data. It is thus possible that the association between the studied polymorphisms and the AMI and SCD risk might be modified by certain other genetic or environmental factors, and some of these factors might vary between the Finnish and other populations, decreasing the importance of the association in the Finnish population. This problem can never be fully resolved in a case-control study of SCD. However, a case-control approach is the only effective way to include a sufficient number of SCD cases needed in a genetic study. The number of cases is always an important issue in association studies as a small study size can leave the possibility of chance as the explanation of any associations found. Even though our study population included all SCD victims occurring in the Oulu

University Hospital district during a 6-year span, the size of the population is still somewhat limited for genetic studies leading to a low value in the power calculations. However, one could argue that were these polymorphisms to have an important influence on the health of the general Finnish population, then they should have been recognized in the present study population.

The development of CAD and its consequences, such as stable angina pectoris, myocardial infarction, and SCD, is a complex and multifactorial process, and therefore there are several genes that may modify the risk of an ischemic event and its consequences (AMI or SCD) (Sing *et al*. 1992). Since the initiation of the present study, time has surpassed studies concerning one to few random variants for candidate genes and a qualitative phenotype. The recent developments in the field of genetic studies have lead to an era of genome-wide association analysis. Modern genotyping platforms offering more than one million SNPs make it possible to conduct a systematic search for inherited components of complex diseases. Several chromosomal loci have already been associated with the premature development of CAD (Samani *et al.* 2007, Helgadottir *et al.* 2007). In this respect, victims of SCD from an acute coronary event should be included in future genome-wide association analysis.

7 Conclusions

This study showed that risk for SCD from an acute coronary event in general population could be assessed by generally available clinical methods. The clustering of SCD in several families points to a genetic background that needs to be assessed in future genetic studies. The specific findings in the studies were as follows:

1. A family history of SCD increased the risk of experiencing a cardiac arrest during an acute coronary event. In particular, the risk of SCD appeared to be high if two or more first-degree relatives have suffered SCD. A family history of SCD differentiated victims of SCD from the survivors of an acute coronary event. The clustering of SCD in certain families points to a genetic background of an ischemic SCD (II).
2. In addition to a family history of SCD, male gender, current smoking, cardiac hypertrophy and three-vessel coronary disease were the specific risk markers for succumbing to SCD during an acute coronary event. Accumulation of these risk factors significantly increased the risk and in fact 100% mortality from an acute coronary event was observed when all these five risk factors were present (III).
3. When subjects with and without a family history of SCD were compared in this series of autopsy verified victims of SCD, family history associated with advanced coronary artery disease but not with a higher prevalence of common coronary risk factors. The finding suggests that genetic factors affecting the rapid progression of CAD may play an important role in familial SCD subsequent to an acute coronary event (IV).
4. Genetic polymorphisms associated with thrombosis did not increase the risk for sudden cardiac death from an acute coronary event when the victims of SCD were compared to the AMI survivors and to a random sample of controls (I, A).

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Original publications

I Kaikkonen KS, Kakko S, Kortelainen ML, Tapanainen JM, Savolainen MJ, Kesäniemi YA, Huikuri HV & Savolainen ER (2005). The –1C to T polymorphism in the Annexin A5 gene is not associated with the risk of acute myocardial infarction or sudden cardiac death in middle-aged Finnish males. Scand J Clin Lab Invest 65: 133–40.

II Kaikkonen KS, Kortelainen ML, Linna E & Huikuri HV (2006). Family history and the risk of sudden cardiac death as a manifestation of an acute coronary event. Circulation 114: 1462–7.

III Kaikkonen KS, Kortelainen ML & Huikuri HV (2008). Comparison of risk profiles between survivors and victims of sudden cardiac death from an acute coronary event. Ann Med 41: 120–7.

IV Kaikkonen KS, Kortelainen ML & Huikuri HV. Comparison of coronary risk factors and autopsy findings of subjects with and without a family history of sudden cardiac death caused by an acute coronary event. Manuscript.

Addendum to publication I

A Kaikkonen KS, Kortelainen ML, Kesäniemi YA, Huikuri HV. Polymorphisms of genes affecting thrombosis and risk of myocardial infarction and sudden cardiac death from an acute coronary event. Manuscript

Original publications are not included in the electronic version of the dissertation.

990. Saarenpää, Ismo (2008) Extracapsular hip fractures—aspects of intramedullary and extramedullary fixation

991. Rantavuori, Kari (2008) Aspects and determinants of children's dental fear

992. Kunnari, Anne (2008) Genetic, epidemiological and cell culture studies on human resistin

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994. Tuomisto, Anne (2008) The role of collagen XIII in B-cell lymphoma development, and characterization of its biosynthesis and tissue distribution

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