# Cardiogenetics: the role of genetic testing for inherited arrhythmia syndromes and sudden death

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**Abstract**

There have been remarkable advances in our knowledge of the underlying heritability of cardiac arrhythmias. Long QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, progressive cardiac conduction disease and the short QT syndrome comprise the inherited arrhythmia syndromes (IASs). Pathogenic variants in cardiac ion channel and calcium handling protein genes lead to these conditions, usually in the absence of overt structural cardiac disease. Diagnosis is contingent on the ECG phenotype but genetic testing may help to confirm the diagnosis and provide information on the mechanism of arrhythmogenesis that may guide treatment and provide prognostic information in relation to the risk of sudden arrhythmic death. Clinical genetic testing uses 'panels' of genes that are the likely culprits for the IASs being investigated. An International Consortium (Clinical Genome Resource) has curated gene panels based on genetic and experimental evidence of causation of inherited conditions and that have a role in clinical genetic testing. A 'single gene' or monogenic basis for IASs exists but in future, missing heritability and incomplete penetrance will be uncovered by association of common variants through genome-wide association studies. Novel rare variants will also be detected through whole-genome sequencing. The formulation of polygenic risk scores will likely help to predict phenotypic expression and response to treatments/risk stratification and move genetic testing very much to the fore of the diagnostic process.

## Introduction

Remarkable advances in our knowledge of the heritability of cardiac arrhythmias has stemmed from dissection of the inherited arrhythmia syndromes (IASs): the long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), progressive cardiac conduction disease (PCCD) and the short QT syndrome (SQTS). Rare variants in cardiac ion channel and calcium handling protein genes lead to these conditions in the absence of structural heart disease. These variants have been investigated functionally for deleterious effects on protein translation, trafficking and function (figure 1). Experimental models have recapitulated the phenotypic expression witnessed in the clinic (figure 2 and figure 3). This review will focus primarily on the genotype-phenotype associations of the IASs and the clinical utilisation of genetic testing in this setting, particularly given its underuse across Europe.[1] Beyond the 'single gene' or monogenic basis for these conditions exist other patients with similar phenotype but elusive for a genetic cause. This is likely to represent a more common polygenic basis for the phenotype and may be dissected by genome-wide association studies (GWAS).

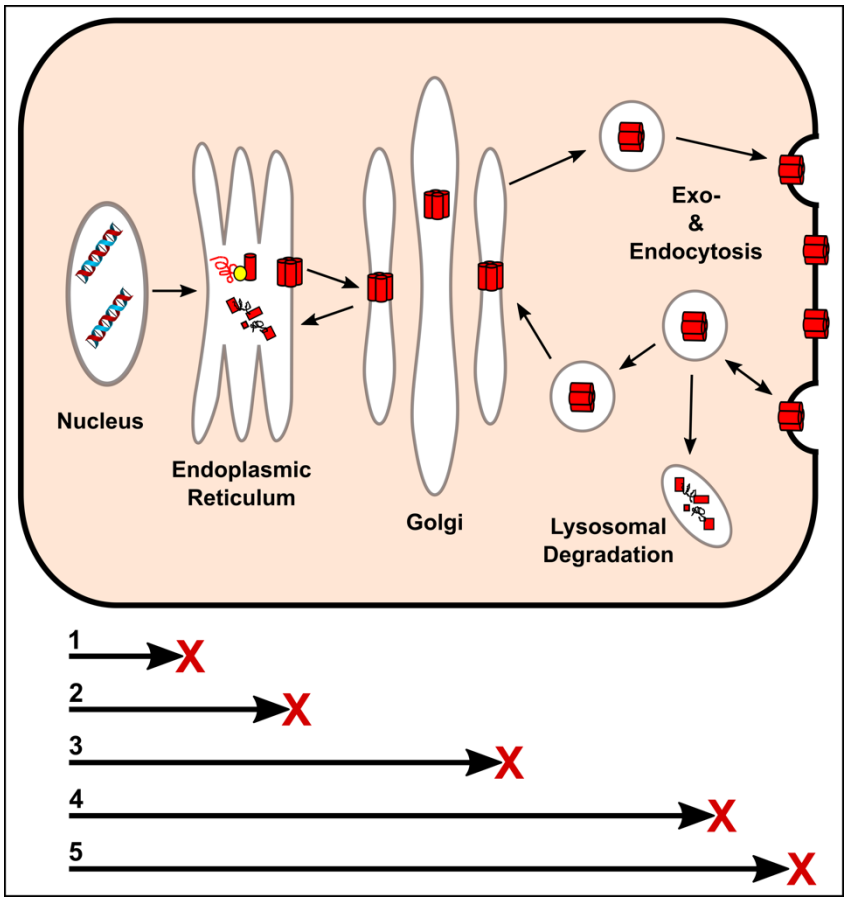


**Figure 1. Disease mechanisms of channelopathies.** Delivery of normally functioning ion channels at the cell membrane can be halted or disturbed at various checkpoints. Pathogenic variants can lead to: (1) defective transcription or translation such that channel proteins are not synthesised at all; (2) aberrant folding of channel proteins into their tertiary and quaternary structures that is recognised by chaperone proteins in the endoplasmic reticulum (ER) leading to degradation and failure to exit the ER; (3) further quality control in the Golgi complex where channels recognised as faulty are retranslocated back to the ER or assigned for degradation; (4) defective cycling to and from the membrane through exocytosis and endocytosis; (5) channels pass through all checkpoints and are trafficked to the membrane but display abnormal gating and/or kinetics, or abnormal responses to modulatory pathways.

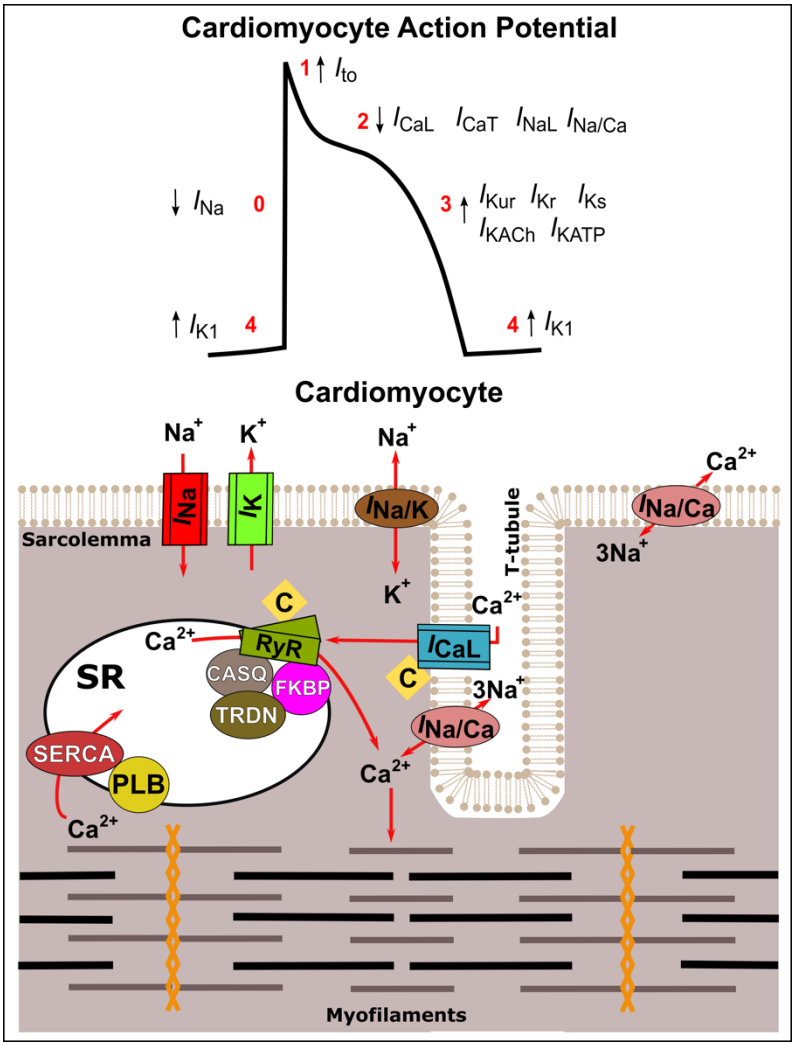


**Figure 2. The cardiomyocyte action potential and excitation-contraction coupling.** The cardiac action potential is the summation of the flow of sodium ($Na^+$), potassium ($K^+$) and calcium ($Ca^{2+}$) ions through their respective ion channels and exchange proteins. This consists of 4 phases (numbered red, ionic flow inwards to the cell down arrows and outwards up arrows). The ionic flow occurs across the cardiomyocyte sarcolemma and the intracellular sarcoplasmic reticulum (SR). Depolarisation transforms the cell membrane at rest from a negative potential difference to a positive one by inward $Na^+$ and $Ca^{2+}$ ions. This leads to release of $Ca^{2+}$ ions from the SR. The binding of $Ca^{2+}$ to intracellular myofilaments then leads to cardiomyocyte contraction. $Na^+$ and $Ca^{2+}$ ions are then extruded, $Ca^{2+}$ ions sequestrated into the SR, and potassium ions flow out to repolarise the cell. C, calmodulin; CASQ, calsequestrin; $I_{Na}$ ,inward sodium current; $I_{NaL}$, late persistent sodium current; $I_{CaL}$, L-type inward calcium current; $I_{CaT}$, T-type inward calcium current; $I_{Na/Ca}$, sodium-calcium exchange current; $I_{to}$, transient outward potassium current; $I_{Ks}$, slowly activating delayed rectifier potassium current; $I_{Kr}$, rapidly activating delayed rectifier potassium current; $I_{Kur}$, ultra-rapidly activating delayed rectifier potassium current; $I_{K1}$, inward rectifier potassium current; $I_{KACh}$, acetylcholine-activated potassium current; $I_{KATP}$, ATP-sensitive potassium channel current; $I_{Na/K}$, sodium-potassium exchanger; PLB, phospholamban; SERCA, sarco-endoplasmic reticulum $Ca^{2+}$

transport ATPase; SR, sarcoplasmic reticulum; TRDN, triadin; RyR, ryanodine receptor; FKBP, FK-506 binding protein.

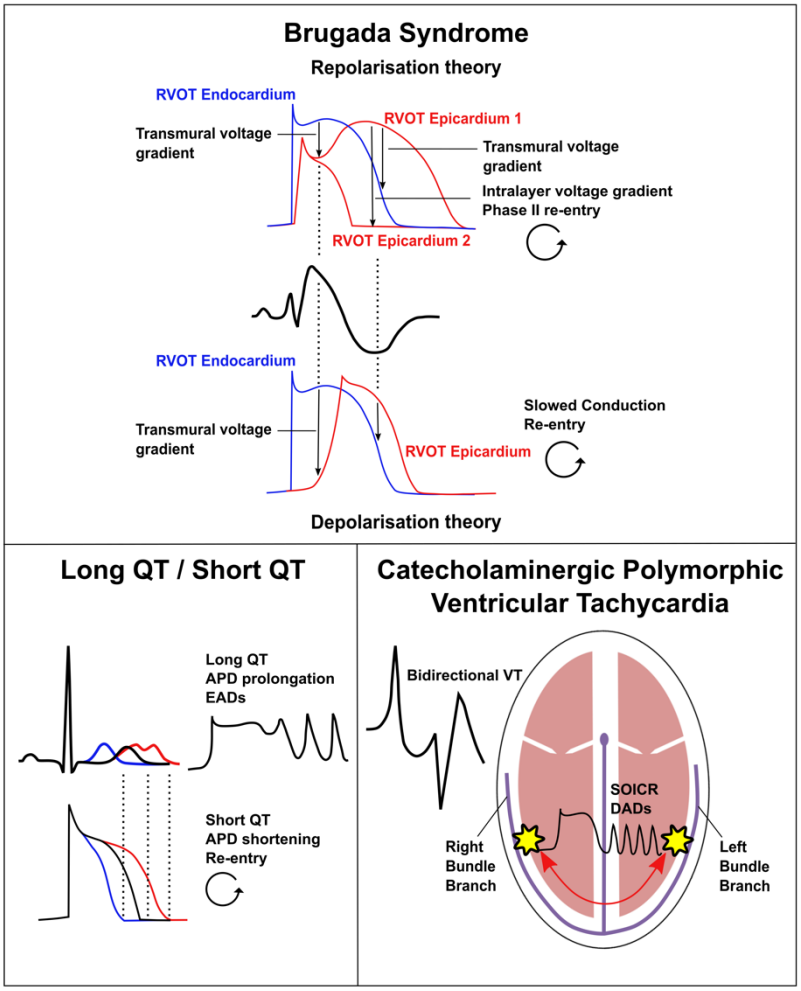


**Figure 3. Mechanisms behind ECG phenotypes of inherited arrhythmia syndromes.** The spatial dispersion across the heart of action potential morphology and duration (APD), together with timing variations of activation and repolarisation, produces the composite of the surface ECG. In long QT and short QT syndromes, action potential prolongation/ shortening leads to QT prolongation/shortening. The mechanism of polymorphic ventricular tachycardia (pVT)/ventricular fibrillation (VF) in long QT syndrome is due to early after-depolarisations (EADs), where a prolonged phase II and phase III leads to re-activation of the inward calcium current and subsequent forward mode inward current from the $I_{Na/Ca}$ exchanger. This leads to a new action potential and premature ventricular contractions that can lead to pVT/VF. In short QT, APD shortening and re-entry is the arrhythmic mechanism. The repolarisation Brugada theory places early repolarisation in some areas of the right ventricular outflow tract (RVOT) epicardium, due to a higher density of $I_{to}$ current opposing a reduced inward current in action potential phase I, to cause J-point elevation and a domed ST segment. It also places a voltage gradient between epicardial layers to cause T wave inversion and 'phase II re-entry' between the heterogeneous epicardial layers leading to arrhythmia. The depolarisation theory suggests a delayed activation of the RVOT epicardium, due to $I_{to}$-opposed reduced inward current, to underpin the ECG phenotype, and where slowed conduction drives re-entry. The classic bidirectional VT of catecholaminergic polymorphic ventricular tachycardia is produced by alternating delayed after-depolarisations (DADs) between the right and left bundle branches. Purkinje cell cardiomyocytes display a greater propensity to develop abnormalities in $Ca^{2+}$ handling and $Ca^{2+}$ loading. Variants lead to

leakiness of sarcoplasmic reticulum (SR) $Ca^{2+}$ handling proteins invoking adrenergically driven store overload-induced $Ca^{2+}$ releases (SOICR) from the SR of Purkinje cardiomyocytes. This leads to DAD-induced oscillating ventricular ectopy from within the His-Purkinje system and degeneration to polymorphic VT and VF can then ensue.[23]

## The clinical utility of genetic testing

Clinical genetic testing uses 'panels' of genes that are the likely culprits for the IASs being investigated. These panels are now defined increasingly by the Clinical Genome Resource (ClinGen). ClinGen is an international consortium curating genes for genetic and experimental evidence of causation of inherited conditions.[2] Table 1 lists the genes implicated in IASs as curated by ClinGen to date. Clinical validity level of gene-disease relationships is categorised based on the quality of genetic studies (familial/ population co-segregation vs small number candidate gene studies with no controls) and the interpretation of experimental functional in vitro assays and model organism studies. ClinGen also uses ClinVar, a freely available database funded by the National Institutes of Health that aggregates genomic variant data and their relationship to phenotype submitted by clinical testing labs, researchers, expert panels and societies. Table 2 summarises the major genes curated by ClinGen for the strongest evidence for causation of IASs, the proteins they encode and the proposed mechanisms involved.

The aim of genetic testing is to identify a pathogenic (class 5) disease-causing variant which must satisfy criteria based on a consensus developed by the American College of Medical Genetics.[3] Many variants (class 3) are labelled a 'variant of uncertain significance' (VUS) as they cannot be defined as disease causing without further investigation of association of phenotype (co-segregation) with the variant in a family. This represents a common problem for clinicians as families are usually too small or accessible to allow co-segregation and uncertainty may remain pending intermittent re-evaluation of variant data.[4] 'Likely pathogenic' (class 4) variants have less incontrovertible supportive data but can still be used diagnostically, although with caution.

In the clinic, a diagnosis is driven primarily by the presentation of the patient and/or the ECG. Detection of a class 4/5 variant may help to confirm the diagnosis and provide information on the mechanism of arrhythmogenesis that may guide treatment and provide prognostic information. However, the yield of class 4/5 variants varies considerably between different IASs as knowledge of causative genes and their genetic architecture is incomplete. A negative result therefore does not refute the diagnosis if a phenotype is present.

Clinical testing panels may still use genes curated by ClinGen as weak/disputed for gene-disease relationship. If a strong and clear phenotype prompts the use of genetic testing, variants identified in weak/disputed genes can be investigated further through familial co-segregation and functional experimental study. This may provide evidence to support upgrading the gene in future curation. In this vein, the finding of a variant in a weak/disputed gene, or a VUS, should prompt referral and assessment to a specialist clinic with access to a multidisciplinary team involving inherited arrhythmia specialists and geneticists.[5] An in-depth discussion and counselling is needed regarding family testing, which will still rely on clinical phenotypic evaluation until the variant is further assessed.

Carrying a class 4/5 variant in a causative gene does not guarantee expression of the associated disorder, known as 'incomplete penetrance'. This is most evident within families where relatives harbouring the same variant do not necessarily express disease. This is likely to be due in part to 'modifier' variants that are common in the general population. Thus, a class 4/5 variant is most useful in the context of family testing where family members without the variant can be discharged from clinic and those harbouring it subjected to clinical assessment.

| Entity | Genetic evidence strong/moderate for causation | | Genetic evidence weak/disputed for causation | |
|---|---|---|---|---|
| **LQTS** | *KCNQ1* (AD/AR) | (LQT1 / JLNS) | *ANK2* | (LQT4) |
| | *KNCH2* | (LQT2) | *KCNE1** (AD/AR) | (LQT5 / JLNS) |
| | *SCN5A* | (LQT3) | *KCNE2* | (LQT6) |
| | *CALM1* | | *CAV3* | (LQT9) |
| | *CALM2* | | *SCN4B* | (LQT10) |
| | *CALM3* | | *AKAP9* | (LQT11) |
| | *TRDN* (AR) | | *SNTA1* | (LQT12) |
| | *KCNJ2* | (LQT7 Andersen-Tawil Syndrome) | *KCNJ5* | (LQT13) |
| | *CACNA1C* | (LQT8 Timothy Syndrome) | | |
| **CPVT** | *RYR2, CASQ2* (AD/AR), *TRDN* (AR), *TECRL* (AR), *CALM1-3* | | *KCNJ2, SCN5A, PKP2, ANK2* | |
| **BrS** | *SCN5A* | | *ABCC9, ANK2, CACNA1C, CACNA2D1, CACNB2, FGF12, GPD1L, HCN4, KCND3, KCNE3, KCNE5, KCNH2, KCNJ8, RANGRF, PKP2, SCN10A, SCN1B, SCN2B, SCN3B, SEMA3A, SLMAP, TRPM4* (?AR) | |
| **SQTS** | *KCNQ1, KCNH2, KCNJ2, SLC4A3* | | *CACNA1C, CACNA2D1, CACNB2, SLC22A5* (AR), *SCN5A* | |

**Table 1. ClinGen curated gene panels for inherited arrhythmia syndromes.[2]** Autosomal dominant inheritance unless stated. * May be considered a milder or modifying form of LQTS that in autosomal recessive form can cause a milder form of JLNS. AD, autosomal dominant; AR, autosomal recessive; BrS, Brugada syndrome; ClinGen, Clinical Genome Resource; CPVT, catecholaminergic polymorphic ventricular tachycardia; JLNS, Jervell and Lange-Nielsen syndrome; LQTS, long QT syndrome; SQTS, short QT syndrome.

| Gene | Protein | Function | Proposed Effect | Entity | Inheritance | Ref. |
|---|---|---|---|---|---|---|
| *KCNQ1* | $K_V7.1$ | Pore subunit: slowly activating delayed outward rectifier potassium current, $I_{Ks}$ | Loss of function<br>Loss of function<br>Gain of function | LQT1<br>JLNS<br>SQTS | AD<br>AR<br>AD | [6]<br>[6]<br>[35] |
| *KCNH2* | $K_V11.1$ | Pore subunit: rapidly activating delayed outward rectifier potassium current, $I_{Kr}$ | Loss of function<br>Gain of function | LQT2<br>SQTS | AD<br>AD | [6]<br>[35] |
| *SCN5A* | $Na_V1.5$ | Pore subunit: cardiac sodium current, $I_{Na}$ | Gain of function<br>Loss of function | LQT3<br>BrS | AD<br>AD | [6]<br>[27] |
| *KCNJ2* | Kir2.1 | Pore subunit: inward rectifier potassium current which sets the resting membrane potential, $I_{K1}$ | Loss of function<br>Gain of function | Andersen-Tawil Syndrome Type 1 (ATS1; LQT7)<br>SQTS | AD<br>AD | [7]<br>[35] |
| *CACNA1C* | $Ca_V1.2$ | Pore subunit: inward calcium current, $I_{CaL}$ | Gain of function | Timothy Syndrome (TS; LQT8) | AD | [8] |
| *CALM1-3* | Calmodulin | Calcium-dependent signalling to an array of cardiac ion channels including $Ca_V1.2$<br>RyR modulation | Downstream modulation and increase in $I_{CaL}$<br>SOICR | *CALM*-LQTS<br>*CALM*-CPVT | AD<br>~90% are *de novo* variants and germline mosaicism has also been described<br>AD | [9]<br>[9] |
| *TRDN* | Triadin | Forms part of the sarcoplasmic reticulum (SR) calcium release unit<br>SR-Calsequestrin localisation | Downstream increase in $I_{CaL}$ via impaired calcium-dependent inactivation from reduced SR $Ca^{2+}$ release<br>SOICR | *TRDN*-LQTS<br>*TRDN*-CPVT | AR (Homozygous; Compound heterozygous)<br>AR | [10]<br>[10] |
| *RYR2* | Ryanodine receptor | Forms part of the sarcoplasmic reticulum (SR) calcium release unit | SOICR | CPVT | AD | [23] |
| *CASQ2* | Calsequestrin | Forms part of the sarcoplasmic reticulum (SR) calcium release unit | SOICR | CPVT | AD & AR forms | [23] |
| *TECRL* | trans-2,3-enoyl-CoA reductase-like protein | Modulates RyR | SOICR | CPVT | AR | [25] |
| *SLC4A3* | cardiac chloride-bicarbonate exchanger | Acid-base modulation | Inhibition of $I_{CaL}$ | SQTS | AD | [36] |

**Table 2. Genes curated as strong/moderate for causation in IASs: function and mode of disease.** AD, autosomal dominant; AR, autosomal recessive; ATS1, Andersen-Tawil syndrome type 1; CPVT, catecholaminergic polymorphic ventricular tachycardia; JLNS, Jervell and Lange-Nielsen syndrome; LQTS, long QT syndrome; RyR, ryanodine receptor; SOICR, SR luminal $Ca^{2+}$ store overload induced $Ca^{2+}$ release (see also figure 3); SQTS, short QT syndrome; SR, sarcoplasmic reticulum.

## Clinical presentations

IASs may present in a variety of ways including incidental findings; family evaluation following diagnosis of an index case (the proband); symptomatically with presyncope or syncope; aborted cardiac arrest or as part of a syndromic condition with extra-cardiac features. Table 3 summarises the clinical presentations, precipitating factors for arrhythmia and management of the conditions.

| Entity | Prevalence / Incidence | Diagnosis* | Arrhythmic Presentation | Management |
|---|---|---|---|---|
| Long QT Syndrome[6,21] | ~1/2000<br><br>Syndromic presentations rare | QTc > 480ms<br><br>a LQTS risk score > 3<br><br>QTc > 460ms and syncope<br><br>Pathogenic variant<br><br>Jervell and Lange-Nielsen Syndrome (JLNS):<br>severe paediatric disease with congenital deafness<br><br>Andersen-Tawil Syndrome Type 1 (ATS1; LQT7):<br>triad of periodic $K^+$-sensitive muscle paralysis, dysmorphic features (scaphocephaly, clinodactyly, brachydactyly, syndactyly), cardiac repolarisation abnormalities (prominent U waves V2-V4)/arrhythmias<br><br>Timothy Syndrome (LQT8):<br>Arrhythmias, syndactyly, facies, and neurodevelopmental delay | Torsades de Pointes/VF causing syncope or CA<br><br>LQT1:<br>During adrenergic stress<br><br>LQT2:<br>In response to abrupt stimuli eg. Alarm clocks<br>Increased risk in post-partum females<br><br>LQT3:<br>At times of increased vagal tone | Avoid QT prolonging drugs (www.crediblemeds.org) and electrolyte disturbance (hypokalaemia, hypomagnesaemia)<br><br>Beta-blockers preferably nadolol<br><br>Mexiletine: LQT3 and add-on LQT2<br><br>ICD for aborted SCD or arrhythmic syncope despite drug treatment<br><br>Left cardiac sympathetic denervation (LCSD) for recurrent syncope/arrhythmia/shocks despite medical therapy, or ICD refused/contraindicated |
| Catecholaminergic Polymorphic VT[6,21] | ~1/10000 | Bidirectional VT and/or pVT during exertion or adrenergic drive<br><br>Pathogenic variant | Bidirectional VT, pVT/VF causing syncope or CA<br><br>Male children higher risk<br><br>SCD first presentation ~30% | Nadolol +/- flecainide<br><br>LCSD for recurrent syncope/arrhythmia/shocks despite medical therapy<br><br>ICD for aborted SCD but high risk of inappropriate therapies and may be deleterious |
| Brugada Syndrome[6,32] | ~1/2000 | Spontaneous type 1 Brugada ECG pattern in one or more leads among V1 or V2 in the $2^{nd}$, $3^{rd}$ or $4^{th}$ intercostal space<br><br>Shanghai Score System:<br>Drug-provoked or fever-induced Br Type 1 ECG require other factors (symptom history, family history or genetic status) | pVT/VF usually at rest or during sleep, periods of increased vagal tone.<br><br>Atrial arrhythmias in up to 20%<br><br>Conduction disease with *SCN5A* disease (sinus node disease and AV block) | Avoid provoking drugs (www.brugadadrugs.org) and electrolyte disturbance, and treat fever.<br><br>ICD for aborted SCD or arrhythmic syncope<br><br>Hydroquinidine for atrial arrhythmia or to suppress VT/VF<br><br>Isoprenaline for VT/VF storm<br><br>Ablation of RVOT epicardial sites displaying slow conduction for storm or repeat shocks |
| Early Repolarisation (ER) Syndrome[21,32] | Uncertain<br><br>Male > Female | Unexplained VF arrest (see IVF) with inferior or infero-lateral early repolarisation pattern:<br>J-point elevation (notched or slurred) ≥ 0.1mV in 2 contiguous inferior or lateral ECG leads (excluding V1-V3) | pVT/VF usually at rest or during sleep, periods of increased vagal tone | ICD<br><br>Hydroquinidine<br><br>Isoprenaline for VA storm |

| | | | | |
|---|---|---|---|---|
| Idiopathic VF[21,32,35,50] | 1.2% of OHCA survivors presenting with a shockable rhythm | Unexplained VF with normal ECG, stress test, coronary anatomy, imaging and drug provocation | Can exhibit short-coupled PVCs <350ms | ICD<br><br>Hydroquinidine<br><br>Isoprenaline, intravenous verapamil for VA storm<br><br>Ablation of the triggering PVC for storm or repeat shocks |
| SADS[37] | 1-2/100,000 per annum in 1-35 year olds | Unexplained death without prior cardiovascular disease within 1h of symptom onset or an unwitnessed death with the individual being seen in good health within 24 h of death; no cause of death is identifiable on comprehensive coronial and cardiac autopsy or on toxicological analyses | Male with death at rest/sleep most common | History and family history<br><br>Specialist cardiac post-mortem<br><br>Molecular autopsy<br><br>Familial evaluation |
| Progressive Cardiac Conduction Defect and structurally normal heart[35] | Rare, uncertain | Unexplained progressive conduction disease <50 years old without skeletal myopathy or cardiomyopathy +/- family history of PCCD | Bradyarrhythmias<br>Bundle branch block<br>Heart block | Permanent Pacemaker |
| Short QT Syndrome[6,21] | Very rare, uncertain | QTc < 340ms<br><br>QTc < 360ms in the context of VT/VF, family history and/or pathogenic variant | pVT/VF 4% in the first year of life, then 1% per year to 40 years of age<br><br>80% during rest or sleep<br><br>20% atrial arrhythmia | ICD following VF or sustained VT<br><br>Quinidine or sotalol to suppress VT/VF |

**Table 3. Clinical presentations of the inherited arrhythmia syndromes, precipitating factors for arrhythmia and management.** *In the absence of reversible or structural cause. ATS1, Andersen-Tawil syndrome type 1; CA, cardiac arrest; ICD, implantable cardioverter defribrillator; LCSD, left cardiac sympathetic denervation; LQTS, long QT syndrome; OHCA, out-of-hospital cardiac arrest; PCCD, progressive cardiac conduction disease; PVC, premature ventricular contraction; pVT, polymorphic VT; RVOT, right ventricular outflow tract; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia.

## The Long QT Syndrome

*Genetics*

LQTS is usually inherited in an autosomal dominant (AD) fashion in the absence of extra-cardiac features (Romano-Ward Syndrome; LQT1-6/9-13). Approximately 75% of patients with LQTS harbour a variant in LQTS susceptibility genes (table 1).[6] Of these, over 90% are found in the three major LQTS genes *KCNQ1; KCNH2; SCN5A* (LQT1–3; table 2).[6]

JLNS is a rare autosomal recessive (AR) form accompanied by deafness in childhood (*KCNQ1; KCNE1*; see all tables).

Andersen-Tawil syndrome type 1 (ATS1; *KCNJ2*; LQT7; tables 2 and 3) is a rare AD multisystem disorder, although non-cardiac manifestations are not always apparent.[7]

Timothy syndrome (*CACNA1C;* LQT8; tables 2 and 3) is also a rare AD multisystem syndrome disorder, although non-syndromic forms of LQT8 are also recognised.[8]

*CALM1–3* all encode the same highly conserved protein calmodulin. AD variants lead to calmodulinopathies either a *CALM*-LQTS (sometimes with functional 2:1 AV block) or *CALM*-CPVT phenotype usually in childhood (table 2).[9]

*TRDN* encodes triadin which forms part of the sarcoplasmic reticulum (SR) calcium release unit (figure 2). Expression of a LQTS or CPVT phenotype is due to homozygous or compound heterozygous AR inheritance.[10]

Other candidate genes have been implicated in LQTS (table 1) but where genetic/experimental data are not as strong.[2] For example, *KCNE1* (LQT5) and *KCNE2* (LQT6) encode the beta-subunits of $K_V7.1$ and $K_V11.1$, respectively and have been downgraded to either mild disease (*KCNE1*) or modifier or susceptible variants (*KCNE1* and *KCNE2*).[11]

Between 4% and 28% of patients deemed initially to have acquired LQTS due to QT prolonging effects of drugs or electrolyte disturbances may be unmasking LQTS due to an underlying class 4/5 variant.[12]

*Genotype-Phenotype Correlation*

$I_{Ks}/I_{Kr}$ are activated on membrane depolarisation. $I_{Ks}$ is recruited through the positive downstream effects of adrenergic drive, accumulating to abbreviate repolarisation at higher heart rate.[13] Thus, the LQT1 phenotype and cardiac events, tend to be precipitated during adrenergic drive (exercise) and, classically, present with a broad-based T wave.

$I_{Kr}$ is the predominant outward potassium current during rest or transient abrupt increases in heart rate.[13] Thus, in LQT2, deficiencies in $I_{Kr}$ lead to arrhythmia during, for example, standing abruptly or precipitated by a loud auditory stimulus.[14] The T wave tends to be notched.

$I_{NaL}$ is a persistent inward sodium current in phases 2 and 3 of the action potential due to a population of $Na_V1.5$ channels that fail to inactivate or reactivate after recovery from

inactivation. Gain-of-function *SCN5A* variants causing an increased and prolonged $I_{NaL}$ lead to a late peaked T wave in LQT3.[15] Accentuations of QT prolongation and cardiac events in LQT3 occur predominantly at times of slow heart rate such as during sleep.[14]

Calmodulinopathies and ATS1 often present with prominent U waves and LQT may be considered a misnomer in ATS1. In *CALM*-LQTS, calcium overloading of the SR from increased $I_{CaL}$ and prolonged action potential duration may lead to spontaneous calcium release events and, thus, delayed after-depolarisations (DADs). Critically timed activation from a DAD can trigger arrhythmia. Prolonged repolarisation in ATS1 can also lead to a similar phenomenon and exertionally induced arrhythmia.[16]

Triadin ensures co-localisation of SR proteins (ryanodine receptor (RyR), calsequestrin, junctin) at the SR cisternae in juxtaposition with $Ca_V1.2$ channels in a complex known as the dyad (see CPVT).[10] Triadin loss disturbs this architecture and leads to reduced SR calcium release during adrenergic drive and impaired calcium-dependent inactivation of $Ca_V1.2$ channels, prolonging their activation and the QT interval, and eventually calcium overloaded myocytes and arrhythmias.[10]

*Genotype Based Risk*

While the general hierarchy of higher risk can be indicated by a QTc > 500ms, LQT3/LQT2 genotype and adult women,[6] there can be additional insights from genetics that may assist in tailoring an approach.

Potassium channel pores are made up of a tetramer of subunits and missense variants on one allele (LQT1 and LQT2) produce dominant negative effects on the whole channel and portend higher risk, whilst haploinsufficiency from non-missense variants have a milder phenotype.[17] Compound heterozygous variants can also have additive effects to produce a more aggressive phenotype, and LQT1 patients harbouring variants in the $K_V7.1$ C-loop region (voltage sensor) have been shown to be at higher risk for arrhythmic events.[17] LQT2 $K_V11.1$ pore-loop variants carry a higher risk profile, especially in men with an up to 6-fold higher hazard ratio.[17] LQT3 in general demonstrates the highest risk of life-threatening arrhythmic events, although some variants, such as E1784K and D1790G, may have a milder phenotype.[18]

Calmodulinopathies, and Triadin knockout syndrome carry a very high risk from the early years of life.[9,10] Whereas ATS1 may have been considered a lower risk entity previously, recent data suggest the opposite with a high incidence of breakthrough events despite beta-blocker therapy.[19]

*Genotype Based Therapy*

Patients with LQT1 are best advised to avoid emotional stress or strenuous exercise, and can be given an exercise prescription based on the results of controlled stress testing on beta-blockers.[20] LQT2 patients should avoid unexpected auditory stimuli.[14]

There are no randomised clinical trials examining pharmacological therapies in LQTS. Observational data support empirical therapy with beta-blockers for all LQT subtypes including asymptomatic patients and genotype-positive phenotype-negative family members, improving outcomes and freedom from SCD by up to ~60%.[6]

The greatest benefit of beta-blocker therapy is in LQT1 where nadolol is the preferred agent.[21] The sodium channel blocker mexiletine has shown positive results for LQT2/LQT3 patients as add-on therapy to beta-blockers, or monotherapy if particularly sensitive to low heart rate.[21]

Recently in vitro correction of trafficking defects has been demonstrated using drug molecular chaperones like lumacaftor already in clinical use for cystic fibrosis, raising hopes of a new approach to therapy.[22]

## Catecholaminergic Polymorphic Ventricular Tachycardia

*Genetics and Phenotype Correlation*

The pathogenesis of CPVT was first explained by gain-of-function variants in *RYR2*, the gene encoding RyR accounting for ~60% of patients with a clear diagnosis.[23] AD inherited missense rare variants increase the sensitivity of RyR to SR luminal $Ca^{2+}$ store overload induced $Ca^{2+}$ release (SOICR) and this incurs the development of DADs (figures 2 and 3).[23]

Other genes have been implicated in ~1-2% of patients. A rare AR severe form was identified in a Bedouin kindred in Israel exhibiting a missense variant in *CASQ2* encoding calsequestrin-2.[24] Further rare *CASQ2* nonsense and missense variants have been identified that lead to either reduced calsequestrin-2 expression, defects in $Ca^{2+}$ binding capacity or disturbances in modulation of RyR.[23] This all predisposes to impaired $Ca^{2+}$ handling and DAD formation.

AR loss-of-function *TRDN* variants have also been associated with a phenotype more in keeping with CPVT than LQTS.[10] A *CALM*-CPVT phenotype is seen in about a third of Calmodulinopathy patients.[9] *TECRL* encodes trans-2,3-enoyl-CoA reductase-like protein that modulates RyR activity and recently novel recessive variants have been associated with a CPVT-like phenotype.[25] Other genes have been associated with a CPVT phenotype but are not considered to have strong evidence (table 1).

*Risk Stratification and Management*

*CASQ2* variants lead to a severe form of CPVT when inherited in a homozygous or compound heterogeneous manner.[23] Beyond this risk stratification and management is clinically based on arrhythmic burden (see table 3).

## The Brugada Syndrome

*SCN5A* was the first gene implicated in BrS. *SCN5A* variants were identified with loss-of-function that was exacerbated by higher temperatures, consistent with fever as a clinically recognised provocation.[26] This expanded to include several hundred associated loss-of-function variants, the majority missense, but including nonsense, frameshift, splice-site, and in-frame insertion/deletions transmitted in an AD fashion. Worsening phenotypes correlate with the degree of loss-of-function, including complete loss-of-function or failure to traffic to the sarcolemma leading to a deficit of half of functional product known as haploinsufficiency.[27] Loss-of-function sodium channels that still traffic also lead to slower

cardiac conduction.[27] Overall ~20% of BrS families harbour *SCN5A* disease but with PR prolongation >200ms this rises to ~40%.[28]

Other genes have been implicated in BrS although they remain disputed for diagnostic purposes (table 1).[29] *SCN10A* encodes a neuronal sodium channel pore subunit $Na_V1.8$ with expression in intracardiac neuronal tissue, and possibly cardiomyocytes.[30] A common variant with loss-of-function has been associated with BrS.[31] Mechanistic effects of *SCN10A* variants could, however, be related to consequent altered expression of *SCN5A,* or altered expression of a $Na_V1.5$-interacting shortened *SCN10A* transcript protein $Na_V1.8$-short.[30]

Another putative paradigm is loss-of-function variation in *CACNA1C* and *CACNB2*, encoding the $I_{CaL}$ alpha-subunit and beta-subunit respectively, implicated in a crossover syndrome of a type 1 Brugada ECG pattern and short QT intervals.[32] The proposed mechanism is akin to sodium channel loss-of-function (figure 3) but data are insufficiently robust for a routine role in clinical testing.[29] At present recommendations for therapeutics (table 3) do not require genetic data although this may change.[6,27]

## Progressive Cardiac Conduction Disease and Structurally Normal Heart

The gene with the most definitive evidence for familial PCCD is *SCN5A* through dominant loss-of-function variants that commonly lead to overlap conditions with BrS, though AR or compound heterozygous forms have also been described.[33] *TRPM4* encodes a $Ca^{2+}$-dependent non-selective monovalent cation channel (TRPM4) where loss-of-function variants, and a gain-of-function rare variant due to impaired endocytosis, have been implicated in isolated PCCD although the mechanism is unclear.[34]

## The Short QT Syndrome

Inheritance in SQTS is AD but its heritability is only explainable in 15-25% of families.[6] Gain-of-function variants in the potassium channel genes *KCNH2, KCNQ1* and *KCNJ2* have been associated and lead to an increase in repolarising current.[35] A heterozygous missense rare variant in *SLC4A3* encoding the cardiac chloride-bicarbonate exchanger was found to co-segregate with phenotype in two unrelated families with SQTS.[36] The mechanism of phenotype is not clear but may be representative of acid-base disturbance on ion channel function.[36] Other genes have also been detected but are rare and insufficiently robust (table 1). There is no gene-specific risk stratification evident to date.

## Sudden Arrhythmic Death Syndrome, Idiopathic VF and The Early Repolarisation Syndrome

In SADS (Sudden Arrhythmic Death Syndrome; table 3) no cause of death is identifiable on comprehensive coronial and cardiac autopsy or on toxicological analyses.[37] Genetic testing using a wide panel of IAS and cardiomyopathy genes (molecular autopsy) in SADS probands leads to a 13% yield of class 4/5 variants that are immediately useful clinically. Coupled with clinical evaluation of family members, this leads to a diagnosis in ~40% of families,[37] highlighting the immense importance of cardiac pathological assessment and molecular autopsy from retained tissue from the decedent.

After an out-of-hospital VF arrest, survivors will commonly undergo a series of initial tests including ECG, echocardiography, coronary angiography, signal-averaged ECG, MRI, exercise testing and provocative drug testing. This to investigate for an occult cardiomyopathy, IAS or coronary artery spasm. In approximately 50% , the cause of the VF arrest remains unexplained (Idiopathic VF, IVF) but applying genetic testing to the IVF group via a wide panel of cardiomyopathy and IAS genes can lead to a class 4/5 variant in ~20%.[38] More specifically, a haplotype (a collection of linked variants) at the *DPP6* gene co-segregated with IVF with short-coupled ventricular ectopic triggers in an AD fashion in three Dutch families.[39] *DPP6* encodes dipeptidyl-aminopeptidase-like protein 6 and is a putative accessory subunit of $K_V4.3$ carrying the $I_{to}$ current. The haplotype associates with a particularly malignant phenotype that justifies empirical ICD implant amongst heterozygotes.[39]

The early repolarisation (ER) pattern (table 3) was first associated with IVF in 2008, termed the early repolarisation syndrome (ERS). It is present in ~20% of relatives of SADS victims (vs 8% controls) and 25% of relatives of IVF survivors, indicating heritability.[32,40] Loss-of-function Class 4/5 *SCN5A* variants have been identified in up to 10% of ERS survivors.[32] However, other associated genes have less robust data and are likely to be disputed when assessed by ClinGen.

## Atrial Fibrillation (AF)

At present there is no clinically applicable approach to genetic testing for the management of AF. Propensity to the arrhythmia is present in other IASs such as BrS. To date five major premature AF-associated genes have been implicated via familial linkage analysis or GWAS: *KCNQ1*, *NPPA*, *TBX5. MYL4*, and *TTN* .[41]

## Future Directions for Genetic Testing

*Genome Wide Association Studies and Polygenic Risk Scores*

The missing heritability in IASs is likely to be explained, in some part, by common variants present with a frequency of more than 1% in the general population. Their effect sizes can be estimated via GWAS and polygenic risk scores (PRSs), derived thereof by combining variants whether protective or predisposing. A GWAS of BrS probands vs healthy controls uncovered common variants with genome-wide significance at three loci (genomic locations) near *SCN5A*, *SCN10A* and *HEY2*.[42] These common variants have been employed in PRSs to explain phenotype.[43] A higher PRS may explain in part incomplete penetrance within families harbouring *SCN5A* rare variants, such that even genotype negative family members can demonstrate a positive sodium channel blocker challenge.[43] A larger GWAS has associated 12 loci with BrS and explained more of its heritability.[44] Furthermore, a GWAS in LQTS has uncovered 3 loci associated with LQTS consisting of common variants that also associate with the QT interval at *NOS1AP*, *KCNQ1* and *KLF12*.[45] A PRS derived from common variants associated with QT interval also explained some of the heritability underlying LQTS as well as being highest in LQTS patients without class 4/5 variants.[45]

Thus, polygenic susceptibility to IASs may act as a modifier of rare variant expressivity or may be sufficiently potent to lead to phenotypic expression. It therefore appears possible that PRSs may play a diagnostic role in the future. Furthermore, the future approach of genetic evaluation in common arrhythmias such as AF is likely to be driven via PRSs with an initial focus on predicting the response to anti-arrhythmic drugs and catheter ablation.[46]

*Whole-Genome Sequencing*

Conventional exome-based genetic testing may miss some rare variants. Intronic rare variants in the known LQTS genes have been uncovered using whole-genome sequencing in previously gene elusive pedigrees.[47] It can also identify structural genetic variants more readily including inversions, duplications, translocations, as well as large deletions and insertions and will interrogate common variation applicable for PRSs.

*To Causality*

Common variants associated with a disease via GWAS are often at loci in a region of interest with nearby genes, rather than in the coding region of a specific gene. Their effects may be on nearby gene expression and can be investigated via RNA expression in specific tissues via expression quantitative trait loci mapping (eQTL). This approach can only develop with collaboration given the need for access to large tissue banks. Delving more deeply will require single cell RNA expression analysis as cardiac cellular composition involves a multitude of different cell types.[48]

Functional assays of novel genes and variants using whole heart methods are not conducive to a high throughput approach. Cellular approaches using gene editing and human inducible pluripotent stem cells can help to overcome this by profiling electrophysiology using high throughput patch clamp for ionic currents, calcium fluorescence imaging, staining for structural analysis and deep mutational scanning of large-scale libraries of mutated variants in a single experiment.[49] This will aid development of new therapies.

Finally, machine learning approaches will be able to analyse large datasets from the output of genotyping and genome studies, together with clinic biomarkers such as ECG, echocardiography and blood markers. This may lead to enhanced diagnostics and prognostication.

## Conclusions

At present the diagnosis of underlying propensity to arrhythmia is predominantly a clinical approach. In some circumstances, identification of a pathogenic rare variant leads to a diagnosis even in the absence of the phenotype. This can inform treatment to reduce risk even prior to phenotypic expression. However, in the large part the clinical utility of genetic testing is to support a diagnosis and improve subsequent cascade familial evaluation, and in some cases provide prognostication. In future, missing heritability will be uncovered by association of common variants through GWASs and novel rare variants through whole genome sequencing. PRSs will likely help to predict phenotypic expression and response to treatments/risk stratification. High throughput functional assays will be needed to support causality of associated variants and develop new therapies.

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