

# Modifier genes for sudden cardiac death

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Genetic conditions, even those associated with identical gene mutations, can present with variable clinical manifestations. One widely accepted explanation for this phenomenon is the existence of genetic factors capable of modifying the consequences of disease-causing mutations (modifier genes). Here, we address the concepts and principles by which genetic factors may be involved in modifying risk for cardiac arrhythmia, then discuss the current knowledge and interpretation of their contribution to clinical heterogeneity. We illustrate these concepts in the context of two important clinical conditions associated with risk for sudden cardiac death including a monogenic disorder (congenital long QT syndrome) in which the impact of modifier genes has been established, and a complex trait (life-threatening arrhythmias in acute myocardial infarction) for which the search for genetic modifiers of arrhythmic risk is more challenging. Advances in understanding the contribution of modifier genes to a higher or lower propensity towards sudden death should improve patient-specific risk stratification and be a major step towards precision medicine.

## Keywords

Genetics • Long QT syndrome • Acute myocardial infarction • Genetic variants • Genetic modifiers

## Introduction

'Why is it that within one family, among members sharing the same disease-causing mutation, some suffer cardiac arrest and die suddenly while others go through life without any symptoms?'. This is one of the most intriguing questions faced by any experienced cardiologist managing patients affected by genetic disorders associated with life-threatening cardiac arrhythmias. Given an identical, disease-causing mutation one would expect a similar clinical course, but reality is often different. While it is probably true that different behaviours or environments may impact on the development of a phenotype, most current efforts to address this question are focused on the role of modifier genes. This term is used to describe genetic factors capable of modifying in either direction the consequences of disease-causing mutations.

The search for modifier genes is challenging because not all diseases are equally suitable for a successful investigation. It is much more difficult to identify a modifier in a complex genetic trait than in a monogenic disorder as illustrated by the different progress made in the congenital long QT syndrome (LQTS)<sup>1</sup> vs. in ischaemic heart disease. Indeed, the incomplete penetrance and variable expressivity of LQTS<sup>2</sup> associated with congenital arrhythmia susceptibility has contributed to the emerging concept that even Mendelian disorders can

have genetic complexity. Unravelling the underlying causes for this variability in disease expression may offer new ways to stratify and mitigate sudden cardiac death (SCD) risk in genetically susceptible hosts.

The genetic basis of SCD in general terms has been reviewed previously.<sup>3,4</sup> Our objective here is to review the current knowledge about modifier genes related or potentially related to SCD including their mechanism(s) of action where known, and their impact on cardiovascular diseases associated with life-threatening arrhythmias with emphasis on their value for risk stratification and clinical management.<sup>5</sup> We will focus particularly on the areas where progress has been made or is being meaningfully attempted, namely LQTS and ischaemic heart disease, exemplified by acute myocardial infarction (AMI).

## Concepts and principles of modifier genes

Clinical heterogeneity is a common feature of many monogenic disorders including those associated with congenital arrhythmia susceptibility. In some families segregating a monogenic disorder, members that share an identical primary disease-causing mutation may have no

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evidence of the disease or have clinical presentations of varying severity. These phenomena are referred to as incomplete penetrance and variable disease expressivity.<sup>2</sup> Penetrance refers to the proportion of people with a specific mutation who exhibit signs and symptoms of a genetic disease. Incomplete penetrance is said to exist when some people with the mutation do not develop the disorder. Variable expressivity refers to the diversity of signs and symptoms, including severity, that occur in different people with the same genetic disease. Incomplete penetrance and variable expressivity may result from combinations of known and unknown genetic, environmental and lifestyle factors. These phenomena confound interpretation of a discovered genotype and make prediction of an individual's genetic risk more challenging.

The concept of modifier genes has been put forth to partially explain incomplete penetrance and variable expressivity of monogenic disorders. Modifier genes may promote higher risk for symptomatic disease and/or more severe disease expression, or might protect an individual from developing the disease. Furthermore, different genetic variants within the same gene might confer opposite effects. Genetic factors that can impact arrhythmia risk may include genes encoding determinants of the primary cellular substrate for abnormal cardiomyocyte excitability such as proteins contributing to the balance of inward and outward currents operating during the cardiac action potential, those governing intracellular calcium cycling or involved with the trafficking of cellular and membrane proteins. Some candidate modifier genes are themselves monogenic arrhythmia susceptibility genes,<sup>5</sup> such as cardiac ion channel subunits or regulatory proteins.

Inter-individual differences in the propensity for arrhythmia triggers may also have a genetic basis and certain genes involved are candidate modifiers. The balance between sympathetic and parasympathetic tone as well as autonomic reflexes that are crucial to determining the onset of life-threatening arrhythmias, and the magnitude of catecholamine responses to stress and exercise varies among individuals. Some of this variability may have a genetic basis, and genes that participate in these autonomic responses are logical candidate modifiers of arrhythmia susceptibility.

Genetic variation with the potential for modifying arrhythmia susceptibility may occur within the coding or noncoding regions of a gene (intragenic) or fall within the vast genomic landscape between genes (intergenic). Further, genetic variability occurs in many forms from single nucleotide and copy number variants to larger chromosomal disruptions. Genetic modifiers may exist in populations at widely different frequency from ultra-rare (e.g. less than 1 in 20 000 persons) to common (e.g. greater than 1% of a population). Genetic variants that modify arrhythmia predisposition could have any population allele frequency. As a general rule, rare variants including disease-causing mutations have greater impact (e.g. effect size) on disease expression than common variants. However, the probability and feasibility of identifying common variants as disease modifiers is much greater than that for rare or ultra-rare variants.

## Genetic modifiers of long QT syndrome

The congenital LQTS offers special opportunities to investigate the contributions of modifier genes to genotype–phenotype correlation

for several reasons. With a prevalence of approximately one in 2000, LQTS is uncommon but not rare.<sup>6</sup> The diagnosis of LQTS is relatively straightforward.<sup>1</sup> The disorder is clinically characterized by two easily determined features, the QT interval and occurrence of cardiac events (syncope, cardiac arrest, or sudden death), which represent hard endpoints useful for assessing the impact of modifiers.<sup>1</sup> In 4–8% of LQTS cases, more than one primary disease-causing mutation help explain exaggerated disease severity compared with other family members carrying single alleles,<sup>7,8</sup> but other explanations are needed to understand why some mutation-positive subjects have less arrhythmia risk. One approach used to identify genetic modifiers of LQTS has focused on candidate genes that fall into two categories: (i) genes and genomic variants that modify the underlying arrhythmogenic substrate including genes associated with variability of QT interval in healthy populations and (ii) genetic factors that affect the probability of arrhythmia-triggering events. By design, candidate gene approaches are biased by currently available knowledge and specific hypotheses. In contrast, genome-wide association studies (GWAS) are best for discovery of previously unsuspected genetic markers of disease risk without the bias of prior knowledge. Unbiased approaches using combinations of genomic and physiological investigations have also emerged recently as successful strategies.<sup>9</sup> Table 1 summarizes the known genetic modifiers of LQTS according to their deleterious or protective effect.

Common non-synonymous coding variants in the same gene as the primary mutation can act as intragenic modifiers. For example, we reported a family segregating a moderate loss-of-function mutation in *KCNH2* (p.A1116V) associated with a latent form of LQTS.<sup>10</sup> Whereas most family members with this mutation were asymptomatic, a 44-year-old woman had cardiac arrest. In addition to the primary *KCNH2* mutation, she also carried a common nonsynonymous coding variant resulting in the amino acid substitution p.Lys897Thr (K897T) on the opposite *KCNH2* allele. The heterologous co-expression of the primary mutation with K897T in an *in vitro* expression assay demonstrated a markedly lower level of rapid delayed rectifier potassium current ( $I_{Kr}$ ) consistent with LQTS pathogenesis. A similar observation was made for another *KCNH2* mutation (c.2775insG; p.P926AfsX14) discovered in a woman with asymptomatic LQTS who was evaluated for early postnatal death of a child and a separate occurrence of intrauterine foetal demise.<sup>11</sup> The deceased offspring both inherited the primary *KCNH2* mutation in combination with the K897T variant transmitted from the father. *KCNH2*-K897T also aggravates LQTS associated with *KCNQ1* mutation. In a group of Finnish LQTS cases caused by mutation *KCNQ1*-G589D, the presence of *KCNH2*-K897T was associated with a longer QT interval during maximal exercise.<sup>12</sup> A common variant in the cardiac sodium channel gene (*SCN5A*-H558R) may also impact the severity of LQTS when present on the same allele as a primary mutation.<sup>13,14</sup> The latter observations led to the discovery of intersubunit interactions in voltage-gated sodium channels.<sup>15</sup>

The aforementioned anecdotal observations suggest that *KCNH2*-K897T may impair repolarization reserve in the setting of LQT1 or LQT2 mutations. The impact of this common variant in the general population may be more complex as evidence by three reports demonstrating an association of this variant with shorter QT intervals in European subjects<sup>16–18</sup> and one report showing association with longer QT intervals in the Framingham study population.<sup>19</sup>

**Table 1** List of SNPs regarded as genetic modifiers for the long QT syndrome

SNP	Gene	Localization	Nucleotide Substitution	Modifier Allele	Effect of the Modifier Allele	References
Detrimental SNPs						
rs4657139	<i>NOS1AP</i>	Intronic	A > T	A	↑ QT ↑ ACA, SD	37,45
rs16847548	<i>NOS1AP</i>	Intronic	T > C	C	↑ QT ↑ CA, SCD ↑ risk of cardiac events	23,37,45
rs10494366	<i>NOS1AP</i>	Intronic	T > G	G	↑ cardiac events	45
rs12143842	<i>NOS1AP</i>	Intronic	C > T	T	↑ QT	23
rs2880058	<i>NOS1AP</i>	Intronic	A > G	G	↑ QT	23
rs2519184	<i>KCNQ1</i>	3' UTR	G > A	A	Inconsistently associated with ↑ QT and ↑ risk of cardiac events	25–28
rs8234	<i>KCNQ1</i>	3' UTR	A > G	G	Inconsistently associated with ↑ QT and ↑ risk of cardiac events	25–28
rs10798	<i>KCNQ1</i>	3' UTR	A > G	G	Inconsistently associated with ↑ QT and ↑ risk of cardiac events	25–28
rs1805123	<i>KCNH2</i>	Exonic (c.269A>C: p.Lys897Thr)	A > C	C	↑ symptoms in LQT2 ↑ I <sub>Kr</sub> current reduction ↑ QT in LQT1-G589D founder population	10–12
rs1805128	<i>KCNE1</i>	Exonic (c.253G>A: p.Asp85Asn)	G > A	A	↑ QT ↑ risk of cardiac events	18,23,20–22
rs11772585	<i>AKAP9</i>	Intronic	C > T	T	↑ risk of cardiac events	39
rs7808587	<i>AKAP9</i>	Intronic	A > G	G	↑ risk of cardiac events	39
rs2961024	<i>AKAP9</i>	Intronic	T > G	G	↑ QT	39
rs8014119	<i>REM2</i>	Exonic (Gly96Ala)	G > C	C	↑ QT ↑ risk of cardiac events	9
Protective SNPs						
rs2282972	<i>AKAP9</i>	Intronic	C > T	T	↓ risk of cardiac events	39
rs2074238	<i>KCNQ1</i>	Intronic	C > T	T	↓ QT ↓ symptoms	23,41
rs1805124	<i>SCN5A</i>	Exonic (c.1673A>G: p.His558Arg)	A > G	G	Rescue normal phenotype in LQT3	13,14
rs10947804	<i>KCNK17</i>	Exonic (Ser21Gly)	T > C	C	↓ QT ↓ symptoms	9

The table shows for each SNP the corresponding gene, the alternative alleles (the minor allele in the general population, according to NCBI-dbSNP, is reported as second), the modifier allele and the corresponding associated effect. The references in the last column refer to the published studies describing the modifier effect of the SNPs.

Common variants in genes encoding ion channel modulators or auxiliary subunits have also been implicated as genetic modifiers of arrhythmia syndromes. In particular, a common nonsynonymous coding variant in *KCNE1* (p.Asp85Asn, also known as D85N) encoding a regulatory subunit of the *KCNQ1*-encoded potassium channel has been demonstrated to predispose to both congenital and drug-induced LQTS.<sup>20,21</sup> This variant has also been associated with variation in QT interval duration among healthy individuals.<sup>18</sup> In Finnish LQTS families segregating the founder mutation *KCNQ1*-p.Gly589Asp, *KCNE1*-p.Asp85Asn was associated with greater QT interval prolongation in males but not females suggesting that it acts as a sex-specific genetic modifier.<sup>22</sup> Similarly, *KCNE1*-p.Asp85Asn was nominally associated with longer QT intervals and higher probability of cardiac events in cases of type 2 LQTS caused by *KCNH2* mutations.<sup>23</sup> These observations can be explained by impaired repolarizing currents ( $I_{Ks}$ ,  $I_{Kr}$ ) when this *KCNE1* variant interacts with *KCNQ1* or *HERG* channels.<sup>20</sup> The clinical counterpart is that, when in asymptomatic individuals presenting exclusively with borderline QT prolongation only this variant is found, we do not make diagnosis of LQTS but believe that avoidance of  $I_{Kr}$  blocking drugs should be strongly considered.

Interindividual variability in the level of LQTS gene expression may also contribute to variable impact of the mutant allele. Specifically, variants affecting transcriptional control or mRNA stability of the normal allele may partially offset the contribution of a mutation by changing the ratio of wild-type and mutant proteins. Many autosomal dominant LQTS-associated mutations, especially those in multimeric potassium channel genes, are known to exert dominant-negative effects on the wild-type subunit.<sup>24</sup> Hence, the balance between transcription or translation of wild-type and mutant alleles may modify the probability of forming dysfunctional ion channel complexes.

This intriguing concept has been explored for variants within the 3'-untranslated region (3'-UTR) of the *KCNQ1* gene. It is widely known that the 3'-UTR of genes may contain noncoding elements important for mRNA stability and translation. An international collaborative study examined the impact of three common *KCNQ1* variants affecting nucleotides within the 3'-UTR on QTc duration and symptoms in several LQT1 families.<sup>25</sup> The investigation sought to determine if 3'-UTR variants were associated with opposite effects on arrhythmic risk and QT interval duration depending on whether they were in *cis* or *trans* to the mutant allele. *In vitro* analyses demonstrated that variant 3'-UTR haplotypes promoted lower expression of a

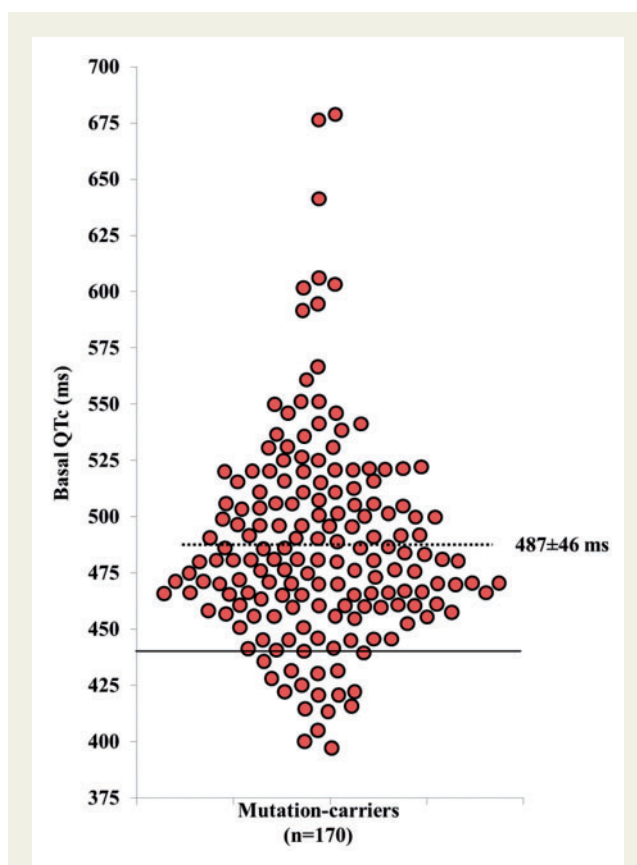
coupled reporter gene (luciferase). Genetic evaluation documented significant associations with longer QT intervals when 3'-UTR variants were in *trans* with the wild type *KCNQ1* allele, which suggested a lower ratio of wild type to mutant *KCNQ1* subunits. Extrapolation to the general population did not reveal that 3'-UTR variants in *KCNQ1* impact the QT interval suggesting that this effect may only be relevant in the context of heterozygous *KCNQ1* mutation. Attempts to replicate these findings in three LQT1 founder populations failed<sup>26</sup> possibly because of differences in genetic architecture of the 3'-UTR, different statistical approaches or the nature of the primary mutations.<sup>27,28</sup> Additional investigations are needed to determine the robustness of these findings.

## Discovery of genetic modifiers in long QT syndrome founder populations

Even though serendipitous observations within a single family may provide important clues regarding the identity of genetic modifiers, as it was the case for the *KCNH2*-K897T variant discussed above,<sup>10</sup> a structured approach exploiting founder populations<sup>29–33</sup> has offered additional opportunities for modifier gene discovery. Given that genetic modifiers have quantitative effects smaller than that of a disease-causing mutation, their discovery is facilitated by avoiding the confounding effect of different mutations (each of which would have its own and variable impact) and by examining instead a large number of individuals carrying the same mutation. An approach is the study of members of founder populations, which are characterized by a large number of individuals and families related to a single ancestor affected by the disease and who thereby carry the same disease-causing mutation.<sup>30</sup> Founder populations are not found everywhere in the world; there are countries or selected areas where they are more easily encountered, and this almost always relates to specific historic or geographic aspects.<sup>33</sup> For example, founder populations are more prevalent in South Africa,<sup>29,30</sup> in Finland,<sup>31</sup> Sweden,<sup>32</sup> Quebec,<sup>34</sup> and the Netherlands.<sup>35</sup>

We previously investigated a large LQT1 cohort comprised of approximately 500 members of whom approximately 200 are heterozygous for the *KCNQ1*-A341V mutation. An ancestor of this founder population moved from the Netherlands to South Africa in A.D. 1690 and genetic mapping studies of several LQT1 families in the region eventually connected an extended pedigree.<sup>29</sup> *KCNQ1*-A341V confers a highly penetrant, clinically severe mutation in the South African cohort and in other mutation-positive families throughout the world.<sup>36</sup> However, phenotypic heterogeneity was evident in the South African population with respect to the presence or absence of cardiac events and highly variable QT interval duration (Figure 1), thus indicating that something besides the primary *KCNQ1* mutation influenced disease expression and penetrance.

Studying the South African LQT1 founder population led us to demonstrate that *NOS1AP* is a genetic modifier of LQTS.<sup>37</sup> This discovery and related work in the field are discussed separately below. This unique LQT1 population was also valuable for demonstrating that variants in *AKAP9*, encoding the protein kinase A anchoring protein *ytiaio* important for regulating  $I_{Ks}$ ,<sup>38</sup> are associated with greater



**Figure 1** Basal QTc in *KCNQ1*-A341V mutation carriers. The uninterrupted line represents the upper limit of normal values for men (440 ms). The dotted line represents the mean QTc value of the 170 mutation carriers. If the QTc prolongation were determined only by the A341V mutation, the QTc values should be uniformly prolonged with modest variability. The large spectrum of QTc values points to the presence of additional variants affecting ventricular repolarization. Modified from Brink et al.<sup>29</sup> with permission.

risk for cardiac events in *KCNQ1*-A341V carriers and longer rate-corrected QT interval duration.<sup>39</sup> The specific variants tested in this study have uncertain effects on gene expression or protein function but may exist in linkage disequilibrium with functional variation in the gene.

The biological basis for the modifier effects of *AKAP9* variants may involve dysregulation of  $I_{Ks}$  by cAMP-dependent pathways such as adrenergic receptor-mediated signalling. Genetic variation in modulating the probability of triggered arrhythmic events may also be important in this LQT1 cohort. Two variants in genes encoding adrenergic receptors (*ADRA2C*-Del322-325 and *ADRB1*-R389) that have been implicated with exaggerated adrenergic responses were associated with enhanced autonomic reflexes, faster heart rates, and a trend for more cardiac events in the South African LQT1 founder population.<sup>40</sup> These findings correlated with clinical evidence that carriers of the *KCNQ1*-A341V mutation who exhibited faster resting heart rates and brisk autonomic responses had a greater probability of being symptomatic.<sup>40</sup> These preliminary observations suggest that



quantitatively greater release of epinephrine, norepinephrine, or even acetylcholine, may predispose to a higher probability of initiating either re-entrant or triggered arrhythmias in LQT1.

A conceptually important departure from the previous observations, in which variants were associated with increased arrhythmic risk, came from an international case–control study examining 112 LQTS duos.<sup>41</sup> The duos were discordant pairs of one symptomatic and one asymptomatic first degree relatives, both carriers of the same *KCNQ1* or *KCNH2* mutation. Twenty five variants previously associated with QTc duration in healthy subjects or variable adrenergic responsiveness were investigated as potential modifiers of QTc duration and cardiac events. The minor allele of one intronic *KCNQ1* variant (rs2074238, T allele) was associated with a lower arrhythmic risk and a shorter QTc. These findings were validated in 336 LQT1 subjects from two founder populations (South African, Finnish) and provided evidence for a protective genetic modifier.<sup>41</sup> An independent study that considered *KCNQ1* rs2074238 in a LQT2 cohort demonstrated a nominal association with shorter QTc duration but no association with cardiac events.<sup>23</sup>

## NOS1AP is an important genetic modifier of long QT syndrome

Genome wide association studies previously identified single nucleotide variants in *NOS1AP* encoding a nitric oxide synthase adaptor protein associated with QT interval duration in the general population and these findings contributed to the initial search for modifier genes in LQTS.<sup>42–44</sup> In the South African LQT1 founder population, we demonstrated that two common noncoding *NOS1AP* variants were associated with elevated risk of life-threatening arrhythmias.<sup>37</sup> Our finding was subsequently validated in heterogeneous populations of LQTS patients<sup>45</sup> and in a cohort of LQT2 families studied in the Netherlands.<sup>23</sup> The consistency of these findings also indicates that *NOS1AP* can be regarded as a valid genetic modifier of LQTS severity. Interestingly, *NOS1AP* variants are also associated with the risk for developing drug-induced long-QT syndrome.<sup>46</sup> These studies collectively demonstrate that modifier genes discovered in a founder population may be relevant to heterogeneous groups with the same or related disorders.

Despite the strong genetic evidence of association with QT duration and LQTS severity, there are conflicting data regarding the physiological effects of *NOS1AP* variants on cardiomyocyte repolarization. The minor alleles of common *NOS1AP* variants associated with longer QT duration in populations have been correlated with higher levels of gene expression in human ventricular myocardium obtained opportunistically from extracted pacemaker or defibrillator leads.<sup>47</sup> Similar findings were reported for putative functional *NOS1AP* variants within a defined *cis*-regulatory (‘enhancer’) element within in the gene.<sup>48</sup> Modulating expression levels of *NOS1AP* in experimental systems have provided conflicting results. Two groups have reported shortening of action potential duration (APD) with over expression of *NOS1AP* in either isolated adult guinea pig ventricular myocytes<sup>49</sup> or cultured neonatal rat ventricular cardiomyocytes.<sup>48</sup> In contrast, shortened APD was evoked by suppressing *NOS1AP* expression in embryonic zebrafish heart.<sup>50</sup> These contradictory biological effects of *NOS1AP* expression on cardiomyocyte

repolarization may be due to species differences, but which model system best represents human cells is unclear. A conundrum exists with these observations in that *NOS1AP* variant alleles that are associated with longer QT intervals in populations are also correlated with elevated *NOS1AP* expression in human ventricular tissue. Therefore, shortened APD evoked by overexpression in guinea pig or rat myocytes is not consistent with human data. Reconciling these observations may require human cardiomyocyte model systems.

## Discovery of long QT syndrome modifiers using a physiological genomics approach

While candidate gene approaches have had some success in identifying plausible modifier genes in LQTS, additional unbiased approaches are needed to discover previously unrecognized genetic factors. An example of a new approach is illustrated by recent work of Chai *et al.*<sup>9</sup> that combined whole exome sequencing and electrophysiological investigations of induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) from members of a large family with type 2 LQTS. In this family, LQTS was associated with a pathogenic mutation in *KCNH2* but there were notable differences in disease expression among mutation carriers. Electrophysiological measurements made from iPSC-CM from severely affected and mildly affected family members recapitulated this genotype–phenotype discordance. Specifically, only iPSC-CM from severely affected individuals exhibited prolonged APD despite the fact that all cells had lower levels of rapid delayed rectifier current ( $I_{Kr}$ ) consistent with a loss of function mutation in *KCNH2*. This finding implied that there was either a protective factor blunting the effect of *KCNH2* mutation on APD or that an aggravating factor was necessary to express impaired repolarization. Unexpectedly, iPSC-CM from the severely affected subjects exhibited larger L-type calcium current ( $I_{Ca,L}$ ) amplitude, which was pharmacologically confirmed when nisoldipine shortened the APD in these cells. Hence, genetic factors responsible for enhanced  $I_{Ca,L}$  were considered as potential LQTS modifiers.

In parallel investigations, exome sequencing revealed variants in the 2-pore domain potassium channel gene *KCNK17* and in a GTP-binding protein encoded by *REM2*, a suspected physiological modulator of  $I_{Ca,L}$ . The *KCNK17* variant was demonstrated to be gain-of-function in heterologous cells, and was absent in all severely affected family members. Silencing this gene in iPSC-CM from a mildly affected subject caused APD prolongation. This finding suggested that this *KCNK17* gain-of-function variant was a protective allele. In contrast, the *REM2* variant was carried by all severely affected subjects and overexpression of the variant in control iPSC-CM potentiated  $I_{Ca,L}$ . Hence, this was considered a plausible aggravating allele. Correction of the *REM2* variant in affected cells using genome editing technology (CRISPR/Cas9) reversed the cellular phenotype. This elegant study demonstrates the synergy of combining modern genetic and cellular technologies to discover novel modifier genes in LQTS.

The practical importance of ‘protective modifiers’ goes beyond their relevance for risk stratification as it suggests the potential of identifying, through the understanding of their mechanism of action, totally novel approaches to therapy.

## Applying genetic modifiers in clinical practice

The search for modifier genes in monogenetic congenital arrhythmia susceptibility represents an important research endeavour that may pay benefits in the future for management of affected individuals. Conceptually, subjects with a primary LQT1 mutation who are also carriers of well-validated *NOS1AP* risk alleles may require more vigilant monitoring for arrhythmic events or more aggressive therapy than those without these variants. The next step in evaluating the significance of modifier genes and their impact on clinical practice will be a prospective analysis to determine long-term outcomes and their association with risk alleles. Efforts to derive and test multilocus genetic risk scores<sup>42–44,51</sup> may be valuable in this regard. However, there are challenges in applying genetic markers of risk deduced from population studies to individuals unless validated by a prospective analysis. The study by Strauss et al.<sup>51</sup> provides an example of prospective testing to determine the value of a population-based genetic risk score applied to individuals. In this randomized, double-blind and placebo-controlled crossover trial of 3 QT-prolonging drugs, a genetic QT risk score comprising 61 common genetic variants previously identified by GWAS correlated with drug-induced QTc prolongation. Applying this genetic QT risk score to LQTS mutation carriers in a similar prospective study might help determine the clinical value of this predictive index in congenital arrhythmia susceptibility.

## Acute myocardial infarction

Sudden cardiac death in association with an AMI represents a major public health problem and a condition for which there are likely genetic risk modifiers. Even though approximately 5% of subjects with a first AMI do not arrive alive to the hospital due to ventricular fibrillation (VF),<sup>52,53</sup> the clinical features that might distinguish those with from those without VF have not been identified. Clinical features including infarct size, location, pump function, age, or sex fail to differentiate the two groups. The hypothesis that genetic variation might contribute to modifying the VF risk in the setting of an AMI has been supported by clinical and experimental studies. In the Paris Prospective Study involving almost 7000 French men followed for 26 years, SCD of one parent increased the relative risk of dying suddenly by 2.6 times, whereas SCD of both parents increased this risk by 9.4 times.<sup>54</sup> A Dutch case-control study showed that familial SCD had occurred significantly more frequently among patients with a first myocardial infarction (MI) complicated by VF than in patients with a first MI without VF (43.1% and 25.1%, respectively; odds ratio 2.72, 95% confidence interval 1.84–4.03).<sup>55</sup> A Finnish study with an almost identical design provided similar results.<sup>56</sup> These three studies together provided evidence that SCD during a first MI has an important familial and likely genetic component. Further support for this concept comes from a canine model for SCD in which depressed baroreflex sensitivity (BRS) allows the early identification of post-MI dogs at high risk for SCD,<sup>57</sup> a finding confirmed in >1000 post-MI patients.<sup>58</sup> As the depression in BRS was evident even prior to the experimental MI, this suggests a genetic component.

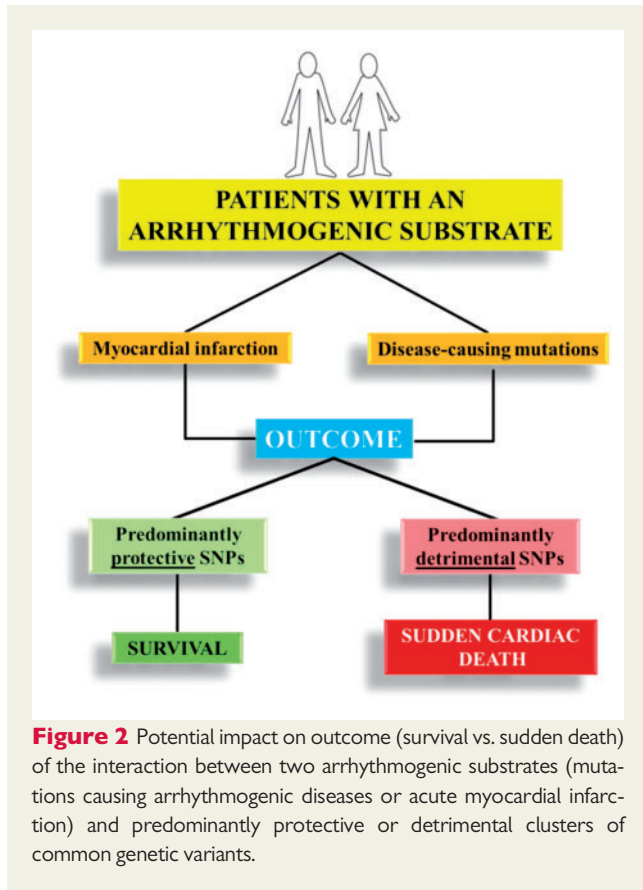
These observations fostered the design of studies aiming at identifying either rare or common genetic variants that favour or hinder

the development of VF in AMI. The first such studies were based on a candidate-gene approach. Small studies tested the role of rare variants in arrhythmia-susceptibility genes and suggested a role for *SCN5A* rare variants.<sup>59,60</sup> Other studies evaluated the role of candidate SNPs. Specifically, *NOS1AP* variants were associated with higher risk for SCD in white adults<sup>61</sup>; an association confirmed in the Rotterdam Study, which however did not exclusively include AMI.<sup>62</sup> The *KCNH2*-K897T polymorphism, previously identified as a genetic modifier of arrhythmic risk in LQTS,<sup>10,11</sup> was subsequently associated with life-threatening arrhythmias following AMI.<sup>63</sup> More recently, 257 ST-elevation myocardial infarction cases with VF and 537 controls (ST-elevation myocardial infarction without VF) of the Danish GEVAMI cohort were analysed for 27 SNPs previously associated with SCD and VF in different cohorts of patients not limited to AMI. One SNP located in intron 1 of *SCN5A* (rs11720524) showed an association with arrhythmic risk.<sup>64</sup>

However, when the field moved from candidate genes to GWAS, none of the SNPs or the genes previously associated with life-threatening arrhythmias in different clinical settings and during AMI showed a significant signal. Two GWAS reported an association of common genetic variants with VF/SCD at a genome-wide statistical significance ( $P < 5 \times 10^{-8}$ ). One study, which compared patients with or without VF during a first AMI (AGNES population), identified a significant signal at a SNP near the *CXADR* gene.<sup>65</sup> This gene, encoding the coxsackie and adenovirus receptor, is highly expressed in heart tissue. Mice haploinsufficient for this gene have delayed cardiac conduction and greater arrhythmia vulnerability during ischaemia.<sup>66</sup> The other study detected an association signal in *BAZ2B*, which encodes the bromodomain adjacent zinc finger domain 2B.<sup>67</sup> *BAZ2B* and other genes in proximity of the risk variant are expressed in the heart, however, it is not yet known how they might influence arrhythmic risk. Although these two GWAS uncovered associations at genome-wide statistical significance, replication efforts have been limited and findings have been inconsistent.<sup>68</sup>

The significant complexities inherent with such studies include the multiplicity of factors that may contribute to the development of VF during AMI, e.g. autonomic activation, extent of ischaemia, electrolyte imbalance. Among them, a most important one is the activation of sympathetic reflexes that occurs during acute myocardial ischaemia,<sup>69</sup> and which through the localized release of norepinephrine at the neural endings in the ventricles, accentuates the heterogeneity of ventricular repolarization to promote re-entrant arrhythmias.<sup>70</sup> Indeed, the Paris prospective study identified excessive sympathetic activation and abnormal heart rate responses as major risk factors for SCD in victims of post-MI SCD.<sup>71</sup> Similarly, it has been repeatedly demonstrated that prolongation of the QT interval is a major risk factor for SCD in post-MI patients<sup>72,73</sup> and in the general population.<sup>74</sup> This, in turn, has led to the suggestion that – if medically appropriate – drugs devoid of  $I_{Kr}$  activity should be preferred always and not just in LQTS patients.<sup>75</sup>

The recognition of these complexities has impacted the design of three similar ongoing studies in Europe seeking to identify genetic risk factors of SCD in the setting of AMI. These studies focus on the cleanest and most well defined phenotypes (first MI with or without VF) and have avoided reliance on surrogate markers (e.g. ICD discharges) that may be confounded by concomitant heart disease independently promoting arrhythmia risk through non-genetic



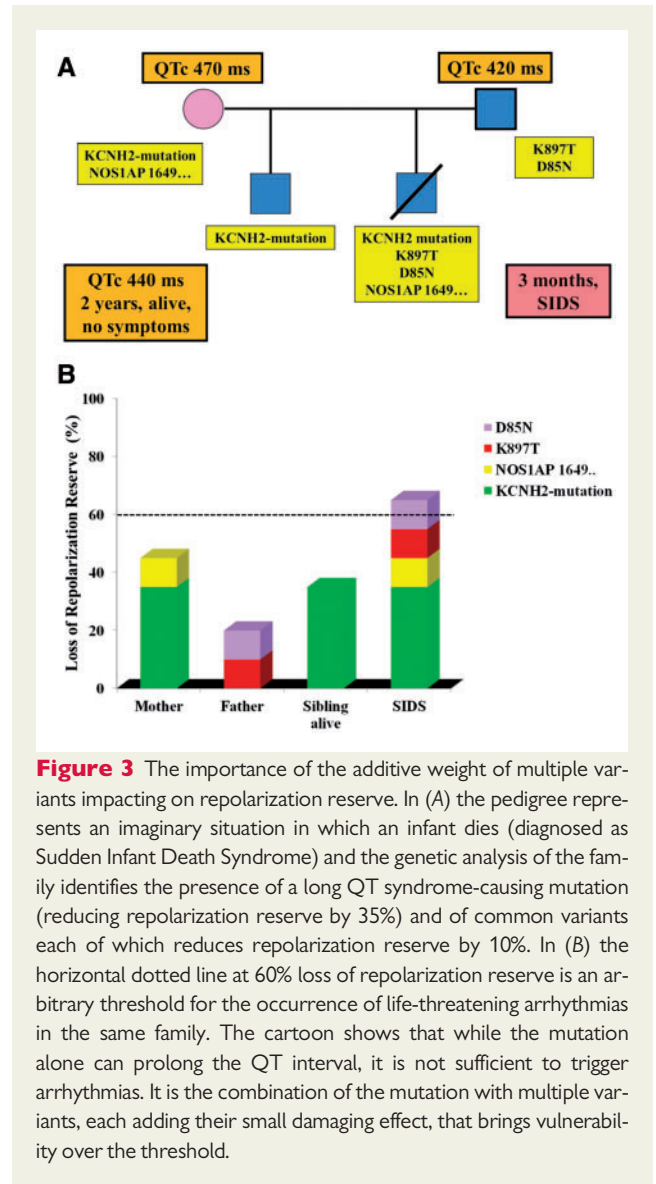
**Figure 2** Potential impact on outcome (survival vs. sudden death) of the interaction between two arrhythmogenic substrates (mutations causing arrhythmogenic diseases or acute myocardial infarction) and predominantly protective or detrimental clusters of common genetic variants.

mechanisms. These ongoing prospective studies (AGNES,<sup>65,66</sup> GEVAMI,<sup>64</sup> and PREDESTINATION<sup>76</sup>) have already enrolled 1400 cases and 2200 controls for a GWAS and, having now joined their databases in an EU Horizon 2020 programme project (ESCAPE-NET),<sup>77</sup> they represent excellent opportunities to identify genetic modifiers of SCD risk in ischaemic heart disease.

## Conclusions

Remarkable progress has been made during the last 20 years in identifying modifier genes in clinical situations with elevated risk for SCD. Clinical observations that were previously difficult to reconcile with the presence of a given disease-causing mutation are now conceptually more easily explained. This has resulted in a concept relevant to both monogenic and polygenic diseases, recently expressed in a consensus statement on SCD.<sup>78</sup> Namely, that if an individual inherits both protective and arrhythmogenic SNPs, but their sum acts predominantly in one direction, there may be an important effect on clinical outcome (Figure 2). Conversely, if by the play of chance another individual inherits by the parents not only a disease-causing mutation but also multiple variants each with even modest detrimental effect (i.e. losses in repolarization reserve), this could create the setting for life-threatening arrhythmias (Figure 3).

The growing recognition that certain specific, and relatively common, genetic variants are able to modulate arrhythmic risk is promoting a more patient-specific risk stratification with the potential for



**Figure 3** The importance of the additive weight of multiple variants impacting on repolarization reserve. In (A) the pedigree represents an imaginary situation in which an infant dies (diagnosed as Sudden Infant Death Syndrome) and the genetic analysis of the family identifies the presence of a long QT syndrome-causing mutation (reducing repolarization reserve by 35%) and of common variants each of which reduces repolarization reserve by 10%. In (B) the horizontal dotted line at 60% loss of repolarization reserve is an arbitrary threshold for the occurrence of life-threatening arrhythmias in the same family. The cartoon shows that while the mutation alone can prolong the QT interval, it is not sufficient to trigger arrhythmias. It is the combination of the mutation with multiple variants, each adding their small damaging effect, that brings vulnerability over the threshold.

clinical management to become progressively better tailored to the entire genetic background of an individual patient. The progress is more rapid for monogenic diseases and will take more time for complex polygenic disorders, but it should now be clear that, thanks to the smart use of genetic information, clinical management of arrhythmic disorders has taken a turn and precision medicine is in sight.<sup>79</sup>

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