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**GENETIC VARIANTS PREDISPOSING TO
CARDIAC ARRHYTHMIA DISORDERS
AND SUDDEN CARDIAC DEATH**

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ACADEMIC DISSERTATION

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are referred to in the text by Roman numerals I-VI. In addition, some unpublished data are presented.

- I Lahtinen AM*, Lehtonen A*, Kaartinen M, Toivonen L, Swan H, Widén E, Lehtonen E, Lehto VP, Kontula K. Plakophilin-2 missense mutations in arrhythmogenic right ventricular cardiomyopathy. *International Journal of Cardiology* 2008; 126(1):92-100.
- II Lahtinen AM, Lehtonen E, Marjamaa A, Kaartinen M, Heliö T, Porthan K, Oikarinen L, Toivonen L, Swan H, Jula A, Peltonen L, Palotie A, Salomaa V, Kontula K. Population-prevalent desmosomal mutations predisposing to arrhythmogenic right ventricular cardiomyopathy. *Heart Rhythm* 2011; 8(8):1214-1221.
- III Lahtinen AM, Marjamaa A, Swan H, Kontula K. *KCNE1* D85N polymorphism – a sex-specific modifier in type 1 long QT syndrome? *BMC Medical Genetics* 2011; 12:11.
- IV Noseworthy PA*, Havulinna AS*, Porthan K, Lahtinen AM, Jula A, Karhunen PJ, Perola M, Oikarinen L, Kontula KK, Salomaa V, Newton-Cheh C. Common genetic variants, QT interval, and sudden cardiac death in a Finnish population-based study. *Circulation Cardiovascular Genetics* 2011; 4(3):305-311.
- V Lahtinen AM*, Noseworthy PA*, Havulinna AS, Jula A, Karhunen PJ, Kettunen J, Perola M, Kontula K, Newton-Cheh C, Salomaa V. Common genetic variants associated with sudden cardiac death: the FinSCDgen study. *PloS One* 2012; 7(7):e41675.
- VI Lahtinen AM, Havulinna AS, Noseworthy PA, Jula A, Karhunen PJ, Perola M, Newton-Cheh C, Salomaa V, Kontula K. Prevalence of arrhythmia-associated gene mutations and risk of sudden cardiac death in the Finnish population. Submitted.

* Equal contribution.

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ABBREVIATIONS

ARVC	arrhythmogenic right ventricular cardiomyopathy
ARVD	arrhythmogenic right ventricular dysplasia
<i>CACNA1C</i>	voltage-gated L-type calcium channel α_{1C} subunit gene
<i>CASQ2</i>	calsequestrin 2 gene
<i>CDKN2A, 2B</i>	cyclin-dependent kinase inhibitor 2A and 2B genes
CHD	coronary heart disease
CI	confidence interval
CPVT	catecholaminergic polymorphic ventricular tachycardia
<i>DSC2</i>	desmocollin-2 gene
<i>DSG2</i>	desmoglein-2 gene
<i>DSP</i>	desmoplakin gene
ECG	electrocardiogram
<i>GPD1L</i>	glycerol-3-phosphate dehydrogenase 1-like gene
GWA	genome-wide association
HR	hazard ratio
HSDS	Helsinki Sudden Death Study
$I_{Ca,L}$	L-type calcium current
I_{K1}	inward rectifier potassium current
I_{Kr}	rapidly activated delayed rectifier potassium current
I_{Ks}	slowly activated delayed rectifier potassium current
I_{Na}	sodium current
I_{NCX}	sodium-calcium exchanger current
I_{to}	transient outward potassium current
<i>JUP</i>	plakoglobin gene
<i>KCNE1, 2, 3</i>	voltage-gated potassium channel, Isk-related family, member 1, 2, and 3 genes
<i>KCNH2</i>	voltage-gated potassium channel, subfamily H (eag-related), member 2 gene
<i>KCNJ2, 5</i>	inwardly-rectifying potassium channel, subfamily J, member 2 and 5 genes
<i>KCNQ1</i>	voltage-gated potassium channel, KQT-like subfamily, member 1 gene
LQTS	long QT syndrome
minK	voltage-gated potassium channel, subfamily E, member 1
MiRP1	minK-related peptide 1
MLPA	multiplex ligation-dependent probe amplification
<i>NOS1AP</i>	nitric oxide synthase 1 (neuronal) adaptor protein gene
PCR	polymerase chain reaction
PIRA	primer-induced restriction analysis
<i>PITX2</i>	paired-like homeodomain 2 gene
<i>PKP2</i>	plakophilin-2 gene
<i>PLN</i>	phospholamban gene
QTc	QT interval corrected for heart rate according to Bazett's formula
QT _{Nc}	QT interval nomogram-corrected for heart rate
QT _{score}	QT genotype score calculated for each individual to aggregate the genetic information of a number of QT interval-prolonging variants
RR	relative risk
<i>RYR2</i>	cardiac ryanodine receptor gene
SCD	sudden cardiac death

ABBREVIATIONS

<i>SCN1B</i>	voltage-gated sodium channel, type I, β subunit gene
<i>SCN5A</i>	voltage-gated sodium channel, type V, α subunit gene
SNP	single nucleotide polymorphism
TASTY	Tampere Autopsy Study
<i>TGFB3</i>	transforming growth factor β 3 gene
<i>TMEM43</i>	transmembrane protein 43 gene
<i>WDR48</i>	WD repeat domain 48 gene

In addition, standard one-letter abbreviations are used for nucleotides and amino acids.

ABSTRACT

Arrhythmogenic right ventricular cardiomyopathy (ARVC) and long QT syndrome (LQTS) are inherited cardiac arrhythmia disorders that predispose to ventricular tachycardia and sudden cardiac death (SCD). In ARVC, structural and electrical abnormalities of the heart occur together with progressive replacement of the right ventricular myocardium by adipose and fibrous tissue. Mutations in desmosomal cell adhesion genes are estimated to account for approximately half of all ARVC cases. LQTS is a cardiac channelopathy manifesting with a prolonged QT interval in a structurally normal heart. Disease-causing mutations delay the repolarization of the ventricular myocardium by disturbing the function of cardiac ion channels. The aims of this study were to identify genetic variants predisposing to ARVC, LQTS, and SCD and to assess their prevalence and clinical significance in the Finnish population.

A total of 33 ARVC probands were screened for mutations in desmosomal genes by direct sequencing. Six mutations, five not previously reported in ARVC, were identified in 18% of the cases. Immunohistochemistry and electron microscopy revealed disorganization of the intercalated disk structure of mutation carriers, but ARVC families demonstrated reduced disease penetrance. The combined carrier frequency of the desmosomal mutations identified in this study was 1:250 in four Finnish population cohorts (total n = 27 670). One in 340 individuals in the general population carried the Finnish ARVC founder mutation *PKP2* Q59L. Compared with the proposed ARVC population prevalence of 1:1000-1:5000, an unexpectedly large number of individuals could be at risk of developing ARVC, and thus, potentially life-threatening arrhythmias in Finland. However, another trigger is likely to be needed for disease expression.

KCNE1 D85N is associated with a 10-ms QT interval prolongation in the general population. To study its effect on the LQTS phenotype, its presence was assayed in 712 carriers of the four Finnish LQTS founder mutations *KCNQ1* G589D, *KCNQ1* IVS7-2A>G, *KCNH2* L552S, and *KCNH2* R176W. *KCNE1* D85N was associated with a 26-ms prolongation of QT interval in males with *KCNQ1* G589D, representing thus a potential sex-specific disease-modifying factor in LQTS.

Associations between 14 QT-prolonging single nucleotide polymorphisms (SNPs), QT interval, and SCD were investigated in two Finnish population cohorts (total n = 6808). The QT_{score} aggregating the genetic information of the 14 QT-associated SNPs explained 8.6% of the variation in QT interval. A 10-ms prolongation of QT interval was associated with a 19% increased risk of SCD, and the association between a diagnostic QT interval threshold (>450 ms in males and >470 ms in females) and risk of SCD was verified. No association between QT_{score} and risk of SCD was, however, observed.

The association of 28 common and 10 rare candidate gene variants with SCD was studied in four Finnish population samples and two series of forensic autopsies (total n = 28 323). Two novel common variants, rs41312391 in *SCN5A* and rs2200733 in 4q25 near *PITX2*, were associated with risk of SCD. In addition, the associations for rs2383207 in 9p21 as well as for clinical risk factors for coronary heart disease were replicated. Rare arrhythmia-associated mutations in desmosomal and ion channel genes had a combined carrier frequency of 1:130 in the Finnish population and were detected in individual SCD victims.

In conclusion, the high prevalence and reduced disease penetrance of desmosomal mutations should be considered in counselling of ARVC patients and family members. In LQTS, *KCNE1* D85N provides a potential sex-specific disease-modifying factor for risk stratification. In addition, two novel genetic risk markers for SCD were identified in this study, providing novel information for SCD risk prediction and prevention.

INTRODUCTION

Cardiac arrhythmia disorders present a major risk factor for cardiac arrest and sudden cardiac death (SCD). Disturbance of heart rhythm may occur due to structural or electrical heart disease or non-cardiac causes. Inherited forms of arrhythmia disorders are rare but often severe, and they are involved in a significant proportion of premature sudden deaths of young adults (Cross et al. 2011). Cardiomyopathies, i.e. disorders of the cardiac muscle, and channelopathies, i.e. ion channel disorders, represent the major forms of inherited cardiac arrhythmia disorders.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited arrhythmia disorder characterized by progressive adipose and fibrous tissue replacement of the right ventricular myocardium (Marcus et al. 2010). It is a common cause of SCD in young athletes (Thiene et al. 1988). Since the initial description of ARVC, then termed right ventricular dysplasia (Frank et al. 1978), several pathogenic theories have been suggested. The first disease locus was identified in 1994 in a linkage study (Rampazzo et al. 1994), but the reduced penetrance and variable expressivity of ARVC have complicated the discovery of disease-causing mutations. Autosomal recessive cardiocutaneous syndromes helped in the establishment of desmosomal gene mutations underlying ARVC (McKoy et al. 2000). Thereafter, several desmosomal cell adhesion genes have been associated with the autosomal dominant form of this disorder, but the exact prevalence and significance of these mutations at the population level have not previously been assessed.

Long QT syndrome (LQTS) is a cardiac channelopathy, first described in the 1950s, when patients with prolonged QT interval and increased risk of SCD associated with congenital deafness were documented (Jervell and Lange-Nielsen 1957). The first ion channel genes involved in LQTS were discovered in 1995 using linkage mapping (Curran et al. 1995, Wang et al. 1995). Today, 13 loci affecting the function of cardiac ion channels have been shown to be associated with LQTS. However, as the disease-causing mutations show a significantly reduced penetrance (Priori et al. 1999), current research efforts have focused on additional disease-modifying factors in the risk stratification of LQTS.

Approximately half of all cardiovascular deaths occur suddenly (Fox et al. 2004a). In addition to cardiomyopathies and primary electrical disorders of the heart, coronary heart

disease (CHD) is the most common disorder underlying SCD (Chugh et al. 2008). For example, myocardial infarction may provide a substrate for ventricular tachycardia, which may lead to ventricular fibrillation and cardiac arrest. Risk of SCD is heritable (Jouven et al. 1999, Friedlander et al. 2002), but the genetic variants conveying susceptibility are largely unknown. Recent advances in molecular genetics have enabled the use of genome-wide approaches in the discovery of novel candidate genes for SCD (Alders et al. 2009, Arking et al. 2010, Bezzina et al. 2010, Arking et al. 2011).

The aims of the present study were to identify genetic variants predisposing to cardiac arrhythmia disorders ARVC and LQTS and to assess the prevalence of arrhythmia susceptibility variants and their association with risk of SCD in the Finnish population.

REVIEW OF THE LITERATURE

1. Molecular basis of inherited cardiac arrhythmia disorders

1.1. Cardiomyopathies

Cardiomyopathies are disorders of the cardiac muscle that may cause heart failure, ventricular arrhythmias, and sudden cardiac death (SCD). Inheritable cardiomyopathies are divided into five distinct disease entities: dilated cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy (ARVC), restrictive cardiomyopathy, and left ventricular noncompaction cardiomyopathy (Watkins et al. 2011). Their inheritance is often autosomal dominant, but also autosomal recessive, X-linked, and mitochondrial inheritance have been reported (Watkins et al. 2011). Various mutations in a single gene may underlie different disease phenotypes, and even within families, carriers of the same mutation may suffer from different types of cardiomyopathies (Mogensen et al. 2003).

Dilated cardiomyopathy manifests with left ventricular dilatation, systolic dysfunction, and myocardial fibrosis. Many disease genes have been identified and they involve distinctive cellular functions, such as nuclear envelope (lamin A and C) (Fatkin et al. 1999), sarcomere structure (β -myosin heavy chain, troponin T, actin) (Olson et al. 1998, Kamisago et al. 2000), force transduction (Cypher/ZASP) (Vatta et al. 2003), cytoskeleton (desmin, δ -sarcoglycan) (Li et al. 1999, Tsubata et al. 2000), cell adhesion (desmoplakin, metavinculin) (Norgett et al. 2000, Olson et al. 2002), calcium handling (phospholamban) (Haghighi et al. 2003, Schmitt et al. 2003), transcription (Schönberger et al. 2005), and messenger RNA splicing (Brauch et al. 2009). Despite their functional divergence, many of these mutations lead to impaired generation or transmission of force and ultimately protein and organelle degradation and apoptosis (Watkins et al. 2011).

Patients with hypertrophic cardiomyopathy show left ventricular hypertrophy, often involving the interventricular septum, and impaired diastolic relaxation. Characteristic features also include myocyte disarray and fibrosis. Disease-causing mutations have been detected in genes encoding sarcomeric proteins (e.g. β -myosin heavy chain, cardiac myosin-binding protein C, cardiac troponin T, and α -tropomyosin) (Geisterfer-Lowrance et al. 1990, Thierfelder et al. 1994, Bonne et al. 1995, Watkins et al. 1995), and genes involved in

energy sensing (γ 2 subunit of adenosine monophosphate-activated protein kinase) (Blair et al. 2001) and production (mitochondrial transfer RNAs) (Merante et al. 1994) and myogenic differentiation (muscle LIM protein) (Geier et al. 2008). In contrast to the sarcomeric mutations observed in dilated cardiomyopathy, those associated with hypertrophic cardiomyopathy cause increased contractility and energy consumption (Watkins et al. 2011). The alterations in cardiomyocyte energetics, calcium handling, and signalling pathways ultimately lead to reduced myocyte relaxation and increased myocyte growth (Watkins et al. 2011).

Restrictive cardiomyopathy presents with reduced ventricular diastolic volume without abnormalities in systolic function and cardiac morphology. A single sarcomeric mutation in cardiac troponin I may lead to either restrictive or hypertrophic cardiomyopathy (Mogensen et al. 2003). Sarcomeric mutations associated with restrictive cardiomyopathy have also been reported in troponin T (Peddy et al. 2006), α -cardiac actin (Kaski et al. 2008), and β -myosin heavy chain (Karam et al. 2008). Desmin mutations may be detected in patients with both skeletal and cardiac myopathy (Goldfarb et al. 1998, Arbustini et al. 2006).

Clinical findings in left ventricular noncompaction cardiomyopathy are trabeculations of the left ventricular myocardium and segmental left ventricular wall thickening due to thickened endocardial layer and thin epicardial layer. Mutations in sarcomeric proteins (α -cardiac actin, β -myosin heavy chain, and cardiac troponin T) may cause this disorder as well as other types of cardiomyopathies (Hoedemaekers et al. 2007, Klaassen et al. 2008). Disease-associated mutations have also been detected in the cytoskeletal protein α -dystrobrevin (Ichida et al. 2001), as well as in lamin A and C (Hermida-Prieto et al. 2004), Cypher/ZASP (Vatta et al. 2003), and taffazin (Ichida et al. 2001) proteins.

1.2. Cardiac ion channel disorders

Channelopathies, i.e. ion channel disorders, are caused by mutations in genes encoding ion channels, their subunits, or associated regulatory proteins. In contrast to cardiomyopathies, manifesting with structural changes of the heart, cardiac channelopathies involve mainly electrical instability of the heart, predisposing to ventricular tachyarrhythmias and SCD. The inheritance is usually autosomal dominant or recessive in nature, but the penetrance may be variable (e.g. Swan et al. 1999a, Lahat et al. 2001). Cardiac channelopathies include long

QT syndrome (LQTS), short QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), and atrial fibrillation (Figure 1). In addition, mutations in the cardiac sodium channel gene *SCN5A* may cause progressive cardiac conduction defect (Schott et al. 1999), sick sinus syndrome (Benson et al. 2003), dilated cardiomyopathy (Bezzina et al. 2003a), and idiopathic ventricular fibrillation (Akai et al. 2000). Also a mutation in the potassium channel gene *KCNJ8* has been identified in a patient with ventricular fibrillation and early repolarization (Haïssaguerre et al. 2009).

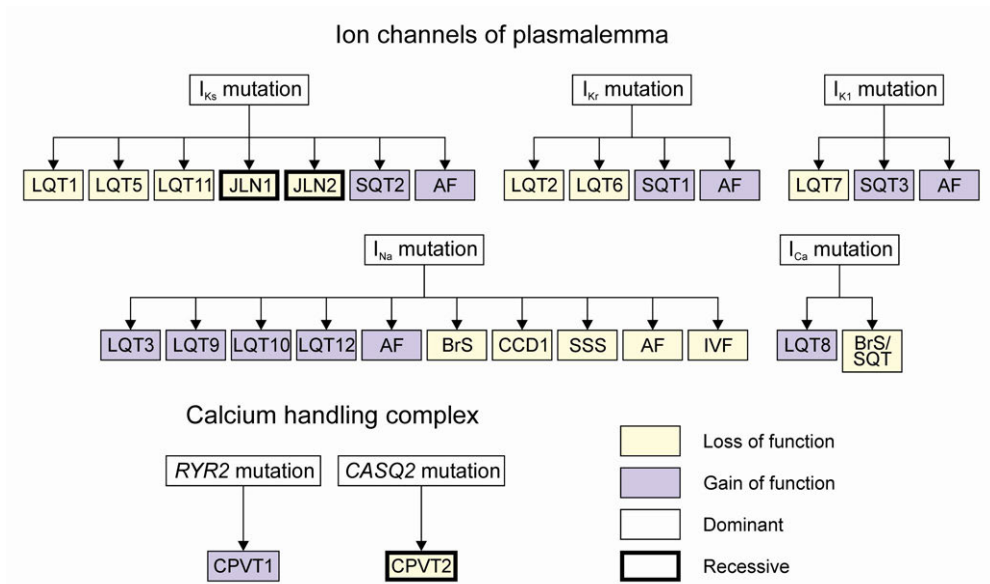


Figure 1. Spectrum of cardiac channelopathies caused by different types of ion channel mutations. AF = atrial fibrillation; BrS = Brugada syndrome; CCD = cardiac conduction defect; CPVT = catecholaminergic polymorphic ventricular tachycardia; IVF = idiopathic ventricular fibrillation; JLN = Jervell and Lange-Nielsen syndrome; LQT = long QT syndrome; SQT = short QT syndrome; SSS = sick sinus syndrome. Adapted from Ruan et al. 2009.

Short QT syndrome is characterized by short QT interval and tall and peaked T waves in electrocardiogram (ECG) (Gussak et al. 2000). This disorder of shortened cardiac repolarization predisposes to atrial fibrillation, ventricular tachycardia, and SCD (Gaita et al. 2003). Causative mutations have been reported in the potassium channel genes *KCNH2* (Brugada et al. 2004), *KCNQ1* (Belloq et al. 2004), and *KCNJ2* (Priori et al. 2005). In short QT syndrome, the potassium channel mutations cause a gain-of-function defect, whereas loss of function of the same channels may lead to long QT syndrome, manifesting with prolonged cardiac repolarization (Curran et al. 1995, Wang et al. 1996, Plaster et al. 2001).

Brugada syndrome presents with elevated ST segments and inverted T waves in the right precordial leads of ECG, associated with increased risk of ventricular fibrillation and SCD (Brugada and Brugada 1992). Loss-of-function mutations in *SCN5A* have been reported in approximately 20% of Brugada syndrome patients (Chen et al. 1998, Kapplinger et al. 2010). Mutations in *GPD1L* gene encoding glycerol-3-phosphate dehydrogenase 1-like protein may also cause Brugada syndrome (London et al. 2007). These mutations decrease inward sodium current by reducing cell surface expression of sodium channels (London et al. 2007). Disease-causing mutations have also been reported in *SCN1B* and *SCN3B*, leading to decreased sodium current (Watanabe et al. 2008, Hu et al. 2009), as well as in *KCNE3* and *KCND3*, leading to increased I_{to} potassium current (Delpón et al. 2008, Giudicessi et al. 2011). Brugada syndrome associated with short QT interval is caused by loss-of-function mutations in the calcium channel genes *CACNA1C*, *CACNB2b*, and *CACNA2D1* (Antzelevitch et al. 2007, Burashnikov et al. 2010).

CPVT is a severe disorder causing stress-induced polymorphic ventricular tachycardia without structural abnormalities of the heart (Leenhardt et al. 1995). The baseline ECG is typically normal, while exercise stress test shows premature ventricular complexes in a rate-dependent fashion characteristic of CPVT. After initial mapping to chromosome 1q42-q43 (Swan et al. 1999a), this disorder was revealed to be caused by reduced threshold for calcium-induced calcium release from the sarcoplasmic reticulum due to dominant mutations in the *RYR2* gene encoding the cardiac ryanodine receptor (Laitinen et al. 2001, Priori et al. 2001). Recessive and dominant mutations in *CASQ2*, which encodes the cardiac calcium-binding protein calsequestrin, may also cause CPVT (Lahat et al. 2001, Postma et al. 2002).

Atrial fibrillation is the most common cardiac arrhythmia characterized by rapid fibrillation of atria and consequent irregular ventricular rate. It is often associated with other cardiovascular risk factors such as hypertension, heart failure, and valvular disease (Benjamin et al. 1994). Lone atrial fibrillation, which occurs without overt cardiovascular disease in patients under 60 years of age, is more rare but has a greater heritability (Fox et al. 2004b), and therefore, genetic studies have mainly focused on this form of disease. Atrial fibrillation-associated gene mutations have been reported in several ion channels, but also in other types of proteins. Gain-of-function mutations in the potassium channel genes *KCNQ1* (Chen et al. 2003), *KCNE2* (Yang et al. 2004), *KCNJ2* (Xia et al. 2005), *KCNH2* (Hong et

al. 2005), *KCNE3* (Lundby et al. 2008), and *KCNE5* (Ravn et al. 2008) lead to atrial arrhythmia, presumably by shortening the atrial action potential and reducing the effective refractory period (Roberts and Gollob 2010). Loss-of-function mutations in the potassium channel gene *KCNA5* (Olson et al. 2006) and the sodium channel genes *SCN5A* (Ellinor et al. 2008), *SCN1B*, and *SCN2B* (Watanabe et al. 2009) cause the disease by a different mechanism, probably by prolonging the atrial action potential and predisposing to early afterdepolarizations (Roberts and Gollob 2010). Also gain-of-function mutations have been reported in *SCN5A*, leading to hyperexcitability (Makiyama et al. 2008, Li et al. 2009b). Different types of genes associated with atrial fibrillation are *GJA5*, encoding the gap junction protein connexin 40 (Gollob et al. 2006), *NPPA*, encoding atrial natriuretic peptide (Hodgson-Zingman et al. 2008), and *NUP155*, encoding a nucleoporin protein (Zhang et al. 2008). In addition to these rare mutations, several common variants are associated with increased risk of atrial fibrillation, including those in the chromosomal region 4q25 near *PITX2* (Gudbjartsson et al. 2007), *ZFHX3* in 16q22 (Benjamin et al. 2009, Gudbjartsson et al. 2009), and *KCNN3* in 1p21 (Ellinor et al. 2010).

2. Arrhythmogenic right ventricular cardiomyopathy (ARVC)

2.1. Cell-cell junctions of cardiomyocytes

The intercalated disks, which connect adjacent cardiomyocytes, consist of three types of adhering junctions: gap junctions, adherens junctions, and desmosomes. Gap junctions couple the cells electrically, whereas adherens junctions and desmosomes anchor the cardiomyocytes mechanically by connecting the myofibrils and cytoskeletons of neighbouring cells. In cardiomyocytes, the components of the different types of junctions may localize together and form junctions of mixed type called *area composita* (Borrmann et al. 2006, Franke et al. 2006).

Gap junctions

Gap junctions are groups of gap junction channels, each composed of two connexons located in the cell membranes of adjacent cells. Each connexon consists of six connexin molecules surrounding the central pore (Yeager and Gilula 1992). Ions and small molecules of up to 1 kD, such as second messengers, can pass through the pore (Elfgang et al. 1995). The selective permeability is regulated by membrane voltage (Bennett and Verselis 1992), intracellular pH (Spray et al. 1981), calcium ion concentration (Rose and Loewenstein 1975), and connexin phosphorylation (Swenson et al. 1990). Gap junctions are responsible for spreading electrical excitation in the heart (Barr et al. 1965). In this organ, three main types of connexins are expressed: connexin 40, connexin 43, and connexin 45. Connexin 43 is the predominant type in ventricles, but also connexin 40 and connexin 45 are detected in the atria and atrioventricular conduction system (Vozzi et al. 1999). Mutations in connexin 43 may lead to complex heart malformations (Britz-Cunningham et al. 1995) and mutations in connexin 40 to atrial fibrillation (Gollob et al. 2006).

Adherens junctions

Adherens junctions attach cells together mechanically and connect the myofibrils to the cell membrane (Geiger et al. 1980). Components of adherens junctions also participate in signal transduction and gene expression regulation in the nucleus. For example, β -catenin may be involved in cell growth control, development, and differentiation (Funayama et al. 1995). N-cadherin is a transmembrane glycoprotein, which mediates calcium-dependent intercellular adhesion by homophilic interactions (Nose et al. 1990). N-cadherin interacts with α -catenin,

β -catenin, and plakoglobin (γ -catenin) by its catenin-binding domain (Stappert and Kemler 1994), and α -catenin can bind to the actin filament either directly (Rimm et al. 1995) or via α -actinin (Knudsen et al. 1995) or vinculin (Watabe-Uchida et al. 1998). The function of adherens junctions may be regulated by controlling cadherin expression (Steinberg and Takeichi 1994), lateral clustering of cadherin complexes (Yap et al. 1997), protein-protein interactions (Reynolds et al. 1994), and protein phosphorylation (Hamaguchi et al. 1993). Dysfunction of adherens junctions may lead to dilated or hypertrophic cardiomyopathy (Olson et al. 2002, Vasile et al. 2006a, Vasile et al. 2006b).

Desmosomes

Desmosomes form dense membrane-associated plaques that anchor intermediate filaments of the cytoskeleton to the cell membrane (Figure 2). These cell-cell junctions are abundant in tissues subject to mechanical stress such as the myocardium and epidermis. Desmosomal components also participate in signalling pathways involved in cell proliferation, differentiation, and apoptosis (Allen et al. 1996, Hakimelahi et al. 2000, Chidgey et al. 2001, Merritt et al. 2002). Desmosomal cadherins desmoglein and desmocollin are transmembrane glycoproteins involved in either heterophilic or homophilic interaction with cadherins of the neighbouring cell (Chitaev and Troyanovsky 1997, Marcozzi et al. 1998, Syed et al. 2002). This adhesion is calcium-dependent, but calcium-independent interaction occurs in the hyperadhesive state of desmosomes (Garrod et al. 2005). Armadillo proteins plakoglobin and plakophilin interact directly with desmosomal cadherins and desmoplakin (Witcher et al. 1996, Kowalczyk et al. 1997, Chen et al. 2002). Desmoplakin, in turn, functions as a link between the desmosomal plaque and intermediate filaments such as desmin in the myocardium (Kouklis et al. 1994). Plakoglobin and plakophilin are detected also in the nucleus, where they participate in the Wnt/ β -catenin signalling pathway and transcriptional regulation (Kolligs et al. 2000, Mertens et al. 2001, Chen et al. 2002). In the cytoplasm, plakophilin may regulate translation initiation (Wolf et al. 2010) and actin cytoskeleton organization (Hatzfeld et al. 2000). Desmosomes are regulated by growth factors and serine and tyrosine phosphorylation by protein kinases (Amar et al. 1999, Gaudry et al. 2001, Miravet et al. 2003). Desmosomal proteins are also targets for caspase cleavage directing the cell to apoptosis (Weiske et al. 2001). Mutations in desmosomal genes may cause ARVC as well as disorders of the skin and hair (McGrath et al. 1997, Armstrong et al. 1999, Gerull et al. 2004).

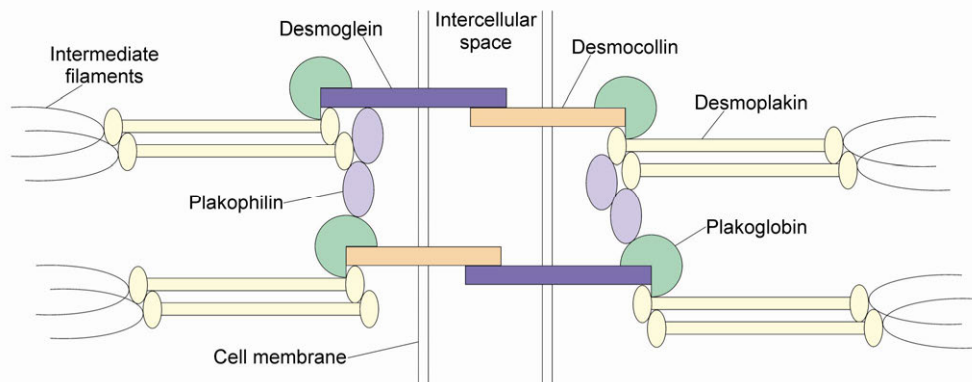


Figure 2. Schematic representation of desmosomal structure. Adapted from Green and Gaudry 2000.

2.2. Clinical features of ARVC

Arrhythmogenic right ventricular cardiomyopathy (ARVC), also called arrhythmogenic right ventricular dysplasia (ARVD), is a severe disorder of the myocardium. In ARVC, ventricular cardiomyocytes are progressively replaced by adipose and fibrous tissue (Nava et al. 1988, Thiene et al. 1988). This substitution is associated with structural and functional changes involving predominantly the right ventricle. The structural manifestations include ventricular dilatation and thinning, hypokinesia, and aneurysms of the ventricular wall, which are often concentrated in the right ventricular inflow, outflow, and apical regions, designated the “triangle of dysplasia” (Frank et al. 1978, Marcus et al. 1982, Blomström-Lundqvist et al. 1988, Lobo et al. 1992, Fontaine et al. 1998). The prevalence of ARVC is estimated to be between 1:1000 and 1:5000 (Rampazzo et al. 1994, Peters et al. 2004), but this condition may be underdiagnosed because of its progressive nature and variable expressivity. The mean age at diagnosis is approximately 30 years, and males are more often affected than females, with an estimated gender ratio of 1.6:1 (Nava et al. 2000).

Along with the structural changes of the myocardium, ARVC manifests with electrical instability of the heart. T-wave inversion in right precordial leads, epsilon waves, and widening of the QRS complex may be detected in resting ECG, and late potentials in signal-averaged ECG (McKenna et al. 1994). Frequent ventricular premature complexes may be recorded in Holter monitoring (McKenna et al. 1994). Ventricular tachycardia originating from the right ventricle is characteristic for ARVC patients and may lead to ventricular fibrillation and SCD (Marcus et al. 1982, Thiene et al. 1988). The diagnosis is based on

classification of clinical findings into major and minor criteria according to the revised Task Force diagnostic procedure (Marcus et al. 2010), as described in Table 1. Definitive diagnosis requires fulfilment of 2 major, 1 major plus 2 minor, or 4 minor criteria from different categories. Borderline diagnosis requires fulfilment of 1 major plus 1 minor, or 3 minor criteria, and possible diagnosis fulfilment of 1 major or 2 minor criteria.

Table 1. Revised Task Force criteria for diagnosis of ARVC (Marcus et al. 2010)

Major criteria	Minor criteria
I. Global or regional dysfunction and structural alterations	
2D echo: regional RV akinesia, dyskinesia, or aneurysm; and 1 of the following: -PLAX RVOT ≥ 32 mm (corrected for body size [PLAX/BSA] ≥ 19 mm/m ²) -PSAX RVOT ≥ 36 mm (corrected for body size [PSAX/BSA] ≥ 21 mm/m ²) -fractional area change $\leq 33\%$ MRI: regional RV akinesia or dyskinesia or dyssynchronous RV contraction; and 1 of the following: -ratio of RV end-diastolic volume to BSA ≥ 110 ml/m ² (male) or ≥ 100 ml/m ² (female) -RV ejection fraction $\leq 40\%$ RV angiography: regional RV akinesia, dyskinesia, or aneurysm	2D echo: regional RV akinesia or dyskinesia; and 1 of the following: -PLAX RVOT ≥ 29 to < 32 mm (corrected for body size [PLAX/BSA] ≥ 16 to < 19 mm/m ²) -PSAX RVOT ≥ 32 to < 36 mm (corrected for body size [PSAX/BSA] ≥ 18 to < 21 mm/m ²) -fractional area change $> 33\%$ to $\leq 40\%$ MRI: regional RV akinesia or dyskinesia or dyssynchronous RV contraction; and 1 of the following: -ratio of RV end-diastolic volume to BSA ≥ 100 to < 110 ml/m ² (male) or ≥ 90 to < 100 ml/m ² (female) -RV ejection fraction $> 40\%$ to $\leq 45\%$
II. Tissue characterization of wall	
Residual myocytes $< 60\%$ by morphometric analysis (or $< 50\%$ if estimated), with fibrous replacement of the RV free wall myocardium in ≥ 1 sample, with or without fatty replacement of tissue on endomyocardial biopsy	Residual myocytes 60% to 75% by morphometric analysis (or 50% to 65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥ 1 sample, with or without fatty replacement of tissue on endomyocardial biopsy
III. Repolarization abnormalities	
Inverted T waves in right precordial leads (V1-V3) or beyond in individuals > 14 years of age (in the absence of complete RBBB QRS ≥ 120 ms)	Inverted T waves in leads V1 and V2 in individuals > 14 years of age (in the absence of complete RBBB) or in V4, V5, or V6 Inverted T waves in leads V1-V4 in individuals > 14 years of age in the presence of complete RBBB

Major criteria	Minor criteria
IV. Depolarization/conduction abnormalities	
Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V1-V3)	<p>Late potentials by SAECG in ≥ 1 of 3 parameters in the absence of a QRS duration of ≥ 110 ms on standard ECG</p> <p>Filtered QRS duration (fQRS) ≥ 114 ms</p> <p>Duration of terminal QRS < 40 μV (low-amplitude signal duration) ≥ 38 ms</p> <p>Root-mean-square voltage of terminal 40 ms ≤ 20 μV</p> <p>Terminal activation duration of QRS ≥ 55 ms measured from the nadir of the S wave to the end of the QRS, including R', in V1, V2, or V3, in the absence of complete RBBB</p>
V. Arrhythmias	
Non-sustained or sustained VT of LBBB morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL)	<p>Non-sustained or sustained VT of RV outflow configuration, LBBB morphology with inferior axis (positive QRS in leads II, III, and aVF and negative in lead aVL) or of unknown axis</p> <p>> 500 ventricular extrasystoles per 24 h (Holter)</p>
VI. Family history	
ARVC confirmed in a first-degree relative who meets current Task Force criteria	History of ARVC in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current Task Force criteria
ARVC confirmed pathologically at autopsy or surgery in a first-degree relative	Premature sudden death (< 35 years of age) due to suspected ARVC in a first-degree relative
Pathogenic mutation (associated or probably associated with ARVC) in the patient under evaluation	ARVC confirmed pathologically or by current Task Force criteria in a second-degree relative

BSA = body surface area; 2D echo = two-dimensional echocardiography; ECG = electrocardiography; LBBB = left bundle branch block; MRI = magnetic resonance imaging; PLAX = parasternal long-axis view; PSAX = parasternal short-axis view; RBBB = right bundle branch block; RV = right ventricular; RVOT = RV outflow tract; SAECG = signal-averaged ECG; VT = ventricular tachycardia.

Due to the progressive nature of ARVC, four different clinicopathological phases of disease can be recognized in the patients (Blomström-Lundqvist et al. 1987, Corrado et al. 1997, Corrado et al. 2000b). In the concealed phase, only subtle right ventricular abnormalities are present and the patients are asymptomatic but nevertheless at risk for SCD. In the overt electrical phase, the patients develop arrhythmias and functional and morphological abnormalities of the right ventricle. The third phase involves right ventricular failure. Ultimately, the disorder may lead to biventricular heart failure in the most advanced phase.

However, left ventricular involvement may be detected also in earlier phases of the disease (Sen-Chowdhry et al. 2008). The patients are treated with medication for arrhythmias and cardiac insufficiency, implantable cardioverter-defibrillator, catheter ablation, and ultimately cardiac transplantation (Wichter et al. 1992, Corrado et al. 2000a). ARVC patients are also advised to avoid extreme physical exertion (Sen-Chowdhry et al. 2004).

2.3. Genetics of ARVC

ARVC has been reported to occur familiarly in 30-70% of cases (Hamid et al. 2002, Dalal et al. 2005, van Tintelen et al. 2006). The mode of inheritance is usually autosomal dominant, with markedly reduced penetrance (Nava et al. 1988). However, compound heterozygosity and digenic heterozygosity are often detected in patients with severe disease (Bhuiyan et al. 2009, den Haan et al. 2009, Bauce et al. 2010). Environmental factors, such as oestrogen, athletic activity, and viral infections, are suggested to affect the disease penetrance in addition to genetic variants (Awad et al. 2008). ARVC is a disorder of the desmosome, as mutations in each of the components of the cardiac desmosomes, plakophilin-2, desmoplakin, desmoglein-2, desmocollin-2, and plakoglobin, have been documented in ARVC patients (Table 2). In addition, several chromosomal loci with non-desmosomal or unknown disease-associated genes have been identified in individual ARVC families.

Several disease mechanisms have been suggested in the pathogenesis of ARVC. Firstly, the disruption of desmosomal organization by mutations in the desmosomal components may lead to loss of myocyte adhesion, and consequently, cell death, which is enhanced by physical strain (Awad et al. 2008, Delmar and McKenna 2010). The myocytes have limited regenerative capacity, and therefore, their death might lead to a repair mechanism by fibrous and adipose tissue replacement. The right ventricle may be especially vulnerable to this loss of myocyte adhesion because of its thin walls and its high ability to dilate (Awad et al. 2008, Delmar and McKenna 2010). Secondly, desmosomal mutations lead to redistribution of plakoglobin to the nucleus, where it suppresses the canonical Wnt/ β -catenin signalling pathway (Garcia-Gras et al. 2006, Asimaki et al. 2009). This causes increased expression of transcriptional regulators of adipogenesis, which has been suggested to lead to differentiation of cardiac progenitor cells into adipocytes instead of cardiomyocytes (Garcia-Gras et al. 2006, Lombardi et al. 2009). Suppression of Wnt/ β -catenin signalling also leads to increased apoptosis (Longo et al. 2002), which is detected in the myocardium of ARVC

patients (Mallat et al. 1996). The third possible disease mechanism involves impairment of the localization and conductivity of the gap junctional protein connexin 43 due to decreased expression of plakophilin-2 (Oxford et al. 2007) or disrupted interaction with desmocollin-2 (Gehmlich et al. 2011). This gap junctional remodelling might lead to an increased propensity for arrhythmias.

ARVC pathogenesis may also involve altered calcium homeostasis or sodium current. A gain-of-function mutation in plakoglobin creates a novel interaction with histidine-rich calcium-binding protein, as detected in a yeast-two-hybrid screen (Asimaki et al. 2007). If a similar defect occurs also in patient cardiomyocytes, it could promote arrhythmias by disturbed calcium signalling. Plakophilin-2 interacts with the α subunit of the cardiac sodium channel (Sato et al. 2009). Loss of plakophilin-2 leads therefore to alterations of the amplitude and voltage-gating kinetics of the sodium current, which may predispose desmosomal mutation carriers to reentrant arrhythmias (Sato et al. 2009).

Table 2. Chromosomal loci and genes identified in linkage and association studies of ARVC

ARVC subtype	Locus	Gene	Protein	Reference
Autosomal dominant				
ARVC1	14q24	<i>TGFB3</i>	transforming growth factor β 3	Rampazzo et al. 1994, Beffagna et al. 2005
ARVC2	1q43	<i>RYR2</i>	cardiac ryanodine receptor	Rampazzo et al. 1995, Tiso et al. 2001
ARVC3	14q12-q22	N/A	N/A	Severini et al. 1996
ARVC4	2q32.1-q32.3	N/A	N/A	Rampazzo et al. 1997
ARVC5	3p23	<i>TMEM43</i>	transmembrane protein 43	Ahmad et al. 1998, Merner et al. 2008
ARVC6	10p12-p14	N/A	N/A	Li et al. 2000
ARVC7	10q22.3	N/A	N/A	Melberg et al. 1999
ARVC8	6p24	<i>DSP</i>	desmoplakin	Rampazzo et al. 2002
ARVC9	12p11	<i>PKP2</i>	plakophilin-2	Gerull et al. 2004
ARVC10	18q12	<i>DSG2</i>	desmoglein-2	Awad et al. 2006a, Pilichou et al. 2006
ARVC11	18q12	<i>DSC2</i>	desmocollin-2	Syrris et al. 2006b
ARVC12	17q21	<i>JUP</i>	plakoglobin	Asimaki et al. 2007
ARVC13	2q35	<i>DES</i>	desmin	Klauke et al. 2010
Autosomal recessive				
Naxos disease	17q21	<i>JUP</i>	plakoglobin	McKoy et al. 2000
Syndromic ARVC	6p24	<i>DSP</i>	desmoplakin	Alcalai et al. 2003
Syndromic ARVC	18q12	<i>DSC2</i>	desmocollin-2	Simpson et al. 2009

N/A = not available (gene unknown).

Plakophilin-2

Plakophilin-2 belongs to the armadillo family of proteins and contains an amino-terminal head domain and nine armadillo repeat motifs (Mertens et al. 1996). It is expressed in most desmosome-containing tissues, and in cardiomyocytes, it is the only desmosomal plakophilin (Mertens et al. 1996, Mertens et al. 1999). This protein is essential for heart morphogenesis and localization of desmoplakin to cell junctions in mice (Grossmann et al. 2004). Mutations in *PKP2* constitute a common cause of ARVC, accounting for approximately 30% of reported cases (Gerull et al. 2004, Antoniadis et al. 2006, Dalal et al. 2006, Pilichou et al. 2006, Syrris et al. 2006a, den Haan et al. 2009, Qiu et al. 2009, Christensen et al. 2010, Fressart et al. 2010, Xu et al. 2010, Cox et al. 2011). Most mutations are dominant with significantly reduced penetrance, but recessive and compound heterozygous mutations have also been identified in several patients (Awad et al. 2006b, Xu et al. 2010). Large exonic deletions in *PKP2* can also be detected in a small number of patients (Cox et al. 2011).

Desmoplakin

Desmoplakin is a member of the plakin family and forms homodimers via its coiled-coil alpha-helical rod domain (Kowalczyk et al. 1994). It is expressed in all desmosome-containing tissues (Leung et al. 2002). Complete loss of desmoplakin is lethal in mice (Gallicano et al. 1998). Cardiac-restricted heterozygous deletion of desmoplakin leads to a phenotype resembling ARVC in a mouse model (Garcia-Gras et al. 2006), as does overexpression of a desmoplakin missense mutation (Yang et al. 2006). *DSP* mutations can be detected in approximately 5% of ARVC cases, many of them featuring left ventricular involvement (Pilichou et al. 2006, Yang et al. 2006, den Haan et al. 2009, Christensen et al. 2010, Fressart et al. 2010, Xu et al. 2010, Cox et al. 2011).

Desmoglein-2

Desmoglein-2 and desmocollin-2 are expressed in all desmosome-containing tissues and are the only desmosomal cadherins expressed in the heart (Schäfer et al. 1994, Nuber et al. 1995). Desmoglein-2 is needed for embryonic stem cell proliferation in mice (Eshkind et al. 2002). Mice overexpressing a *DSG2* missense mutation manifest with features resembling ARVC and develop myocyte necrosis (Pilichou et al. 2009). *DSG2* mutations are detected in approximately 7% of ARVC patients (Awad et al. 2006a, Heuser et al. 2006, Pilichou et al.

2006, Syrris et al. 2007, den Haan et al. 2009, Christensen et al. 2010, Fressart et al. 2010, Xu et al. 2010, Cox et al. 2011).

Desmocollin-2

DSC2 knockdown in zebrafish embryos leads to desmosomal dysfunction and myocardial contractility defects, suggesting that desmocollin-2 is needed for cardiac morphogenesis and function (Heuser et al. 2006). *DSC2* mutations are rare in ARVC, accounting for only 2% of reported cases (Heuser et al. 2006, Syrris et al. 2006b, den Haan et al. 2009, Christensen et al. 2010, Fressart et al. 2010, Xu et al. 2010, Cox et al. 2011).

Plakoglobin

Plakoglobin (γ -catenin) is a member of the armadillo protein family and contains 13 armadillo repeat motifs (Franke et al. 1989). It is located in both desmosomes and adherens junctions as well as in the nucleus. Homozygous deletion of *JUP* is lethal and leads to severe heart defects in mice (Bierkamp et al. 1996, Ruiz et al. 1996). Heterozygous plakoglobin-deficient mice develop an ARVC-like phenotype, which is precipitated by endurance training (Kirchhof et al. 2006). Dominant *JUP* mutations can be detected in approximately 1% of ARVC cases (den Haan et al. 2009, Christensen et al. 2010, Fressart et al. 2010, Xu et al. 2010, Cox et al. 2011).

Other genes associated with ARVC

Mutations in cardiac ryanodine receptor have been identified in families with effort-induced polymorphic tachycardias (Rampazzo et al. 1995, Tiso et al. 2001), a phenotype resembling *RYR2*-linked CPVT. Mutations in the untranslated region of *TGFB3*, encoding the multifunctional cytokine transforming growth factor β 3, have been identified in two ARVC probands (Beffagna et al. 2005). However, no mutations in the protein-coding region of *TGFB3* have yet been reported in ARVC. Transmembrane protein 43 is a nuclear membrane protein in many cell types, but in cardiomyocytes, it localizes to the cell membrane (Bengtsson and Otto 2008, Christensen et al. 2011). Mutations of *TMEM43* have been identified in a fully penetrant and lethal form of ARVC (Merner et al. 2008) and in Emery-Dreifuss muscular dystrophy-related myopathy (Liang et al. 2011). Mutations in the intermediate filament protein desmin are associated with skeletal and cardiac myopathy (Goldfarb et al. 1998), but also with ARVC without skeletal muscle involvement (Klauke et

al. 2010). Recently, mutations in *TTN*, located near the *ARVC4* locus and encoding the sarcomeric protein titin (Taylor et al. 2011), and in *PLN*, encoding phospholamban (van der Zwaag et al. 2012), were reported in families with ARVC.

2.4. Syndromic forms of ARVC

Syndromic forms of ARVC involving skin and hair abnormalities follow an autosomal recessive mode of inheritance and are associated with mutations in the desmosomal genes. Naxos disease patients suffer from non-epidermolytic palmoplantar keratoderma, woolly hair, and ARVC (Protonotarios et al. 1986). A homozygous deletion in the last armadillo repeat of plakoglobin has been reported in all affected cases (McKoy et al. 2000). A homozygous missense mutation in plakoglobin has been identified in a similar syndrome with palmoplantar keratoderma, alopecia, and ARVC (Erken et al. 2011). Symptoms of Carvajal syndrome include epidermolytic palmoplantar keratoderma, woolly hair, and dilated cardiomyopathy with predominant left ventricular involvement (Carvajal-Huerta 1998). Affected patients are homozygous for a deletion in the carboxy terminus of desmoplakin (Norgett et al. 2000). A similar syndrome with pemphigous-like skin disorder, woolly hair, and cardiomyopathy described as ARVC due to predominantly right ventricular involvement is caused by a homozygous missense mutation in the carboxy terminus of desmoplakin (Alcalai et al. 2003). A homozygous mutation in desmocollin-2 has been identified in patients with ARVC, mild palmoplantar keratoderma, and woolly hair (Simpson et al. 2009).

3. Long QT syndrome (LQTS)

3.1. Cardiac ion channels

Cardiac action potential is generated by sequential opening and closing of ion channels located in the plasma membrane of cardiomyocytes (Figure 3). The resting membrane potential of ventricular cardiomyocytes is negative, approximately -85 mV (Amin et al. 2010). Depolarization is caused by the inward sodium current (I_{Na}). Early repolarization by the transient outward potassium current (I_{to}) is followed by a plateau phase, in which potassium outflow by the rapidly (I_{Kr}) and slowly (I_{Ks}) activated delayed rectifier currents is balanced by the L-type inward calcium current ($I_{Ca,L}$). The negative membrane potential is restored in the repolarization phase by the delayed outward rectifier potassium currents after the inactivation of the $I_{Ca,L}$ channels. In the resting phase, the negative membrane potential is maintained by the inward rectifier potassium current (I_{K1}). Calcium influx in the plateau phase initiates calcium-induced calcium release from the sarcoplasmic reticulum through activation of the cardiac ryanodine receptor complex. The elevation of intracellular calcium concentration couples excitation with contraction in cardiomyocytes (Amin et al. 2010).

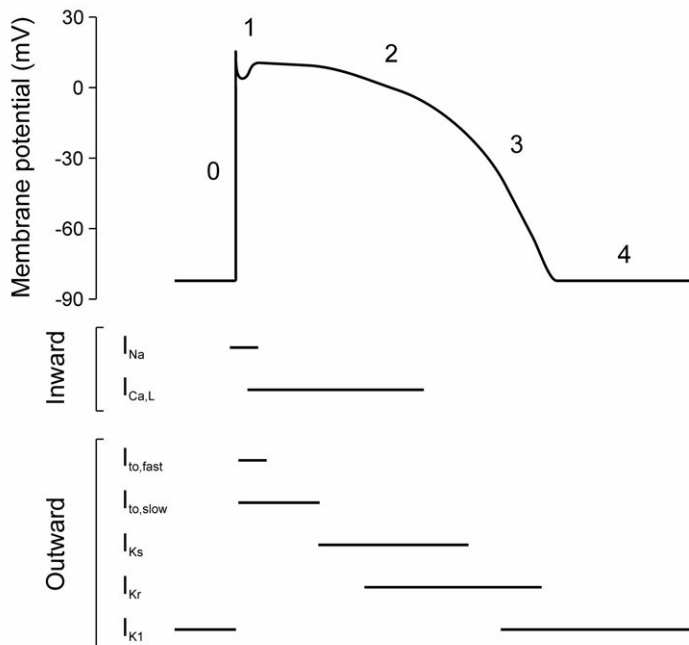


Figure 3. Action potential of a ventricular cardiomyocyte and the main ion currents contributing to each phase (0-4). Adapted from Amin et al. 2010.

Cardiac ion channels consist of pore-forming α -subunits and accessory β -subunits. The α -subunit of sodium and calcium channels comprises four repeats of a domain structure (DI-DIV), each containing six transmembrane segments (S1-S6) (Gellens et al. 1992). The pore loop of the channel is located between S5 and S6, and segment S4 is responsible for voltage-dependent activation (Stühmer et al. 1989). I_{to} , I_{Kr} , and I_{Ks} channels consist of a single domain with six transmembrane segments, and I_{K1} of a single domain with two transmembrane segments (MacKinnon 1991). Voltage-gated potassium channel α -subunits co-assemble to form a functional tetramer structure (MacKinnon 1991).

The main ion channels of ventricular cardiomyocytes are listed in Table 3. Most of these channels have several β -subunits or regulatory proteins affecting their function. For example, minK encoded by *KCNE1* and MiRP1 encoded by *KCNE2* are required for generation of I_{Ks} and I_{Kr} currents (Barhanin et al. 1996, Sanguinetti et al. 1996, McDonald et al. 1997, Abbott et al. 1999). In addition, cardiac ion channels may be regulated by membrane voltage, ion concentrations, phosphorylation, second messengers, ligand binding, channel-blocking agents, and microRNAs (Amin et al. 2010).

Table 3. Main ion currents during the action potential of ventricular cardiomyocytes

Current	α -subunit	α -subunit gene	Reference
I_{Na}	Na _v 1.5	<i>SCN5A</i>	Gellens et al. 1992
$I_{to,fast}$	K _v 4.3	<i>KCND3</i>	Kong et al. 1998
$I_{to,slow}$	K _v 1.4	<i>KCNA4</i>	Tamkun et al. 1991
$I_{Ca,L}$	Ca _v 1.2	<i>CACNA1C</i>	Schultz et al. 1993
I_{Kr}	K _v 11.1	<i>KCNH2</i>	Warmke and Ganetzky 1994
I_{Ks}	K _v 7.1	<i>KCNQ1</i>	Wang et al. 1996
I_{K1}	Kir2.1	<i>KCNJ2</i>	Raab-Graham et al. 1994
I_{NCX}	NCX1	<i>SLC8A1</i>	Komuro et al. 1992

3.2. Clinical features of LQTS

Long QT syndrome (LQTS) is a severe electrical disorder of the heart manifesting with risks of ventricular arrhythmias and SCD despite a structurally normal heart. Jervell and Lange-Nielsen syndrome was the first reported form of LQTS (Jervell and Lange-Nielsen 1957). This autosomal recessive disorder features also congenital deafness due to a potassium secretion defect in the inner ear (Vetter et al. 1996). The estimated prevalence of Jervell and Lange-Nielsen syndrome is 1:55 000-1:200 000 (Tranebjaerg et al. 1999). Romano-Ward

syndrome (Romano et al. 1963, Ward 1964), the autosomal dominant form of LQTS, is more common, with prevalence estimates ranging from 1:2000 to 1:5000 (Goldenberg and Moss 2008, Schwartz et al. 2009). The average age of onset is 12 years, but symptoms may begin any time between early childhood and the age of 40 years (Priori et al. 2003).

LQTS is characterized by prolonged QT interval on ECG, an indication of delayed repolarization. Ventricular tachycardia typically occurs in the form of *torsades de pointes*, which can be seen in ECG as twisting of the QRS axis and may lead to ventricular fibrillation and ultimately to SCD (Viskin et al. 1996). The extent of QT prolongation predicts the risk of cardiac events (Zareba et al. 1995). Since QT interval duration is rate-dependent, it is generally corrected for heart rate according to Bazett's formula ($QTc = QT/\sqrt{RR}$) (Bazett 1920). $QTc \leq 440$ ms has often been considered normal (Vincent et al. 1992). A specific diagnostic scoring system, which takes into account both ECG findings and clinical and family history, is suitable for individuals with borderline prolonged QT interval or those without symptoms (Schwartz et al. 1993). Exercise stress test is especially useful for differentiating the subtypes LQT1 and LQT2 (Swan et al. 1999b).

The expressivity and penetrance of LQTS are variable and the symptoms may vary considerably even within a single family (Priori et al. 1999). Patient history, including syncope and cardiac arrest, family history, QTc interval duration, and sex, as well as information on mutated locus and mutation type can be used for risk stratification of LQTS patients (Moss et al. 2000, Moss et al. 2002, Priori et al. 2003). Arrhythmic events of LQTS patients can be prevented with beta blocker therapy, or more rarely with left cardiac sympathetic denervation, and treated with an implantable cardioverter-defibrillator (Zipes et al. 2006). Lifestyle advice includes avoidance of QT-prolonging medication, electrolyte disturbances, and adrenergic stimuli (Zipes et al. 2006). Patients with LQT1 are recommended to refrain from competitive sports and swimming, and patients with LQT2 to avoid exposure to auditory stimuli, especially during sleep (Schwartz et al. 2001).

Acquired LQTS may be caused by environmental factors, such as QT-prolonging medication or electrolyte disturbances, which affect the function of cardiac ion channels (Roden 2004). It may also arise from ischaemic or structural heart disease or congestive heart failure (Roden 2004). Although acquired LQTS is not usually inherited, certain genetic

variants may predispose to it in the presence of an additional trigger (Abbott et al. 1999, Napolitano et al. 2000, Sesti et al. 2000, Lehtonen et al. 2007).

3.3. Genetics of LQTS

LQTS results from defective function of cardiac ion channels. Thus far, 13 genes have been reported to be associated with LQTS (Table 4), but many of them are mutated only in individual families (Bokil et al. 2010). These genes encode the α -subunits of cardiac ion channels (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNJ2*, *CACNA1C*, and *KCNJ5*), but also the regulatory β -subunits (*KCNE1*, *KCNE2*, and *SCN4B*), ion-channel scaffolding proteins (*AKAP9* and *SNTA1*), and proteins targeting the ion channels to the cell membrane or altering the biophysical properties of ion channels (*ANK2* and *CAV3*). Mutations in these genes are detected in up to 70% of LQTS cases, which is suggestive of additional disease loci (Napolitano et al. 2005, Bai et al. 2009). Most mutations are of missense type, but also frameshift, nonsense, and splice site mutations occur frequently. Recently, large copy number variants in *KCNQ1* and *KCNH2* were reported to account for approximately 3% of LQTS cases without point mutations in the known LQTS genes (Barc et al. 2011).

Most LQTS mutations cause either a loss of function in the repolarizing potassium currents or a gain of function in the depolarizing sodium or calcium currents. Both types of defects result in prolongation of the repolarization phase. It is also possible that these defects lead to reduced repolarization reserve manifesting in LQTS symptoms only after an additional stimulus, such as adrenergic stimulation, bradycardia, or use of QT-prolonging medication, affects repolarization (Roden 1998). Delayed repolarization predisposes to early and delayed afterdepolarizations, which trigger a *torsades de pointes* type of arrhythmia (Antzelevitch 2007). Arrhythmias may also be caused by a reentry mechanism due to increased transmural dispersion of repolarization (Antzelevitch 2007).

Table 4. Genes and related functional defects associated with LQTS

LQTS subtype	Gene	Protein	Functional defect	Trigger of symptoms	Reference
Autosomal dominant					
LQT1	<i>KCNQ1</i>	K _v 7.1	I _{Ks} ↓	Exercise, swimming, emotion	Wang et al. 1996
LQT2	<i>KCNH2</i>	K _v 11.1	I _{Kr} ↓	Sound, emotion	Curran et al. 1995
LQT3	<i>SCN5A</i>	Na _v 1.5	I _{Na} ↑	Sleep, rest, emotion	Wang et al. 1995
LQT4	<i>ANK2</i>	Ankyrin B	I _{Na,K} , I _{NCX} , I _{Na} ↓	Exercise	Mohler et al. 2003
LQT5	<i>KCNE1</i>	minK	I _{Ks} ↓	Exercise, emotion	Splawski et al. 1997
LQT6	<i>KCNE2</i>	MiRP1	I _{Kr} ↓	Rest, exercise	Abbott et al. 1999
LQT7 (AS)	<i>KCNJ2</i>	Kir2.1	I _{K1} ↓	Rest, exercise	Plaster et al. 2001
LQT8 (TS)	<i>CACNA1C</i>	Ca _v 1.2	I _{Ca,L} ↑	Exercise, emotion	Splawski et al. 2004
LQT9	<i>CAV3</i>	M-Caveolin	I _{Na} ↑	Rest, sleep	Vatta et al. 2006
LQT10	<i>SCN4B</i>	Na _v β4	I _{Na} ↑	Exercise	Medeiros-Domingo et al. 2007
LQT11	<i>AKAP9</i>	Yotiao	I _{Ks} ↓	Exercise	Chen et al. 2007
LQT12	<i>SNTA1</i>	α1-Syntrophin	I _{Na} ↑	Rest	Ueda et al. 2008
LQT13	<i>KCNJ5</i>	Kir3.4	I _{KAch} ↓	Exercise, emotion	Yang et al. 2010
Autosomal recessive					
JLNS1	<i>KCNQ1</i>	K _v 7.1	I _{Ks} ↓	Exercise, swimming, emotion	Tyson et al. 1997
JLNS2	<i>KCNE1</i>	minK	I _{Ks} ↓	Exercise, swimming, emotion	Tyson et al. 1997

AS = Andersen's syndrome; JLNS = Jervell and Lange-Nielsen syndrome; TS = Timothy syndrome.

In Finland, the four founder mutations *KCNQ1* G589D, *KCNQ1* IVS7-2A>G, *KCNH2* L552S, and *KCNH2* R176W account for the majority of the known genetic spectrum of LQTS (Piippo et al. 2001, Fodstad et al. 2004). Only 23-38% of the carriers of these mutations are symptomatic, indicating a role for other genetic and environmental disease-modifying factors (Fodstad et al. 2004). These founder mutations have clustered in the Finnish population due to the unique population history of Finland, with small founder populations, bottleneck effects, and geographic and cultural isolation (Sajantila et al. 1996, Peltonen et al. 1999). One in 250 Finns carry one of these four mutations, which prolong the QT interval by 22-50 ms when studied at the population level (Marjamaa et al. 2009b).

LQT1

The first LQTS locus was assigned to chromosome 11p15.5 in a linkage study (Keating et al. 1991). Positional cloning revealed *KCNQ1* (also called *KVLQT1*) as the disease-associated gene (Wang et al. 1996). Mice with a homozygous disruption of the *Kcnq1* gene show a shaker/waltzer phenotype and deafness in addition to prolongation of QT and JT intervals and abnormal T- and P-wave morphologies, a cardiac phenotype comparable to Jervell and Lange-Nielsen syndrome (Casimiro et al. 2001). Approximately 50% of LQTS patients with a genetic diagnosis harbour mutations in *KCNQ1* (Napolitano et al. 2005).

LQT2

Chromosome 7q35-q36 was identified as the second loci linked to LQTS (Jiang et al. 1994). Mapping of *KCNH2* (also called *HERG*, the human ether-a-go-go-related gene) to this region and detection of mutations in this gene in LQTS patients revealed *KCNH2* as the disease-associated gene in LQT2 (Curran et al. 1995). Homozygous deletion of the homologous gene in mice leads to elimination of the I_{Kr} current and episodic sinus bradycardia (Lees-Miller et al. 2003). Approximately 40% of genetically defined LQTS patients have mutations in *KCNH2* (Napolitano et al. 2005). Mutations located in the pre-forming region have been associated with a more severe disease phenotype than other mutations (Moss et al. 2002).

LQT3

LQT3 locus in 3p21 was reported together with LQT2 in a linkage study (Jiang et al. 1994). In a subsequent study, an intragenic deletion of *SCN5A* was detected as the disease-causing mutation in two families (Wang et al. 1995). A corresponding heterozygous deletion in mice causes sudden accelerations in heart rate, lengthening of the action potential, and ventricular arrhythmias (Nuyens et al. 2001), whereas homozygous disruption of *Scn5a* leads to intrauterine lethality (Papadatos et al. 2002). Mutations in *SCN5A* account for approximately 10% of LQTS patients with a genetic diagnosis (Napolitano et al. 2005).

3.4. Genetics of QT interval and LQTS modifier genes

QT interval has a heritability of 35-40% in the general population (Newton-Cheh et al. 2005, Li et al. 2009a). Previously, genetic studies of QT interval concentrated mainly on candidate genes identified through association with LQTS. Consequently, several common variants in the LQTS genes, such as *KCNE1* D85N (rs1805128) and *KCNH2* K897T (rs1805123), were reported to be associated with QT interval duration in the general population (Pietilä et al. 2002, Bezzina et al. 2003b, Gouas et al. 2005, Pfeufer et al. 2005, Newton-Cheh et al. 2007, Marjamaa et al. 2009a). More recently, genome-wide association (GWA) studies have enabled the hypothesis-free search of QT interval-associated loci. This approach has revealed several associated loci of unknown function in addition to the known LQTS loci (Table 5). The *NOS1AP* locus in 1q23 consistently shows the statistically strongest association with QT interval (Arking et al. 2006, Marroni et al. 2009, Newton-Cheh et al. 2009b, Nolte et al. 2009, Pfeufer et al. 2009). *NOS1AP* encodes the neuronal nitric oxide synthase 1 adaptor protein, which modulates cardiac repolarization by inhibition of $I_{Ca,L}$ current and enhancement of I_{Kr} current (Chang et al. 2008). Together, the known loci explain only up to 10% of the heritability of QT interval (Newton-Cheh et al. 2009b, Jamshidi et al. 2010), suggesting a role for additional still unknown QT interval-modifying loci.

Table 5. QT interval-associated loci identified in GWA studies ($p < 5 \times 10^{-8}$)

Chr.	Position*	Nearest gene	SNP	Coded allele [†]	CAF
1p36	6 201 957	<i>RNF207</i>	rs846111	C	0.28
1q23	160 300 514	<i>NOS1AP</i>	rs12143842	T	0.26
1q24	167 366 107	<i>ATP1B1</i>	rs10919071	A	0.87
2p22	40 611 295	<i>SLC8A1</i>	rs13017846	A	0.58
3p22	38 568 397	<i>SCN5A</i>	rs12053903	T	0.66
6q22	118 787 067	<i>PLN</i>	rs11970286	T	0.44
7q36	150 268 796	<i>KCNH2</i>	rs4725982	T	0.22
11p15	2 441 379	<i>KCNQ1</i>	rs2074238	C	0.94
13q13	34 095 789	<i>NBEA</i>	rs885170	G	0.19
13q14	47 060 559	<i>SUCLA2</i>	rs2478333	A	0.33
16p13	11 599 254	<i>LITAF</i>	rs8049607	T	0.49
16q21	57 124 739	<i>CNOT1</i>	rs37062	A	0.76
17q12	30 348 495	<i>LIG3</i>	rs2074518	C	0.54
17q24	66 006 587	<i>KCNJ2</i>	rs17779747	G	0.65
21q22	34 743 550	<i>KCNE1</i>	rs1805128	A	0.01

References: Marroni et al. 2009, Newton-Cheh et al. 2009b, Nolte et al. 2009, Pfeufer et al. 2009, Kim et al. 2012.

*According to NCBI genome build 36.

[†]Allele associated with QT prolongation.

CAF = coded allele frequency in the original study; Chr. = chromosome; GWA = genome-wide association; SNP = single nucleotide polymorphism.

The QT interval-associated single nucleotide polymorphisms (SNPs) provide intriguing candidates for studies of LQTS modifier genes that affect the large phenotypic variation among LQTS mutation carriers. The *KCNE1* D85N (rs1805128) minor allele, which prolongs QT interval up to 10 ms at the population level (Marjamaa et al. 2009a), has been shown to reduce both I_{Ks} and I_{Kr} currents *in vitro* and to occur as a second variant in several LQTS patients (Westenskow et al. 2004, Nishio et al. 2009). In addition, variants in *NOS1AP* have been associated with increased risk of cardiac events in LQTS patients (Crotti et al. 2009, Tomás et al. 2010). A common polymorphism in a LQTS gene may also directly affect the phenotype caused by a mutation in the same gene. For example, the *KCNH2* K897T minor allele has been suggested to accentuate the I_{Kr} reduction caused by a more severe *KCNH2* mutation (Crotti et al. 2005), but the findings on cardiac repolarization are conflicting both *in vitro* and *in vivo* (Laitinen et al. 2000, Scicluna et al. 2008). Interestingly, the minor allele of *SCN5A* H558R was shown to rescue the phenotype of a LQTS-causing *SCN5A* mutation (Ye et al. 2003).

4. Sudden cardiac death (SCD)

4.1. Epidemiology and clinical risk factors of SCD

Sudden cardiac death (SCD) is generally defined as an unexpected death occurring within one hour of the onset of symptoms or, when unwitnessed, within 24 hours of being seen alive and well (Chugh et al. 2008). Exclusion of non-cardiac causes of death, such as pulmonary embolism, aortic rupture, or stroke, is essential for the diagnosis. The estimated yearly incidence of SCD is 50:100 000-80:100 000 in Western countries (Chugh et al. 2008), and the corresponding figure in Finland has been estimated to be 57:100 000 (Hookana et al. 2011). The incidence of SCD has declined over the past decades due to the improvement of prevention and treatment strategies for cardiovascular disease, but at the same time, the occurrence of SCD as a proportion of overall cardiovascular deaths has increased (Fox et al. 2004a). SCD is estimated to account for approximately half of all cardiovascular deaths (Salomaa et al. 2003, Fox et al. 2004a). Coronary heart disease (CHD) underlies up to 80% of SCDs (Chugh et al. 2004a, Hookana et al. 2011). Cardiomyopathy is detected in 10-15% of SCD cases and a congenital abnormality or a structurally normal heart, indicating a primary arrhythmogenic disorder, is reported in 5-10% (Chugh et al. 2008). Familial investigation of autopsy-negative SCD cases reveals inherited arrhythmia disorders in approximately half of the families, including LQTS, Brugada syndrome, CPVT, ARVC, and hypertrophic cardiomyopathy (Tan et al. 2005, Behr et al. 2008).

Males have a higher risk of SCD than females, and the occurrence peaks in early childhood and after the age of 45 (Chugh et al. 2004a). Clinical risk factors for CHD predispose also to SCD. These include smoking, obesity, lack of physical activity, hypertension, diabetes, hypercholesterolaemia, and family history of CHD (Wannamethee et al. 1995, Jouven et al. 1999). After myocardial infarction, the risk of SCD is highest during the first 30 days, decreasing gradually thereafter (Adabag et al. 2008), and atrial fibrillation is known to increase the risk (Pedersen et al. 2006). Other risk factors for SCD are heart failure, left ventricular dysfunction and hypertrophy, reduced pulmonary vital capacity, elevated heart rate, abnormal ECG, and abnormal autonomic markers such as decreased heart rate variability (Adabag et al. 2010a). Prolonged QT interval predisposes to SCD in the general population (Straus et al. 2006). J-point elevation (Tikkanen et al. 2009) and QRS complex

widening (Dhar et al. 2008, Kurl et al. 2012) are also known risk factors for SCD. The SCD risk is increased in people with low socioeconomic status (Reinier et al. 2006).

SCD is the first manifestation of cardiovascular disease in approximately 50% of cases (Fox et al. 2004a). In addition, most of the clinical risk factors have a low positive predictive value for SCD. High-risk criteria, such as myocardial infarction or left ventricular dysfunction, reveal only a small proportion of potential victims, and the majority of SCDs occur in risk groups with the lowest incidence (Figure 4) (Myerburg et al. 1998, Huikuri et al. 2001, Noseworthy and Newton-Cheh 2008). Prevention of fatal arrhythmic events with, for example, an implantable cardioverter-defibrillator is feasible only in the high-risk groups (Zipes et al. 2006). Therefore, identification of individuals with a markedly elevated risk of SCD is essential. Genetic risk markers could provide a means for better risk prediction together with the clinical risk factors.

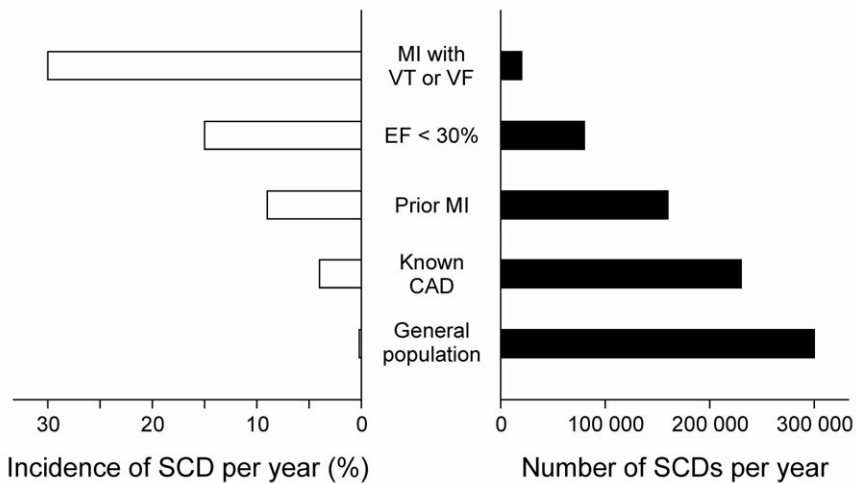


Figure 4. Incidence and total number of events for SCD in the different risk groups in the USA. CAD = coronary artery disease; EF = ejection fraction; MI = myocardial infarction; SCD = sudden cardiac death; VF = ventricular fibrillation; VT = ventricular tachycardia. Adapted from Myerburg et al. 1998, Huikuri et al. 2001, and Noseworthy and Newton-Cheh 2008.

4.2. Genetics of SCD

The risk of SCD is heritable, but the genes involved are largely unknown. Parental history of SCD approximately doubles the risk of SCD, but if both parents have died suddenly, the risk of sudden death is 9-fold (Jouven et al. 1999, Friedlander et al. 2002). Sudden death in a first-degree relative is also associated with an increased risk of ventricular fibrillation during myocardial infarction and with an elevated risk of dying suddenly during an acute coronary event (Dekker et al. 2006, Kaikkonen et al. 2006), indicating that genetic variants may predispose to fatal arrhythmic events during myocardial infarction.

Inherited arrhythmia disorders, such as LQTS, CPVT, and ARVC, may lead to SCD. Rare mutations in the LQTS genes *KCNQ1*, *KCNH2*, and *SCN5A*, as well as in the CPVT gene *RYR2*, have also been detected in SCD victims without a previously diagnosed electrical disorder (Table 6). Together, mutations in these genes may occur in up to one-third of sudden unexplained death victims (Tester et al. 2004, Tester and Ackerman 2007). Recently, mutations in the ARVC gene *PKP2* were also reported in cases of sudden unexplained death with negative autopsy findings (Zhang et al. 2012). In addition to the rare mutations, also common variants in the *KCNQ1* and *SCN5A* ion channel genes are associated with increased risk of SCD (Burke et al. 2005, Albert et al. 2010). Accordingly, common variants in the CPVT-associated *CASQ2* gene, the Brugada syndrome-associated *GPDIL* gene, and *NOS1AP*, which has previously been associated with QT interval duration, have also been reported to predispose to SCD (Kao et al. 2009, Westaway et al. 2011). Of the common variants associated with QRS complex duration, one SNP in the *TKT-CACNA1D-PRKCD* locus was reported to be associated also with risk of SCD (Arking et al. 2011).

Sympathetic activation is involved in generation of ventricular arrhythmias and may ultimately influence the risk of SCD. Therefore, variants in genes affecting the function of the autonomic nervous system may predispose to SCD. The Q27E polymorphism in β_2 -adrenergic receptor alters the agonist-mediated down-regulation of receptor expression (Green et al. 1994) and is associated with risk of SCD (Sotoodehnia et al. 2006). α_{2B} -adrenergic receptor is involved in vasoconstriction, and the variant form with deletion of three glutamate residues shows impaired agonist-promoted desensitization and increased risk of SCD (Snapir et al. 2003). Genes involved in angiotensin-converting enzyme-related pathways may also contribute to the inherited risk of SCD (Sotoodehnia et al. 2009).

Table 6. Genetic variants associated with risk of SCD

Chr.	Nearest gene	Variant	Study design*	Phenotype	Reference
Common variants (identified in population-based or case-control studies)					
1p13	<i>CASQ2</i>	rs17500488	1	SCD with CAD	Westaway et al. 2011
		rs3010396	1	SCD with CAD	Westaway et al. 2011
		rs7366407	1	SCD with CAD	Westaway et al. 2011
1q23	<i>NOS1AP</i>	rs10918859	1	SCD with CAD	Westaway et al. 2011
		rs12084280	1	SCD with CAD	Westaway et al. 2011
		rs12567209	1	SCD	Kao et al. 2009
		rs16847548	1	SCD	Kao et al. 2009
1q24	<i>SELP</i>	V168M	1	VF during MI	Elmas et al. 2010
2q11	<i>ADRA2B</i>	Ins/Del	1	SCD	Snapir et al. 2003
2q24	<i>BAZ2B</i>	rs4665058	2	SCD	Arking et al. 2011
3p21	<i>TKT</i>	rs4687718	1	SCD	Arking et al. 2011
3p22	<i>GPD1L</i>	rs9862154	1	SCD with CAD	Westaway et al. 2011
3p22	<i>SCN5A</i>	rs11720524	1	SCD	Albert et al. 2010
		S1103Y	1	SCD	Burke et al. 2005
3q24	<i>AGTR1</i>	rs1492099	1	SCA	Sotoodehnia et al. 2009
3q27	<i>KNG1</i>	rs710448	1	SCA in women	Sotoodehnia et al. 2009
5q33	<i>ADRB2</i>	Q27E	1	SCD	Sotoodehnia et al. 2006
7q22	<i>SERPINE1</i>	Ins/Del	1	SCD with CAD	Anvari et al. 2001
9p21	<i>CDKN2BAS</i>	rs10757274	1	SCD	Newton-Cheh et al. 2009a
		rs2383207	1	SCD	Newton-Cheh et al. 2009a
11p15	<i>KCNQ1</i>	rs2283222	1	SCD	Albert et al. 2010
11q23	<i>IL18</i>	rs187238	1	SCD in men	Hernesniemi et al. 2008
13q31	<i>GPC5</i>	rs3864180	2	SCA with CAD	Arking et al. 2010
13q34	<i>F7</i>	R353Q	1	SCD in men	Mikkelsen and Karhunen 2002
15q22	<i>LIPC</i>	-480C>T	1	SCD in men	Fan et al. 2007
17p13	<i>GP1BA</i>	T145M	1	SCD in men <55 y	Mikkelsen et al. 2001
17q21	<i>ITGB3</i>	L33P	1	SCD in men <50 y	Mikkelsen et al. 2000
21q21	<i>CXADR</i>	rs2824292	2	MI with VF	Bezzina et al. 2010
Rare variants (identified in individual patients or families)					
1q43	<i>RYR2</i>	Several mutations	1	SCD, SUD	Tester et al. 2004, Marjamaa et al. 2011
3p22	<i>SCN5A</i>	Several mutations	1	SCD, SUD	Tester and Ackerman 2007, Albert et al. 2008, Adabag et al. 2010b
7q36	<i>DPP6</i>	N/A	3	VF	Alders et al. 2009
7q36	<i>KCNH2</i>	Several mutations	1	SCD, SUD	Chugh et al. 2004b, Tester and Ackerman 2007, Adabag et al. 2010b
11p15	<i>KCNQ1</i>	Several mutations	1	SUD	Tester and Ackerman 2007
12p11	<i>PKP2</i>	Several mutations	1	SUD	Zhang et al. 2012

*Study design: 1 = candidate gene study, 2 = genome-wide association study, 3 = genome-wide haplotype-sharing study.

CAD = coronary artery disease; Chr. = chromosome; Del = deletion; Ins = insertion; MI = myocardial infarction; N/A = not available (variant unknown); SCA = sudden cardiac arrest; SCD = sudden cardiac death; SUD = sudden unexplained death; VF = ventricular fibrillation; y = years.

Genes controlling thrombosis and atherosclerosis are apparent candidate genes for myocardial infarction and SCD. A variant in factor VII of the coagulation cascade has been suggested to be associated with SCD (Mikkelsen and Karhunen 2002), and a deletion variant in the *SERPINE1* gene encoding the plasminogen activator inhibitor-1, which regulates endogenous fibrinolysis, has been associated with SCD in patients with coronary artery disease (Anvari et al. 2001). Several variants involved in platelet activation are associated with ventricular fibrillation during myocardial infarction and SCD (Mikkelsen et al. 2000, Mikkelsen et al. 2001, Elmas et al. 2010). A promoter variant in the gene encoding hepatic lipase predisposes to SCD by a mechanism that may involve elevated total and high-density lipoprotein cholesterol levels (Fan et al. 2007). A promoter variant in the interleukin 18 gene decreases the expression of this atherogenic cytokine and reduces the risk of SCD (Hernesniemi et al. 2008). In the 9p21 chromosomal region, the cyclin-dependent kinase inhibitor genes *CDKN2A* and *CDKN2B* as well as the *CDKN2B* antisense RNA gene *CDKN2BAS* appear to be potential candidate genes for myocardial infarction and SCD (Helgadóttir et al. 2007, Newton-Cheh et al. 2009a).

In addition to these associations detected in studies of previously known candidate genes, GWA studies have revealed novel genes that may contribute to risk of SCD or ventricular fibrillation during myocardial infarction (Arking et al. 2010, Bezzina et al. 2010, Arking et al. 2011). The exact pathogenetic mechanisms associated with these variants are still unknown. Glypican 5, encoded by *GPC5*, is a member of the heparan sulphate proteoglycan family of proteins that modulates vasculogenesis and angiogenesis after ischaemic injury (Arking et al. 2010). *CXADR* encodes a viral receptor protein that may be involved in viral myocarditis and cardiac conduction (Bezzina et al. 2010). *BAZ2B* is a bromodomain-containing gene with an unknown function (Arking et al. 2011), and *DPP6* encodes a putative component of the cardiac I_{to} channel (Alders et al. 2009).

5. Methods of studying the genetics of cardiac arrhythmia and SCD

5.1. Candidate gene approach

The candidate gene approach is used for investigating the association between variants in a previously identified gene and a disorder or other trait of interest. Candidate genes may be selected on the basis of functional similarity to genes with a previously detected association with the same phenotype. In addition to the exact function of the proteins, this similarity may concern the cellular compartment, the expression pattern, the regulatory network, or the interaction partners of the proteins. In ARVC, identification of disease-causing mutations in plakoglobin and desmoplakin (McKoy et al. 2000, Rampazzo et al. 2002) led to the discovery of this disorder being commonly caused by desmosomal mutations (Gerull et al. 2004). Similarly, initial recognition of ion channel gene mutations associated with LQTS (Curran et al. 1995, Wang et al. 1995, Wang et al. 1996) revealed many suitable candidate genes with a similar function.

Candidate genes may also be identified through a known association with another disorder that shows phenotypic similarities to the disorder being investigated. For example, after the identification of LQTS-causing mutations in *SCN5A* (Wang et al. 1995), this gene has been associated with many other electrical disorders of the heart (Chen et al. 1998, Schott et al. 1999, Akai et al. 2000, Benson et al. 2003, Ellinor et al. 2008). Also genes associated with a risk factor for a disorder provide potential candidates for future genetic studies. Accordingly, genes associated with QT interval or QRS complex duration or J-point elevation could potentially be associated with fatal arrhythmias. For example, *NOS1AP*, a QT-modulating gene, has been associated with SCD (Kao et al. 2009).

Initially, candidate genes may be identified based on positional information gained from linkage studies, as in the case of *TMEM43* mutations in ARVC (Merner et al. 2008) and *KCNQ1* mutations in LQTS (Wang et al. 1996). Another possibility is to identify the causative mutation for the disorder first in an animal model and subsequently to localize a homologous gene in the human genome. For example, striatin could be considered a novel candidate gene in ARVC as it was found to be mutated in the canine model of this disorder (Meurs et al. 2010). The candidate gene approach enables the identification of small relative risks, but is restricted by the current knowledge of the pathogenetic mechanisms.

5.2. Linkage and association studies

Linkage studies are conducted to identify a genetic locus that cosegregates with the trait of interest in pedigrees. This approach takes advantage of the positional information of genetic loci in chromosomes. The likelihood of linkage between the genetic marker and the genetic locus to be found is compared with the likelihood of independent assortment between the two loci by calculating a logarithm of odds score (Morton 1955). Linkage analysis can be utilized to map both quantitative and qualitative trait loci. Parametric linkage analysis requires information on the mode of inheritance, trait and marker allele frequencies, penetrance, and probability of phenocopies. For complex disorders or traits with unknown parameters of the genetic model, testing increased allele-sharing among affected relative pairs using non-parametric linkage analysis may be more suitable. Linkage studies can be restricted to previously defined chromosomal loci or they can cover the whole genome. For example, the ARVC8 locus was mapped to 6p24 in a genome-wide linkage scan using microsatellite markers (Rampazzo et al. 2002), and more recently, the LQT13 locus was mapped to 11q24 with a similar approach (Yang et al. 2010).

Association studies compare genotype frequencies between, for example, cases and controls or in a family-based approach. Association may be tested directly between a causative variant and a phenotype, but commonly this type of study takes advantage of linkage disequilibrium between alleles of a causative variant and alleles of linked genetic markers. In this way, location of the causative variant near a genetic marker may be assessed based on the association between the phenotype of interest and a genetic marker. Association studies may be targeted at a set of candidate genes or at the whole genome. A GWA study is a hypothesis-free statistical approach suitable for identifying common genetic variants associated with a complex trait (Manolio 2010). In this approach, a large set of genetic markers representing the whole genome is screened for associations with a selected trait, and modest effect sizes may be discovered using large sample sizes. Several candidate genes for SCD have been located in GWA studies (Arking et al. 2010, Bezzina et al. 2010, Arking et al. 2011).

5.3. Future directions

Development of tools for human genetic studies has benefited from the rapid progress achieved by international projects aimed at revealing the human genomic sequence and variation. The Human Genome Project assembled the consensus sequence of the human genome, providing the exact positional and sequence information of each gene (International Human Genome Sequencing Consortium 2004). The International HapMap Project established a haplotype map of the human genome as well as a high-resolution map of common SNPs and copy number polymorphisms (Altshuler et al. 2010). The 1000 Genomes Project aims to provide a more comprehensive map of human genomic variation, including rare variants with an allele frequency of $\geq 1\%$, by low-coverage whole-genome sequencing of 2500 samples (1000 Genomes Project Consortium 2010). The 1000 Genomes Exon Pilot Project collected deep-coverage exome data to identify rare variants with $< 1\%$ allele frequency (Marth et al. 2011). The achievements of these international projects clear the path for future human genetic studies, including identification of novel disease-causing mutations by whole-genome and whole-exome sequencing. These large-scale sequencing methods, together with traditional linkage and association studies, provide a powerful tool for discovering new disease genes and mechanisms in cardiac arrhythmias and SCD.

AIMS OF THE STUDY

This study aimed to identify genetic variants predisposing to cardiac arrhythmia disorders, specifically ARVC and LQTS, as well as their most severe end-point, SCD. Specific aims were as follows:

1. To reveal the desmosomal mutation spectrum in Finnish ARVC patients and to assess the pathophysiological consequences of these mutations (Studies I and II).
2. To estimate the population prevalence of Finnish ARVC-associated desmosomal mutations and to analyse associated electrocardiographic abnormalities (Study II).
3. To investigate potential modifier genes in LQTS, focusing on a common variant with the largest known QT interval-prolonging effect in the general population (Study III).
4. To evaluate the association of QT interval and QT genotype score (QT_{score}) with SCD in the Finnish population (Study IV).
5. To investigate the role of common genetic variants predisposing to arrhythmia and related ECG abnormalities in risk of SCD (Study V).
6. To assess the association of rare Finnish LQTS and ARVC mutations with SCD (Study VI).

MATERIALS AND METHODS

1. Patient and control samples (I-III)

In Studies I and II, the ARVC patient sample consisted of 29 consecutive probands diagnosed according to the International Task Force criteria (McKenna et al. 1994) between 1998 and 2004 at the Department of Cardiology, University of Helsinki. In addition, unpublished data were available from four ARVC probands diagnosed between 2004 and 2009. Available family members (n = 42) of five probands were diagnosed according to the International Task Force criteria or the modified criteria for first-degree family members (Hamid et al. 2002).

In Study III, the LQTS patient sample comprised 712 carriers of the four Finnish LQTS founder mutations from 126 families referred to the Laboratory of Molecular Medicine, University of Helsinki, between 1999 and 2008. This material included all available carriers of *KCNQ1* G589D (n = 492), *KCNQ1* IVS7-2A>G (c.1129-2A>G, n = 66), *KCNH2* L552S (n = 73), and *KCNH2* R176W (n = 88), excluding those with QT-prolonging medication at the time of ECG recording.

Over 250 DNA samples from ethnically matched blood donors were used as controls to estimate genotype frequencies in the Finnish background population in Studies I and II. The studies were approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa. Written informed consent was obtained from all participating patients and their family members.

2. Population cohorts and autopsy materials (II, IV-VI)

Health 2000 (Studies II and IV-VI) is a two-stage stratified cluster sample (n = 8028) representative of the adult (age ≥ 30 years) Finnish population and was collected between 2000 and 2001 (Heistaro 2008). The Mini-Finland Health Survey (Studies IV-VI) is a similar sample (n = 8000) initially collected between 1978 and 1980 from the Finnish population (Aromaa et al. 1989). DNA was available from a follow-up study of 985 individuals conducted in 2001. FINRISK 1992 (n = 6051), FINRISK 1997 (n = 8446), and FINRISK 2002 (n = 8648) (Studies V and VI) are Finnish population-based cohorts

collected independently of each other at 5-year intervals from the age group of 25-74 years (Vartiainen et al. 2010). Gene-expression analysis in Study V was performed in a sample of 510 unrelated Finnish individuals recruited as an extension of the FINRISK 2007 study.

The Helsinki Sudden Death Study (HSDS) and the Tampere Autopsy Study (TASTY) are series of forensic autopsies utilized in Studies V and VI. HSDS comprised all out-of-hospital deaths of previously healthy men aged 35-69 years ($n = 300$) autopsied in Helsinki between 1991 and 1992 (Tynnelä et al. 2009). TASTY included 740 consecutive medico-legal autopsies of individuals aged ≤ 97 years performed in Tampere between 2002 and 2004 (Kok et al. 2009). Subjects aged >80 or <25 years were excluded from the statistical analyses of Studies IV-VI.

3. Phenotypic characterization (I-VI)

The information on proband or family member status, occurrence of syncope, use of beta blocker medication, and pacemaker or implantable cardioverter-defibrillator in Study III was based on questionnaires at baseline and at follow-up in 2006. The clinical information in the Health 2000 and FINRISK studies (Studies II and IV-VI) was collected from health questionnaires and clinical assessment at baseline as well as from registry-based information on medications, hospitalizations, and causes of death. In the population cohorts, the causes of death were classified as either probable SCD, possible SCD, unlikely SCD, or unknown cause of death by two independent physicians reviewing data from baseline examinations, the Causes of Death Registry, the Hospital Discharge Registry, the Drug Reimbursement Registry, and the Pharmacy Database as described in Studies IV-VI. In cases of discrepancy, two additional physicians reviewed the data independently, and final adjudication was achieved by consensus of all four physicians. In the series of forensic autopsies, causes of death were classified accordingly based on autopsy results. Probable and possible SCDs were pooled for the analyses.

Standard 12-lead electrocardiography was used in all ECG measurements. QT interval was measured manually in lead II in the LQTS patient sample (Study III) and automatically with manual confirmation as a mean of 12 leads in the Health 2000 study (Studies II and IV). QT interval was corrected for heart rate by using either linear regression (Studies II and III), Bazett's formula (Bazett 1920) (Studies II and III), or the nomogram-correction method

(Karjalainen et al. 1994) (Study IV). Heart rate, PQ interval, and QRS duration were measured automatically as described in Study II.

4. Molecular genetic studies (I-VI)

DNA was extracted from peripheral blood lymphocytes using standard methods (Studies I-III): the phenol-chloroform method (Blin and Stafford 1976) or the salting-out method using PureGene DNA Purification Kit (Gentra, Minneapolis, MN, USA). Genomic DNA was amplified using polymerase chain reaction (PCR) (Mullis et al. 1986) with a primer pair specific for each exon or mutation under investigation (Studies I-VI).

Disease-causing mutations were searched for in 29 unrelated ARVC patients using direct sequencing (Sanger et al. 1977) of *PKP2b* (NM_004572), *DSP* isoform I (NM_004415), *DSG2* (NM_001943), *DSC2a* (NM_024422), and *DSC2b* (NM_004949) exons (Studies I and II). Four additional probands were screened for mutations in *PKP2b* exons (Lahtinen AM et al. unpublished data). PCR products were purified with exonuclease I and shrimp alkaline phosphatase and sequenced with BigDye Terminator v3.1 and ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Electropherograms were analysed with Sequencher 4.5-4.8 software (Gene Codes Corporation, Ann Arbor, MI, USA).

In Study II, large deletions and duplications were screened using multiplex ligation-dependent probe amplification (MLPA) detecting relative copy number changes by measuring the hybridization of sequence-specific probes (Schouten et al. 2002). The Salsa MLPA kit P168 (MRC Holland, Amsterdam, the Netherlands) includes probes for all *PKP2* exons and for selected exons in *DSP*, *JUP*, *RYR2*, and *TGFB3*.

In Study I, haplotype analysis of *PKP2* was performed by genotyping of six polymorphic repeat markers using PCR with fluorescent-labelled primers and capillary electrophoresis with ABI 3730 DNA Analyzer.

Known genetic variants were detected by a restriction enzyme digestion method in Studies I-III. In this method, PCR products are cleaved with a restriction endonuclease recognizing and cleaving either the wild-type or mutated sequence. Resulting DNA fragments are separated in agarose gel or polyacrylamide gel electrophoresis. In Studies I and II, those

variants with no suitable recognition sequence were detected by primer-induced restriction analysis (PIRA) creating an artificial recognition sequence in the PCR product (Kumar and Dunn 1989).

Large population cohorts and autopsy materials were genotyped using Sequenom MALDI-TOF mass spectrometry (Storm et al. 2003) (MassARRAY Analyzer Compact, Sequenom Inc., San Diego, CA, USA) in Studies II and IV-VI. The Sequenom iPLEX assay can be used to genotype up to 36 SNPs in a single well by single-base extension of a hybridized primer and subsequent mass analysis. *KCNH2* R176W was genotyped using Custom TaqMan SNP Genotyping Assay (Applied Biosystems) in Study VI. The discrimination of alleles with fluorescent-labelled probes was performed using 7900HT Fast Real-Time PCR System and SDS2.3 software (Applied Biosystems).

In Study V, gene expression analysis was performed to detect potential effects of SNPs on gene expression regulation. Genome-wide RNA level quantification was achieved in RNA samples extracted from peripheral blood using Illumina HumanHT-12 Expression BeadChips (Illumina Inc., San Diego, CA, USA), as described previously (Inouye et al. 2010). Expression intensity of probes within 2 Mb of the SNP in interest were analysed using linear regression.

5. Microscopic analyses (I, II)

Endomyocardial biopsy samples were obtained from two ARVC patients and two control patients with hypertrophic cardiomyopathy and CPVT, respectively. Immunohistochemistry with mouse desmoglein-2, plakophilin-2, plakoglobin, desmoplakin 1/2 (Progen, Heidelberg, Germany), mouse N-cadherin (Sigma-Aldrich, St. Louis, MO, USA), and rabbit connexin 43 antibodies (Sigma-Aldrich) was performed using the Advance HRP system (DakoCytomation, Glostrup, Denmark) or the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Quantification of the immunoreaction was achieved using Alexa Fluor 594 detection (Invitrogen, Carlsbad, CA, USA) and measuring the integrated signal density using the ImageJ program. Electron microscopy was performed on endomyocardial tissue samples retrieved from paraffin blocks using a Jeol JEM 1200EX or Jeol JEM 1400 electron microscope.

6. Statistical analyses (I-VI)

Hardy-Weinberg equilibrium was confirmed by Chi-square test or, in the case of rare variants, by Fisher's exact test. Variants with Hardy-Weinberg p value below the selected threshold (Studies I-III and VI: 0.05; Study IV: 0.0001; Study V: 0.002) were excluded from the study. In the genotyping quality control of large population cohorts, selected thresholds were applied for the genotyping success of variants (Study IV: 80%; Study V-VI: 90%) and samples (Study IV: 57%; Studies V-VI: 80%). Genotyping quality was also monitored by using sex markers, duplicate samples, and positive control samples.

Discrete variables were tested by Chi-square test or Fisher's exact test. The effect of genotype was analysed using an additive model (number of minor alleles coded as 0, 1, 2). In Study II, prevalence estimates of mutations were calculated from the weighted study population, as described previously (Aromaa and Koskinen 2004). In Study VI, prevalence estimates were calculated with survey-specific sampling weights, and the estimation was stratified by study, sex, study region, and 10-year age group. In Studies IV and V, QT_{score} was calculated for each individual to aggregate the information of 12-14 QT interval-prolonging SNPs. QT_{score} represents the predicted effect of genotype on QT interval. It was calculated by multiplying the previously reported effect estimate of each SNP in ms (Newton-Cheh et al. 2009b, Pfeufer et al. 2009) by the number of coded alleles and finally calculating the sum over all SNPs.

In Studies II and III, the association of genotype with PR interval, QRS duration, QT interval, and heart rate was investigated by linear regression, adjusting for age, sex, and heart rate. In Study III, an additional model included also a multiplicative interaction term between genotype and sex. In Study IV, the association of genotype or QT_{score} with QT interval nomogram-corrected for heart rate (QT_{Nc}) was evaluated by linear regression, adjusting for age, sex, and geographic region. In Studies IV and V, the association of genotype, QT_{score} , or QT_{Nc} with SCD was studied by Cox proportional hazards model, using age as the time scale and adjusting for sex and geographic region. In the SCD analyses, additional adjustments included QT-prolonging and QT-shortening medication, prevalent CHD, established cardiovascular risk factors, and prevalent heart failure. In Study V, the autopsy series were analysed using logistic regression by comparing probable and possible SCDs to unlikely SCDs and adjusting for age at death and sex. The risk estimates were

pooled using inverse variance-weighted, fixed-effects meta-analysis. When significant heterogeneity occurred ($I^2 > 0.5$), random-effects meta-analysis was applied. In Study V, a similar meta-analysis was performed for all-cause and cardiac mortality, adjusting for sex and geographic region. The association between baseline cardiovascular risk factors and SCD was also investigated in Study V using Cox proportional hazards regression. In Study VI, the association between rare mutations and SCD was analysed using Fisher's exact test. Statistical analyses were performed with SPSS 11.0-17.0 (SPSS Inc., Chicago, IL, USA) and R version 2.11. Two-tailed $p < 0.05$ was considered statistically significant. Bonferroni-corrected significance threshold was applied in Studies IV and V.

RESULTS

1. Desmosomal mutations in ARVC patients and families

Out of the 29 unrelated ARVC patients in Studies I and II, three carried mutations in *PKP2*, one in *DSG2*, and one in *DSP* (patients A-E, Table 7). No mutations were identified in *DSC2*. Occurrence of large genomic rearrangements in *PKP2* was excluded using MLPA. One of the four additional patients (patient F, Table 7) carried a mutation in *PKP2* (Lahtinen AM et al. unpublished data). In total, six (18%) of the 33 probands carried a desmosomal mutation. *PKP2* mutations accounted for two-thirds of these mutations. All mutations occurred in an evolutionary conserved region and were absent in 250 control samples. Pedigree data are presented in Figure 5.

Table 7. ARVC patients with desmosomal mutations

Proband	Mutation			Age [†]	Sex	Symptoms
	Gene	Nucleotide*	Amino acid			
A	<i>PKP2</i>	184C>A	Q62K	32	M	Presyncope at exercise, VT in LBBB morphology
	<i>PKP2</i>	1839C>G	N613K			
B	<i>PKP2</i>	176A>T	Q59L	39	F	Syncope at exercise, VT in LBBB morphology
C	<i>PKP2</i>	176A>T	Q59L	42	M	Syncope at exercise, VT in LBBB morphology
D	<i>DSG2</i>	3059_3062 delAGAG	E1020AfsX18	24	M	Stress-related VT in LBBB morphology
E	<i>DSP</i>	4117A>G	T1373A	29	M	VT in LBBB morphology at exercise
F	<i>PKP2</i>	563delT	L188PfsX2	29	F	VT in LBBB morphology at exercise originating from right ventricular apex

*Numbering of nucleotides starts from the methionine translation initiation codon.

[†]Age at onset of symptoms.

F = female; LBBB = left bundle branch block; M = male; VT = ventricular tachycardia.

Proband A was a compound heterozygous carrier of two *PKP2* missense mutations, Q62K and N613K. This patient presented with arrhythmia, adipose and fibrous tissue replacement of the myocardium, and right ventricular structural abnormalities typical of ARVC. The family data suggested a pathogenic role for the novel N613K mutation, but uncertain pathogenicity for Q62K, which has been reported as an unclassified variant also in other populations (van Tintelen et al. 2006, Xu et al. 2010). *PKP2* Q59L was detected in two unrelated probands, B and C. Both of them featured arrhythmia, ECG abnormalities, and

right ventricular structural alterations. Families B and C included a total of ten mutation carriers, of which two (20%) fulfilled the Task Force diagnostic criteria and one (10%) the modified diagnostic criteria for first-degree family members. *PKP2* Q59L was linked with an identical haplotype in both families, indicating a common founder individual for these probands (Study I). *PKP2* 563delT was detected in a proband who suffered from episodes of ventricular tachycardia and was treated with an implantable cardioverter-defibrillator (Lahtinen AM et al. unpublished data). The mutation was also present in her mother, whose ECG showed inverted T waves in leads V1-V3.

DSG2 3059_3062delAGAG and *DSP* T1373A were detected in probands D and E, respectively. The *DSG2* four-nucleotide deletion generates a frameshift that deletes the 99 carboxy-terminal amino acids of desmoglein-2. Both of these probands presented with arrhythmia, ECG abnormalities, and right ventricular structural alterations. Proband D also featured adipose and fibrous tissue replacement. Of the five deletion carriers in Family D, three (60%) fulfilled either the Task Force or the modified diagnostic criteria for ARVC.

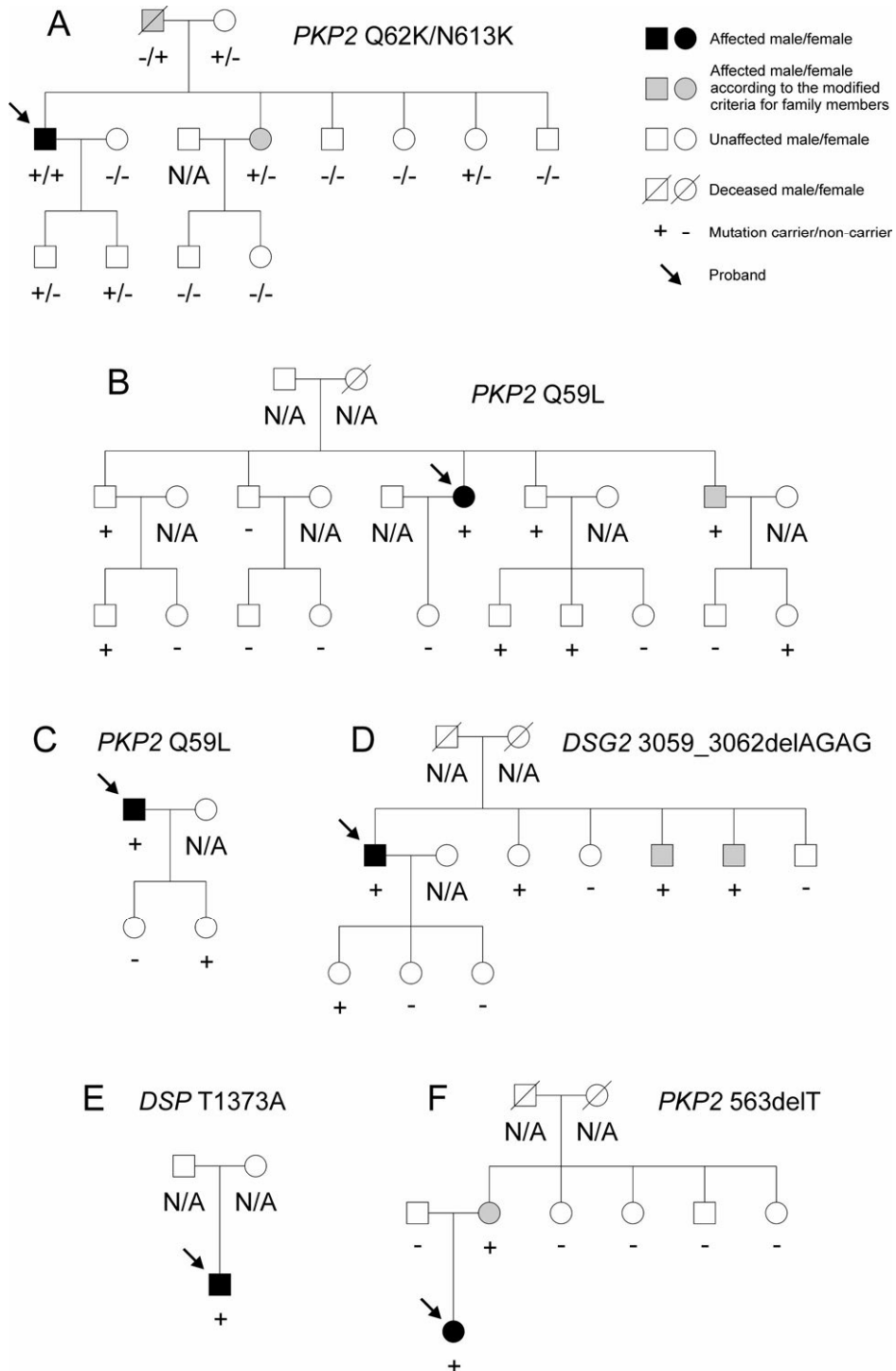


Figure 5. Family data of six ARVC probands with desmosomal mutations (Studies I and II, and Lahtinen AM et al. unpublished data). N/A = no clinical or genetic information available.

2. Effects of desmosomal mutations at the cellular level

Endomyocardial biopsy samples of two ARVC probands (A and D) were analysed in Studies I and II by immunohistochemical staining of desmosomal proteins and electron microscopy. The samples of both patients showed adipose and fibrous tissue replacement of cardiac myocytes characteristic of ARVC.

Plakophilin-2 staining of the sample of proband A with mutations *PKP2* Q62K and N613K showed mild reduction of plakophilin-2 immunoreactive signal as well as less linearly organized intercalated disk structure. Immunohistochemical stainings of the sample of proband D with *DSG2* deletion showed reduced immunoreactive signal for all desmosomal proteins assessed: desmoglein-2, plakophilin-2, desmoplakin, and plakoglobin (Figure 6). N-cadherin, a marker of tissue quality, and the gap junctional protein connexin 43 showed no reduction compared with the control samples.

Electron microscopic analysis of the intercalated disk area revealed more vacuolated intercalated disks in both ARVC samples than in the control samples. In the sample of proband A, fewer desmosomes were detected and some of the desmosomal junctions appeared small and irregularly oriented. In the sample of proband D, occasional disorganization of the cell-cell junctions was observed.

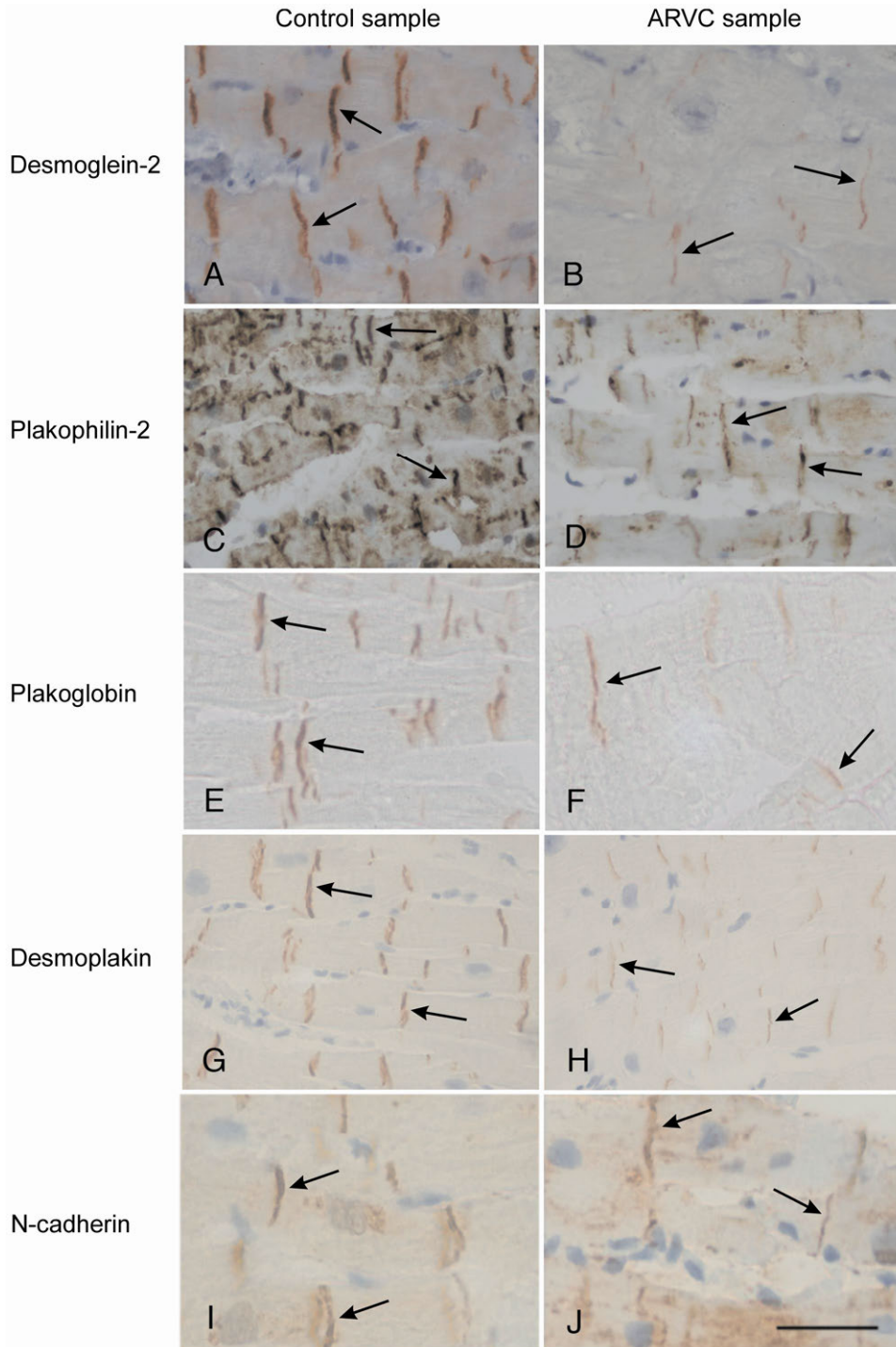


Figure 6. Immunohistochemical staining of a control sample and the ARVC sample with *DSG2* 3059_3062delAGAG for desmoglein-2 (A-B), plakophilin-2 (C-D), plakoglobin (E-F), desmoplakin (G-H), and N-cadherin (I-J). The arrows point at selected intercalated disk structures. Bar: 50 μ m.

3. Desmosomal variants in the Finnish population

The population prevalence and clinical phenotypes of five desmosomal ARVC-related mutations identified in Studies I and II were investigated in Studies II and VI. In addition, possible phenotypic associations of two common *PKP2* polymorphisms were evaluated in the Health 2000 population sample in Study II. The combined prevalence of the five desmosomal mutations was 48 per 10 000 (95% confidence interval [CI] 33-71 per 10 000) in the Health 2000 study (Study II). A similar prevalence estimate (39 per 10 000, 95% CI 31-50 per 10 000) was detected in Study VI using both Health 2000 and FINRISK population cohorts (Table 8). The most prevalent mutation was *PKP2* Q59L, which was carried by 29 per 10 000 Finns (95% CI 22-39 per 10 000). The carriers of this mutation clustered in Southeastern Finland, further supporting the hypothesis that *PKP2* Q59L is a founder mutation in the Finnish population.

Arrhythmia (self-reported or physician-diagnosed) occurred in 11 (35%) of the total of 31 mutation carriers in the Health 2000 study (Study II). ECG abnormalities characteristic of ARVC (T-wave inversion in leads V2 and V3 or QRS complex duration ≥ 110 ms) were detected in 6 (19%) carriers. Arrhythmia or ECG abnormalities occurred altogether in 16 carriers (52%), and only one mutation carrier featured both types of clinical characteristics. A *PKP2* Q59L carrier had a diagnosis of ventricular tachycardia and encountered SCD at the age of 46 years. The ECG of one *PKP2* Q62K carrier showed frequent premature ventricular complexes, and one *DSP* T1373A carrier was diagnosed with paroxysmal tachycardia. *DSP* T1373A was associated with PR interval prolongation of 33 ms in the population sample ($p = 0.005$).

The two *PKP2* polymorphisms L366P and I531S had similar allele frequencies in the Health 2000 population sample (19.5% and 2.2%, respectively) as in the ARVC patient material (17% and 2%, respectively). I531S was not associated with arrhythmia or ECG abnormalities in Study II. The minor allele of L366P was associated with PR interval prolongation ($p = 0.036$) as well as reduced occurrence of arrhythmia in clinical examination ($p = 0.028$) and T-wave inversion in ECG ($p = 0.040$).

Table 8. Desmosomal mutations in the Finnish population

Gene	Mutation	Study II: Health 2000 (n = 6334)			Study VI: Health 2000, FINRISK 1992, 1997, and 2002 (n = 28 465)	
		n	n (%) with arrhythmia or ECG abnormalities*	Prevalence per 10 000 (95% CI)	n	Prevalence per 10 000 (95% CI)
<i>PKP2</i>	Q59L	19	10 (53)	30.1 (18.3-49.4)	85	29.3 (22.2-38.6)
<i>PKP2</i>	Q62K	6	2 (33)	8.5 (3.7-19.6)	12	4.3 (2.3-8.1)
<i>PKP2</i>	N613K	0	-	-	0	-
<i>DSG2</i>	3059_3062 delAGAG	1	1 (100)	1.7 (0.2-12.2)	5	2.7 (1.0-7.5)
<i>DSP</i>	T1373A	5	3 (60)	8.0 (3.4-19.0)	10	3.8 (1.6-8.7)
Total of all mutations		31	16 (52)	48.5 (33.2-70.8)	112	39.3 (31.0-49.9)

*Arrhythmia in clinical examination, self-reported arrhythmia, T-wave inversion (V2-V3), or QRS complex ≥ 110 ms.

CI = confidence interval; ECG = electrocardiography.

4. *KCNE1* D85N as a sex-specific disease-modifying variant in LQTS

Study III assessed the clinical significance of a common QT-prolonging variant, *KCNE1* D85N, in 712 carriers of the four Finnish LQTS founder mutations (*KCNQ1* G589D, *KCNQ1* IVS7-2A>G, *KCNH2* L552S, and *KCNH2* R176W). In this combined patient group, *KCNE1* D85N was associated with a 13-ms prolongation of QT interval (standard error 6.0 ms, $p = 0.028$). *KCNQ1* G589D was the most prevalent founder mutation (n = 492). In males with *KCNQ1* G589D, *KCNE1* D85N was associated with a QT interval prolongation of 26 ms (standard error 8.6 ms, $p = 0.003$) (Figure 7). Confining the analysis to males ≤ 16 years of age did not change the result. In females with *KCNQ1* G589D, no association was observed ($p = 0.935$). The multiplicative interaction term for *KCNE1* D85N and sex attained a significance of $p = 0.028$, which indicates that the effect of D85N on QT interval may be sex-specific in this Finnish LQTS founder mutation group.

The association of *KCNE1* D85N with clinical variables reflecting disease severity was studied in *KCNQ1* G589D mutation carriers. The percentage of probands was higher in *KCNE1* D85N heterozygotes (31%) than in non-carriers (12%, $p = 0.042$) and the percentage of patients using beta blocker medication was higher in *KCNE1* D85N heterozygotes (81%) than in non-carriers (47%, $p = 0.010$) (Figure 8). The percentage of patients having experienced syncope and patients with pacemaker or implantable cardioverter-defibrillator did not differ significantly between the *KCNE1* D85N heterozygotes (44% and 6.3%, respectively) and non-carriers (36% and 4.5%, respectively).

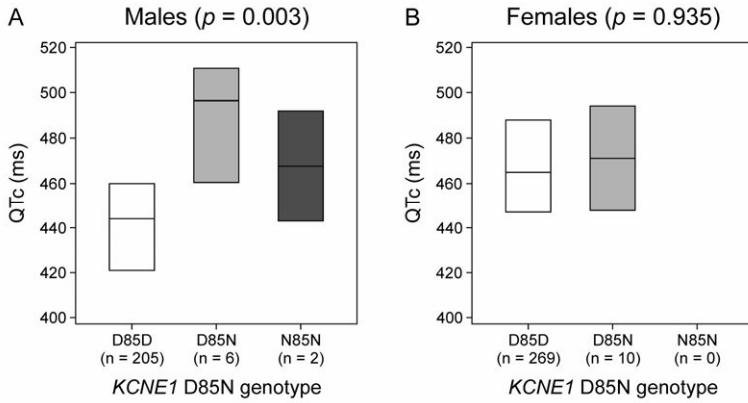


Figure 7. Heart rate-corrected QT interval (QTc) in the different *KCNE1* D85N genotype classes in *KCNQ1* G589D carrier males (A) and females (B). Box plots show medians and interquartile ranges.

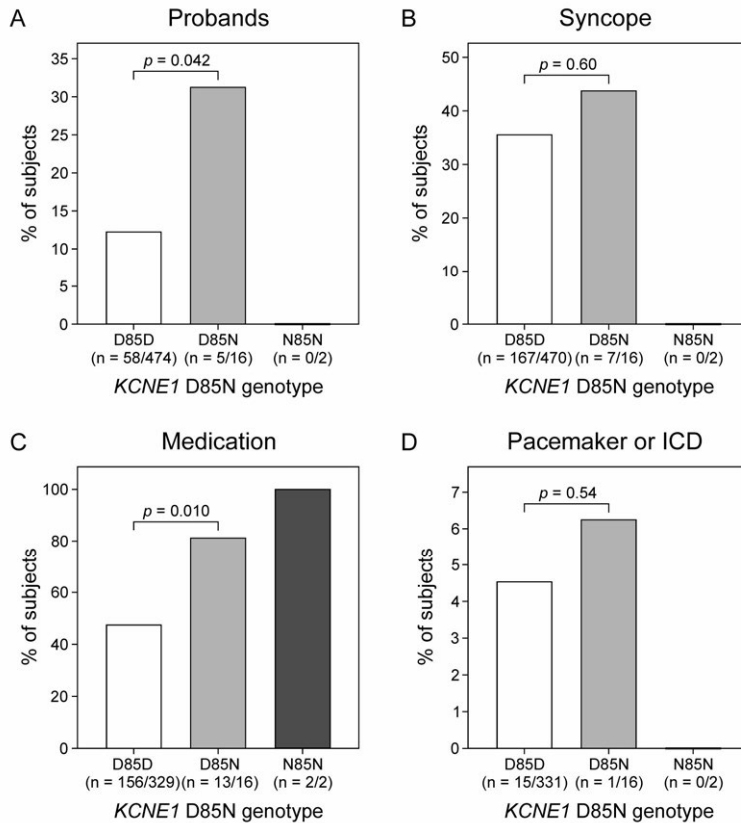


Figure 8. Occurrence of selected clinical variables in the different *KCNE1* D85N genotype classes in *KCNQ1* G589D carriers. Percentage of probands (A), patients having experienced syncope (B), patients with beta blocker medication (C), and patients with pacemaker or implantable cardioverter-defibrillator (ICD) (D). N indicates the number of individuals with the selected clinical feature out of all individuals in the corresponding genotype group.

5. QT interval and QT_{score} in SCD

The association of QT_{score} , a genotype score representing the predicted effect of 14 QT interval-associated SNPs, with SCD was investigated in the Health 2000 and Mini-Finland population cohorts ($n = 6808$, n of SCDs = 116) in Study IV. In addition, the relationships between QT_{score} and QT_{Nc} as well as between QT_{Nc} and SCD were investigated in the Health 2000 cohort ($n = 6091$, n of SCDs = 99). Thirteen of the QT_{score} SNPs were independently associated with QT_{Nc} ($p < 0.007$, Table 9). The effect sizes of individual SNPs ranged from 1 ms to 10 ms. The association between rs17779747 near *KCNJ2* and QT_{Nc} was statistically non-significant after correction for multiple testing ($p = 0.039$). The linear QT_{score} was associated with QT_{Nc} ($p < 10^{-107}$) and explained 8.6% of QT_{Nc} variation after adjustment for age, sex, and geographic region.

A 10-ms increase in QT_{Nc} was associated with a 19% increased risk of SCD in the Health 2000 study (95% CI for hazard ratio [HR] 1.07-1.32, $p = 0.002$). The association of a diagnostic threshold for prolonged QT interval ($QT_{Nc} > 450$ ms for males and > 470 ms for females) with risk of SCD was also confirmed in this study. The risk of SCD was 1.3% below and 24% above the threshold QT interval (HR 13.3, 95% CI 4.7-37.7, $p = 1 \times 10^{-6}$). Adjustment for prevalent CHD and use of QT-altering medication did not change the results.

The QT_{score} SNPs were not associated with SCD independently (Table 9) and neither was the linear QT_{score} , which combines the QT-prolonging effects of these SNPs. The risk of SCD was suggestively increased in the highest QT_{score} quintile compared with the middle quintile in Study IV (HR 1.92, 95% CI 1.05-3.58, $p = 0.04$), but this result was not replicated in Study V in the meta-analysis of four population cohorts and two series of forensic autopsies (total $n = 28\,323$, n of SCDs = 716).

Table 9. Effects of individual SNPs on QT interval and SCD

Chr.	Position*	Nearest gene	SNP	Coded allele	CAF	QT _{Nc}		SCD	
						β (ms)	p	HR	p
1p36	6 201 957	<i>RNF207</i>	rs846111	C	0.25	2.9	8.9×10^{-12}	1.29	0.08
1q23	160 300 514	<i>NOS1AP</i>	rs12143842	T	0.24	5.0	5.1×10^{-31}	1.26	0.12
1q24	167 366 107	<i>ATP1B1</i>	rs10919071	A	0.89	3.3	7.5×10^{-8}	0.89	0.58
3p22	38 568 397	<i>SCN5A</i>	rs12053903	T	0.59	1.3	6.0×10^{-4}	1.02	0.89
6q22	118 759 897	<i>PLN</i>	rs12210810	G	0.97	3.6	5.9×10^{-4}	0.96	0.92
6q22	119 100 335	<i>PLN</i>	rs11756440	A	0.47	1.9	1.2×10^{-7}	0.99	0.93
7q36	150 268 796	<i>KCNH2</i>	rs4725982	T	0.28	2.0	2.4×10^{-6}	1.23	0.16
7q36	150 276 467	<i>KCNH2</i>	rs1805123	A	0.82	2.6	1.3×10^{-7}	1.12	0.50
11p15	2 441 379	<i>KCNQ1</i>	rs2074238	C	0.91	6.3	1.9×10^{-24}	0.87	0.51
11p15	2 458 895	<i>KCNQ1</i>	rs12576239	T	0.16	2.6	4.6×10^{-8}	0.98	0.91
16p13	11 601 037	<i>LITAF</i>	rs735951	G	0.58	1.4	1.6×10^{-4}	1.05	0.72
16q21	57 124 739	<i>CNOT1</i>	rs37062	A	0.74	2.4	1.0×10^{-8}	0.94	0.69
17q24	66 006 587	<i>KCNJ2</i>	rs17779747	G	0.75	0.88	0.039	1.19	0.29
21q22	34 743 550	<i>KCNE1</i>	rs1805128	A	0.01	10.4	9.0×10^{-11}	1.11	0.85

*According to NCBI genome build 36.

β = effect size; CAF = coded allele frequency; Chr. = chromosome; HR = hazard ratio; QT_{Nc} = QT interval nomogram-corrected for heart rate; SCD = sudden cardiac death; SNP = single nucleotide polymorphism.

6. Common variants and cardiovascular risk factors in SCD

Study V explored the association of 28 common SNPs with increased risk of SCD in four population cohorts and two series of forensic autopsies (total $n = 28\,323$, n of SCDs = 716). The candidate SNPs were selected based on previously reported associations with arrhythmia or related ECG phenotypes such as QT interval. Two SNPs were associated with SCD after Bonferroni correction for multiple testing: *SCN5A* rs41312391 (relative risk [RR] 1.27 per minor T-allele, 95% CI 1.11-1.45, $p = 3.4 \times 10^{-4}$) and rs2200733 in 4q25 (RR 1.28 per minor T-allele, 95% CI 1.11-1.48, $p = 7.9 \times 10^{-4}$) (Figure 9, Table 10). No heterogeneity between the different study cohorts was detected for the effect sizes of these two SNPs ($I^2 = 0.00$). Confining the phenotype to probable SCD did not change the relative risk estimates (RR 1.28, 95% CI 1.11-1.48, $p = 6 \times 10^{-4}$ for rs41312391 and RR 1.27, 95% CI 1.08-1.49, $p = 0.003$ for rs2200733). We also replicated the previously reported association of rs2383207 in 9p21 near the *CDKN2A* and *CDKN2B* genes with SCD (RR 1.13 per G-allele, 95% CI 1.01-1.26, $p = 0.036$).

Gene expression analysis in peripheral blood suggested that the minor allele of rs2200733 could be associated with increased expression of the nearest gene *PITX2* ($p = 0.013$), and

the minor allele of rs41312391 with increased expression of *WDR48* ($p = 0.037$). Both SNPs, rs41312391 and rs2200733, remained significantly associated with SCD after additional adjustment for cardiovascular risk factors (high-density lipoprotein-total cholesterol concentration ratio, systolic blood pressure, prevalent diabetes, body mass index, current and former cigarette smoking, and leisure-time physical activity) and prevalent CHD (model 2), QT-prolonging and QT-shortening medication (model 3), and prevalent heart failure (model 4).

Study V also replicated the association of previously reported cardiovascular risk factors, including male gender, high systolic blood pressure, prevalent diabetes, current and former cigarette smoking, Eastern Finnish residency, and low physical activity, with SCD ($p < 0.05$). Prevalent coronary heart disease ($p < 2.2 \times 10^{-16}$) and digoxin use ($p = 2.7 \times 10^{-8}$) were also associated with increased risk of SCD, whereas QT-prolonging medication was not ($p = 0.57$).

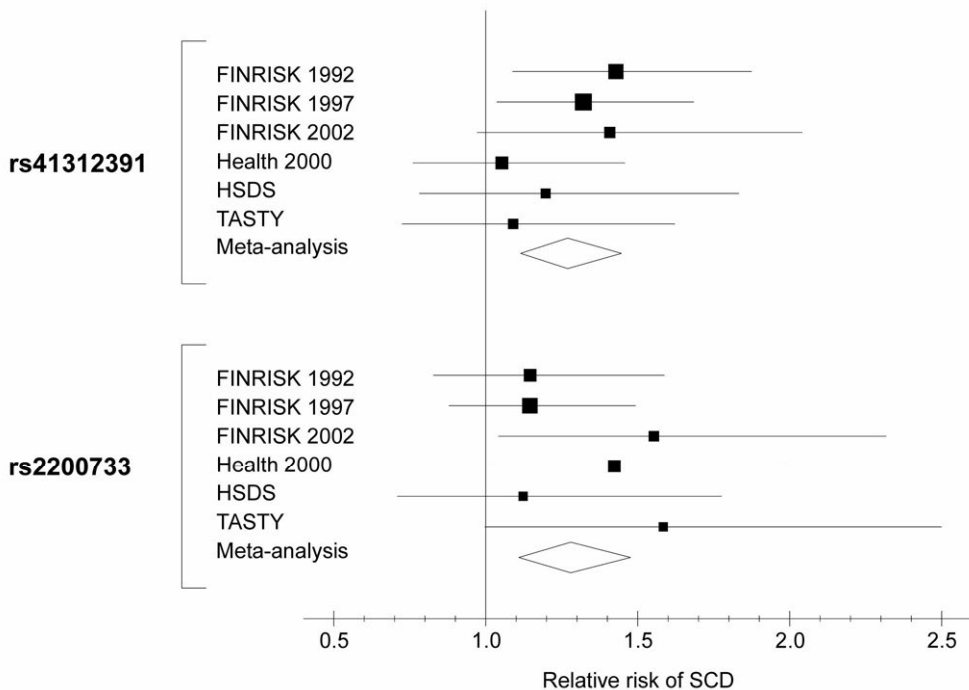


Figure 9. Forest plot of the relative risks of the two common SNPs associated with SCD. Point estimates for relative risk of SCD are represented by squares (area proportional to the inverse-variance weight) and 95% confidence intervals by horizontal lines. The widest point of the diamond represents the relative risk estimate in the meta-analysis and the lateral tips show the 95% confidence interval. HSDS = Helsinki Sudden Death Study; SCD = sudden cardiac death; TASTY = Tampere Autopsy Study.

RESULTS

Table 10. Results from the SCD meta-analysis of common genetic variants

SNP	Coded allele	CAF	FINRISK 1992 HR	FINRISK 1997 HR	FINRISK 2002 HR	Health 2000 HR	HSDS OR	TASTY OR	SCD			
									I ²	RR	95% CI	p
rs846111	C	0.26	0.93	1.05	1.14	1.29	0.88	1.04	0.00	1.06	0.93-1.20	0.39
rs2880058	G	0.36	0.99	1.02	1.19	1.08	0.93	1.16	0.00	1.05	0.94-1.18	0.41
rs12036340	G	0.25	0.95	1.02	1.36	1.29	0.92	1.20	0.00	1.10	0.97-1.25	0.15
rs12143842	T	0.25	0.88	1.06	1.26	1.26	0.91	1.19	0.00	1.08	0.95-1.23	0.25
rs10919071	G	0.11	0.94	0.88	1.48	1.12	0.91	0.80	0.00	1.00	0.83-1.19	0.96
rs1805126	G	0.44	1.22	1.07	1.51	0.99	1.10	1.16	0.00	1.14	1.02-1.28	0.02
rs12053903	C	0.41	1.28	1.04	1.38	0.98	1.12	1.06	0.00	1.12	1.00-1.26	0.045
rs41312391	T	0.20	1.43	1.32	1.41	1.05	1.20	1.09	0.00	1.27	1.11-1.45	3.4x10⁻⁴
rs3922844	T	0.26	0.90	1.24	1.04	1.29	0.88	0.96	0.10	1.08	0.95-1.23	0.22
rs6599219	G	0.37	0.91	1.01	1.09	0.80	1.01	1.05	0.00	0.97	0.86-1.08	0.54
rs7372712	T	0.16	0.90	0.88	1.25	1.16	0.84	1.14	0.00	1.01	0.87-1.18	0.86
rs2200733	T	0.16	1.15	1.15	1.55	1.42	1.12	1.58	0.00	1.28	1.11-1.48	7.9x10⁻⁴
rs10033464	T	0.17	1.28	0.92	1.19	0.94	1.32	1.20	0.00	1.10	0.94-1.28	0.23
rs1042714	G	0.38	0.78	1.22	0.81	0.96	1.07	1.12	0.44	1.00	0.89-1.12	0.99
rs12210810	C	0.03	0.79	0.77	1.77	1.04	0.52	0.68	0.00	0.94	0.68-1.30	0.71
rs4725982	T	0.29	0.73	1.08	1.00	1.23	1.11	1.66	0.62	1.09	0.89-1.34	0.42
rs1805123	G	0.17	1.39	1.06	0.55	0.89	N/A	N/A	0.69	0.97	0.71-1.34	0.87
rs3807375	T	0.44	0.86	1.05	1.09	1.52	1.29	1.49	0.61	1.18	0.98-1.41	0.08
rs2383207	G	0.43	1.04	1.00	1.15	1.06	1.31	1.61	0.31	1.13	1.01-1.26	0.04
rs2074238	T	0.09	0.98	0.77	1.22	1.15	1.03	1.03	0.00	1.00	0.82-1.21	0.98
rs757092	G	0.36	0.89	0.91	0.82	1.25	0.94	0.90	0.03	0.95	0.85-1.07	0.43
rs12576239	T	0.16	1.05	0.73	0.87	0.98	1.17	0.91	0.00	0.92	0.79-1.08	0.32
rs10798	G	0.36	0.89	1.08	1.06	1.15	1.20	0.91	0.00	1.04	0.93-1.17	0.49
rs735951	A	0.42	0.81	1.06	1.00	0.95	0.95	1.00	0.00	0.96	0.86-1.08	0.50
rs37062	G	0.27	1.02	0.95	1.03	1.06	1.27	0.97	0.00	1.03	0.90-1.16	0.71
rs17779747	T	0.25	0.81	1.04	1.03	0.84	0.95	1.05	0.00	0.95	0.83-1.08	0.42
rs1805128	T	0.02	0.89	0.71	0.89	1.11	0.52	1.00	0.00	0.84	0.53-1.34	0.46
rs727957	T	0.17	0.93	0.93	0.89	0.71	0.96	1.11	0.00	0.92	0.79-1.07	0.26

CAF = coded allele frequency; Chr. = chromosome; CI = confidence interval; HR = hazard ratio; HSDS = Helsinki Sudden Death Study; N/A = not available; OR = odds ratio; RR = relative risk; SCD = sudden cardiac death; SNP = single nucleotide polymorphism; TASTY = Tampere Autopsy Study.

7. Rare arrhythmia-associated mutations in the Finnish population

The prevalence and clinical phenotype, including the occurrence of arrhythmia, heart failure, and SCD, of ten arrhythmia-associated gene mutations (Table 11) were studied in the FINRISK 1992, 1997, 2002, and Health 2000 (including the Mini-Finland Health Survey) population cohorts and two series of forensic autopsies, HSDS and TASTY (Study VI, total n = 29 290, n of SCDs = 715). The combined prevalence of these ten mutations was 79 per 10 000 individuals in the Finnish population (Table 11). Within Finland, substantial regional differences in the prevalences of *KCNQ1* G589D, *KCNH2* L552S, *KCNH2* R176W, and *PKP2* Q59L were identified. In addition, the municipality of birth of *DSP* T1373A and *RYR2* R3570W carriers showed marked geographic clustering.

In the population cohorts, 14 (6.5%) of the mutation carriers suffered from arrhythmia and 7 (3.3%) from heart failure based on information obtained from the national health care registries. Of the 715 probable and possible SCD cases in the population and autopsy samples, 7 (1.0%) carried one of the ten arrhythmia-associated mutations (Table 11), but none of the mutations were associated with significantly increased risk of SCD ($p > 0.05$). The yearly incidence of probable and possible SCD was 0.19% for the mutation carriers and 0.18% for non-carriers.

Table 11. Rare arrhythmia mutations in the Finnish population and autopsy samples

Gene	Mutation	FINRISK and Health 2000 population cohorts (n = 28 465)			HSDS and TASTY (n = 825)	
		n	Prevalence per 10 000 (95% CI)	n of SCD cases	n	n of SCD cases
<i>KCNQ1</i>	G589D	28	9.9 (6.6-14.9)	0	0	-
<i>KCNQ1</i>	IVS7-2A>G	1	0.4 (0.05-2.6)	0	0	-
<i>KCNH2</i>	L552S	26	8.0 (5.1-12.6)	0	1	1
<i>KCNH2</i>	R176W	42	17.6 (12.4-24.8)	1	2	2
<i>PKP2</i>	Q59L	85	29.3 (22.2-38.6)	2	1	0
<i>PKP2</i>	Q62K	12	4.3 (2.3-8.1)	0	0	-
<i>PKP2</i>	N613K	0	-	-	0	-
<i>DSG2</i>	3059_3062delAGAG	5	2.7 (1.0-7.5)	0	0	-
<i>DSP</i>	T1373A	10	3.8 (1.6-8.7)	0	1	0
<i>RYR2</i>	R3570W	7	2.6 (1.0-6.3)	1	0	-
Total of all mutations		215*	78.5 (66.8-92.3)	4	5	3

*One subject with both *PKP2* Q59L and *RYR2* R3570W.

CI = confidence interval; HSDS = Helsinki Sudden Death Study; SCD = sudden cardiac death; TASTY = Tampere Autopsy Study.

DISCUSSION

1. Desmosomal defects underlying Finnish ARVC

1.1. Desmosomal mutations and their cellular consequences

Previous studies have revealed desmosomal mutations in approximately half of the ARVC patients examined genetically (Pilichou et al. 2006, den Haan et al. 2009, Christensen et al. 2010, Fressart et al. 2010, Xu et al. 2010, Cox et al. 2011). In the Finnish ARVC patient material, however, only 18% of probands were shown to carry a desmosomal mutation (Studies I and II, and Lahtinen AM et al. unpublished data). This difference may reflect population-specificity in the genetics of ARVC as well as still unidentified genes and pathways in the pathogenesis of this disorder. All mutations in the present study, except *PKP2* Q62K, were novel, which further underlines the population-specific differences in ARVC.

Compound heterozygosity and digenic heterozygosity of desmosomal mutations have increasingly been recognized in ARVC patients (Bhuiyan et al. 2009, den Haan et al. 2009, Bauce et al. 2010). A compound heterozygous patient with *PKP2* Q62K and N613K was reported in Study I. It seems that less severe mutations are unable to cause the disease alone, requiring an additional trigger, such as another mutation, for disease progression. Since ARVC is a common cause of death in young athletes (Thiene et al. 1988) and endurance training accelerates the disease development in a mouse model (Kirchhof et al. 2006), physical exertion may represent an external factor inducing the disease in susceptible patients.

PKP2 Q59L was reported as a Finnish ARVC founder mutation with a penetrance of 20% in Studies I and II. It is located in the conserved HR2 domain within the plakophilin-2 head domain, which mediates interactions with other desmosomal proteins (Chen et al. 2002). Functional studies have shown that this mutation disrupts the interaction between plakophilin-2 and desmoplakin (Hall et al. 2009). Plakophilin-2 with Q62K is degraded more rapidly than wild-type protein and fails to recruit desmoplakin to desmosomes (Hall et al. 2009). *PKP2* N613K resides in a conserved amino acid sequence, which may participate in protein binding (Choi and Weis 2005). Study I demonstrated less linear intercalated disks and irregular desmosomal structures in the cardiomyocytes of the compound heterozygous

carrier of *PKP2* N613K and Q62K. These plakophilin-2 missense mutations may thus lead to decreased cytoskeletal attachment at intercalated disks, which may predispose to tissue disruption during physical stress. *PKP2* 563delT abolishes almost half of the plakophilin-2 head domain and all armadillo repeats, potentially having deleterious consequences on the desmosomal structure and protein interactions.

DSP T1373A may affect the homodimerization of desmoplakin since it is located in the coiled-coil rod domain of this protein (Study II). *DSG2* 3059_3062delAGAG truncates the desmoglein-specific cytoplasmic region, which is involved in interactions with desmosomal proteins residing in the cytoplasm (Kami et al. 2009). This mutation leads to a diminished immunoreactive signal for several desmosomal proteins: desmoglein-2, plakophilin-2, plakoglobin, and desmoplakin (Study II), indicating that the desmosomal structure is affected as an ensemble. This impairment leads to disorganization of the intercalated disk structure, as detected in the electron microscopic analyses in Study II. The reduction of plakoglobin at the desmosomes may disturb Wnt/ β -catenin signalling (Garcia-Gras et al. 2006, Asimaki et al. 2009), which could result in cardiomyocyte apoptosis and replacement by adipose and fibrous tissue (Figure 10).

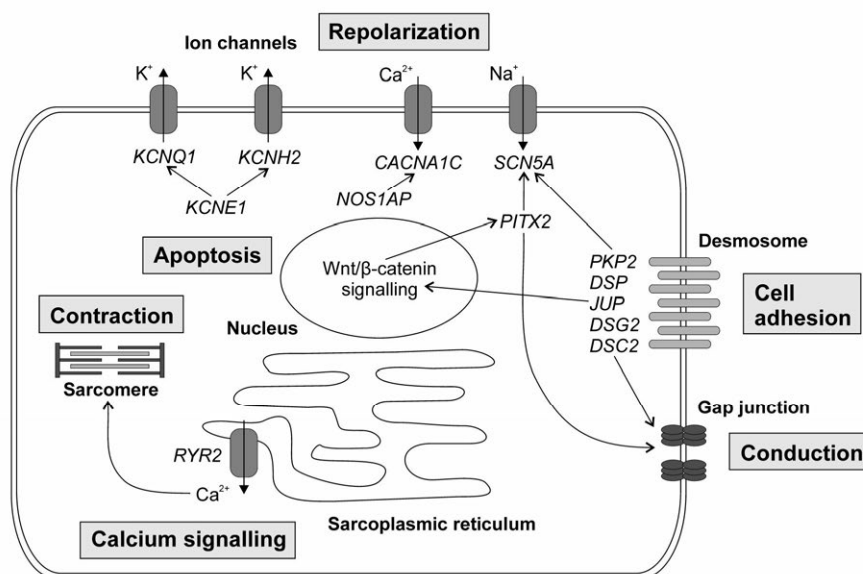


Figure 10. Main cellular processes in cardiomyocytes contributing to arrhythmia susceptibility. Impairment of any of these important cellular functions may predispose to potentially life-threatening arrhythmias. The functions and interplay of the principal genes in this study are also shown. Ca^{2+} = calcium ion; K^{+} = potassium ion; Na^{+} = sodium ion.

1.2. Desmosomal mutations at the population level

The prevalence of desmosomal mutations in the general Finnish population, 1:200-1:250 (Studies II and VI), is considerably higher than the published estimation of ARVC prevalence 1:1000-1:5000 (Rampazzo et al. 1994, Peters et al. 2004). Since the prevalence estimate of desmosomal mutations is based on only five mutations, comprehensive screening of all desmosomal genes in a population sample could reveal a substantially larger percentage of mutation carriers. A recent study reported 69 (16%) of 427 control individuals to carry a rare mutation in a gene associated with ARVC (Kapplinger et al. 2011). The mutations in these control individuals were randomly distributed along the coding sequence of the desmosomal genes, whereas mutations in ARVC patients clustered in the *PKP2* gene and the amino-terminal regions of *DSG2* and *DSP* (Kapplinger et al. 2011). In contrast to these desmosomal mutations in control individuals, which were considered mainly non-pathogenic background noise (Kapplinger et al. 2011), the desmosomal mutations identified in Studies I and II were considered probably or potentially harmful based on functional and family data. Therefore, a large number of individuals in the Finnish population are predicted to be at risk of developing ARVC or related myocardial abnormalities.

PKP2 Q59L was identified as a Finnish ARVC founder mutation, with a prevalence of 1:340 in the general population (Studies I, II, and VI). This founder effect appears to be caused by the unique population history of Finland, including a small founder population, bottleneck effects, and genetic isolation (Sajantila et al. 1996, Peltonen et al. 1999). The prevalence of desmosomal mutations in other populations remains to be elucidated, but since ARVC founder mutations have been reported in Dutch and South African populations (Watkins et al. 2009, van der Zwaag et al. 2010, Kapplinger et al. 2011), prevalent desmosomal mutations may also occur in other populations.

Approximately half of the mutation carriers in the Health 2000 study cohort presented with arrhythmia or ECG abnormalities (Study II). This figure encompasses a wide range of clinical manifestations, including self-reported arrhythmia, ECG alterations suggestive of cardiac abnormalities, and ventricular tachycardia in a case of SCD. *DSP* T1373A could play a role even in atrioventricular conduction since it was associated with PR interval prolongation. Only one of those 11 mutation carriers with arrhythmia also featured ECG alterations characteristic of ARVC, although repolarization abnormalities are usually

considered an early marker of ARVC manifestation. This is in line with the recent finding that T-wave inversion in right precordial leads is not associated with an increased risk of arrhythmic mortality (Aro et al. 2012). Desmosomal mutations may thus be associated with a wide spectrum of cardiac abnormalities and may require an additional genetic or environmental trigger for progression to overt disease. Also protective variants, such as *PKP2* L366P, may affect disease expressivity. Other possible explanations for the low penetrance of ARVC mutations in the Finnish population sample are late onset of disease and lack of comprehensive cardiologic examinations in the Health 2000 study.

2. Common genetic variants modulating QT interval and LQTS phenotype

2.1. Genetic components of QT interval

Two GWA studies reported 15 independent SNPs to be associated with QT interval duration (Newton-Cheh et al. 2009b, Pfeufer et al. 2009). Thirteen of these associations were replicated with effect estimates to the same direction in the Health 2000 population cohort (Study IV). The *LIG3* SNP rs2074518 was excluded in the quality control of genotyping, and the *KCNJ2* SNP rs17779747 could not be replicated, probably due to limited power to detect a small effect size. The QT interval-associated loci include cardiac ion channel genes associated with LQTS: *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNJ2*. Not surprisingly, QT interval-associated SNPs are also found near genes with a known function as regulators of ion channels: *NOS1AP* encoding the nitric oxide synthase 1 adaptor protein, *PLN* encoding phospholamban, an inhibitor of cardiac muscle sarcoplasmic reticulum calcium channel, and *ATP1B1* encoding the β -subunit of the sodium-potassium transporter. *CNOT1*, *LITAF*, and *RNF207* represent candidate genes with unestablished roles in cardiac repolarization.

QT_{score} in Study IV combines the effects of the 14 QT interval-associated SNPs. It explained 8.6% of the variation in QT interval, compared with 5.4-6.5% in the QTGEN Study (Newton-Cheh et al. 2009b). These common SNPs explain only a minority of the heritability of QT interval, which has been estimated at 35-40% (Newton-Cheh et al. 2005, Li et al. 2009a). The highest QT_{score} quintile had a 15.6 ms longer group mean QT interval than the lowest quintile (Study IV), while the corresponding figure was only 10-12 ms in the QTGEN Study (Newton-Cheh et al. 2009b). These differences may be explained by the use

of manually checked QT interval measurements and QT interval nomogram-corrected for heart rate in the Health 2000 study. It is also possible that the genetic components of QT interval in the genetically homogeneous Finnish population slightly differ from those in the QTGEN study populations. Unlike the ECG measurement of QT interval, QT_{score} is not dependent on temporal variation in environmental factors such as hormones or medication. It remains constant throughout a person's lifetime. QT interval-prolonging variants may together influence the repolarization reserve, which reflects the ability of the cell to resist external factors affecting repolarization (Roden 2006). QT_{score} could thus be useful in prediction of individual susceptibility to QT-prolonging medication or other arrhythmogenic stimuli.

2.2. Modifier genes in LQTS

The length of QT interval and the severity of symptoms vary considerably even between carriers of the same LQTS mutation (Fodstad et al. 2004), suggesting the occurrence of other disease-modifying factors. The minor allele of *KCNE1* D85N has been found to prolong QT interval by 10 ms in the general Finnish population (Marjamaa et al. 2009a). It has been reported to reduce both I_{Ks} and I_{Kr} currents (Figure 10) and to be associated with a more severe phenotype in LQTS mutation carriers (Westenskow et al. 2004, Nishio et al. 2009). In Study III, *KCNE1* D85N was shown to prolong QT interval in *KCNQ1* G589D male carriers more than in *KCNQ1* G589D female carriers or in the general population. The data also suggested that *KCNE1* D85N could be associated with a more severe clinical phenotype in *KCNQ1* G589D carriers. The interaction between *KCNQ1* G589D and *KCNE1* D85N may arise from the co-assembly of $K_v7.1$ encoded by *KCNQ1* and minK encoded by *KCNE1* to form the I_{Ks} channel (Barhanin et al. 1996, Sanguinetti et al. 1996) (Figure 10). The effect of *KCNE1* D85N in *KCNH2* founder mutation carriers was non-significant in Study III, but this could be caused by a limited statistical power.

Several potential explanations exist for the suggested sex difference in the effect of *KCNE1* D85N. The baseline QT interval in *KCNQ1* G589D female carriers is longer than in *KCNQ1* G589D male carriers (Piippo et al. 2001, Study III). It is thus possible that *KCNE1* D85N brings out a masked I_{Ks} defect present in males. Another possibility is that sex differences in *KCNE1* expression levels (Drici et al. 2002) may influence the susceptibility to *KCNE1* D85N-mediated QT interval prolongation. *KCNE1* D85N could also interfere with binding

of hormonal or other sex-specific regulators of I_{Ks} and I_{Kr} channels, as sex hormones have been reported to regulate cardiac ion channels and to alter their susceptibility to blocking agents (Busch et al. 1997, Kurokawa et al. 2008).

LQTS modifier variants can be detected in genes encoding cardiac ion channels, such as K897T in *KCNH2* (Crotti et al. 2005) and H558R in *SCN5A* (Ye et al. 2003), but they may also occur in genes with a regulatory role in cardiac function. For example, variants in *NOS1AP*, which participates in the regulation of cardiac repolarization (Chang et al. 2008), influence the phenotype of LQTS mutations (Crotti et al. 2009, Tomás et al. 2010). It is likely that more LQTS modifier genes will be discovered in the future. Large samples of genetically uniform LQTS patients provide an excellent opportunity to study the effects of potential modifier variants on QT interval or arrhythmia susceptibility.

3. Genes, QT interval, and SCD

QT interval duration can be used to predict the risk of SCD (Straus et al. 2006). Study IV showed a 19% increased risk of SCD per 10-ms prolongation of QT interval. The effect was roughly linear, although familial short QT syndrome also predisposes to arrhythmia and SCD (Brugada et al. 2004, Hong et al. 2005). However, a markedly shortened QT interval occurs very rarely in the general population and may not always indicate an increased risk of cardiac death (Anttonen et al. 2007), which could explain the linear relationship. A dichotomous QT interval threshold (>450 ms for males and >470 ms for females) is a widely applied measure of risk of cardiac events in the clinical setting (Straus et al. 2006). Study IV provided further evidence for the utility of this threshold as a SCD risk predictor and demonstrated that QT interval is a suitable intermediate phenotype for the genetic studies of SCD.

The linear QT_{score} was not associated with SCD in Studies IV and V. A suggestive U-shaped association between the QT_{score} quintiles and SCD was noted in Study IV, but this finding was not replicated in the large meta-analysis in Study V. This lack of association could result from limited power to detect small effect sizes (hazard ratio <1.03 per 1-ms change in QT_{score} in Study V). It is also possible that all QT-prolonging alleles do not contribute to increasing the risk of SCD. Alternatively, some QT-shortening alleles may increase the risk of SCD (Brugada et al. 2004) or the effect may depend on the interaction between different

genetic factors (Ye et al. 2003). As depicted in Figure 1, both loss-of-function and gain-of-function types of mutations in ion channel genes may result in severe cardiac disorders predisposing to SCD. Therefore, a risk score based on the direct effect on SCD risk is likely to be more accurate than one based on the QT-prolonging effect of each allele.

4. Genetic arrhythmia susceptibility variants in SCD

4.1. Common genetic variants and SCD

The minor alleles of two novel common variants, rs41312391 and rs2200733, were significantly associated with risk of SCD, showing a 27% and 28% increased risk, respectively (Study V). Additional covariate adjustments indicated that these SNPs may predispose to fatal arrhythmias independently of CHD and its risk factors, QT-modulating medication, and heart failure. In addition, the association of rs2383207 with SCD was replicated ($p = 0.036$).

The SNP rs41312391 (IVS24+116G>A) is located in an intron of *SCN5A*. Evidence on the association between this SNP and cardiac repolarization is conflicting, as the minor allele has been associated with QT interval prolongation in one study (Aydin et al. 2005) and QT interval shortening in another study (Gouas et al. 2007). This variant is nevertheless in low linkage disequilibrium with rs12053903 ($r^2 = 0.36$) and rs1805126 ($r^2 = 0.32$), whose minor alleles are associated with QT interval shortening (Newton-Cheh et al. 2009b, Pfeufer et al. 2009). It is possible that rs41312391 is associated with increased risk of arrhythmia independently of cardiac repolarization. The gene expression analysis in Study V suggested that *WDR48*, a regulator of chromatin structure, might be involved in this association. However, *SCN5A* remains a more likely candidate gene for fatal arrhythmia due to its reported associations with several cardiac disorders (Wang et al. 1995, Chen et al. 1998, Schott et al. 1999, Benson et al. 2003, Bezzina et al. 2003a, Ellinor et al. 2008) and SCD (Burke et al. 2005, Tester and Ackerman 2007, Albert et al. 2010). In fact, the common *SCN5A* variant S1103Y is associated with risk of SCD (Splawski et al. 2002, Burke et al. 2005) and sudden infant death syndrome (Plant et al. 2006) in African Americans.

The variant rs2200733 was selected as a candidate SNP based on its previously reported association with atrial fibrillation (Gudbjartsson et al. 2007), which has been shown to

predispose to SCD after acute myocardial infarction (Pedersen et al. 2006). The results of Study V provide the first evidence of the association of rs2200733 with SCD. This SNP is located in 4q25 near *PITX2*. The gene expression analyses suggested that the minor allele of rs2200733 could be associated with increased expression of *PITX2*, which encodes a homeobox transcription factor involved in the generation of left-right asymmetry in cardiac development (Franco and Campione 2003) and sinoatrial node formation (Mommersteeg et al. 2007). *PITX2* is regulated by the Wnt/ β -catenin signalling pathway involved in cell proliferation and apoptosis (Kioussi et al. 2002). Deletion of *Pitx2c* in mice leads to gene expression changes in several cellular pathways, including apoptosis, cell adhesion, gap junctions, and cardiac ion channels (Chinchilla et al. 2011, Kirchhof et al. 2011). The disturbance of these cellular processes implicates a potential link between *PITX2* expression and life-threatening arrhythmia (Figure 10).

The SNP rs2383207 is located in 9p21 and has previously been linked to increased risk of myocardial infarction and SCD (Helgadottir et al. 2007, Newton-Cheh et al. 2009a). The proximal cyclin-dependent kinase inhibitor genes *CDKN2A* and *CDKN2B* are involved in proliferation of aortic smooth muscle cells and CHD (Visel et al. 2010). Adjustment for risk factors for CHD attenuated the association (Study V), and therefore, it seems likely that the association between this SNP and SCD is conveyed through development of CHD.

The *NOS1AP* variants rs2880058, rs12036340, and rs12143842, previously reported to be associated with QT interval duration (Marjamaa et al. 2009a, Newton-Cheh et al. 2009b, Pfeufer et al. 2009), were not significantly associated with SCD in Study V. In the course of Study V, several other *NOS1AP* SNPs were reported to be associated with SCD (Kao et al. 2009, Westaway et al. 2011), and the effect of these variants remains to be examined in the Finnish population. The previously reported association of *ADRB2* Q27E (rs1042714) with SCD (Sotoodehnia et al. 2006) was not replicated in Study V. This could result from differences in the genetic structure of the study populations or in the SCD case adjudication protocol. The power to detect a hazard ratio of more than 1.3 was over 99% in Study V, but limited power could explain the lack of an association for SNPs with a more modest effect size. In general, varying availability of witness reports and autopsy data has increased heterogeneity in case definition between different SCD studies, thus complicating the replication of genetic findings.

4.2. Rare arrhythmia-associated mutations and SCD

The ten rare arrhythmia-associated mutations located in coding regions of the *KCNQ1*, *KCNH2*, *PKP2*, *DSG2*, *DSP*, and *RYR2* genes had a combined carrier frequency of 79 per 10 000 individuals in Finland (Study VI). The prevalence of the four Finnish LQTS founder mutations (36 per 10 000) corresponded to that previously reported in the general population (Marjamaa et al. 2009b), and the prevalence of the five Finnish ARVC mutations (39 per 10 000) was in the same range as in Study II. According to these results, as many as 1 in 130 Finns may carry a mutation increasing the susceptibility to severe arrhythmias. The geographic clustering of these mutations may be caused by founder effects during the population history of Finland (Peltonen et al. 1999). Long-term genetic drift may also have shaped the geographic differences in mutation prevalences (Palo et al. 2009). Only a small proportion of the mutation carriers suffered from arrhythmia (6.5%) or heart failure (3.3%) based on causes of death, hospitalization records, and special reimbursement eligibility for specific medications. Thus, the mutation penetrances seem significantly reduced, although data from extensive cardiologic examinations were not available in Study VI.

In previous studies, mutations in cardiac ion channels *KCNQ1*, *KCNH2*, *SCN5A*, and *RYR2* (Chugh et al. 2004b, Tester et al. 2004, Tester and Ackerman 2007, Albert et al. 2008, Adabag et al. 2010b, Marjamaa et al. 2011), and recently also in the desmosomal gene *PKP2* (Zhang et al. 2012), have been identified in victims of SCD. In Study VI, 1% of the SCD victims carried one of the ten rare Finnish arrhythmia-associated mutations. These mutations seem to be involved in SCD only in rare cases, and future studies may require a longer follow-up time and a particular focus on cases with sudden arrhythmic death, which was not feasible in the population-based approach in Study VI. Of the rare mutations, *RYR2* R3570W and *KCNH2* R176W have been reported in several Finnish SCD cases. *RYR2* R3570W, which causes a gain-of-function defect in the cardiac ryanodine receptor, was initially reported in two Finnish SCD victims (Marjamaa et al. 2011). The exact clinical significance of this mutation remains uncertain, however, as clinical findings among the surviving relatives were scarce (Marjamaa et al. 2011). *KCNH2* R176W is a potentially disease-causing LQTS variant that has been found to prolong QT interval by 22 ms in the general Finnish population (Marjamaa et al. 2009b) and by 32 ms in LQTS families (Fodstad et al. 2006). Altogether three SCD victims carried *KCNH2* R176W in Study VI, but further studies are needed to confirm the potential role of this mutation in SCD.

5. SCD risk prediction

Only a minority of SCDs involve patients classified into high-risk groups, including those with previously diagnosed myocardial infarction and ventricular tachycardia (Huikuri et al. 2001). Therefore, identification of more sensitive risk markers is essential for more effective prevention of SCDs. Traditional risk factors for CHD predispose to SCD in the general population (Wannamethee et al. 1995, Jouven et al. 1999). This was also observed in Study V, in which male gender, higher systolic blood pressure, prevalent diabetes, current and former cigarette smoking, low leisure-time physical activity, prevalent CHD, Eastern Finnish residency, and digoxin use were shown to be associated with increased risk of SCD. These risk factors may reveal increased risk of cardiovascular disease underlying SCD, but are not effective in evaluating individual risk of sudden death due to their low positive predictive value (Huikuri et al. 2001). At the population level, however, treatment of underlying cardiovascular disease reduces the occurrence of SCD.

Heart failure and left ventricular dysfunction, measured by reduced left ventricular ejection fraction, can be used to predict risk of SCD from arrhythmia, but these risk markers are not specific to arrhythmic causes of death (The Multicenter Postinfarction Research Group 1983). Premature ventricular depolarizations and non-sustained ventricular tachycardia may reflect underlying heart failure, but are not specific markers for SCD (Caruso et al. 1997). However, sustained polymorphic ventricular tachycardia and ventricular fibrillation predict high risk of SCD (Huikuri et al. 2001). ECG is useful in diagnosing structural heart disease and electrophysiological conditions, such as LQTS and ARVC, which predispose to SCD (Jervell and Lange-Nielsen 1957, Thiene et al. 1988). Even without a diagnosis of a specific cardiac disorder, several ECG markers provide additional information on arrhythmia susceptibility. For example, QT interval prolongation, QRS complex widening, and J-point elevation are associated with increased risk of SCD (Straus et al. 2006, Dhar et al. 2008, Tikkanen et al. 2009, Kurl et al. 2012, Study IV). Signal-averaged ECG may also indicate susceptibility to arrhythmia, but its positive predictive value is low (McClements and Adgey 1993). Electrophysiologic testing is a valuable tool for risk stratification in patients with high risk of ventricular arrhythmias (Moss et al. 1996, Buxton et al. 1999).

Family history of SCD remains a risk factor for SCD even after adjustment for myocardial infarction and CHD risk factors (Friedlander et al. 1998, Jouven et al. 1999, Friedlander et

al. 2002). This finding indicates the involvement of independent genetic risk factors predisposing to SCD. At the individual level, molecular screening of mutations in cardiac ion channel genes may help in evaluating the cause of sudden unexplained death (Lunetta et al. 2003, Tester et al. 2004, Tester and Ackerman 2007). In this case, identification of a rare ion channel mutation may also prove effective in evaluating the SCD risk in surviving family members. Common SCD risk variants are associated with a more modestly increased risk (Study V), but could be used to predict risk together with clinical risk factors. QT_{score} did not appear to be a useful predictor of SCD risk in Studies IV and V, but development of a risk score based on common SCD-associated variants could prove more informative along with accumulating data on the genetic components of SCD.

6. Study limitations

In Study I, only mutations in the exons of four desmosomal genes were searched for in the ARVC patients, and therefore, the occurrence of other types of mutations cannot be excluded. In Study II, only five desmosomal mutations and two common polymorphisms were assayed in the Health 2000 population sample. Thus, the total prevalence of all desmosomal mutations in the Finnish population is expected to be higher than 1:250. The participants of the Health 2000 and FINRISK studies did not undergo comprehensive cardiologic examinations, and thus, the exact disease penetrance in the mutation carriers could not be assessed. Future functional and population studies are needed to reveal the exact clinical significance of the desmosomal mutations identified in Studies I and II.

In Study III, the power to identify an association between *KCNE1* D85N and clinical outcome in LQT2 was limited due to the small number of mutation carriers with D85N. Despite the large sample size in Studies IV-VI, the power to identify modest effect sizes was limited. Multiple health care registries and autopsy data were utilized to reliably identify SCDs, but the association between genetic variants and sudden arrhythmic death could not be specifically investigated in this population-based approach. As the population samples consisted only of individuals aged over 25 years, the prevalence and clinical significance of arrhythmia-associated variants in children and young adults remain to be explored. Replication studies in independent samples are needed to confirm the novel findings of Studies II, III, and V. In particular, confirmation of the gene expression results would require a larger sample size and RNA quantification in cardiac tissue.

CONCLUSIONS

Mutations in genes encoding desmosomal proteins account for only approximately one-fifth of ARVC cases in Finland. Desmosomal mutations are associated with reduced disease penetrance, and compound heterozygosity may contribute to disease progression. At sites of cell adhesion, a mutation in a desmosomal protein may disturb the attachment of other desmosomal proteins and lead to disorganization of the intercalated disk structure. *PKP2* Q59L is a novel ARVC founder mutation showing an estimated prevalence of 1:340 in Finland. In total, at least 1:250 Finns carry a desmosomal mutation predisposing to ARVC or related functional abnormalities of the heart, but the reduced disease penetrance associated with these mutations should be considered in counselling of mutation carriers. Including both desmosomal and ion channel mutations, as many as 1:130 Finns may carry a mutation increasing the susceptibility to severe arrhythmias.

KCNE1 D85N presents a potential sex-specific disease-modifying factor in LQTS. This common genetic variant seems to prolong QT interval in males with LQT1, but not in females with LQT1. It may also be associated with increased disease severity. In the general population, *KCNE1* D85N together with 13 other SNPs explain less than 10% of the variation in QT interval. These QT interval-associated SNPs as well as still undiscovered variants present potential LQTS modifiers, and ultimately, this information could be used in assessment of individual susceptibility to QT-prolonging medication and LQTS.

A 10-ms prolongation of QT interval is associated with a 19% increased risk of SCD in the Finnish population. A risk score based on QT interval-associated SNPs does not, however, directly contribute to SCD risk prediction. In contrast, a novel variant in *SCN5A* and another in 4q25 near *PITX2*, as well as a previously identified variant in 9p21 near the *CDKN2A* and *CDKN2B* genes, are associated with increased risk of SCD. Rare mutations in *KCNH2*, *RYR2*, and *PKP2* are carried by individual SCD victims, but future studies are needed to reveal their significance in sudden death. In the future, a panel of genetic and clinical risk markers could provide useful information for SCD risk stratification and prevention.

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