Genetic risk factors for common and rare cardiac rhythm disorders

Marsman, R.F.J.

Publication date
2014

ATGGAATTCCCCATTGGATCCCTCGAAACTAACAACTTCCGTCGCTTTACTCCGGAGTCACTGGTGG
AGATAGAGAAGCAAATTGCTGCCAAGCAGGGAACAAAGAAAGCCAGAGAGAAGCATAGGGAGCA
GAAGGACCAAGAAGAGAAGCCTCGGCCCCAGCTGGACTTGAAAGCCTGCAACCAGCTGCCCAAG
TTCTATGGTGAGCTCCCAGCAGAACTGATCGGGGAGCCCCTGGAGGATCTAGATCCGTTCTACAGC
ACACACCGGACATTTATGGTGCTGAACAAAGGGAGGACCATTTCCCGGTTTAGTGCCACTCGGGCC
CTGTGGCTATTCAGTCCTTTCAACCTGATCAGAAGAACGGCCATCAAAGTGTCTG**T**CCACTCGTGGT
TCAGTTTATTTATTACGGTCACTATTTTGGTTAATTGTGTGTGCATGACCCGAACTGACCTTCCAGAG
AAAATTGATATGTCTTCACTGTCATTTACACCTTTGAAGCCTTGATAAAGATACTGGCAAGAGGATTT
TGTCTAAATGAGTTCACGTACCTGAGAGATCCTTGGAACTGGCTGGATTTTAGCGTCATTACCCTGGT

CHAPTER 02

Genetics of sudden cardiac death caused by ventricular arrhythmias

Nature Reviews Cardiology 2014;11(2):96-111

R.F.J. Marsman, H.L. Tan, C.R. Bezzina

ABSTRACT

Sudden cardiac death (SCD) resulting from ventricular tachyarrhythmia is a major contributor to mortality. Clinical management of SCD, currently based on clinical markers of SCD risk, can be improved by integrating genetic information. The identification of multiple disease-causing gene variants has already improved patient management and increased our understanding of the rare Mendelian diseases associated with SCD risk in the young, but marked variability in disease severity suggests that additional genetic modifiers exist. Next-generation DNA sequencing could be crucial to the discovery of SCD-associated genes, but large data sets can be difficult to interpret. SCD usually occurs in patients with an average age of 65 years who have complex cardiac disease stemming from multiple, common, acquired disorders. Heritable factors are largely unknown, but are likely to have a role in determining the risk of SCD in these patients. Numerous genetic loci have been identified that affect electrocardiogram indices, which are regarded as intermediate phenotypes for tachyarrhythmia. These loci could help to identify new molecules and pathways affecting cardiac electrical function. These loci are often located in intergenic regions, so our evolving understanding of the noncoding regulatory regions of the genome are likely to aid in the identification of novel genes that are important for cardiac electrical function and possibly SCD.

KEY POINTS

- Sustained ventricular tachyarrhythmias are the most-common cause of sudden cardiac death (SCD), and traditional risk stratification methods could be improved with genetic testing
- Multiple genes affecting SCD risk in young patients have been uncovered, but our understanding of the variability in disease severity is incomplete, and interpreting genetic test results is difficult
- Next-generation sequencing in families that have inherited patterns of SCD, but are mutation-negative for the known disease genes, is expected to uncover novel genes for these rare diseases
- Genome-wide association studies (GWAS) have identified genomic loci that affect electrocardiogram indices, SCD risk in the general population, and risk of rare cardiac diseases such as Brugada syndrome
- The unavailability of large, deeply phenotyped SCD cohorts has precluded the identification of genetic causes of risk for SCD in patients with acquired cardiac disease
- The mechanisms underlying loci identified through GWAS could involve functional regulatory elements in the noncoding region of the genome, and understanding them is important to understanding SCD

INTRODUCTION

Sudden cardiac death (SCD) is a major contributor to mortality in the general population, accounting for almost 20% of all-cause mortality in industrialized countries.[1,2] Accordingly, substantial efforts have been made to develop risk stratification methods aimed at identifying individuals at increased risk of SCD and finding preventive and therapeutic interventions. These efforts have been wide-ranging, directed at various types of patients at increased risk of SCD (both those with common acquired diseases, such as ischaemic heart disease or heart failure, and those with rare, inherited SCD-associated diseases), and have examined the appropriate use of drugs and implantable cardioverter-defibrillators (ICDs). Thus far, risk stratification has mostly revolved around clinical indicators for increased risk. The basis of SCD is, however, heterogeneous, involving numerous different diseases, and the indicators used for risk stratification could be disease-specific. For example, reduced left ventricular ejection fraction is useful to identify risk of SCD in patients with ischemic heart disease. These efforts have been widely implemented in clinical practice, and have been clearly successful. For example, the introduction of ICDs into routine clinical care of individuals at increased risk of SCD in the Netherlands accounted for 33% of the decline in the incidence of resuscitations for out-of-hospital cardiac arrest.[3] However, much can still be gained by improving risk stratification. For instance, ICD use in routine clinical care is also hampered by the potential for ICD-associated adverse events. In 2013, our group, at a referral centre for inherited cardiac diseases, reported the sobering finding that ICDs implanted for primary prevention in patients with particular diseases carried a 35% adverse event rate, and delivered virtually no appropriate shocks.[4] Clearly, risk stratification must be further refined, and individuals who do not benefit from preventive interventions such as ICDs should be identified.

SCD most often results from ventricular tachyarrhythmias, notably ventricular fibrillation (VF). Thus, systematic efforts to improve risk stratification for SCD must be rooted in an understanding of the molecular mechanisms and pathways underlying the occurrence of VF. One potential approach to unravel the molecular determinants of VF risk is based on the identification of the underlying genes. This approach has become possible because of our current understanding of the genome and the development of new cost-effective methodologies for genetic testing at the molecular level. Unravelling the molecular determinants of VF, however, remains a challenging task. VF risk is likely to be governed by multiple pathophysiological pathways, and the nature of these pathways and their relative contributions to the determination of risk are likely to vary between different cardiac pathologies. These efforts could still dramatically improve our understanding of VF, and this gene-based strategy might complement the clinical indicator strategy currently used for risk stratification. Moreover, identification of the genetic and molecular determinants of VF provides new targets that could spur the development of new therapies. In this Review, we describe the progress made in the genetic studies of VF and associated cardiac electrical traits. Insights gained from these studies are already starting to change our conceptual framework for SCD, but their direct applications in routine clinical care do not yet exist. We also discuss the potential to decipher the molecular mechanisms underlying loci identified by genome wide association studies (GWAS) using genomic resources that are becoming increasingly available.

EPIDEMIOLOGY

The reported incidence of SCD in the general population varies, because different methodologies are used to identify cases. For instance, in the USA, the incidence estimates range from 180,000 to >450,000 cases annually.[5] Given the size of the population of the USA (313,933,954 in 2012), this figure amounts to a yearly incidence of 0.6 to >1.4 per 1,000 individuals.[6] This estimate is consistent with the incidence (1.0 per 1,000 individuals) reported in a prospective community-based study performed in the Netherlands.[2] Although SCD is approximately four times more common in men than in women, VF is also the most-common arrhythmia in women with SCD.[7,8] SCD is estimated to account for approximately 50% of all cardiovascular mortality and is the first clinically identified sign of cardiac disease in 50% of cases.[2,9]

SCD occurs in a broad range of pathological cardiac settings. SCD in the general population occurs in individuals with an average age of 65 years[10] who have complex disease stemming from multiple common acquired disorders, especially coronary artery disease (CAD) and associated conditions such as myocardial infarction (MI), ischaemia, post-MI myocardial scarring, and ischaemic cardiomyopathy.[11] Some cases of SCD are caused by other disorders, including acquired infiltrative disorders, valvular and congenital structural heart diseases, heritable cardiomyopathies and heritable primary electrical disease.[12] In young individuals (aged <35 years), in whom the incidence of SCD (estimated incidence of 0.005–0.2 per 1,000 individuals per year)[13] is lower than in the general adult population congenital structural heart disease, heritable cardiomyopathies, and heritable primary electrical disease have a prominent role.[13–15] Nevertheless, even in these young individuals with heritable diseases, SCD does not always occur, because disease severity is heterogeneous (as discussed below).

HERITABILITY AND GENETIC ARCHITECTURE

Mendelian inheritance patterns

Disorders associated with SCD in the young, such as cardiomyopathies and primary electrical disease are present from birth and typically have a Mendelian pattern of inheritance with clear transmission from one generation to the next. In Mendelian disease, phenotypes are, by definition, usually determined by rare variants that have large effects. Thus, in these disorders, the contribution of genetic factors to the risk of the disease — and consequently to SCD - is large (**Figure 1**). Using classic genetic linkage analysis strategies and candidate gene studies in families affected by these disorders, multiple genes containing rare variants that confer large effects on risk have been identified (**Figure 2**).[16]

Complex inheritance patterns

CAD generally results from atherosclerosis and develops over a long period of time, typically decades, eventually culminating in myocardial ischaemia and an increased risk of SCD. Although genetic factors for the development of CAD have been described,[17] CAD is generally regarded as an acquired disorder, largely dependent on lifestyle factors such as smoking and metabolic diseases such as diabetes mellitus and dyslipidaemia. Thus, the relative contribution of genetic factors to the risk of SCD is weaker for patients with CAD than for those with Mendelian disease, and transmission patterns within families are

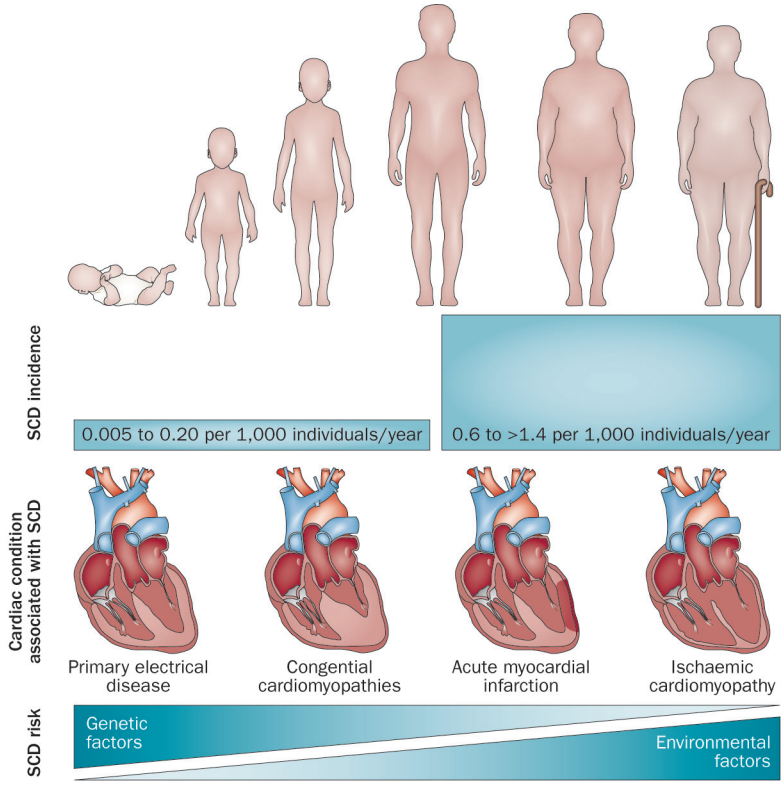

Figure 1 | SCD in various age groups. The relative contribution of genetic factors to the pathogenesis of SCD is greatest in young people. In this age group, SCD mainly occurs in the setting of disorders that are present from birth and display a clear inheritance pattern within families. In patients aged >40 years, SCD mainly occurs in cardiac diseases that arise as a consequence of coronary artery disease. Coronary artery disease develops over a prolonged period and is influenced by environmental factors, such as lifestyle. Abbreviation: SCD, sudden cardiac death.

less clear. Nevertheless, heritable factors have also been demonstrated to have a role in determining the risk of SCD in the community, where most SCD occurs as sequela of CAD. Here, similarly to many late-onset, complex, cardiovascular-associated diseases (such as CAD,[18] hypertension,[19] and diabetes[20]), family history of SCD is an independent risk factor for SCD. Evidence of a heritable component for risk of SCD was first found in two population-based studies published in the late 1990s.[21,22] In a case–control study, family history of MI or SCD was associated with SCD (relative risk 1.57), independently of classic cardiovascular risk factors such as diabetes and hypertension.[23] Subsequently, these investigators reanalysed their data, differentiating between family history of MI and family history of SCD. After adjustment for other risk factors and family history of MI, a positive family history of early-onset SCD (aged <65 years) was associated with a 2.7-fold increased risk of SCD.[22] Likewise, in the Paris Prospective I study, a long-term (>20 years) cohort study of >7000 middle-aged French men, parental history of SCD was a predisposing factor for SCD.[21] The relative risk of SCD increased 1.8-fold if one parent had SCD (the proportion of one-parental SCD was 10.7% [759/7,079] in the total cohort and 18.6% [22/118] in the SCD cohort), and 9.4-fold if both parents had SCD (the proportion of two-parental SCD in the total cohort was 0.3%).

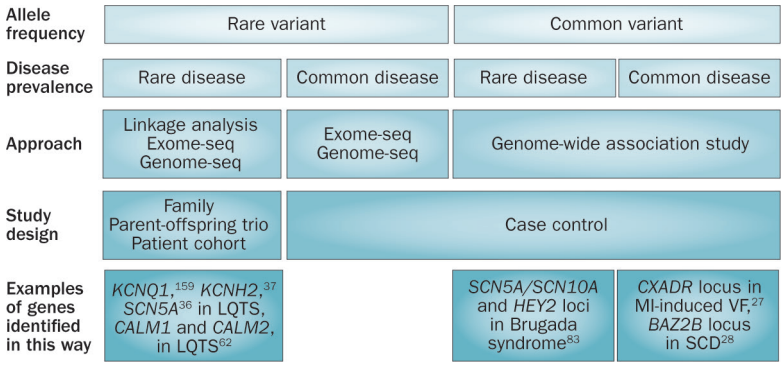

Figure 2 | Unbiased approaches for mapping of genetic variants predisposing to sudden cardiac death. The expected genetic architecture of the disease and the expected characteristics of the disease-associated allele, namely its expected frequency in the population and effect size, determine which approach and study design are most useful for the identification of disease-associated genes. Examples of genes that contribute to the risk of SCD that have been identified through each of these approaches are indicated. Abbreviations: LQTS, long QT syndrome; MI, myocardial infarction; SCD, sudden cardiac death; VF, ventricular fibrillation.

These initial studies did not distinguish between the various cardiac pathologies in which SCD occurred. SCD in the general population most often occurs in patients with CAD, so we designed the AGNES study specifically to analyse risk factors for VF in patients with a first acute ST-segment elevation MI (STEMI). By comparing patients with STEMI who also had VF with those with STEMI who did not have VF, we identified SCD among first-degree relatives as an independent risk factor for VF (odds ratio 2.72).[24] Family history of CAD, infarct location, and culprit artery were not different between the two patient groups. Similar evidence was obtained in a Finnish case–control study published around the same time, in which patients who experienced SCD during a first acute coronary event were more likely to have a family history of SCD than acute MI survivors (odds ratio 1.6) and healthy controls (odds ratio 2.2).[25]

These findings provide a strong rationale for the search for SCD risk loci in patients with CAD. Inherited susceptibility for SCD in this setting is expected to be similar to the susceptibility for other complex traits and is thus likely to be attributable to a spectrum of variants of different frequencies and effect sizes, ranging from high frequency variants with small incremental effects on risk to low frequency variants that individually confer high risk.[26] In theory, low frequency variants could exist that would be deleterious in the setting of acute ischaemia but silent under baseline conditions. The role of common variants has started to be addressed in the past three years in GWAS,[27,28] as described in detail below.

SCD-ASSOCIATED DISORDERS IN THE YOUNG

Traditionally, rare genetic disorders that predispose young individuals to SCD are categorized into those associated with a structurally normal heart (primary electrical diseases), and those associated with structural heart disease (cardiomyopathies). These disorders are outlined in more detail in the following sections.

Primary electrical diseases

The primary electrical diseases, which include long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia (CPVT), are often characterized by specific electrocardiogram (ECG) abnormalities either at baseline or during particular conditions such as exercise (CPVT, LQTS), fever (Brugada syndrome) or pharmacological challenge (Brugada syndrome). To date, most genes associated with these disorders encode cardiac ion channel subunits or proteins that interact with and regulate ion channels.[16] In these disorders, dysfunctional ion channels cause abnormalities in the electrical properties of the heart, resulting in arrhythmia. For instance, in LQTS, an increased influx of depolarizing sodium ions or calcium ions, or a decreased efflux of potassium ions, causes an imbalance between inward and outward currents during cardiomyocyte repolarization, thereby prolonging the duration of the action potential and the QT interval on the ECG. Action potential prolongation increases susceptibility to early afterdepolarizations and ensuing *torsades de pointes* arrhythmia. Mutations in the gene encoding sodium channel protein type 5 subunit α (*SCN5A*) increase sodium ion influx; mutations in the gene encoding voltage-dependent L-type calcium channel subunit α-1C (*CACNA1C*) increase calcium ion influx; and mutations in the genes encoding potassium voltage-gated channel subfamily KQT member 1 (*KCNQ1)*, potassium voltage-gated channel subfamily H member 2 (*KCNH2)*, potassium voltage-gated channel subfamily E member 1 or 2 (*KCNE1* and *KCNE2)*, or inward rectifier potassium channel 2 (*KCNJ2*) increase potassium efflux.

Inherited cardiomyopathies

The inherited cardiomyopathies are categorized as hypertrophic cardiomyopathy, dilated cardiomyopathy, or arrhythmogenic right ventricular cardiomyopathy (also known as arrhythmogenic cardiomyopathy).[29–31] Hypertrophic cardiomyopathy is characterized by left ventricular hypertrophy, myocyte disarray, and fibrosis, and has been linked to genes encoding sarcomeric proteins. Dilated cardiomyopathy is characterized by left ventricular dilatation, systolic dysfunction, myocyte death, and fibrosis, and this disease has been associated with mutations in genes encoding cytoskeletal proteins, nuclear membrane proteins, or proteins involved in calcium homeostasis. Arrhythmogenic right ventricular cardiomyopathy, characterized by fibrofatty replacement of the myocardium, has been mainly associated with genes encoding desmosomal proteins. The abnormal structure of the heart likely forms the substrate for arrhythmia in patients with cardiomyopathies. However, the risk of SCD in patients carrying mutations associated with these disorders can also be elevated in the absence of marked structural abnormalities, suggesting that other mechanisms also contribute to arrhythmia susceptibility.[32] Changes in calcium handling might confer a predisposition to arrhythmias in troponin T-associated hypertrophic cardiomyopathy.[33] Similarly, decreased sodium channel function and conduction slowing in patients with arrhythmogenic right ventricular cardiomyopathy (as a consequence of desmosomal gene mutation) can precede the onset of cardiomyopathic changes.[34,35]

IMPLICATIONS OF GENE DISCOVERY

Gene discovery for the rare cardiac disorders associated with SCD has provided insight into the pathophysiological mechanisms, allowing for more rational use of existing therapies and an improved understanding of risk factors. The demonstration that genotypic information could rationalize drug therapy in the primary electrical disorders was made very soon after the first gene discoveries for these disorders.[36–38] In a clinical study conducted in patients with LQTS, the sodium channel blocker mexiletine shortened the QT-interval of patients with a mutation in *SCN5A,* but not of patients with a mutation in *KCNH2*.[38] The biological basis for this differential effect on the QT-interval is that sodium channels harbouring mutations causing LQTS are selectively targeted by mexiletine, which has a high affinity for the pathological late opening state of these channels.[39]

Another example of genotype-specific therapy is associated with the use of β-blockers. β-Blocker therapy is highly effective in patients with mutations in *KCNQ1*.[40] Patients with missense mutations in the gene regions encoding the cytoplasmic loops of this potassium channel had a higher risk of ventricular arrhythmias and benefitted more from β-blocker therapy than patients with missense mutations in other regions of the channel or nonmissense mutations.[41] These clinical observations are supported by findings from *in vitro* electrophysiological studies conducted on a series of missense mutations located in various regions of the channel. In these studies, all mutations led to decreased basal slowly activating delayed rectifier potassium current (I_{Ks}), but only missense mutations in the cytoplasmic loop regions had a dramatically impaired response to the protein kinase A (PKA) activator forskolin. This observation suggests a blunted PKA-mediated activation of the I_{Ks} current upon β-adrenoceptor stimulation in patients with mutations in the cytoplasmic domains of the channel,[40] which could account for the difference in response to β-blockers.[41] Notably, disrupted β-adrenoceptor stimulation has also been seen in patients with a mutation in *KCNQ1* that results in a alanine to valine substitution substitution at residue 341, an amino acid located at the end of the S6 transmembrane segment;[42] this region has been suggested to interact with the S4-S5 cytoplasmic loop.[43]

Another example of rationalized, mechanism-based therapy is the use of the sodium channel blocker flecainide in the arrhythmia-suppression regime of patients with CPVT.[44,45] The majority of patients with CPVT carry a mutation in the gene encoding ryanodine receptor 2 (*RYR2*), a calcium channel on the sarcoplasmic reticulum. CPVT-causing mutations in this channel result in spontaneous diastolic calcium release, which can trigger ventricular arrhythmias. In initial studies conducted in a mouse model of CPVT, flecainide inhibited calcium release from the ryanodine receptor 2 channels, reduced spontaneous calcium release events and triggered beats, and prevented ventricular tachycardia.[44] Subsequent implementation of this therapy in patients with CPVT reduced their exercise-induced arrhythmias.[44,45]

DNA DIAGNOSTICS AND GENE DISCOVERY

Gene discovery has enabled genetic testing for the rare rhythm disorders. Genetic testing is, as yet, largely restricted to tertiary referral centres and its use is more relevant for some disorders than for others.[46,47] Nevertheless, genetic testing has had a major influence on

Box 1 | Projects and resources for genetic studies

Encyclopedia of DNA Elements (ENCODE): Maps all transcribed and regulatory functional elements, including RNA transcripted regions, transcription factor binding sites, chromatin structure, and histone modification sites.[144,155] Thus far, 80% of the components of the human genome have been assigned at least one biochemical function.

Exome Sequencing Project (ESP): A multi-institutional project which aims to discover novel genes and pathways contributing to heart, lung, and blood disorders by generating exome sequencing data from diverse, well-phenotyped cohorts. Variants identified in this project are publically available via the Exome Variant Server.[51]

The 1000 Genomes Project: An international collaboration aiming to produce an extensive public catalogue of human genetic variation. The genomes of about 2,500 individuals from over 25 different human populations with Asian, African, or European ancestry will be sequenced. Data on combined and population-specific allele frequencies are publically available.[156]

patient care and has affected diagnosis, choice of therapy, and prognosis, and has prompted presymptomatic cascade testing of relatives of mutation carriers. However, substantial challenges exist in the interpretation of genetic test results in the setting of DNA diagnostics. The degree of allelic heterogeneity (multiple variants in the same gene) at the known disease-associated genes creates a challenge for the interpretation of DNA diagnostic test results. The degree of heterogeneity has become increasingly clear in the past 2 years, as exome and genome sequencing projects such as the 1000 Genomes Project[48,49] and the NIH Heart, Lung and Blood Institute's Exome Sequencing Project[50,51] have generated nearly complete maps of genetic variation in thousands of human exomes and genomes (Box 1). The availability of these databases has brought with it the realization that genomes of 'control' individuals could also contain rare or private genetic variants in arrhythmia-associated genes. Conversely, particular genetic variants, previously deemed pathogenic on the basis of their absence in a few hundred control individuals, are actually present in the general population as observed in these large samples.[52,53]

TOOLS FOR DIAGNOSTIC TESTING

In most DNA diagnostic laboratories, testing of large, multigene panels (for example those encompassing all genes known to be mutated in patients with primary electrical disease or cardiomyopathy) by next-generation sequencing (NGS) is increasingly replacing the screening of single genes or small panels of genes.[54] NGS refers to high-throughput DNA sequencing technologies that allow for rapid sequencing of large stretches of DNA, the entire exome, or the entire genome in a highly efficient and cost-effective manner (as compared with the conventional Sanger sequencing method). This change in procedure has introduced additional interpretive challenges. Of the tens of variants identified using multigene panels, most variants are classified as 'likely benign' by virtue of their prevalence in the general population. However, each test can yield several variants that are novel or that occur at a very low prevalence in the general population. The identification of such variants of unknown significance in one or more disease genes is not uncommon. In the absence of genetic datasets encompassing large families for extensive and robust cosegregation

analysis, which is usually the case, distinguishing deleterious disease-causing variants from innocuous variants remains a major challenge.[55]

Several computational strategies that estimate the potential deleteriousness of identified gene variants are available.[55] One category (called 'sorting tolerant from intolerant')[56] employs amino acid sequence alignments to determine the degree of evolutionary conservation. The underlying premise is that the highly evolutionarily conserved amino acid positions tend to be intolerant to substitution, whereas those with a low degree of conservation tolerate most substitutions. Some of these programs incorporate additional features such as structural information and biochemical data, including the accessible surface area and hydrophobic propensity of an amino acid (for example in the PolyPhen-2 tool).[57]

In a somewhat different approach, multiple amino acid sequence alignments of LQTS gene paralogues have been used to identify important amino acids.[58] Paralogues are genes that are descended from a common ancestral gene and have diverged after a genomic duplication event. They encode proteins with similar functions; for example *KCNQ1,* which encodes a potassium channel expressed in heart and, when mutated, is associated with LQTS, is a paralogue of *KCNQ4*, which encodes a potassium channel expressed in the inner ear and, when mutated, is associated with deafness. The disease-causing amino acid substitutions in the LQTS paralogues were used to predict functionally important residues encoded in LQTS-associated genes, substitutions of which are potentially disease-causing.[58] Computational strategies based on nucleotide sequence alignments and evolutionary conservation have also been developed.[59] Such *in silico* methods are used by some diagnostics laboratories to assess the deleteriousness of the variants of unknown significance that they identify in patients. These laboratories sometimes suggest the significance of these variants, as determined through *in silico* analyses, in the report of the DNA test result. However, the interpretation of the output of such analyses must be viewed in light of their limitations and caveats. Unfortunately, functional studies, which can sometimes shed light on the causality of amino acid-changing variants, cannot usually be done in the clinical setting, as these studies require a specialized research setting and are labour-intensive and expensive.

Interpreting the importance of DNA variants in the diagnostic setting is difficult. The cardiac community must, therefore, standardize genetic testing platforms and the depth of clinical phenotyping. Standardization is required to support the exchange of genotype and phenotype data across centres, which is necessary to further our understanding of the contribution of such variants to these rare diseases. Though indispensable, such an effort in variant annotation in extended patient sets is likely to take years of careful studies before information on these variants can be used in routine clinical care. This effort will also benefit from the systematic study of control populations wherein genomic data are annotated with detailed clinical information and physiologically relevant assays.[60]

EXOME AND WHOLE-GENOME SEQUENCING

NGS could also be used to identify novel disease-associated genes in patients with rare cardiac disease in whom no mutations in the known disease-associated genes are identified. Exome sequencing (and possibly genome sequencing, as this becomes increasingly available) is likely to have a crucial role in discovering new disease-associated genes.[61] Around 20,000 variants are found in comparisons of exome sequence data from one individual with

the reference genome, amplifying the issue of variant interpretation as discussed in the previous section. This large number of variants is subsequently reduced to a few hundred by filtering against catalogues of already-identified variants[49,51] to find variants that are novel or rare. The ensuing analysis to determine the causal mutation or mutations depends on the suspected mode of inheritance.

Employing the *de novo* mutation paradigm, exome sequencing was performed in trios consisting of an affected child and unaffected parents.[62] Mutations in two genes encoding calmodulin (*CALM1* and *CALM2)* were identified in infants affected by severe QT-interval prolongation and recurrent cardiac arrest (the mutation in each child was not present in the unaffected parents).[62] These genetic defects would have been impossible to discover before the introduction of NGS. Each individual carries only a limited number of variants that are not also found in their parents (around 50–100 *de novo* mutations, approximately one of which could be amino acid-altering).[63] Thus, the analysis of a few unrelated parent–child trios with sporadic presentation of the same presumed autosomal dominant disorder can be sufficient to identify *de novo* mutations in disease-associated genes.

In the search for genes involved in rare diseases, exome sequencing has been most successful in the identification of *de novo* mutations and of genes for recessive disorders (which involves a search for compound heterozygous mutations or homozygous mutations in consanguineous individuals, which also substantially reduces the number of remaining candidate variants). By contrast, genes responsible for autosomal dominant disorders are difficult to identify by exome sequencing.[61] To identify these genes, heterozygous gene variants shared among affected relatives are compared, which typically still leaves a considerable number of variants, particularly in small families in whom cosegregation analysis cannot be effectively performed. Two exome sequencing studies of autosomal dominant disorders with reduced penetrance published in 2013 - one from our group in which a mutation in *CALM1* was found in patients with idiopathic ventricular fibrillation,[64] and one in which a mutation in *CACNA1C* was discovered in patients with autosomal dominant LQTS[65] - employed additional downstream strategies for variant prioritization, such as the cardiovascular gene ontology annotation,[64] to home in on disease-associated mutations. Interestingly, in both studies, distinct phenotypes were observed for mutations in known arrhythmia-causing genes. The detection of mutations in the same gene in unrelated individuals or families is an important means of gene validation. In the absence of this observational evidence, support for causality needs to come from functional studies. The increasing accessibility of whole-genome sequencing and our increasing knowledge of regulatory elements in the noncoding region of the genome, discussed in detail below, will ultimately encourage the search for causal variants in the noncoding region of the genome. The noncoding region is an unchartered territory in rare disease genetics in general.

GENETIC FEATURES OF THE RARE DISORDERS

Incomplete penetrance

Clinical management of patients with rare cardiac Mendelian disorders is complicated by the variability in disease severity among mutation carriers, as is the case for most Mendelian disorders (**Figure 3**). In 1980, even before the identification of genes associated with LQTS, the disease spectrum of LQTS was proposed to be larger than expected, and was thought

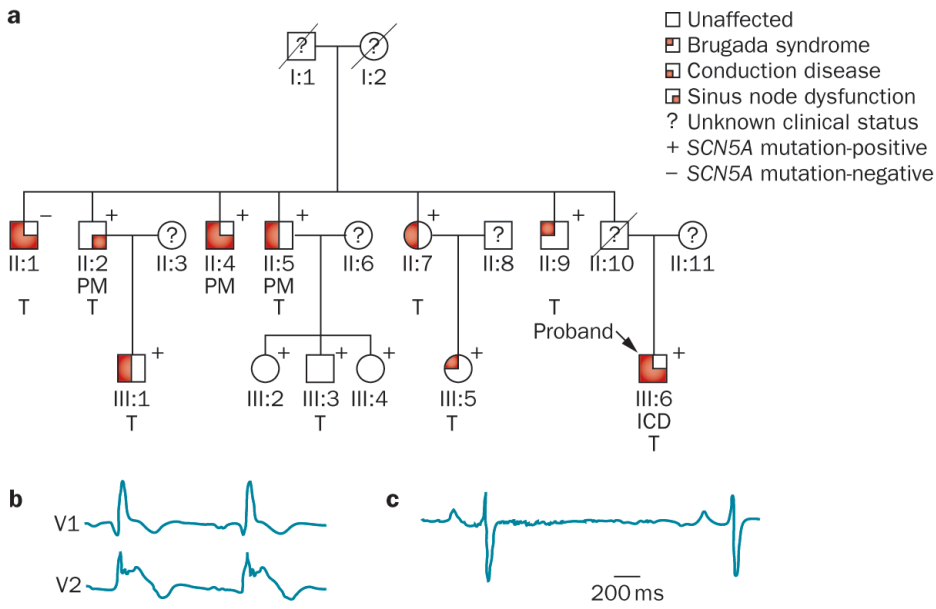

Figure 3 | Family with primary electrical disease displaying reduced penetrance and variable disease expression. **(A)** Pedigree of a family harboring a mutation in *SCN5A*. This mutation resulted in loss of sodium channel function (shown by electrophysiological studies) consistent with the phenotype. The index patient (III:6, male, aged 37 years) presented with palpitations. Notably, one individual (II:1) presented with Brugada syndrome and sinus node dysfunction despite testing negative for the familial mutation. Age and sex likely modulate disease manifestations in this family, but additional genetic factors could also have a role. The mode of inheritance for these disorders is likely more complex then previously suspected. **(B)** An ECG diagnostic of Brugada syndrome was observed in the index patient after sodium channel blocker challenge with flecainide. **(C)** Holter monitoring strip results of the index pateint showing sinus node dysfunction and conduction abnormalities.

likely to include individuals with a normal QT interval.[66] Variable disease severity is probably best appreciated in large pedigrees or in multiple families harbouring founder mutations, as phenotype variability is most easily observed among a large number of carriers of the same primary genetic defect (excluding effects on clinical severity stemming from different genetic defects).[67,68] Certain mutation carriers might not display clinical signs of the disease (such as ECG abnormalities in patients with primary electrical disease), even though they carry the familial mutation (commonly referred to as reduced penetrance), whereas others who are affected have variable disease severity (variable expressivity).[69,70] Crucially, only a fraction of mutation carriers develops a life-threatening arrhythmia.[71]

In the primary electrical disorders, additional genetic factors are suspected to contribute to clinical disease variability together with established modulators such as sex,[72] age,[73] heart rate,[74] and drug use.[75] The occurrence of compound mutations[76,77] (>1 mutation in ≥1 gene; a scenario that occurs in as many as 9% of patients with LQTS[78,79]) and the particular type and location of the amino acid alteration in the resulting protein[80,81] can explain disease severity in some cases. However, additional genetic factors, known as genetic modifiers, are also suspected to contribute to variability in disease severity. The identification of genetic modifiers is of considerable interest, and is expected to lead to

improved risk stratification.[68,82,83] The rare cardiac disorders associated with SCD have been widely assumed to be Mendelian, monogenic disorders to date, caused by the inheritance of a single, large-effect, penetrant or nearly penetrant variant that is very rare in the general population. However, in addition to the large variability in disease severity, several other observations, including the occurrence of sporadic cases and our inability to identify new disease-associated genes by classic linkage analysis, suggest an oligogenic mode of inheritance for at least some of these rare disorders. Such a model would involve the inheritance of multiple variants, possibly of different effect size, in different genes.

Genetic modifiers

For the primary electrical diseases in particular, the obvious candidate modulatory variants for ECG manifestations or the occurrence of arrhythmia are the common single nucleotide polymorphisms (SNPs) that have been identified by GWAS as modulators of ECG indices in the general population (**Table 1**). Common variants in the genes encoding carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein *NOS1AP*[68,82] and *KCNQ1,*[84] identified as QT-interval modifiers by GWAS on the basis of ECG indices (ECG-GWAS),[85–87] modify the QT-interval and risk of arrhythmias in patients with LQTS. The use of these variants for routine clinical management is probably not imminent owing to the small effect size that is typical of such common genetic variants. Future studies in which additional variants identified by ECG-GWAS are investigated will likely inform us of the usefulness of genotyping multiple small-effect variants that might collectively explain a larger part of the interindividual variability than individual SNPs do.

In a search for genetic modifiers in patients with mutations in *KCNQ1*, we and others hypothesized that SNPs in the 3´ untranslated region (3´UTR) of *KCNQ1* modify the relative expression of the normal and mutation-containing alleles and thereby contribute to disease variability.[88] In our analysis, patients carrying specific *KCNQ1* 3'UTR variants (SNPs rs2519184, rs8234 and rs10798) on the same allele as the LQTS-associated *KCNQ1* mutation had a less severe phenotype than those who carried these variants on the other allele. These allele-specific effects are presumably caused by differences in microRNA binding at the 3'UTR of the respective transcript. In support of an allele-specific effect of these variants, genetic variation at one of the SNPs implicated in this study (rs10798) was associated with differential allelic expression in lymphoblastoid cell lines.[89] This work highlights another level of complexity in the interpretation of data from genetic modifier studies—the necessity to consider allele-specific, disease-modifying effects of genetic variation. This consideration is particularly important for ion channel disorders that are caused by mutations in channels made up of multimers from subunits encoded by both alleles.

Brugada syndrome

An oligogenic model of inheritance is strongly suspected in Brugada syndrome,[90] which is causally associated with mutations in *SCN5A* in approximately 20% of cases.[91] In 2013, we published the results of a GWAS in which we examined the contributions of common genetic variants to risk of Brugada syndrome, and identified three independent SNPs associated with an increased risk (rs10428132 and rs11708996, both at the *SCN5A/SCN10A* locus, and rs9388451 near *HEY2*, which encodes hairy/enhancer-of-split related with YRPW motif protein 2).[83] Of note, we found that disease risk increased consistently with increasing numbers of risk alleles carried at these three loci, with an estimated odds ratio of 21.5 with

more than four risk alleles compared with fewer than two. Although further validation is required in additional independent sets of patients with Brugada syndrome, these findings suggest, for the first time, that the genetic architecture of at least some of the rare rhythm disorders could be different to that assumed thus far. Furthermore, the associations at the *SCN5A/SCN10A* locus demonstrate that genetic polymorphisms previously shown to modulate cardiac conduction in ECG-GWAS in the general population[92–95] (**Table 1**) can also influence susceptibility to a rare primary electrical disorder. Mechanistically, because *HEY2* encodes a transcriptional repressor, altered transcriptional programming during cardiac development could also contribute to the pathogenesis of Brugada syndrome.[96] The hypothesis that changes in *HEY2* expression at SNP rs9388451 increase the risk of Brugada syndrome is supported by evidence in mice that *Hey2* regulates cardiac ion channel gene expression and electrical activity.[83]

RISK VARIANTS FOR SCD IN THE COMMUNITY

Candidate gene studies

In contrast to the genetic discoveries made for the rare cardiac disorders associated with SCD, very little progress has been made in identifying genetic risk factors for complex arrhythmias occurring in the setting of acquired cardiac diseases in the general population. In a few candidate gene studies, common[97–101] and rare[99,102,103] variants in genes encoding ion channel subunits already implicated in the primary electrical disorders (*SCN5A*, *KCNQ1*, and *KCNH2*) were found to contribute to SCD risk in this setting, although independent replication of these findings is lacking for most studies.

Some studies have also addressed the possible role of common variants at loci with predicted biological relevance for SCD, including genes implicated in the sympathetic nervous system (such as the β2-adrenergic receptor, encoded by *ADRB2*)[104–106], platelet activity and coagulation (such as glycoprotein 1bα and integrin β3, encoded by *GP1BA*, and *ITGB3,* respectively)[107,108], the renin–angiotensin–aldosterone system (such as type I and type II angiotensin II receptors, encoded by *AGTR1* and *AGTR2,* respectively),[109] loci implicated in schizophrenia and epilepsy,[110] and the myocardial infarction locus on chromosome 9p21.[111] The MetaboChip[112] custom array was used in a 2012 publication to compare the frequency of almost 120,000 SNPs that were previously found to be nominally associated with a variety of metabolic, cardiovascular, and anthropometric traits in large-scale meta-analyses of several GWAS.[113] In this analysis, two SNPs (rs6730157 on chromosome 2q21, and, rs2077316 on chromosome 10q21) were associated with SCD in the context of CAD at genome-wide statistical significance; however, similar to most SNPs identified in candidate genes studies, these SNPs currently await validation in additional patient sets.

Genome-wide association studies

Since 2010, systematic analysis of variants for associations with SCD has been performed at a genome-wide level. To date, four GWAS studies on VF and SCD have been published;[27,28,114,115] associations at the stringent P-value threshold of $<5\times10^{-8}$, corresponding to genome-wide statistical significance, were reported in two of these studies (**Table 2**).[27,28] The first of these two studies was conducted by our group in the AGNES case–control set, in which we compared individuals with or without VF in the setting of a first STEMI.[27] The most-

Table 1 | GWAS loci containing genes associated with cardiac electrical function and/or arrhythmia

SNP[$]	Chromosome	Candidate gene	Other associated cardiac traits
Heart rate			
rs6882776[129]	5q34	*NKX2.5*	PR interval,[129] QRS interval,[129] QT interval, [129] AF[129]*
rs11154022,[130] rs9398652 or rs1015451[129–131]	6q21-q23.2	*GJA1*	QRS interval,[129] QT interval,[129] AF[129]
rs281868,[131] rs11153730[129]	6q22	*PLN*	QRS interval,[95,129]* QT interval, [86,87,129]* PR interval,[129] AF[129]
rs223116[131]	14q11.2	*MYH7*	None
rs365990[93,129,131]	14q11.2	*MYH6, miRNA 208a*	PR interval,[93,154] QT interval,[154]
rs4489968[129]	15q24.1	*HCN4*	AF[129]*
PR interval			
rs3922844,[133] rs11708996,[94] rs6599222,[133] rs6795970 or rs6801957[92–94,133]	3p22.2	*SCN5A, SCN10A*	QT interval,[86,87,95] QRS interval,[93,95,138]* P-wave duration,[92] AF,[94,138] pacemaker implantation,[93] Brugada syndrome,[83] MI-induced VF,[92] cardiac arrhythmias[138]
rs251253[94]	5q35.1	*NKX2.5*	AF[94]
rs3807989[93,94]	7q31.1	*CAV1, CAV2*	AF[93,94]
rs1896312,[94] rs7312625,[133] rs3825214[93]	12q24.21	*TBX3, TBX5*	QRS interval,[93,95]* AF,[93] advanced atrioventricular block,[93] QT interval[93]
QRS interval			
rs4074536[95]	1p13.3-p11	*CASQ2*	None
rs4687718[95]	3p14.3	*CACNA1D*	QT interval,[86] SCD[28]
rs6795970 or rs6801957,[93,95,138] rs2051211,[95] rs10865879,[95,138] rs11710077,[95] rs11708996,[95] rs9851724[95]	3p22.2	*SCN5A, SCN10A*	PR interval,[92–95,133]* P-wave duration,[92] AF,[94,138] pacemaker implantation,[93] Brugada syndrome,[83] MI-induced VF,[92] cardiac arrhythmias,[138] QT interval [86,87,95]*
rs11153730[95]	6q22	*PLN*	Altered heart rate,[129,131]* QT interval,[86,87,129]* and PR interval,[129] AF[129]
rs1362212[95]	7p14.3	*TBX20*	None
rs883079,[95,133] rs10850409,[95] rs3825214[95]	12q24.21	*TBX3, TBX5*	PR interval,[133]* AF,[93] advanced atrioventricular block[93] QT interval[93]
rs9912468[95]	17q22-q23.2	*PRKCA*	QT interval[95]

QTc interval			
rs10919071[86]	1q24.2	*ATP1B1*	None
rs11129795[86,87]	3p22.2	*SCN5A*	QRS interval[95,138]*
rs11970286,[85] rs11756438[86]	6q22	*PLN*	Heart rate,[129,131]* QRS interval,[95,129]* PR interval,[129] QT interval,[129]* AF[129]
rs2968863,[86] rs4725982[87]	7q36.1	*KCNH2*	None
rs12296050,[86] rs12576239,[87] rs2074238,[87]	11p15.5	*KCNQ1*	None
rs17779747[86]	17q24.3	*KCNJ2*	None
rs1805128[87]	21q22.12	*KCNE1*	None

$Only association signals that were identified at $P<5\times10^{-8}$ are listed. *Association at genome-wide statistical significance ($P<5\times10^{-8}$). Abbreviations: AF, atrial fibrillation; GWAS, genome-wide association studies; MI, myocardial infarction; SCD, sudden cardiac death; SNP, single nucleotide polymorphism; QTc, corrected QT interval; VF, ventricular fibrillation.

significant association with VF was found at chromosome 21q21 (SNP rs2824292). This association signal occurred in a gene-poor region; however, of the two genes located within 1 Mb of the signal, *CXADR*, which encodes coxsackievirus and adenovirus receptor (CAR), is a plausible candidate for the observed effect (the other potential candidate in the region is *BTG3*). CAR is a cell adhesion molecule predominantly located at the intercalated disc.[116] The protein has been long-recognized for its involvement in virus-mediated myocarditis, but mice deficient for *cxadr* also have defects in atrioventricular conduction through aberrant gap junction γ-1 (also known as Cx45) protein localization at the intercalated disk.[117,118] Interestingly, the expression level of gap junction α-1 protein (also known as Cx43), the predominant gap junction molecule in the ventricular myocardium, was decreased in hearts of *car*-deficient mice.[118] This finding highlights the intriguing possibility that CAR also has a role in ventricular conduction and susceptibility to ventricular arrhythmia.

An association with SCD at genome-wide statistical significance was also reported in a meta-analysis of GWAS data on individuals with SCD and controls from the general population from five community-based cohort studies, with follow-up genotyping of selected SNPs in additional SCD patients and controls. In this study, an association signal was identified at chromosome 2q24.2 (SNP rs4665058).[28] The lead SNP at this locus maps to an intron within the *BAZ2B* gene, which encodes bromodomain adjacent zinc finger domain 2B, and the haplotype tagged by this SNP extends to the *WDSUB1* and *TANC1* genes (encoding WD repeat, SAM and U-box domain-containing protein 1 and protein TANC1, respectively). All three genes are expressed in heart; however, their possible involvement in cardiac electrical function and susceptibility to SCD remains unknown. Furthermore, other genes are located in the proximity of the associated haplotype. Interestingly, the risk allele at rs4665058 has a low frequency in European ancestry populations (minor allele frequency 1.4%), underscoring the possibility that relatively rare variants have a role in determining SCD risk. Although the above studies have discovered association signals for VF and SCD, replication efforts have been very limited, both in the original studies and in the few studies that have followed. Furthermore, consistent associations across studies are difficult to find, including those from GWAS. For example, although the association between VF and the 21q21

Table 2 | Genetic loci associated with SCD through GWAS

SNP	Locus	Nucleotide in risk allele	Risk allele frequency* (%)	Odds ratio (95% CI)	P value	Nearest gene(s)	Study
rs2824292	21q21	G	46	1.78 (1.47-2.13)	3.36×10^{-10}	*CXADR*, *BTG3*, *C21orf91*	Bezzina *et al.* (2010)[27]
rs4665058	2q24.2	A	1	1.92 (1.57-2.34)	1.8×10^{-10}	*BAZ2B*	Arking *et al.* (2011)[28]

*** Individuals of European descent. Abbreviations: GWAS, genome-wise association studies; SCD, sudden cardiac death; SNP, single nucleotide polymorphism, SSS, sick sinus node disease.**

(rs2824292) locus detected in the AGNES GWAS was replicated in a small case–control set in the original study,[26] it was not detected in another small case–control set.[119] Similarly, the *BAZ2B* locus was not found to affect VF risk in the AGNES case–control set.[28] These inconsistencies could be caused by several factors, most of which are likely associated with fundamental difficulties and limitations of genetic studies on the VF or SCD phenotype (**Box 2**). Of note, the SNPs associated with VF or SCD in these studies had small effect sizes on risk and are not currently useful for risk stratification or to direct clinical management. Common genetic variants generally have small effect sizes, as has been observed in most GWAS conducted thus far across various traits.[120] We hope, however, that these studies indicate as-yet-unknown arrhythmia mechanisms that could be targeted in intervention strategies. Moreover, although rare genetic variants with large effects, which could be useful in risk prediction, are suspected to be involved in SCD and VF, the identification of such variants by NGS is hampered by the small size of the SCD populations currently available for study. This limitation could be solved by ongoing efforts to expand such data sets.

GWAS OF ECG INDICES

Arrhythmia is a complex phenotype and is likely governed by multiple interacting biological and environmental factors. Among the biological factors, intrinsic cardiac electrical function is likely to have a crucial role. Thus, ECG indices are regarded as intermediate phenotypes of arrhythmia.[121] In support of this concept, prolongation of particular ECG intervals (such as QT and QRS duration) could be a risk factor for SCD, both among individuals from the general population and in patients with specific cardiac diseases.[122–126] Many researchers have, therefore, focused on the identification of genetic factors that govern interindividual variability in cardiac electrical function as measured by surface ECG. ECG indices are very useful in genetic studies because they display marked heritability[93,127,128] and can be accurately measured in large groups of individuals.

Large international consortia have, over the past 7 years, successfully employed the GWAS approach in large samples of the general population to identify common genetic variants that modulate heart rate and ECG indices of conduction (PR and QRS duration) and repolarization (QT duration) (**Table 1**).[85–87,92–95,129–134] As expected, these studies identified genetic variation within or close to genes already linked to these traits: for example *HCN4* and *NKX2-5* (which encode potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 and homeobox protein Nkx-2.5, respectively), were associated with heart rate; *TBX3*,

TBX5 (which encode T-box transcription factor TBX3 and TBX5, respectively), and *SCN5A* were associated with PR and QRS duration; and *KCNH2*, *KCNQ1*, *KCNJ2*, and *KCNE1* were associated with QT duration. However, loci that do not harbour genes previously known to have a role in determining ECG parameters were also identified (**Table 3**). Thus, despite the small effect size in these GWAS signals, many untapped avenues for research have been identified in these studies and are likely to provide us with a broader understanding of the biology of these traits.

Although the effect of common variants on ECG indices is highly reproducible, and the role of these variants as mediators of risk is plausible, studies that have directly assessed the effect of these variants on SCD risk have been generally disappointing. Most reports have not detected an association with SCD or have yielded inconsistent findings.[28,135–137] Perhaps one exception is rs6795970 (in high linkage disequilibrium with rs10428132 and rs6801957 at the *SCN5A*/*SCN10A* locus, see below), associated with PR and QRS duration in GWAS, which has been associated with atrial fibrillation in two studies,[94,138] and with VF in another.[92] Of note, the PR-prolonging and QRS-prolonging allele was associated with a protective effect in all three studies.

The difficulty in detecting effects of ECG-GWAS SNPs on SCD risk could have multiple causes. First, the studies designed to attempt to link these genetic variants to SCD risk have the same limitations as studies designed to try to link variants to the SCD phenotype directly (**Box 2**).

BOX 2 | Challenges in conducting genetic studies on VF or SCD in the community

Cases

One difficulty in conducting genetic studies on patients with VF or SCD is the paucity of patients from whom DNA and deep phenotyping data have been collected. The statistical power for genetic discovery and the potential to replicate findings are, therefore, limited. SCD occurs suddenly (by definition) and usually unexpectedly, and VF is lethal within minutes if left untreated. The few DNA biobanks of such patients that exist are still small. The varying extent of the phenotypic and clinical characterization of SCD cases in the existing biobanks, particularly the VF/SCD phenotype itself, and the lack of definition of the underlying cardiac pathology in which it occurs could also render findings inconsistent. Attribution of the death to SCD often relies solely on the suddenness of the death, as few studies include ECG documentation of VF.[24,157] Some cases of sudden death, such as stroke or aneurysm could be incorrectly classified as SCD. VF occurs in a broad spectrum of cardiac pathologies, so VF might stem from different mechanisms in different groups of patients, each with different genetic underpinnings, rendering deep phenotyping crucial to distinguish the various mechanisms underlying VF. Additional information from autopsies or the patients' medical history would provide information about the underlying cardiac substrate, but entails considerable effort.

Controls

Some studies have employed population-based controls, whereas other studies have sought to employ similarly exposed controls (same cardiac pathology but no VF or SCD) such as patients with a first acute MI but without VF,[27] or with MI and CAD.[113] The rationale is that MI or CAD controls would enable the discovery of genetic associations related to VF and SCD, but independent of CAD, thus avoiding confounding and increasing the statistical power for detection of VF-associated SCD variants.

Abbreviations: CAD, coronary artery disease; MI, myocardial infarction; SCD, sudden cardiac death; VF, ventricular fibrillation.

Table 3 | Loci without genes previously associated with electrical function

SNP[$]	Chromosome	Nearest gene(s)	Association with other cardiac traits
Heart rate			
rs2745967[131]	1q32	*CD34*	None
rs12731740[129,130]	1q32.2	*CD46, CD34, PLXNA2*	None
rs11576175[130]	1q21.2	*CTSS*	None
rs17362588[129]	2q31	*CCDC141*	QRS interval[129]
rs4140885[129]	2q32	*TFPI*	None
rs13030174[129]	2q37	*B3GNT7*	None
rs7612445[129]	3q26	*GNB4*	None
rs9647379[129]	3q26	*FNDC3B*	None
rs12110693[130]	6q22.31	*LOC644502*	None
rs180242[129]	7q21	*GNG11*	None
rs314370[131], rs13245899[129]	7q22	*EPHB4, SLC12A9, SRRT, UFSP1, ACHE*	None
rs2350782[129]	7q33	*CHRM2*	None
rs174549[129,131]	11q13	*FADS1*	QRS interval,[129] QT interval[129]*
rs7980799[129]	12p12	*SYT10*	None
rs17287293[129,131]	12p12	*SOX5, BCAT1*	PR interval,[94]* AF[94]
rs826838[129]	12q12	*CPNE8*	None
rs2067615[129]	12q23	*RFX4*	None
rs885389[134]	12q24.33	*GPR133*	None
rs17796783[129]	14q31	*FLRT2*	None
rs6127471[129]	20q11	*KIAA1755*	None
PR interval			
rs10865355[94,133]	2p14	*MEIS1*	None
rs7660702[93,94]	4q22.1	*ARHGAP24*	None
rs4944092[94]	11q13.5	*WNT11*	None
rs11047543[94,129]	12p12.1	*SOX5, BCAT1*	Heart rate,[131]* AF[94]

QRS interval			
rs17391905[95]	1p32	*C1orf185, RNF11, CDKN2c, FAF1*	PR interval[95]
rs9436640[95]	1p31	*NFIA*	None
rs7562790[95]	2p21	*CRIM1*	None
rs17020136[95]	2p22.2	*HEATR5B, STRN*	None
rs2242285[95]	3p14.1	*LRIG, SLC25A26*	None
rs13165478[95]	5q33	*HAND1, SAP30L*	None
rs1321311,[93,95] rs9470361[93,95]	6p21.2	*PI16, CDKN1A*	None
rs7784776[95]	7p12.3	*IGFBP3*	None
rs1733724[93,95]	10q11.2	*DKK1*	None
rs7342028[95]	10q25.2	*VTI1A*	None
rs1886512[95]	13q22	*KLF12*	None
rs11848785[95]	14q24.2	*SIPA1L1*	None
rs17608766[95]	17q21	*GOSR2*	None
rs991014[95]	18q21.1	*SETBP1*	None
QTc interval			
rs12143842[86,87] rs2880058,[92,134] rs10494366[85,134] rs12029454,[87] rs16857031,[87] rs4657178[85]	1q23.3	*NOS1AP*	QRS interval,[95] SCD,[135] QTc[82] and arrhythmia[68,82] in long QT syndrome
rs2478333[134]	13q13	*SUCLA2*	None
rs8049607[86,87]	16p13.13	*LITAF, CLEC16A, SNN, ZC3H7A, TNFRSF17*	None
rs7188697[86,87] or rs37062[86,87]	16q21	*CNOT1, GINS3, NDRG4, SLC38A7, GOT2*	None
rs2074518[87]	17q11.2-q12	*LIG3, RFFL*	None

[$]Only association signals that were identified at $P<5\times10^{-8}$ are listed. *Association at genome-wide statistical significance (P <5×10-8). Abbreviations: AF, atrial fibrillation; GWAS, genome-wide association studies; SCD, sudden cardiac death; SNP, single nucleotide polymorphism; QTc, corrected QT interval.

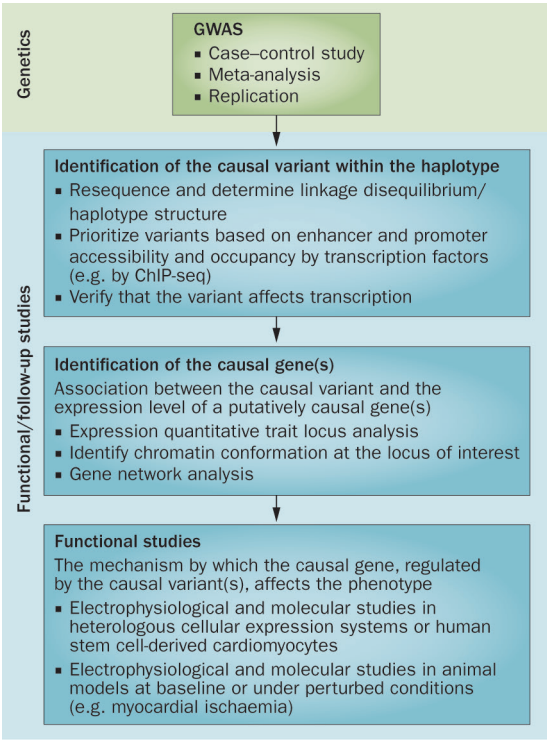

Figure 4 | GWAS and post-GWAS strategies. Genetic regions that could affect cardiac ECG parameters (as a measure of cardiac electrical function) and/or the risk of sudden cardiac death can be identified through GWAS. Follow-up studies aim to determine which genetic variation at the identified locus is likely to cause the association, and how that variation affects gene expression or protein function and thereby the disease. Abbreviations: GWAS, genome-wide association studies; ChIP-seq, Chromatin immunoprecipitation-sequencing.

In addition, the effect of ECG-GWAS SNPs is generally in the order of a few milliseconds or even a fraction of a millisecond (that is, allele effect sizes of 0.4–0.9 ms for QRS duration, and 1–4 ms for QT duration). This effect size might not be important relative to the large effects on cardiac electrophysiological properties that are induced by common acquired disorders, such as acute ischaemia or remodelling induced in the setting of heart failure.[139,140] In addition, although testing of the effect of variants in aggregate seems attractive, the process is complicated by the possibility that ECG-SNPs have pleiotropic effects, and that these multiple effects have opposing directions. For instance, index SNPs at the *SCN5A-SCN10A* and *PRKCA* loci seem to affect both QT duration and QRS duration, but in opposite directions —they shorten the QT interval and prolong the QRS interval.[95]

IDENTIFYING DISEASE MECHANISMS VIA GWAS

The effect size of the common SNPs identified by the GWAS studies on ECG indices, VF and SCD discussed above is small, and mapping these SNPs is thus unlikely to provide useful information for clinical decision-making in the short term. However, their identification in these studies has provided the scientific community with numerous leads (**Tables 1** and **3**) to initiate basic studies aimed at identifying novel molecular mechanisms and pathways underlying cardiac electrical function. In particular, the large number of association signals identified by ECG-GWAS might suggest novel biological pathways involved in arrhythmia. These pathways could be tested in animal models, and could eventually be used for

pharmacological intervention. Genomic loci discovered by GWAS are important to follow up functionally since, beyond the knowledge of the molecular counterparts of the cardiac ionic currents (the ion channels themselves), little knowledge of the higher-order regulators of cardiac electrical function (such as the regulators of ion channels) exists.

In our continued attempts to discover the biological underpinnings of SNP associations, however, we are inevitably confronted with the inherent limitation of GWAS: it illuminates a region in the genome that is associated with a trait, but does not inform us about the exact genetic variant and gene mediating that effect. Firstly, SNPs are highly correlated (in high linkage disequilibrium) with neighbouring SNPs, thereby forming haplotypes. Therefore, any SNP on a given haplotype could be responsible for the effect observed in GWAS. Similarly to the majority (~88%) of SNPs identified in GWAS in general,[141] SNPs associated with ECG parameters, VF, or SCD are usually located within the noncoding regions of the genome and thus likely exert their effect through changes in gene expression. Accordingly, with the exception of those rare cases where a candidate amino-acid-changing variant is located on the risk haplotype (and could be directly tested in functional or electrophysiological studies), linking a GWAS signal to a functional mechanism ideally involves three components (**Figure 4**). Firstly, the causal variant within the susceptibility haplotype should be identified. Secondly, the association between the causal variant and the expression level of a cognate

Box 3 | Definitions of biological concepts and methodologies

Enhancer: A regulatory DNA element that promotes gene expression distally from both the transcription start site and promoter elements directly upstream of a gene. Enhancers often function in a cell-type-specific or tissue-type-specific manner. Enhancer sequences are composed of clusters of transcription factor binding sites to which activating and repressive factors can bind. These elements can be located in introns and intergenic regions, and can be kilobases apart from their target genes.[158]

Linkage disequilibrium: Alleles at two or more neighbouring loci that do not segregate independently. Linkage disequilibrium is highest within short distances (5–20 kilobases), but can occur at distances of >100 kilobases. The pattern of linkage disequilibrium varies among human populations.

MicroRNA: Short, noncoding RNA molecules implicated in gene regulation by posttranscriptional repression of mRNA translation.

Promoter: A regulatory DNA element that promotes and initiates gene expression proximal to a gene. Promoter sequences are composed of RNA polymerase and transcription factor binding sites and are generally located at the 5´end of the transcription initiation site of a gene.

Chromatin immunoprecipitation-sequencing (ChIP-seq): A technique to identify enhancers and histone modifications. Protein–DNA complexes are isolated using antibodies that recognize specific DNA-binding proteins, followed by massive parallel sequencing, thereby scanning the entire genome for potential transcription factor binding sites and histone modification sites.[145]

Exome sequencing: A targeted approach to selectively sequence the approximately 1% of the genome that codes for proteins (the exome) in order to identify protein-altering genetic variants.

Expression quantitative trait locus (eQTL): Genomic locus that controls transcript abundance. Systematic eQTL mapping involves combining quantitative assays of gene expression (e.g. microarrays or RNA sequencing) with genetic variation (e.g. SNPs) on a genome-wide basis.

Genome sequencing: High-throughput sequencing of the entire genome (~3.2 billion nucleotides).

gene should be investigated by means of expression quantitative trait locus (eQTL) analysis to identify a candidate gene for functional studies (**Box 3**). Finally, functional studies will provide the potential mechanism through which the candidate gene, under the control of the causal variant, affects the phenotype (**Figure 4**).[142,143]

The ENCODE project[144] and individual laboratories are rapidly providing insight into the functional regulatory elements (such as enhancers and promoters) in the noncoding part of the genome. These developments are crucial to furthering our understanding of the genetic mechanisms associated with GWAS loci in noncoding regions, because they provide a means to prioritize the potentially causal variants in haplotypes for further studies. The identification of such regulatory regions has been made possible by new technologies that have become available in the past decade that locate the DNA sites where specific proteins, including transcription factors and transcriptional coactivators, are bound (Box 3). Pioneering work using *in vivo* chromatin immunoprecipitation sequencing (ChIP-seq) for the enhancer-associated coactivator p300 in mouse embryonic heart identified >3,000 potential cardiac enhancers.[145] A subsequent study from the same laboratory using an antibody that recognizes both p300 and the closely related CBP coactivator protein mapped >6,000 potential human cardiac enhancer sequences in foetal and adult human cardiac tissue.[146]

Strictly defined patterns of transcriptional activity, such as those involved in tissue-specific gene expression, are thought to be achieved, at least in part, by co-occupancy of distinct enhancer elements by multiple transcription factors. Chromatin co-occupancy by the transcription factors GATA4, NKX-2.5, TBX5, serum response factor (SRF), and myocyte-specific enhancer factor 2A (MEF2A), for example, has been used to identify transcriptional enhancers active in heart.[147] A clear example of how such endeavours can help understand the mechanisms underlying GWAS signals came from a study[148] which overlaid the binding sites for TBX3, NKX-2.5, GATA4, and p300 that were identified by ChIP-seq in cardiac tissue. A G>A nucleotide change in SNP rs6801957 (at the *SCN5A/SCN10A* locus), was found to reside in a consensus T-box transcription factor binding site within a cardiac enhancer.[148] This G>A change reduced T-box transcription factor binding to the enhancer, affected the stimulation and repression, by TBX5 and TBX3, respectively, of a reporter in *in vitro* assays, and reduced the activity of the enhancer *in vivo*, thereby providing strong evidence that it is the causal variant within the rs6795970/rs10428132-tagged haplotype.[148] This haplotype is implicated in the regulation of PR duration[92–94] and QRS duration,[95] susceptibility to atrial fibrillation[94,138] and ischaemia-induced VF,[92] and risk of Brugada syndrome.[83] However, although this nucleotide change is expected to affect the regulation of *SCN5A* or *SCN10A* expression by TBX3 and TBX5 in human heart, data on variations in these transcripts as a function of genotype at the rs6801957 SNP site (the eQTL effect) are lacking.[148,149] Although eQTL resources are available for a variety of tissues, they are not yet available for the human heart, hindering our ability to correlate GWAS SNPs with the expression of genes as a means of deducing candidate causal genes.

Despite the strong evidence for rs6801957 in mediating the pathophysiological effect of the haplotype on which it occurs, through differences in transcription factor binding, SNP rs6795970 is also on this haplotype (r^2=0.93 with rs6801957) and encodes a nonsynonymous substitution (Ala1073Val) in the *SCN10A*-encoded sodium channel protein type 10 subunit α (also known as $Na_v1.8$). This protein was originally reported to be highly expressed in the nociceptive sensory neurons of the dorsal root ganglia and cranial sensory ganglia.[150] However, subsequent studies in mice have shown that this sodium channel subunit

is expressed in cardiac neurons,[151] the working myocardium,[92,152] and the specialized conduction system.[95,153] These data indicate a possible role for $Na_v1.8$ in cardiac electrical function. Therefore, at least some of the effect of the rs6801957/rs6795970/rs10428132-tagged haplotype at the *SCN5A/SCN10A* region could occur through alteration of sodium channel protein type 10 subunit α function (through the Ala1073Val amino acid change) or level of expression. However, the relative amounts of *SCN5A* and *SCN10A* mRNA and the proteins they encode in human heart, and the relative contributions of these two channels to the total sodium current in human cardiomyocytes, are currently completely unknown.

CONCLUSIONS

Much has been learned about the genetic underpinnings of the rare Mendelian diseases associated with SCD. These insights have been useful for clinical management. Nevertheless, the observations that some individuals do not carry mutations in the known genes, and that marked variability in clinical disease expression among mutation carriers exists, highlight the necessity to search for additional genetic factors that contribute to risk. Discovery of additional genetic factors might also help us understand the genetic basis of SCD in patients without Mendelian inheritance patterns for the disease, where progress has been very limited. In this situation, the absence of large and comprehensively-phenotyped SCD cohorts (caused by the difficulties associated with studying SCD) present a challenge for gene discovery. SCD occurs suddenly (by definition), usually unexpectedly, and VF is rapidly fatal, precluding DNA collection and phenotyping. Although GWAS have discovered many genetic loci associated with ECG traits as intermediate phenotypes of arrhythmias, these loci have yet to be linked to SCD. These issues must be addressed by the research community if we are to identify genetic risk factors for SCD. Genomic loci identified by ECG-GWAS can help to increase our understanding of the molecular underpinnings of these traits, and might also identify new pathways, the relevance of which could be tested in animal models of arrhythmia. New technologies, such as NGS and the generation of genome-wide maps of functional elements in the noncoding region of the genome, are likely to help to identify new loci and mechanisms for cardiac electrical function and SCD risk.

ACKNOWLEDGEMENTS

The authors are supported by grants from the Netherlands Organization for Scientific Research (NWO, grant ZonMW Vici 918.86.616 to H.L.T.), the Netherlands Heart Foundation (NHS2007B202 and NHS2007B010 to C.R.B.), the Center for Translational Molecular Medicine (CTMM-COHFAR project, C.R.B.) and the CardioVascular Onderzoek Nederland and Netherlands Heart Foundation project PREDICT (C.R.B. and H.L.T.). We thank Yuka Mizusawa for providing the electrocardiograms in Figure 3.

REFERENCES

1. Zipes DP, Camm a J, Borggrefe M, Buxton AE, Chaitman B, Fromer M, Gregoratos G, Klein G, Moss AJ, Myerburg RJ, Priori SG, Quinones M a, Roden DM, Silka MJ, Tracy C, Smith SC, Jacobs AK, Adams CD, Antman EM, Anderson JL, Hunt S a, Halperin JL, Nishimura R, Ornato JP, Page RL, Riegel B, Blanc J-J, Budaj A, Dean V, Deckers JW, Despres C, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo JL, Zamorano JL. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of for Practice G. *J Am Coll Cardiol*. 2006;48:e247–346.
2. De Vreede-Swagemakers JJ, Gorgels a P, Dubois-Arbouw WI, van Ree JW, Daemen MJ, Houben LG, Wellens HJ. Out-of-hospital cardiac arrest in the 1990's: a population-based study in the Maastricht area on incidence, characteristics and survival. *J Am Coll Cardiol*. 1997;30:1500–5.
3. Hulleman M, Berdowski J, de Groot JR, van Dessel PFHM, Borleffs CJW, Blom MT, Bardai A, de Cock CC, Tan HL, Tijssen JGP, Koster RW. Implantable cardioverter-defibrillators have reduced the incidence of resuscitation for out-of-hospital cardiac arrest caused by lethal arrhythmias. *Circulation*. 2012;126:815–21.
4. Olde Nordkamp LR a, Wilde A a M, Tijssen JGP, Knops RE, van Dessel PFHM, de Groot JR. The ICD for primary prevention in patients with inherited cardiac diseases: indications, use, and outcome: a comparison with secondary prevention. *Circ Arrhythm Electrophysiol*. 2013;6:91–100.
5. Kong MH, Fonarow GC, Peterson ED, Curtis AB, Hernandez AF, Sanders GD, Thomas KL, Hayes DL, Al-Khatib SM. Systematic review of the incidence of sudden cardiac death in the United States. *J Am Coll Cardiol*. 2011;57:794–801.
6. Commerce UD of. US and world population clock [Internet]. 2013;Available from: http://www.census.gov/popclock
7. Cobb LA, Fahrenbruch CE, Olsufka M, Copass MK. Changing Incidence of Out-of-Hospital Ventricular Fibrillation, 1980-2000. *JAMA*. 2002;288:3008–3013.
8. Albert CM, Chae CU, Grodstein F, Rose LM, Rexrode KM, Ruskin JN, Stampfer MJ, Manson JE. Prospective study of sudden cardiac death among women in the United States. *Circulation*. 2003;107:2096–101.
9. Myerburg RJ, Kessler KM, Castellanos a. Sudden cardiac death: epidemiology, transient risk, and intervention assessment. *Ann Intern Med*. 1993;119:1187–97.
10. Bardai A, Lamberts RJ, Blom MT, Spanjaart AM, Berdowski J, van der Staal SR, Brouwer HJ, Koster RW, Sander JW, Thijs RD, Tan HL. Epilepsy is a risk factor for sudden cardiac arrest in the general population. *PLoS One*. 2012;7:e42749.
11. Huikuri H V, Castellanos A, Myerburg RJ. Sudden death due to cardiac arrhythmias. *New Engel J Med*. 2001;345:1473–1482.
12. Zheng Z-J, Croft JB, Giles WH, Mensah G a. Sudden Cardiac Death in the United States, 1989 to 1998. *Circulation*. 2001;104:2158–2163.
13. Meyer L, Stubbs B, Fahrenbruch C, Maeda C, Harmon K, Eisenberg M, Drezner J. Incidence, causes, and survival trends from cardiovascular-related sudden cardiac arrest in children and young adults 0 to 35 years of age: a 30-year review. *Circulation*. 2012;126:1363–72.
14. Corrado D, Basso C, Thiene G. Sudden cardiac death in young people with apparently normal heart. *Cardiovasc Res*. 2001;50:399–408.
15. Tan HL, Hofman N, van Langen IM, van der Wal AC, Wilde A a M. Sudden unexplained death: heritability and diagnostic yield of cardiological and genetic examination in surviving relatives. *Circulation*. 2005;112:207–13.
16. George Jr AL. Molecular and genetic basis of sudden cardiac death. *J Clin Invest*. 2013;123:75–83.
17. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J, Goldstein BA, Stirrups K, König IR, Cazier J-B, Johansson A, Hall AS, Lee J-Y, Willer CJ, Chambers JC, Esko T, Folkersen L, Goel A, Grundberg E, Havulinna AS, Ho WK, Hopewell JC, Eriksson N, Kleber ME, Kristiansson K, Lundmark P, Lyytikäinen L-P, Rafelt S, Shungin D, Strawbridge RJ, Thorleifsson G, Tikkanen E, Van Zuydam N, Voight BF, Waite LL, Zhang W, Ziegler A, Absher D, Altshuler D, Balmforth AJ, Barroso I, Braund PS, Burgdorf C, Claudi-Boehm S, Cox D, Dimitriou M, Do R, Doney ASF, El Mokhtari N, Eriksson P, Fischer K, Fontanillas P, Franco-Cereceda A, Gigante B, Groop L, Gustafsson S, Hager J, Hallmans G, Han B-G, Hunt SE, Kang HM, Illig T, Kessler T, Knowles JW, Kolovou G, Kuusisto J, Langenberg C, Langford C, Leander K, Lokki M-L, Lundmark A, McCarthy MI, Meisinger C, Melander O, Mihailov E, Maouche S, Morris AD, Müller-Nurasyid M, Nikus K, Peden JF, Rayner NW, Rasheed A, Rosinger S, Rubin D, Rumpf MP, Schäfer A, Sivananthan M, Song C, Stewart AFR, Tan S-T, Thorgeirsson G, van der Schoot CE, Wagner PJ, Wells GA, Wild PS, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*. 2013;45:25–33.
18. Myers RH, Kiely DK, Cupples LA, Kannel WB. Parental history is an independent risk factor for coronary artery disease: the Framingham Study. *Am Heart J*. 1990;120:963–9.
19. Stamler R, Stamler J, Riedlinger WF, Algera G, Roberts RH. Family (parental) history and prevalence of hypertension. Results of a nationwide screening program. *JAMA*. 1979;241:43–6.

20. Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes*. 2000;49:2201–7.
21. Jouven X, Desnos M, Guerot C, Ducimetiere P. Predicting Sudden Death in the Population : The Paris Prospective Study I. *Circulation*. 1999;99:1978–1983.
22. Friedlander Y, Siscovick DS, Arbogast P, Psaty BM, Weinmann S, Lemaitre RN, Raghunathan TE, Cobb L a. Sudden death and myocardial infarction in first degree relatives as predictors of primary cardiac arrest. *Atherosclerosis*. 2002;162:211–6.
23. Friedlander Y, Siscovick DS, Weinmann S, Austin M a., Psaty BM, Lemaitre RN, Arbogast P, Raghunathan TE, Cobb L a. Family History as a Risk Factor for Primary Cardiac Arrest. *Circulation*. 1998;97:155–160.
24. Dekker LRC, Bezzina CR, Henriques JPS, Tanck MW, Koch KT, Alings MW, Arnold AER, de Boer M-J, Gorgels APM, Michels HR, Verkerk A, Verheugt FW a, Zijlstra F, Wilde A a M. Familial sudden death is an important risk factor for primary ventricular fibrillation: a case-control study in acute myocardial infarction patients. *Circulation*. 2006;114:1140–5.
25. Kaikkonen KS, Kortelainen M-L, Linna E, Huikuri H V. Family history and the risk of sudden cardiac death as a manifestation of an acute coronary event. *Circulation*. 2006;114:1462–7.
26. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TFC, McCarroll SA, Visscher PM. Finding the missing heritability of complex diseases. *Nature*. 2009;461:747–53.
27. Bezzina CR, Pazoki R, Bardai A, Marsman RF, de Jong JSSG, Blom MT, Scicluna BP, Jukema JW, Bindraban NR, Lichtner P, Pfeufer A, Bishopric NH, Roden DM, Meitinger T, Chugh SS, Myerburg RJ, Jouven X, Kääb S, Dekker LRC, Tan HL, Tanck MWT, Wilde A a M. Genome-wide association study identifies a susceptibility locus at 21q21 for ventricular fibrillation in acute myocardial infarction. *Nat Genet*. 2010;42:688–91.
28. Arking DE, Junttila MJ, Goyette P, Huertas-Vazquez A, Eijgelsheim M, Blom MT, Newton-Cheh C, Reinier K, Teodorescu C, Uy-Evanado A, Carter-Monroe N, Kaikkonen KS, Kortelainen M-L, Boucher G, Lagacé C, Moes A, Zhao X, Kolodgie F, Rivadeneira F, Hofman A, Witteman JCM, Uitterlinden AG, Marsman RF, Pazoki R, Bardai A, Koster RW, Dehghan A, Hwang S-J, Bhatnagar P, Post W, Hilton G, Prineas RJ, Li M, Köttgen A, Ehret G, Boerwinkle E, Coresh J, Kao WHL, Psaty BM, Tomaselli GF, Sotoodehnia N, Siscovick DS, Burke GL, Marbán E, Spooner PM, Cupples LA, Jui J, Gunson K, Kesäniemi YA, Wilde A a M, Tardif J-C, O'Donnell CJ, Bezzina CR, Virmani R, Stricker BHCH, Tan HL, Albert CM, Chakravarti A, Rioux JD, Huikuri H V, Chugh SS. Identification of a sudden cardiac death susceptibility locus at 2q24.2 through genome-wide association in European ancestry individuals. *PLoS Genet*. 2011;7:e1002158.
29. Rizzo S, Pilichou K, Thiene G, Basso C. The changing spectrum of arrhythmogenic (right ventricular) cardiomyopathy. *Cell Tissue Res*. 2012;348:319–23.
30. Watkins H, Ashrafian H, Redwood C. Inherited cardiomyopathies. *N Engl J Med*. 2011;364:1643–56.
31. Basso C, Corrado D, Marcus FI, Nava A, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. *Lancet*. 2009;373:1289–300.
32. Varnava AM, Elliott PM, Baboonian C, Davison F, Davies MJ, McKenna WJ. Hypertrophic cardiomyopathy: histopathological features of sudden death in cardiac troponin T disease. *Circulation*. 2001;104:1380–4.
33. Schober T, Huke S, Venkataraman R, Gryshchenko O, Kryshtal D, Hwang HS, Baudenbacher FJ, Knollmann BC. Myofilament Ca sensitization increases cytosolic Ca binding affinity, alters intracellular Ca homeostasis, and causes pause-dependent Ca-triggered arrhythmia. *Circ Res*. 2012;111:170–9.
34. Rizzo S, Lodder EM, Verkerk AO, Wolswinkel R, Beekman L, Pilichou K, Basso C, Remme CA, Thiene G, Bezzina CR. Intercalated disc abnormalities, reduced Na(+) current density, and conduction slowing in desmoglein-2 mutant mice prior to cardiomyopathic changes. *Cardiovasc Res*. 2012;95:409–18.
35. Cerrone M, Noorman M, Lin X, Chkourko H, Liang F-X, van der Nagel R, Hund T, Birchmeier W, Mohler P, van Veen TA, van Rijen H V, Delmar M. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. *Cardiovasc Res*. 2012;95:460–8.
36. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*. 1995;80:805–11.
37. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell*. 1995;80:795–803.
38. Schwartz PJ, Priori SG, Locati EH, Napolitano C, Cantù F, Towbin JA, Keating MT, Hammoude H, Brown AM, Chen LS. Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential responses to Na+ channel blockade and to increases in heart rate. Implications for gene-specific therapy. *Circulation*. 1995;92:3381–6.
39. Wang DW, Yazawa K, Makita N, George AL, Bennett PB. Pharmacological targeting of long QT mutant sodium channels. *J Clin Invest*. 1997;99:1714–20.
40. Vincent GM, Schwartz PJ, Denjoy I, Swan H, Bithell C, Spazzolini C, Crotti L, Piippo K, Lupoglazoff J-M, Villain E, Priori SG, Napolitano C, Zhang L. High efficacy of beta-blockers in long-QT syndrome type 1: contribution of

noncompliance and QT-prolonging drugs to the occurrence of beta-blocker treatment “failures”. *Circulation*. 2009;119:215–21.
41. Barsheshet A, Goldenberg I, O-Uchi J, Moss AJ, Jons C, Shimizu W, Wilde A a, McNitt S, Peterson DR, Zareba W, Robinson JL, Ackerman MJ, Cypress M, Gray D a, Hofman N, Kanters JK, Kaufman ES, Platonov PG, Qi M, Towbin J a, Vincent GM, Lopes CM. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to β-blocker therapy in type 1 long-QT syndrome. *Circulation*. 2012;125:1988–96.
42. Heijman J, Spätjens RLHMG, Seyen SRM, Lentink V, Kuijpers HJH, Boulet IR, de Windt LJ, David M, Volders PGA. Dominant-negative control of cAMP-dependent IKs upregulation in human long-QT syndrome type 1. *Circ Res*. 2012;110:211–9.
43. Choveau FS, Rodriguez N, Abderemane Ali F, Labro AJ, Rose T, Dahimène S, Boudin H, Le Hénaff C, Escande D, Snyders DJ, Charpentier F, Mérot J, Baró I, Loussouarn G. KCNQ1 channels voltage dependence through a voltage-dependent binding of the S4-S5 linker to the pore domain. *J Biol Chem*. 2011;286:707–16.
44. Watanabe H, Chopra N, Laver D, Hwang HS, Davies SS, Roach DE, Duff HJ, Roden DM, Wilde A a M, Knollmann BC. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat Med*. 2009;15:380–3.
45. Van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, Shimizu W, Sumitomo N, Fish F a, Bhuiyan Z a, Willems AR, van der Veen MJ, Watanabe H, Laborderie J, Haïssaguerre M, Knollmann BC, Wilde A a M. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. *J Am Coll Cardiol*. 2011;57:2244–54.
46. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, Camm a J, Ellinor PT, Gollob M, Hamilton R, Hershberger RE, Judge DP, Le Marec H, McKenna WJ, Schulze-Bahr E, Semsarian C, Towbin J a, Watkins H, Wilde A, Wolpert C, Zipes DP. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Europace*. 2011;13:1077–109.
47. Wilde AAM, Behr ER. Genetic testing for inherited cardiac disease. *Nat Rev Cardiol*. 2013;10:571–83.
48. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491:56–65.
49. Consortium 1000 Genomes Project. 1000 Genomes [online] [Internet]. 2013;Available from: http://www.1000genomes.org/
50. Tennessen JA, Bigham AW, O’Connor TD, Fu W, Kenny EE, Gravel S, McGee S, Do R, Liu X, Jun G, Kang HM, Jordan D, Leal SM, Gabriel S, Rieder MJ, Abecasis G, Altshuler D, Nickerson DA, Boerwinkle E, Sunyaev S, Bustamante CD, Bamshad MJ, Akey JM. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science*. 2012;337:64–9.
51. NHLBI GO Exome Sequencing Project (ESP). Exome Variant Server [Internet]. 2013;Available from: http://evs.gs.washington.edu/EVS/
52. Jabbari J, Jabbari R, Nielsen MW, Holst AG, Nielsen JB, Haunsø S, Tfelt-Hansen J, Svendsen JH, Olesen MS. New Exome Data Question the Pathogenicity of Genetic Variants Previously Associated with Catecholaminergic Polymorphic Ventricular Tachycardia. *Circ Cardiovasc Genet*. 2013;
53. Risgaard B, Jabbari R, Refsgaard L, Holst A, Haunsø S, Sadjadieh A, Winkel B, Olesen M, Tfelt-Hansen J. High prevalence of genetic variants previously associated with Brugada syndrome in new exome data. *Clin Genet*. 2013;
54. Rehm HL. Disease-targeted sequencing: a cornerstone in the clinic. *Nat Rev Genet*. 2013;14:295–300.
55. Cooper GM, Shendure J. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. *Nat Rev Genet*. 2011;12:628–40.
56. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4:1073–81.
57. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–9.
58. Ware JS, Walsh R, Cunningham F, Birney E, Cook S a. Paralogous annotation of disease-causing variants in long QT syndrome genes. *Hum Mutat*. 2012;33:1188–91.
59. Cooper GM, Stone EA, Asimenos G, Green ED, Batzoglou S, Sidow A. Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res*. 2005;15:901–13.
60. Katsanis SH, Katsanis N. Molecular genetic testing and the future of clinical genomics. *Nat Rev Genet*. 2013;14:415–26.
61. Boycott KM, Vanstone MR, Bulman DE, Mackenzie AE. Rare-disease genetics in the era of next-generation sequencing: discovery to translation. *Nat Rev Genet*. 2013;14:681–91.
62. Crotti L, Johnson CN, Graf E, De Ferrari GM, Cuneo BF, Ovadia M, Papagiannis J, Feldkamp MD, Rathi SG, Kunic JD, Pedrazzini M, Wieland T, Lichtner P, Beckmann B-M, Clark T, Shaffer C, Benson DW, Kääb S, Meitinger T, Strom TM, Chazin WJ, Schwartz PJ, George AL. Calmodulin mutations associated with recurrent cardiac arrest

in infants. *Circulation*. 2013;127:1009–17.
63. Lynch M. Rate, molecular spectrum, and consequences of human mutation. *Proc Natl Acad Sci U S A*. 2010;107:961–8.
64. Marsman RF, Barc J, Beekman L, Alders M, Dooijes D, van den Wijngaard A, Ratbi I, Sefiani A, Bhuiyan ZA, Wilde AAM, Bezzina CR. A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. *J Am Coll Cardiol*. 2014;63:259-66
65. Boczek NJ, Best JM, Tester DJ, Giudicessi JR, Middha S, Evans JM, Kamp TJ, Ackerman MJ. Exome sequencing and systems biology converge to identify novel mutations in the L-type calcium channel, CACNA1C, linked to autosomal dominant long QT syndrome. *Circ Cardiovasc Genet*. 2013;6:279–89.
66. Schwartz PJ. The long QT syndrome. In: Kulbertus HE, Wellens HJJ, editors. Sudden death. The Hague/Boston/London: Martinus Nijhoff Publishers; 1980. p. 358–378.
67. Bezzina C, Veldkamp MW, van Den Berg MP, Postma A V, Rook MB, Viersma JW, van Langen IM, Tan-Sindhunata G, Bink-Boelkens MT, van Der Hout AH, Mannens MM, Wilde AA. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. *Circ Res*. 1999;85:1206–13.
68. Crotti L, Monti MC, Insolia R, Peljto A, Goosen A, Brink PA, Greenberg DA, Schwartz PJ, George AL. NOS1AP is a genetic modifier of the long-QT syndrome. *Circulation*. 2009;120:1657–63.
69. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. *Circulation*. 1999;99:529–33.
70. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Brignole M, Giordano U, Giovannini T, Menozzi C, Bloise R, Crotti L, Terreni L, Schwartz PJ. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: A prospective evaluation of 52 families. *Circulation*. 2000;102:2509–15.
71. Brink PA, Crotti L, Corfield V, Goosen A, Durrheim G, Hedley P, Heradien M, Geldenhuys G, Vanoli E, Bacchini S, Spazzolini C, Lundquist AL, Roden DM, George AL, Schwartz PJ. Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. *Circulation*. 2005;112:2602–10.
72. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D. Risk stratification in the long-QT syndrome. *N Engl J Med*. 2003;348:1866–74.
73. Postema PG, Christiaans I, Hofman N, Alders M, Koopmann TT, Bezzina CR, Loh P, Zeppenfeld K, Volders PGA, Wilde AAM. Founder mutations in the Netherlands: familial idiopathic ventricular fibrillation and DPP6. *Neth Heart J*. 2011;19:290–6.
74. Schwartz PJ, Vanoli E, Crotti L, Spazzolini C, Ferrandi C, Goosen A, Hedley P, Heradien M, Bacchini S, Turco A, La Rovere MT, Bartoli A, George AL, Brink PA. Neural control of heart rate is an arrhythmia risk modifier in long QT syndrome. *J Am Coll Cardiol*. 2008;51:920–9.
75. Roden DM. Drug-induced prolongation of the QT interval. *N Engl J Med*. 2004;350:1013–22.
76. Schwartz PJ, Priori SG, Napolitano C. How really rare are rare diseases?: the intriguing case of independent compound mutations in the long QT syndrome. *J Cardiovasc Electrophysiol*. 2003;14:1120–1.
77. Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound mutations: a common cause of severe long-QT syndrome. *Circulation*. 2004;109:1834–41.
78. Giudicessi JR, Ackerman MJ. Prevalence and potential genetic determinants of sensorineural deafness in KCNQ1 homozygosity and compound heterozygosity. *Circ Cardiovasc Genet*. 2013;6:193–200.
79. Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, Wilde AAM, Ackerman MJ. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm*. 2009;6:1297–303.
80. Moss AJ, Shimizu W, Wilde AAM, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, Hofman N, Seth R, Kamakura S, Miyamoto Y, Goldenberg I, Andrews ML, McNitt S. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation*. 2007;115:2481–9.
81. Shimizu W, Moss AJ, Wilde AAM, Towbin JA, Ackerman MJ, January CT, Tester DJ, Zareba W, Robinson JL, Qi M, Vincent GM, Kaufman ES, Hofman N, Noda T, Kamakura S, Miyamoto Y, Shah S, Amin V, Goldenberg I, Andrews ML, McNitt S. Genotype-phenotype aspects of type 2 long QT syndrome. *J Am Coll Cardiol*. 2009;54:2052–62.
82. Tomás M, Napolitano C, De Giuli L, Bloise R, Subirana I, Malovini A, Bellazzi R, Arking DE, Marban E, Chakravarti A, Spooner PM, Priori SG. Polymorphisms in the NOS1AP gene modulate QT interval duration and risk of arrhythmias in the long QT syndrome. *J Am Coll Cardiol*. 2010;55:2745–52.
83. Bezzina CR, Barc J, Mizusawa Y, Remme CA, Gourraud J-B, Simonet F, Verkerk AO, Schwartz PJ, Crotti L, Dagradi F, Guicheney P, Fressart V, Leenhardt A, Antzelevitch C, Bartkowiak S, Schulze-Bahr E, Zumhagen S, Behr ER, Bastiaenen R, Tfelt-Hansen J, Olesen MS, Kääb S, Beckmann BM, Weeke P, Watanabe H, Endo N, Minamino T, Horie M, Ohno S, Hasegawa K, Makita N, Nogami A, Shimizu W, Aiba T, Froguel P, Balkau B, Lantieri O, Torchio M, Wiese C, Weber D, Wolswinkel R, Coronel R, Boukens BJ, Bézieau S, Charpentier E, Chatel S, Despres A, Gros F, Kyndt F, Lecointe S, Lindenbaum P, Portero V, Violleau J, Gessler M, Tan HL, Roden DM, Christoffels VM, Le Marec H, Wilde AA, Probst V, Schott J-J, Dina C, Redon R. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. *Nat Genet*.

2013;9:1044–9.
84. Duchatelet S, Crotti L, Peat RA, Denjoy I, Itoh H, Berthet M, Ohno S, Fressart V, Monti MC, Crocamo C, Pedrazzini M, Dagradi F, Vicentini A, Klug D, Brink PA, Goosen A, Swan H, Toivonen L, Lahtinen AM, Kontula K, Shimizu W, Horie M, George AL, Trégouët D-A, Guicheney P, Schwartz PJ. Identification of a KCNQ1 Polymorphism Acting as a Protective Modifier Against Arrhythmic Risk in Long-QT Syndrome. *Circ Cardiovasc Genet*. 2013;6:354–61.
85. Arking DE, Pfeufer A, Post W, Kao WHL, Newton-Cheh C, Ikeda M, West K, Kashuk C, Akyol M, Perz S, Jalilzadeh S, Illig T, Gieger C, Guo C-Y, Larson MG, Wichmann HE, Marbán E, O'Donnell CJ, Hirschhorn JN, Kääb S, Spooner PM, Meitinger T, Chakravarti A. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet*. 2006;38:644–51.
86. Pfeufer A, Sanna S, Arking DE, Müller M, Gateva V, Fuchsberger C, Ehret GB, Orrú M, Pattaro C, Köttgen A, Perz S, Usala G, Barbalic M, Li M, Pütz B, Scuteri A, Prineas RJ, Sinner MF, Gieger C, Najjar SS, Kao WHL, Mühleisen TW, Dei M, Happle C, Möhlenkamp S, Crisponi L, Erbel R, Jöckel K-H, Naitza S, Steinbeck G, Marroni F, Hicks A a, Lakatta E, Müller-Myhsok B, Pramstaller PP, Wichmann H-E, Schlessinger D, Boerwinkle E, Meitinger T, Uda M, Coresh J, Kääb S, Abecasis GR, Chakravarti A. Common variants at ten loci modulate the QT interval duration in the QTSCD Study. *Nat Genet*. 2009;41:407–14.
87. Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PIW, Yin X, Estrada K, Bis JC, Marciante K, Rivadeneira F, Noseworthy P a, Sotoodehnia N, Smith NL, Rotter JI, Kors J a, Witteman JCM, Hofman A, Heckbert SR, O'Donnell CJ, Uitterlinden AG, Psaty BM, Lumley T, Larson MG, Stricker BHC. Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat Genet*. 2009;41:399–406.
88. Amin AS, Giudicessi JR, Tijsen AJ, Spanjaart AM, Reckman YJ, Klemens C a, Tanck MW, Kapplinger JD, Hofman N, Sinner MF, Müller M, Wijnen WJ, Tan HL, Bezzina CR, Creemers EE, Wilde A a M, Ackerman MJ, Pinto YM. Variants in the 3' untranslated region of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity in patients with type 1 long QT syndrome in an allele-specific manner. *Eur Heart J*. 2012;33:714–23.
89. Serre D, Gurd S, Ge B, Sladek R, Sinnett D, Harmsen E, Bibikova M, Chudin E, Barker DL, Dickinson T, Fan J-B, Hudson TJ. Differential allelic expression in the human genome: a robust approach to identify genetic and epigenetic cis-acting mechanisms regulating gene expression. *PLoS Genet*. 2008;4:e1000006.
90. Mizusawa Y, Wilde AAM. Brugada syndrome. *Circ Arrhythm Electrophysiol*. 2012;5:606–16.
91. Crotti L, Marcou CA, Tester DJ, Castelletti S, Giudicessi JR, Torchio M, Medeiros-Domingo A, Simone S, Will ML, Dagradi F, Schwartz PJ, Ackerman MJ. Spectrum and prevalence of mutations involving BrS1- through BrS12-susceptibility genes in a cohort of unrelated patients referred for Brugada syndrome genetic testing: implications for genetic testing. *J Am Coll Cardiol*. 2012;60:1410–8.
92. Chambers JC, Zhao J, Terracciano CMN, Bezzina CR, Zhang W, Kaba R, Navaratnarajah M, Lotlikar A, Sehmi JS, Kooner MK, Deng G, Siedlecka U, Parasramka S, El-Hamamsy I, Wass MN, Dekker LRC, de Jong JSSG, Sternberg MJE, McKenna W, Severs NJ, de Silva R, Wilde A a M, Anand P, Yacoub M, Scott J, Elliott P, Wood JN, Kooner JS. Genetic variation in SCN10A influences cardiac conduction. *Nat Genet*. 2010;42:149–52.
93. Holm H, Gudbjartsson DF, Arnar DO, Thorleifsson G, Thorgeirsson G, Stefansdottir H, Gudjonsson SA, Jonasdottir A, Mathiesen EB, Njølstad I, Nyrnes A, Wilsgaard T, Hald EM, Hveem K, Stoltenberg C, Løchen M-L, Kong A, Thorsteinsdottir U, Stefansson K. Several common variants modulate heart rate, PR interval and QRS duration. *Nat Genet*. 2010;42:117–22.
94. Pfeufer A, van Noord C, Marciante KD, Arking DE, Larson MG, Smith AV, Tarasov K V, Müller M, Sotoodehnia N, Sinner MF, Verwoert GC, Li M, Kao WHL, Köttgen A, Coresh J, Bis JC, Psaty BM, Rice K, Rotter JI, Rivadeneira F, Hofman A, Kors J a, Stricker BHC, Uitterlinden AG, van Duijn CM, Beckmann BM, Sauter W, Gieger C, Lubitz S a, Newton-Cheh C, Wang TJ, Magnani JW, Schnabel RB, Chung MK, Barnard J, Smith JD, Van Wagoner DR, Vasan RS, Aspelund T, Eiriksdottir G, Harris TB, Launer LJ, Najjar SS, Lakatta E, Schlessinger D, Uda M, Abecasis GR, Müller-Myhsok B, Ehret GB, Boerwinkle E, Chakravarti A, Soliman EZ, Lunetta KL, Perz S, Wichmann H-E, Meitinger T, Levy D, Gudnason V, Ellinor PT, Sanna S, Kääb S, Witteman JCM, Alonso A, Benjamin EJ, Heckbert SR. Genome-wide association study of PR interval. *Nat Genet*. 2010;42:153–9.
95. Sotoodehnia N, Isaacs A, de Bakker PIW, Dörr M, Newton-Cheh C, Nolte IM, van der Harst P, Müller M, Eijgelsheim M, Alonso A, Hicks AA, Padmanabhan S, Hayward C, Smith AV, Polasek O, Giovannone S, Fu J, Magnani JW, Marciante KD, Pfeufer A, Gharib SA, Teumer A, Li M, Bis JC, Rivadeneira F, Aspelund T, Köttgen A, Johnson T, Rice K, Sie MPS, Wang YA, Klopp N, Fuchsberger C, Wild SH, Mateo Leach I, Estrada K, Völker U, Wright AF, Asselbergs FW, Qu J, Chakravarti A, Sinner MF, Kors JA, Petersmann A, Harris TB, Soliman EZ, Munroe PB, Psaty BM, Oostra BA, Cupples LA, Perz S, de Boer RA, Uitterlinden AG, Völzke H, Spector TD, Liu F-Y, Boerwinkle E, Dominiczak AF, Rotter JI, van Herpen G, Levy D, Wichmann H-E, van Gilst WH, Witteman JCM, Kroemer HK, Kao WHL, Heckbert SR, Meitinger T, Hofman A, Campbell H, Folsom AR, van Veldhuisen DJ, Schwienbacher C, O'Donnell CJ, Volpato CB, Caulfield MJ, Connell JM, Launer L, Lu X, Franke L, Fehrmann RSN, te Meerman G, Groen HJM, Weersma RK, van den Berg LH, Wijmenga C, Ophoff RA, Navis G, Rudan I, Snieder H, Wilson JF, Pramstaller PP, Siscovick DS, Wang TJ, Gudnason V, van Duijn CM, Felix SB, Fishman GI, et al. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat Genet*. 2010;42:1068–76.

96. Fischer A, Gessler M. Hey genes in cardiovascular development. *Trends Cardiovasc Med*. 2003;13:221–6.
97. Albert CM, MacRae C a, Chasman DI, VanDenburgh M, Buring JE, Manson JE, Cook NR, Newton-Cheh C. Common variants in cardiac ion channel genes are associated with sudden cardiac death. *Circ Arrhythm Electrophysiol*. 2010;3:222–9.
98. Westaway SK, Reinier K, Huertas-Vazquez A, Evanado A, Teodorescu C, Navarro J, Sinner MF, Gunson K, Jui J, Spooner P, Kaab S, Chugh SS. Common variants in CASQ2, GPD1L, and NOS1AP are significantly associated with risk of sudden death in patients with coronary artery disease. *Circ Cardiovasc Genet*. 2011;4:397–402.
99. Stecker EC, Sono M, Wallace E, Gunson K, Jui J, Chugh SS. Allelic variants of SCN5A and risk of sudden cardiac arrest in patients with coronary artery disease. *Hear Rhythm*. 2006;3:697–700.
100. Burke A, Creighton W, Mont E, Li L, Hogan S, Kutys R, Fowler D, Virmani R. Role of SCN5A Y1102 polymorphism in sudden cardiac death in blacks. *Circulation*. 2005;112:798–802.
101. Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, Cappuccio FP, Sagnella G a, Kass RS, Keating MT. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science*. 2002;297:1333–6.
102. Albert CM, Nam EG, Rimm EB, Jin HW, Hajjar RJ, Hunter DJ, MacRae C a, Ellinor PT. Cardiac sodium channel gene variants and sudden cardiac death in women. *Circulation*. 2008;117:16–23.
103. Crotti L, Hu D, Barajas-Martinez H, De Ferrari GM, Oliva A, Insolia R, Pollevick GD, Dagradi F, Guerchicoff A, Greco F, Schwartz PJ, Viskin S, Antzelevitch C. Torsades de pointes following acute myocardial infarction: evidence for a deadly link with a common genetic variant. *Heart Rhythm*. 2012;9:1104–12.
104. Sotoodehnia N, Siscovick DS, Vatta M, Psaty BM, Tracy RP, Towbin JA, Lemaitre RN, Rea TD, Durda JP, Chang JM, Lumley TS, Kuller LH, Burke GL, Heckbert SR. Beta2-adrenergic receptor genetic variants and risk of sudden cardiac death. *Circulation*. 2006;113:1842–8.
105. Tseng ZH, Aouizerat BE, Pawlikowska L, Vittinghoff E, Lin F, Whiteman D, Poon A, Herrington D, Howard TD, Varosy PD, Hulley SB, Malloy M, Kane J, Kwok P-Y, Olgin JE. Common beta-adrenergic receptor polymorphisms are not associated with risk of sudden cardiac death in patients with coronary artery disease. *Heart Rhythm*. 2008;5:814–21.
106. Gavin MC, Newton-Cheh C, Gaziano JM, Cook NR, VanDenburgh M, Albert CM. A common variant in the β2-adrenergic receptor and risk of sudden cardiac death. *Heart Rhythm*. 2011;8:704–10.
107. Mikkelsson J, Perola M, Penttila a., Karhunen PJ. Platelet Glycoprotein Ib HPA-2 Met/VNTR B Haplotype as a Genetic Predictor of Myocardial Infarction and Sudden Cardiac Death. *Circulation*. 2001;104:876–880.
108. Mikkelsson J, Perola M, Laippala P, Penttilä a, Karhunen PJ. Glycoprotein IIIa Pl(A1/A2) polymorphism and sudden cardiac death. *J Am Coll Cardiol*. 2000;36:1317–23.
109. Sotoodehnia N, Li G, Johnson CO, Lemaitre RN, Rice KM, Rea TD, Siscovick DS. Genetic variation in angiotensin-converting enzyme-related pathways associated with sudden cardiac arrest risk. *Heart Rhythm*. 2009;6:1306–14.
110. Huertas-Vazquez A, Teodorescu C, Reinier K, Uy-Evanado A, Chugh H, Jerger K, Ayala J, Gunson K, Jui J, Newton-Cheh C, Albert CM, Chugh SS. A common missense variant in the neuregulin1 gene is associated with both schizophrenia and sudden cardiac death. *Hear Rhythm*. 2013;10:994–8.
111. Newton-Cheh C, Cook NR, VanDenburgh M, Rimm EB, Ridker PM, Albert CM. A common variant at 9p21 is associated with sudden and arrhythmic cardiac death. *Circulation*. 2009;120:2062–8.
112. Voight BF, Kang HM, Ding J, Palmer CD, Sidore C, Chines PS, Burtt NP, Fuchsberger C, Li Y, Erdmann J, Frayling TM, Heid IM, Jackson AU, Johnson T, Kilpeläinen TO, Lindgren CM, Morris AP, Prokopenko I, Randall JC, Saxena R, Soranzo N, Speliotes EK, Teslovich TM, Wheeler E, Maguire J, Parkin M, Potter S, Rayner NW, Robertson N, Stirrups K, Winckler W, Sanna S, Mulas A, Nagaraja R, Cucca F, Barroso I, Deloukas P, Loos RJF, Kathiresan S, Munroe PB, Newton-Cheh C, Pfeufer A, Samani NJ, Schunkert H, Hirschhorn JN, Altshuler D, McCarthy MI, Abecasis GR, Boehnke M. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS Genet*. 2012;8:e1002793.
113. Huertas-Vazquez A, Nelson CP, Guo X, Reinier K, Uy-Evanado A, Teodorescu C, Ayala J, Jerger K, Chugh H, Wtccc, Braund PS, Deloukas P, Hall AS, Balmforth AJ, Jones M, Taylor KD, Pulit SL, Newton-Cheh C, Gunson K, Jui J, Rotter JI, Albert CM, Samani NJ, Chugh SS. Novel loci associated with increased risk of sudden cardiac death in the context of coronary artery disease. *PLoS One*. 2013;8:e59905.
114. Arking DE, Reinier K, Post W, Jui J, Hilton G, O'Connor A, Prineas RJ, Boerwinkle E, Psaty BM, Tomaselli GF, Rea T, Sotoodehnia N, Siscovick DS, Burke GL, Marban E, Spooner PM, Chakravarti A, Chugh SS. Genome-wide association study identifies GPC5 as a novel genetic locus protective against sudden cardiac arrest. *PLoS One*. 2010;5:e9879.
115. Aouizerat BE, Vittinghoff E, Musone SL, Pawlikowska L, Kwok P-Y, Olgin JE, Tseng ZH. GWAS for discovery and replication of genetic loci associated with sudden cardiac arrest in patients with coronary artery disease. *BMC Cardiovasc Disord*. 2011;11:29.
116. Noutsias M, Fechner H, Jonge H De, Wang X, Dekkers D, Houtsmuller AB, Pauschinger M, Bergelson J, Warraich R, Yacoub M, Hetzer R, Lamers J, Schultheiss H, Poller W. Human coxsackie-adenovirus receptor is colocalized

with integrins αvβ3 and αvβ5 on the cardiomyocyte sarcolemma and upregulated in dilated cardiomyopathy: implications for cardiotropic viral infections. *Circulation*. 2001;104:275–280.
117. Lim B, Xiong D, Dorner A, Youn T, Yung A, Liu TI, Gu Y, Dalton ND, Wright AT, Evans SM, Chen J, Peterson KL, Mcculloch AD, Yajima T, Knowlton KU. Coxsackievirus and adenovirus receptor (CAR) mediates atrioventricular-node function and connexin 45 localization in the murine heart. *J Clin Invest*. 2008;118:2758–2770.
118. Lisewski U, Shi Y, Wrackmeyer U, Fischer R, Chen C, Schirdewan A, Jüttner R, Rathjen F, Poller W, Radke MH, Gotthardt M. The tight junction protein CAR regulates cardiac conduction and cell-cell communication. *J Exp Med*. 2008;205:2369–79.
119. Bugert P, Elmas E, Stach K, Weiss C, Kälsch T, Dobrev D, Borggrefe M. No evidence for an association between the rs2824292 variant at chromosome 21q21 and ventricular fibrillation during acute myocardial infarction in a German population. *Clin Chem Lab Med*. 2011;49:1237–9.
120. Park J-H, Wacholder S, Gail MH, Peters U, Jacobs KB, Chanock SJ, Chatterjee N. Estimation of effect size distribution from genome-wide association studies and implications for future discoveries. *Nat Genet*. 2010;42:570–5.
121. Kolder ICRM, Tanck MWT, Bezzina CR. Common genetic variation modulating cardiac ECG parameters and susceptibility to sudden cardiac death. *J Mol Cell Cardiol*. 2012;52:620–9.
122. Straus SMJM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MCJM, Stricker BHC, Witteman JCM. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J Am Coll Cardiol*. 2006;47:362–7.
123. Schwartz PJ, Wolf S. QT interval prolongation as predictor of sudden death in patients with myocardial infarction. *Circulation*. 1978;57:1074–7.
124. Chugh SS, Reinier K, Singh T, Uy-Evanado A, Socoteanu C, Peters D, Mariani R, Gunson K, Jui J. Determinants of prolonged QT interval and their contribution to sudden death risk in coronary artery disease: the Oregon Sudden Unexpected Death Study. *Circulation*. 2009;119:663–70.
125. Kashani A, Barold SS. Significance of QRS complex duration in patients with heart failure. *J Am Coll Cardiol*. 2005;46:2183–92.
126. Zimetbaum PJ, Buxton AE, Batsford W, Fisher JD, Hafley GE, Lee KL, O'Toole MF, Page RL, Reynolds M, Josephson ME. Electrocardiographic predictors of arrhythmic death and total mortality in the multicenter unsustained tachycardia trial. *Circulation*. 2004;110:766–9.
127. Singh JP, Larson MG, O'Donnell CJ, Tsuji H, Evans JC, Levy D. Heritability of heart rate variability: the Framingham Heart Study. *Circulation*. 1999;99:2251–4.
128. Friedlander Y, Lapidos T, Sinnreich R, Kark JD. Genetic and environmental sources of QT interval variability in Israeli families: the kibbutz settlements family study. *Clin Genet*. 1999;56:200–9.
129. Den Hoed M, Eijgelsheim M, Esko T, Brundel BJJM, Peal DS, Evans DM, Nolte IM, Segrè A V, Holm H, Handsaker RE, Westra H-J, Johnson T, Isaacs A, Yang J, Lundby A, Zhao JH, Kim YJ, Go MJ, Almgren P, Bochud M, Boucher G, Cornelis MC, Gudbjartsson D, Hadley D, van der Harst P, Hayward C, den Heijer M, Igl W, Jackson AU, Kutalik Z, Luan J, Kemp JP, Kristiansson K, Ladenvall C, Lorentzon M, Montasser ME, Njajou OT, O'Reilly PF, Padmanabhan S, St Pourcain B, Rankinen T, Salo P, Tanaka T, Timpson NJ, Vitart V, Waite L, Wheeler W, Zhang W, Draisma HHM, Feitosa MF, Kerr KF, Lind PA, Mihailov E, Onland-Moret NC, Song C, Weedon MN, Xie W, Yengo L, Absher D, Albert CM, Alonso A, Arking DE, de Bakker PIW, Balkau B, Barlassina C, Benaglio P, Bis JC, Bouatia-Naji N, Brage S, Chanock SJ, Chines PS, Chung M, Darbar D, Dina C, Dörr M, Elliott P, Felix SB, Fischer K, Fuchsberger C, de Geus EJC, Goyette P, Gudnason V, Harris TB, Hartikainen A-L, Havulinna AS, Heckbert SR, Hicks AA, Hofman A, Holewijn S, Hoogstra-Berends F, Hottenga J-J, Jensen MK, Johansson A, Junttila J, Kääb S, Kanon B, Ketkar S, Khaw K-T, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. *Nat Genet*. 2013;45:621–31.
130. Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban H-J, Yoon D, Lee MH, Kim D-J, Park MSM, Cha S-H, Kim J-W, Han B-G, Min H, Ahn Y, Han HR, Jang H-Y, Cho EY, Lee J-KJ-EJ-YJ-K, Cho NH, Shin C, Park T, Park JW, Cardon L, Clarke G, McCarthy MI, Oh B, Kim H-L. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet*. 2009;41:527–34.
131. Eijgelsheim M, Newton-Cheh C, Sotoodehnia N, de Bakker PIW, Müller M, Morrison AC, Smith A V, Isaacs A, Sanna S, Dörr M, Navarro P, Fuchsberger C, Nolte IM, de Geus EJC, Estrada K, Hwang S-J, Bis JC, Rückert I-M, Alonso A, Launer LJ, Hottenga JJ, Rivadeneira F, Noseworthy PA, Rice KM, Perz S, Arking DE, Spector TD, Kors JA, Aulchenko YS, Tarasov K V, Homuth G, Wild SH, Marroni F, Gieger C, Licht CM, Prineas RJ, Hofman A, Rotter JI, Hicks AA, Ernst F, Najjar SS, Wright AF, Peters A, Fox ER, Oostra BA, Kroemer HK, Couper D, Völzke H, Campbell H, Meitinger T, Uda M, Witteman JCM, Psaty BM, Wichmann H-E, Harris TB, Kääb S, Siscovick DS, Jamshidi Y, Uitterlinden AG, Folsom AR, Larson MG, Wilson JF, Penninx BW, Snieder H, Pramstaller PP, van Duijn CM, Lakatta EG, Felix SB, Gudnason V, Pfeufer A, Heckbert SR, Stricker BHC, Boerwinkle E, O'Donnell CJ. Genome-wide association analysis identifies multiple loci related to resting heart rate. *Hum Mol Genet*. 2010;19:3885–94.
132. Nolte IM, Wallace C, Newhouse SJ, Waggott D, Fu J, Soranzo N, Gwilliam R, Deloukas P, Savelieva I, Zheng

D, Dalageorgou C, Farrall M, Samani NJ, Connell J, Brown M, Dominiczak A, Lathrop M, Zeggini E, Wain L V, Newton-Cheh C, Eijgelsheim M, Rice K, de Bakker PIW, Pfeufer A, Sanna S, Arking DE, Asselbergs FW, Spector TD, Carter ND, Jeffery S, Tobin M, Caulfield M, Snieder H, Paterson AD, Munroe PB, Jamshidi Y. Common genetic variation near the phospholamban gene is associated with cardiac repolarisation: meta-analysis of three genome-wide association studies. *PLoS One*. 2009;4:e6138.

133. Smith JG, Magnani JW, Palmer C, Meng YA, Soliman EZ, Musani SK, Kerr KF, Schnabel RB, Lubitz SA, Sotoodehnia N, Redline S, Pfeufer A, Müller M, Evans DS, Nalls MA, Liu Y, Newman AB, Zonderman AB, Evans MK, Deo R, Ellinor PT, Paltoo DN, Newton-Cheh C, Benjamin EJ, Mehra R, Alonso A, Heckbert SR, Fox ER. Genome-wide association studies of the PR interval in African Americans. *PLoS Genet*. 2011;7:e1001304.
134. Marroni F, Pfeufer A, Aulchenko YS, Franklin CS, Isaacs A, Pichler I, Wild SH, Oostra BA, Wright AF, Campbell H, Witteman JC, Kääb S, Hicks AA, Gyllensten U, Rudan I, Meitinger T, Pattaro C, van Duijn CM, Wilson JF, Pramstaller PP. A genome-wide association scan of RR and QT interval duration in 3 European genetically isolated populations: the EUROSPAN project. *Circ Cardiovasc Genet*. 2009;2:322–8.
135. Kao WHL, Arking DE, Post W, Rea TD, Sotoodehnia N, Prineas RJ, Bishe B, Doan BQ, Boerwinkle E, Psaty BM, Tomaselli GF, Coresh J, Siscovick DS, Marbán E, Spooner PM, Burke GL, Chakravarti A. Genetic variations in nitric oxide synthase 1 adaptor protein are associated with sudden cardiac death in US white community-based populations. *Circulation*. 2009;119:940–51.
136. Noseworthy P a, Havulinna AS, Porthan K, Lahtinen AM, Jula A, Karhunen PJ, Perola M, Oikarinen L, Kontula KK, Salomaa V, Newton-Cheh C. Common genetic variants, QT interval, and sudden cardiac death in a Finnish population-based study. *Circ Cardiovasc Genet*. 2011;4:305–11.
137. Pazoki R, de Jong JSSG, Marsman RF, Bruinsma N, Dekker LRC, Wilde A a M, Bezzina CR, Tanck MWT. SNPs identified as modulators of ECG traits in the general population do not markedly affect ECG traits during acute myocardial infarction nor ventricular fibrillation risk in this condition. *PLoS One*. 2013;8:e57216.
138. Ritchie MD, Denny JC, Zuvich RL, Crawford DC, Schildcrout JS, Bastarache L, Ramirez AH, Mosley JD, Pulley JM, Basford M a, Bradford Y, Rasmussen L V, Pathak J, Chute CG, Kullo IJ, McCarty C a, Chisholm RL, Kho AN, Carlson CS, Larson EB, Jarvik GP, Sotoodehnia N, Manolio T a, Li R, Masys DR, Haines JL, Roden DM. Genome- and phenome-wide analyses of cardiac conduction identifies markers of arrhythmia risk. *Circulation*. 2013;127:1377–85.
139. Janse MJ, Wit AL. Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischemia and infarction. *Physiol Rev*. 1989;69:1049–1169.
140. Nattel S, Maguy A, Le Bouter S, Yeh Y-H. Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. *Physiol Rev*. 2007;87:425–56.
141. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci U S A*. 2009;106:9362–7.
142. Freedman M, Monteiro A, Gayther S, Coetzee G, Risch A, Plass C, Casey G, De Biasi M, Carlson C, Duggan D, James M, Liu P, Tichelaar J, Vikis H, You M, Mills I. Principles for the post-GWAS functional characterisation of risk loci. *Nat Preced*. 2011;
143. Kathiresan S, Srivastava D. Genetics of Human Cardiovascular Disease. *Cell*. 2012;148:1242–1257.
144. Dunham I, Kundaje A, Aldred SF, Collins PJ, Davis CA, Doyle F, Epstein CB, Frietze S, Harrow J, Kaul R, Khatun J, Lajoie BR, Landt SG, Lee B-KB-K, Pauli F, Rosenbloom KR, Sabo P, Safi A, Sanyal A, Shoresh N, Simon JM, Song L, Trinklein ND, Altshuler RC, Birney E, Brown JB, Cheng C, Djebali S, Dong X, Ernst J, Furey TS, Gerstein M, Giardine B, Greven M, Hardison RC, Harris RS, Herrero J, Hoffman MM, Iyer S, Kelllis M, Kheradpour P, Lassmann T, Li Q, Lin X, Marinov GK, Merkel A, Mortazavi A, Parker SCJSL, Reddy TE, Rozowsky J, Schlesinger F, Thurman RE, Wang J, Ward LD, Whitfield TW, Wilder SP, Wu W, Xi HS, Yip KY, Zhuang J, Bernstein BE, Green ED, Gunter C, Snyder M, Pazin MJ, Lowdon RF, Dillon LAL, Adams LB, Kelly CJ, Zhang J, Wexler JR, Good PJ, Feingold EA, Crawford GE, Dekker J, Elinitski L, Farnham PJ, Giddings MC, Gingeras TR, Guigó R, Hubbard TJTJ, Kellis M, Kent WJ, Lieb JD, Margulies EH, Myers RM, Starnatoyannopoulos JA, Tennebaum SA, Weng Z, White KP, Wold B, Yu Y, Wrobel J, Risk BA, Gunawardena HP, Kuiper HC, Maier CW, Xie L, et al. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489:57–74.
145. Visel A, Blow MJ, Li Z, Zhang T, Akiyama J a, Holt A, Plajzer-Frick I, Shoukry M, Wright C, Chen F, Afzal V, Ren B, Rubin EM, Pennacchio L a. ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature*. 2009;457:854–8.
146. May D, Blow MJ, Kaplan T, McCulley DJ, Jensen BC, Akiyama J a, Holt A, Plajzer-Frick I, Shoukry M, Wright C, Afzal V, Simpson PC, Rubin EM, Black BL, Bristow J, Pennacchio L a, Visel A. Large-scale discovery of enhancers from human heart tissue. *Nat Genet*. 2012;44:89–93.
147. He A, Won S, Ma Q, Pu WT. Co-occupancy by multiple cardiac transcription factors identifies transcriptional enhancers active in heart. *Proc Natl Acad Sci U S A*. 2011;108:5632–5637.
148. Boogaard M Van Den, Wong LYE, Tessadori F, Bakker ML, Dreizehnter LK, Wakker V, Bezzina CR, Hoen PAC, Bakkers J, Barnett P, Christoffels VM. Genetic variation in T-box binding element functionally affects SCN5A /

SCN10A enhancer. *J Clin Invest*. 2012;122:2519–2530.
149. Arnolds DE, Liu F, Fahrenbach JP, Kim GH, Schillinger KJ, Smemo S, Mcnally EM, Nobrega MA, Patel V V, Moskowitz IP. TBX5 drives Scn5a expression to regulate cardiac conduction system function. *J Clin Invest*. 2012;122.
150. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature*. 1996;379:257–62.
151. Verkerk AO, Remme CA, Schumacher CA, Scicluna BP, Wolswinkel R, de Jonge B, Bezzina CR, Veldkamp MW. Functional Nav1.8 channels in intracardiac neurons: the link between SCN10A and cardiac electrophysiology. *Circ Res*. 2012;111:333–43.
152. Yang T, Atack TC, Stroud DM, Zhang W, Hall L, Roden DM. Blocking Scn10a channels in heart reduces late sodium current and is antiarrhythmic. *Circ Res*. 2012;111:322–32.
153. Pallante BA, Giovannone S, Fang-Yu L, Zhang J, Liu N, Kang G, Dun W, Boyden PA, Fishman GI. Contactin-2 expression in the cardiac Purkinje fiber network. *Circ Arrhythm Electrophysiol*. 2010;3:186–94.
154. Holm H, Gudbjartsson DF, Sulem P, Masson G, Helgadottir HT, Zanon C, Magnusson OT, Helgason A, Saemundsdottir J, Gylfason A, Stefansdottir H, Gretarsdottir S, Matthiasson SE, Thorgeirsson GM, Jonasdottir A, Sigurdsson A, Stefansson H, Werge T, Rafnar T, Kiemeney LA, Parvez B, Muhammad R, Roden DM, Darbar D, Thorleifsson G, Walters GB, Kong A, Thorsteinsdottir U, Arnar DO, Stefansson K. A rare variant in MYH6 is associated with high risk of sick sinus syndrome. *Nat Genet*. 2011;43:316–20.
155. ENCODE [online] [Internet]. Nat. Publ. Gr. 2013;Available from: http://www.nature.com/encode/threads
156. 1000 Genomes Browser [online] [Internet]. 2013;Available from: http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/
157. Berdowski J, Blom MT, Bardai A, Tan HL, Tijssen JGP, Koster RW. Impact of onsite or dispatched automated external defibrillator use on survival after out-of-hospital cardiac arrest. *Circulation*. 2011;124:2225–32.
158. Kleinjan D, van Heyningen V. Long-range control of gene expression: emerging mechanisms and disruption in disease. *Am J Hum Genet*. 2005;76:8–32.
159. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, Schwartz PJ, Toubin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, Keating MT. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet*. 1996;12:17–23.