Eeva Hookana

CHARACTERISTICS OF VICTIMS OF NON-ISCHEMIC SUDDEN CARDIAC DEATH

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU, FACULTY OF MEDICINE,
INSTITUTE OF CLINICAL MEDICINE,
DEPARTMENT OF INTERNAL MEDICINE, DIVISION OF CARDIOLOGY;
DEPARTMENT OF DIAGNOSTICS, DEPARTMENT OF FORENSIC MEDICINE
EEVA HOOKANA

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Abstract

A non-ischemic etiology of sudden cardiac death (SCD), mostly due to various cardiomyopathies (CMP), accounts for about 20% of all SCDs. Most of the major studies of risk factors for SCD have focused on coronary artery disease (CAD). The aim of the present study was to clarify the characteristics of non-ischemic SCD in Northern Finland.

In this study, consecutive victims of SCD (n=2661) were prospectively collected, and among whom post-mortem examinations were performed between 1998 and 2007. Information about the SCD victims was obtained from a combination of available medical records, postmortem examination reports, medication used at the time of SCD, and standardized questionnaire filled out by the closest family members of the victims of SCD. We also screened the candidate genes from a Finnish family in which fatal arrhythmias was first manifestation of a cardiac disease. The collagen content of the myocardium from histological samples in victims of SCD due to idiopathic myocardial fibrosis (IMF) was also evaluated.

CAD was the most common cause of death (2082 victims, 78.2%). The prevalence of non-ischemic SCDs was 21.8% of all the SCDs. After sub-grouping the non-ischemic SCDs into various categories, the most common cause of death was CMP related to obesity (23.7%), followed by alcoholic CMP (19.0%), hypertensive CMP (15.5%) and IMF (13.6%). The association of SCD with IMF is notably frequent among victims <40 years old (28.3%). The prevalence of family history of SCD was significantly higher in the victims of ischemic (34.2%) than non-ischemic SCD (13.4%, P<0.001) or controls (17.6%, P<0.001). Lamin A/C gene mutation R541C was found from Finnish SCD family, in which the IMF was predominant pathologic-anatomic finding. Myocardial type I collagen synthesis was increased in victims of SCD due to IMF.

In conclusion, the characteristics of non-ischemic SCD in Finland differ from those reported previously. Higher prevalences of CMP-associated SCDs related to obesity, IMF and alcoholic CMP were observed as clinical and/or pathologic bases for non-ischemic SCD. The family history of SCD is not significantly increased in victims of non-ischemic SCD, suggesting a larger role of sporadic occurrence than inherited traits as the cause of non-ischemic SCD. Replacement of cardiac myocytes by fibrosis can be responsible for fatal cardiac arrhythmias in subjects with the lamin A/C gene mutation. The victims of SCD due to IMF have increased myocardial type I collagen synthesis.

Keywords: cardiomyopathy, collagen type I, family history, idiopathic myocardial fibrosis, lamin A/C gene, sudden cardiac death
Ei-iskeemisen sydänperäisen äkkikuoleman tunnuspiirteet.

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta, Kliinisen lääketieteen laitos, Kardiologian osasto, Sisäaudit; Diagnostiikan laitos, Oikeuslääketiede, PL 5000, 90014 Oulun yliopisto


Tiivistelmä

Ei-iskeeminen sydänperäinen äkkikuolema aiheuttaa noin 20 % kaikista sydänperäisistä äkkipuoleista. Suurin osa ei-iskeemistä sydänperäisistä äkkikuolemista johtuu erilaisista sydänhihassaarauksista, kardiomyopatiorissa. Useimmat sydänperäisen äkkikuoleman riskitekijöitä kartottavista tutkimuksista ovat keskittyneet sepelvaltimoihin. Tämän tutkimuksen tarkoituksena oli selvittää ei-iskeemisen sydänperäisen äkkikuoleman tunnuspiirteitä pohjoissuomalaisessa väestössä.

Tutkimuksessa käytettiin potilasaineistona sydänperäiseen äkkipuoleen menehtyneitä vainajia (n=2661), joille on tehty oikeuslääketieteellinen ruumiinavaus. Tiedot vainajista saatiin saatavilla olevista potilaskertomuksista, ruumiinavauspyytäkertoimista, äkkikuoleman aikaisesta lääkityksestä ja lähiomaisille lähetetyistä standardisoidusta kyselylomakkeesta. Kandidattingeitutututtiin pohjoissuomalaisesta perheestä, jossa ensimmäinen oire sydänhauskeudesta oli hengenvaarallinen rytmihäiriö. Lisäksi sydänhihaksen kollageenikoostumus analysoitiin histologisista näytteistä vainajista, joiden sydänperäinen äkkipuolema johtui idiopaattisesta sydänhihaksen sidekudoskasvuun.

Sepelvaltimoihin oli yleisin sydänperäisen äkkikuoleman aiheuttaja (n=2082, 78,2 %). Ei-iskeemisten sydänperäisten äkkipuoleiden osuus oli 21,8 % (n=579) kaikista sydänperäisistä äkkipuoleista. Ei-iskeemiset sydänperäiset äkkipuolot jotka ajoitiin alarhymmiin, joista yleisimmin olivat lihavuuteen liittyvät kardiomyopatia (23,7 %), alkoholikardiomyopatia (19,0 %), korkeaan verenpaineeseen liittyvät kardiomyopatia (15,5 %) sekä idiopaattinen sydänhihaksen sidekudoskasvu (13,6 %), joka myös oli yleisin ei-iskeemiseen sydänperäiseen äkkipuoleen johtava syy alle 40-vuotiailla (28,3 %). Positiivinen sydänperäisen äkkikuoleman kohun historia oli tilastollisesti merkittävästi yleisempiä iskeemisillä (34,2 %) kuin ei-iskeemisillä (13,4 %) sydänperäisen äkkijuoleman uhreilla. Lamin A/C – geenin mutaatio löytyi pohjoissuomalaisesta äkkikuolemaperheestä, jossa idiopaattinen sydänhihaksen sidekudoskasvu todettiin pääasiallisesti patologiseksi löydöksiksi. Tyypin I kollageeni synteesi todettiin kohonneeksi idiopaattiseen sydänhihaksen sidekudoskasvuun menehtyneillä vainajilla.

Yhteenvetona voidaan todeta, pohjoissuomalaisen väestön ei-iskeemisen sydänperäisen äkkikuoleman tunnuspiirteet eroavat aiemmin raportoituista; lihavuuteen liittyvät kardiomyopatia, alkoholikardiomyopatia, sekä idiopaattinen sydänhihaksen sidekudoskasvu olivat aiempaa yleisempiä ei-iskeemiseen äkkikuoleman aiheuttajia. Positiivinen sydänperäisen äkkikuoleman sukun historia ei ollut tilastollisesti merkittävästi kohonnut ei-iskeemisen sydänperäiseen äkkikuolemaan menehtyneillä. Tämä tarkoittaa, että perinnellinen syy ei-iskeemiseen sydänperäiseen äkkikuoleman aiheuttajana on luultava herinnaisempi. Lamin A/C – geenin mutaation kantajilla sydänhihaksujen korvauminen sidekudoskella todettiin hengenvaarallisen rytmihäiriön aiheuttajaksi. Lisäksi, tyypin I kollageenin synteesi todettiin kohonneeksi idiopaattiseen sydänhihaksen sidekudoskasvun menehtyneillä vainajilla.

Asiasanat: idiopaattinen sydänhihas-fibroosi, kardiomyopatia, lamin A/C geenin, sukun historia, sydänperäinen äkkikuolema, tyypin I kollageeni
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Eeva Hookana
Abbreviations

AMI  acute myocardial infarction
ARVC  arrhythmogenic right ventricular cardiomyopathy
AV  atrio-ventricular
BMI  body mass index
BrS  Brugada syndrome
BSA  body surface area
CAD  coronary artery disease
CHF  congestive heart failure
CMP  cardiomyopathy
DCM  dilated cardiomyopathy
ECG  electrocardiogram
EF  ejection fraction
EP  electrophysiological
FDR  first-degree relative
GEE  generalized estimating equations
GWA  genome wide association
HCM  hypertrophic cardiomyopathy
HDL  high density lipoprotein
HOCM  hypertrophic obstructive cardiomyopathy
ICD  implantable cardioverter defibrillator
ICTP  carboxy-terminal telopeptide of collagen type I
IVS  end-diastolic interventricular septum thickness
IIINTP  aminoterminal telopeptide of type III collagen
IMF  idiopathic myocardial fibrosis
LMNA  lamin A/C gene
LQTS  long QT syndrome
LV  left ventricle
LVH  left ventricle hypertrophy
LVID  end-diastolic left ventricular internal dimension
LVM  left ventricle mass
LVMII  left ventricle mass index
MI  myocardial infarction
MRI  magnetic resonance imaging
PINP  aminoterminal propeptide of type I collagen
PIIINP  aminoterminal propeptide of type III collagen
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<td>end-diastolic posterior wall thickness</td>
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<td>RV</td>
<td>right ventricle</td>
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<td>SCD</td>
<td>sudden cardiac death</td>
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<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>SQTS</td>
<td>short QT syndrome</td>
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<td>STR</td>
<td>short tandem repeat</td>
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<td>VF</td>
<td>ventricular fibrillation</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1 Introduction

Sudden cardiac death (SCD) is the major cause of death in Western countries. Despite recent progress in treatment and prevention of coronary artery disease (CAD), SCD remains a major public health problem. Around the world, the estimated annual burden of SCD is in the range of 4–5 million cases per year (Chugh et al. 2008). SCD accounts for about 50% of all CAD deaths and 15% to 20% of all deaths (Gillum 1990, Myerburg et al. 2004). Since CAD accounts for about 80% of SCDs in Western societies, most of the major studies of risk factors for SCD have focused on this etiologic category. The epidemiological patterns of SCD among patients with various forms of cardiomyopathy (CMP) are largely unknown.

The incidence of SCD has remained unchanged for decades, affecting younger people in general (mean age approximately 65 years) than other causes of cardiovascular mortality (Huikuri et al. 2001). A number of reasons have been identified as being responsible for the inability to significantly improve the outcome of patients at risk for premature unexpected SCD, such as our poor understanding of the basic mechanisms behind fatal arrhythmias, the inability to unravel the contributions of additional genetic and environmental modifiers of the phenotype and the lack of good risk stratification parameters. Lifestyle patterns, decreased physical activity, and socioeconomic development, are just some of the other factors that have changed significantly in recent decades, and they are thought likely to be affecting the epidemiologic patterns of SCD in the current era. Obesity is now a worldwide epidemic (World Health Organization 1998) and is recognized as a risk factor for cardiac diseases. In addition to these behavioural changes, both the primary and secondary prevention of cardiovascular diseases, such as treatment strategies and diagnostic methods, have changed (Huikuri et al. 2003). For example, in the last 20 years, genetics has started to play an increasingly important role in the progress of medical science. The impact of increasing knowledge and new techniques has been felt also in cardiology. While the role of genes in rare monogenic heart diseases is more clearly established, the contribution of a person’s genetic make-up in determining his/her risk of sudden death due to the more common form of sudden death secondary to CAD is less clear.

A comprehensive evaluation may help reveal indications about the clinical, anatomical, and genetic risk factors for SCD. The overall aim of this thesis was to clarify the characteristics of non-ischemic SCD in Northern Finland. The Finnish
Genetic Study of Arrhythmic Events (FinGesture) was designed to prospectively collect the data from a consecutive series of victims of SCD who were autopsied in the province of Oulu. This present study has reviewed the deceased’s clinical records, conducted close relative interviews and performed autopsy, toxicology testing, and genetic analysis for mutations in genes associated with SCD, to fill in the gaps in our knowledge about the prevalence and causes of non-ischemic SCD.
2 Review of the literature

2.1 Epidemiology of SCD

Sudden cardiac death is described as natural, unexpected death from a cardiovascular cause in a person with or without pre-existing heart disease, occurring within 1 h of the onset of final symptoms. The time passing between the onset of final symptoms and death is controversial, ranging from 6 h or 1 h according to different definitions. Pathologists tend to use a broader definition describing a death as sudden when it occurs within 24 h of a person known to be alive and functioning normally. This broader time frame allows for the inclusion of unwitnessed SCD victims. The published incidence of SCD ranges between 36 and 128 deaths per 100 000 population annually (Chugh et al. 2004, Cobb et al. 2002, de Vreede-Swagemakers et al. 1997, Escobedo & Zack 1996, Moore et al. 2006, Myerburg 2002, Tokashiki et al. 1999, Zheng et al. 2001). The incidence of SCD in any population fluctuates depending on several factors e.g. gender, age, nationality, screening methods to detect sudden death, and attempts to prevent or avert SCD pharmacologically, surgically, and/or by the use of external or implantable devices (Fishbein 2010).

There are two well-established peaks in the age-related prevalence of SCD, one smaller during early childhood (between birth and 6 months of age) representing the sudden infant death syndrome and the second, more sizable peak, in the elderly age group. Major advances in understanding the etiology and mechanisms of cardiovascular diseases have been achieved, these being associated with a concomitant reduction in age-adjusted cardiovascular disease-related mortality. Nonetheless, the relative incidence of SCD has remained unchanged in the middle-aged and older population. In addition, SCD continues to account for a greater proportion of deaths than other mechanisms of cardiovascular mortality among younger individuals (Schatzkin et al. 1984). SCD is unfortunately often the first and last manifestation of the cardiovascular disease making it particularly difficult to prevent. Furthermore, most of these deaths occur in people considered to be at low risk for such an event (Myerburg et al. 1997). This is purely because there are many more individuals in this low-risk group than in higher-risk groups.

Even though the incidence of SCD increases significantly with age, the proportion of deaths that are sudden is higher in the younger age groups (Becker
et al. 1993). At all ages, women have a lower incidence of SCD than men. Earlier studies had reported a 25:75 ratio of females to males, but a more recent study has demonstrated that women account for approximately 40% of all SCD cases (Chugh et al. 2004). There are also ethnic differences in the occurrence of SCD that are not well understood; blacks have higher SCD rates than whites (Becker et al. 1993). Moreover, survival rates after MI are also lower for blacks than whites. The incidence of SCD is also likely affected by socioeconomic factors (Mensah et al. 2005, Rozanski et al. 1999). Risk factors for CAD such as lack of physical activity, smoking, hyperlipidemia, hypertension, obesity and diabetes are more common among individuals with lower socioeconomic status.

2.2 Causes of SCD

The majority of SCDs are caused by a fatal cardiac arrhythmia, either ventricular tachycardia/fibrillation or severe bradycardia/pulseless electrical activity. Ventricular fibrillation (VF) is the presenting arrhythmia in the vast majority of SCD cases (Greene 1990). The pathophysiology of SCD is complex; it is believed to require an interaction between a transient event and an underlying substrate, e.g. a heart disease either acquired or genetically determined. This interaction may trigger some kind of electric instability and lethal ventricular arrhythmias followed by hemodynamic collapse. In general, acute myocardial infarction (AMI) is considered to be the most common cause triggering fatal arrhythmias (Myerburg et al. 1993). In addition to ischemia, a number of other arrhythmia inducing mechanisms have been recognized, including systemic metabolic and hemodynamic alterations, neurochemical and neurophysiological factors, and exogenous toxic or pharmacologic effects (Myerburg et al. 1993, Myerburg et al. 1997). These factors have a tendency to increase sympathetic activity, which in turn may possibly trigger arrhythmias and SCD. These triggers are transient and dynamic events, and are often difficult to measure and quantify.

Previous studies have detected a circadian pattern to the occurrence of SCD and out-of-hospital cardiac arrest (Muller et al. 1987). The highest frequency of SCDs occurs in the morning hours from 6 AM to noon (Cohen et al. 1997). It has been hypothesized that the greater risk of SCD in the first hours after awakening may be partially due to the morning elevation in blood pressure, heart rate and changes in heart rate variability (Muller et al. 1987). Lower socioeconomic status, depression, social isolation, and anxiety have all been linked to an increase in cardiovascular mortality in different populations (Mensah et al. 2005, Rozanski et
Moreover, emotional and psychological stress can increase SCD in populations during devastating disasters such as earthquakes or wars. It is also well known that SCD occurs with a higher-than-average frequency during or shortly after strenuous physical exertion (Kohl et al. 1992), despite the long-term benefits of exercise. These triggering settings can interact with ischemia, cardiac structural abnormalities, or primary electrophysiological abnormalities, resulting in a complex of factors that can produce sudden death from arrhythmia.

No symptoms have been identified as being specific for SCD in those patients who do develop symptoms before the event, SCD can also become manifest in the complete absence of symptoms. The patients may describe a wide variety of symptoms such as palpitations, chest discomfort, dyspnoea, pre-syncope or syncope. There is also a considerable overlap between the risk factors for related conditions, such as acute coronary syndrome and congestive heart failure (CHF), which makes it difficult to identify these patients who are likely to suffer a SCD.

2.2.1 Ischemic SCD

In the vast majority of cases (about 80%), SCD is caused by an ischemic cause (Figure 1). CAD is the underlying anatomic substrate of ischemic SCD due to atherosclerotic changes occurring in the coronary arteries. From a pathological point of view, CAD is defined as a heart showing with at least one of three coronary arteries narrowed to 75% or more by an atherosclerotic plaque and/or thrombosis (de la Grandmaison 2006). SCD has been reported to be the first clinical manifestation of CAD in as many as 20% of patients with coronary heart disease (Priori et al. 2003).

Atherosclerosis exists in all humans at advancing age and progresses during the lifetime, but its degree and progression is dependent on many factors; the classical environmental risk factors of CAD are now well established and are mainly associated with lifestyle. Systolic blood pressure, serum cholesterol, cigarette smoking, obesity, diabetes and a family history of CAD predispose to early CAD. For example, patients with high blood pressure frequently have abnormalities of cardiac structure or function, including systolic and diastolic dysfunction and in extreme cases, evident heart failure (Lip et al. 2000). At the histological level, cardiac myocytes of the left ventricle (LV) are enlarged in hypertensive heart disease (Anversa et al. 1990). In addition, fibrosis is another feature of the adverse structural remodelling found in the hypertensive heart (Campbell et al. 1993). Also diabetes is associated with an increased incidence of
heart failure, even after controlling for CAD and hypertension (Rubler et al. 1972). Increased LV mass is an independent risk factor for heart failure and may occur independently of arterial blood pressure in Type 2 diabetics, and may contribute to reduced myocardial compliance (Aneja et al. 2008). The Framingham study reported a significant increase in LV wall thickness in women with diabetes (Gelderisi et al. 1991). Apoptosis and necrosis have been reported to be increased in all cell populations within the heart in myocardial biopsies taken from diabetic subjects with heart failure and these changes could not be attributed to myocardial ischemia. (Frustaci et al. 2000).

The progression from conventional risk factors of CAD to arrhythmogenesis and SCD can be represented as a cascade of events: atherogenesis, changes in atherosclerotic plaque anatomy, disruption of an active plaque, activation of the thrombotic cascade and acute occlusion, followed by acute changes in myocardial electrophysiology which become the immediate trigger for arrhythmogenesis and SCD (Montagnana et al. 2008).

CAD predisposes to SCD in 3 general settings: (1) AMI, (2) ischemia without infarction, and (3) structural alterations such as scar formation or ventricular dilatation secondary to an earlier infarction or chronic ischemia. In the Framingham study, pre-existing CAD was associated with a 2.8- to 5.3-fold increase in risk of SCD, and CHF was associated with a 2.6- to 6.2-fold increased risk (Cupples et al. 1992). A previous myocardial infarction (MI) increases the risk of SCD 4- to 10-fold higher in women and men (Albert et al. 2003, Kannel et al. 1998). AMI occurs due to an occlusion of a coronary artery resulting in an imbalance between blood flow demand and supply to the myocardium.
2.2.2 Non-ischemic SCD

It has been estimated that non-ischemic causes of SCD account for about 15–20% of all SCDs (Huikuri et al. 2001). Various CMPs are the most common cause of death in non-ischemic SCDs. The European Society of Cardiology Working Group on Myocardial and Pericardial Diseases defined CMP as a myocardial disorder in which the heart muscle is structurally and functionally abnormal, in the absence of CAD, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality (Elliott et al. 2008). CMP represents a diverse group of heart muscle disorders, which are further subdivided on the basis of their anatomic and hemodynamic findings. The classifications for CMPs have proved to be extremely complex (Maron et al. 2006) since a variety of systematic classifications have been presented over the years. An inevitable limitation of any classification is the substantial overlap encountered between categories into which the diseases have been segregated. General etiologic classifications of CMPs are problematical, given that diseases with the same (or similar) phenotypes can arise from diverse origins and mechanisms. Determining the correct cause of the SCD is important, as the cause of SCD may determine the extent of risk to which the victim’s family members are exposed. (Maron et al. 2006).
Hypertrophic cardiomyopathy (HCM) is characterized morphologically and defined by a hypertrophied, nondilated LV in the absence of another systemic or cardiac disease that is able to produce the extent of wall thickening evident (for example, systemic hypertension, aortic valve stenosis) (Maron et al. 2006). The most characteristic feature in histological evaluation is myocardial fiber disarray with cardiomyocytes thickened up to 100 μm arranged in whirls and branched. It is assumed that the loss of contractility associated with this cellular disarray and interstitial fibrosis activates the myocardial hypertrophy process. Left ventricular hypertrophy (LVH) in the absence of hypertension and valve disease occurs in approximately 1:500 of the general population (Maron et al. 2003). The principal method to diagnose LVH is echocardiography, by which the thickness of the muscle of the heart can be measured. The left ventricular internal diameters and wall thickness are measured from M-mode recordings under 2-D guidance according to the recommendations of the American Society of Echocardiography (ASE) (Sahn et al. 1978). The left ventricular mass (LVM) is calculated from the ASE measurements according to the corrected equation (Devereux et al. 1986):

\[
LVM(g) = 0.8 \times \{1.04 \times [(IVS + LVID + PWT)^3 - LVID^3]\} + 0.6, \\
\]

where IVS is the end-diastolic interventricular septum thickness, LVID is the end-diastolic left ventricular internal dimension and PWT is the end-diastolic posterior wall thickness. The left ventricular mass index (LVMI) is calculated by dividing LVM (g) by body surface area (BSA) (m²). BSA is determined by the Dubois equation (Du Bois & Du Bois 1989):

\[
BSA(m^2) = 0.007184 \times \text{[weight (kg)]}^{0.425} \times \text{[height (cm)]}^{0.725} \\
\]

The LVH cut-off points are > 134 g/m² in men and > 110 g/m² in women (Devereux et al. 1984). There are also few sets of criteria used to diagnose LVH via electrocardiography (ECG):

- Sokolow-Lyon index (SOKOLOW & LYON 1949): sum of S wave in lead V1 + R wave in lead V5 or lead V6 > 3.5 mV; and R wave in lead aVL ≥ 11 mm
- Cornell voltage index (Casale et al. 1987): men: R wave in lead aVL + S wave in lead V5 > 2.8 mV; women: R wave in lead aVL + S wave in lead V5 > 2.0 mV.

LVH may be either eccentric or concentric (2 × LV posterior wall thickness relative to LV end diastolic dimension ≤ 0.42 or > 0.42, respectively) (Lang et al. 2005) and, although hypertensive patients with either type of LVH typically have an increased LVMI, which reflects the degree of LVH, eccentric and concentric...
LVH have different LV geometries, including LV wall thickness and LV cavity size. The thickening of the LV wall relative to the internal cavity is referred to as concentric LVH. Less common is the disproportionate (relative to the posterior wall) thickening of the intraventricular septum, referred as eccentric LVH. (Lip et al. 2000).

The range of symptoms experienced by HCM patients varies from asymptomatic to severe restrictions. Dyspnea and angina pectoris under stress are the most common symptoms. Palpitations are common and often associated with light-headedness, dizziness and occasionally with syncope (Braunwald et al. 1964). The most dramatic symptom is SCD, which is most common in young and previously asymptomatic patients. Death occurs predominantly during or after strenuous physical exercise and is attributable to malignant dysrhythmias. Maron et al. described HCM as the most common cause (36%) of SCD in competitive athletes over a 10-year period in the United States (Maron et al. 1996).

Dilated cardiomyopathy (DCM) is defined by the presence of LV dilatation and LV systolic dysfunction in the absence of abnormal loading conditions (hypertension, valve disease) or CAD adequate enough to cause a global systolic impairment. Echocardiography is the most important investigation for establishing the diagnosis of DCM, by defining the presence and severity of LV dilatation and dysfunction. Diagnostic criteria have relied on the identification of the ejection fraction (EF) < 45%, and/or a fractional shortening < 25%, in association with a LV end-diastolic dimension > 112% predicted value corrected for age and BSA. (Thomas et al. 2009).

At least 25% of DCM patients in Western populations have evidence for familial disease with predominantly autosomal dominant inheritance (Burkett & Hershberger 2005, Dec & Fuster 1994, Mestroni et al. 1999). The DCM phenotype with sporadic occurrence may develop from a particularly broad range of primary (and secondary) causes, including infectious agents, particularly viruses, often producing myocarditis (coxsackievirus, adenovirus). Other causes include toxins; chronic excessive consumption of alcohol and chemotherapeutic agents (Maron et al. 2006). In addition, early studies with relatively small numbers of obese patients, suggested that they displayed predominantly dilated hearts (Alexander 1985, Alpert et al. 1995). Obesity refers to excess body fat and may be defined as body weight which is 20% above the ideal body weight. Adverse cardiac effects of obesity include LVH, chronic volume overload, systolic and diastolic dysfunction and left atrial enlargement with atrial fibrillation (Lavie et al. 2009). In a study by Kasper et al. (Kasper et al. 1992),
the most common finding on endomyocardial biopsy in an obese group with CHF was mild myocyte hypertrophy (67%). It has been debated whether there is a true cardiomyopathy of obesity or whether the cardiac changes in obese individuals are a result from other comorbid conditions (Owan & Litwin 2007).

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a rare form of inheritable heart muscle disease with a rather recent description (Marcus et al. 1982). ARVC involves primarily the right ventricle where there is a progressive loss of myocytes and fatty or fibrofatty tissue substitution, resulting in regional or global abnormalities. The clinical features include ventricular arrhythmias with left bundle branch morphology and ventricular fibrillation which might lead to sudden death (Thiene et al. 1988). Although uncommon (estimated prevalence 1:5000), ARVC is a frequent cause of death in young people in some areas of Europe, and a familial occurrence has been reported in about 50% of cases. (Thiene et al. 2007). In the Finnish population, ARVC is inherited as an autosomal dominant trait with variable penetrance. The ARVC in Finland presents with distinct arrhythmic and RV dilative subtypes. The sporadic disease is similar to the familial type which may reflect low penetrance in relatives. The proportion of familial ARVC in Finland is comparable to that noted elsewhere in Europe. (Kaartinen et al. 2007).

A small proportion of non-ischemic SCDS display no obvious morphological abnormalities at post-mortem examination that can be unequivocally defined. These deaths may be due to the ion-channel disorders, ‘channelopathies’. A growing number of channelopathies have been described in the past two decades, being responsible for several cases of unexplained arrhythmias in young individuals which were attributable to distinct heritable disorders such as the long QT syndrome (LQTS), the short QT syndrome (SQTS), and the Brugada syndrome (BrS) (Priori 2010). LQTS is characterized by an excessive prolongation of cardiac repolarization (QTc > 480ms) thought to lead to ventricular arrhythmias (Moss & Kass 2005). Symptomatic individuals frequently manifest with stress-induced syncopal episodes or cardiac arrest. At present, 13 different variants of LQTS have been described in the literature, even though most of the genotyped patients belong to the first three variants (LQT1, LQT2, and LQT3). Cardiac arrhythmias are often induced by stress and emotion in LQTS patients, although in some cases they may also occur at rest or during sleep. In contrast to LQTS, SQTS is characterized by a shorter than normal QT interval (< 350 ms). This also may lead to ventricular and atrial arrhythmias, and SCD (Gussak et al. 2000). It seems that the occurrence of SCD as the first
manifestation is not uncommon, indicating that only a limited number of SQTS patients have been reported.

The BrS is a channelopathy characterized by a peculiar ECG pattern; a coved-type ST-segment elevation of the right precordial leads of the 12-lead ECG (Brugada & Brugada 1992). The most common clinical manifestations of BrS are syncope or SCD caused by ventricular tachyarrhythmias mostly occurring during sleep or at rest. Fever is also another well recognized triggering factor in these patients (Rossenbacker & Priori 2007). The prevalence of BrS is 1:1000 in Asian countries, and probably somewhat lower elsewhere. The clinical phenotype of patients with BrS is 8 to 10 times more prevalent in males than in females. The SCN5A gene mutation is found in approximately 20% of BrS cases. (Rossenbacker & Priori 2007).

Coronary anomalies and valvular heart disease account for a small proportion of non-ischemic SCD causes. The high-risk anomalies mainly consist of four types; anomalous origin of one or more coronary arteries arising from the pulmonary trunk; anomalous origin of one or more coronary arteries from aorta; single coronary ostium from aorta; and hypoplastic coronary arteries (Virmani et al. 2001). Anomalous coronary arteries are suspected to cause myocardial ischemia due to the reduction in the regional blood flow (Basso et al. 2000, Taylor et al. 1997). Valvular heart disease is characterized by damage to or a defect in one of the four heart valves: the mitral, aortic, tricuspid or pulmonary. In valvular heart disease, the valves become too narrow and hardened (stenotic) to open fully, or are unable to close completely. The most common congenital abnormality of the heart is the bicuspid aortic valve. (Maganti et al. 2010). Serious complications will develop in at least one-third of patients with bicuspid aortic valve, and this can be responsible for more deaths and morbidity than the combined effects of all the other congenital heart defects (De Mozzi et al. 2008).

2.2.3 Medico-legal autopsy

The definition of SCD is still not internationally standardized. The incidence of SCD also depends upon how the diagnosis is made. Death certificate data have been used as a replacement for national examination of SCD, but the accuracy of this method may be limited (Myerburg 2002), which might be a reason for these diverse assessments. According to one estimate, retrospective studies utilizing death certificate review may possibly overestimate SCD occurrence by 200- 300% (Arking et al. 2004). Irrespective of where within this range the actual quantity
lies, this represents a major epidemiological problem that deserves serious attention and attempts to achieve solutions.

The quality of the cause-of-death definition system is of a high standard and consistent throughout Finland (Nayha 1981). There are national laws and comprehensive guidelines about death investigation to ensure the reliability, accuracy and comprehensiveness, i.e. the quality, of death investigation (Lahti et al. 1998). In the Finnish Act on the Inquest into the Cause of Death (459/73), the 7th paragraph states: “Police shall make an investigation when 1) It is not known that death has been caused by disease, or a physician has not treated the deceased during his/her last illness, 2) Crime, accident, suicide, poisoning, occupational disease or medical treatment (malpractice) has caused the death or there is reason to suspect that death has resulted from such a cause, 3) Death has been otherwise unexpected. In the investigation, a medical doctor must be consulted if necessary.”

In Finland, medicolegal autopsies are performed by forensic pathologists, with standardized university training and qualifications, in the Departments of Forensic Medicine in five university hospitals and in ten central hospitals. All dissections are complete autopsies; no partial dissections are performed. This point is very important for ensuring the correctness of cause-of-death determination, particularly for medico-legal deaths due to natural causes (Asnaes & Paaske 1980, Saukko 1995).

Cause-of-death classification is categorized and coded according to the International Classification of Diseases (ICD), adopted by World Health Organization (WHO). Since the beginning of 1996, the tenth revision of the ICD (ICD-10) and its national adaptation “Tautuluokitus ICD-10” has been used in Finland. Statistics Finland uses the original ICD-10, with physicians relying on the national version. The ICD rules determine the selection of the underlying cause of death for primary mortality tabulation.

The total autopsy rate in Finland has been at its highest, around 38%, in the 1970’s. During the 1980’s, the national autopsy rate has shown an overall declining trend, but since the rate of medico-legal autopsies have increased, this has led to stabilization of the total autopsy rate at a level of 30 to 31% since the beginning of the 1990’s. In 2010, the overall autopsy rate was in Finland 29.8%, 6.6% for clinical autopsies and 23.2% for medico-legal autopsies (Anonymous ). The decline in autopsy rate has been a worldwide phenomenon during the last three or four decades. In the US, a decline in the overall autopsy rate from about 15% in 1980 to 9.4% by 1994. Autopsies were reported as being performed on 7.7
percent of deaths that occurred in 2003 (Hoyert et al. 2007). In the Nordic
countries, there has also been a declining tendency. In Denmark, the medico-legal
autopsy rate has been historically and is still very low, around 2.5% in 1992. In
Sweden, the medico-legal rate has fallen to the level of about 6% after 1980’s.
(Saukko 1995). Thus, because of the overall high autopsy rates and death
investigation laws, autopsy rates for natural deaths are rather high in Finland.

Previous studies have demonstrated the importance of autopsy in the correct
diagnoses of cause of deaths. For example, relatively infrequent causes of sudden
death, such as various CMPs, and valvular heart diseases, have been infrequently
correctly identified on the death certificate, unless there was a specific prior
history and diagnosis (Tavora et al. 2008). In the study of Corrado et al. (Corrado
et al. 2001), macroscopic heart features were normal in nearly one-third of young
SCD victims. In 79% of these cases, a histological study revealed pathologic
substrates such as cardiomyopathy or focal myocarditis. Thus, medico-legal
autopsies can provide histopathological and toxicological data that cannot be
obtained by other methods.

2.3 Genetics of sudden cardiac death

2.3.1 Mode of inheritance

SCD is likely to have a strong genetic component, but only a small fraction of the
genetic variants that underlie the risk are known. Ischemic heart disease most
commonly arises from complex and intricate interactions between genetics,
lifestyle habits, and environment. Instead, a considerable number of cases of non-
ischemic heart disease – including CMP and arrhythmias, are due to inherited
traits. Hereditary diseases have been classified by (Campuzano et al. 2009):

- Monogenic diseases, which are caused by a single gene and follow a
  Mendelian pattern of inheritance.
- Complex or polygenic diseases, which are due to the interaction of different
genomes.
- Chromosomal disorders, with the deletion or addition of a part of a
  chromosome or an entire chromosome.

The human genome is composed of a sequence of approximately 3 billion
nucleotide base pairs and approximately 20,000 genes have been identified. There
is considerable nucleotide variation within the genome. The most common form of genetic variation is a single nucleotide polymorphism (SNP), which is attributable to the variation of a single nucleotide and occurs in more than 1% of the population. A SNP occurs in approximately one out of every 1000 base pairs, thus there are estimated to be more than 10 million SNPs in the human genome. The majority of SNPs are allelic variants that do not affect either expression or function of a protein. These kinds of SNPs are frequently used as genetic markers to localize nearby disease-causing variations in linkage and association analyses. SNPs that have a direct effect on phenotype may be located within coding or regulatory regions of genes. Variations located in coding regions of a gene may alter the amino acid and consequently the 3-dimensional structure and the function of the protein that it encodes (Campuzano et al. 2009). A mutation is defined as any change in a DNA sequence away from normal. This indicates that there is a normal allele, a form of a genetic locus, that is prevalent in the population and that the mutation changes this to a rare and abnormal variant. The arbitrary cut-off point between a mutation and a polymorphism is 1%; to be classified as a polymorphism, the least common allele must have a frequency of 1% or more in the population. If the frequency is lower than 1%, the allele is regarded as a mutation. A nonsense mutation is a single nucleotide mutation in a sequence of DNA that results in a premature stop codon, and usually in a nonfunctional protein product. It differs from a missense mutation, which is a mutation where a single nucleotide is changed leading to a substitution of a different amino acid. Silent mutations are DNA mutations that do not result in a change to the amino acid sequence of a protein. (Buckingham 1990).

A chromosome mutation is any change in the structure or arrangement of the chromosomes. When the chromosome's structure is altered, this can take several forms (Shaw & Lupski 2004):

- Deletions: A portion of the chromosome is missing or deleted.
- Duplications: A portion of the chromosome is duplicated, resulting in extra genetic material.
- Translocations: A portion of one chromosome is transferred to another chromosome.
- Inversions: A region of DNA on the chromosome can flip its orientation with respect to the rest of the chromosome.
- Insertions: A portion of one chromosome has been deleted from its normal place and inserted into another chromosome.
2.3.2 Monogenic heart diseases

Monogenic diseases exhibit one of the following four Mendelian inheritance patterns: autosomal dominant, autosomal recessive, sex-related or mitochondrial. In autosomal dominant diseases, only one defective allele (one of two forms of a gene or a genetic locus) is needed to cause a disease. Two defective alleles are required for the disease to arise in autosomal recessive diseases. Sex-related diseases are governed by genes in the sex-chromosomes; X and Y. X-linked traits are maternally inherited from carrier mothers or from an affected father. Mitochondrial diseases are most usually transmitted along the female line because the mitochondrial chromosome always originates from the mother. The mitochondrial DNA syndromes are maternally inherited, when the primary defect is a mutation or deletion in mitochondrial DNA, and autosomally inherited when the mitochondrial DNA defect is secondary to a mutation in a nuclear gene that is required for mitochondrial DNA maintenance. (Ylikallio & Suomalainen 2012)

CMP is one of the clinical phenotypes associated with mitochondrial DNA mutations (Anan et al. 1995, Marin-Garcia et al. 1998, Sato et al. 1994, Zeviani et al. 1991). Most commonly, the CMP associated with these mutations is hypertrophic. Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) is a distinct clinical syndrome characterized by short stature, seizures, hemiparesis, hemianopia and cortical blindness (Pavlakis et al. 1984). Structural and functional abnormalities of the heart were common in Finnish MELAS patients with mitochondrial mutation 3243A>G which was almost exclusively associated with LVH. (Majamaa-Voltti et al. 2002).

Most of the genetic forms of non-ischemic cardiac diseases are inherited as a monogenic trait (Table 1). These diseases result from mutations in the genes that are mainly encoded by three large protein families (Campuzano et al. 2009):

1. Sarcomeric proteins, which are responsible for generating force in cardiac myocytes; these mutations are associated with the etiology of HCM (Marian & Roberts 1995). In addition, mutations in calcium-handling and metabolic regulatory protein encoding genes have been identified. It has been shown that approximately 50% to 60% of HCM cases are familial, and an autosomal dominant pattern of inheritance is the most common form. At present, over 30 genes and hundreds of different mutations have been described (Brion et al. 2010). In a Finnish study by Jääskeläinen et al. (Jaaskelainen et al. 2004), collectively six mutations were found in sarcomeric genes MYBPC3, TPM1
and MYH7 that accounted for 40% of all HCM cases in the study group. The genetic profile of HCM in eastern Finland is unique, characterized by a few founder mutations. The phenotypic expression of HCM in patients from eastern Finland, as well as that related to the identified disease causing mutations, is relatively benign.

2. Cytoskeletal proteins, which are responsible for transmitting this force to the surrounding cells and which are associated with the etiology of DCM (Olson et al. 1998). Mutations in genes encoding proteins of cell nucleus have also been described to be responsible for DCM. About 20% to 35% of DCM cases have been reported as being familial, although with incomplete and age-dependent penetrance, and linked to a diverse group of more than 20 loci and genes (Campuzano et al. 2009). The mutations most frequently associated with DCM have been reported in the lamin A/C gene (LMNA) (Arbustini et al. 2002, Brodsky et al. 2000, Fatkin et al. 1999). The Ser143Pro mutation in the LMNA gene was found to be quite common in Finnish patients with familial DCM. Haplotype analysis strongly suggested that the Ser143Pro mutation is a founder mutation, probably the first founder mutation described for DCM (Karkkainen & Peuhkurinen 2007). A recent study reported that titin gene (TTN) mutations are also relatively frequent in DCM patients (Kimura 2011).

3. Proteins that encode ion channels, which are responsible for maintaining intracellular and extracellular electrolyte balance and which cause inherited arrhythmias (Roden et al. 1996).

This coarse classification is not a clear cut, as there is a significant overlap between diseases and genes (Cirino & Ho 2006). For example, a mutation in sarcomeric MYH7 (β-myosin heavy chain gene) can cause both DCM and HCM. Furthermore, even traits inherited in a “simple” Mendelian manner are sometimes affected by factors that exert a significant influence on phenotype. There might be genetic heterogeneity (same phenotype caused by polymorphisms in different genes), allelic heterogeneity (same phenotype caused by different alleles in the same gene), phenotypic heterogeneity (variability in expressivity or clinical manifestations of a genetic disease), epistasis (the effect of a genotype depends on the presence of another genotype) and gene-environment interactions (the effect of a genotype depends on the presence of an environmental factor). These are just some of the modifications that may have an effect on the phenotype (Brion et al. 2010). For example, genetic studies of HCM have revealed that about 25% of genetically affected adults are healthy carriers without displaying any ECG or
echocardiographic abnormalities (Maron et al. 2001). It is presently unknown whether or not healthy mutation carriers are at risk of developing a later form of disease. Further confusion might arise over the terms genetic and familial. A familial disease is hereditary, passed on from one generation to the next. It resides in a genetic mutation that is transmitted by mother or father (or both) through the germ cells (sperm or egg) to their offspring. However, not all genetic disorders are familial, because the mutation (de novo mutation) may arise for the first time during the formation of the germ cells or during the early development of the fetus. Such a child will have a genetic abnormality, though the parents themselves do not have this abnormality.

Mutations can be divided by the effect they have on function. For example, most of the mutations identified in the genes encoding ion channel subunits disrupt the function of channels and modify their biophysical properties. These mutations are called “loss-of-function” defects in order to distinguish them from a minority of the genetic defects called “gain-of-function” mutations that result in a hyperactive ion channel. LQTS and SQTS represent a typical example of how different defects in the same genes may cause opposite phenotypes. Gain-of-function mutation causes SQTS in the same gene (KCNJ2), which causes LQTS through loss-of-function mutation (Priori et al. 2005).

Infrequently, premature CAD is caused by Mendelian disorders (Table 1), such as familial hypercholesterolemia and familial defective apolipoprotein B. These diseases are caused by mutations in genes involved in sterol metabolism, high density lipoprotein (HDL) concentration regulation, cholesterol efflux in macrophages and homocysteine concentration regulation (Soutar & Naoumova 2007, Tybjaerg-Hansen et al. 1990).

The linkage study approach has been efficacious in the identification of underlying variants for conditions with strong heritability, particularly monogenic Mendelian diseases. In linkage analysis, one undertakes the genotyping of large pedigrees in which the disease of interest is prevalent in an attempt to identify regions of genetic variations that are shared among the affected individuals in the pedigree more often than predicted by Mendelian segregation or chance.
<table>
<thead>
<tr>
<th>Cardiovascular disease</th>
<th>Phenotype</th>
<th>Involved genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ischemic cardiomyopathies</td>
<td>HCM</td>
<td>MYH7, TNNT2, TPM1, MYBPC3, PRKAG2, TNNI3, MYL3, TTN, MYL2, ACTC1, CSRP3, LAMP2</td>
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<td>DCM</td>
<td>MYBPC, MLP, ACTN2, PLN, ZASP, MYH6, ABCC, TNNC1, TCAP, EYA4, LMNA, SCN5A, DMD, TAZ, TNNI3</td>
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<tr>
<td>ARVC</td>
<td>JUP, DSP, PKP2, DSG2, DSC2, RYR2, TGFB3</td>
<td></td>
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<tr>
<td>Channelopathies</td>
<td>Long QT syndrome</td>
<td>SCN5A, SCN4B, KCNQ1, KCNH2, KNE1, KNE2, KCNJ2, ANK2, CAV3</td>
</tr>
<tr>
<td>Brugada syndrome</td>
<td>SCN5A, SCN1B, GPD1L, CACNA1C, CACNB2b</td>
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<td>Short QT syndrome</td>
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<td></td>
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<tr>
<td>CAD, myocardial infarction</td>
<td>Mendelian inheritance</td>
<td>LDLR, APOB, ABCG5, ABCG8, APOA1, ABCA1, CBS</td>
</tr>
<tr>
<td>Complex disease</td>
<td>9p21, SH2B3, MRPS5-SLC5A3-KCNE, PHACTR1, CELSR2-PSRC1-SORT, CXCL12, MIA3, PCSK9</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ARVC, arrhythmogenic right ventricular dysplasia; CAD indicated coronary artery disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy.

### 2.3.3 Complex heart diseases

Over the past 50 years, information has accumulated on the role of environmental and genetic factors predisposing to atherosclerosis and to its clinical sub-phenotypes. SCD in patients with CAD is a result of ventricular tachycardia/fibrillation secondary to acute ischemia or due to myocardial scarring. Thus, a great number of genetic factors that affect an individual’s susceptibility to atherosclerosis, coronary thrombosis, electrical or autonomic instability can apparently affect the risk for sudden death. Consequently, pathways that affect cholesterol metabolism, atherosclerotic plaque formation, the coagulation cascade, ion channels, gap junctions, receptor and signaling pathways, central and autonomic neural networks as well as many other factors might contribute to the occurrence of sudden death. The evidence that genes have a role to play in sudden death due to CAD has been gathered from family studies. Recent evidence from these studies has identified the presence of familial clustering of SCD as a clinical expression of CAD (Friedlander et al. 1998, Jouven et al. 1999, Kaikkonen et al. 2006). In the study of Jouven et al. (1999), multivariate analysis revealed that the occurrence of SCD in a parent or first-degree relative results in a 1.6- to 1.8-fold
increase in SCD susceptibility after controlling for traditional risk factors for CAD.

A large number of studies have been designed to unravel the molecular mechanisms underlying the heritability of CAD. Linkage analysis studies suffer from a lack of statistical power to identify genetic variations with low penetrance and with a small influence on the predisposition towards complex diseases, and hence have produced only a few reproducible results for CAD (Helgadottir et al. 2004). Early phase case–control studies have focused on the differences in carrier frequency of genetic variations in “candidate genes” between cases and controls. Genes are designated as “candidate” based on the fact that they are known to have an effect on traditional risk factors (i.e., genes involved in lipid metabolism) or are part of pathways with a postulated role in atherogenesis. In recent years, several genome-wide association (GWA) studies with CAD as the outcome have been published (Helgadottir et al. 2007, Samani et al. 2007, Vasan et al. 2009). These studies largely focus on populations, rather than on families, and are carried out under the hypothesis that common variants are shared by subjects with a pre-specified phenotype. A GWA study can be concisely defined as a method that analyses a large number of SNP markers, that are distributed all over the genome, for an association with a specific disease or condition by comparing relative frequencies between affected cases and controls. The basis for GWA studies is the “common disease, common variant” hypothesis in which a limited number of genetic variants with a high frequency (typically in more than 1% to 5%) in the general population contribute to susceptibility for a disease (Manolio 2010). A GWA study does not require any previous knowledge of the disease pathways, in contrast to the candidate gene approach. This makes it possible to scan the genome in an unbiased way, permitting the identification of novel susceptibility factors.

Large-scale GWA studies have greater power than linkage studies in their ability to identify genetic variants with small effects on CAD susceptibility but large sample sizes are required to detect genes with statistically significant associations that contribute a small effect (Wang et al. 2005). In particular, an association between CAD and a region on chromosome 9 (9p21) was first identified in 2007 by Helgadottir et al. This result has been replicated in 25 subsequent studies. A recent meta-analysis of 16 of these 25 studies reported a statistically significant association between 9p21 polymorphisms and CAD (Palomaki et al. 2010). The region 9p21 has been the most replicated and has been shown to have the strongest association, thus there have been many attempts
to determine the molecular mechanism underlying this relationship. The risk haplotype for cardiovascular disease on 9p21 is located in an area without any annotated genes, close to cluster INK4a/ARF which is composed of the tumor suppressor genes cyclin-dependent kinase inhibitor 2A (CDKN2A) and cyclin-dependent kinase inhibitor 2B (CDKN2B). These genes have been implicated in the development of a variety of cancers. Next to these two genes, an antisense noncoding RNA gene known as ANRIL has been identified (Pasmant et al. 2007). Gene expression studies in 9p21 have revealed that the genes CDKN2A, CDKN2B, and ANRIL are expressed in atherosclerotic tissue and are transcriptionally co-regulated (Holdt et al. 2010). The ANRIL gene has several transcripts whose expression levels demonstrate a stronger correlation with risk SNPs genotypes in 9p21 than with CDKN2A/2B. Nevertheless, a clear functional interpretation is still lacking.

Even though the GWA has proved to be a powerful tool in recognizing strong associations between many SNPs and traits, much further work will still be needed to define the functional basis for the observed associations so that appropriate interventions can be developed. Much remains to be learned about how variations in intronic and intergenic regions, where the vast majority of SNP–trait associations are located, can influence gene expression, protein coding, and disease phenotypes (Hardy & Singleton 2009).

The analysis of phenotypes, such as CAD or MI, is complicated by the substantial impact of the environment on disease development, by the multiplicity of pathways involved in the response to environmental stress in different phases of disease evolution, and the multiplicity of clinical sub-phenotypes, such as hypertension and hypercholesterolemia. The complexity of phenotypes can lead to non-replications (Moonesinghe et al. 2008). For example, the cardiovascular phenotypes have been classified on the basis of observed disease outcomes in GWA studies. Even a relatively distinct phenotype may be traced to different underlying pathophysiologies. MI can be triggered by several types of acute pathophysiologies, such as plaque rupture, erosion or haemorrhage. Furthermore, SCD has been broadly defined as death occurring within a certain time frame after the onset of symptoms. This definition clearly includes individuals with very different disease etiologies and probably with different genetic and biological risk factors. It is possible that in the years to come, with the refinement of samples and the development of new methods, data unravelling the complexity of CAD will be easier to obtain.
2.4 Role of fibrosis in SCD

Cardiac cells are embedded in a network that consists mainly of fibrillar collagen. Collagen is a major insoluble fibrous protein present in the extracellular matrix and in connective tissue. This collagen network provides cardiac strength and participates in intercellular communication. In the heart, collagen types I (80%) and III (11%) are the most abundant types out of all the 29 different collagen forms that have been described (Medugorac & Jacob 1983, Weber et al. 1988). Lesser amounts of types IV and V are detected in the basement membrane of the myocytes, and in the perivascular and pericellular spaces (Eghbali & Weber 1990). The amount of collagen, a normal component of the heart muscle, increases in the majority of heart diseases, this accumulation of collagen is called fibrosis. The main collagen type in cardiac fibrosis is type I, similar to that found in the healthy myocardium (Swynghedauw 1999, Weber et al. 1995). The collagen type I/type III ratio is increased in several cardiac diseases (Pauschinger et al. 1999).

The formation of fibrosis can be activated by tissue injuries or stresses such as myocardial ischemia, inflammation, and hypertensive overload (Zannad & Radauceanu 2005). Fibrosis is most frequently found in the setting of an ischemic scar, but there is increasing recognition that diffuse myocardial fibrosis can occur as a distinct entity in a variety of conditions, even in the absence of ischemia, including hypertensive heart disease, HCM, DCM and diabetic heart. Regan et al. (1977) found a significant increase in collagen deposition around intramural vessels and between myofibers in heart biopsies from diabetic patients. In addition, a significant increase in collagen type III but not type I or VI was found in endomyocardial biopsies obtained from patients with Type 2 diabetes, who did not have significant CAD and hypertension (Shimizu et al. 1993). Furthermore, diastolic dysfunction detected in a population of uncomplicated Type 2 diabetes correlated with pro-collagen type I carboxy-terminal peptide (Gonzalez-Vilchez et al. 2005, Ihm et al. 2007), suggesting a mechanistic role for myocardial fibrosis in myocardial dysfunction in diabetes.

A small but distinct subgroup of patients with SCD, have idiopathic myocardial fibrosis (IMF), i.e. fibrosis in the absence of other cardiac diseases. IMF is generally identified in 1% to 3% of overall cases among autopsy series of SCD (Chugh et al. 2000, Maron et al. 1996). Previously, IMF has been attributed to the residual effects of myocarditis or age-related degenerative changes (Lecomte et al. 1993, LEV 1964). However, the post-mortem diagnosis of myocarditis in humans requires the documentation of inflammatory infiltrates
consisting mainly of lymphocytes, in addition to myocyte necrosis and significant replacement fibrosis (Lecomte et al. 1993). Since in previous studies no inflammatory infiltrates were observed, prior myocarditis is unlikely to have led to IMF (John et al. 2004). Thus, a detailed characterization of this condition still needs to be undertaken.

Cardiac fibrosis may evoke electrical heterogeneity and act as a substrate for arrhythmogenicity, which may cause SCD. Fibrosis can be reparative or reactive (Jellis et al. 2010). Reparative, also called replacement fibrosis, is defined as increased interstitial/perivascular collagen with evidence of myocyte loss (John et al. 2004). Since myocytes are thought to be terminally differentiated, myocytes lost due to necrosis or apoptosis, for example after MI, are replaced by collagen. This collagen deposition is necessary to preserve the cardiac structure and morphology after myocyte loss. However, fibrous tissue formation can also occur without the loss of cells, for example, there can be collagen formation in the perivascular arterioles, increased amount of collagen between viable myocytes, and thickening of existing collagen fibers. This type of fibrosis is called reactive or interstitial fibrosis, and it is defined as increased interstitial and/or perivascular collagen without any evidence of myocyte loss. In DCM, reactive fibrosis predominates, whereas replacement fibrosis has been found in HCM (Swynghedauw 1999). In addition, the amount of fibrosis differs in various diseases as well as with time after onset of the disease. The study of Tanaka et al. reported that the percentage area of fibrosis was 10.5% ± 4.3% in the hearts with HCM, whereas the amount of fibrosis was 1.1% ± 0.5% in normal hearts (Tanaka et al. 1986).

In recent years, many studies have sought biomarkers for cardiac remodelling which can be assayed from blood. Fibrotic biomarkers that can be used for the detection of collagen synthesis are the propeptides of collagens. The propeptides are differentiated into either aminoterminal propeptides (aminoterminal propeptide of type I collagen (PINP) and aminoterminal propeptide of type III collagen (PIIIINP)) or carboxyterminal telopeptides (carboxy-terminal telopeptide of collagen type I (ICTP)) or aminoterminal telopeptides (aminoterminal telopeptide of type III collagen (IINTP)). PINP and PIIIINP represent the newly synthesized type I and III collagens, IINTP is a marker of cross-linked mature collagen III, whereas ICTP detect can be used to a degradation product of cross-linked type I collagen (Bode et al. 2000, Risteli & Risteli 1995). Differences between patients and controls in these biomarker levels have been reported in many studies (Biolo et al. 2009, Gonzalez et al. 2010). In contrast to serologic
collagen studies, there have been far fewer histological studies investigating the content of myocardial fibrosis. The study of Polyakova et al. (2011) reported discordant changes in the content of collagen types I and III in the failing myocardium. These results suggest that the structural alterations of collagen content are rather specific for each type of cardiac disease rather than simply due to heart failure.

2.5 Summary

In summary, SCD can be traced to an ischemic cause in about 80% of cases; non-ischemic causes of SCD account for about 15–20% of all SCDs. Various CMPs are the most common causes of death in non-ischemic SCDs. CMP represents a diverse group of heart muscle disorders, which can be further subdivided on the basis of their anatomic and hemodynamic findings. CMPs have proved to be extremely difficult to classify and a variety of systematic classifications have been presented through the years. The epidemiological patterns of SCD among patients with various forms of CMP are largely unknown.

Previous studies have highlighted the importance of autopsy in the correct diagnoses of cause of death. However, the few studies have had access to meticulous autopsy examination data. Therefore, it was useful to perform this comprehensive evaluation of the clinical, anatomical, and genetic risk factors in patients who succumbed to non-ischemic SCD.
3 Purpose of the present study

The overall aim of the present study was to clarify the characteristics of non-ischemic SCD in Northern Finland. The specific aims were:

1. To identify the genetic factor causing SCD from a Finnish family in which the mother was resuscitated from ventricular fibrillation and the daughter died suddenly without any prior cardiac symptoms (I)
2. To classify the autopsy-verified causes of non-ischemic SCD in different age groups in the current era (II)
3. To study whether non-ischemic SCD has a familial background indicative of a genetic predisposition for sudden death (III)
4. To evaluate the collagen content of the myocardium from histological samples of victims of SCD due to idiopathic fibrosis (IV)
4  Materials and methods

4.1  Study populations

4.1.1  SCD family: Study population I

In this Finnish family, the mother had been resuscitated from VF and the daughter had died suddenly without any prior symptoms (Fig 2). A clinical examination, 12-lead ECG and a transthoracic echocardiographic examination were performed in all first-degree and second-degree family-members. Measurements of left atrium diameter, LV end diastolic and end systolic diameters, LV fractional shortening and Doppler analysis of valve function were obtained from echocardiographic examination. All subjects with any signs of a structural cardiac abnormality in echocardiography were also examined with cardiac magnetic resonance imaging (MRI).

The mother (subject II-3) also underwent an electrophysiological (EP) evaluation after resuscitation from VF. The atrial, atrio-ventricular (AV) nodal and right ventricular (RV) refractory periods and AV conduction time were measured according to standard pacing protocols. The inducibility of ventricular tachyarrhythmias was tested by twice-threshold stimulation at RV apex and outflow tract at 600 and 400 ms basic cycle length with a maximum of three extra stimuli. The shortest cycle length of the extra stimuli was 200ms. The subject who died suddenly (III-5) was subjected to a thorough medico-legal autopsy with appropriate post-mortem chemical laboratory analysis of the blood and tissues. Standard histo-pathological analyses were conducted on all cardiac biopsies.
Fig. 2. Pedigree of the family. Filled symbols indicate affected subjects, while clear symbols indicate unaffected family members. The proband is indicated with an arrow. Diagonal line indicates that the subject is deceased. The symbol “+” indicates mutation positive individuals, “-” mutation negative individuals and “?” the study subject who refused to undergo the genetic evaluation.

4.1.2 FinGesture and other study populations

The FinGesture study population (studies II, III and IV) was derived from 2,661 consecutive victims of SCD in the Province of Oulu, Northern Finland, in whom post-mortem examinations had been performed in the Department of Forensic Medicine of the University of Oulu between 1998 and 2007. The FinGesture database contains 6557 instances of noncardiac causes of sudden death. Victims with non-cardiac causes of sudden death were excluded, also victims of pulmonary embolism were excluded. Altogether 29,610 deaths occurred in the Province of Oulu between 1998 and 2007 and medico-legal autopsy was performed for 9,218 victims.

Since post-mortem studies are mandatory in Finland whenever SCD is not due to a known disease, the deceased had not been treated by a physician during his/her last illness, or when death was otherwise unexpected (Act on the Inquest into the Cause of Death, 459/1973, 7th paragraph: Finnish Law) (Lahti 2005), and thus selection bias of forensic studies in victims with unexpected SCD is minimal. Information about the SCD victims was obtained from a combination of the available medical records, post-mortem examination reports, the medication used
at the time of SCD (Kaikkonen et al. 2006), and a standardized questionnaire to the closest family members of the victims of SCD.

To clarify the prevalence of family history (study III) of SCD in the general population, a control group without a history of coronary heart disease, AMI, or aborted cardiac arrest was included in the study. The controls were subjects from the OPERA (Oulu Project Elucidating Risk of Atherosclerosis) study on randomly selected subjects from the Social Insurance Register covering the entire population of the city of Oulu (Rantala et al. 1999). The mean age of subjects at the beginning of the study was 51 years. The survey on family history was performed 12 years later, when the mean age of the controls did not differ from those of SCD victims. Subjects who died of any cardiac cause or who experienced AMI during the follow-up before the end of 2004, were omitted from the present study. Altogether, 809 controls were included.

In study IV, 10 cases with idiopathic fibrosis as the cause of death were selected from the FinGesture population for the analysis. Control subjects were also collected from the Province of Oulu, Northern Finland, among whom post-mortem examinations were performed at the Department of Forensic Medicine of the University of Oulu. Controls included were cases in whom the death was defined as suicide after toxicologic examination, or caused by a traffic accident, and no cardiac or non-cardiac disease could be found as a cause of unexpected death at autopsy. Seventeen such cases with the same age range as the IMF cases were identified in 2009, which served as a control group.

### 4.2 Determination of cause of death

Definition of the cause of death was based on a combination of medical records, autopsy data, and the results of a questionnaire, and it was classified according to ICD-10 code classes. Furthermore, the classification described in Table 2 was used for more detailed descriptions of the underlying cardiac disease based on post-mortem findings, in conjunction with data obtained from medical records and specific questionnaires of relatives. A histological examination was performed in all cases of SCD. A toxicological investigation was performed when the autopsy findings were insufficient to define a cause of death, or if there was suspicion of a toxic exposure or cause. Obesity was defined as body weight which is 20% above the ideal body weight. Limited genotyping for Finnish founder mutations of long QT syndrome was performed in those victims without any definable structural basis for SCD. The study complied with the Declaration of
Helsinki and the Ethics Committee of the University of Oulu approved the study. The National Authority for Medicolegal Affairs (Valvira) approved the review of post-mortem data by the investigators. Investigation system for sudden death and the means by which a history was obtained are presented in Figure 3.

Fig. 3. Investigation system for sudden death and the means by which the history was obtained.
<table>
<thead>
<tr>
<th>Adjudicated Causes of Death</th>
<th>Descriptive Causes of Death from Autopsy</th>
<th>Autopsy findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary atherosclerosis</td>
<td>CAD with active plaque, thrombosis, or acute myocardial infarction</td>
<td>Chronic atherosclerotic lesions, occlusive or non-occlusive thrombi, acute myocardial infarction, old myocardial scars or diffuse fibrosis.</td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
<td>CAD with healed scar or fibrosis</td>
<td>Chronic atherosclerotic lesions, with old myocardial scars or diffuse fibrosis.</td>
</tr>
<tr>
<td>Chronic ischemic heart disease</td>
<td>Anomalous coronary arteries</td>
<td>Anomalies of origination and course, anomalies of intrinsic coronary arterial anatomy, anomalies of coronary termination, anomalous collateral vessels.</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>Valvular heart disease</td>
<td>Aortic valve calcification, mitral valve calcification.</td>
</tr>
<tr>
<td>Inflammatory heart disease</td>
<td>Inflammatory heart disease</td>
<td>Myocarditis: inflammatory cell infiltrate of the myocardium with necrosis and/or degeneration of the adjacent myocytes.</td>
</tr>
<tr>
<td>Structurally normal heart</td>
<td>No cardiac abnormalities at autopsy</td>
<td>Macroscopically normal heart, normal microscopic findings, with or without identified genetic abnormality; Long QT mutation KCNQ1 Gly589Asp.</td>
</tr>
<tr>
<td>Non-ischemic cardiomyopathy</td>
<td>DCM</td>
<td>Left ventricular dilatation with inadequate degree of LVH, in later stages pale and flabby myocardium and dilatation of both ventricles and atria, unspecific fibrosis and focal atrophy / hypertrophy of myocytes.</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>HCM</td>
<td>Concentric LVH, with myocyte disarray accompanied by various degrees of interstitial fibrosis.</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>HOCM</td>
<td>LVH, with asymmetric septal hypertrophy, myocyte disarray and various degrees of interstitial fibrosis.</td>
</tr>
<tr>
<td>Right ventricular dysplasia/CMP</td>
<td>ARVC</td>
<td>Right ventricular dilation, atrophy of the right ventricular myocardium with fibrofatty replacement of myocytes.</td>
</tr>
<tr>
<td>Other cardiomyopathies</td>
<td>IMF</td>
<td>Interstitial, diffuse or patchy myocardial fibrosis without LVH, myocardial scarring, or other structural abnormalities.</td>
</tr>
<tr>
<td></td>
<td>Hypertensive CMP</td>
<td>Increased heart weight, left ventricular hypertrophy, unspecific fibrosis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other organ changes related to hypertension, e.g. medial hypertrophy and intimal fibrosis in renal arterioles.</td>
</tr>
</tbody>
</table>
4.3 Family history

Familial background of SCD in the first-degree patients was ascertained by mail or telephone call to the control subjects, and by using a previously described questionnaire forwarded to the closest relatives of the victims of SCD (Kaikkonen et al. 2006). The reported SCD cases in the first degree relatives (FDR) were confirmed from death certificates from the Central Statistics Office in Finland and the Causes of Death Register. Complete family history could be obtained from relatives of 223 (38.5%) of 579 non-ischemic SCD victims, 596 (28.6%) of 2082 ischemic SCD victims, and 475 (58.7%) of 809 control subjects.

4.4 Genetic analysis

In study I, blood samples for genotype analysis were taken from the proband (mother), the victim of sudden death (daughter) and from all first- and second-degree family members. Ninety-six healthy subjects served as controls for genetic testing. Screening for mutations for several candidate genes for structural heart diseases was carried out by sequencing DNA extracted from the peripheral blood. The candidate genes used in this study are described in Table 3. DNA samples were amplified by polymerase chain reaction and sequenced with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits using the ABI Prism
3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA). The paternity for the proband was genetically confirmed by typing short tandem repeats (STR) with the ABI PRISM™ 3100 sequencer (Applied Biosystems, Foster City, CA). The results were analyzed with the GeneMapper® Software version 4.0 (Applied Biosystems, Foster City, CA).

Table 3. Candidate genes.

<table>
<thead>
<tr>
<th>Candidate gene</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac troponin T2</td>
<td>TNNT2</td>
</tr>
<tr>
<td>Titin</td>
<td>TTN</td>
</tr>
<tr>
<td>Essential myosin light chain</td>
<td>MYL3</td>
</tr>
<tr>
<td>Cardiac troponin C</td>
<td>TNNC1</td>
</tr>
<tr>
<td>Cardiac myosin binding protein C</td>
<td>MYBPC3</td>
</tr>
<tr>
<td>Regulatory myosin light chain</td>
<td>MYL2</td>
</tr>
<tr>
<td>Beta myosin heavy chain</td>
<td>MYH7</td>
</tr>
<tr>
<td>Alpha myosin heavy chain</td>
<td>MYH6</td>
</tr>
<tr>
<td>Cardiac actin</td>
<td>ACTC</td>
</tr>
<tr>
<td>Alpha tropomyosin</td>
<td>TPM1</td>
</tr>
<tr>
<td>Cardiac troponin I</td>
<td>TNNI3</td>
</tr>
<tr>
<td>Lamin A/C</td>
<td>LMNA</td>
</tr>
<tr>
<td>Desmin</td>
<td>DES</td>
</tr>
<tr>
<td>Delta sarcoglycan</td>
<td>SGCD</td>
</tr>
<tr>
<td>Desmoplakin</td>
<td>DSP</td>
</tr>
<tr>
<td>Phospholamban</td>
<td>PLN</td>
</tr>
<tr>
<td>Cypher/ZASP</td>
<td>LDB3</td>
</tr>
<tr>
<td>Cardiac muscle LIM protein</td>
<td>CLP</td>
</tr>
<tr>
<td>ATP-sensitive K channel</td>
<td>ABCC9</td>
</tr>
<tr>
<td>Cardiotrophin 1</td>
<td>CTF1</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>DMD</td>
</tr>
<tr>
<td>Tafazzin</td>
<td>TAZ</td>
</tr>
<tr>
<td>Cardiac ryanodine receptor</td>
<td>RYR2</td>
</tr>
<tr>
<td>Plakophilin-2</td>
<td>PKP2</td>
</tr>
<tr>
<td>Telethonin</td>
<td>TELT</td>
</tr>
<tr>
<td>Dystrobrevin, alpha</td>
<td>DTNA</td>
</tr>
<tr>
<td>Syntrophin, alpha 1</td>
<td>SNTA1</td>
</tr>
<tr>
<td>FK506 binding protein 1A</td>
<td>FKBP1A</td>
</tr>
</tbody>
</table>
4.5 Transmission electron microscopy

In study I, heart specimens were primary fixed post-mortem in 10% neutral formalin for 24 hours and then transferred to 1% glutaraldehyde 4% formaldehyde mixture in 0.1 M phosphate buffer for 24 hours. Tissue samples were postfixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Epon LX 112 (Ladd Research Industries, Vermont, USA). Thin sections were cut with Leica Ultracut UCT ultramicrotome, stained in uranyl acetate and lead citrate and examined in Philips CM100 transmission electron microscope. Images were captured by CCD camera equipped with TCL-EM-Menu version 3 from Tietz Video and Image Processing Systems GmbH (Gaunting, Germany).

4.6 Immunohistochemistry

In study IV, formalin-fixed paraffin-embedded heart tissues were obtained from the archives of the Institute of Diagnostics, Department of Forensic Medicine, University of Oulu, Finland. Tissue sections (5 µm) were deparaffinized and rehydrated before epitope-retrieval. Immunohistochemical staining was carried out by Autostainer Plus (Dako) using EnVision (Peroxidase/DAB) detection system (Dako). Polyclonal antibodies recognizing PINP and type IV collagen (Bode et al. 2000) were used at 1:10,000 and 1:1,000 dilution, respectively. In addition, polyclonal antibodies recognizing ICTP, PIINP and IIINTP were used at 1:2,000, 1:4,000 and 1:4,000 dilutions, respectively. Furthermore, additional dilution series 1:500, 1:1,000 and 1:1,000 were tested for ICTP, PIINP and IIINTP, respectively. Slides were counterstained with hematoxylin.

4.7 Statistical analysis

All analyses were performed with the Statistical Package for Social Studies version 13.0 (SPSS Inc, Chicago, Ill). \( p < 0.05 \) was considered statistically significant.

In study II, two-sided \( t \) test and \( \chi^2 \) analyses were used for comparisons between study groups. When comparisons were made between three age groups, analysis of variance with Bonferroni post-hoc analysis was used for multiple comparisons.

In study III, two-sided \( t \) test and \( \chi^2 \) analyses were used for comparisons between study groups. Logistic regression analysis was used to assess the
significance of family history of SCD between the groups after adjustment for age, gender, smoking, BMI, history of diabetes mellitus, hypercholesterolemia, hypertension or angina pectoris, number of first-degree relatives, and rate of adequate information on the mode of death of the family members in each group. The GEE-analysis (Generalized Estimating Equations), which adjusts for the correlation among family members, was also utilized. \( \chi^2 \) analysis was used for comparison of SCD among FDR between the victims of ischemic SCD with or without an infarct scar at autopsy.

In addition, in study IV, two-sided t test and \( \chi^2 \) analyses were used for comparisons between study groups, respectively.
5 Results

5.1 SCD family (I)

5.1.1 Clinical and cardiac imaging findings

The 40-year-old female proband (Subject II-3) with no prior cardiac or systemic symptoms suffered a sudden collapse while playing volleyball in 2005. Cardiopulmonary resuscitation was initiated immediately by lay persons and the patient was successfully converted from VF to sinus rhythm by DC-shock performed by the paramedics. Cardiac troponin levels measured in the emergency room of the Oulu University Hospital were normal. The 12-lead ECG displayed sinus rhythm with normal PR interval and mildly inverted T-waves in the precordial leads V1-V6. The coronary angiogram and the results of EP study (e.g., AV conduction) were normal. No sustained tachyarrhythmias were inducible by programmed ventricular stimulation. Endomyocardial biopsies taken from the right ventricular septum did not reveal any structural abnormalities.

A local infero-posterior hypokinesia was observed on echocardiography and MRI. In this area, the cardiac wall had thinned to 2–3 mm compared to 9 mm thickness of the septum and 8 mm thickness of the lateral wall. The global LV function (EF 54%) and size (end-diastolic dimension 57 mm) were within the normal limits. A single chamber ventricular rate responsive (VVIR) implantable cardioverter defibrillator (ICD) was implanted. The bradycardia pacing lower rate was programmed for an ICD 30 bpm. The ventricular tachycardia detection zone was set to 170–200 bpm (monitor only) and the VF detection setting was 200 bpm (31J shock). During the follow-up of 2 years, her LV function and size have remained unchanged, and no ICD therapies either appropriate or inappropriate have occurred.

Subject III-5 was the 14-year-old daughter of subject II-3. She had no prior symptoms or any medical history cardiac related conditions. She experienced a sudden witnessed collapse while standing on the street with her friends in the evening. No resuscitation attempts were initiated and she died before help arrived on the scene.

The other family members did not exhibit any echocardiographic abnormalities or changes in the MRI or ECG and had suffered no symptoms attributable to arrhythmias or heart failure.
5.1.2 Medico-legal autopsy

In the autopsy of subject III-5, macroscopic findings from the heart revealed a thin and fibrotic infero-posterior LV wall. Fibrosis also extended partly to the basal area and the apex of the LV and up to 1 centimeter from the posterior mitral ring. The valves and coronary arteries were normal and the skeletal muscles were also normal in both macroscopic and histo-pathological examinations. The organ specific examinations and chemical laboratory analysis uncovered no other cause of death. The hematoxylin and eosin staining of the cardiac biopsies from the autopsy revealed fibrosis in the LV posterior wall (Fig 4A) and mild diffuse fibrosis in the RV (Fig 4B). At the ultrastructural level, nuclei were irregular in shape (Fig 5A), having nuclear membrane infoldings (Fig 5B) and focal densities in the peripheral areas of nuclei (Fig 5C). Nuclear pore structures were difficult to detect. The cause of death was determined as sudden cardiac death due to unspecified cardiomyopathy.

Fig. 4. Subject III-5. (A) Left ventricle posterior wall of subject III-5. Hematoxylin and eosin staining of cardiac biopsy of LV posterior wall. Biopsy was taken during the autopsy. Replacement of myocytes by fibrosis was the predominant finding. There was also a loss of normal cell-to-cell coupling of the myocytes. (B) Right ventricle of subject III-5. Hematoxylin and eosin staining of cardiac biopsy of right ventricle. Biopsy was taken during the autopsy.
5.1.3 Genetic analyses

Several candidate genes (Table 3) (TNNT2, TNNI3, MYH7, DSP, PKP2, DTNA, SNTA1, LDB3, TAZ, and FKBP1A) were sequenced before the lamin A/C gene. Sequencing of the lamin A/C gene revealed a C to T transition in nucleotide position 1621. This results in a change of an arginine to a cysteine in amino acid position 541. Mutation R541C was found in the two affected subjects, but not in any other first- or second-degree family members. One family member (III-7) refused to undergo the genetic evaluation. In addition, the mutation was not found in 96 normal unrelated subjects. Paternity testing by typing the polymorphic STR markers confirmed the paternity for the proband.

5.2 Characteristics of Fin Gesture population

The study data were derived from a total of 2,661 SCD victims, whose deaths occurred between 1998 and 2007. Based upon a population of 467,190 in the Province of Oulu in the year 2007 (Statistics Finland), the annual incidence of SCD was established at 56.9 deaths per 100,000 inhabitants (0.06 percent per year). A non-ischemic cause of SCD was found in 579 victims (21.8% of all the SCDs). The characteristics of the total study population, including both ischemic and non-ischemic SCD are summarized in Table 4.
Table 4. Characteristics of study subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Victims of non-ischemic SCD (n = 579)</th>
<th>Victims of ischemic SCD (n = 2082)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54.7 (± 12.1)</td>
<td>65.1 (± 11.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>452 (78.1)</td>
<td>1674 (80.1)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>29.5 (± 8.1)</td>
<td>26.8 (± 5.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total heart weight, g</td>
<td>503.6 (± 152.8)</td>
<td>477.8 (± 119.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>182 (33.9)</td>
<td>697 (38.1)</td>
<td>0.041</td>
</tr>
<tr>
<td>Diabetes type I or II</td>
<td>82 (15.1)</td>
<td>366 (19.3)</td>
<td>0.014</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>37 (6.9)</td>
<td>174 (9.4)</td>
<td>0.036</td>
</tr>
<tr>
<td>Location at the time of SCD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td>81.6%</td>
<td>71.0%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Public location</td>
<td>18.4%</td>
<td>29.0%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Time of death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12am-12pm</td>
<td>60.3%</td>
<td>51.5%</td>
<td>0.004</td>
</tr>
<tr>
<td>12pm-6pm</td>
<td>20.8%</td>
<td>29.4%</td>
<td>0.001</td>
</tr>
<tr>
<td>6pm-12am</td>
<td>18.9%</td>
<td>19.1%</td>
<td>NS</td>
</tr>
<tr>
<td>Prior cardiac history (no)</td>
<td>66.0%</td>
<td>54.8%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SD) or number of subjects (%).

BMI indicates body mass index; SCD, sudden cardiac death.

The mean age of non-ischemic SCD victims was 54.7 years (± 12.1) with the majority (78.1%) being males. The age of ischemic SCD victims was significantly higher (65.1 years ± 11.4, p < 0.000). There also were statistically significant differences between the two groups in terms of total heart weight, the history of hypertension, diabetes and hypercholesterolemia, respectively. The vast majority of all SCDs occurred in the home, with a higher proportion among non-ischemic SCD victims (81.6%), compared to ischemic SCDs (71.0%). In addition, among non-ischemic SCD victims, 60.3% of the deaths occurred during the 12-hour period between midnight and noon, compared to 51.5% of the ischemic SCDs. There was a lower SCD event rate (~19%) between 6 pm and midnight in both groups. SCD was also more frequently the first cardiac event among those with non-ischemic compared to the situation in the ischemic SCD victims (66% vs. 55%, p < 0.001).

5.3 Causes of non-ischemic SCD (II)

The causes of SCD are presented in Table 5. CAD was the most common cause of death (2082 victims, 78.2%). The prevalence of non-ischemic SCDs was 21.8%
of all the SCDs. After sub-grouping the non-ischemic SCDs into various categories, the most common cause of death was CMP related to obesity (23.7%), followed by alcoholic CMP (19.0%), hypertensive CMP (15.5%) and IMF (13.6%). All nine cases with no cardiac abnormalities at autopsy were screened for Finnish founder mutations for long QT syndrome. The $KCNQ1$ (potassium voltage-gated channel, KQT-like subfamily, member 1 gene) mutation G589D was identified in two SCD victims.

### Table 5. Underlying cardiac disease of victims of SCD.

<table>
<thead>
<tr>
<th>Cause of non-ischemic SCD</th>
<th>Victims of non-ischemic SCD (n = 579)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anomalous coronary arteries</td>
<td>3 (0.5)</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>29 (5.0)</td>
</tr>
<tr>
<td>Inflammatory cardiac disease</td>
<td>24 (4.1)</td>
</tr>
<tr>
<td>Structurally normal heart</td>
<td>9 (1.6)</td>
</tr>
<tr>
<td>DCM</td>
<td>23 (4.0)</td>
</tr>
<tr>
<td>HCM</td>
<td>11 (1.9)</td>
</tr>
<tr>
<td>HOCM</td>
<td>4 (0.7)</td>
</tr>
<tr>
<td>ARVC</td>
<td>7 (1.2)</td>
</tr>
<tr>
<td>IMF</td>
<td>79 (13.6)</td>
</tr>
<tr>
<td>Hypertensive CMP</td>
<td>90 (15.5)</td>
</tr>
<tr>
<td>Alcoholic CMP</td>
<td>110 (19.0)</td>
</tr>
<tr>
<td>CMP related to obesity</td>
<td>137 (23.7)</td>
</tr>
<tr>
<td>Non-specified CMP</td>
<td>53 (9.1)</td>
</tr>
</tbody>
</table>

Values are expressed as number of subjects (%).

Abbreviations: ARVC, arrhythmogenic right ventricular dysplasia; CMP, cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; IMF, idiopathic myocardial fibrosis.

Causes of non-ischemic SCD were analyzed separately for subjects under 40 years of age, between 40 and 59, and over 60 (Table 6). Only 53 of 579 non-ischemic SCDs (9.2%) occurred in those individuals under 40 years old. IMF was the most common association, observed in 28.3% of SCDs, and CMP associated with obesity was adjudicated as the cause of death in 26.4% of the victims. Among those between the ages of 40 and 59 years, the incidence of alcoholic CMPs increased to 25.8% and in CMP the association of SCD with CMP in the presence of obesity was observed in 23.7% of the victims. In victims over 60 years of age, the prevalence of CMP related to obesity was 22.8% and hypertensive CMPs accounted for 20.6%.
Table 6. Underlying cardiac disease of victims of SCD at different age groups.

<table>
<thead>
<tr>
<th>Cause of non-ischemic sudden cardiac death</th>
<th>0–39 years (n = 53)</th>
<th>40–59 years (n = 337)</th>
<th>&gt; 60 years (n = 189)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anomalous coronary arteries (n = 3; 0.5%)</td>
<td>1/53 (1.9)</td>
<td>1/337 (0.3)</td>
<td>1/189 (0.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Valvular heart disease (n = 29; 5.0%)</td>
<td>1/53 (1.9)</td>
<td>10/337 (3.0)</td>
<td>18/189 (9.5)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Inflammatory cardiac disease (n = 24; 4.1%)</td>
<td>2/53 (3.8)</td>
<td>15/337 (4.5)</td>
<td>7/189 (3.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Structurally normal heart (n = 9; 1.6%)</td>
<td>2/53 (3.8)</td>
<td>6/337 (1.8)</td>
<td>1/189 (0.5)</td>
<td>NS</td>
</tr>
<tr>
<td>DCM (n = 23; 4.0%)</td>
<td>3/53 (5.7)</td>
<td>9/337 (2.7)</td>
<td>11/189 (5.8)</td>
<td>NS</td>
</tr>
<tr>
<td>HCM (n = 11; 1.9%)</td>
<td>3/53 (5.7)</td>
<td>6/337 (1.8)</td>
<td>2/189 (1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>HOCM (n = 4; 0.7%)</td>
<td>0/53 (0.0)</td>
<td>2/337 (0.6)</td>
<td>2/189 (1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>ARVC (n = 7; 1.2%)</td>
<td>2/53 (3.8)</td>
<td>2/337 (0.6)</td>
<td>3/189 (1.6)</td>
<td>NS</td>
</tr>
<tr>
<td>IMF (n = 79; 13.6%)</td>
<td>15/53 (28.3)</td>
<td>43/337 (12.8)</td>
<td>21/189 (11.1)</td>
<td>0.004†</td>
</tr>
<tr>
<td>Hypertensive CMP (n = 90; 15.5%)</td>
<td>4/53 (7.5)</td>
<td>47/337 (13.9)</td>
<td>39/189 (20.6)</td>
<td>0.031</td>
</tr>
<tr>
<td>Alcoholic CMP (n = 110; 19.0%)</td>
<td>3/53 (5.7)</td>
<td>87/337 (25.8)</td>
<td>20/189 (10.6)</td>
<td>&lt; 0.001‡</td>
</tr>
<tr>
<td>CMP related to obesity (n = 137; 23.7%)</td>
<td>14/53 (26.4)</td>
<td>80/337 (23.7)</td>
<td>43/189 (22.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Non-specified CMP (n = 53; 9.1%)</td>
<td>3/53 (5.7)</td>
<td>29/337 (8.6)</td>
<td>21/189 (11.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as number of subjects (%). * indicates statistically significant p-value between the age groups 40–59 years and > 60 years; † indicates statistically significant p-value between the age groups 0–39 years and > 60 years; ‡ indicates statistically significant p-value between the age groups 40–59 years and > 60 years.

Abbreviations: ARVC, arrhythmogenic right ventricular dysplasia; CMP, cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; IMF, idiopathic myocardial fibrosis.

5.4 Family history of SCD (III)

5.4.1 Patient characteristics

Table 7 presents the demographic data and some clinical features of victims SCD and controls. Age and BMI differed between the groups with non-ischemic victims being younger and they had higher BMI values than ischemic SCD victims or controls. Smoking status also differed between the groups, with the number of current smokers being significantly higher in the non-ischemic and ischemic SCD victims in comparison with controls. The prevalences of hypertension and diabetes were more common in non-ischemic SCD victims compared to controls. The same was true in the comparison of ischemic SCD victims and controls. Only about one third of the non-ischemic SCD victims had a prior cardiac history, thus in ~70% of the victims, death was the very first
manifestation of cardiac disease. In ischemic SCDs, a prior cardiac history existed in 40% of the victims.

Table 7. Characteristics of study subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Victims of non-ischemic SCD (N = 223)</th>
<th>Victims of ischemic SCD (N = 596)</th>
<th>Controls (N = 459)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>53.4 (11.3)*†</td>
<td>64.9 (11.2)</td>
<td>62.5 (5.8)</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>176/223 (78.9)</td>
<td>483/596 (81.0)†</td>
<td>252/459 (54.9)</td>
</tr>
<tr>
<td>BMI</td>
<td>30.0 (7.9)*†</td>
<td>26.7 (4.9)</td>
<td>27.2 (4.3)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>72/223 (32.3)‡</td>
<td>204/596 (34.2)‡</td>
<td>202/406 (49.8)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>26/223 (11.7)*†</td>
<td>126/596 (21.2)</td>
<td>99/406 (24.3)</td>
</tr>
<tr>
<td>Current</td>
<td>125/223 (56.0)*†</td>
<td>286/596 (44.6)‡</td>
<td>105/406 (25.9)</td>
</tr>
<tr>
<td>Diabetes type I or II</td>
<td>50/223 (22.4)†</td>
<td>153/596 (25.6)‡</td>
<td>6/424 (1.4)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>106/223 (47.5)†</td>
<td>368/596 (61.8)‡</td>
<td>147/421 (34.9)</td>
</tr>
<tr>
<td>Prior cardiac history</td>
<td>75/223 (32.3)*†</td>
<td>246/596 (41.3)</td>
<td></td>
</tr>
</tbody>
</table>

BMI indicates body mass index. Values are expressed as mean (SD) or number of subjects (%). Probability values refer to one-way ANOVA and logistic regression analyses between groups.

*p < 0.001 non-ischemic SCD vs ischemic SCD; †p < 0.001 non-ischemic SCD vs control; ‡p < 0.001 ischemic SCD vs control.

5.4.2 Causes of non-ischemic SCD

CMP related to obesity was the most common cause of death (45.8%) among the non-ischemic SCDs (Table 8). IMF (20.0%), hypertensive CMP (13.8%) and alcoholic CMP (9.3%) were other frequent causes of non-ischemic SCDs.

Table 8. Causes of non-ischemic death.

<table>
<thead>
<tr>
<th>Cause of non-ischemic death</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive CMP</td>
<td>31/223 (13.8)</td>
</tr>
<tr>
<td>Inflammatory cardiac disease</td>
<td>1/223 (0.4)</td>
</tr>
<tr>
<td>DCM</td>
<td>9/223 (4.0)</td>
</tr>
<tr>
<td>Alcoholic CMP</td>
<td>21/223 (9.3)</td>
</tr>
<tr>
<td>HCM</td>
<td>9/223 (4.0)</td>
</tr>
<tr>
<td>CMP related to obesity</td>
<td>103/223 (45.8)</td>
</tr>
<tr>
<td>ARVC</td>
<td>4/223 (1.8)</td>
</tr>
<tr>
<td>IMF</td>
<td>45/223 (20.0)</td>
</tr>
</tbody>
</table>

Abbreviations: ARVC, arrhythmogenic right ventricle dysplasia; CMP, cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; IMF, idiopathic myocardial fibrosis.
5.4.3 Family history of SCD

The prevalence of SCD among the FDRs of non-ischemic SCD victims with various underlying causes of cardiac disease is described in Table 9. Only those with a HCM tended to have a higher prevalence of SCD in FDRs when compared to the other groups or controls, the prevalence being within the same range as in those with ischemic SCD.

In a subgroup analysis of ischemic SCD victims with and without autopsy evidence of old infarct scar, there was no difference in the family history of SCD between those with (33.1%) or without an infarct scar (29.9%) (p = 0.222).

Table 9. Family history of SCD in non-ischemic SCDs.

<table>
<thead>
<tr>
<th>Cause of non-ischemic SCD (N = 223)</th>
<th>Family history of SCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive CMP (N = 31)</td>
<td>3/31 (9.7)</td>
</tr>
<tr>
<td>Inflammatory cardiac disease (N = 1)</td>
<td>1/1 (100.0)</td>
</tr>
<tr>
<td>DCM (N = 9)</td>
<td>0/9 (0.0)</td>
</tr>
<tr>
<td>Alcoholic CMP (N = 21)</td>
<td>3/21 (14.3)</td>
</tr>
<tr>
<td>HCM (N = 9)</td>
<td>3/9 (33.3)</td>
</tr>
<tr>
<td>CMP related to obesity (N = 103)</td>
<td>13/103 (12.6)</td>
</tr>
<tr>
<td>ARVC (N = 4)</td>
<td>0/4 (0.0)</td>
</tr>
<tr>
<td>IMF (N = 45)</td>
<td>7/45 (15.6)</td>
</tr>
</tbody>
</table>

Abbreviations: ARVC, arrhythmogenic right ventricle dysplasia; CMP, cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; IMF, idiopathic myocardial fibrosis.

The total number of SCD cases among the total number of FDRs of the non-ischemic SCD victims (3.2%) did not differ from control subjects (2.4%) (Table 10). However, the number of SCDs among FDRs was significantly higher in ischemic (5.8%) than non-ischemic (3.2%) SCD victims (OR 2.9, 95% CI 1.9 to 4.5, p < 0.001; adjusted OR 2.1, 95% CI 1.2 to 3.7, p = 0.011). Furthermore, the number of SCD cases among the FDRs of the ischemic SCD victims (5.8%) was higher than in control subjects (2.4%) (OR 2.1, 95% CI 1.6 to 2.8, p < 0.001; adjusted OR 1.6, 95% CI 1.1 to 2.5, p = 0.027).

Table 10. History of SCD among first-degree relatives.

<table>
<thead>
<tr>
<th>Family History</th>
<th>FDR of non-ischemic SCD (N = 1770)</th>
<th>FDR of ischemic SCD (N = 5651)</th>
<th>FDR of controls (N = 3671)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD among FDR</td>
<td>56/1770 (3.2) *</td>
<td>328/5651 (5.8) †</td>
<td>87/3671 (2.4)</td>
</tr>
</tbody>
</table>

FDR indicates first-degree relatives. Values are number of subjects (%).

*p < 0.001 non-ischemic SCD vs ischemic SCD; †p < 0.001 ischemic SCD vs control.
The prevalence of a positive family history of SCD was more common in ischemic (34.2%) than in non-ischemic (13.4%) SCD cases (OR 4.3, 95% CI 2.1 to 8.7, \( p < 0.001 \); adjusted OR 5.3, 95% CI 2.0 to 14.4, \( p = 0.001 \)) (Table 11). The history of SCD in 2 or more FDRs was also higher in ischemic (14.7%) than non-ischemic SCD (4.0%) victims (OR 4.3, 95% CI 2.1 to 8.7, \( p < 0.001 \); adjusted OR 6.2, 95% CI 1.8 to 21.5, \( p = 0.004 \), respectively). Similarly, ischemic SCD victims had a higher prevalence of SCD in FDRs as compared to controls (17.6%) (OR 12.5, 95% CI 5.4 to 28.9, \( p < 0.001 \); adjusted OR 13.5, 95% CI 4.3 to 42.8, \( p < 0.001 \)).

When non-ischemic SCD victims (14.7%) were compared to controls, a positive family history of SCD was observed somewhat more often in controls, but the difference disappeared after adjustments for confounding variables (17.6%) (OR 0.5, 95% CI 0.3 to 0.9, \( p = 0.020 \); adjusted OR 0.9, 95% CI 0.4 to 1.7, \( p = 0.641 \)) (Table 11). However, a history of SCD in 2 or more FDRs was higher in non-ischemic SCD victims (4.0%) as compared with controls (1.3%) (OR 2.9, 95% CI 1.1 to 8.5, \( p = 0.040 \); adjusted OR 19.0, 95% CI 1.2 to 308.6, \( p = 0.039 \)).

### Table 11. Family history of SCD.

<table>
<thead>
<tr>
<th>SCD among FDR</th>
<th>Victims of non-ischemic SCD (N = 223)</th>
<th>Victims of ischemic SCD (N = 596)</th>
<th>Controls (N = 459)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>30/223 (13.4)†</td>
<td>204/596 (34.2)‡</td>
<td>81/459 (17.6)</td>
</tr>
<tr>
<td>Yes, &gt; 1 SCD among FDR</td>
<td>9/223 (4.0)†</td>
<td>88/596 (14.7)‡</td>
<td>6/459 (1.3)</td>
</tr>
</tbody>
</table>

FDR indicates first-degree relative. Values are number of subjects (%). Probability values refer to logistic regression analysis between groups.

\( \ast p < 0.001 \) non-ischemic SCD vs ischemic SCD; \( \dagger p < 0.05 \) non-ischemic SCD vs control; \( \ddagger p < 0.001 \) ischemic SCD vs control.

Dependence of correlation among family members to the presence of family history were taken into account with GEE analysis; the family members of ischemic SCD victims were more likely to have another SCD in their family, compared to family members of non-ischemic SCD victims (OR 1.8, 95% CI 1.1 to 2.9, \( p = 0.011 \)). The difference was not statistically significant between the family members of the non-ischemic SCD victims and the family members of control cases (OR 1.3, 95% CI 0.8 to 2.2, \( p = 0.234 \)), whereas the prevalence was statistically significant between the family members of ischemic SCD victims and the family members of control cases (OR 2.4, 95% CI 1.9 to 3.1, \( p < 0.001 \)).
5.5 The collagen content of the diseased myocardium (IV)

5.5.1 Patient characteristics

The detailed patient characteristics and description of the fibrosis are presented in Table 12. The age, gender and body mass index (BMI) distribution was not different between cases and controls (age 43.1 (SD 17.2) vs. 41.9 (16.1) years ($p = 0.865$), male gender 50.0% vs. 76.5% ($p = 0.162$), BMI 24.2 (3.3) vs. 22.7 (0.8) ($p = 0.438$)). Different formations of fibrosis were observed in various locations of the myocardium (Table 12). One of the victims (patient # 4) had lamin A/C gene mutation R541C (15). In subsequent genetic testing, the same LMNA mutation was not observed in any other cases of IMF. Two of the SCD victims had a family history of SCD.

Table 12. Detailed patient characteristics.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age</th>
<th>BMI</th>
<th>Heart weight (g)</th>
<th>Description of the fibrosis and other information</th>
<th>Prior cardiac history</th>
<th>Family history of SCD</th>
<th>Cardiac medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>male</td>
<td>38</td>
<td>25.4</td>
<td>409</td>
<td>Focal fibrosis in LV</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient 2</td>
<td>female</td>
<td>65</td>
<td>23.4</td>
<td>331</td>
<td>Patchy fibrosis in septum</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient 3</td>
<td>female</td>
<td>30</td>
<td>22.8</td>
<td>335</td>
<td>Patchy fibrosis in LV</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Patient 4</td>
<td>female</td>
<td>14</td>
<td>23.3</td>
<td>260</td>
<td>Focal fibrosis in LV posterior wall, diffuse fibrosis in RV, LMNA mutation</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Patient 5</td>
<td>female</td>
<td>43</td>
<td>17.8</td>
<td>212</td>
<td>Perivascular fibrosis and diffuse fibrosis in LV</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient 6</td>
<td>male</td>
<td>50</td>
<td>29.7</td>
<td>410</td>
<td>Diffuse fibrosis in LV</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient 7</td>
<td>male</td>
<td>43</td>
<td>26.3</td>
<td>416</td>
<td>Diffuse fibrosis in LV</td>
<td>Alcohol abuse related atrial fibrillation</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient 8</td>
<td>male</td>
<td>37</td>
<td>22.1</td>
<td>418</td>
<td>Severe patchy fibrosis in whole myocardium</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient 9</td>
<td>male</td>
<td>75</td>
<td>21.0</td>
<td>363</td>
<td>Patchy perivascular fibrosis and diffuse fibrosis in LV</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient 10</td>
<td>female</td>
<td>36</td>
<td>30.3</td>
<td>322</td>
<td>Diffuse fibrosis in LV and mild perivascular fatty degeneration</td>
<td>Small VSD</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; LV, left ventricle; RV, right ventricle.
5.5.2 Immunohistochemistry

The amount of PINP was clearly increased in the fibrotic tissue of the IMF cases in comparison with control samples in all cases when the 1:10,000 antibody dilution was used (Figure 6A and 6B). The amount of ICTP, the peptide form that reflects collagen type I degradation, did not differ between the fibrosis cases and control myocardium (Figure 7A and 7B). Immunohistochemical staining of PIIINP did not reveal any differences between the victims of SCD and controls with 1:4,000 antibody dilution. However, by using the antibody dilution of 1:2,000, small amounts of PIIINP could be detected in two IMF cases with the most evident fibrosis in macroscopic evaluation, including the lamin A/C mutation case (Figure 8A and (B); in other IMF cases, the staining remained negative. The amount of IIINTP did not differ between the SCD cases and control myocardium (Figure 9A and 9B) indicating that there is no increased production of mature cross-linked collagen type III.

Fig. 6. Immunohistochemical staining of PINP in myocardium of idiopathic fibrosis case (A) and in control case (B). Magnification x200.
Fig. 7. Immunohistochemical staining of ICTP in myocardium of idiopathic fibrosis case (A) and in control case (B). Magnification x200.

Fig. 8. Immunohistochemical staining of PIIINP in myocardium of lamin A/C mutation case (A) and in control case (B). Magnification x200.

Fig. 9. Immunohistochemical staining of IIINTP in myocardium of idiopathic fibrosis case (A) and in control case (B). Magnification x200.
6 Discussion

6.1 Clinical presentations of patients with lamin A/C mutation (I)

Study I investigated a family where the first clinical manifestation of the lamin A/C gene mutation R541C was cardiac arrest. The mutation was detected in only the two affected family members (mother – the proband, and the deceased daughter) and was absent in the DNA of other first-degree family members, including the mother and father of the proband. The paternity for the proband was genetically confirmed. Thus, the mutation had arisen de novo in this family. De novo mutations have been previously reported to be quite frequent in the lamin A/C gene (Bonne et al. 1999, Bonne et al. 2000).

The phenotype of the two affected subjects was somewhat different from that of the previously described lamin A/C mutations. Usually, clinically affected lamin A/C mutation carriers exhibit signs of a DCM with AV conduction disturbances at middle age with mild to moderate impairment of LV contraction and they display the symptoms of heart failure (Becane et al. 2000, Fatkin et al. 1999, Karkkainen et al. 2004). Most of the mutations in these patients and families have been located in exons 1, 2 and 3 of the lamin A/C gene. The lamin A/C mutation observed in the present family (R541C) was located in exon 10. These findings suggest that the location of the mutation in the lamin A/C gene may at least in part determine the phenotype and clinical presentation in the patients. The same mutation as observed here has been previously described only in a French family, where it manifested as an LV aneurysm and ventricular tachyarrhythmias (Forissier et al. 2003).

It is evident that various mutations in the lamin A/C gene may result in heterogeneous cardiac abnormalities as well as a variable clinical presentation. DCM-associated lamin A/C mutations have been reported to cause very similar phenotypes (Arbustini et al. 2002, Fatkin et al. 1999, Karkkainen et al. 2004) characterised by global LV involvement rather than localized LV abnormalities. In contrast, the R541C mutation observed here and in the previously reported family (Forissier et al. 2003) resulted in a more localized involvement of the LV with no significant changes in other parts of the heart. The local abnormality may also create heterogeneity in cardiac electrical properties and perhaps explain the arrhythmia vulnerability related to this specific mutation of the lamin A/C gene.
6.1.1 Pathologic-anatomic findings of patients with lamin A/C mutations

Lamins A and C belong to the intermediate filament protein family and are structural components of the nuclear lamina. The mechanism by which mutations of the lamin A/C gene lead to the above described pathologies is not well understood. It has been proposed that nuclear fragility and alterations in the regulation of gene expression related to the lamin A/C mutations may result in a dysfunction of the nuclear membrane and cell death (Franz et al. 2001, Novelli & D'Apice 2003). Fibrosis, fatty infiltration, and degenerative myocyte changes have also been observed in the AV junctions of the affected subjects.

To our knowledge, there has been no autopsy data reported from deceased subjects with a lamin A/C R541C mutation. Previous pathologic-anatomic data of the patients with lamin A/C mutation in other exons have shown four-chamber dilatation, myocyte hypertrophy and fibrosis without inflammation (Fatkin et al. 1999). A histo-pathological study of the conduction system of a lamin A/C mutation carrier revealed fibrosis and fatty metamorphosis in the sinoatrial and AV node, as well as in the AV bundle branches (Bharati et al. 1992). In addition, disruption of the nuclear membrane has been observed in samples taken from the hearts of lamin A/C mutation carriers (Verga et al. 2003). In the case of the 14-year-old victim of SCD described here, some abnormalities at the ultrastructural level were detected, such as nuclear membrane infoldings and focal densities in the peripheral areas of the nuclei. In addition, nuclear pore structures were difficult to detect, but it is unclear whether this difficulty was due to post-mortem specimen deterioration. The autopsy of the victim of SCD revealed thinning and fibrosis of the LV. The most prominent feature was localization of the cardiac abnormality to the infero-posterior area of the heart with only a minor involvement of the other areas, including the RV. In the histo-pathological examination, the typical finding was a loss of myocytes with their replacement by fibrosis. A strong association between myocardial fibrosis and sudden death in otherwise normal hearts has been described previously by Chugh et al. (2000) and John et al. (2004). John et al. also reported an interesting finding that fibrosis was most prominent in the infero-posterior LV wall in patients with IMF and sudden death.
6.2 Underlying cardiac disease in victims of non-ischemic SCD (II)

The results of study II confirm, in part, the previous data on the proportions of CAD and CMP as causes of SCD in the general population in the current era. The novel finding in study II is the observation of higher prevalences of CMP-associated SCDs related to obesity, alcoholic CMP and myocardial fibrosis as clinical and/or pathological bases for non-ischemic SCDs. Obesity is a well-recognized risk factor for coronary atherosclerosis and an association with ischemic SCD would be anticipated. However, the stronger association with non-ischemic SCD in this study was not anticipated, and is still yet unexplained. The greater frequency of nocturnal SCD in the non-ischemic group, in connection with the obesity data, supports a possible contribution of a sleep apnea-related mechanism of SCD. The incidence of 56.9 SCDs per 100,000 inhabitants is in line with other similar studies in western countries (Byrne et al. 2008, Priori et al. 2001). As reported earlier, a large proportion of SCDs occur in the home (75%), compared to public places (de Vreede-Swagemakers et al. 1997). A minor circadian pattern of SCD was noted, with a lower rate in the evening hours, but the proportion was evenly distributed across the other 6 hour intervals between midnight and 6 PM. SCD was also more commonly a first event of cardiac disease (2/3 of the victims) in the group with a non-ischemic SCD compared to ischemic SCD.

The most prevalent subtypes of CMP among victims of non-ischemic SCD were alcoholic CMP, IMF and CMP related to obesity (Table 5). The association of CMP with obesity was relatively common even in subjects under the age 40 years in the present study. The CMP subtype related to obesity is characterized by cardiomegaly, LV dilatation, and myocyte hypertrophy in the absence of interstitial fibrosis or evidence of CAD (Duflou et al. 1995). Obesity has become a global epidemic and is recognized as a risk factor for cardiac diseases. The existence of CMP of obesity, as a distinct disease association causing SCD has not been generally recognized (Owan & Litwin 2007) and a better understanding and improved identification of this CMP subtype are needed in view of the increasing obese patient population. We feel that the clinical entity of CMP related to obesity (without CAD) is not very well recognized by either clinicians or investigators in this field, but is well known among the pathologists who perform large amounts of autopsies in victims of SCD (Kortelainen & Porvari 2011), at least in Finland where the autopsy rate is much higher than in the majority of other western countries. In addition, the recent study of Tavora et al.
(2012) reported that among adults with a mean age of about 50 years, cardiomegaly is a frequent cause of SCD, and is clearly associated with obesity.

Alcoholic CMP is a heart muscle disease characterized by dilated LV, normal or reduced LV wall thickness, and increased LV mass. A diagnosis of alcoholic CMP is made only if other causes of death are excluded. The key factor in the diagnosis is a long-term history of heavy alcohol abuse and findings of other organ changes related to excessive long-term alcohol consumption (Table 2). If there is no history of alcohol abuse but there is a distinct alcohol disease at autopsy, for example liver cirrhosis or evidence of prior pancreatitis in addition to CMP, the diagnosis of alcoholic CMP can be inferred after careful consideration. The prevalence of alcoholic CMP among alcoholics is variable, from 23 to 40%, and it occurs more frequently in men than in women (Gavazzi et al. 2000, Piano 2002). The prevalence of alcoholic CMP as a distinct disease etiology causing SCD in the general population has been evaluated only in few previous studies (Bowker et al. 2003, Byrne et al. 2008) with a much lower prevalence, perhaps because it may have often been affiliated as a part of DCM (Fabre & Sheppard 2006).

Non-specific fibrotic CMP i.e. IMF, defined as unexplained myocardial fibrosis without any evidence of other macroscopic or histological abnormalities, was also a more common underlying cardiac abnormality at autopsy than that reported previously. Concurrent with the present study, Chugh et al. (2003) described several victims of SCD whose hearts were structurally normal except for the presence of myocardial fibrosis on histological examination. The increase in fibrosis was exclusively interstitial, without evidence of myocyte necrosis or stigmata of myocarditis, similar to the present study. Genetic factors, such as mutations in LMNA gene (Hookana et al. 2008) and overexpression of transforming growth factor β1 (John et al. 2004), might be potential mediators of interstitial remodeling leading to myocardial fibrosis, but the exact reasons of myocardial fibrosis leading to SCD are largely unknown.

The role of other causes of SCD, such as coronary anomalies, valvular heart disease, inflammatory cardiac disease or suspected primary electrical abnormalities combined, was much smaller than that reported earlier, representing only 2.4% of all the SCDs and 11.2% of the non-ischemic SCDs. In previous studies among Caucasians, the prevalence of structurally normal heart at autopsy in victims of SCD has varied considerably, between 4% and 52% (Bowker et al. 2003, Fabre & Sheppard 2006). The high prevalence is most probably due to a lack of meticulous histological examination. It is also possible that even in
Caucasian populations the causes of SCD may vary significantly from one country to another.

6.2.1 Causes of SCD in victims under the age of 40 years

The reported causes of SCD in young people are also variable among different populations. In an Italian population, CAD was the most frequent cause (21%) and ARVC was the second most common cause (14%) of SCD in a study population aged 1–35 years (Corrado et al. 2001). In contrast, in an Australian population, presumed primary arrhythmia was the most common cause of SCD (29%) in persons aged 5–35 years (Puranik et al. 2005). In the present study, IMF was the most common association with SCD followed by CMP related to obesity in subjects under 40 years of age. The prevalence of ARVC and structurally normal hearts were relatively low, highlighting the possible geographical differences in the causes of SCD in young subjects. Even if one limits the data to young athletes, the incidence and the causes of SCD vary depending on the country in which the individuals live. Maron et al. (1996) described HCM as the most common cause (36%) of SCD in competitive athletes over a 10-year period in the United States, while two Italian studies identified ARVC as the most common cause (25%) of SCD in young competitive athletes (Corrado et al. 1990, Thiene et al. 1988).

6.2.2 Possible reasons for geographical differences in the causes of SCD

The divergent results, especially about the young SCD victims, may be related to differences in data gathering, definitions of SCD, autopsy techniques, or it may be an outcome of genetic and ethnic diversity. Owing to its few initial inhabitants, national isolation and population bottlenecks (Peltonen et al. 1999), some haplotypes have become enriched in the Finnish population. These features of the Finnish population, e.g. in regard to the non-structural genetic arrhythmia syndromes, may not be generalizable to other areas of the world. However, the known prevalence of those disorders is sufficiently low such that it does not override the general observations in this report. The lack of meticulous autopsy examinations in many previous studies, including histology, has probably resulted in an underestimation of the etiological factors of SCD e.g. IMF and CMP related
to obesity. These present findings emphasize the importance of undertaking careful histologic studies in the postmortem evaluation of SCD victims.

The specific socioeconomic and cultural background of the individual countries may also have an impact on the etiology of SCD (Reinier et al. 2006). For example, differences in alcohol consumption habits may partly explain the excess alcoholic CMP as a cause of SCD in the present series. In a recent study, alcoholic CMP was established to be an under-diagnosed disease in Finland (Hilden et al. 2006). In the United States, long-term heavy alcohol consumption is the leading cause of a non-ischemic DCM (Vital and Health Statistics. Hyattsville, MD. 1995), ethanol consumption per capita having been ~8.7 litres in 2007 (NIAAA. 2010). In Finland, ethanol consumption per capita was as high as 10.4 litres in 2008, respectively (STAKES. 2010), and it has been continuously on the increase in recent years.

6.3 Family history (III)

The results of the study III support the concept that even though a part of non-ischemic heart disease may be due to inherited traits, the vast number of non-ischemic heart diseases has a sporadic occurrence. It was also confirmed in this study in a large sample of victims of SCD with autopsy verified CAD that ischemic SCD has a strong familial background, that one out of every three SCD victims has had one or more SCDs in their FDRs, this being the case in victims both with and without a previous AMI.

Some demographic and clinical variables differed between the groups. Victims of non-ischemic SCD were younger and their BMI was higher than in the ischemic SCDS or controls. The number of current smokers was also highest in the non-ischemic SCD group. A history of hypertension and diabetes mellitus was more common in the SCD victims compared with controls. Thus, the risk profile of SCD victims differed in several aspects from those of control subjects. When the prevalence of SCD in FDRs was adjusted with these features in the statistical analyses, the main results remained the same, suggesting that the strong family history of SCD in those with ischemic heart disease was not simply the result of inheritance of specific risk factors of SCD, such as tendency to smoke, suffer from hypertension or diabetes; thus some other genetic factors are probably involved in ischemic SCD.
6.3.1 Family history of SCD in non-ischemic heart disease

In previous studies, it has been claimed that in subjects with well-defined SCD syndromes, having a family history noticeably increases the risk of SCD. For example, a family history of SCD in patients with HCM is associated with a 5-fold elevated risk of SCD (Elliott et al. 2000). In the analysis of various subtypes of non-ischemic SCD in the present study, a family history of SCD was also relatively common (33%) in those SCD victims with evidence of HCM at autopsy. However, the number of cases with HCM was relatively low in the present population of victims of SCD. Consequently, the overall prevalence of family history of SCD remained low in the total sample of non-ischemic cases. HCM has been reported to be the most frequently occurring CMP and the most common monogenic cardiac disorder in the United States (Maron et al. 1995). A previous study also indicated HCM to be the most common cause of SCD in the young (Maron et al. 1996). It is evident that the reported causes of non-ischemic SCD are variable among different populations; this may be due to differences in the genetic profiles and features of acquired heat disease between the various countries. In addition, the autopsy rates and reporting of autopsy results are clearly quite different between countries.

As stated before, the causes of non-ischemic SCD of the present studies differ from those reported previously (Chugh et al. 2000, Fabre & Sheppard 2006); obesity, alcoholic CMP, and IMF were more prevalent causes of non-ischemic SCD than the causes of non-ischemic SCD reported previously. This may partly explain the lack of familial clustering of non-ischemic SCD in our population. The inheritance of the most prevalent causes of non-ischemic SCD does not seem to be as straightforward as that of other specific arrhythmia syndromes, such as LQTS or ARVC and HCM which seem to be more prevalent causes of SCD in other populations. It should be also noted that we excluded here those subjects with a normal autopsy, i.e. SCD victims caused by inherited ion channel disorders, such as long QT or Brugada syndrome were excluded.

Despite the lack of differences in SCD between the total number of FDRs of the victims of non-ischemic SCD and controls (Table 10), the number of > 1 SCD in the FDRs was somewhat higher in the non-ischemic group than controls (Table 11). This may be due to inherited monogenic mutations with a high penetrance of disease in some families with non-ischemic SCD. However, the number of these cases was also relatively small, resulting in a low overall prevalence of family histories of SCD in the non-ischemic cases.
6.3.2 Family history of SCD in ischemic heart disease

We have previously reported an increased family history of SCD in a series of 138 victims of SCD due to an acute coronary event as compared to survivors of such an event (Kaikkonen et al. 2006). The results from study III confirm and extend these results by investigating a larger sample size. Here a subgroup analysis of family history of SCD was performed in those with and without an old infarct scar at autopsy, because the pathophysiological mechanism behind the SCD encountered in these two entities may differ. Ventricular tachycardia related to the presence of an infarct scar has been proposed to be the primary initiating arrhythmia leading to cardiac arrest in subjects with an old infarct scar whereas the primary ventricular fibrillation has been considered to be the major mechanism of cardiac arrest in individuals without an infarct scar. However, no differences were observed in the family history of SCD between these two groups suggesting that genetic factors may play a similar role in SCD in those who suffer cardiac arrest as the first manifestation of their cardiac disease and in those with a prior history of cardiac disease.

6.4 The collagen content of the fibrotic myocardium (IV)

The results of study IV show that myocardial type I collagen synthesis is increased in victims of SCD due to IMF. These results suggest an increased accumulation of newly synthetized collagen type I in the diseased myocardium. No significant difference was observed in the myocardium of IMF SCD cases and controls in the amount of IIINTP levels in the initial analysis. The PIIINP staining with the more concentrated antibody dilution revealed small amounts of type III collagen in two IMF cases with the most evident fibrosis, including the lamin A/C mutation case, which might be a clinically different entity than the other IMF cases. A detailed histology data from medico-legal autopsy of this R541C mutation carrier has been described in study I (Hookana et al. 2008). The same mutation was not observed in other cases of IMF indicating that the excess accumulation of collagen type I in the myocardium is not only related to this specific mutation, but may result from various disorders. Thus, there seems to be excess accumulation of collagen type I in the myocardium of SCD victims with IMF, but there is some variation in the amount of collagen type III.

As stated before, it is evident that lack of histological examination in many previous studies has underestimated the possibility of myocardial fibrosis as a
potential factor leading to SCD. The texture of fibrosis plays an essential role in propagation of the electrical impulse, in addition to its total amount. Fibrosis may be interstitial, compact, patchy or diffuse (Kawara et al. 2001). This intermingling of fibrosis and myocardium creates an arrhythmogenic substrate. In study IV, different textures of fibrosis were observed in various locations of the myocardium in different victims of SCD.

Initial reports have suggested that myocarditis may play a role in IMF (Lecomte et al. 1993). However, in this study, the SCD victims had no clinical history of prior myocarditis, and there was no histological evidence of it (diagnosed according by the presence of inflammatory infiltrates of the myocardium with the degeneration and/or necrosis of adjacent myocytes).

Biomarkers for cardiac fibrosis have been intensively studied in recent years specifically in DCM and CHF. Patients with DCM (Klappacher et al. 1995), and CHF (Barasch et al. 2009, Martos et al. 2007) have higher serum PIIINP levels than healthy controls. On the other hand, the levels of PINP have been claimed to be increased in patients with HCM. Additionally, elevated serum levels of PICP, also reflecting increased collagen type I synthesis, have been observed in sarcomere-mutation carriers without any obvious HCM (Ho et al. 2010). Another previous study reported that the serologic test of type I collagen turnover (the PINP/ICTP ratio) was associated with resting diastolic dysfunction in HCM patients (Shim et al. 2009). Additionally, no significant difference was observed in serum PINP levels between controls and CHF patients (Alla et al. 2006), nor with hypertensive patients with or without diastolic CHF (Martos et al. 2007). These results from previous studies are in concordance with those found in study IV; the accumulation of collagen type I seems to be associated with arrhythmogenic heart diseases; SCD attributable to IMF and HCM. It is possible that collagen type I is specifically overexpressed in the myocardium of subjects with various gene mutations coding for structural proteins, such as lamin and sarcomere. An increased amount of collagen type III is more typically found in acquired heart diseases; such as CHF due to various causes.

The direct correlation between biomarkers and histological myocardial collagen deposits has only been explored in a few studies (Klappacher et al. 1995, Polyakova et al. 2011). The serum concentrations of extracellular matrix proteins were higher in patients with DCM than in control subjects, and they reflected the concomitant increase in their myocardial tissue analogues to some extent. Nevertheless, there are some reservations that a non-invasive serologic test could be able to correctly estimate the degree of myocardial fibrosis. Direct examination
of cardiac tissues represents the most reliable method for the evaluation of collagen metabolism and the quantification of myocardial fibrosis. On the other hand, the feasibility of direct tissue examinations is limited. Therefore studies on the correlations of biomarker levels and direct or indirect evidence of increased myocardial fibrosis by histology or gadolinium late enhancement cardiac MRI are needed.

6.5 Study limitations

There are some limitations in all of the present studies that have to be addressed when evaluating the overall results. Classifications of CMPs have proved to be exceedingly complex and in many respects, contradictory. Recently, some revisions have been presented to the existing classification schemes (Elliott et al. 2008, Maron et al. 2006). Nonetheless, general etiologic classifications of CMPs are problematic, given that diseases with the same (or similar) anatomic and histological findings can arise from diverse origins and mechanisms. For example, establishing a comprehensible link between obesity and SCD is problematical (Owan & Litwin 2007). It is not known whether cardiac hypertrophy in obese patients is directly caused by increased adiposity or from the effects of comorbid conditions such as hypertension, diabetes and sleep-disordered breathing. In addition, it is not clear whether in obese patients functional changes (such as mild reductions in systolic and diastolic function) develop over time to the stage where they trigger SCD or other cardiac conditions. A substantial proportion of especially victims of CMP associated with obesity may have had undiagnosed diabetes or pre-diabetes but the current autopsy study design was unable to diagnose these cases. CMP associated with obesity, hypertensive CMP and alcoholic CMP may be related to each other. For instance, there is an established relationship between obesity and hypertension (Hsu et al. 1977, Kannel et al. 1967, Stamler et al. 1978). In addition, many cross-sectional studies have found a positive association between alcohol consumption and body weight or measures of abdominal adiposity, especially in heavy drinkers and binge drinkers (Arif & Rohrer 2005, Breslow & Smothers 2005, Wannamethee et al. 2005). The observed findings may partly be a result of a combination of these factors. However, the cases were diagnosed in only the one most prominent abnormality according to these categories. In those with IMF the cases with obesity and known alcohol abuse were excluded. It also should be mentioned that the criteria used in this autopsy series may differ from clinical diagnostics in many respects.
In addition, all findings must be considered in the context of prior medical records and the circumstances of SCD, in order to distinguish between association and causation.

The existing diagnostic testing of various CMPs may improve in the future, following the recognition of relevant genetic abnormalities as well as other novel causes, and thus the sub-grouping of CMPs might be extensively revised. In order to define the most probable cause of SCD, post-mortem examination, including histological examination, is the most reliable method to confirm or exclude antemortem structural disease and to define with accuracy the cause of death (Lahti et al. 1998). In appropriate cases, post-mortem genetic studies may add to clarification of causes that cannot be identified anatomically. The lack of DNA analyses in determining the genetic CMPs in this study is a limitation. The great genetic heterogeneity of different CMPs means that genetic analyses very challenging. On the other hand, a family history of SCD was not significantly increased in victims of non-ischemic SCD, pointing to a larger role of sporadic occurrence rather than inherited traits as the cause of non-ischemic SCD.

It is notable that resuscitated cardiac arrest victims were not a part of the FinGesture study. Distances in Northern Finland are long and therefore a kind of rural effect may cause a bias in cardiac arrest survival. Nevertheless, most of victims were found dead at home and resuscitation attempts were mostly in vain. In addition, coronary spasm cannot be excluded by autopsy. Especially in some of the obese and smoking non-ischemic SCD victims, the possibility of vasospasm and ischemic VF cannot be entirely excluded. However, all of the major coronary branches were dissected and analysed for coronary plaques followed by a histological analysis, if there was any suspicion of even minor coronary plaque complication, minimizing the possibility for a false negative result in the classification of ischemic SCD. In addition, there is a possibility that some cases with no cardiac abnormalities at autopsy might have escaped from this study setting, and have been diagnosed to be something other than SCD.

Despite the collection of consecutive victims of SCD over a long time period in the FinGesture study, it was possible to gather a relatively small sample number of victims of non-ischemic SCD. When the study setting is retrospective, relatives of subjects with recent sudden death might more often selectively report familial diseases, true or supposed, compared with control subjects. The lack of a reliable family history from the closest relatives of the victims of SCD, including the review of death certificates, was the main obstacle preventing the collection of a larger sample. In particular, when various sub-groups of the non-ischemic SCD
victims were being compared in studies II and III, the number of cases in each group was small which hindered reliable statistical comparisons. One must also acknowledge that the reported causes of non-ischemic SCD are variable among different populations, and these features of the Finnish population with regard to the family history of non-ischemic SCDs may not be generalizable to other parts of the world.

In addition, in study IV, the number of victims of SCD with IMF was also relatively small limiting the generalizability of the results. However, a large sample size would be difficult to obtain within a reasonable time frame. Regarding the selection of control group, the cases included were those in whom the death was defined as suicide after toxicologic examination, or caused by a traffic accident, and no cardiac or non-cardiac disease could be found as a cause of unexpected death at autopsy. Autopsy of these cases is relatively rare, because the cause of death can be defined without the autopsy in many cases. There are about 10–15 annually of these kinds of cases which undergo medico-legal autopsy and are finally defined as a death caused by suicide (toxicologic analysis). Consecutive series of autopsy verified suicides or accidents were collected from our institution, where the heart was stored for further analysis. The age appeared to be rather similar, although there were some gender differences between the groups. To our knowledge, there have been no studies about gender-related differences in the extent of cardiac fibrosis.

In study IV, no other extensive genotyping was performed, e.g. for mutations of HCM genes, in addition to the lamin A/C mutation. Furthermore, the use of immunohistochemistry alone is a limitation due to the lack of quantification of the collagen peptides although different antibody dilutions were used to find the most abundant collagen types with the initial dilution. Furthermore, a healed myocarditis as a cause of IMF cannot be completely excluded despite the lack of histologic signs of active inflammation. Despite these limitations it is reasonable to conclude that, the results of study IV are likely to stimulate further research interest into this area, especially on studies of biological surrogate serum biomarkers of arrhythmogenic cardiac fibrosis, such as type I collagen.

6.6 Summary

In summary, the results of studies I-IV show that characteristics of non-ischemic SCD in Finland differ from those reported previously. Higher prevalences of CMP-associated SCDs related to obesity, IMF and alcoholic CMP were observed.
as the clinical and/or pathologic bases for non-ischemic SCD. A family history of SCD was not significantly increased in victims of non-ischemic SCD, pointing to a larger role of sporadic occurrence rather than inherited traits as the cause of non-ischemic SCD. Replacement of cardiac myocytes by fibrosis can be responsible for fatal cardiac arrhythmias in subjects with the lamin A/C gene mutation. The victims of SCD due to IMF have increased myocardial type I collagen synthesis.
7 Conclusions

1. CMP associated with obesity, IMF and alcoholic CMP are commonly associated with non-ischemic SCD in Finland in the current era. The association of SCD with IMF is rather frequent among victims < 40 years old. The results of this study also confirm, in part, the previous data about the proportions of CAD and CMPs as causes of SCD among the general population in the current era. (II).

2. Even though a part of non-ischemic heart disease may be due to inherited traits, a vast number of non-ischemic heart diseases have a sporadic occurrence. The causes of non-ischemic SCD of the present study differ from those reported previously; this may partly explain the lack of familial clustering of non-ischemic SCD in this population. Ischemic SCD has a strong familial background; one out of three SCD victims has one or more SCDs in their FDRs both in those with and without a previous AMI (III).

3. Fatal or near-fatal cardiac arrhythmia may be the first clinical manifestation of a de novo mutation R541C of the lamin A/C gene. The R541C mutation resulted in a localized involvement of the LV, with no significant changes in the other parts of the heart. Replacement of cardiac myocytes by fibrotic cells seems to be the predominant pathologic-anatomic finding (I).

4. The victims of SCD due to IMF have increased myocardial type I collagen synthesis. In contrast, the amount of type III collagen was not as frequently altered in the fibrotic areas. The texture of fibrosis seems to play an important role in the propagation of the electrical impulse, i.e. it is not simply the amount of fibrosis. In the present study, different textures of fibrosis were observed in various locations of the myocardium in different victims of SCD (IV).
References


Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ, Chamber Quantification Writing Group, American Society of Echocardiography's Guidelines and Standards Committee & European Association of Echocardiography (2005) Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 18(12): 1440–1463.


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