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AN INTERNATIONAL COMPENDIUM OF MUTATIONS IN THE SCN5A-ENCODED CARDIAC SODIUM CHANNEL IN PATIENTS REFERRED FOR BRUGADA SYNDROME GENETIC TESTING

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BACKGROUND: Brugada syndrome (BrS) is a common heritable channelopathy. Mutations in the SCN5A-encoded sodium channel (BrS1) culminate in the most common genotype.

OBJECTIVE: To perform a retrospective analysis of BrS databases from 9 centers that have each genotyped > 100 unrelated cases of suspected BrS.

METHODS: Mutational analysis of all 27 translated exons in SCN5A was performed. Mutation frequency, type, and localization were compared between cases and 1300 ostensibly healthy volunteers including 649 whites and 651 non-whites (blacks, Asians, Hispanics, and others) that were genotyped previously.

RESULTS: 2111 unrelated patients (78% males, mean age = 39 ± 15 years) were referred for BrS genetic testing. Rare mutations/variants were more common among BrS cases than controls (438/2111, 21% vs. 11/649, 1.7% whites and 31/651, 4.8% non-whites, respectively, $p < 10^{-55}$). The yield of BrS1 genetic testing ranged from 11% to 28% ($p = 0.0017$). Overall, 293 distinct mutations were identified in SCN5A: 193 missense, 32 nonsense, 38 frameshift, 21 splice-site, and 9 in-frame deletions/insertions. The 4 most frequent BrS1-associated mutations were E1784K (14x), F861WfsX90 (11x), D356N (8x), and G1408R (7x). Most mutations localized to the transmembrane-spanning regions.

CONCLUSIONS: This international consortium of BrS genetic testing centers has added 200 new BrS1-associated mutations to the public domain. Overall, 21% of BrS probands have mutations in SCN5A compared to the 2-5% background rate of rare variants reported in healthy controls. Additional studies drawing on the data presented here may help further distinguish pathogenic mutations from similarly rare but otherwise innocuous ones found in cases.

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EXPRESSION OF A COMMON LQT1 MUTATION IN FIVE APPARENTLY UNRELATED FAMILIES IN A REGIONAL INHERITED ARRHYTHMIA CLINIC

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BACKGROUND: The Inherited Arrhythmia Clinic at the University of Western Ontario services a catchment area of 1.5 million people and follows families with inherited arrhythmia syndromes.

METHODS: Patients referred for evaluation of long-QT Syndrome (LQTS) are evaluated with resting and standing ECGs, and treadmill exercise testing. Patients with findings consistent with LQTS are offered comprehensive genetic testing with screening of all first-degree relatives of genotype-positive patients.

RESULTS: Among 31 probands with disease-causing LQTS mutations, 5 probands from apparently unrelated families of Irish descent were found to have an identical disease causing transmembrane mutation in KCNQ1 (Leu266Pro). Systematic screening of 33 first-degree relatives of genotype-positive individuals detected 15 unaffected and 18 asymptomatic affected family members. Symptoms in 6 patients occurred later in life than reported LQT1 populations (61 +/- 18 years, range 44-89). In this cohort, several family members presented with cardiac arrest during acute myocardial ischemia (n = 2), sudden death, unexplained drowning, and torsade de pointes during exercise testing. There was no identifiable common relative for this cohort after pedigree construction of the previous 4-7 generations. Affected patients had mild QT prolongation at rest with dramatic QT prolongation with exercise.

Conclusions: Genetic testing in this LQTS population suggests a common KCNQ1 Leu266Pro founder effect, with the descendants clustering in our geographical region even though no common relative has been identified. The observations highlight the utility of genotypic and phenotypic correlation and a specialized clinic.

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GENETIC TESTING FOR LONG-QT SYNDROME: DISTINGUISHING PATHOGENIC MUTATIONS FROM BENIGN VARIANTS

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BACKGROUND: Genetic testing for long-QT syndrome (LQTS) has diagnostic, prognostic, and therapeutic implications. Hundreds of causative mutations in 12 known LQTS-susceptibility genes have been identified. Genetic testing that includes the 3 most commonly mutated genes is available clinically. Distinguishing pathogenic mutations from innocuous rare variants is critical to the interpretation of test results. We sought to quantify the value of mutation type and gene/protein region in determining the probability of pathogenicity for mutations.

METHODS AND RESULTS: Type, frequency, and location of mutations across KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3) were compared between 388 unrelated "definite" (clinical diagnostic score ≥ 4 and/or QTc ≥ 480 ms) cases of LQTS and >1300 healthy controls for each gene. From these data, estimated predictive values (percent of mutations found in definite cases that would cause LQTS) were determined according to mutation type and location. Mutations were 10 times more common in cases than controls (0.58 per case versus 0.06 per control). Missense mutations were the most common, accounting for 78%, 67%, and 89% of mutations in KCNQ1, KCNH2, and SCN5A in cases and >95% in controls. Nonmissense mutations have an estimated predictive value >99% regardless of location. In contrast, location appears to be critical for characterizing missense mutations. Relative frequency of missense mutations between cases and controls ranged from approximately 1:1 in the SCN5A interdomain linker to infinity in the pore, transmembrane, and linker in KCNH2. These correspond to estimated predictive values ranging from 0% in the interdomain linker of SCN5A to 100% in the transmembrane/linker/pore regions of KCNH2. The estimated predictive value is also high in the linker, pore, transmembrane, and C terminus of KCNQ1 and the transmembrane/linker of SCN5A.

CONCLUSIONS: Distinguishing pathogenic mutations from rare variants is of critical importance in the interpretation of genetic testing in LQTS. Mutation type, mutation location, and ethnic-specific **BACKGROUND:** should be viewed as variants of uncertain significance and prompt further investigation to clarify the likelihood of disease causation. However, mutations in regions such as the transmembrane, linker, and pore of KCNQ1 and KCNH2 may be defined confidently as high-probability LQTS-causing mutations. These findings will have implications for other genetic disorders involving mutational analysis.



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SPECTRUM AND PREVALENCE OF MUTATIONS FROM THE FIRST 2,500 CONSECUTIVE UNRELATED PATIENTS REFERRED FOR THE FAMILION LONG QT SYNDROME GENETIC TEST

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BACKGROUND: Long QT syndrome (LQTS) is a potentially lethal, highly treatable cardiac channelopathy for which genetic testing has matured from discovery to translation and now clinical implementation.

OBJECTIVES: Here we examine the spectrum and prevalence of mutations found in the first 2,500 unrelated cases referred for the FAMILION LQTS clinical genetic test.

METHODS: Retrospective analysis of the first 2,500 cases (1,515 female patients, average age at testing 23 +/- 17 years, range 0 to 90 years) scanned for mutations in 5 of the LQTS-susceptibility genes: KCNQ1 (LQT1), KCNH2 (LQT2), SCN5A (LQT3), KCNE1 (LQT5), and KCNE2 (LQT6).

RESULTS: Overall, 903 referral cases (36%) hosted a possible LQTS-causing mutation that was absent in >2,600 reference alleles; 821 (91%) of the mutation-positive cases had single genotypes, whereas the remaining 82 patients (9%) had >1 mutation in > or =1 gene, including 52 cases that were compound heterozygous with mutations in >1 gene. Of the 562 distinct mutations, 394 (70%) were missense, 428 (76%) were seen once, and 336 (60%) are novel, including 92 of 199 in KCNQ1, 159 of 226 in KCNH2, and 70 of 110 in SCN5A.

CONCLUSION: This cohort increases the publicly available compendium of putative LQTS-associated mutations by >50%, and approximately one-third of the most recently detected mutations continue to be novel. Although control population data suggest that the great majority of these mutations are pathogenic, expert interpretation of genetic test results will remain critical for effective clinical use of LQTS genetic test results.

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PREVALENCE OF EARLY-ONSET ATRIAL FIBRILLATION IN CONGENITAL LONG QT SYNDROME

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BACKGROUND: The prevalence of atrial fibrillation (AF) in the young (age <50 years) is 0.1%, or 1:1,000 persons. Mutations in KCNQ1-, KCNH2-, and KCNA5-encoded potassium channels and SCN5A-encoded sodium channels have been reported in familial AF. A mechanism of atrial torsade has been suggested to occur in patients with congenital long QT syndrome (LQTS).

OBJECTIVE: The purpose of this study was to determine the prevalence of AF in patients with congenital LQTS.

METHODS: History of documented AF was sought from two independent cohorts. One cohort consisted of 252 consecutive patients (146 females and 106 males, average age at diagnosis 23 +/- 16 years, QTc 465 +/- 51 ms) with genetically proven LQTS seen at Mayo's LQTS Clinic. The second cohort consisted of 205 consecutive patients (133 females and 72 males, average age at testing 23 +/- 16 years, QTc 479 +/- 51 ms) with a positive FAMILION genetic test (PGxHealth) for LQTS.

RESULTS: Early-onset AF was documented in 8 (1.7%) of 457 patients, including 6 (2.4%) of 252 patients seen at Mayo and 2 (1%) of 205 patients with a positive FAMILION test. Five (2.4%) of 211 patients with LQT1-susceptibility mutations had documented AF, compared to 0 of 174 patients with LQT2, 1 of 59 patients with LQT3, 1 of 1 patient with Andersen-Tawil syndrome, and 1 of 34 patients with multiple mutations. The average age at diagnosis of AF of the six patients evaluated at Mayo was 24.3 years (range 4-46 years). Early-onset AF (age <50 years) was significantly more common in patients with LQTS compared to population-based prevalence statistics (P <.001, relative risk 17.5).

CONCLUSION: Compared to the background prevalence of 0.1%, early-onset AF was observed in almost 2% of patients with genetically proven LQTS and should be viewed as an uncommon but possible LQT-related dysrhythmia. Clinical complaints of palpitations warrant thorough assessment in patients with LQTS.

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ALLELIC DROPOUT IN LONG QT SYNDROME GENETIC TESTING: A POSSIBLE MECHANISM UNDERLYING FALSE-NEGATIVE RESULTS

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BACKGROUND: Genetic testing for congenital long QT syndrome (LQTS) has been performed in research laboratories for the past decade. Approximately 75% of patients with high clinical probability for LQTS have a mutation in one of five LQTS-causing cardiac channel genes. Possible explanations for the remaining genotype-negative cases include LQTS mimickers, novel LQTS-causing genes, unexplored regions of the known genes, and genetic testing detection failures.

OBJECTIVES: The purpose of this study was to explore the possibility of allelic dropout as a possible mechanism underlying false-negative test results.

METHODS: The published primers currently used by many research laboratories to conduct a comprehensive analysis of the 60 translated exons in the KCNQ1 (LQT1), KCNH2 (LQT2), SCN5A (LQT3), KCNE1 (LQT5), and KCNE2 (LQT6) genes were analyzed for the presence of common intronic single nucleotide polymorphisms (SNPs). Repeat mutational analysis, following primer/amplicon redesign using polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing, was performed on a cohort of 541 consecutive, unrelated patients referred for LQTS genetic testing.

RESULTS: Common (>1% minor allele frequency) intronic SNPs were discovered within the primer sequences of five of 60 translated exons. Following primer redesign to eliminate the possibility of allelic dropout, four previously genotype-negative index cases were found to possess LQTS-causing mutations: R591H-KCNQ1 and R594Q-KCNQ1 for exon 15 and E229X-KCNH2 in two unrelated cases. Repeat examination of these two amplicons in 400 reference alleles did not identify these or any additional amino acid variants.

CONCLUSION: Allelic dropout secondary to intronic SNP-primer mismatch prevented the discovery of LQTS-causing mutations in four cases. Considering that many LQTS genetic testing research laboratories have used these primers, patients who reportedly are genotype negative may benefit from re-examination of those regions susceptible to allelic dropout due to primer-disrupting SNPs, particularly exon 15 in KCNQ1 and exon 4 in KCNH2.



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PHARMACOGENETIC ISSUES IN THROUGH QT TRIALS

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Drug-induced QT prolongation (DI-LQT), through its associated arrhythmias, is a leading cause of drugs being withdrawn from the market. As a consequence, the US FDA and other regulatory agencies are mandating that all new drugs go through a so-called 'Thorough QT' (TQT) study to evaluate the potential for 'QT liability', specifically the potential for a drug to cause a discernible increase in the QT interval. Several genetic factors that modulate the risk of DI-LQT have been discovered. These are genes responsible for the congenital long QT syndrome, drug metabolism genes (mainly CYP2D6 and CYP3A4), and genes in other regulatory pathways. Here, we briefly review the links between genetic variants and drug-induced QT risk, and propose approaches to consider for using pharmacogenetics in planning and analyzing TQT studies.