MOLECULAR GENETICS OF
SEVERE VENTRICULAR ARRHYTHMIAS

Kirsi Piippo

Academic dissertation

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals.
In addition, some unpublished data are presented.


Publication V appears also in the thesis of Heikki Swan (1999).
ABBREVIATIONS

AD  autosomal dominant inheritance
AP  action potential
AR  autosomal recessive inheritance
ARVD arrhythmogenic right ventricular dysplasia
bp  base pair
BWS Beckwith-Wiedeman syndrome
cDNA complementary DNA
cM  centiMorgan
DCM  dilated cardiomyopathy
DNA  deoxyribonucleic acid
ECG electrocardiogram, electrocardiographic
FH familial hypercholesterolemia
FPVT familial polymorphic ventricular tachycardia
HCM hypertrophic cardiomyopathy
HERG human ether á-go-go related gene (protein)
I_{Ca-L}  L-type calcium current
I_{Ca-T}  T-type calcium current
I_C  chloride current
I_{Kr}  potassium plateau current
I_{Kr}  rapidly activated delayed rectifier potassium current
I_{Ks}  slowly activated delayed rectifier potassium current
I_{Kur} ultra rapid potassium current
I_Na  natrium current
I_{to}  transient outward potassium current
JLN1 type 1 Jervell and Lange-Nielsen syndrome
JLN2 type 2 Jervell and Lange-Nielsen syndrome
KCNQ1 KCNQ potassium channel gene family member 1 (protein)
LCSD left cardiac sympathetic denervation
Iod  logarithm of odds
LQTS long QT syndrome
LQT1 type 1 long QT syndrome (Romano-Ward syndrome)
LQT2 type 2 long QT syndrome (Romano-Ward syndrome)
LQT3 type 3 long QT syndrome (Romano-Ward syndrome)
LQT4 type 4 long QT syndrome (Romano-Ward syndrome)
LQT5 type 5 long QT syndrome (Romano-Ward syndrome)
LQT6 type 6 long QT syndrome (Romano-Ward syndrome)
MH malignant hyperthermia
minK minimal potassium channel (gene)
MiRP minK related peptide (gene)
NPL non-parametric linkage analysis
PCCD progressive cardiac conduction defect
PCR polymerase chain reaction
PIRA primer-induced restriction assay
QTc QT interval corrected for heart rate
RNA ribonucleic acid
RWS Romano-Ward syndrome
SCN5A natrium channel alpha subunit number 5 (gene)
SIDS sudden infant death syndrome
SSCP single-strand conformation polymorphism
wt wild type

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

Long QT syndrome (LQTS) is a prototype of congenital arrhythmias characterized by ventricular tachycardia, such as torsade de pointes, and risk for sudden death. LQTS may have either autosomal dominant (Romano-Ward syndrome, RWS) or recessive (Jervell and Lange-Nielsen syndrome, JLNS) inheritance. These two diseases are allelic and caused by mutations in genes encoding potassium channel subunits KCNQ1 or minK; in addition, mutations in two other genes encoding K+ channel subunits (HERG and MiRP1) or the gene encoding Na+ channel SCN5A may lead to RWS. Defective channels result in prolongation of the cardiac repolarization phase, indicated by lengthening of the QT interval on electrocardiogram and risk for arrhythmias. Several mutations have been described in the five LQTS genes, and typically each family carries a unique gene defect.

Polymorphic ventricular tachycardias may occur as a dominantly inherited trait and represent a highly malignant disorder manifesting as exercise-induced ventricular arrhythmias at a young age. The mortality is high. At the beginning of this study, two Finnish families were diagnosed with this disease, and several reminiscent cases were reported in other populations. The molecular pathology of the disease was unknown.

In this work, 114 Finnish LQTS families were studied using molecular genetic methods. Mutations were screened using either a single-strand conformation polymorphism technique or direct DNA sequencing. The nucleotide variants were confirmed with a specific test, typically based on restriction enzyme digestion. For particular mutations, functional studies were also carried out using a patch-clamp technique. The mapping of the familial polymorphic ventricular tachycardia locus was performed using polymorphic microsatellite markers.

Altogether eight novel mutations in three LQTS genes were described. Six of them were located in the KCNQ1 gene (Y171X, G269S, S277del, T311I, D317N and G589D or KCNQ1-Fin) and one in both HERG (L552S or HERG-Fin) and SCN5A (V1667I) genes. All but two of the mutations (KCNQ1-Fin and HERG-Fin) were present in one family only.
The KCNQ1-Fin mutation, located in the C-terminus of the KCNQ1 gene, caused LQTS in 34 out of 114 (30%) Finnish families. It was also present in homozygous form in two siblings with JLNS and in compound heterozygous form in one unrelated JLNS patient. In vitro KCNQ1-Fin showed a marked increase in the voltage threshold of activation, but no dominant negative suppression when co-expressed with the wild type. The phenotype of KCNQ1-Fin heterozygotes, based on QTc length (mean 460 ± 40 ms) and occurrence of symptoms, appeared to be milder than in LQT1 patients in general. The relative enrichment of this mutation in Finland suggested a founder effect. Indeed, a common haplotype around the KCNQ1 locus was detected in nearly all probands and the birthplaces of the ancestors of the probands were situated in the same geographical area.

The HERG-Fin mutation, modifying the S5 transmembrane region of the HERG subunit, was present in six LQTS families. In addition, two sisters with a severe cardiac phenotype but no other abnormalities were recognized to be homozygous for the HERG-Fin mutation. In vitro HERG-Fin activated and deactivated more rapidly than the wild-type channel. Phenotypically, HERG-Fin heterozygotes had a prolonged QTc interval (mean 466 ± 47 ms) and the majority (90%) of those symptomatic were female. A founder effect could be inferred based on both haplotype and geographical data.

The locus for familial polymorphic tachycardia was mapped to chromosome 1q42-q43. The 9 cM region of interest contains several possible candidate genes. Two of them, one coding for the potassium channel TWIK1 and the other for actin-binding protein ACTN2, were sequenced, but no mutations were found. This is the first step in characterizing the causative gene and molecular pathology of this disorder.

These findings have immediate clinical benefits. A molecular diagnostic tool is now available for approximately 50% of Finnish LQTS families. Early and accurate diagnosis of LQTS is of paramount importance as the different LQTS types differ in triggers for symptoms and response to treatment. Carriers of the founder mutations form a genetically uniform population in which effects of different genetic and non-genetic factors on LQTS phenotype can be studied.
1. INTRODUCTION

Inherited cardiac arrhythmias, although rare at the population level, are an important cause of sudden death in young and otherwise healthy individuals. Long QT syndrome (LQTS) is the best characterized arrhythmic disorder manifesting as a prolonged QT interval on ECG and ventricular arrhythmias (Chiang and Roden 2000). Other hereditary diseases causing severe ventricular arrhythmias include familial forms of cardiomyopathies, arrhythmogenic right ventricular dysplasia, Brugada syndrome (Priori et al. 1999a) and polymorphic ventricular tachycardia (Leenhardt et al. 1995).

Long QT syndrome is a monogenic disorder with autosomal dominant or recessive inheritance (Chiang and Roden 2000). Molecular genetic studies were initiated in 1991, when the first locus for LQTS was mapped to chromosome 11 (Keating et al. 1991). The first three LQTS genes were identified within a year during 1995-1996 by the same research group in the United States (Curran et al. 1995; Wang et al. 1995, 1996a). Thus far, over 200 mutations of five different genes have been described to cause LQTS (Slawski et al. 2000). The underlying molecular pathology is defective cardiac voltage-gated potassium or sodium channels that cause disturbance in electrical signalling of the heart, resulting in prolongation of cardiac repolarization and risk for arrhythmias (Chiang and Roden 2000). During recent years, LQTS has been shown to have incomplete penetrance, which can vary markedly between families and even between generations within the same family (Priori et al. 1999b). Therefore, it is highly likely that some intrinsic or extrinsic factors modify the outcome of LQTS.

A few transgenic mice models have been developed to study the pathophysiology of cardiac arrhythmias in vivo (Gehrmann and Berul 2000). Heterozygous and homozygous mice overexpressing the N-terminus of a rat delayed rectifier K+ channel Kv1.1 (Kv1.1N206Tag) have prolonged QT intervals and ventricular arrhythmias, providing good models of LQTS (London et al. 1998; Jeron et al. 2000). Recent molecular data have yielded valuable basic knowledge applicable in clinical management of LQTS (Priori et al. 1999a,c).

Familial polymorphic ventricular tachycardia (FPVT) is a highly malignant disorder with unknown etiology. It is characterized by exercise-induced polymorphic ventricular tachycardia in
structurally normal hearts (Leenhardt et al. 1995). The onset of disease is in adolescence, and the prognosis is poor. FPVT appears to be inherited in an autosomal dominant fashion, but nothing is known about its molecular background.

In this work, two distinct congenital disorders causing severe ventricular arrhythmias, long QT syndrome and familial polymorphic ventricular tachycardia, were studied in Finnish families using molecular genetic methods.
2. REVIEW OF THE LITERATURE

2.1. Ion channels

2.1.1. General characteristics

Ion channels are protein complexes that form a route across the lipid bilayer to enable different ions to pass through the cell membrane. They are present in nearly every cell type, and regulate salt balance inside the cells and in extracellular space (Lehman-Horn and Jurkat-Rott 1999). In excitable cells, such as neurons and myocytes, ion channels generate the electrical impulses. The function of channels can be activated in different ways; voltage-gated ion channels are opened by voltage changes, whereas various extracellular ligands, e.g. cAMP, activate other channels. The conducting part of the channel is often highly selective for specific ions and is used to classify it as a potassium, sodium, calcium or chloride channel. Typical of all voltage-gated cation channels is a voltage sensor in the fourth transmembrane domain (S4 region) and a pore region with a selectivity filter between the S5 and S6 domains (Lehman-Horn and Jurkat-Rott 1999). Functionally, a common feature is one open state and two closed states of the channel, the latter further described as either an inactivated closed or a resting closed state (Katz 1993). When the channel is inactivated (inactivated closed state), a recovery time is needed (resting closed state) before opening again. The inactivation can occur through two different mechanisms, fast (N-type) or slow (C-type) inactivation, or can be a combination of both (Lehman-Horn and Jurkat-Rott 1999).

Homologous genes encode ion channels that are expressed in different tissues. These channels have similar structure and function. Potassium channels are considered to be the most primitive of voltage-gated channels because of their early evolution and structural similarity to ancestral channels (Katz 1993). The large variability within potassium channels is explained by long-lasting evolution and monomeric, non-covalent (unstable) structure. Still today, marked evolutionary conservation exists between species as different as human, mouse and Drosophila (Warmke and Ganetzky 1994).

The function of ion channels can be followed in vitro using a patch-clamp technique (Neher and Sakmann 1976). In this method, a pipette containing a small electrode is pressed tightly against
the cell membrane to record currents. Three different patch-clamping modes can be used: 1) the cell-attached mode used for single channel recordings, 2) the cell-free inside-out or outside-out mode, where a patch of membrane is ripped off with the pipette and 3) the whole-cell mode, where the suction of the pipette disrupts the membrane and allows measuring of the entire intracellular space (Ogden and Standfield 1993). With the whole-cell patch-clamp technique, different ion channels expressed in the cell can be measured by changing the intracellular and extracellular environments.

2.1.2. Major ion currents in the heart

A cardiac action potential (AP) is the combined effect of different ion channels in the heart, when a balance between inward and outward currents is achieved (Figure 1). Fast inflow of positively charged sodium ions depolarizes the cell membrane and initiates an AP. Inactivation of Na+ channels and outflow of potassium ions repolarize the cell.

![Diagram of ion currents and genes](image)

**Figure 1.** Ion currents (left) and underlying genes (right) that are responsible for a cardiac action potential (top). Inward currents are shown below the line and outward currents above the line. Current amplitudes are not drawn to scale. The currents and genes relevant for this work are shown in bold. (modified from Priori et al. 1999c.)
The delayed rectifier potassium current is most important in determining repolarization, return to resting potential, and shape of the cardiac action potential. Calcium inward currents (\(I_{\text{Ca-L}}\), \(I_{\text{Ca-T}}\)) and transient outward potassium currents (\(I_{\text{TO}}\)) also modulate the repolarization phase. In fact, at least ten different K+ currents, three different Ca2+ currents and a Na+ current have been recognized in the mammalian heart (Roden and George 1996).

**The delayed rectifier potassium current** consists of two biophysically and pharmacologically different components: the slowly activating \(I_{Ks}\) and the rapidly activating \(I_{Kr}\) (Sanguinetti and Jurkiewicz 1990) (Figure 1). \(KCNQ1\) gene (formerly \(KVLQT1\)) encodes the \(\alpha\)-subunit, and \(minK\) gene (also called \(KCNE1\)) the \(\beta\)-subunit of the \(I_{Ks}\) channel (Barhanin et al. 1996; Sanguinetti et al. 1996a). The mature channel is assembled in the endoplasmic reticulum of four identical \(\alpha\)-subunits and an unknown amount of auxiliary \(\beta\)-subunits (Figure 2). The \(minK\) peptide is suggested to contribute to pore formation in \(I_{Ks}\), and also to physically interact with the \(KCNQ1\) pore-forming region (Romey et al. 1997; Sesti and Goldstein 1998; Tai and Goldstein 1998). Characteristics for \(I_{Ks}\) include slow activation after depolarization and slow deactivation (Barhanin et al. 1996; Sanguinetti et al. 1996a). It does not inactivate by a typical N- or C-type mechanism, rather by a combination of both (Pusch et al. 1998). The current is modulated by both protein kinase A and protein kinase C phosphorylation (Lo and Numann 1998). In addition to \(KCNQ1\), four homologous genes (\(KCNQ2-5\)) form the \(KCNQ\) family of genes that encodes potassium channels important in the heart, brain and inner ear (Wang et al. 1996a; Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998; Kubisch et al. 1999; Lerche et al. 2000).

The rapidly activating K+ channel \(I_{Kr}\) is likewise a tetramer encoded by the \(HERG\) gene (Figure 2) (Sanguinetti et al. 1995). Although the \(HERG\) current resembles the native \(I_{Kr}\), a \(\beta\)-subunit MiRP1 (KCNE2) has recently been shown to co-assemble with \(HERG\) in a stable fashion, and modulate it \textit{in vitro} (Figure 2) (Abbott et al. 1999). The HERG/MiRP1 complex is functionally and pharmacologically similar to \(I_{Kr}\), however, no data exist on how the hetero-oligomer is formed \textit{in vivo}. \(I_{Kr}\) is structurally related to the Drosophila Shaker channel (Warmke and Ganetzky 1994) but exhibits unique functional properties, namely, fast inactivation and slow deactivation (Sanguinetti and Jurkiewicz 1990). \(HERG\) is inactivated by a mechanism resembling C-type inactivation (Smith et al. 1996; Spector et al. 1996). \(I_{Kr}\) is regulated by
extracellular potassium (Sanguinetti et al. 1995) and direct phosphorylation through protein kinase A (Thomas et al. 1999).

**Figure 2:** The schematic structure of the five cardiac ion channel subunits underlying the long QT syndrome. The location of the transmembrane segments S1-S6 and the pore region is shown. Four KCNQ1 α-subunits (shown in white) and unknown number of minK β-subunits (black) form the slow component ($I_{Ks}$), and four HERG α-subunits (white) and unknown number of MiRP1 β-subunits (black) the rapid component ($I_{Kr}$) of the delayed rectifier potassium channel. The pore regions are located in the middle of the $I_{Ks}$ and $I_{Kr}$ tetramer forming the ion passage route. SCN5A consists of four similar domains DI-DIV, and it forms the cardiac sodium channel.
A cardiac sodium current is generated by an ion channel, SCN5A, which consists of four similar domains, each containing a pore-forming unit and a voltage sensor, analogous to Shaker potassium channels (Figure 2). Although assembly of this α-subunit with a sodium channel β-subunit (SCN1B) appears in vitro, its relevance in the heart is unclear (Roden and George 1996). Rapid inactivation is typical of all voltage-gated Na+ channels (Roden and George 1996). Cardiac sodium channels are resistant to tetrodotoxin, a blocking toxin, in contrast to Na+ channels expressed in other tissues (Roden and George 1996).

2.1.3. Ion channel disorders

Shared properties among different ion channels also have implications for similar disease mechanisms, which has lead to the concept of ion channelopathies. These disorders can affect different tissues such as heart, brain and skeletal muscle. Features common to all of these diseases are variable phenotype and incomplete penetrance. Studies of naturally occurring mutations have increased the general knowledge of ion channel structure and function.

Cardiac channelopathies include long QT syndrome (LQTS) (see 2.3), idiopathic ventricular fibrillation (Brugada syndrome) and progressive cardiac conduction defect (PCCD). Defective potassium channels underlie LQTS types 1, 2, 5 and 6, while mutations in the cardiac sodium channel gene SCN5A cause LQTS type 3, Brugada syndrome and PCCD (Wang et al. 1995; Chen et al. 1998; Schott et al. 1999). Brugada syndrome is characterized by unique ECG abnormalities, such as right bundle-branch block with ST segment elevation, sudden death and absence of any structural heart disease. Typical for PCCD, in turn, is widening of the QRS complex, right or left branch block in the absence of structural heart disease, syncope and sudden death. The disease mechanisms vary; mutations associated with LQT3 cause a gain in function and constitutive opening of the channel (Bennett et al. 1995), while mutations resulting in Brugada syndrome abolish channel functioning (Chen et al. 1998). A specific mutation has been shown to cause characteristics of both diseases in one family (Bezzina et al. 1999). The molecular pathogenesis of PCCD remains obscure.

Neuronal channelopathies can be caused by mutations in potassium, sodium or calcium channel genes. Defective KCNQ2 and KCNQ3 potassium channels were recently shown to result in a
specific form of epilepsy, benign familial neonatal convulsions (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998). Mutations in the KCNA1 gene, a homologue of the Drosophila Shaker gene, have been described in episodic ataxia 1 patients (Browne et al. 1994). Mutations in genes encoding sodium channels can also cause one form of epilepsy: defective sodium channel β1-subunits (SCNB1) and neuronal sodium channel α-subunits (SCNA1) underlie generalized epilepsy with febrile seizures (Wallace et al. 1998; Escayg et al. 2000). Different types of mutations in a single calcium channel gene, CACNA1A, can cause three distinct neurological disorders. Episodic ataxia 2 results from premature truncation of the protein or post-transcriptional splicing (Ophoff et al. 1996), while the neurodegenerative disease spinocerebellar ataxia is associated with trinucleotide repeat expansion in the protein coding sequence (Riess et al. 1997; Zhuchenko et al. 1997). The third allelic disease is familial hemiplegic migraine, which is caused by several missense mutations (Ophoff et al. 1996).

**Inner ear channelopathies** are caused by modified potassium channel α- or β-subunits and result in hearing loss. Autosomal recessive Jervell and Lange-Nielsen syndrome (JLNS) is characterized by prolonged cardiac QT interval and congenital deafness (Jervell and Lange-Nielsen 1957). Mutations in KCNQ1 or minK genes can cause JLNS. One form of autosomal dominant non-syndromic progressive hearing loss is associated with defective potassium channel KCNQ4 (Kubisch et al. 1999). The most common cause of monogenic hearing loss is defective connexin 26, a channel-forming gap junction protein responsible for K+ recycling to the endolymph (reviewed in Willems 2000).

**Skeletal muscle channelopathies** result from incomplete inactivation of Na+ current or pathological L-type Ca2+ current. Missense mutations in different parts of the SCN4A gene encoding skeletal muscle-specific sodium channel, underlie three autosomal dominant diseases: hyperkalemic periodic paralysis (Ptacek et al. 1991; Rojas et al. 1991), paramyotonia congenita (McClatchey et al. 1992a) and potassium-aggravated myotonia (McClatchey et al. 1992b). Hypokalemic periodic paralysis is caused by mutations in L-type calcium channel gene CACNA1S, also called dihydropyridine receptor (DHPR) (Jurkat-Rott et al. 1994; Ptacek et al. 1994). In rare cases, DHPR mutations may lead to malignant hyperthermia (MH), a pathological response to general anaesthesia (Monnier et al. 1997). Another Ca2+ channel expressed in skeletal muscle is
ryanodine receptor 1 (RYR1), which is functionally coupled to DHPR (Missiaen et al. 2000). Mutations in RYR1 gene may also cause MH (Gillard et al. 1991; Quane et al. 1993) and occasionally central core disease (Quane et al. 1993; Zhang et al. 1993).

In addition to defects in voltage-gated K+, Na+ and Ca2+ channels, mutations in genes encoding other types of ion channels, such as inward rectifying potassium channels, voltage-gated chloride channels and cystic fibrosis transmembrane regulator chloride channel, can cause human diseases (reviewed in Abraham et al. 1999; Lehmann-Horn and Jurkat-Rott 1999; and Ackerman and Clapham 1997, respectively).

### 2.2. Inherited cardiac arrhythmias

Malignant cardiac arrhythmias may appear in relation to several cardiac and non-cardiac disorders. A genetic cause has been shown to be at the base of a small proportion of arrhythmias (Priori et al. 1999a,c). Although rare, inherited arrhythmogenic disorders form a group of severe, often fatal disorders and are a common cause for sudden cardiac death in young otherwise healthy individuals. Most of the familial arrhythmogenic disorders are monogenic and inherited in an autosomal dominant fashion (Table 1). Several genetic loci and causative genes have been identified to underlie these diseases (Table 1). Malignant ventricular arrhythmias are frequently associated with hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular dysplasia (ARVD), familial ventricular fibrillation (Brugada syndrome) (see 2.1.3), long QT syndrome (LQTS) (see 2.3) and familial polymorphic ventricular tachycardia (FPVT). Atrial fibrillation and conduction defects can also be caused by inherited arrhythmogenic diseases (Table 1).

**HCM** is the leading cause of sudden death among young athletes (Maron 1997). The disease is characterized by thickening of the ventricular wall (hypertrophy), and can manifest as ventricular or atrial arrhythmia and sudden death. Both locus and allelic heterogeneity exist; mutations in eight different genes encoding sarcomeric proteins can cause HCM, and in addition, one locus in chromosome 7 has been identified (Table 1). Dysfunctional proteins in the sarcomere are supposed to abolish force generation and subsequent hypertrophy (Chen and Chien 1999).
Recently, mutations in the same genes coding for α-actin, β-myosin heavy chain and cardiac troponin T have been shown to cause both dilated and hypertrophic cardiomyopathy (Table 1).

Table 1: Summary of inherited arrhythmogenic disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Gene/protein</th>
<th>Inheritance</th>
<th>Reference</th>
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<tr>
<td>HCM</td>
<td>1q3</td>
<td>Cardiac troponin T</td>
<td>AD</td>
<td>Thierfelder et al. 1994</td>
</tr>
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<td></td>
<td>3p21.2-p21.3</td>
<td>Ventricular myosin essential light chain 1</td>
<td>AD</td>
<td>Poetter et al. 1996</td>
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<tr>
<td></td>
<td>7q3</td>
<td>?</td>
<td>AD</td>
<td>MacRae et al. 1995</td>
</tr>
<tr>
<td></td>
<td>11p11.2</td>
<td>Cardiac myosin binding protein C</td>
<td>AD</td>
<td>Bonne et al. 1995</td>
</tr>
<tr>
<td></td>
<td>12q23-q24</td>
<td>Ventricular myosin regulatory light chain 2</td>
<td>AD</td>
<td>Watkins et al. 1995</td>
</tr>
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<td>14q11-q12</td>
<td>Beta myosin heavy chain</td>
<td>AD</td>
<td>Poetter et al. 1996</td>
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<td></td>
<td>15q14</td>
<td>Alpha actin</td>
<td>AD</td>
<td>Geisterfer et al. 1990</td>
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<tr>
<td></td>
<td>15q22</td>
<td>Alpha tropomyosin</td>
<td>AD</td>
<td>Mogensen et al. 1999</td>
</tr>
<tr>
<td>DCM</td>
<td>19p13.2-q13.2</td>
<td>Cardiac troponin I</td>
<td>AD</td>
<td>Thierfelder et al. 1994</td>
</tr>
<tr>
<td></td>
<td>1p1-1q21</td>
<td>Lamin A/C</td>
<td>AD</td>
<td>Kimura et al. 1997</td>
</tr>
<tr>
<td></td>
<td>1q3</td>
<td>Cardiac troponin T</td>
<td>AD</td>
<td>Fatkin et al. 1999</td>
</tr>
<tr>
<td></td>
<td>1q32</td>
<td>?</td>
<td>AD</td>
<td>Kamisago et al. 2000</td>
</tr>
<tr>
<td></td>
<td>2q14-q22</td>
<td>?</td>
<td>AD</td>
<td>Durand et al. 1995</td>
</tr>
<tr>
<td></td>
<td>2q31</td>
<td>?</td>
<td>AD</td>
<td>Jung et al. 1999</td>
</tr>
<tr>
<td></td>
<td>2q35</td>
<td>Desmin</td>
<td>AD</td>
<td>Siu et al. 1999</td>
</tr>
<tr>
<td></td>
<td>3p22-p25</td>
<td>?</td>
<td>AD</td>
<td>Li et al 1999</td>
</tr>
<tr>
<td></td>
<td>5q33-q34</td>
<td>Delta sarcoglycan</td>
<td>AD</td>
<td>Olson and Keating 1996</td>
</tr>
<tr>
<td></td>
<td>6q23-q24</td>
<td>?</td>
<td>AD</td>
<td>Tsubata et al. 2000</td>
</tr>
<tr>
<td></td>
<td>9q13-q21</td>
<td>?</td>
<td>AD</td>
<td>Schönberger et al. 2000</td>
</tr>
<tr>
<td></td>
<td>10q21-q23</td>
<td>?</td>
<td>AD</td>
<td>Krajnovic et al. 1995</td>
</tr>
<tr>
<td></td>
<td>14q11-q12</td>
<td>Beta myosin heavy chain</td>
<td>AD</td>
<td>Kamisago et al. 2000</td>
</tr>
<tr>
<td></td>
<td>15q14</td>
<td>Alpha actin</td>
<td>AD</td>
<td>Olson et al. 1998</td>
</tr>
<tr>
<td></td>
<td>Xp21.2</td>
<td>Dystrophin</td>
<td>X</td>
<td>Montoni et al. 1993</td>
</tr>
<tr>
<td></td>
<td>Xq28</td>
<td>G4.5/tafazzin</td>
<td>X</td>
<td>Bione et al. 1996</td>
</tr>
<tr>
<td>ARVD</td>
<td>1q42-q43</td>
<td>?</td>
<td>AD</td>
<td>Rampazzo et al. 1995</td>
</tr>
<tr>
<td>Arrhythmogenic right ventricular dysplasy</td>
<td>2q32.1-q32.2</td>
<td>?</td>
<td>AD</td>
<td>Rampazzo et al. 1997</td>
</tr>
<tr>
<td></td>
<td>3p23</td>
<td>?</td>
<td>AD</td>
<td>Ahmad et al. 1998</td>
</tr>
<tr>
<td></td>
<td>10p12-p14</td>
<td>?</td>
<td>AD</td>
<td>Li et al. 2000</td>
</tr>
<tr>
<td></td>
<td>14q12-q22</td>
<td>?</td>
<td>AD</td>
<td>Severini et al. 1996</td>
</tr>
<tr>
<td></td>
<td>14q23-q24</td>
<td>?</td>
<td>AD</td>
<td>Rampazzo et al. 1994</td>
</tr>
<tr>
<td>(Naxos disease)</td>
<td>17q21</td>
<td>Plakoglobin</td>
<td>AR</td>
<td>McKoy et al. 2000</td>
</tr>
<tr>
<td>Idiopathic ventricular fibrillation (Brugada syndrome)</td>
<td>3p21-p23</td>
<td>SCN5A</td>
<td>AD</td>
<td>Chen et al. 1998</td>
</tr>
<tr>
<td>Familial atrial fibrillation</td>
<td>10q22-q24</td>
<td>?</td>
<td>AD</td>
<td>Brugada et al. 1997</td>
</tr>
<tr>
<td>Progressive cardiac conduction defect</td>
<td>3p21-p23</td>
<td>SCN5A</td>
<td>AD</td>
<td>Schott et al. 1999</td>
</tr>
<tr>
<td>LQT1</td>
<td>11p15.5</td>
<td>KCNQ1 (KVLQT1)</td>
<td>AD</td>
<td>Wang et al. 1996</td>
</tr>
<tr>
<td>LQT2</td>
<td>7q35-q36</td>
<td>HERG</td>
<td>AD</td>
<td>Curran et al. 1995</td>
</tr>
<tr>
<td>LQT3</td>
<td>3p21-p23</td>
<td>SCN5A</td>
<td>AD</td>
<td>Wang et al. 1995</td>
</tr>
<tr>
<td>LQT4</td>
<td>4q25-q27</td>
<td>?</td>
<td>AD</td>
<td>Schott et al. 1995</td>
</tr>
<tr>
<td>LQT5</td>
<td>21q22.1-q22.2</td>
<td>MinK</td>
<td>AD</td>
<td>Splawski et al. 1997b</td>
</tr>
<tr>
<td>LQT6</td>
<td>21q22.1-q22.2</td>
<td>MiR1</td>
<td>AD</td>
<td>Abbott et al. 1999</td>
</tr>
<tr>
<td>JLN1</td>
<td>11p15.5</td>
<td>KCNQ1 (KVLQT1)</td>
<td>AR</td>
<td>Neyroud et al. 1997</td>
</tr>
<tr>
<td>JLN2</td>
<td>21q22.1-q22.2</td>
<td>MinK</td>
<td>AR</td>
<td>Schulze-Bahr et al.1997a</td>
</tr>
</tbody>
</table>

AD = autosomal dominant, X = X-chromosomal, AR = autosomal recessive
LQT1-6 = long QT syndrome types 1-6 (Romano-Ward syndrome), JLN1-2 = Jervell and Lange-Nielsen syndrome types 1-2
DCM is a phenotypically and genetically heterogeneous disorder distinguished by a stretched, thin-walled heart and both ventricular and atrial malignant arrhythmias (Priori et al. 1999b). Familial cases account for up to 25% of all those affected (Chen and Chien 1999), and thus far, eight different genes and seven additional genetic loci have been described (Table 1). In addition to autosomal dominant and X-chromosomal inheritance, dilated cardiomyopathy can be caused by mutations in mitochondrial DNA (Marin-Garcia and Goldenthal 1997). Each of the proteins is located in the cytoskeleton or sarcomere, and when mutated, could affect heart muscle contraction and result in abnormal force transmission (Chen and Chien 1999).

ARVD is characterized by fibrofatty replacement of right ventricular myocardium and presents as ventricular tachycardia, supraventricular arrhythmias, right-sided heart failure and sudden death (Basso et al. 1996; Thienc et al. 1997). Thus far, six autosomal dominant loci and one autosomal recessive locus have been genetically linked to ARVD (Table 1). Very recently, the genetic basis of Naxos disease, the recessive variant of ARVD, was revealed when McKoy et al. (2000) described a homozygous deletion in the plakoglobin gene segregating with the disease in several families. Plakoglobin is important in desmosome formation in many tissues. Disruption of desmosome function caused by mutated plakoglobin during mechanical stress could disrupt myocyte junctions and lead to apoptosis. The cardiac phenotype in Naxos disease is similar to that of ARVD, but in addition, these patients have woolly hair and palmoplantar keratoderma (McKoy et al. 2000).

FPVT was described for the first time in 21 children whose initial symptoms of polymorphic exercise-induced ventricular tachycardia occurred before the age of ten years (Leenhardt et al. 1995). In 30% of those a familial history of syncope or sudden death could be demonstrated. Already in 1975 Reid et al. reported patients in whom any adrenergic stimulation, such as exercise, fright or infusion of isoproterenol, induced polymorphic ventricular complexes.

2.3. Long QT syndromes (LQTS)
2.3.1 History and classification
Originally, long QT syndrome (LQTS) was divided into two entities: the autosomal dominant form, the Romano-Ward syndrome (RWS) (Romano 1965; Ward 1964), and the autosomal
recessive form with congenital deafness, the Jervell and Lange-Nielsen syndrome (JLNS) (Jervell and Lange-Nielsen 1957). In 1957, Jervell and Lange-Nielsen described a family with four deaf siblings sustaining sudden loss of consciousness (syncopes) and sudden death. Later, Romano (1965) and Ward (1964) separately described families with prolonged QT intervals and syncopes but with normal hearing. Although already in 1964 Fraser et al. suggested that these two apparently different disorders could share a genetic background, it was not until 1997 that RWS and JLNS were proven to be allelic diseases caused by mutations of ion channel genes KCNQ1 and minK (Wang et al. 1996; Neyroud et al. 1997; Schulze-Bahr et al. 1997; Splawski et al. 1997a; Tyson et al. 1997). Today, long QT syndrome is known to be caused by mutations at least in five genes encoding ion channels and is classified based on the underlying genotype (LQT1-LQT6, JLN1-JLN2) (Table 1).

2.3.2 Major clinical findings
The long QT syndrome is characterized by delayed ventricular repolarization, detected as a prolonged QT interval in electrocardiogram (ECG) (Schwartz et al. 1975). The abnormal prolongation of the repolarization phase in the cardiac action potential results from defective potassium or sodium channel function, and causes an increased risk for ventricular tachyarrhythmias, particularly torsade de pointes (twisting of the points). The symptoms include syncopal attacks and sudden death due to cardiac arrhythmias. Typically, initial symptoms occur during adolescence in relation to physical or emotional stress. In addition to cardiac manifestation, JLNS is characterized by congenital sensorineural deafness. Cardiac symptoms tend to occur in childhood and be more severe than in RWS (Moss 1985).

Large phenotypic variation is detected in LQTS patients, even within the same family. Some correlation is observed between the cardiac phenotype and LQTS genotype: LQT1 patients appear to have symptoms related to physical activity, especially swimming (Ackerman et al. 1999; Moss et al. 1999; Schwartz et al. 2001) whereas a specific trigger for symptoms is often loud noise in LQT2 patients (Moss et al. 1999; Wilde et al. 1999; Schwartz et al. 2001) and sleep in LQT3 patients (Schwartz et al. 1995, 2001). The risk and frequency of cardiac events are genotype-dependent, with the LQT1 and LQT2 genotypes having the greatest risk, and the LQT1 group, the greatest frequency of symptoms (Zareba et al. 1998).
Currently, most symptomatic LQTS patients are treated efficiently with beta-adrenergic antagonists (Moss et al. 1985, 2000). However, those individuals who have been symptomatic before initiating β-blocker therapy have a high risk for recurrent cardiac events (32% within five years) (Moss et al. 2000). LQT1 and LQT2 patients appear to respond to β-blockers effectively, whereas in LQT3 patients the same therapy has no obvious beneficial effect (Moss et al. 2000). A pacemaker, an implantable cardiac defibrillator or a left cervicothoracic sympathetic ganglionectomy alone or in addition to β-blockers should be considered in individuals who have syncopes despite β-blocker therapy (Chiang and Roden 2000; Moss et al. 2000).

Recent results have suggested that the different LQTS types appear to benefit from different treatments. Thus, LQT3 patients with defective Na+ channels may benefit from sodium channel-blocker mexiletine (Schwartz et al. 1995), while an increased extracellular potassium level and potassium channel openers may be beneficial in LQT2 patients (Compton et al. 1996) and LQT1 patients (Shimizu et al. 1998a), respectively.

### 2.3.3 Diagnostic criteria

Diagnostic criteria of LQTS include a prolonged QT interval corrected for heart rate (QTc) and either documented torsade de pointes, syncope or family history of syncopal spells, sudden death or LQTS (Table 2) (Schwartz et al. 1985, 1993). In addition, post-exercise ECG has been suggested as an auxiliary diagnostic tool for LQT1 and LQT2 patients (Swan et al. 1999). The mean QT intervals for different heart rates in LQT1, LQT2 and control groups differed significantly from each other during recovery from exercise, whereas during exercise this difference was not significant. Recently, genetic diagnosis has become increasingly important, and molecular genetic analysis is recommended for all family members of a genotyped proband (Priori et al. 1999b; Chiang and Roden 2000). However, the use of DNA diagnostics is still restricted because of the high degree of genetic heterogeneity (Priori et al. 1999b).
Table 2: Diagnostic criteria of LQTS\(^1\)

<table>
<thead>
<tr>
<th>Electrocardiographic findings(^2)</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTc(^3)</td>
<td></td>
</tr>
<tr>
<td>&gt;480 ms</td>
<td>3</td>
</tr>
<tr>
<td>460-470 ms</td>
<td>2</td>
</tr>
<tr>
<td>450 ms (men)</td>
<td>1</td>
</tr>
<tr>
<td>Torsade de pointes(^4)</td>
<td>2</td>
</tr>
<tr>
<td>T-wave alternans</td>
<td>1</td>
</tr>
<tr>
<td>Notched T-wave in three leads</td>
<td>1</td>
</tr>
<tr>
<td>Low heart rate for age(^5)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Clinical history

<table>
<thead>
<tr>
<th>Syncope(^4)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>with stress</td>
<td>2</td>
</tr>
<tr>
<td>without stress</td>
<td>1</td>
</tr>
<tr>
<td>Congenital deafness</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Family history\(^6\)

<table>
<thead>
<tr>
<th>Family members with definite LQTS</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexplained sudden cardiac death before age 30 among immediate family members</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Scoring: <1 point = low probability, 2-3 points = intermediate probability, >4 points = high probability of LQTS.

\(^1\)According to Schwartz et al. 1993. \(^2\)In the absence of QT interval-prolonging drugs or disorders. \(^3\)QTc calculated by Bazett's formula (Bazett 1920). \(^4\)Mutually exclusive. \(^5\)Resting heart rate below the second percentile for age. \(^6\)Definite LQTS is defined by score >4.

2.3.4 Prevalence of LQTS

While the actual prevalence of RWS remains unknown, an estimation of 1:10 000 has been suggested (Ackerman 1998; Vincent 1998). The disease has long been underdiagnosed as well as commonly misdiagnosed, which could indicate a higher than expected prevalence (Vincent 1998; Chiang and Roden 2000). The Romano-Ward syndrome is much more common than the allelic Jervell and Lange-Nielsen syndrome. Detailed population studies in Norway have demonstrated a prevalence of 1:55 000 to 1:200 000 for JLNS in their population (Tranebjaerg et al. 1999). Estimates from 1:170 000 to 1:1 000 000 have earlier been suggested in the British Isles (Fraser et al. 1964).

2.3.5 Molecular genetic findings in long QT syndromes

In 1991, Mark Keating and colleagues reported a genetic linkage of RWS to the Harvey ras-1 locus in the short arm of chromosome 11 (Keating et al. 1991). Since then, molecular genetic studies on LQTS have been intense. The RWS locus was refined to 11p15.5, adjacent to the
Harvey ras-1 gene, and the underlying disease gene, \textit{KVLQT1} (now called \textit{KCNQ1}), was identified by positional cloning in 1996 (Wang et al. 1996a). Today, mutations in five different genes are known to cause RWS, and homozygous mutations in two of them can cause JLNS (Table 1). All recognized genes encode cardiac ion channel alpha or beta subunits (Table 3). One additional locus has been linked to RWS, and evidence supports the existence of still unknown genes underlying LQTS (Table 1).

<table>
<thead>
<tr>
<th>Table 3: Characteristics and frequency of different LQTS types.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS type</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>LQT1</td>
</tr>
<tr>
<td>LQT2</td>
</tr>
<tr>
<td>LQT3</td>
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<tr>
<td>LQT4</td>
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<tr>
<td>LQT5</td>
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<tr>
<td>LQT6</td>
</tr>
<tr>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>JLN1</td>
</tr>
<tr>
<td>JLN2</td>
</tr>
</tbody>
</table>

1 in mixed US and European samples of LQTS patients, according to Splawski et al. 2000

The \textit{KCNQ1} gene consists of 16 coding exons and the corresponding genomic DNA is about 400 kb (Splawski et al. 1998). In addition, three exons exist that are included in differently spliced forms (Lee et al. 1997; Neyroud et al. 1999). Several isoforms of \textit{KCNQ1} gene have been detected, but only isoform 1 appears functional (Neyroud et al. 1999). Some confusion was present in nucleotide numbering due to the lack of knowledge of the entire nucleotide sequence, but at the moment, isoform 1 is used as the reference sequence. The gene product is an \(\alpha\)-subunit of the slowly activated delayed rectifier potassium channel \(I_{Ks}\) that is expressed in several tissues, mainly in the heart, kidney, lung, placenta and the stria vascularis of the inner ear (Wang et al. 1996a; Neyroud et al. 1997; Yang et al. 1997). Mutations in the \textit{KCNQ1} gene are the most common cause of LQTS (Table 3): heterozygous mutations result in LQT1 (Wang et al. 1996), and homozygous or compound heterozygous mutations, in JLN1 (Neyroud et al. 1997; Splawski et al. 1997a). However, some homozygous or compound heterozygous \textit{KCNQ1} carriers appear to
have normal hearing (Priori et al. 1998; Larsen et al. 1999). To date, tens of mutations world-wide have been detected in this gene, and as a rule, each mutation is unique for one family only (Splawski et al. 2000). In addition, two mutational hot spots have been suggested to occur in the KCNQ1 gene (Wang et al. 1996a; Murray et al. 1999). Most of the mutations are missense mutations (79%) and are located in the transmembrane domains of the channel (44%) or are intracellular (44%) (Splawski et al. 2000).

The KCNQ1 gene is imprinted implying that only the maternal allele is expressed in the majority of tissues (Lee et al. 1997). However, expression of KCNQ1 in the heart is biallelic. Imprinting is a mechanism of developmental regulation, and when disrupted, often leads to cancer. Chromosomal rearrangements within the KCNQ1 gene result in Beckwith-Wiedemann syndrome (BWS) (Lee et al. 1997) with fetal overgrowth and childhood tumours (reviewed in Reik and Maher 1997). However, the phenotype of a KCNQ1 knockout mouse suggests that the KCNQ1 gene itself is not involved in BWS (Lee et al. 2000).

The HERG gene (KCNH2) is related to the Drosophila ion channel gene EAG (ether á-go-go gene), and belongs to the ERG (ether á-go-go related gene) subfamily (Warmke and Ganetzky 1994). It was originally cloned from a human hippocampal cDNA library by homology to EAG (Warmke and Ganetzky 1994). The genomic DNA covers about 19 kb in chromosome 7q35-q36 (Itoh et al. 1998). The HERG cDNA consists of 15 exons (Itoh et al. 1998) and encodes a rapidly activating delayed rectifier potassium channel alpha subunit (I_K) expressed in the heart, brain, liver and pancreas (Curran et al. 1995). Heterozygosity for HERG mutations causes the LQT2-type of LQTS (Curran et al. 1995), and the first homozygous patients with a severe cardiac phenotype were recently characterized (Study III, Hoortnje et al. 1999). The majority of mutations in the HERG gene are intracellular (60%) (Splawski et al. 2000), with missense mutations being most common (65%), but some frameshift mutations also existing (20%).

MinK (KCNE1) and MiRPI (KCNE2) are small genes located in the same chromosomal locus (21q22.1-q22.2) in opposite frames. Their open reading frames are included in a single exon (Abbott et al. 1999). Both encode a small peptide (129 and 123 amino acids, respectively) with only one transmembrane domain (Takumi et al. 1988; Murai et al. 1989; Abbott et al. 1999).
MinK co-assembles with the KCNQ1 protein to form functional \( I_{Ks} \) in the heart and *stria vascularis* of inner ear (Barhanin et al. 1996; Sanguinetti et al. 1996a). It has also been shown to co-assemble with HERG *in vitro*, but the functional impact of this interaction remains obscure (McDonald et al. 1997). As for *KCNQ1*, heterozygous mutations in *minK* cause RWS (LQT5) (Splaowski et al. 1997b), while homozygous mutations result in JLNS (JLN2) (Schulze-Bahr et al. 1997; Tyson et al. 1997). However, mutations in the *minK* gene are a relatively rare cause of LQTS (Table 3). Mutations affect both the extracellular and intracellular regions as well as the transmembrane segment of the minK protein.

Recently, a novel gene encoding a minK-related peptide (*MiRP1*) was cloned and shown to cause LQTS, subsequently classified as type LQT6 (Abbott et al. 1999). MiRP1 has been shown to co-assemble with HERG and to produce *in vitro* currents closely resembling native \( I_{Kr} \) (Abbott et al. 1999). The *MiRP1* gene is expressed only in the heart. Thus far, only three mutations have been detected in the *MiRP1* gene, and these are located in the extracellular and transmembrane domains of the protein (Splaowski et al. 2000). Like *minK*, *MiRP1* constitutes a rare cause of LQTS (Table 3).

The *SCN5A* gene was cloned in 1992 (Gellens et al. 1992), and the first LQTS-causing mutations in this gene were characterized three years later (Wang et al. 1995). *SCN5A* is a large gene comprising 28 coding exons and spanning 80 kb on chromosome 3p21 (Wang et al. 1996b). *SCN5A* encodes a voltage-gated cardiac sodium channel alpha subunit that is expressed exclusively in the human heart (Gellens et al. 1992). Because of its large size, incomplete mutation screening has been reported, and 14 *SCN5A* mutations are recognized to date (Splaowski et al. 2000). They modify the third or fourth domain of the channel and include both missense (64%) and frameshift (36%) mutations (Splaowski et al. 2000).

Other LQTS causative genes very likely exist. A linkage to chromosome 4q25-q27 was established in one French family with long QT intervals and specific arrhythmogenic features (LQT4) (Schott et al. 1995), but the underlying gene is yet to be determined. Several families show no linkage to known LQTS genes, and in general, genetic screening has revealed mutations in only half of the cases studied (Ackerman 1998; Splaowski et al. 2000). No promoter or other
regulatory sequences of any of the ion channel genes causing LQTS have been characterized, but it is possible that mutations of these sequences cause one part of LQTS.

2.3.6 Molecular pathogenesis

Mutations in the genes encoding sodium or delayed rectifier potassium channels involved in cardiac repolarization cause long QT syndromes (Table 3). Two distinct mechanisms may lead to prolongation of the action potential: defective potassium channel subunits result in loss of K+ current (Sanguinetti et al. 1995, 1996b; Chouabe et al. 1997; Shalaby et al. 1997) and activating mutations of the sodium channel cause a gain in Na+ current (Bennett et al. 1995).

Mutations of the potassium channel genes causing RWS often have a dominant negative effect on channel function when present in a heterozygous form (Sanguinetti et al. 1996b; Chouabe et al. 1997; Shalaby et al. 1997; Nakajima et al. 1998). The mature channel consists of four identical alpha subunits that are randomly assembled in the endoplasmic reticulum. Even one defective part of the tetramer has been proposed to alter or destroy normal channel function by decreasing potassium current. This is called dominant negative suppression. Recently, HERG A561V mutant subunits associated with wild-type subunits were shown to cause misfolding of the channel tetramer, leading to rapid degradation and proteolysis of the complex (Kagan et al. 2000). The existence of a dominant negative effect on potassium current has also been demonstrated in vivo in transgenic mice expressing Kv1.1 N-terminal fragment (London et al. 1998) or HERG G628S mutation (Babij et al. 1998). Mutations causing JLNS are more often small deletions or insertions leading to frameshift and truncation of the protein (Tranebjærg et al. 1999; Tyson et al. 2000). Mutations resulting in truncated protein often cause loss of function rather than a dominant negative effect (Sanguinetti et al. 1996b; Chouabe et al. 1997; Mohammad-Panah et al. 1999). This is largely explained by the inability of a truncated protein to co-assemble with other subunits (Schmitt et al. 2000). Besides defective co-assembly of alpha or beta subunits, improper post-translational modification or protein folding, rapid degradation of mutants or deficient protein trafficking can cause loss of channel function (Franqueza et al. 1999; Furutani et al. 1999).
In summary, prolongation of the cardiac QT interval can obviously occur through several different mechanisms. Delayed repolarization may lead to early after depolarizations, ventricular tachycardia (especially torsade de pointes) and even ventricular fibrillation and sudden death (Viskin 1999).

2.3.7 Acquired long QT syndrome
Several pharmacological agents, such as anti-arrhythmic and antimicrobial drugs, and metabolic abnormalities, such as hypokalemia, may prolong the QT interval (Viskin 1999). This form of QT interval prolongation is often called acquired LQTS to distinguish it from the inherited form. However, gene polymorphisms or mild mutations of ion channel genes have been suggested to predispose to drug-induced LQTS, as well. Recent studies have shown that mutations and polymorphisms of the MirP1 gene in particular are often associated with drug-induced arrhythmias, both in congenital and acquired LQTS patients (Abbott et al. 1999; Sesti et al. 2000). Various pharmacological agents are known to block the rapid delayed rectifier potassium channel IKr, encoded by the HERG gene (Viskin 1999). Mitcheson et al. (2000) suggested that certain HERG-specific amino acids in the S6 transmembrane region of the HERG protein could interact with different drugs including antihistamine terfenadine and gastrointestinal drug cisapride. Indeed, the common denominator behind both congenital and acquired LQTS appears to be the block of potassium current active in cardiac repolarization.

2.4. The population history of Finns
The population history of Finns is characterized by a small number of founding individuals, a relatively recent and rapid population growth and prolonged isolation. The first inhabitants apparently landed in Finland after the last ice age about 9000 years ago (Huurre 1990). According to a dual-origin theory supported by ancestral Y chromosomal haplotypes, an early immigration wave took place about 4000 years ago from the eastern Uralic region (Kittles et al. 1998). The majority of the current population descends from people moving to Finland from the south over the Gulf of Finland and possibly from the Swedish border some 2000 years ago (Nevanlinna 1972; Kittles et al. 1998; Peltonen et al. 1999). The inhabittance was restricted to coastal areas with little internal migration until the 1500s. A relative isolation by distance, culture, religion and language created small subisolates separated from each other. Famine,
epidemics and wars resulted in great reductions to population size, causing natural bottlenecks that can still be observed today in the decrease in genetic heterogeneity of mitochondrial and Y chromosom al DNA (Sajantila et al. 1996). A major population expansion took place in the 18th century, leading to a rapid population increase from 250 000 to five million inhabitants in less than 300 years (Peltonen et al. 1995).

The concept of the Finnish disease heritage, introduced in the 1970s, refers to diseases that are more common in Finland than in other parts of the world, and also to world-wide Mendelian diseases that are nearly absent in Finland (Norio et al. 1973). The majority of these diseases are autosomal recessive and caused by one major mutation (recent review: Peltonen et al. 1999). Rare autosomal dominant diseases, such as familial amyloidosis of the Finnish type caused by a single founder mutation (Levy et al. 1990, Paunio et al. 1992), can also be enriched in isolated populations (de la Chapelle 1993). The unique population history is further reflected in more common inherited diseases such as autosomal dominant familial hypercholesterolemia (FH). In Finland, a few major mutations account for the majority of FH cases, and even more strikingly, in a genetic isolate of North Karelia, a single deletion underlies 90% of FH cases (Koivisto et al. 1992).

Founder mutations of rare inherited diseases as well as some other monogenic disorders clearly occur frequently in Finland. Thus, it was reasonable to ask whether specific arrhythmic syndromes were enriched in the Finnish population and whether the Finns harboured specific mutations in genes regulating cardiac repolarization.

Before this study, three cardiac ion channel genes, namely KCNQ1, HERG and SCN5A, had been recognized to underlie long QT syndrome, with the first mutations of these genes being characterized. Three Finnish families with LQTS had been genetically linked to 11p15.5, the LQT1 locus (Kainulainen et al. 1995).
3. AIMS OF THE STUDY

- To characterize gene defects underlying long QT syndrome in the Finnish population and search for population-prevalent mutations in the cardiac ion channel genes.
- To compare the genotype and phenotype of LQTS patients.
- To genetically map the locus of familial polymorphic ventricular tachycardia and to initiate studies on the molecular pathogenesis of this disorder.
4. MATERIALS AND METHODS

4.1. Patients and controls
In Studies I-IV, apparently unrelated probands of Finnish origin who had clinically established autosomal dominant form of long QT syndrome were examined (Study I: n=44, II:114, III:88, IV:30). In addition, three patients with a diagnosis of an autosomal recessive form of LQTS were included in Study II. In families where a gene defect was detected, all available relatives were studied. Altogether, 73 subjects were studied for the presence of a D317N mutation (Study I), seven subjects for T311I (I), 25 subjects for G269S (unpublished), 13 subjects for S277del (unpublished), 739 subjects for KCNQ1-Fin (II), seven subjects for Y171X (II), 78 subjects for HERG-Fin (III) and 15 subjects for V1667I (IV).

Diagnostic criteria of Romano-Ward syndrome included a prolonged rate-adjusted QT (QTc) interval (>440 ms) and at least one symptomatic episode (syncope or tachyarrhythmia). In family members, diagnosis of RWS additionally included a family history of LQTS. Diagnosis of JLNS required occurrence of prolonged QT interval along with congenital deafness. Detailed questionnaires on medical histories and present symptoms were requested to be filled out by all studied individuals. Each participant gave an informed consent. Apparently healthy adult individuals (either blood donors from the Helsinki area or employees from a local factory) were used as controls (Study I: n=45, II: n=200, III: n=100, IV: n=50). Their female: male ratio was 50:50. This work was approved by the Ethics Review Committee of the Department of Medicine.

Study V examined two families with the phenotype of polymorphic ventricular tachycardia. Affected individuals were characterized as having frequent premature ventricular complexes or ventricular tachycardia during the exercise stress test. Only subjects aged over 18 years and with negative ECG findings were assessed to be unaffected. Individuals under the age of 30 who died suddenly for an unknown reason were considered affected. Nine affected and 16 unaffected subjects from one family, and four affected and six unaffected subjects from the other family fulfilled the above-mentioned criteria and were included in the linkage study.
4.2. DNA extraction
Genomic DNA was isolated from peripheral venous blood samples using a standard phenol extraction method (Blin and Stafford 1976). For some deceased cases, a paraffin-embedded sample from liver tissue was available, and a modified protocol was applied (Díaz-Cano and Brady 1997).

4.3. Polymerase chain reaction (PCR)
PCR (Mullis et al. 1986) was carried out in varying conditions, described in detail in Studies I-V. When possible, intronic primers were used for mutation screening to cover the whole coding sequence and exon-intron boundaries. For genotyping, 5’prime primers were fluorescently labeled, and for solid-phase sequencing, they were biotinylated.

4.4. Mutation analysis in LQTS families
The SSCP technique was applied for mutation screening essentially as described in Orita et al. (1989). PCR amplicons were denatured and run on a 6% bisacrylamide/acrylamide gel containing 10% glycerol in a 1 x tris-borate buffer for 6 h at 35 W or for 16 h at 10 W. In each sample showing an aberrant conformation on SSCP gel DNA sequencing was carried out.

4.4.2. DNA sequencing
Prior to sequencing, PCR products were purified enzymatically (SAPI and exonuclease I) or using the Quiaclick PCR purification kit (Quiagen, Chatsworth, CA). DNA sequencing was carried out using the dyeterminator cycle-sequencing procedure and the ABI Prism 377 automatic DNA sequencer (PE Biosystems, Foster City, CA). In Study I, a solid-phase sequencing method was applied essentially as described in Syyänen et al. (1989), using the T7 Sequenase version 2.0 enzyme (US Biochemicals, Cleveland, OH).

4.4.3. Specific mutation analyses (I-IV, unpublished)
The DNA samples of family members and healthy controls were studied using an appropriate method for each mutation. Assays based on specific restriction enzyme digestion were designed to separate wild-type and mutant alleles (KCNQ1-Fin, Y171X, D317N). An application of
primer-induced restriction analysis (PIRA) (Jacobson and Moskovits 1991) was used when a suitable restriction site was lacking in the PCR-amplified normal or mutant allele (HERG-Fin, T311I, G269S). In this assay, an artificial restriction site was deliberately introduced into the DNA sequence using a novel primer including a mismatch. The presence of the S277del deletion was demonstrated by heteroduplex analysis (Glavac and Dean 1995). A mixture of PCR product and stop solution (95% formamide, 20 mM EDTA, 0.05% xylene cyanole, 0.05% bromophenol blue) was denatured and separated on a 12% polyacrylamide gel. The presence of V1667I substitution was studied by direct DNA sequencing.

4.4.4. Haplotype analysis (II, III)

To study genetic relatedness between families, haplotype analysis with highly polymorphic microsatellite markers close to the locus of interest was performed. Haplotypes were constructed manually.

4.4.5. Functional studies (II, III)

Properties of KCNQ1-Fin and HERG-Fin mutations were studied in vitro. The expression vectors containing the wild-type KCNQ1 (AF000571) and minK (L33815) cDNAs were the kind gifts of Dr. Jacques Barhanin, while HERG (U04270) cDNA was kindly provided by Dr. Gail Robertson. The cDNAs for KCNQ1 and minK were cloned to a bicistronic vector (pIRES-CD8) expressing either KCNQ1 and CD8 antigen or minK and CD8 antigen. The cDNA for HERG was cloned to a pcNA3 expression vector. The in vitro mutagenesis was performed using the QuikChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA) essentially according to manufacturer’s instructions.

Transfections for transient in vitro expression of KCNQ1 were made using the DEAE-dextran precipitate method (reviewed in Schenborn and Goiffon 2000). Transfected cells were detected as CD8-expressing cells with magnetic beads (Dynal, Oslo, Norway). Whole-cell membrane currents (Ogden and Standfield 1993) were measured and analysed using a Biologic RK 400x patch-clamp amplifier and pCLAMP software (Axon Instruments Inc., Foster City, CA). Transient transfections of HERG constructs were made using the Effectene™ Transfection Reagent (Qiagen, Valencia, CA). A green fluorescent protein plasmid was transfected with wild-
type and mutant constructs to identify the transfected cells. Whole-cell membrane currents were measured using an EPC-9 amplifier and Pulse/Pulsefit software (HEKA, Lambrecht, Germany).

4.4.6. Statistical evaluation (I-IV)

The Student’s t-test or analysis of variance (ANOVA) was used to compare different variables, such as mean QTc interval, between groups. Data are given as the mean ± SD (standard deviation), unless otherwise indicated. A p-value < 0.05 was considered statistically significant.

4.5. Genetic mapping of a novel arrhythmic disorder (V, unpublished)

4.5.1. Genotyping

Highly polymorphic di-, tri- or tetranucleotide microsatellite markers were used in genotyping. Markers for a genome-wide scan were obtained from the Cooperative Human Linkage Center (http://www.chlc.org) and from Généthon (Dib et al. 1996). The 31 markers used for scanning chromosome 1 were located approximately every 10 cM, covering the entire chromosome (283 cM), and had a mean heterozygosity of 0.77. Selection of additional markers was based on information from the Genetic Location Database (Collins et al. 1996) and a radiation hybrid map from the Whitehead Institute (http://www-genome.wi.mit.edu). The amplified fragments were separated using an ABI Prism 377™ automatic DNA sequencer (PE Biosystems, Foster City, CA) and analysed with Genescan analysis software (PE Biosystems). The results were forwarded to Genotyper (PE Biosystems) for precise allele determination.

4.5.2. Linkage and haplotype analyses

In data analysis, autosomal dominant inheritance with 90% penetrance for disease and affected allele frequency of 0.0002 were assumed. Linkage analyses were carried out with MLINK and ILINK options of FASTLINK v. 3.0P package (Cottingham et al. 1993; Schaffer et al. 1994) and GENEHUNTER (Kruglyak et al. 1996). MLINK was used to calculate the two-point lod scores at different recombination fractions, ILINK to define the maximal likelihood estimate of theta and GENEHUNTER to calculate multipoint likelihood for both parametric and non-parametric linkage analyses. Marker allele frequencies were either assumed equal (pairwise analysis) or were estimated from the data (multipoint analysis). Multipoint lod scores were calculated in affected only mode in pedigree 1, while in two-point calculations, the whole pedigree was included.
Power calculations were performed using SIMLINK v. 4.11. Haplotypes were constructed manually.

4.5.3. Candidate gene analysis
An assay based on reverse transcription and PCR was applied to directly sequence the TWIK-1 and ACTN2 genes. Primers amplifying cDNA were designed to cover the whole coding region of TWIK-1 (GenBank U33632) and ACTN2 (GenBank M86406). Peripheral lymphocytes were isolated from two affected and two unaffected individuals using Lymphoprep (Nycomed Pharma, Oslo, Norway). RNA was extracted with RNAzo+B (TEL-TEST, Friendswood, TX) and transcribed to cDNA with SuperScript™II Reverse Transcriptase (Gibco BRL, Life Technologies, Paisley, UK). PCR was performed, first, using the cDNA as a template and, secondly, using the first PCR product as a template ("nested PCR"). The nested PCR product was purified with the Quiagene PCR purification kit (Quiagen, Chatsworth, CA) and directly sequenced with an ABI Prism 377 DNA sequencer.
5. RESULTS

5.1. Finnish founder mutations (II, III)

5.1.1. Identification of two common LQTS mutations

In Study II, two male probands with Jervell and Lange-Nielsen syndrome were screened for mutations in the KCNQ1 gene by direct sequencing (Figure 3). Two novel mutations were revealed: a change from G to A at nucleotide 1766 of exon 14, predicted to result in a substitution of aspartic acid for glycine at position 589 (C-terminus of the protein), and a C to G substitution at nucleotide 513 in exon 2, resulting in a premature stop codon at position 171 (between transmembrane domains S2 and S3) and predicted synthesis of a truncated protein (Figure 4). The proband and his deceased brother were homozygous for the G589D mutation, whereas the other proband was a compound heterozygote, carrying both the G589D missense and Y171X nonsense mutations. Specific genetic assays based on DNA amplification and restriction enzyme digestion were set up to study relatives, other probands with Romano-Ward syndrome and healthy controls. Altogether 15 G589D heterozygotes, including parents of the siblings, were characterized in the first family (Figure 3). In addition, the mutation was present in 33 RWS families. In the compound heterozygous JLNS patient, the Y171X mutation was inherited from his mother and the G589D (designated as KCNQ1-Finn because of its relatively common occurrence) from his father (Figure 3). Neither the KCNQ1-Finn nor Y171X mutation was present in control samples from 200 unrelated healthy Finns.

In Study III, a family consisting of two affected daughters with a severe cardiac phenotype of LQTS but normal hearing, and phenotypically normal parents was studied (Figure 3). Sequence analysis of the HERG gene disclosed a novel nucleotide change (T1655C) predicted to result in substitution of a conserved leucine by serine at amino acid position 552. The L552S mutation modifies the cytoplasmic end of the transmembrane domain S5 of the HERG subunit (Figure 4). It was present in a homozygous state in the two affected daughters as well as in a heterozygous state in their parents and five other family members (Figure 3). In all, this substitution was detected in six apparently unrelated families. The L552S mutation (designated as HERG-Finn) was absent in all 100 control samples of healthy Finns.
Figure 3. Pedigrees of families with KCNQ1-Fin homozygotes (A) or HERG-Fin homozygotes (B). The QTc interval in ms is shown under each symbol. In A) half-filled symbols indicate heterozygous KCNQ1-Fin carriers, filled symbols homozygous KCNQ1-Fin carriers and half-striped symbols heterozygous Y171X carriers. In the upper pedigree two patients homozygous for the KCNQ1-Fin mutation have JLNS (IV/6 and IV/8) while in the lower pedigree a compound heterozygote carrying both the KCNQ1-Fin and Y171X mutation has JLNS (III/7). In B) half-filled symbols indicate heterozygous HERG-Fin carriers and filled symbols homozygous HERG-Fin carriers (III/5 and III/6). The HERG-Fin homozygotes have severe cardiac symptoms, but normal hearing.
Figure 4. A schematic illustration of the distribution of the eight mutations in the KCNQ1, HERG and SCN5A channels described in this work. The missense mutations are shown by black circles, the amino acid deletion (S277del) by a black square and the nonsense mutation (Y171X) by a black triangle.

5.1.2. Functional characteristics of the novel mutations

To characterize the properties of the KCNQ1-Fin (G589D) and HERG-Fin (L552S) substitutions in vitro, each mutation was introduced into a corresponding normal cDNA. The wild-type and mutant constructs were transiently expressed in COS cells and analysed in whole-cell patch-clamp experiments.

When co-expressed with minK, KCNQ1-Fin produced functional channels, but the currents were much smaller than those observed for KCNQ1-wt (Table 4). Detectable KCNQ1-Fin currents
were present in only 17% of the cells, while all cells transfected with KCNQ1-wt, or simultaneously with KCNQ1-wt and KCNQ1-Fin in a 1:1 ratio, displayed robust currents. Furthermore, a remarkable rightward shift was present in the voltage of activation, with mean (± SE) half activation voltages of 11.0 ± 2.2 mV for KCNQ1-wt and 41.3 ± 2.9 mV for KCNQ1-Fin (Table 4). No significant differences existed in the mean current amplitudes, activation voltages or activation time constants between cells expressing KCNQ1-wt alone or KCNQ1-wt together with KCNQ1-Fin. KCNQ1-Fin not contributing substantially to the recorded currents when both the KCNQ1-wt and KCNQ1-Fin constructs were present in the cell shows that no dominant negative suppression is induced by the KCNQ1-Fin mutation (Table 4).

<table>
<thead>
<tr>
<th>Property</th>
<th>KCNQ1-Fin</th>
<th>HERG-Fin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form functional channels</td>
<td>Yes, to some extent</td>
<td>Yes</td>
</tr>
<tr>
<td>Current amplitude</td>
<td>Reduced vs. wildtype</td>
<td>Similar to wildtype</td>
</tr>
<tr>
<td>Voltage of activation</td>
<td>Marked rightward shift</td>
<td>Slight leftward shift</td>
</tr>
<tr>
<td>Special characteristics</td>
<td>No dominant negative effect</td>
<td>Rapid deactivation and activation</td>
</tr>
</tbody>
</table>

The HERG-Fin construct produced functional channels in COS7 cells with robust currents similar in amplitude to those generated by the HERG-wt construct (Table 4). HERG-Fin displayed a marked increase in the rate of activation and deactivation, with mean (± SE) rates of activation of 891 ± 116 ms for HERG-wt and 151 ± 28 ms for HERG-Fin at 0 mV and mean (± SE) rates of deactivation of 623 ± 101 ms for HERG-wt and 120 ± 19 ms for HERG-Fin at 60 mV (Table 4). Moreover, the HERG-Fin was activated at slightly lower voltages than HERG-wt. An increase in the HERG deactivation rate is expected to decrease the HERG channel activity at the end of the cardiac action potential, probably prolonging the repolarization.

5.1.3. Phenotypic expression of the mutants
The KCNQ1-Fin homozygotes had a classical phenotype of JLNS with markedly prolonged QT intervals (QTc 661 ms and 592 ms), cardiac symptoms with early onset and congenital deafness. The brother of the proband had died at the age of nine. The phenotype of the subject who was
The two sisters homozygous for the HERG-Fin mutation had markedly prolonged QT intervals (QTc 677 ms and 513 ms) and ventricular arrhythmias already in early infancy. In addition, the younger child had a 2:1 atrioventricular block as a neonate, and she died at the age of four. In both patients, a disturbance of glucose homeostasis was associated with cardiac events. The siblings were carefully examined, but no other abnormal phenotypic characteristics were detected.

The mean QTc interval of the 316 heterozygous KCNQ1-Fin carriers (460 ± 40 ms, range 390-580) was significantly longer than that of the 423 non-carriers (410 ± 20 ms, range 350-490) (p<0.001) (Table 5). Among the KCNQ1-Fin heterozygotes, women had on average a significantly longer QTc interval (470 ± 32 ms) than men (446 ± 38 ms) (p<0.001). There were altogether 83 (26%) symptomatic heterozygous carriers comprising 52 females and 31 males (Table 5). The mean QTc interval of symptomatic mutation carriers (470 ± 30 ms) was longer than that of asymptomatic carriers (450 ± 30 ms) (p<0.001), and the likelihood of occurrence of symptoms increased with increasing QTc value. In 48 cases (58%), the triggering factor for syncope could unequivocally be related to physical or psychological stress, and 17 (20%) had experienced syncopal spells while swimming. Seven patients (8%) had had a cardiac event while using some sort of medication.

Table 5: Phenotypic characteristics of heterozygous mutation carriers and non-carriers.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Y171X</th>
<th>G269S</th>
<th>S277del</th>
<th>T311I</th>
<th>D317N</th>
<th>KCNQ1-Fin</th>
<th>HERG</th>
<th>SCN5A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>30</td>
<td>316</td>
<td>35</td>
<td>9</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-carriers</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>20</td>
<td>174</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Mean QTc</td>
<td>429±7</td>
<td>430±29</td>
<td>474±32</td>
<td>490±80</td>
<td>484±38</td>
<td>460±40</td>
<td>466±47</td>
<td>450±30</td>
</tr>
<tr>
<td>carriers</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>non-carriers</td>
<td>410±33</td>
<td>404±28</td>
<td>NA</td>
<td>NA</td>
<td>406±27</td>
<td>410±20</td>
<td>412±23</td>
<td>423±22</td>
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<tr>
<td>Number of</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>symptomatic (%)</td>
<td>0</td>
<td>2 (25)</td>
<td>1 (13)</td>
<td>2 (50)</td>
<td>12 (40)</td>
<td>83 (26)</td>
<td>10 (29)</td>
<td>2 (22)</td>
</tr>
</tbody>
</table>

QTc = QT interval corrected for heart rate, NA = not available
The mean QTc value of the 35 heterozygous HERG-Fin carriers (466 ± 47 ms, range 400-620) was significantly higher than that of the 43 non-carriers (412 ± 23 ms, range 370-475) (p<0.001) (Table 5). There was a trend towards a longer mean QTc interval in heterozygous female mutation carriers (471 ± 47 ms) than in male carriers (447 ± 43 ms). Ten (nine females and one male) heterozygous carriers of the HERG-Fin mutation were symptomatic (29%), with the majority (90%) experiencing symptoms at rest (Table 5). Only one individual had had an exercise-induced syncope, but none had one in relation to drug use. The mean QTc interval of the symptomatic mutation carriers (500 ± 59 ms) was significantly longer than that of asymptomatic carriers (452 ± 34 ms) (p<0.001). However, a marked female preponderance was present among the symptomatic carriers.

5.1.4. Prevalence and geographical clustering suggest a founder effect

A total of 34 out of the 114 (30%) unrelated RWS probands were heterozygous for the KCNQ1-Fin mutation. Over 700 family members of the 34 G589D-positive probands had been screened for the presence of this mutation, with identification of 316 heterozygotes. The occurrence of the KCNQ1-Fin mutation in one-third of LQTS patients studied suggested a founder effect. To validate this hypothesis, polymorphic markers adjacent to the KCNQ1 locus were analysed in affected probands to construct possible disease-associated haplotypes. Using markers D11S860, D11S1318 and TH, an underlying common haplotype could indeed be detected in 25 out of the 26 individuals in which the analysis was feasible. The whole haplotype was conserved in half (n=13) of the cases, while in the remainder telomeric and centromeric historical recombinations defined a minimal genetic region of 1500 kb between markers D11S860 and D11S1318. Family data were traced back to the level of the most distant obligate mutation carrier in each family. The birthplaces of the affected ancestors clustered in a zone from North-Karelia to Northern Ostrobothnia (Figure 5). These data suggest that the KCNQ1-Fin mutation was introduced into the population some 500-750 years, or 25-35 generations, ago (Varilo et al. 1996).
5.2. KCNQ1 mutations in single families (I, II, unpublished)

DNA samples from probands of a total of 59 (44 families in Study I and 15 unpublished families) Finnish LQTS families were screened using the SSCP technique with attention to the region encoding the putative transmembrane regions S2-S6 and the pore region of the KCNQ1 gene (Wang et al. 1996a). This analysis revealed four mobility shifts that were confirmed by sequencing. Three novel nucleotide substitutions (D317N [former D188N], T311I [former T182I], G269S) and an amino acid deletion (S277del) were characterized. At the time of
publication of study I the N-terminus of KCNQ1 gene was not sequenced in completion, thus resulting in false codon numbering. This has been corrected later when the whole gene structure was revealed (Chouabe et al. 1997; Yang et al. 1997).

The D317N mutation was identified in 30 individuals of one large family. The T311I mutation was identified in the proband and three family members. The presence of the S277del deletion was demonstrated in seven persons, and the G269S mutation in seven relatives in addition to the proband himself. None of these mutations were found in 50 apparently healthy unrelated random controls, nor in other families, except the index families. All mutations lie in highly conserved regions: the D317N and T311I substitutions in the pore region and the S277del and G269S mutations in the S5 transmembrane domain of the KCNQ1 subunit (Figure 4).

The mean QTc in D317N carriers (484 ± 38 ms, range 432-615) was significantly higher than the corresponding value in non-carriers (406 ± 27 ms, 348-480) (p<0.001) (Table 5). A syncopal attack related to exercise or anxiety was experienced by 40% of the documented or inferred D317N carriers, and by two (50%) out of four T311I carriers (mean QTc 490 ± 80 ms, range 438-629) (Table 5). None of the subjects had had symptoms during sleep. Patients with the S277del deletion had markedly prolonged QTc intervals (mean QTc 474 ± 32 ms, range 442-515) but were asymptomatic, with the exception of the propositus of the family, who had neonatal flabbiness (Table 5). The QT intervals of patients with the G269S mutation appeared to be normal or only marginally prolonged (mean QTc 430 ± 29 ms, range 385-464), however, a syncopal spell had been experienced by two (25%) out of eight carriers, one of whom had succumbed (Table 5). This case was the index patient, a 12-year-old boy, who died suddenly while playing football. Two years earlier, he had lost his consciousness while swimming but the diagnosis was missed.

The Y171X nonsense mutation detected in study II was present in heterozygous state in four family members in addition to the compound heterozygous JLNS patient. The Y171X heterozygotes had normal to marginally prolonged QTc intervals (mean QTc 429 ± 7 ms, range 419-436 ms) and no reported symptoms. The location of the Y171X mutation corresponds to the first codon after the S2 transmembrane domain (Figure 4). The truncated Y171X subunit is not
expected to be able to form functional channels. Clinical data are in accordance with this assumption: similar phenotypes were observed in all three JLNS patients, whether homozygotes for the KCNQ1-Fin or compound heterozygotes for the KCNQ1-Fin and Y171X mutations, and individuals heterozygous for the Y171X mutation were asymptomatic.

5.3. LQT3 and drug-induced arrhythmia (IV)

Mutational analysis using direct sequencing of the SCN5A gene was performed in 30 probands with long QT syndrome and an as yet unestablished genotype. Because the previously reported mutations appeared to cluster in exons 23, 26 and 28 of the SCN5A gene (Splatzki et al. 2000), these exons were selected as targets for initial screening. In one sample, a nucleotide transition, G4999A, predicted to substitute isoleucine for valine at codon 1667, was detected. The V1667I mutation is located in the S5 region of the fourth domain of the sodium channel in a highly conserved region (Figure 4).

The V1667I mutation was present in nine members of the index family but was absent in the remaining probands and in 50 healthy controls. Two of the mutation carriers were symptomatic: both the proband, a 16-year-old boy with clinically established LQTS, and his 40-year-old mother had experienced documented torsade de pointes during therapy with the antimalarial drug halofantrine. In both patients, regular doses of halofantrine resulted in recurrent syncopal spells within 24 hours of administration. Compared to basal values, halofantrine prolonged the QTc interval from 500 to 740 ms and from 460 to 580 ms, respectively, with transient Mobitz II-type heart block in the son. The mean QTc value of all heterozygotes was 450 ± 30 ms (Table 5).

5.4. Genetic assignment of familial polymorphic ventricular tachycardia locus to chromosome 1q42-q43 (V, unpublished)

5.4.1. Genetic mapping of the disease locus

As an initial attempt to localize the gene defect underlying familial polymorphic ventricular tachycardia, the loci linked to the different forms of long QT syndrome were screened in one large family. Highly polymorphic microsatellite markers in 11p15.5 (KCNQ1), 7q35-q36 (HERG), 3p21-p24 (SCN5A) and 4q25-27 were tested, but linkage of the disease phenotype to
LQTS loci could not be demonstrated in any case. In addition, the whole coding region of the minK gene was sequenced, but no mutations were found.

After exclusion of the currently known LQTS loci, a genome-wide scan to localize the causative gene was initiated. Highly polymorphic microsatellite markers, located approximately every 10 cM, were used in genotyping the 25 (nine affected and 16 unaffected) members of the large family. The analysis started with chromosome 1 and, upon demonstration of significantly positive lod scores, the scanning was not continued further. A promising signal was achieved with the marker D1S179. To determine the positive region more precisely, a denser map was made by selecting highly polymorphic markers both centromeric (D1S2800) and telomeric (D1S235, ACTN2, D1S2680, D1S2670, AFM214Xe11, D1S184) to D1S179. A maximum two-point lod score (4.13, θ = 0) was achieved with the marker D1S179, and a maximum multipoint lod score of 2.98 (NPL score of 22.6, p-value of 0.0005) was given by marker D1S2680 in GENEHUNTER analysis. The same eight markers were tested similarly in the second family, also revealing a linkage (maximal lod of 1.64 in both pairwise and multipoint analysis). When the results from both pedigrees were combined, a maximal pairwise lod score of 4.74 (θ = 0) was revealed with marker D1S2670, and a maximal multipoint lod score of 4.62, NPL score of 17.10 and p-value of 0.0005 with marker D1S2680 were achieved. A tight linkage was also demonstrated with the marker ACTN2, which is a CA repeat within the α-actinin gene and has a heterozygosity index of 0.44 (Beggs et al. 1992a). A recombination event between markers D1S2800 and D1S179 was observed in one unaffected individual in the large pedigree and between markers D1S2670 and AFM214Xe11 in one unaffected subject in both pedigrees, which limited the positive region telomeric to D1S2800 but centromeric to AFM214Xe11 (Figure 6).

The haplotypes shared by the affected individuals differed from each other in different pedigrees: in one pedigree it was 10-1-1-1-4 and in the other 6-1-1-5-3 (Figure 6). One clinically unaffected subject (aged 30 years) had inherited the affected haplotype from his affected mother. This suggests an incomplete penetrance of the disease. In conclusion, the locus for FPVT was genetically mapped to the telomere of chromosome 1 (1q42-43), between markers D1S2800 and AFM214Xe11, to a region of approximately 9 cM.
<table>
<thead>
<tr>
<th>cen</th>
<th>D1S2800</th>
<th>D1S179</th>
<th>D1S235</th>
<th>ACTN2</th>
<th>D1S2680</th>
<th>D1S2670</th>
<th>AFM214XE11</th>
<th>D1S184</th>
<th>tel</th>
<th>status</th>
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<td>unaff</td>
<td>1</td>
<td>2</td>
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</tbody>
</table>

**Figure 6:** Haplotypes of affected and unaffected subjects in pedigrees 1 and 2. The box with solid line shows the haplotype in affected individuals and that with dotted line the possible segment of affected haplotype seen in unaffected individuals. The affected haplotype 10-1-1-1-4 was also seen in one unaffected individual. Markers are shown in their relative order from centromere to telomere of the long arm of chromosome 1. aff = affected, unaff = unaffected, n = number of individuals with the specific haplotype, PED = pedigree

### 5.4.2. A study of candidate genes in the critical region

The chromosomal area 1q42-q43 contains several putative candidate genes. *TWIK-1* (Tandem of P domains in a Weak Inward rectifying K+ channel) is supposed to be responsible for background potassium current in several tissues (Lesage et al. 1996a,b). *ACTN2* codes for α-actinin, which associates with actin in microfilaments in the heart and is the major component of Z-discs in cardiac muscle (Beggs et al. 1992b). The entire coding region of the *TWIK-1* gene and the majority of that of the *ACTN2* gene were sequenced from patient cDNA, but no mutations or polymorphisms were identified.
6. DISCUSSION

6.1. Molecular genetics of LQTS in Finland (I-IV, unpublished)

Altogether six *KCNQ1* mutations, nine *HERG* mutations and one *SCN5A* mutation have been identified in Finland to date (Table 6). The two apparent founder mutations *KCNQ1*-Fin and *HERG*-Fin together account for over one-third of LQTS in Finland, with the prevalence of *KCNQ1*-Fin alone being 30% (Figure 7). The situation is exceptional when compared with other countries, in which each mutation is typically present in a single family only (Splawski et al. 2000). Previously, a LQTS founder mutation has been suggested to exist in South-Africa and a JLNS founder mutation in Norway, where five families and seven families, respectively, shared an identical haplotype around the *KCNQ1* locus (de Jager et al. 1996; Tranebjærg et al. 1999). The high frequency of the *KCNQ1*-Fin mutation in the Finnish LQTS probands may be attributed to the relatively benign nature of the mutation as well as to the population history of the Finns. It would be interesting to find out whether the *KCNQ1*-Fin mutation exists in other countries as well. According to a recent survey of LQTS mutations in 262 North-American and European families, no carriers of the *KCNQ1* G589D mutation were disclosed (Splawski et al. 2000).

Because of the highly prevalent *KCNQ1*-Fin mutation, the majority of Finnish LQTS families appear to represent the LQT1 type of disease (46/62; 74%) (Table 6, Figure 7). Mutations of the *HERG* gene constitute the second most common cause of LQTS in Finland. So far, no mutations have been identified in genes encoding minimal potassium channels minK and MiRP1 in Finnish families. The proportion of different LQTS types in Finland appears similar to those in other European countries and in the United States (Splawski et al. 2000).

In accordance with other surveys (Splawski et al. 2000), most gene defects characterized thus far are missense mutations (75%) and located in transmembrane regions (63%) of the protein (Table 6). However, because the emphasis in the present study and in many other studies has been on transmembrane domains, results may have been biased. The promoter and regulator sequences of LQTS genes are currently unknown and may contain undetected mutations. Methods used, including SSCP analysis and direct sequencing of exons, may also have missed certain types of mutations such as large deletions or insertions.
Table 6: Occurrence of LQTS mutations in Finland.\(^1\)

<table>
<thead>
<tr>
<th><strong>KCNQ1</strong> gene</th>
<th>Coding effect</th>
<th>Mutation type</th>
<th>Channel region</th>
<th>Exon</th>
<th>Number of families</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>513C&gt;G</td>
<td>Y171X</td>
<td>nonsense</td>
<td>S2-S3</td>
<td>2</td>
<td>1 [JLNS+RWS]</td>
<td>II</td>
</tr>
<tr>
<td>805G&gt;A</td>
<td>G269S</td>
<td>missense</td>
<td>S5</td>
<td>6</td>
<td>1 (1) RWS</td>
<td>unpublished, Ackerman et al. 1999</td>
</tr>
<tr>
<td>828-830delCTC</td>
<td>S277del</td>
<td>aa deletion</td>
<td>S5</td>
<td>6</td>
<td>1 RWS</td>
<td>unpublished</td>
</tr>
<tr>
<td>932C&gt;T</td>
<td>T311I</td>
<td>missense</td>
<td>pore</td>
<td>7</td>
<td>1 RWS</td>
<td>I</td>
</tr>
<tr>
<td>1766G&gt;A</td>
<td>G589D</td>
<td>missense</td>
<td>C-terminus</td>
<td>14</td>
<td>39 RWS, 2 JLNS+RWS</td>
<td>II</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th><strong>HERG</strong> gene</th>
<th>Coding effect</th>
<th>Mutation type</th>
<th>Channel region</th>
<th>Exon</th>
<th>Number of families</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>453delC</td>
<td>P150fs/14</td>
<td>frameshift</td>
<td>N-terminus</td>
<td>3</td>
<td>1 RWS</td>
<td>Laitinen et al. 2000</td>
</tr>
<tr>
<td>526C&gt;T</td>
<td>R176W</td>
<td>missense</td>
<td>N-terminus</td>
<td>4</td>
<td>1 RWS</td>
<td>Laitinen et al. 2000</td>
</tr>
<tr>
<td>1352C&gt;T</td>
<td>P451L</td>
<td>missense</td>
<td>S2</td>
<td>6</td>
<td>1 RWS</td>
<td>Laitinen et al. 2000</td>
</tr>
<tr>
<td>1631delAG</td>
<td>S543fs/82</td>
<td>frameshift</td>
<td>S5</td>
<td>7</td>
<td>1 RWS</td>
<td>Laitinen et al. 2000</td>
</tr>
<tr>
<td>1655T&gt;C</td>
<td>L552S</td>
<td>missense</td>
<td>S5</td>
<td>7</td>
<td>7 (1) RWS</td>
<td>III, Splawski et al. 2000</td>
</tr>
<tr>
<td>1705T&gt;C</td>
<td>Y569H</td>
<td>missense</td>
<td>S5</td>
<td>7</td>
<td>1 RWS</td>
<td>Laitinen et al. 2000</td>
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<tr>
<td>1750G&gt;A</td>
<td>G584S</td>
<td>missense</td>
<td>S5-pore</td>
<td>7</td>
<td>1 (1) RWS</td>
<td>Laitinen et al. 2000, Splawski et al. 2000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SCN5A</strong> gene</th>
<th>Coding effect</th>
<th>Mutation type</th>
<th>Channel region</th>
<th>Exon</th>
<th>Number of families</th>
<th>Study</th>
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<tr>
<td>4999G&gt;A</td>
<td>V1667I</td>
<td>missense</td>
<td>S5</td>
<td>28</td>
<td>1 RWS</td>
<td>IV</td>
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JLNS = Jervell and Lange-Nielsen syndrome, RWS = Romano-Ward syndrome, aa = amino acid, fs = last amino acid unaffected by a frameshift (number after fs is number of amino acids before termination)\(^1\)

\(^1\)The situation as of 12/2000. No mutations have been detected in minK or MiR1 genes in Finnish patients.

\(^2\)All nucleotide numbering is started from transcription initiation codon.

\(^3\)Number of Finnish families, number of non-Finnish families in parentheses.

\(^4\)Two homozygous patients with severe cardiac phenotype have also been identified.

To date, LQTS-causing mutations have been detected in almost half (62/133) of the Finnish index families, with altogether 544 molecularly defined carriers (as of 12/2000) (Figure 7). Assuming that an approximately equal number of affected individuals is present in the remaining
71 families, a minimum prevalence of 1:5000 for LQTS in the Finnish population of five million people is obtained. This rate is considerably higher than rates (1:10 000) suggested for other populations (Ackerman 1998, Vincent 1998).

![Circle diagram showing the relative proportion of Finnish LQTS mutations. Other mutations include the 14 mutations, excluding KCNQ1-Fin and HERG-Fin, listed in Table 6.](image)

**Figure 7:** The relative proportion of Finnish LQTS mutations. Other mutations include the 14 mutations, excluding KCNQ1-Fin and HERG-Fin, listed in Table 6.

### 6.2. Genotype-phenotype correlations in LQTS

#### 6.2.1 LQTS type and triggers for symptoms (I-III)

Previous studies have suggested that the phenotypes of the three most common LQTS types, LQT1, LQT2 and LQT3, differ from each other. Typically, the triggering factor for symptoms in LQT1 is emotional or physical stress, especially swimming (Ackerman et al. 1999; Moss et al. 1999; Schwartz et al. 2001), while in LQT2, auditory stimuli or rest may provoke symptoms (Moss et al. 1999; Wilde et al. 1999; Schwartz et al. 2001). In LQT3, symptoms typically appear during sleep (Schwartz et al. 1995, 2001). In accordance with earlier findings among LQT1
patients, swimming was an important risk factor for KCNQ1-Fin carriers, being related to symptoms in 20% of cases. All symptomatic patients carrying the KCNQ1-D317N mutation (n=12) had experienced syncope while exercising or during emotional stress. In contrast, the majority (90%) of HERG-Fin carriers had had symptoms at rest, supporting the difference between LQT1 and LQT2 types of disease. Moreover, the occurrence of cardiac events and electrocardiographic characteristics have been suggested to vary between the LQTS types (Zareba et al. 1998; Zhang et al. 2000). LQT3 patients differ from LQT1 and LQT2 patients in occurrence of symptoms: the frequency of cardiac events is lower (18%), but the event is more often lethal, in the LQT3 group (Zareba et al. 1998). However, in this study the number of LQT3 patients characterized was too small to make any reliable comparisons in Finnish patients. Recently, also ST-T-wave patterns were shown to differ between LQT1 and LQT2 patients at least in some international LQTS registry cases (Zhang et al. 2000).

6.2.2 The variable phenotype of KCNQ1-Fin and HERG-Fin carriers (II, III)
The phenotype of heterozygous KCNQ1-Fin and HERG-Fin mutation carriers appeared milder than that of LQT1 or LQT2 patients in general. Approximately one-fourth of the individuals heterozygous for the KCNQ1-Fin mutation were symptomatic, a proportion somewhat lower than the corresponding percentage among carriers of the two mutations (D317N, T311I) of the pore region of the KCNQ1 gene (40-50% in Study I), or in LQT1 patients in general (62% in Zareba et al. 1998). The proportion of symptomatic individuals among heterozygous HERG-Fin carriers was 29%, a figure significantly lower than that estimated earlier for LQT2 patients (46% in Zareba et al. 1998). Likewise, the mean QTc intervals of KCNQ1-Fin (460 ± 40 ms) and HERG-Fin (466 ± 47 ms) heterozygotes were shorter than those reported by Zareba et al. (1998) in large cohorts of genotyped LQT1 (490 ± 43 ms) and LQT2 (495 ± 43 ms) patients. A ”normal” QTc value (<440 ms) has been recorded in 114 (29%) out of the 388 Fin-mutation carriers whose ECG was available (as of 12/2000). The majority (79 individuals or 69%) of these 114 patients were men. Although the phenotype of KCNQ1-Fin and HERG-Fin carriers on average was mild, it varied markedly from patient to patient: the range of QTc values was wide, and synapses had also been experienced by those with normal QTc. Among KCNQ1-Fin carriers with QTc <440 ms, 16% were symptomatic, revealing that mutation carriers are at risk for cardiac events even when their QTc interval is normal.
Detailed questionnaires filled out by the families identified a baby girl who had died at the age of three months of unknown reason while sleeping, and the cause of death was therefore classified as sudden infant death syndrome (SIDS). No family data suggesting LQTS were available at that time. Post-mortem DNA analysis demonstrated heterozygosity for the KCNQ1-Fin mutation. Moreover, the baby’s mother was also a KCNQ1-Fin heterozygote and was diagnosed with a prolonged QTc interval (493 ms). Although the sudden infant death syndrome most likely represents an etiologically heterogeneous disorder, prolongation of the QT interval was strongly associated with SIDS in a prospective study comprising 34 000 newborns (Schwartz et al. 1998). Later, Schwartz et al. (2000) described a case of near-SIDS who had markedly prolonged QTc and was a carrier of the de novo missense mutation of the SCN5A gene. Study II strengthens the assumption that in rare cases inherited forms of LQTS may cause SIDS.

All JLNS patients described in Study II were deaf, had markedly prolonged QT intervals and had cardiac symptoms at an early age. Two of the patients were KCNQ1-Fin homozygotes and one was a compound heterozygote carrying both the KCNQ1-Fin mutation and the nonsense mutation Y171X. The cardiac phenotype of JLNS patients is generally more severe than that of RWS patients, suggesting a gene dosage effect. However, recently a Norwegian patient homozygous for a KCNQ1 mutation was described to have deafness and a prolonged QT interval, but no symptoms until the age of 83 (Tranebjærg et al. 1999). Contrasting examples are a KCNQ1 homozygote (Priori et al. 1998) and a compound heterozygote (Larsen et al. 1999) with cardiac signs of JLNS but without deafness. Two sisters homozygous for HERG-Fin had a cardiac phenotype similar to that in KCNQ1-Fin homozygotes, but no abnormalities in other organs (Study III). In addition, the younger sibling had a 2:1 atrioventricular block, and she died at the age of four. Hoortnje et al. (1999) reported a large duplication of the HERG gene, leading to a severely truncated protein, in a homozygous state in two siblings, one of whom died in utero and the other showed neonatal torsade de pointes and a 2:1 atrioventricular block. Thus, similarly to KCNQ1 homozygotes, HERG homozygotes have a more severe phenotype than the heterozygotes. Collectively, these data suggest that the genotype-phenotype correlations in LQTS are more complex than earlier expected.
Consistent with the results of this study, there is previous evidence that LQT1 patients with C-terminal mutations have milder phenotypes than those with mutations of the pore or transmembrane domains (Donger et al. 1997; Larsen et al. 1999; Swan et al. 1999). In the C-terminus, a 30-amino acid domain adjacent to codon 589 is suggested to be important for subunit assembly (Schmitt et al. 2000). Thus, the underlying disease mechanism in KCNQ1-Fin mutation could be a distorted interaction of subunits, while the mutations in transmembrane domains more often affect channel gating.

The HERG-Fin mutation lies in the amino terminus of the S5 transmembrane domain. The effect of mutation site on phenotypic variation has not been shown for the HERG gene, and a considerable portion of mutations are located outside the transmembrane domains (Splawski et al. 2000). Since the HERG-Fin increased the deactivation and inactivation rates of the channel, the region containing amino acid 552 may be important for these processes (Shieh et al. 1997; Wang et al. 1998).

In LQTS, the relation between genotype and phenotype is complicated because of other intrinsic and extrinsic factors affecting phenotype. It cannot be ruled out that in small proportion of mutation carriers a second mutation or a polymorphism exists that functions as a predisposing or a protecting factor. The common founder mutations provide a unique opportunity to study the effect of modifying factors, such as gene polymorphisms, drugs and metabolic disturbances, on LQTS phenotype.

6.2.3 Drug-induced arrhythmias in congenital LQTS (II, IV)

In Study IV, a novel mutation (V1667I) causing LQT3 was characterized. Two mutation carriers were symptomatic and have had torsade de pointes related to antimalarial therapy with halofantrine. Altogether nine V1667I carriers were detected, with a remarkably shorter mean QTc interval (450 ± 30 ms) than in LQT3 patients in general (510 ± 48 ms) (Zareba et al. 1998). The low symptom frequency among these patients agrees with earlier findings in LQT3 patients (Zareba et al. 1998). Study IV shows for the first time that not only LQT1 and LQT2 patients but also LQT3 patients are susceptible to malignant drug-induced QT prolongation and cardiac events (Table 7). Recently, halofantrine was shown to block HERG channels in vitro (Tie et al.
Thus, it is possible that the acute cardiac events in these two patients are explainable on the basis of a dual ion channel block, one caused by a genetic defect of the SCN5A sodium channel, and the other provoked by a halofantrine-induced block of the HERG potassium channel.

Table 7: Drugs reported to provoke ventricular tachycardia or syncope in the presence of a specific mutation or polymorphism in the LQTS genes.

<table>
<thead>
<tr>
<th>Gene/Channel</th>
<th>Drug</th>
<th>Usage</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>KCNQ1/IKs</td>
<td>mefloquine</td>
<td>antimalarial</td>
<td>Donger et al. 1997</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>disopyramide</td>
<td>anti-arrhythmic</td>
<td>Donger et al. 1997</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>cisapride</td>
<td>cholinergic antagonist</td>
<td>Napolitano et al. 2000</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>terfenadine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>antihistamine</td>
<td>Study II</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>terfenadine+quinine hydrochloride</td>
<td>antihistamine+antimalarial</td>
<td>Study II</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>terfenadine+ketoconazol</td>
<td>antihistamine+antifungal</td>
<td>Study II</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>thioridazine</td>
<td>antipsychotic</td>
<td>Study II</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>haloperidol+chlorproxiin hydrochloride</td>
<td>antipsychotic</td>
<td>Study II</td>
</tr>
<tr>
<td>KCNQ1/IKs</td>
<td>celiprolol+lisinopril+hydrochlorotiazide</td>
<td>antihypertensive+diuretic</td>
<td>Study II</td>
</tr>
<tr>
<td>HERG/IKr</td>
<td>quinidine</td>
<td>anti-arrhythmic</td>
<td>Schulze-Bahr et al. 1997</td>
</tr>
<tr>
<td>SCN5A/INa</td>
<td>halofantrine</td>
<td>antimalarial</td>
<td>Study V</td>
</tr>
<tr>
<td>MiRP1/IKr</td>
<td>clarithromycin</td>
<td>antibiotic</td>
<td>Abbott et al. 1999</td>
</tr>
<tr>
<td>MiRP1/IKr</td>
<td>quinidine</td>
<td>anti-arrhythmic</td>
<td>Sesti et al. 2000</td>
</tr>
<tr>
<td>MiRP1/IKr</td>
<td>sulfametoxazole</td>
<td>antibiotic</td>
<td>Sesti et al. 2000</td>
</tr>
<tr>
<td>MiRP1/IKr</td>
<td>procainamide</td>
<td>anti-arrhythmic</td>
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</tr>
<tr>
<td>MiRP1/IKr</td>
<td>oxatomide</td>
<td>antihistamine</td>
<td>Sesti et al. 2000</td>
</tr>
</tbody>
</table>

<sup>1</sup>Two patients had symptoms with terfenadine alone in this study.

A large variety of drugs have been implicated in prolongation of the QT interval and provocation of ventricular tachyarrhythmias in both the “healthy” population and LQTS patients (Viskin 1999). The majority of them are known to block HERG channels (Vincent 1998), possibly through a common amino acid interaction site (Mitcheson et al. 2000). Recently, mutations in LQTS genes have been linked to some clinical cases of drug-induced arrhythmia (Table 7). In particular, nucleotide variants in the most recently discovered LQTS gene MiRP1 have been shown to be responsible for acquired LQTS (Abbott et al. 1999; Sesti et al. 2000). A relatively common polymorphism of the MiRP1 gene has been shown to associate with the antibiotic sulfametoxazole both <i>in vivo</i> and <i>in vitro</i> (Sesti et al. 2000).
Some (8%) of the KCNQ1-Fin carriers had also experienced synapses related to various drug therapies (Table 7). The most common of these drugs appears to be terfenadine, an antihistamine known to prolong QT interval and induce torsade de pointes. It is important to note that although not every mutation carrier appears phenotypically affected, she or he still has an abnormally functioning ion channel which may predispose to drug-induced arrhythmias (Priori et al. 1999b; Viskin 1999).

6.3. Towards identification of a novel arrhythmia gene (V, unpublished)

FPVT is a rare dominantly inherited disease characterized by exercise-induced polymorphic ventricular tachycardia in structurally normal hearts and normal or marginally prolonged QT interval. The mortality is 30% by the age of 30 years, and the disease appears to have a high penetrance in adulthood.

Linkage and haplotype analyses assigned the FPVT locus to chromosome 1q42-q43 within a 9 cM area in the two families studied. This was the first step on the way to characterizing the underlying disease gene and gene defects as well as the molecular pathogenesis of this fatal arrhythmogenic disorder. At present, haplotype analysis using microsatellite markers within the critical region can be utilized to genotype presymptomatic members in affected families. All inherited arrhythmogenic disorders are genetically heterogeneous and caused by mutations in several genes. Therefore, it is possible that other as yet unknown FPVT loci exist.

Chromosome 1 appears to be relatively rich in structural genes, but fewer genes have been mapped to the telomere of the long arm than elsewhere in the same chromosome (GeneMap99: http://www.ncbi.nlm.nih.gov/genemap99). According to GeneMap99, 26 genes and 113 expressed sequence tags have been localized within the critical region. These include several possible candidate genes, such as the genes encoding ion channels human two-pore domain K+ channel 1 (TWIK-1) (Lesage et al. 1996a,b) and cardiac ryanodine receptor 2 (RYR-2) (Tunwell et al. 1996) as well as actin-binding protein alpha actinin 2 (ACTN2) (Beggs et al. 1992b).

None of the affected individuals had a structurally abnormal heart, which would suggest a molecular pathogenesis involving cardiac electrophysiological abnormalities similar to long QT
syndromes. The TWIK-1 homodimer forms a weak inward rectifier potassium channel expressed in several tissues but predominantly in the brain and heart (Lesage et al. 1996b). It is suggested to be responsible for background K+ conductance of different tissues. However, the function of TWIK-1 in vivo is still largely unknown (Roden and George 1996; Goldstein et al. 1998). Ten two-pore potassium channels have been cloned in mammals to date, but no disease has been linked to any of them. The RYR-2 homotetramer forms a calcium release channel expressed mainly in the heart (Tunwell et al. 1996). It regulates Ca\textsuperscript{2+} outflow from the sarcoplasmic reticulum during cardiac excitation-contraction coupling. Two other genes encoding ryanodine receptors are known: the RYR-1 gene is expressed mainly in the skeletal muscle (Zorzato et al. 1990) and the RYR-3 gene in the brain (Nakashima et al. 1997). Mutations in RYR-1 may cause malignant hyperthermia or central core disease (Missiaen et al. 2000).

Familial polymorphic ventricular tachycardia showed linkage to a CA repeat within the ACTN2 gene. A specific type of arrhythmogenic right ventricular dysplasia (ARVD2), characterized by ventricular tachycardia and fatty fibrous replacement in the heart, has been linked to the ACTN2 gene in one Italian family (Rampazzo et al. 1995). Despite the differences in phenotypes, these two diseases may be allelic. Alpha actinin 2 protein is expressed in cardiac and skeletal muscle, where it is the major component of Z-discs (Beggs et al. 1992b). Mutations in α-actinin 4 have been shown to cause familial renal dysfunction, involving focal and segmental glomerulosclerosis (Kaplan et al. 2000).

Recently, the underlying disease gene for familial polymorphic ventricular tachycardia was described (Laitinen et al. 2001; Priori et al. 2001). Missense mutations were characterized in the cardiac ryanodine receptor gene (RYR-2) in three Finnish families and four Italian probands, respectively. Also, mutations in RYR-2 have been shown to cause ARVD2 (Tiso et al. 2001).

6.4. Applying molecular genetics for diagnosis of cardiac arrhythmias

The diagnosis of LQTS is based on clinical data, electrocardiographic findings and family history (Table 2). However, recent approaches using molecular methods have revealed weaknesses in the sensitivity and specificity of clinical diagnosis (Priori et al. 1999b). The QT interval length shows marked overlap in affected and unaffected individuals, and consequently, a considerable number
of affected subjects are recorded as having a normal QTc (Vincent et al. 1992; Priori et al. 1999b; this study).

DNA-based diagnostics can be applied mainly in four different situations: 1) testing family members of genotyped probands, 2) screening ungenotyped probands for previously established mutations, 3) scanning ungenotyped probands for mutations of known genes, and 4) analysing genetic linkage in large families (Eng and Vijg 1997). Simple PCR-based assays unique for each mutation are easy to use for studying family members. The specificity and sensitivity of diagnosis are close to 100%. On the other hand, study of the ungenotyped probands is problematic in the case of LQTS caused by mutations of several genes. The benefit of linkage analysis in diagnostic purposes is restricted to large families with highly penetrant disease such as familial polymorphic ventricular tachycardia.

In LQTS, genetic testing is particularly useful in borderline cases and in presymptomatic diagnostics of children and adolescents, in whom clinical diagnostic methods are inaccurate (Priori et al. 1999a). Information derived by genotyping can also be utilized for gene-specific therapy and counselling (e.g. avoidance of swimming for LQT1) (Priori et al. 1999a). β-blockers appear to be beneficial in LQT1 and LQT2 patients but less efficient in LQT3 patients (Priori et al. 1996; Shimizu et al. 1998b; Moss et al. 2000). The sodium channel blocker mexiletine has been suggested to shorten the QT interval and prevent torsade de pointes in LQT3 patients (Schwartz et al. 1995; Shimizu et al. 1997). Similar effects have been recorded with rapid pacing in LQT3 (Shimizu et al. 1997). Administration of potassium to LQT1 and LQT2 patients has reduced the QT interval in preliminary studies (Compton et al. 1996). In addition, LQT1 patients have been suggested to benefit from potassium channel openers such as nicorandil (Shimizu et al. 1998a).

In Finland, the disease-causing gene defect has been identified in approximately half of the LQTS families in which simple and rapid genetic test can thus be applied. Indeed, about 550 LQT1, LQT2 and LQT3 Finnish carriers have been characterized to date using modern molecular genetic tools. At present, exclusion of affected status has been established for 840 family members using DNA diagnostics. A combination of two simple PCR tests, one for the KCNQ1-
Fin and another for the HERG-Fin mutation, correctly identifies 35% of Finnish LQTS patients. Today, the assay is used as a first step to screen all new LQTS probands, offering a unique possibility compared with other populations, where mutation screening is restricted to family members of genotyped probands (Chiang and Roden 2000).

LQTS families with an unknown genetic background remain a technical challenge for molecular diagnostics. Scanning for mutations in five genes (over 10 kb) for diagnostic purposes is expensive and time consuming, and fails to provide a 100% exclusion of the disease (Eng and Vijg 1997; Chiang and Roden 2000). Development of efficient and accurate mutation detection methods is, however, important in genotyping new probands. Semiautomated bidirectional nucleotide sequencing, while being highly sensitive and specific, is laborious and expensive (Eng and Vijg 1997). Denaturing high-performance liquid chromatography, in contrast, is inexpensive and appears to have a high accuracy in heterozygote detection (O’Donovan et al. 1998; Choy et al. 1999). In future, DNA microarrays or chips may provide efficient diagnostic tests for large-scale population scanning (Hacia 1999). With DNA chips, tens to hundreds of known mutations can be screened simultaneously with a specifically designed array. However, accuracy of the test in detecting heterozygous mutations is dependent on hybridization conditions and varies between 88% and 98% (Gerhold et al. 1999; Hacia 1999).
7. CONCLUSIONS

Eight novel mutations in three LQTS genes were characterized in 46 Finnish families. The KCNQ1-Fin and HERG-Fin mutations were present in 34 and in 6 individuals, respectively, out of 114 LQTS probands studied. In addition, six mutations were found to be family-specific. The majority of the LQT1 heterozygotes had symptoms related to physical exercise, while the LQT2 patients had symptoms at rest. The phenotypes of heterozygous KCNQ1-Fin and HERG-Fin carriers, based on QTc length and appearance of symptoms, were milder than those in LQT1 or LQT2 disease in general. However, a risk for cardiac events existed even in individuals with normal QTc (<440 ms). Both KCNQ1-Fin and HERG-Fin homozygotes had a more severe phenotype than the corresponding heterozygotes. Among the nine LQT3 patients, only two were symptomatic, having torsade de pointes related to malaria treatment with halofantrine. This is the first study to show that LQT3 patients are also at risk for drug-induced cardiac events.

LQTS founder mutations occur in the Finnish population with a remarkably high prevalence. Both the KCNQ1-Fin and the HERG-Fin mutations appear to be enriched in Eastern Finland, from North-Karelia to Southern Lapland, probably as a result of a founder effect. The existence of common disease mutations have rendered molecular diagnostics of LQTS feasible in Finland. In addition to studying family members of genotyped probands, two simple PCR-based tests can be used to screen novel index cases. In future, these highly prevalent LQTS founder mutations may offer a model for studying the effects of genetic or non-genetic factors, such as polymorphisms, drug or hormone administration, physical activity and psychosocial stress, on the LQTS phenotype.

The locus for familial polymorphic ventricular tachycardia was mapped to chromosome 1q42-q43. This served as the first step in identifying the disease gene, which indeed was recently recognized, by ourselves and by other investigators, to be cardiac ryanodine receptor RYR2 located in this linkage region.
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