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New Insights into the Genetic Basis of Inherited Arrhythmia Syndromes

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Introduction

Inherited arrhythmia syndromes encompass a number of different diseases including long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), short QT syndrome (SQTS), idiopathic ventricular fibrillation (IVF) and progressive cardiac conduction system disease (PCCD).[1] The heart is typically structurally normal with no evidence of disease macroscopically. They are an important cause for sudden cardiac death (SCD) in the young and an autopsy is typically negative.[2, 3]

Ventricular arrhythmias are due to mutations of ion channels and their interacting proteins, predominantly involving potassium, sodium and calcium handling.[4] Genetic studies have identified the specific genetic abnormalities that underpin these diseases even permitting diagnosis in the deceased using post-mortem genetic testing (the "molecular autopsy").[3] Most arrhythmia syndromes are inherited in an autosomal dominant manner, such that first degree family members have a 50% chance of inheriting the disease. Identification of the mutation allows for predictive genetic testing in other living family members.[4] 'Variable penetrance' is common in all arrhythmia syndromes, the same mutation in the same family causing wide variation in phenotype.[4] This suggests that other factors such as genetic modifiers and environmental factors may influence the phenotype.

This review will highlight the latest developments in understanding the genetic basis of inherited arrhythmia syndromes and discusses the new opportunities and challenges faced with evolving genetic technologies including determining pathogenicity and the utility of large genetic databases. Finally, we will discuss newly described entities that continue the evolving theme of genetic syndromes with phenotypic overlap. Early views that a single genotype

associates with a particular phenotype continue to be challenged by our greater understanding of the genotype phenotype relationship.

Inherited Arrhythmia Syndromes

Long QT Syndrome

Congenital LQTS is diagnosed in the presence of a prolonged corrected QT (QTc) interval after secondary causes (e.g. QT-prolonging medications or electrolyte abnormalities) are excluded.[1] The 2013 HRS/EHRA/APHRS guidelines recommended that LQTS also be diagnosed in the presence of an LQTS risk (Schwartz) score ≥3.5 or in the presence of an unequivocally pathogenic variant in one of the known LQTS genes.[1] Patients with unexplained syncope and a borderline QTc between 480-499ms in recurrent ECGs, or who were asymptomatic but had repeated measurements of over 500ms, may also be diagnosed with LQTS.[1] The more recent 2015 ESC guidelines softened these criteria to QTc≥480ms in an asymptomatic patient or a QTc ≥460ms in the presence of unexplained syncope.[5] It was argued that higher values equated to high risk LQTS and were therefore too conservative.

QT prolongation results from ion channel dysfunction that prolongs cellular repolarisation.[6, 7] There are currently 15 genes known to cause congenital LQTS, however the main 3 genotypes account for >90% of genetically confirmed LQTS (**Table 1**).[1, 4] The genetic cause remains elusive in 25% of LQTS families.[12] LQT1 is caused by loss of function mutations affecting *KCNQ1* the gene encoding for I_{Ks} (slow) channel.[4] Both haploinsufficiency and dominant-negative mechanisms of *KCNQ1* mutations are described.[13, 14] LQT2 is caused by loss of function mutations in *KCNH2* the gene encoding for the I_{Kr} (rapid) channel predominantly due to failure of trafficking to the cell membrane surface.[15, 16] Gain of function mutations in *SCN5A*, the gene encoding for I_{Na}, causes failed inactivation and increased late current leading to LQT3.[17]

Whilst most LQTS is inherited in an autosomal dominant manner, the rare recessive form (Jervell and Lange-Nielsen Syndrome [JLNS]) leads to a severe LQTS phenotype and associated sensorineural deafness.[18] Indeed, greater severity is typical when more than one mutation is identified.[4]

A recent study has suggested that LQTS may be preferentially transmitted from the maternal allele, with higher than expected maternal transmission and inheritance, particularly with LQT1.[19] The authors also found that this phenomenon was not linked to locus-specific grandparental origin allele transmission distortion and in fact appears to be related to the severity of channel dysfunction. The authors postulate that that altered potassium channel function may modulate reproduction, particularly *KCNQ1* which is expressed in the granulosa and trophoblastic cells of the ovaries.[19] LQTS-associated potassium channel dysfunction has also been proposed recently as a possible mechanism for some stillbirths given that LQTS susceptibility variants can be identified in some cases.[20] This hypothesis follows on from evidence that some sudden infant death syndrome (SIDS) may also be due to LQTS.[4]

Common variants (single nucleotide polymorphism or SNPs) at the *NOS1AP* locus have been shown to modify phenotype by affecting both the QT interval and the likelihood of symptoms in LQTS, whilst common variants in *KCNQ1* within the 3'untranslated region may also influence disease severity.[21-23] A recent genome-wide association and replication study identified a total of 35 SNP tagged loci associated with QT interval amongst around 100,000 individuals of European ancestry, 6 of which were found to have rare coding variants in LQTS patients absent in controls.[24] Whilst this does not equate to pathogenicity, it is likely that these loci may also modify the LQTS phenotype to some extent.

Genotyping in LQTS is useful diagnostically, therapeutically and to a lesser extent,

prognostically.[1, 12] Beta-blockers are the mainstay of therapy, with LQT1 patients, particularly those with mutations in cytoplasmic loops, most protected regardless of drug and LQT2 patients responding best to nadolol.[25, 26] Sodium channel blockers (e.g. mexiletine) can be useful in addition to betablockers for patients who have LQT3.[27] High risk features include severe QT prolongation, boys, female adults, prior cardiac arrest and syncope despite beta-blocker therapy. However only LQT2 females and those with high risk genetic profiles (i.e. multiple mutations or JLNS) have been associated with sufficient additional risk to suggest a potential risk-stratifying role.[5] Additionally the biophysical consequences of the mutation may offer alternative methods for stratification.[28]

Brugada Syndrome

BrS was first formally described in 1992, whereby Brugada et al reported 8 patients with characteristic cove-shaped ST elevation in the right precordial leads with associated SCD due to ventricular fibrillation (VF).[29] Diagnostic criteria from a recent consensus report require the patient to have a spontaneous type 1 Brugada pattern ECG defined as ≥2mm ST elevation with type 1 morphology in ≥1 right precordial lead V1 or V2 in either 2nd, 3rd or 4th intercostal space.[30] Unlike the earlier 2013 Guidelines,[1] if the type 1 pattern is only revealed after sodium channel blocker challenge, the diagnosis of BrS also requires one of the following: documented VF or polymorphic ventricular tachycardia (VT), suspected arrhythmic syncope, family history of SCD with negative autopsy, BrS ECG in family members, or nocturnal agonal respiration. This follows recent data suggesting a higher than expected yield of positive results in ajmaline provocation tests in an apparently healthy control population.[31]

Traditionally BrS has been proposed as a primary electrical disease involving a relative impairment of the inward sodium current compared to the transient outward potassium current

(I_{to}) in the right ventricular outflow tract (RVOT).[30] However a transgenic mini-pig model of a truncating *SCN5A* mutation showed evidence of conduction disease but without a type 1 ECG pattern.[32] This may reflect that haploinsufficiency leads to an isolated conduction disease phenotype without the necessary further genetic variation associated with BrS in humans (see below); the young age of the pigs given that BrS exhibits age related penetrance; and/or the absence of I_{to} in the pig myocardium. The diagnosis of BrS has also required the exclusion of overt structural heart disease and the pathophysiology has been attributed to transmural and epicardial heterogeneity of repolarization. However, there is mounting evidence that BrS may represent one end of a spectrum of subtle structural disease.[33] In particular imaging studies have suggested abnormalities of the right ventricle and RVOT.[34, 35] Epicardial ablation studies have identified fractionated signals consistent with slowed conduction and fibrosis which have been confirmed by other investigators both invasively and using ECG imaging.[36-38] Subsequent pathological studies in open surgical ablation and sudden death victims from BrS families have confirmed subtle epicardial fibrosis and reduced connexin-43 in the RVOT.[39] An experimental perfused canine wedge model has however proposed that these fractionated potentials may result from phase II re-entry.[40]

BrS has traditionally been considered as an autosomal dominant inherited disease. However, this convention has also been challenged. The most common associated genetic abnormality is a loss of function mutation in *SCN5A*. However, a mutation is identified in only one fifth of patients.[4] Strong linkage studies associating *SCN5A* with BrS do not exist, with the exception of families with overlap phenotypes with LQTS and PCCD.[41] Additionally, the genotype-phenotype correlation within families shows worrying mismatch. Probst et al, described 5 out of 13 large BrS families with known *SCN5A* mutations with several individuals

with a type 1 Brugada ECG pattern *within the same family* who were mutation-negative.[42] Mutations in 22 other genes encoding components and interacting proteins of the sodium channel, calcium channel, and potassium channels have also been implicated in BrS (**Table 2)** however few are based on strong linkage studies and most are rare.[4, 43]

Furthermore, a genome-wide association study identified three SNPs with additive effects on the likelihood of BrS irrespective of the presence of an *SCN5A* mutation.[44] These included two SNPs at the *SCN5A* and *SCN10A* locus; *SCN10A* encoding the alpha subunit of the neuronal Nav1.8 current associated with nociception. This prompted investigation by Hu et al that suggested that mutations in *SCN10A* are present in up to 16% of BrS patients.[45] A contemporaneous study assessed the burden of rare coding variants in BrS associated genes in patients compared to a control group and only showed enrichment of *SCN5A.* This concluded that previously reported genes including *SCN10A* do not account for BrS cases in patients of European ancestry.[46] Additionally, a subsequent study of 45 known cardiac genes in *SCN5A* negative cases showed enrichment of the *DSC2* gene linked to arrhythmogenic right ventricular cardiomyopathy (ARVC) but not the other BrS genes.[47] These data reinforced the possible role for ARVC associated rare variants in BrS that had already been proposed for the *PKP2* gene in an earlier study.[48] Another study of potential novel candidate genes in *SCN5A* negative cases showed a similar low yield although a probable mutation was identified in *TBX5*, a transcription factor for *SCN5A* and *SCN10A*.[49] The study also confirmed previous findings that SNPs at the *SCN10A* locus associates with BrS but rare variation was not significantly associated. Collectively these studies support the theory that BrS is an oligogenic disease with the potential that ion channel and structural rare variants may have modulatory influences upon a 'common substrate'. Together with other factors such as age, gender and environmental exposures, these

affect the susceptibility for the BrS phenotype and potentially contribute to the subtle structural disease that has been associated with the disorder. This may also account for the unexpectedly high yield of ajmaline provocation in the general population, whereby susceptibility to BrS is partly due to genetic variation prevalent in the general population.[31] The role for diagnostic genetic testing is therefore relatively limited at this time until we are able to dissect the interaction of rare and common variation.

Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is a highly lethal, rare inherited arrhythmia syndrome characterised by bidirectional and polymorphic VT which is adrenergically stimulated, typically through physical exertion.[50, 51] The diagnostic criteria require a structurally normal heart and normal baseline ECG, with the development of polymorphic or bidirectional VT following exercise or catecholamine, usually in a patient aged <40yrs.[1] CPVT can also be diagnosed in patients who are found to be carriers of a known clearly pathogenic genetic variant as well as in family members of an index case who develop premature ventricular contractions (PVCs) during exercise.[1]

The pathophysiology of CPVT is dependent upon abnormal intracellular calcium handling leading to triggered activity.[52] Linkage studies in 2 large Finnish families with autosomal dominantly inherited CPVT first identified linkage at chromosome 1q42-43.[53] Subsequent studies indicated the gene encoding the cardiac ryanodine receptor gene (*RYR2*) to be responsible (CPVT1).[1, 4] In CPVT, *RYR2* is 'leaky' under adrenergic activation, causing an excess of calcium release into the cytosol in diastole resulting in increased activation of the sodium-calcium exchanger and delayed after-depolarisations.[54] A rarer form of autosomal recessive CPVT (CPVT2) is known to result from mutations in the cardiac calsequestrin gene (*CASQ2*) an important calcium storage and buffering protein in the sarcoplasmic reticulum

interacting with *RYR2*.[4] A recent study has also shown linkage to a heterozygous *CASQ2* variant in autosomal dominant CPVT for the first time.[55]

Collectively, these two genes combined account for around 50-65% of cases of CPVT.[12] Other genes, including Calmodulin (*CALM1)*, Triadin (*TRDN*), *TECLR*, Ankyrin-B (*Ank2)* and *KCNJ2* account for rare cases of CPVT.[4, 56] Whether *Ank2* and *KCNJ2* represent phenocopies or overlap syndromes with LQT4 and 7 respectively remains a moot point (**Table 3**).

Short QT Syndrome

SQTS is a rare inherited arrhythmia syndrome characterised by syncope or sudden cardiac death associated with a shortened QTc interval. The diagnosis requires the presence of a very short QTc ≤330ms, or a QTc ≤360ms in association with one of the following: family history of SQTS, pathogenic mutation, family history of sudden death at age ≤40 years; and/or survival of a VT/VF episode in the absence of heart disease.[1] SQTS was first described in 2000[57] and since then candidate gene studies have linked SQTS to missense mutations causing gain of function in potassium channels (*KCNH2*- SQT1, *KCNQ1*- SQT2, *KCNJ2*- SQT3).[4, 58] There is also an association with loss of function mutations in calcium channels (*CACNA1C* and *CACNB2b*) in association with a BrS phenotype.[4, 58] SQTS is very rare with the largest series of 73 patients demonstrating a low genetic yield of 14% despite familial disease being evident in 44%.[58] It is unclear whether there may be an overlap with idiopathic VF (IVF) cases with only moderate QT shortening leading to underdiagnosis.

Idiopathic Ventricular Fibrillation

IVF describes patients who experience a resuscitated cardiac arrest, ideally with documented VF in the absence of other causes for VF i.e. metabolic, toxicological, cardiac (including other channelopathies and structural heart disease), respiratory and infectious.[1] The arrhythmias are

believed to be precipitated by short-coupled premature ventricular contractions (PVCs) due to Purkinje fibre potentials leading to polymorphic VT.[59-61]

Whilst IVF remains largely unexplained, the genetic basis for disease has been proposed in some cases. Alders et al linked disease with *DPP6* gene though a genome-wide haplotype sharing analysis in three distantly related Dutch families. Carriers demonstrated high penetrance with 50% experiencing an episode of VF or sudden death before age 58.[62] Marsman et al used exome sequencing to link IVF to *CALM1* in a family of Moroccan descent.[63] A recent report described a family with a likely IVF phenotype, with short-coupled PVCs at rest and a novel *RYR2* mutation H29D causing diastolic calcium leak at rest.[64] Finally a very recent report highlights *IRX3* as a putative gene in humans and animal models.[65] Collectively these reports suggest potential monogenic causes for IVF. However due to the lack of large IVF families it remains possible that the disease may be oligogenic.[66] Additionally, the prevalence of IVF is declining as more patients initially diagnosed with IVF are subsequently diagnosed with a different inherited arrhythmia syndrome such as BrS.[66]

Progressive Cardiac Conduction System Disease (PCCD)

Progressive cardiac conduction system disease (PCCD) is diagnosed in young patients (<50 years) exhibiting abnormalities of conduction with and without a structurally normal heart in the absence of skeletal myopathies.[1] When there is a clear familial pattern to disease it is typically autosomal dominant and genetic testing has diagnostic utility.[1] The majority of familial cases with a structurally normal heart are attributed to mutations in *SCN5A* and *TRPM4*.[12] Cases due to *SCN5A* mutations can be associated with a BrS overlap syndrome.[12, 41, 67] There are also rare associations with other genes (*SCN1B, SCN10A, KCNK17*).[68] PCCD with congenital heart disease is associated with transcription factor mutations (e.g. NKX2.5, GATA4)[69] and PCCD

with dilated cardiomyopathy or LV dysfunction is associated with lamin A/C (*LMNA*) or desmin (*DES*) variants.[12] The presence of a *LMNA* mutation carries prognostic utility and may influence ICD implantation.[1]

Genetic Testing and Next Generation Sequencing

Identification of the likely pathogenic variant in a family permits predictive (cascade) testing in family members. Those who are not found to carry the variant are released from regular clinical screening.[70] The best approach to improving the pre-test probability of a pathogenic variant being identified is to ensure the clinical phenotype is clearly defined, whether there is a family history of disease and that only genes which are plausible for the phenotype are assessed.[12]

Diagnostic testing had traditionally relied upon Sanger sequencing and disease causation or pathogenicity was often inferred only by absence of a variant in relatively small control populations. Since 2005, next-generation sequencing (NGS) methods have become increasingly available including cardiac gene panel testing, whole exome and whole genome sequencing. These allow large volumes of data to be analysed quickly and comprehensively at lower cost and have helped identify causative genes in disease where traditional methods have previously failed.[71] These differing approaches as well as their relevant advantages and disadvantages are shown in **Supplementary Material**.

NGS has also facilitated large public databases of genetic variation such as the 1000 Genomes project, the Exome Sequencing Project (ESP) or Exome Aggregation Consortium (ExAC) that have indicated rare variants to be more common than expected.[30] For example, in *SCN5A* there is a 3-5% background 'noise' of rare variants amongst healthy individuals.[72] Therefore up to 1 in 20 so-called positive genetic tests for BrS could in fact be false-positives.[30] The current 'signal-to-noise ratio' for the top four inherited arrhythmia genes ranges between

4.5:1 (*SCN5A* for LQT3) and 70:1 (*KCNQ1* for LQT1).[73] Additionally, variants previously thought of as being pathogenic in early Sanger studies are now accepted to be too common to be causative of rare disease and are therefore false positives. For example, 1 in 23 individuals in the ESP database carried a previously published BrS-associated variant.[74] Similarly Refsgaard et al identified 33 rare variants previously associated with LQTS in the ESP database suggesting a disease prevalence of 1 in 31 rather than 1 in 2000.[75] Ghouse et al showed that 10 variants previously associated with LQTS were in fact found frequently amongst a Danish population sample.[76] Finally, Van Driest et al showed no significant difference in arrhythmic phenotype between those with and without rare *KCNH2* or *SCN5A* variants in an unselected population. [77] They also showed poor concordance amongst laboratories when designating rare variants as pathogenic.

Thus in order to strengthen utility of contemporary genetic testing there are stringent criteria to determine pathogenicity. It is no longer considered a binary variable, but instead a probabilistic spectrum of pathogenicity as shown in **Figure 1**.[70, 78, 79] Criteria have been published by the American College of Medical Genetics and Genomics (ACMG) and are currently being updated with cardiac specific recommendations.[80]

Assessing Pathogenicity

Online databases of previously reported pathogenic variants have provided important tools for recognising unequivocally pathogenic variants although they are only as robust as the data entered and require regular updating and stringent curation. The ClinVar (*www.ncbi.nlm.nih.gov/clinvar/*) and ClinGen (*www.clinicalgenome.org*) initiatives offer important opportunities for global harmonisation of such data.[81]

Rarity can still be helpful when assessing likelihood of pathogenicity.[82] The public databases are integral to determining which variants are truly rare or novel and which are part of normal background genetic variation within cardiac inherited arrhythmia genes.[30] The prevalence of the disease being tested for should, however, be considered. For example, the disease prevalence of CPVT is 1/10,000; therefore, a variant with a population frequency of 0.0001, whilst rare, would potentially account for all CPVT cases globally and is therefore too common to be pathogenic. Novelty or substantially lower allele frequency than the disease prevalence is therefore required to support pathogenicity.

Loss of function is a known mechanism of disease pathogenesis for most inherited arrhythmia syndromes and an important step in assessing pathogenicity is reviewing the predicted effect of the variant on protein function. This is relatively straightforward for nonsense, frameshift or splice-site mutations but less so for missense variants. *In silico* software tools and functional studies, either *in vivo* or *in vitro*, can then also be used to assess the physiological effect of the variant and predict likelihood of pathogenicity although they are not always reliable.[30, 80, 82] Kapplinger et al used multiple *in silico* tools and ion channel topography to improve variant classification as benign or pathogenic.[72] All tools bar the Grantham score showed some predictive value although none showed strong independent utility.

Despite these efforts variants of uncertain significance (VUS) have become increasingly problematic. These are typically absent from population databases and may have computational evidence suggestive of pathogenicity. Lack of functional or segregation data however means that the variant cannot be classified as likely pathogenic or benign. Genetic counselling is critical to ensure the patients understand this potential complication of genetic testing.[12] Predictive testing

should therefore only be performed in monogenic disease when a variant has been classified as pathogenic or likely pathogenic.[70]

The Changing Landscape of Inherited Arrhythmia Syndromes

Inherited arrhythmia syndromes have typically been attributed to autosomal dominant inheritance due to monogenic abnormalities. As discussed above, these diseases may be genetically more complex and oligogenic, particularly in BrS (**Figure 2A)**. The simple genotype equals phenotype equation is also increasingly questioned. This was first evidenced by *SCN5A* overlap syndrome of BrS, PCCD and LQTS[41] followed by association with other phenotypes,[67] as well as dilated cardiomyopathy and atrial fibrillation.[4] There is also evidence of CPVT and LQTS overlap phenotypes due to different *ANK2* and *KCNJ2* mutations. The evolving relationship between genotype and phenotype is depicted in **Figure 2B**.

New Entities

Calmodulinopathies

Calmodulin is a ubiquitous calcium binding protein.[83] The importance of calmodulin binding to KCNQ1 for correct IK_s channel function was first highlighted using patch-clamp experiments 10 years ago.[84] Shamgar et al showed that LQTS mutations located near the C-terminal affect calmodulin binding, channel gating and assembly as well as calcium-sensitive IK_s-current stimulation. Defective calcium binding due to mutant calmodulin causes ventricular action potentials to be significantly prolonged.[85, 86] This cellular work has translated to the bedside with the evolution of the "calmodulinopathies".

Human calmodulin is encoded by three separate genes (*CALM1, CALM2, CALM3*), located on three different chromosomes, with the same amino acid sequences present in the three protein products of the genes.[87] Mutations in *CALM1* and *CALM2* were first identified through

whole exome sequencing in two unrelated infants with severe QT prolongation and recurrent cardiac arrest in infancy.[10] This was confirmed in two additional similar cases.[10] A large linkage study in a Swedish family with a dominantly inherited CPVT-like phenotype established linkage to a heterozygous *CALM1* mutation.[11] A second CPVT patient was identified with a different de novo missense mutation in *CALM1*.[11] A recent exome sequencing study assessed the prevalence of *CALM1-3* variants in 38 gene-negative LQT patients and found a significant proportion of calmodulin variants in this cohort compared to ExAC (13.2% vs 0.04%).[9] These cases had a young age of onset, high rate of cardiac arrest and severe QTc interval prolongation. Functional characterisation of one calmodulin variant (E141G) revealed a reduction in calcium binding affinity by a factor of 11 and functionally dominant loss of inactivation of L-type calcium channels with mild effects on late sodium current. *CALM1* has also been linked to familial idiopathic VF.[63] Currently *CALM1* and *CALM2* are known as LQT14 and LQT15 genes (**Table 1**) whilst *CALM1* is also CPVT4 gene (**Table 3**). *CALM3* has not yet been classified. It seems the term "calmodulinopathies" encompasses an LQTS/CVPT/IVF overlap syndrome with severe arrhythmic phenotype demonstrated from a young age (**Figure 2B**).

Triadin Knockout Syndrome

Triadin (TRDN) is an important protein in the formation of the macromolecular calcium release complex, working with junctin to anchor CASQ2 to RYR2.[88] Mouse models with complete knockout of the *TRDN* gene have significantly altered structure and function of the calcium release complex.[89] The first study of *TRDN* was performed in a cohort of 97 patients with genotype negative CPVT and identified three mutations in two families with recessive CPVT.[88] Two mutations were premature truncations whilst the third was a missense mutation with subsequent functional studies confirming mutant TRDN protein. More recently triadin knockout

syndrome has also been associated with a distinct autosomal recessive LQTS phenotype.[8] Exome sequencing in a trio of unaffected parents with a severely affected 10-year-old daughter identified a homozygous frameshift mutation. Subsequent sequencing in a cohort of 33 additional unaffected patients with gene negative LQTS revealed 4 additional patients with homozygous or compound heterozygous *TRDN* frameshift mutations. All patients carried the same distinct phenotype of extensive T-wave inversion in the precordial ECG leads V1-V4 (similar to ARVC), QTc prolongation and severe disease expression at a young age, with arrhythmias particularly precipitated by exercise.[8] The mechanism underpinning the *TRDN* knockout syndrome appears to be reduction in negative feedback on the L-type calcium channel leading to increased intracellular calcium, increased frequency of sarcoplasmic reticulum calcium release and subsequent VT, particularly with adrenergic stimulation.[89] L-type calcium channel blocking agents have been proposed as potential therapeutic options in patients with this distinct syndrome.[8] The triadin knockout syndrome appears to be an overlap syndrome with a LQTS/CPVT/ARVC overlap phenotype (**Figure 2B**).

Gene Therapies for Inherited Arrhythmia Syndromes

Recent work has highlighted that it may be possible to cure arrhythmia syndromes in the not too distant future. A *CASQ2* knockout mouse model of CPVT was rescued with CASQ2 via an adeno-associated viral vector (AAV9). The arrhythmic phenotype and ultrastructural abnormalities were also rescued with absence of arrhythmias in the rescued mice.[90] Subsequent work showed similar success in rescuing the phenotype from a knock-in murine model with CASQ2 R33Q homozygous mutation with administration of AAV9-CASQ2 at birth. The mice continued to display no evidence of CPVT at 1 year of age. Whilst still early in evolution, these animal models provide an exciting insight into where potential gene therapy options may arise.

Conclusion

Our understanding of inherited arrhythmia syndromes continues to evolve. Clinicians must continue to use caution when interpreting genetic variants and when using genetic results to determine management of patients and families with inherited arrhythmia syndromes. There is increasing genetic and phenotypic heterogeneity and mounting evidence that BrS is an oligogenic disorder. The era of NGS has evolved rapidly providing challenges including attributing pathogenicity, background genetic noise and increased detection of VUS; as well as new opportunities, including identification of new inherited arrhythmia entities such as the calmodulinopathies and Triadin disease. Our early understanding that a particular genotype causes a specific phenotype is further challenged with these new entities.

Disclosures: None

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Table 1: Genes associated with long QT syndrome (modified from Wilde and Behr)[4, 8-11]

LQTS type	Gene	Protein	Current	Frequency
LQT1	*KCNQ1*	Kv7.1	IKs↓	40-45%
LQT2	*KCNH2*	KV11.1	IKr↓	30-35%
LQT3	*SCN5A*	Nav1.5	INa↑	10%
LQT4	*ANK2*	Ankyrin-B	Na+/K+↓	1%
LQT5	*KCNE1*	MinK	IKs↓	1%
LQT6	*KCNE2*	MiRP1	IKr↓	Rare
LQT7	*KCNJ2*	Kir2.1	IK1↓	Rare
LQT8	*CACNA1C*	CaV1.2	ICa-L↑	Rare
LQT9	*CAV3*	Caveolin3	INa↑	Rare
LQT10	*SCN4B*	SCNβ4subunit	INa↑	Rare
LQT11	*AKAP9*	Yotiao	IKs↓	Rare
LQT12	*SNTA1*	Syntrophin-α1	INa↓	Rare
LQT13	*KCNJ5*	Kir3.4	IKACH↓	Rare
LQT14	*CALM1*	Calmodulin1	Calcium signalling	Rare
LQT15	*CALM2*	Calmodulin2	Calcium signalling	Rare
LQT16	*TRDN*	Triadin	ICa-L↑	Rare
Jervell and Lange-Nielsen syndrome (Autosomal Recessive)				
JLN1	*KCNQ1*	Kv7.1	IKs↓	Rare
JLN2	*KCNE1*	MinK	IKs↓	Rare

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Table 2: Genes Associated with Brugada Syndrome (Modified from Wilde and Behr; and Gourraud et al.)[4, 43]

Name	Gene	Protein	Prevalence
BrS1	*SCN5A*	α-subunit Nav1.5 Sodium channel	20-25%
BrS2	*GPD1L*	Glycerol-3-phosphate dehydrogenase 1-like	rare
BrS3	*CACNA1C*	α-subunit α1C Cav1.2 Calcium channel	1-2%
BrS4	*CACNB2b*	β-subunit Cavβ2b calcium channel	1-2%
BrS5	*SCN1b*	β-subunit Navβ1 sodium channel	rare
BrS6	*KCNE3*	β-subunit MiRP2 potassium channel	rare
BrS7	*SCN3b*	β-subunit Navβ3 sodium channel	rare
BrS8	*HCN4*	Hyperpolarization-activated cyclic nucleotide-gated channel 4	rare
BrS9	*KCND3*	α-subunit KV4.3 potassium channel	rare
BrS10	*KCNJ8*	α-subunit KIR6.1 potassium channel	rare
BrS11	*CACNA2D1*	δ-subunit Cavα2δ1 calcium channel	rare
BrS12	*KCNE5*	β-subunit potassium channel	rare
BrS13	*RANGRF*	RAN guanine nucleotide release factor	rare
BrS14	*KCND2*	α-subunit KV4.2 potassium channel	rare
BrS15	*TRPM4*	Calcium-activated non-selective ion channel	rare
BrS16	*SCN2B*	β-subunit Navβ2 sodium channel	rare
BrS17	*PKP2*	Plakophilin 2	rare
BrS18	*ABCC9*	ATP-sensitive potassium channels	rare
BrS19	*SLMAP*	Sarcolemma-associated protein	rare
BrS20	*KCNH2*	α-subunit of HERG potassium channel	rare
BrS21	*SCN10A*	α-subunit Nav1.8 sodium channel	1-16%
BrS22	*FGF12*	Fibroblast growth factor 12	rare
BrS23	*SEMA3A*	Semaphorin family protein	rare

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Table 3: Genes associated with CPVT (Modified from Wilde and Behr)[4]

Name	Gene	Protein	Frequency
CPVT1	*RYR2*	Cardiac Ryanodine Receptor 2	50-60%
CPVT2	*CASQ2*	Cardiac Calsequestrin	~5%
CPVT3	*TECLR*	Originally mapped to chromosome 7 p14-p22, now reallocated to chromosome 4	Rare
CPVT4	*CALM1*	Calmodulin	Rare
CPVT5	*TRDN*	Triadin	Rare
? LQT4 overlap	*ANK2*	Ankyrin B	Rare
? LQT7 overlap	*KCNJ2*	Potassium Inwardly Rectifying Channel Kir2.1	Rare

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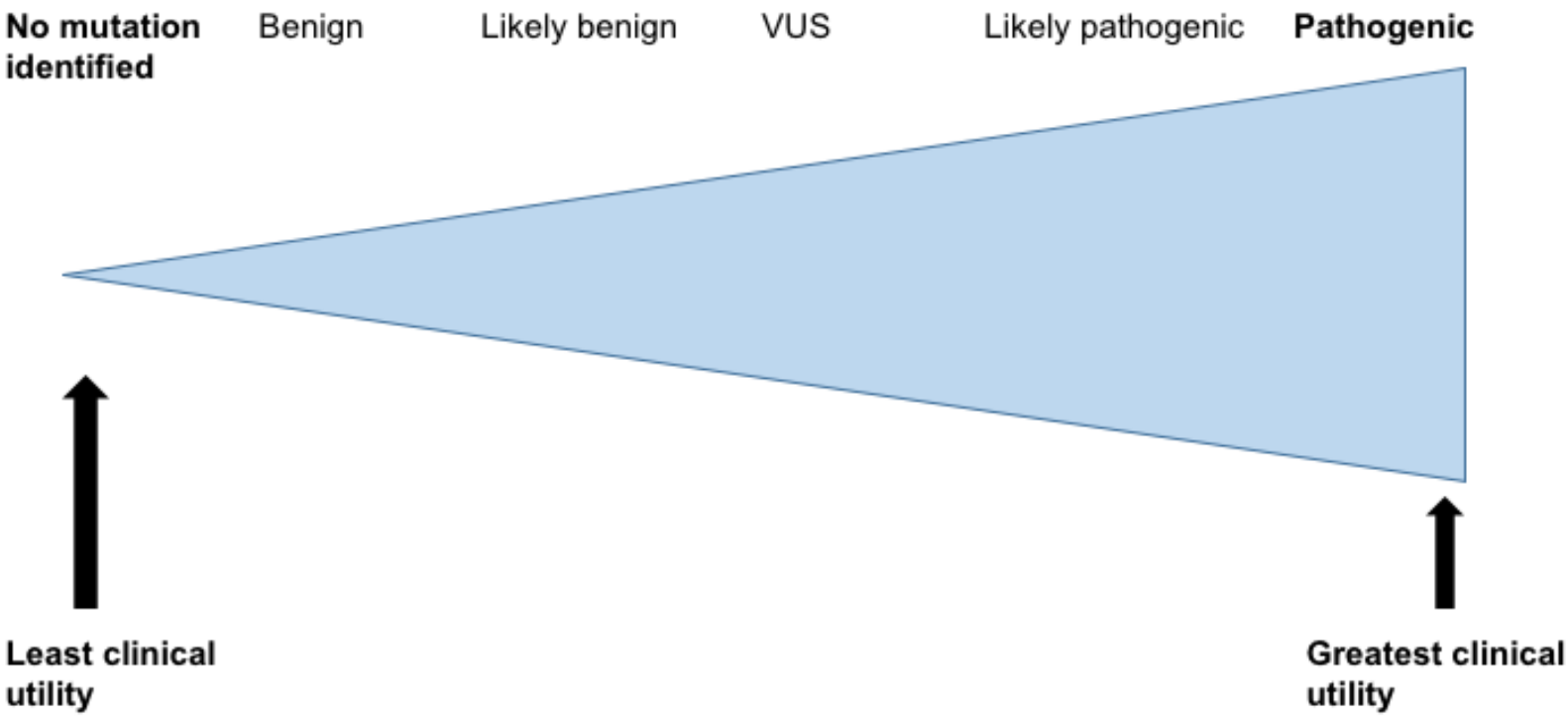
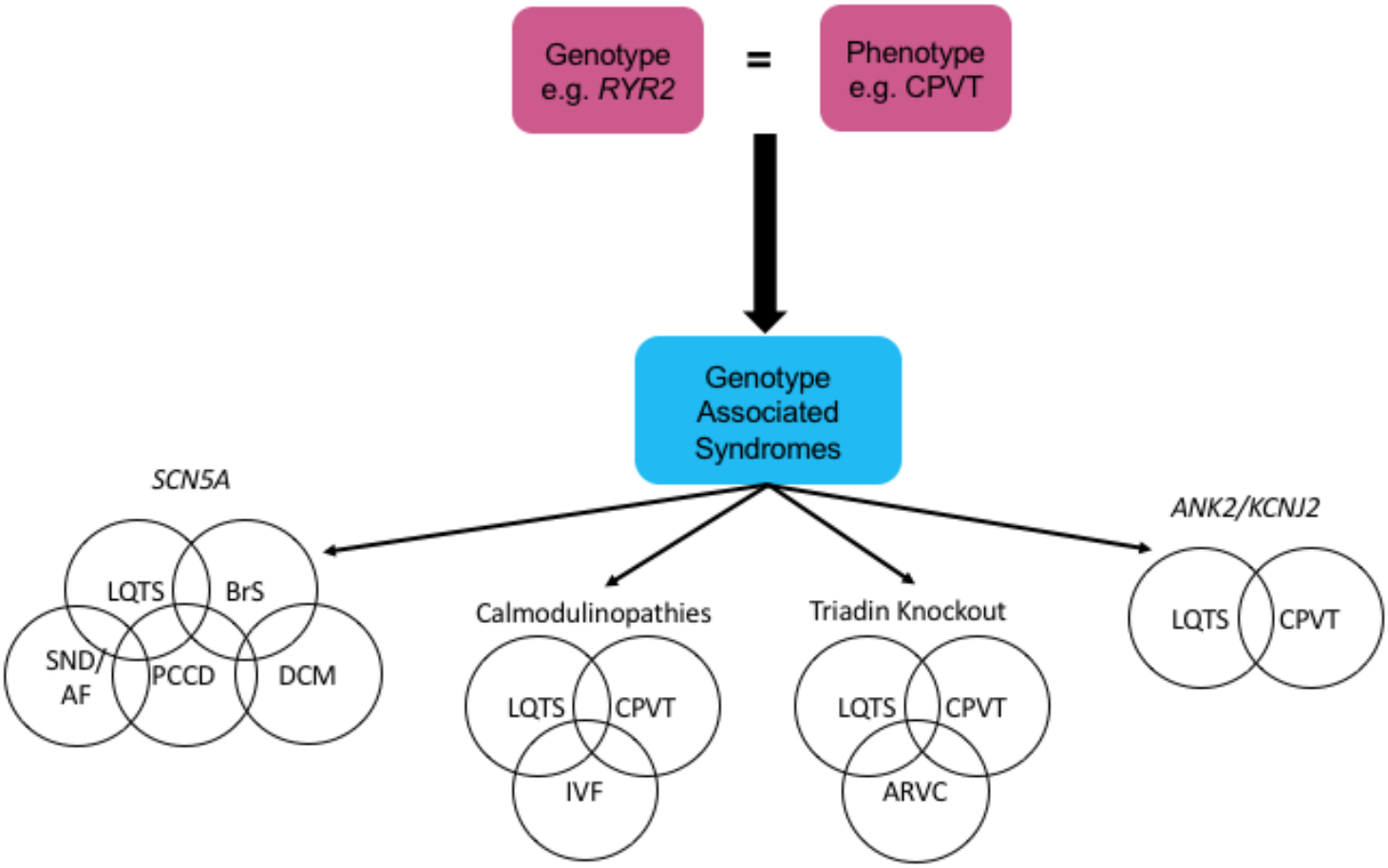
Figure Legends:

Figure 1: Probabilistic nature of genetic results (Modified with permission from Maron et al)[78]

Figure 2: (**A**) Spectrum of underlying genetic abnormalities in inherited arrhythmia syndromes (**B**) Changing landscape of genotype-phenotype interaction in inherited arrhythmia syndromes


No mutation identified
Benign
Likely benign
VUS
Likely pathogenic
Pathogenic
Least clinical utility
Greatest clinical utility


CPVT
SQTS
PCCD
LQTS
IVF
BrS
MONOGENIC
Mendelian inheritance
OLIGOGENIC
Multiple genes
Genetic modifiers


Genotype
e.g. RYR2
=
Phenotype
e.g. CPVT
Genotype
Associated
Syndromes
SCN5A
LQTS
BrS
SND/
AF
PCCD
DCM
Calmodulinopathies
LQTS
CPVT
IVF
Triadin Knockout
LQTS
CPVT
ARVC
ANK2/KCNJ2
LQTS
CPVT