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The genetic architecture of long QT syndrome: A critical reappraisal

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Abstract

Collectively, the completion of the Human Genome Project and subsequent development of high-throughput next-generation sequencing methodologies have revolutionized genomic research. However, the rapid sequencing and analysis of thousands upon thousands of human exomes and genomes has taught us that most genes, including those known to cause heritable cardiovascular disorders such as long QT syndrome, harbor an unexpected background rate of rare, and presumably innocuous, non-synonymous genetic variation. In this Review, we aim to reappraise the genetic architecture underlying both the acquired and congenital forms of long QT syndrome by examining how the clinical phenotype associated with and background genetic variation in long QT syndrome-susceptibility genes impacts the clinical validity of existing gene-disease associations and the variant classification and reporting strategies that serve as the foundation for diagnostic long QT syndrome genetic testing.

Keywords

Arrhythmia; Genetic testing; Genetic variation; Long QT syndrome; Sudden cardiac death

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tcm.2018.03.003.

Introduction

Long QT syndrome (LQTS) is a genetically and phenotypically heterogeneous disorder of cardiac repolarization characterized clinically by prolongation of the heart rate-corrected QT interval (QTc) on the 12-lead electrocardiogram (ECG) and an increased propensity for torsadogenic syncope/seizures and sudden cardiac death (SCD) [1,2]. Following the sentinel discovery of the canonical LQTS-susceptibility genes (*KCNQ1, KCNH2,* and *SCN5A*) in 1995 and 1996 [3–5]and completion of the Human Genome Project in 2003, rapid advances in deoxyribonucleic acid (DNA) sequencing technology allowed for the discovery of new Mendelian disease-susceptibility genes, including most of the 14 minor LQTS-susceptibility genes [6–12], at a dizzying pace.

However, the high-throughput DNA sequencing and subsequent analysis of tens-to-hundreds of thousands human exomes and genomes have exposed the stark reality that one in eight protein-encoding base pairs hosts a genetic variant and many Mendelian disease-susceptibility genes, including the canonical LQTS-susceptibility genes, are surprisingly tolerant to non-synonymous genetic variation [13–15]. Unfortunately, this burden of rare non-synonymous background genetic variants not only complicates variant interpretation [16–18], but for some minor LQTS-susceptibility genes, also calls into question the veracity of longstanding gene-disease associations.

In this Review, we (i) detail current paradigms regarding the molecular and cellular underpinnings of LQTS, (ii) reappraise the veracity of the QT phenotype associated with specific minor LQTS subtypes, (iii) examine ongoing attempts to classify and curate the clinical validity of existing gene-disease associations, and (iv) assess the limitations of current variant interpretation and reporting standards in an effort to highlight how a critical reappraisal of LQTS genetic architecture can help preserve, and hopefully enhance, the clinical validity of diagnostic LQTS genetic testing. To assist those readers with limited genetics/genomics backgrounds or those in need of a quick refresher, a glossary of terminology used throughout this Review is included in the online supplementary material.

Long QT syndrome: cellular mechanisms, genetic underpinnings, and prevailing paradigms

The electromechanical function of the heart is dependent on the coordinated activation and inactivation of inward depolarizing [sodium (Na^+) and calcium ($Ca2^+$)] and outward repolarizing [potassium (K^+)] currents that underlie the five major phases of the cardiac action potential (Fig. 1) [19,20]. Genetic or acquired defects that accentuate depolarizing Na^+ and $Ca2^+$ currents (I_{Na} and $I_{Ca,L}$) or attenuate repolarizing K^+ currents (I_{Ks}, I_{Kr}, and I_{K1}) (Fig. 1a) can prolong the ventricular cardiac action potential (Fig. 1b), as reflected by a prolonged QT interval on the surface 12-lead ECG (Fig. 1c). Current joint expert consensus guidelines define a prolonged QTc value as >450 ms in males and >460 ms in females [21]. However, ~5–10% of individuals within the general population have a QTc > 460 ms on screening ECG [22]. As such, sex-specific 99.5th percentile QTc values (>460 ms for prepubertal males and females, >470 ms for postpubertal males, and >480 ms for postpubertal females) are used commonly as a cutoff to identify those who might benefit

from cLQTS genetic testing [23]. Practically, a baseline QTc value ≥ 500 ms is considered definitely abnormal and if observed, in the absence of one or more QT prolonging risk factors, should compel strong clinical suspicion for congenital LQTS (cLQTS) and a class I recommendation to proceed with LQTS genetic testing [23].

Regardless of the underlying mechanism of QT prolongation [acquired/drug-induced LQTS (aLQTS) vs. cLQTS], increased cardiomyocyte refractoriness generates an electrical substrate that can give rise to frequent early afterdepolarizations (EADs) mediated by re-activation of L-type Ca^{2+} and sodium-calcium exchange currents during phase 2 and 3 of the cardiac action potential. Triggered activity from focal EADs coupled with EAD augmented electrical heterogeneity in adjacent regions of myocardium likely generates the electrical substrate needed for the initiation, propagation, and maintenance of *torsades de pointes* (TdP), the hallmark form of self-sustaining polymorphic ventricular tachycardia observed in patients with LQTS (Fig. 1d) [24].

During the early-to-mid 1990s, a series of now classical linkage-analysis studies identified the *KCNQ1* -encoded K_v 7.1 K^+ channel, the *KCNH2*-encoded K_v 11.1/hERG K^+ channel, and the *SCN5A*- encoded Na_v 1.5 Na+ channel pore-forming α-subunits as genetic substrates for congenital LQTS [3–5]. Among clinically definitive LQTS cases (i.e. QTc ≥ 480 ms and “Schwartz score” ≥ 3.5) [25], ~75% harbor a pathogenic variant in one of the three canonical LQTS-susceptibility genes *[KCNQ1* (~35%), *KCNH2* (~30%), and *SCN5A* (~10%)] (Table 1) [26]. An additional ~5–10% of LQTS cases, including patients that may display prolonged QTc values as part of distinct multisystem syndromes [27–29] such as Timothy syndrome (TS1 and *CACNA1C),* Andersen-Tawil Syndrome (ATS1 and *KCNJ2),* and Triadin Knockout Syndrome (TKOS and *TRDN),* are anticipated to harbor a pathogenic variant in one of at least 14 additional “minor” LQTS-susceptibility genes that have been published in the literature to date (Table 1).

A case of mistaken identity? Reappraising the QT phenotype associated with Andersen Tawil syndrome (ATS) and ankyrin-B syndrome (ABS)

Disease severity in most forms of LQTS, including the degree of QT prolongation, is influenced strongly by the phenomena of incomplete penetrance and variable expressivity. However, since the sentinel clinical description of both ATS [30] and Ankyrin-B syndrome (ABS) [31], subsequent evidence suggests that individuals with both disorders rarely display baseline QTc values consistent with cLQTS. As such, the following paragraphs briefly examine if ATS and ABS still merit designation as bona fide LQTS subtypes and whether *KCNJ2* or *ANK2* should remain on diagnostic LQTS genetic testing panels.

ATS was first described in 1971 as a clinical triad of periodic paralysis, ventricular ectopy/arrhythmia, and variable developmental abnormalities of the head, face, and limbs [30]. Due to the presence of prominent U waves and resulting QT-U interval prolongation on 12-lead ECG, ATS was labelled, initially and erroneously, as a multisystem form of cLQTS and the ATS-causative *KCNJ2* gene given the historical designation as LQT7 [32].

However, once the abnormal U wave is excluded, ATS patients almost always display normal QT intervals (<440 ms) [33]. Furthermore, the burden of complex ventricular ectopy at rest and biventricular tachycardia (a rare subtype of polymorphic ventricular tachycardia observed in catecholaminergic polymorphic ventricular tachycardia, but not LQTS) observed in ATS are highly uncharacteristic of LQTS [33]. As such, it is difficult to find substantive evidence to support the ongoing use of the historical LQT7 convention to describe ATS or the inclusion of *KCNJ2* on diagnostic LQTS genetic testing panels, other than to catch the rare case of ATS incorrectly diagnosed as LQTS due to a failure to recognize the multi-system disorder's distinct clinical and electrocardiographic profile.

Unlike ATS, ABS is a cardiac-only disorder characterized clinically by a range of variably present arrhythmia phenotypes including sinus node dysfunction, supraventricular and ventricular arrhythmias, and SCD [34]. Interestingly, overt QT prolongation (average QTc 490 ± 30 ms) was a characteristic of affected individuals in the multigenerational French pedigree that led to the first description of ABS (historically termed LQT4) and the discovery of the locus containing *ANK2* [31,35]. However, subsequent studies have demonstrated that baseline QTc prolongation is at best inconsistently observed in ABS [34,36,37]. Like ATS, this initial discrepancy may be caused, in part, by the inclusion of prominent U waves/sinusoidal T-U abnormalities in QTc calculations. As a result, QTUc values may appear markedly prolonged in ABS, but true QTc values typically reside in the normal-to-borderline range as more commonly reported [34,36,37].

That said, the recent description of a novel *ANK2* variant, p.Ser646Phe-ANK2, that appears to be more consistently associated with true QTc prolongation (average QTc 475 ± 40 ms) [38] and the prior association of common *ANK2* variants with QTc duration in the general population [39] support a role for *ANK2* in cardiac repolarization/LQTS pathogenesis. As such, it would be premature, at least on the basis of clinical phenotype alone, to call for the removal of *ANK2* from diagnostic LQTS genetic testing panels. However, when taken in context of (i) the weak QT phenotype inconsistently observed in ABS [34,36,37], (ii) failure of subsequent larger genome-wide association studies (GWAS) to reproduce the association between *ANK2* and QTc duration in the general population [40,41], and (iii) public exome data presented in the ensuing sections, there is clearly impetus to reappraise the role of *ANK2* in the pathogenesis of monogenic cLQTS.

Minor LQTS-susceptibility genes: time for a critical reappraisal?

In the 15 years following the release of the completed human genome, advances in sequencing technology facilitated the discovery of new disease-susceptibility genes in scenarios (i.e. singletons and small pedigrees) not feasible with classical linkage analysis. As a result, nearly half of the gene-disease associations in existence today [42], including the majority of the minor LQTS-susceptibility genes (Table 1) [6–12], were discovered over the last ~10 years using largely hypothesis-driven, mutational analysis of biologically plausible genes and their gene-encoded proteins. However, many of these genes were discovered in an era before the burden of background non-synonymous genetic variation was appreciated fully. As such, the strength of evidence behind existing gene-disease associations is widely variable. In the following paragraphs, we explore the minor non-syndromic LQTS-

susceptibility genes as a prototype to examine ongoing efforts to assess the validity of gene-disease associations and detail the potential impact of these efforts on clinical LQTS genetic testing.

Reappraising the non-syndromic minor LQTS-susceptibility genes

Due to a relative paucity of gene- and variant-level evidence, virtually every **novel** ultra-rare missense variant in a nonsyndromic, minor LQTS-susceptibility gene (Table 1) is likely to receive, at best, a variant of uncertain significance (VUS) designation. Not suprisingly, the identification of an ambiguous VUS in so-called weak or preliminary evidence genes is rarely clinically informative, increases the risk of genetic test misinterpretation, and ultimately can result in potentially harmful diagnostic miscues. In an effort to improve the validity of genes included on clinical genetic tests, the National Institutes of Health funded Clinical Genome Resource (ClinGen) has developed a semi-quantitative framework designed to standardize how the strength of genetic and functional evidence supporting a given gene-disease association is assessed systematically [43].

Although official ClinGen curations for the majority of LQTS-and other inherited cardiac channelopathy-susceptibility genes are eagerly awaited, independent application of the open access ClinGen gene-disease association framework suggests that 6/9 (66.7%) of the minor non-syndromic LQTS-susceptibility genes will receive *(ANK2, KCNE2, KCNJ5/* and *SNTA1)* or have already received *(AKAP9* and *SCN4B)* [43] disputed-evidence gene or limited-evidence gene designations (Table 3) [43].

For *AKAP9, SCN4B*, and *SNTA1*, where only a single qualifying functional ultra-rare missense variant is present in the literature (Table 2), there is simply not enough genetic evidence to elevate these genes beyond a limited-evidence gene regardless of the strength of experimental evidence available (Table 3). Of note, the putative LQTS-causative variants within both *AKAP9* and *SNTA1* used to establish their initial gene-disease associations are present, albeit at extremely low frequency (<10 out of >10 0,00 0 individuals), in pubic exomes (Table 2). Although this finding rightfully questions the nature of *AKAP9* and *SNTA*'s association with penetrant mongogenic cLQTS, the identification of additional LQTS-associated variants, more sophisticated functional characterization of existing and newly discovered variants such as using patient-specific and their variant-corrected induced pluripotent stem cell-derived cardiac cell lines to assess both necessity and sufficiency of an implicated genetic variant, and development of publication avenues that encourage the pursuit and reporting of these findings is needed urgently to determine if *AKAP9, SCN4B/* and *SNTA1* are candidates for elevation from a limited-evidence gene to a *bona fide* LQTS-susceptibility gene or further demotion to a disputed-evidence gene.

Already, the body of evidence for some of the other minor non-syndromic LQTS genes compel their demotion (Table 3). In a recent analysis, those variants in *KCNE2*-encoded MinK-related peptide 1 (MiRP1) initially deemed to be KCNE2-LQTS (historically designated as LQT6)-causative [7] were (i) collectively observed in 1.4% of public exomes, (ii) not found to co-segregate with a LQTS phenotype in either the literature or a large multicenter cohort of *KCNE2* rare variant-positive subjects, and (iii) rarely associated with

QT prolongation, TdP, or SCD in the absence of a secondary stressor (electrolyte abnormalities, QT prolonging medications, etc.) [44]. This collective evidence led to the conclusion that rare loss-of-function *KCNE2* variants confer an underlying arrhythmia-susceptiblity that may manifest as QT prolongation, TdP, and SCD when exacerbated by additional environmental and/or genetic risk factors, but likely do not cause *bona fide* cLQTS in isolation [44]. As such, *KCNE2* appears poised to receive a disputed-evidence gene designation when the official ClinGen gene-disease association classifications are released (Table 3).

Similarly, a majority of the *ANK2* -encoded ankyrin-B loss-of-function variants previously deemed to be ABS-causative [34,35,45] are observed at unacceptably high minor allele frequencies (MAF) in public exomes (Table 2). Furthermore, as discussed above, over time, it has become increasingly clear that QT prolongation is not a consistent feature of the cardiac phenotype observed in ABS [36]. Although the disruption of multiple ion channels/transporters and resulting arrhythmia-susceptibility conferred by loss-of-function *ANK2* variants is not in question, the high frequency of putative ABS-causative variants in public exomes and nature of the ABS cardiac phenotype certainly question the ongoing designation of *ANK2* as a self-sufficient cLQTS-susceptibility gene. That said, the aforementioned recent discovery of a loss-of-function *ANK2* variant (p.Ser646Phe-ANK2) in the Gitxsan First Nations founder population that appears to be more consistently associated with QT prolongation and is absent from public exomes may save *ANK2* from receiving a disputed-evidence gene designation (Table 3) [38].

Lastly, a single trafficking-defective variant, p.Gly387Arg-KCNJ5, in the KCNJ5-encoded G-protein-coupled inward rectifier potassium channel subtype 4 (GIRK4; Kir3.4) that partially conducts the acetylcholine-induced potassium current (IiKACh) current has been linked to cLQTS through a multi-generational Chinese pedigree [12]. On one hand, *KCNJ5* represents one of the only nonsyndromic, minor LQTS-susceptibility genes to be discovered by classical linkage analysis [12]. On the o present in 47/9424 (0.005%) East Asian public exomes/genomes and evidence to support a definitive role for I_{KACh} in human ventricular repolarization is lacking currently [46]. Furthermore, a closer analysis of the clinical phenotype of p.Gly387Arg-KCNJ5-positive individuals revealed that overt QT prolongation was only observed in individuals with additional non-modifiable QT prolongation risk factors such as heart failure, bradycardia, and diabetes [47]. Therefore, it is not clear whether *KCNJ5* functions as a self-sufficient but weakly penetrant cLQTS-susceptibility gene or more likely confers an underlying arrhythmia-susceptibility that can manifest as QT prolongation in the presence of multiple additional QT prolongation risk factors. Regardless, in light of the aformentioned population frequency data (Table 2) and lingering questions regarding the role of I_{KACh} in ventricular repolarization, it would come as little suprise if *KCNJ5* receives a disputed-evidence gene designation (Table 3).

While *AIKAP9, ANK2, KCNE2, KCNJ5, SCN4B,* and *SNTA1* appear headed for demotion to either limited- or disputed-evidence gene status, it is important to remember that our understanding of LQTS genetic architecture is not static. A gene demoted today in the setting of a prevailing monogenic paradigm may well find new life tomorrow as our understanding of the oligogenic/polygenic nature of LQTS continues to evolve. As such, the

temptation to simply lock these genes up and throw away the key must be resisted and the fluid nature of gene-disease association assessment must be embraced.

Genetic testing panels: is bigger really better?

From the onset of clinical genetic testing for LQTS and other Mendelian disorders, the mindset regarding the size of genetic testing panels has largely followed a “bigger is better” mentality. As new disease-susceptibility genes were discovered, the race between clinical laboratories to rapidly expand their respective diagnostic genetic testing panels was on. In recent years, a natural extension of this “bigger is better” mantra has been the development of massive pan-disease diagnostic genetic testing panels (i.e. pan-arrhythmia, pan-cardiac, etc.) that often encompass hundreds of genes with highly variable gene-disease association strengths [18]. When combined with an inherently high rate of background genetic variation, the use of these large and relatively non-specific genetic testing panels has become a recipe for the identification of an enormous number of VUS destined for so-called “genetic purgatory” [17]. Consequently, the unintentional mishandling of VUS has resulted in diagnostic miscues that range in severity from the misguided initiation of predictive cascade screening to the inappropriate implantation of prophylactic implantable cardioverterdefibrillators [16,17].

As those genes ultimately designated as disputed-evidence genes and limited-evidence genes (i.e. genes of uncertain significance or GUS) are likely to also contribute a substantial number of VUS, there are bound to be calls to remove some of the minor LQTS-susceptibility genes designated officially as either disputed/limited-evidence genes from genetic testing panels. However, the assumption that variants residing within such a gene cannot impact clinical decision making may be flawed.

For example, unless current estimates of LQTS/LQTS subtype prevalence represent vast underestimates, functional rare variants in *KCNE2* (i.e. p.Thr10Met-KCNE2, p.Met54Thr-KCNE2, and p.Ile57Thr-KCNE2) [44,48] are simply observed too frequently in public exomes to support even a weakly penetrant, but selfsufficient, role in the pathogenesis of a LQTS subtype widely believed to account for ≤1% of LQTS cases [26,36,49]. However, each of these *KCNE2* rare variants have been observed in individuals who exhibited a prolonged QT phenotype, including se vere manifestations such as TdP and SCD, in the presence of additional endogenous and exogenous risk factors [44]. Thus, despite an uncertain role in the pathogenesis of fully penetrant monogenic cLQTS, it still remains possible that rare, functionally pro-arrhythmic, variants in an otherwise disputed/limited-evidence gene (from a monogenetic disease-causing perspective) can potentially contribute to oligogenic/polygenic LQTS or predispose to acquired forms of LQTS. Therefore, some variants in these genes have the potential to impact clinical decision-making in some clinical scenarios.

This begs the question of how to ensure that ordering healthcare providers are made aware of the presence of potentially clinically-impactful, functional rare variants termed **functional risk alleles** that may contribute to oligogenic, polygenic, or acquired forms of LQTS residing within genes believed to have a limited or disputed role in penetrant monogenic

LQTS without needlessly increasing the number of novel rare VUSs. At first glance, the removal of disputed-evidence genes and perhaps even limited-evidence genes from diagnostic genetic testing panels might be the best solution. However, as our understanding of the genetic architecture underlying LQTS continues to evolve, we may quickly discover that the removal of some limited/disputed-evidence genes has resulted in a genetic test that now fails to convey/capture an individual's true genetic predilection for disease. As such, once the formal ClinGen classifications for LQTS and other SCD-predisposing cardiovascular disorders are released, it will be up to research community at large to develop more definitive means of assessing the potential pathogenic contributions of the minor LQTS-susceptibility genes that have been demoted, including potential oligogenic/polygenic contributions, as well as determining formal criteria for the fluid inclusion of genes on clinical genetic tests of all shapes and sizes.

Historical LQTS conventions: farewell old friend?

Unlike genes, which are granted an official name and symbol by a formal committee [the Human Genome Organization (HUGO) Gene Nomenclature Committee (HGNC)], there is and never was a gold standard by which the genetics and genomics community was expected to adhere when naming genetic disorders. The result has been a hodge-podge of naming conventions: (i) major sign of the disorder (e.g. LQTS), (ii) responsible genetic/biochemical defect (e.g. Ankyrin-B syndrome, ABS), (iii) surname(s) of the disorder's discover(s) (e.g. Andersen and Tawil Syndrome, ATS, Jervell and Lange-Nielsen Syndrome, JLNS, and Timothy Syndrome, TS), and (iv) the adoption of a rigid numerical approach (i.e. LQT1, LQT2, etc.) to the naming of disease subtypes.

With the likely demotion of previously published monogenic cLQTS-causative genes due to either (i) contradictory clinical evidence (KCNJ2/LQT7), (ii) genetic evidence (AKAP9/LQT11, KCNE2/LQT6, KCNJ5/LQT13, SCN4B/LQT10, and *SNTA1*/LQT12), or (iii) a combination of both (*ANK2*/LQT4), gaps in the classical/historical LQT1–17 subtype nomenclature are bound to emerge. As such, the time to reappraise existing LQTS nomenclature and institute substantive changes has never been better.

To this end, the adoption of a system that allows the canonical, irrefutable, and common disease-susceptibility genes to retain their historical LQT1–3 designations and then assigns a gene-derived convention for the minor non-syndromic LQTS-susceptibility genes (i.e. CACNA1C-LQTS) is preferable. Such a system (i) communicates directly the gene/minor LQTS subtype in question, (ii) eliminates the need to recall additional/unnecessary associations (LQT15 is caused by what again?), and (iii) allows genes to fluidly enter or exit as the strength of evidence and/or prevailing paradigms dictate regarding LQTS genetic architecture.

Reappraisal of variant interpretation and reporting strategies in diagnostic LQTS genetic testing

As a result of inter-laboratory heterogeneity in the classification and reporting of genetic variants, the American College of Medical Genetics and Genomics (ACMG) and the

Association for Molecular Pathology (AMP) released updated variant classification and reporting standards in 2015 [50]. In response, most commercial genetic testing companies, including the majority of those that perform diagnostic LQTS genetic testing, have adopted variant classification and reporting strategies that closely align with the 2015 ACMG/AMP guidelines. However, these general “one-size-fits-all” guidelines were not intended to address the intricacies and idiosyncrasies associated with genetic testing for specific disorders such as LQTS [51]. As such, the following paragraphs examine specific limitations of the 2015 ACMG/AMP guidelines that have already negatively impacted diagnostic LQTS genetic testing.

Potentially pro-arrhythmic common genetic variants (functional risk alleles): missing in action or not clinically actionable?

Unfortunately, the 2015 ACMG/AMP guidelines specifically avoided guidance on how or whether common genetic variants, that may modify cLQTS phenotypic severity and/or serve as genetic risk factors for aLQTS, should be classified and incorporated into diagnostic LQTS genetic testing reports. In turn, this lack of guidance gave those clinical genetic testing laboratories, that adopted ACMG/AMP-based schemes, the latitude to interpret (or misinterpret) the 2015 guidelines in a manner that has resulted in the exclusion of common variants with the potential to impact clinical decision making (Table 4) from diagnostic LQTS genetic testing reports [51].

As recently reviewed in detail elsewhere [51], the population frequency of common variants such as p.Asp85Asn-KCNE1 and p.Ser1103Tyr-SCN5A (Table 4) easily exceed cLQTS prevalence. However, these variants have substantial epidemiologic and experimental evidence to suggest they are capable of generating a circumstance-dependent, pro-arrhythmic state in the setting of QT prolonging medications, electrolyte abnormalities, structural heart disease, and repolarization reserve-deficient genetic backgrounds [51]. Thus, these common variants are likely not benign as frequently classified (Table 4). Rather, they represent a newly defined class of variants termed “functional risk alleles” capable of mimicking the sequelae of ultra-rare penetrant cLQTS-causative pathogenic variants, including conferring susceptibility for TdP and SCD, under specific conditions [51]. Therefore, the practice of withholding or limiting information regarding the presence of so-called functional risk alleles to a supplement that must be requested by healthcare providers has likely resulted in LQTS genetic tests that fail to accurately reflect their patient’s true genetic risk of SCD-predisposing disease.

As such, consideration should be given to reporting those variants that meet the epidemiologic (i.e. odds ratio >5 in case-control studies) and experimental (i.e. well-established functional evidence such as *in vitro* cellular electrophysiology data) to be classified as **functional risk alleles** (Table 4) under a distinct “Other Reportable” category with an accompanying disclaimer such as “This variant is not a self-sufficient, disease-causative mutation in isolation. However, in the setting of either disease-causative mutations or acquired QT-aggravating risk factors, the presence of this risk allele can potentially increase the patient’s risk of a potentially life threatening ventricular arrhythmia” [51].

This approach differentiates functional risk alleles from truly pathogenic/likely pathogenic variants and ambiguous VUS and limits the risk of misinterpretation and downstream diagnostic mis-cues. Furthermore, this approach highlights how a critical reappraisal of current variant classification and reporting strategies can improve the state of diagnostic LQTS genetic testing by ensuring patients and healthcare providers are informed of functional risk alleles and afforded every opportunity to institute the simple interventions [i.e. QT prolonging drug avoidance (www.crediblemeds. org), hydration/potassium supplementation, judicious use of antipyretics, etc.] needed to mitigate the small but increased risk of SCD that the presence of functional risk alleles may confer [51].

LQTS-lite: reappraising how weakly penetrant “pathogenic” genetic variants are classified and reported

Residing somewhere between functional risk alleles (Table 4) and penetrant ultra-rare cLQTS-causative pathogenic variants (an LQT1-causative variant for example) is a class of functional rare variants deemed previously to be LQTS-causative, but whose frequency in public exomes exceed expected cLQTS subtype prevalence (1:7,143 for LQT1, 1:10,000 for LQT2, 1:25,0 00 for LQT3, and 1:250,0 0 0 for minor LQTS subtypes). In a heterozygous state, these variants, which are perhaps best exemplified by Finnish (p.Arg176Trp-KCNH2 and p.Leu552Ser-KCNH2) [52] and Norwegian/Swedish (p.Arg518Ter-KCNQ1) [53] founder variants, are associated with a subclinical/concealed LQTS phenotype consisting of borderline QTc prolongation (~450–460 ms) and low SCD risk. Functionally, these rare variants result in haploinsufficiency (p.Arg518Ter-KCNQ1 and all truncating *KCNQ1* variants for that matter) or confer modest reductions in I_{Kr} current density (p.Arg176Trp-KCNH2 and p.Leu552Ser-KCNH2) [54–57]. Furthermore, the pro-arrhythmic potential of these variants is further highlighted by their frequent contribution to the severe LQTS phenotype observed in JLNS/compound heterozygosity [53,56] and/or association with drug-induced LQTS/TdP risk [58]. As such, these weakly penetrant, but functional, rare variants represent well- known examples of an emerging class of LQTS-causative variants, that includes many *KCNQ1* prematurely truncating nonsense and frame-shift variants [59], which collectively result in a latent and more common form of LQTS perhaps best referred to as “LQTSLite”.

Based on public exome-derived population frequencies, LQTSLite-causative variants are anticipated to be amongst those variants most frequently encountered during diagnostic LQTS genetic testing [13]. Although classification of LQTS-Lite-causative variants as “pathogenic variants” or “likely pathogenic variants” is appropriate, these weakly penetrant variants highlight the fact that current ACMG/AMP guidelines were not designed to communicate blatant differences in the strength/penetrance of pathogenic variants. As a result of this “all-or-nothing” approach, healthcare providers with little experience with genetic heart diseases may subject extremely low risk asymptomatic individuals with LQTS-Lite-causative variants to overly aggressive treatment on the basis of a “positive” genetic test. As such, the best management strategies for patients with LQTS-Lite-causative variant(s) and the development of variant classification and reporting mechanisms capable of

differentiating between pathogenic variants with intrinsically lower/higher risk and/or penetrance represent areas in need of ongoing reappraisal.

LQTS genetic architecture: truly a monogenic disease?

The number of minor LQTS-susceptibility gene variants observed at unacceptably high frequency in public exomes (Table 2) suggests that the role many of these genes play in LQTS pathogenesis may be more limited, or alternatively more complicated, than believed initially. When viewed in the context of pro-arrhythmic common variants/functional risk alleles (Table 4) and weakly pen etrant LQTS-Lite-causative rare variants in the canonical LQTS-susceptibility genes, it appears that the spectrum of genetic variants capable of contributing to the genetic architecture of LQTS (Fig. 2) may be far more complicated than envisioned previously.

On the severe end of the LQTS genetic spectrum are ultra-rare, self-sufficient pathogenic/likely pathogenic variants that substantially reduce cardiac repolarization reserve and underlie relatively penetrant monogenic cLQTS (Fig. 2). Due to the strong negative selective pressure exerted against these variants, they are likely rarely, if ever, observed at frequencies that exceed the estimated prevalence of cLQTS in the general population [1:2,500 or a minor allele frequency (MAF) < 0.00 04%] [60].

The opposite (benign) end of the LQTS genetic spectrum features common genetic variants (MAF > 1%), often identified through large-scale genome wide association studies associated with QT interval duration in the general population (Fig. 2) [40,41,61]. Although benign in isolation, the aggregate effect of multiple QT-influencing common genetic variants, as assessed by weighted-effect genetic risk scores, are associated with inter-individual variability in baseline QTc duration [62] and the exaggerated QTc response/TdP risk following exposure to a medication with known QT prolonging potential [63] observed in acquired/drug-induced LQTS (Fig. 2). In theory, the burden of QT-influencing, common genetic variants within an individual's genetic background (i.e. repolarization reserve rich vs. deficient) could also contribute to the phenomena of incomplete penetrance and variable expressivity observed in many LQTS pedigrees as well as the ~20% of LQTS cases that remain genotype-negative.

Lastly, in the middle of the LQTS genetic variation spectrum are relatively rare and comparatively more common genetic variants (MAF ranging from >0.00 04 to < 1%) that we have designated herein as either LQTS-Lite-causative rare variants or func tional risk alleles, respectively (Fig. 2). These variants result in mild-to-moderate reductions in cardiac repolarization reserve that may manifest as borderline or transient QT prolongation, but rarely cause LQTS-triggered syncope/seizures or SCD in the absence of additional QT prolonging genetic variants or endogenous/exogenous risk factors. As such, these variants may serve as primary drivers of so-called oligogenic cLQTS when present in genetic backgrounds that contain other QT-related genetic modifiers such as common variants in *NOS1AP* [64,65], the 3′untranslated region of *KCNQ1* [66], and perhaps an increased burden of those common variants shown to influence QTc duration in health. Furthermore, LQTS-Lite-causative variants within this class likely explain, in part, the observation that

functional rare variants previously associated with cLQTS are observed at unexpectedly high frequency in public exome/genome databases [67].

As the dust settles: practical implications for the clinician

As outlined in the preceding sections, the stage is set for several paradigm shifts, notably the possible demotion/reclassification of ~40% of LQTS-susceptibility genes and the transition away from a rigid monogenic model of disease, with the potential to radically alter our view of the genetic architecture underlying LQTS and other heritable cardiovascular disorders. For those charged with the care of patients with LQTS, whether it be on a daily basis or once in a blue moon, it is anticipated that this Review will generate a litany of questions, and perhaps some appropriate anxiety, regarding the current role of genetics in the diagnosis, risk-stratification, and management of LQTS.

Once formal ClinGen gene-disease association classifications are released, it will take time for (i) the field to reach a consensus regarding if/how variants in demoted/reclassified genes are reported and (ii) clinical laboratories to implement these recommendations. As genetic testing is, and should remain, a class I recommendation for LQTS [23], clinicians should continue to utilize the current generation of diagnostic LQTS genetic tests. However, ordering healthcare providers should assure they are receiving a complete genetic risk profile on every patient by requesting the supplemental information needed to determine if functional risk alleles are present and handling variants identified in genes that are poised for demotion/reclassification with extra caution and skepticism.

During this transition period, clinicians may find it helpful to utilize a tiered approach, independent from commercial classification and reporting schemes, to prioritize the analysis of diagnostic LQTS genetic testing results (Fig. 3). Under such an approach, findings in the canonical [*KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3)] and moderate-to-strong evidence minor LQTS-susceptibility genes (CACNA1C-LQTS, CALM1/2/3-LQTS, CAV3-LQTS, KCNE1-LQTS, and TRDN-LQTS/TKOS) are prioritized and represent the area where generalists should focus their efforts. Until the dust settles, it is recommended that patients with either a VUS or variants labelled, previously or actively, as pathogenic/likely pathogenic in limited/disputed evidence genes be referred to dedicated Cardiovascular Genetics Clinics to decrease the risk of diagnostic miscues. Furthermore, any vexing questions related to LQTS genetic testing, including appropriateness of cascade screening, VUS resolution, and the possibility of oligogenic/polygenic disease, are best addressed in a similar setting.

Conclusion

As illustrated in this Review, the unexpectedly high rate of background genetic variation in the canonical and minor LQTS-susceptibility genes is beginning to re-shape our understanding of the genetic architecture underlying LQTS. In short order, a number of the minor LQTS-susceptibility genes, previously believed to be responsible for ~5–10% of non-syndromic LQTS cases, could be demoted to either limited-evidence or disputed-evidence gene status and, at best, be relegated to roles as oligogenic/polygenic contributors.

Meanwhile, the presence of functional and potentially clinically impactful genetic variation in LQTS-susceptibility genes whose population frequencies easily exceed overall LQTS and/or LQTS subtype prevalence exposes inherent limitations in our current variant classification and reporting strategies. Unfortunately, these issues are not unique to LQTS and are anticipated to impact all heritable cardiovascular disorders, including the aortopathies, cardiomyopathies, and the other cardiac channelopathies, to some degree. As these issues continue to challenge current paradigms, it is paramount that the field continually reappraise whether (i) a larger than anticipated number of LQTS cases, including many currently labelled as minor gene-positive or genotype-negative, are actually oligogenic or polygenic in nature, (ii) previous estimates of cLQTS prevalence are indeed accurate, and (iii) existing variant classification and reporting strategies accurately reflect the full spectrum of genetic variation that may contribute to LQTS pathogenesis and therefore convey an individual's true genetic risk of disease. Hopefully, ongoing efforts, to address these and other pressing issues (i.e. "the VUS crisis"), will continue to improve the state of diagnostic LQTS genetic testing and ensure that patients have access to accurate genotype-guided approaches to the diagnosis, risk-stratification, and clinical management of this potentially fatal, but highly treatable, genetic disorder [68].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ABS	ankyrin-B syndrome
ACMG	American College of Medical Genetics and Genomics
aLQTS	acquired/drug-induced LQTS
AMP	Association for Molecular Pathology
Ca^{2+}	calcium
ClinGen	Clinical Genome Resource
cLQTS	congenital LQTS
DNA	deoxyribonucleic acid
EAD	early afterdepolarization
ECG	electrocardiogram
GWAS	genome-wide association study

I KACh	acetylcholine- induced potassium current
K^+	potassium
LQTS	long QT syndrome
MAF	minor allele frequency
Na^+	sodium
QTc	heart rate-corrected QT interval
SCD	sudden cardiac death
TdP	torsades de pointes
VUS	variant of uncertain significance

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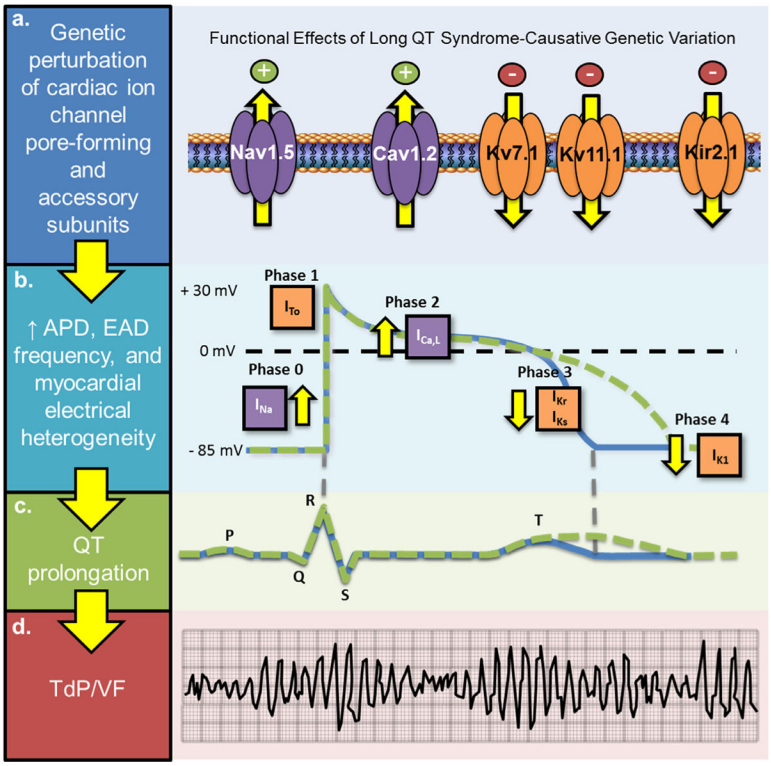

Fig. 1.

Current paradigm for the molecular and cellular basis of congenital long QT syndrome. (a) Genetic variation in pore-forming α-subunits or accessory β-subunits that results in cardiac potassium channel (*KCNQ1*-encoded Kv7.1, *KCNH2*-encoded Kv11.1, and *KCNJ2*-encoded Kir2.1) loss-of-function, cardiac sodium channel (*SCN5A*-encoded Nav1.5) gain-of-function, or cardiac L-type calcium channel (*CACNA1C*-encoded Cav1.2) gain-of-function. (b) A genetically-mediated increase in outward depolarizing currents (purple channels/boxes) and/or inward repolarizing currents (orange channels/boxes) prolongs the action potential duration (APD) generating the substrate needed for an increased frequency of early after depolarizations (EADs). (c) An increase in APD manifests as heart-rate corrected QT (QTc) interval prolongation on 12-lead surface electrocardiogram. (d) EAD-triggered action potentials can precipitate *torsades de pointes* (TdP) which may degenerate into ventricular fibrillation. Adapted from Giudicessi et al. [69] with permission. Copyright © 2018, Wiley. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

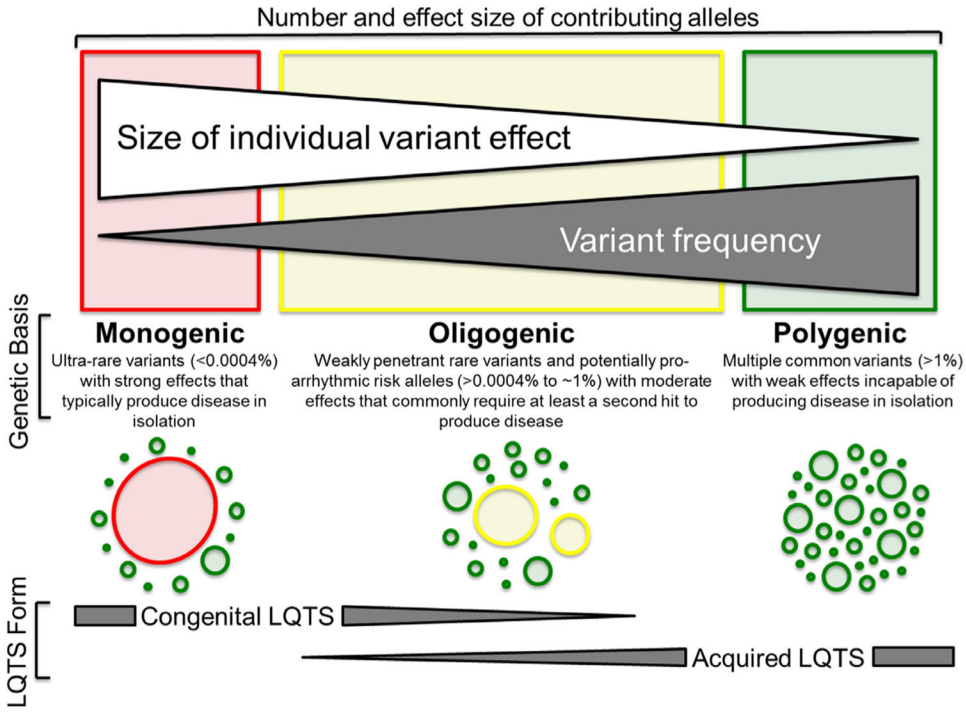

Fig. 2.

Spectrum of genetic variation underlying the acquired and congenital forms of long QT syndrome. At the severe (red) end of the spectrum are ultra-rare congenital long QT syndrome (cLQTS)-causative pathogenic variants with strong effects on gene function. In the middle of the spectrum (yellow) are weakly penetrant rare variants previously labelled as cLQTS-causative, but whose minor allele frequencies in public exomes exceed anticipated cLQTS/cLQTS subtype prevalence, and comparatively more common functional risk alleles (e.g. p.Asp85Asn-KCNE1) that exhibit moderate effects on gene function and rarely produce overt QT prolongation in the absence of additional endogenous or exogenous hits to cardiac repolarization and therefore result in so-called LQTS-Lite. Lastly, at the benign (green) end of the spectrum are common variants with weak effects on gene function, largely discovered through large genome wide association studies of QT prolongation in the general population, that are incapable of producing disease in isolation, but have been shown to result in acquired LQTS (aLQTS) risk when multiple QT-prolonging common variants are present within the genome of an individual with additional endogenous and exogenous QT prolonging risk factors. In recognition that the genetic basis of aLQTS and cLQTS is variable, dark gray triangles denote the spectrum of genetic variation underlying both LQTS forms. Adapted from Giudicessi and Ackerman [60] with permission. Copyright © 2013, Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

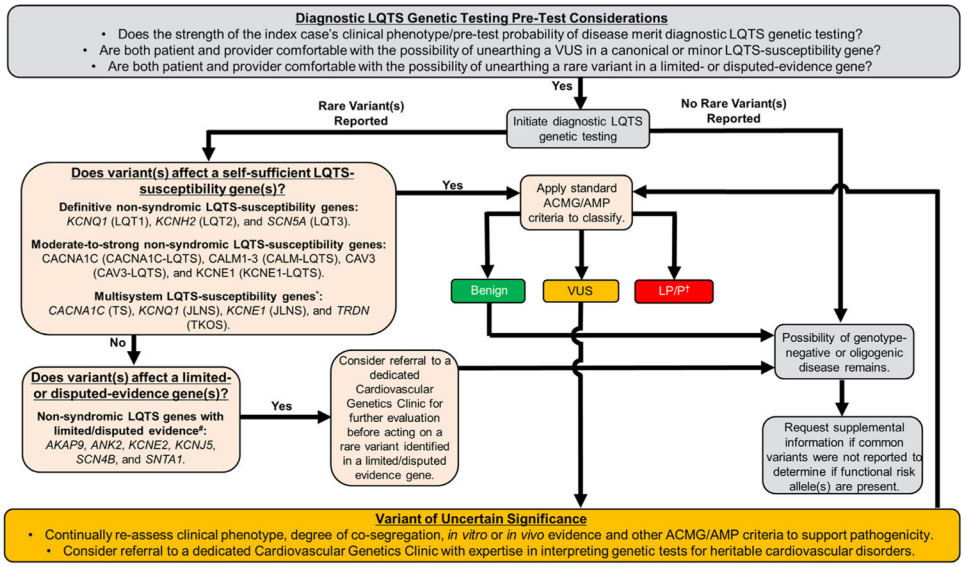

Fig. 3.

A rational tiered approach to LQTS genetic testing initiation, variant interpretation, and ongoing re-assessment of variants of unknown/uncertain significance (VUS). Light gray boxes denote basic considerations pertaining to the initiation of LQTS genetic testing. Light orange boxes denote a tiered approach to the assessment of rare variants in LQTS-susceptibility genes with variable gene-disease association evidence strength. Gold boxes denote basic considerations pertaining to the identification of a rare VUS in self-sufficient LQTS-susceptibility genes that currently lacks sufficient evidence to classify as either benign or pathogenic. *Due to the lack of a true QT prolongation phenotype, the authors recommend against the routine inclusion of *KCNJ2* on diagnostic LQTS testing panels. # Although the self-sufficient role of these genes in LQTS pathogenesis requires further investigation, specific rare variants in these genes may still confer arrhythmia-susceptibility and/or contribute to an oligogenic/polygenic form of LQTS. Referral to a dedicated Cardiovascular Genetics Clinic is recommended.† Current ACMG/AMP guidelines are not equipped to assess or communicate the strength of likely pathogenic/pathogenic variants. Outside of beta-blocker use, management of low-risk asymptomatic/concealed individuals should not be made on genetic test results alone. Abbreviations: ACMG, American College of Medical Genetics and Genomics; AMP, Association for Molecular Pathology; JLNS, Jervell and Lange-Nielson syndrome; LP, likely pathogenic; LQTS, long QT syndrome; P, pathogenic; TKOS, triadin knock-out syndrome; TS, Timothy syndrome; and VUS, variant of uncertain significance. Adapted from Giudicessi et al. [51] with permission. Copyright © 2018, Lippincott Williams & Wilkins with permission. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Current paradigms regarding the genetic basis of non-syndromic and multisystem LQTS subtypes.

Gene	LQTS subtype	Historical convention[a]	OMIM	Protein	Functional effect	Mode of inheritance	Frequency	Ref.
Non-syndromic long QT syndrome (major)								
KCNQ1	LQT1	LQT1	192500	Kv7.1	Reduced I_{Ks}	AD; AR	~30–35%	[4,70]
KCNH2	LQT2	LQT2	613688	Kv11.1	Reduced I_{Kr}	AD	~25–30%	[3]
SCN5A	LQT3	LQT3	603830	Nav1.5	Increased I_{Na}	AD	~5–10%	[5]
Non-syndromic long QT syndrome (minor)								
AKAP9[b]	AKAP9-LQTS	LQT11	611820	Yotiao	Reduced I_{Ks}	AD	<1%	[11]
CACNA1C	CACNA1C-LQTS	LQT8	N/A	Cav1.2	Increased $I_{Ca,L}$	AD	~1–2%	[71]
CALM1	CALM1-LQTS	LQT14	616247	Calmodulin 1	Increased $I_{Ca,L}$ (defective CDI)	Sporadic	~1–2%	[72]
CALM2	CALM2-LQTS	LQT15	616249	Calmodulin 2	Increased $I_{Ca,L}$ (defective CDI)	Sporadic	~1%	[72]
CALM3	CALM3-LQTS	LQT16	114183	Calmodulin 3	Likely increased $I_{Ca,L}$ (defective CDI)	Sporadic	<1%	[73]
CAV3	CAV3-LQTS	LQT9	611818	Caveolin 3	Increased I_{Na}	AD	<1%	[8]
KCNE1	KCNE1-LQTS	LQT5	613695	MinK	Reduced I_{Ks}	AD	<1%	[6]
KCNE2[b]	KCNE2-LQTS	LQT6	613693	MiRP1	Reduced I_{Kr}	AD	<1%	[7]
KCNJ5[b]	KCNJ5-LQTS	LQT13	613485	Kir3.4	Reduced $I_{K,Ach}$	AD	<1%	[12]
SCN4B[b]	SCN4B-LQTS	LQT10	611819	Nav1.5 /β4-subunit	Increased I_{Na}	AD	<1%	[9]
SNTA1[b]	SNTA1-LQTS	LQT12	612955	Syntrophin-α1	Increased I_{Na}	AD	<1%	[10]
Cardiac-only Timothy syndrome								
CACNA1C	COTS	N/A	N/A	Cav1.2	Increased $I_{Ca,L}$	AD	~1%	[74]
Jervell and Lange–Nielson syndrome								
KCNQ1	JLNS1	JLNS1	220400	Kv7.1	Reduced I_{Ks}	AR	Very rare	[75]
KCNE1	JLNS2	JLNS2	612347	MinK	Reduced I_{Ks}	AR	Very rare	[76]
Ankyrin-B syndrome								

Gene	LQTS subtype	Historical convention[a]	OMIM	Protein	Functional effect	Mode of inheritance	Frequency	Ref.
ANK2[c,b]	ABS	LQT4	600919	Ankyrin B	Aberrant ion channel/ transporter localization	AD	<1%	[35]
Andersen–Tawil Syndrome								
KCNJ2[C]	ATS1	LQT7	170390	Kir2.1	Reduced I_{K1}	AD	<1%	[28]
Timothy syndrome								
CACNA1C	TS1	LQT8	601005	Cav1.2	Increased $I_{Ca,L}$ (slowed VDI)	Sporadic; AD mosaicism	Very rare	[29,77]
Triadin knockout syndrome								
TRDN	TKOS	N/A	615441	Triadin	Likely increased I_{CaL} (disruption of CRU)	AR; sporadic	~2%	[27]

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; ABS, Ankyrin-B syndrome; ATS, Andersen Tawil syndrome; CDI, calcium-dependent inactivation; COTS, cardiac-only Timothy syndrome; CRU, calcium release unit; LQTS, long-QT syndrome; TS, Timothy syndrome, and VDI, voltage-dependent inactivation.

[a]Due to potential demotion of some genes previously alleged to be LQTS-causative, the authors strongly recommend limiting the use of historical numerical conventions to *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3).

[b]Genes that already have or likely will receive limited or disputed evidence gene designations.

[c]Genes that merit removal from diagnostic LQTS genetic testing panels due to lack of true association with QT prolongation.

Table 2

Functional rare variants in non-syndromic minor LQTS-susceptibility genes reported in seminal gene-disease association studies.

Gene	Variant	*De novo* status	Number of segregations	gnomAD minor allele frequency	*In vitro* phenotype	Ref.
AKAP9	p.Ser1570Leu	No	1	2/239262	LQT1-like I_{Ks} LOF	[11]
ANK2	p.Ser646Phe	No	14	Absent[a]	Cytosolic Ca^{2+} accumulation	[38]
	p.Glu1458Gly	No	17	143/276786	Cytosolic Ca^{2+} accumulation	[35,45]
	p.Thr3744Asn	No	N/A	176/276914	Cytosolic Ca^{2+} accumulation	[45]
	p.Leu3740Ile	No	N/A	877/276944	Cytosolic Ca^{2+} accumulation	[45]
	p.Arg3906Trp	No	N/A	293/27830	Cytosolic Ca^{2+} accumulation	[45]
	p.Glu3931Lys	No	N/A	762/276920	Cytosolic Ca^{2+} accumulation	[45]
CACNA1C	p.Ala28Thr	No	2	7/246970	$I_{Ca,L}$ GOF	[78]
	p.Ala582Asp	No	N/A	Absent	$I_{Ca,L}$ GOF	[79]
	p.Leu762Phe	No	N/A	Absent	$I_{Ca,L}$ GOF	[80]
	p.Pro857Arg	No	3	1/245530	$I_{Ca,L}$ GOF	[71]
	p.Arg860Gly	No	N/A	Absent	$I_{Ca,L}$ GOF	[78]
	p.Ile1166Thr	No	N/A	Absent	$I_{Ca,L}$ GOF	[78]
	p.Ile1166Val	No	3	Absent	$I_{Ca,L}$ GOF	[78]
	p.Ile1475Met	No	1	Absent	$I_{Ca,L}$ GOF	[78]
	p.Glu1496Lys	No	N/A	Absent	$I_{Ca,L}$ GOF	[78]
	p.Gly1783Cys	No	N/A	Absent	$I_{Ca,L}$ GOF	[79]
CAV3	p.Phe97Cys	Yes	N/A	Absent	LQT3-like late I_{Na} GOF	[8]
	p.Ser141Arg	Yes	N/A	Absent	LQT3-like late I_{Na} GOF	[8]
KCNE1	p.Val47Phe	No	N/A	Absent	LQT1-like I_{Ks} LOF	[81]
	p.Leu51His	No	N/A	Absent	LQT1-like I_{Ks} LOF	[81]
	p.Gly52Arg	No	5	Absent	LQT1-like I_{Ks} LOF	[82]
	p.Ser74Leu	No	1	5/277126	LQT1-like I_{Ks} LOF	[6]
	p.Asp76Asn	No	2	19/277116	LQT1-like I_{Ks} LOF	[6,81]
	p.Tyr81Cys	No	N/A	2/246202	LQT1-like I_{Ks} LOF	[83]
	p.Trp87Arg	No	N/A	Absent	LQT1-like I_{Ks} LOF	[81]

Gene	Variant	*De novo* status	Number of segregations	gnomAD minor allele frequency	*In vitro* phenotype	Ref.
	p.Val109Ile	No	1	39/276906	LQT1-like I_{Ks} LOF	[84]
KCNE2	p.Thr10Met	No	N/A	63/277186	LQT2-like I_{Kr} LOF	[44,48]
	p.Met54Thr	No	N/A	66/227224	LQT2-like I_{Kr} LOF	[7]
	p.Ile57Thr	No	N/A	266/277246	LQT2-like I_{Kr} LOF	[7]
KCNJ5	p.Gly387Arg	No	11	47/255978	I_{KACh} LOF	[12]
SCN4B	p.Leu179Phe	No	1	Absent	LQT3-like late I_{Na} GOF	[9]
SNTA1	p.Ala257Gly	No	N/A	500/276612	LQT3-like late I_{Na} GOF	[85]
	p.Aal390Val	No	N/A	6/276632	LQT3-like late I_{Na} GOF	[10]

Abbreviations: GOF, gain-of-function, LOF, loss-of-function; and LQTS, long QT syndrome.

[a]Public exome databases such as ExAC and gnomAD do not contain individuals from the Gitxsan First Nations community. The minor allele frequency for this variant in this unique founder population is not established.

Table 3

Preliminary reappraisal of the evidence supporting gene-disease associations for non-syndromic LQTS minor genes.

Gene	ExAC missense constraint Z score[a]	ClinGen genetic evidence score (# of qualifying variants)[b]	ClinGen experimental evidence score[b]	Anticipated ClinGen classification (total score)[c]	Ref.
AKAP9	−1.03	0.5 (1)	3	Limited evidence (3.5)	[11,43,86,87]
ANK2	+1.06	0.5 (1)	5	Limited evidence (5.5)	[34,35,38,45]
CACNA1C	+6.41	5.0 (10)[d]	2.5[d]	Moderate evidence (7.5)	[71,78–80,88]
CAV3	+1.19	7 (3)	3	Moderate evidence (10)	[8,89]
KCNE1	−0.46	3.0 (6)	5.5	Moderate evidence (8.5)	[6,76,81–84,90–92]
KCNE2	−0.23	0 (0)	5	Disputed evidence (5)	[7,44,48,93–99]
KCNJ5	+1.46	0 (0)	3.5	Disputed evidence (3.5)	[12,46,100]
SCN4B	−0.13	0.5 (1)	3	Limited evidence (3.5)	[9,101,102]
SNTA1	+0.77	0.5 (1)	3	Limited evidence (3.5)	[10,85]

Abbreviations: ClinGen, Clinical Genome Resource; and ExAC, exome aggregation consortium.

[a] A positive Z score indicates fewer missense variants than expected (intolerant to genetic variation) whereas a negative Z score indicates more missense variants where observe than expected (tolerant to genetic variation).

[b] Denotes author calculations using the open-access evidence-based framework proposed and in use by ClinGen curators. Only protein-truncating or functionally characterized missense variants present in ≤ 10 individuals in gnomAD were considered as qualifying variants.

[c] Gene-disease associations are classified according to the ClinGen clinical validity matrix as limited (1–6 points), moderate (7–11 points), strong (12–18 points), and definitive (12–18 points with replication over time) on the basis of the sum of genetic (0–12 points) and experimental (0–6 points) evidence. Currently, official ClinGen curations are only available for *AKAP9* (limited evidence) and *SCN4B* (limited evidence).

[d] Genetic and functional data (cellular electrophysiology, model organism, etc.) related to multisystem Timothy syndrome or the complex cardiac phenotype observed in cardiac-only Timothy syndrome were not included in these calculations.

Table 4

Pro-arrhythmic common variants with the potential to impact clinical decision making.

Variant	Overall MAF (ExAC)	African MAF (ExAC)	Functional Evidence	Epidemiologic evidence	SIFT/PolyPhen prediction	Current ACMG classification	Amended ACMG classification	Refs.
p.Ser1103Tyr-SCN5A	0.8%	10%	LQT3-like I_{Na} GOF	Over-represented in aLQTS, SCD in SHD, and SIDS	Possibly damaging/deleterious	Benign	Functional risk allele[a]	[103,104]
p.Asp85Asn-KCNE1	0.9%	0.2%	LQT1/2-like I_{Ks} and/or I_{Kr} LOF	Over-represented in aLQTS	Benign/deleterious	Likely benign	Functional risk allele[a]	[105–108]

Abbreviations: ACMG, American College of Medical Genetics and Genomics; aLQTS, acquired long QT syndrome; ExAC, Exome Aggregation Consortium; GOF, gain-of-function; IK r , rapid component of the delayed-rectifier potassium current; I_{Na} , inward depolarizing sodium current; LOF, loss-of-function; LQTS, long QT syndrome; MAF, minor allele frequency; SCD, sudden cardiac death; SHD, structural heart disease; SIDS, sudden infant death syndrome.

[a]If included on genetic testing variants, these variants should be listed under a distinct “Functional Risk Allele” or “Other Reportable” category with a disclaimer such as “This variant is NOT a self-sufficient LQTS-causative mutation in isolation. However, in the setting of either disease-causative mutations or acquired QT aggravating risk factors, the presence of this particular variant can potentially increase the patient’s risk of a potentially life threatening ventricular arrhythmia.”