

American Heart Association 2009, Abstract 2206

EVIDENCE THAT RARE MISSENSE VARIANTS SEEN IN LONG QT SYNDROME-SUSCEPTIBILITY GENES IN HEALTHY VOLUNTEERS ARE NOT PATHOGENIC

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INTRODUCTION: Long QT syndrome (LQTS) is the most common cardiac channelopathy where genetic testing has matured from discovery to translation and now clinical implementation. Whether rare variants found in ostensibly healthy individuals are benign or are low penetrance LQTS-causing mutations has been questioned. We have taken a statistical approach to address this question.

METHOD: In previous analyses of 1300 ostensibly healthy volunteers including 649 whites and 651 non-whites (blacks, Asians, Hispanics, and others) for the 3 major LQTS genes, 83 missense variants were observed once each. Clinical cases bearing any of these rare control variants were identified in the first 2500 index cases submitted for the FAMILION LQTS genetic test. If these control variants are instead pathogenic, then the yield of "other" mutations in these cases should be dramatically lower than the overall yield of mutations; specifically, it should match the fraction of cases that carry multiple mutations. We therefore contrasted the overall yield and multiple mutation fraction with the yield in these rare-variant-bearing cases.

RESULTS: Of the 83 rare variants, 24 (4 in KCNQ1, 8 in KCNH2, 12 in SCN5A) were observed in 71 of the 2500 cases. Of those 71 cases, 28 (39%) also had a putative mutation not known from controls. This yield is statistically indistinguishable from the 36% overall test yield ($p=0.62$), but is significantly higher than the 9% (82/903) multiple mutation fraction ($p=1.0 \times 10^{-10}$).

CONCLUSIONS: The results of this study suggest that missense variants seen in even one control should, in the absence of overriding evidence of pathogenicity, be considered background variants of no expected clinical significance in LQTS. A case bearing a rare control variant is no less likely to also have a "real" mutation than a case without such a variant. This statistical result is consistent with our expectations given the rarity of LQTS in the general population, and emphasizes the need to evaluate large healthy reference populations as part of genetic mutation testing.

Heart Rhythm 2009, Abstract PO02-160

CASE-CONTROL GENETIC COMPARISON OF THE CARDIAC RYANODINE RECEPTOR IN CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

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INTRODUCTION: Cardiac ryanodine receptor (RYR2) mutations are a cause of catecholaminergic polymorphic ventricular tachycardia (CPVT). Knowledge of normal genetic variation is critical for distinguishing pathogenic mutations from benign variants in clinical genetic tests. Therefore, we sought to determine the spectrum and prevalence of RYR2 genetic variants in ethnically-diverse healthy individuals and suspected CPVT patients.

METHODS: Targeted DNA sequence analysis of 38 RYR2 exons (3, 8-15, 37, 41, 44-50, 83, 87-105) was performed for 427 unrelated, healthy control individuals (28% Asian, 26% Black, 24% White, 10% Hispanic, 12% Other) and 125 unrelated patients (53% male, age at genetic testing 26 ± 17 years) referred to PGxHealth for CPVT genetic testing. Non-synonymous variants identified in cases and controls were analyzed and compared by type, location, frequency, and biochemical features.

RESULTS: Rare protein-altering variants were far more common ($p < 0.0001$) among suspected CPVT patients (22%) than controls (4%). In cases, 28 possible CPVT-causing mutations (22 novel) were identified including two in exons not previously reported to contain mutations (13 and 48), while 16 rare variants (15 novel) were identified in controls. Central region and channel domain variants were identified in 19% of patients compared with 2% of controls ($p < 0.0001$), while variant frequencies were similar in other regions tested (3% in cases vs. 1% in controls; $p = 0.12$). Amino acid substitutions among patients tended to be more radical than in controls but these differences did not achieve statistical significance ($p = 0.09$). Mutation-positive patients were significantly younger than mutation-negative patients (19.4 ± 13 vs. 28.3 ± 18 years; $p < 0.05$).

CONCLUSIONS: This study represents the largest collection of rare RYR2 genetic variants identified in healthy individuals of diverse ethnicity, identified a large number of novel possible CPVT-causing mutations, and elucidated high and low specificity RYR2 regions, which together will help further distinguish benign variants from CPVT-causing mutations in clinical testing.

Heart Rhythm Society 2009, Abstract PO03-4

SPECTRUM AND PREVALENCE OF MUTATIONS FOR THE FIRST 2500 CONSECUTIVE UNRELATED LQTS PATIENTS REFERRED FOR FAMILION[®] GENETIC TESTING

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INTRODUCTION: Long QT syndrome (LQTS) is the most common cardiac channelopathy. The FAMILION[®] LQTS clinical genetic test has an expected yield of ~75% for clinically definite LQTS cases compared to the ~4% background rate of rare nonsynonymous single nucleotide polymorphisms noted in otherwise healthy Caucasians. Here, we examine the spectrum and prevalence of mutations found in the first 2500 unrelated cases referred for LQTS genetic testing.

METHODS: Retrospective analysis of the first 2500 cases [(985 males, average age at testing, 23 ± 17 years (range < 1 – 90 years)] submitted for mutational analysis of the 3 major LQTS susceptibility genes: KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3) plus 2 minor genes: KCNE1 (LQT5) and KCNE2 (LQT6).

RESULTS: Overall, 903/2500 (36%) hosted a possible LQTS causing mutation that was absent in > 2600 reference alleles. 821 (91%) of the genotype-positive patients had single mutations: 386 were LQT1, 288 (LQT2), 115 (LQT3), 24 (LQT5), and 8 (LQT6) while multiple mutations were noted in KCNQ1 (22 cases), KCNH2 (3), SCN5A (8), KCNE1 (2), and KCNE2 (1). Fifty-eight cases had > 1 mutation in > 1 gene. Of the 562 distinct mutations identified, 398 (71%) were missense, 428 (76%) were seen only once, and 336 (60%) are novel including 92/199 mutations in KCNQ1, 159/226 mutations in KCNH2, and 70/110 mutations in SCN5A. The novelty rate has varied during the lifetime of the test ranging from 32% to 35% when comparing groups of 500 cases while the overall frequency of positive genetic test results has not decreased over time (1st 500, 36%, last 500, 39%).

CONCLUSIONS: This cohort will increase the publicly available compendium of novel LQTS-associated mutations from 636 to 972. A saturation point has not yet been reached as the novel mutation discovery rate continues at ~33%. Considering the 4% frequency of rare genetic variants in these LQTS-susceptibility genes, the ~36% frequency of positive genetic test results suggests that approximately 10% of these tests could be “false positives”. Ongoing structure-function studies of these novel mutations should help refine their probability of pathogenicity.

Heart Rhythm 2009, Abstract PO06-6

A WORLDWIDE COMPENDIUM OF PUTATIVE BRUGADA SYNDROME ASSOCIATED MUTATIONS IN THE SCN5A-ENCODED CARDIAC SODIUM CHANNEL

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INTRODUCTION: Brugada syndrome (BrS) is a common heritable channelopathy. Mutations in the SCN5A-encoded sodium channel (BrS1) are the most common genotype. BrS1 genetic testing is clinically available. Here, we sought to assemble a world-wide compendium of putative BrS1-associated mutations.

METHODS: A retrospective analysis of BrS databases from 9 centers throughout the world that have genotyped > 100 unrelated cases of suspected BrS was performed. Mutational analysis of all 27 translated exons in SCN5A was performed using PCR, DHPLC, and/or direct DNA sequencing. In addition, 1300 ostensibly healthy volunteers including 649 whites and 651 non-whites (blacks, asians, and hispanics) were genotyped.

RESULTS: 1959 unrelated patients (76% males) were referred for BrS genetic testing. The average age at testing was 39 ± 15 years. Rare mutations were far more common among BrS cases (482/1959, 22%) than controls (11/649, 1.7% whites and 31/651, 4.8% non-whites, $p < 10^{-58}$). The yield of BrS1 genetic testing ranged from 11% to 32% ($p < 10^{-5}$). Overall, 304 distinct BrS1-associated mutations were identified: 205 missense, 35 nonsense, 36 frameshift, 20 splice-site, and 8 in-frame deletions/insertions. The 5 most frequent BrS1-associated mutations were E1784K (12x, 4 labs), F861fsX90 (11x, 2 labs), D356N (8x, 5 labs), G1408R (7x, 4 labs), and R367H (6x, 4 labs). Thirty-seven (18%) missense mutations localized to the N-terminus and interdomain linker regions, where most rare control variants are found, making some of these likely to be "false positives".

CONCLUSIONS: This worldwide consortium of BrS genetic testing centers has nearly quadrupled the spectrum of BrS1-associated mutations with the addition of 266 new mutations to the public domain. Overall, 22% of probands diagnosed with BrS have rare mutations in SCN5A. This worldwide compendium now identifies a handful of BrS1-associated "hotspots". Given the 2-5% background rate of rare variants and the clustering of mutations to particular domains, additional studies may help further distinguish pathogenic mutations from similarly rare but otherwise innocuous ones found in cases.

Heart Rhythm Society 2008, Abstract AB36-4

DISTINGUISHING LONG QT SYNDROME-CAUSING MUTATIONS FROM “BACKGROUND” GENETIC NOISE

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INTRODUCTION: Genetic testing has diagnostic, prognostic, and therapeutic roles in the evaluation of long QT syndrome (LQTS). Hundreds of causative mutations in now 12 known LQTS genes have been identified. Genetic testing for the 3 most common subtypes is available. Distinguishing pathogenic mutations from innocuous rare variants is critical. We sought to identify informative factors for determining the likelihood of pathogenicity for rare, non-synonymous (NS) mutations.

METHODS: Type, frequency, and location of mutations across *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3) were compared between 388 “definite” (Schwartz score ≥ 4 and/or QTc > 480 ms) cases of LQTS and 1380 unrelated controls. Mutations, defined as protein-altering variants with allelic frequency $< 0.5\%$, were localized to gene region using SwissProt.

RESULTS: Mutations were more common in cases than controls (48.2% vs 6.9%, $p < 0.001$). NS mutations were the most common, with prevalences of 78%, 67%, and 87% in *KCNQ1*, *KCNH2*, and *SCN5A* in cases and $> 95\%$ in controls. Non-NS mutations have a $> 99\%$ likelihood of LQTS-causation regardless of location ($p < 0.001$). In contrast, location is critical for characterizing NS mutations. Relative frequency of rare NS mutations between cases and controls ranged from 0.77 in the *SCN5A* interdomain linker (IDL) to infinity in *KCNH2*'s pore (P), transmembrane (TM), and linker (L, $p < 0.001$). Estimated predictive value (EPV) of the percent of case mutations that are deleterious ranged from $\sim 0\%$ in the IDL of *SCN5A* to 100% in the P, TM, and L regions of *KCNH2*. EPV is also high in *KCNQ1*'s L, P, TM, and C-terminus and the TM of *SCN5A*. When ethnicity is taken into account, EPV increases to 25% for *SCN5A*'s IDL reflecting the lower control mutation rate in whites versus non-whites (4% vs. 9%, $p < 0.05$) but a similar case mutation rate (61% vs. 66%). EPV also increases to 100% in *KCNQ1*'s P and TM and the TM of *SCN5A* in whites only.

CONCLUSIONS: With increasing availability of genetic testing, distinguishing pathogenic mutations from rare variants is of great importance. Mutation type, mutation location, and ethnic-specific background rates are key factors in predicting pathogenicity of novel mutations.

Heart Rhythm Society 2008, Abstract AB3-3

AMINO ACID PHYSICOCHEMICAL DIFFERENCES MAY SERVE AS AN ADJUNCT TO FREQUENCY ANALYSIS TO DETERMINE MUTATION PATHOGENICITY IN LONG QT SYNDROME

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INTRODUCTION: Mutation type and location facilitate distinguishing pathogenic mutations from rare variants in long QT syndrome (LQTS) genetic testing. However, missense mutations localizing to the terminal regions of *KCNQ1* and *KCNH2* and non transmembrane regions of *SCN5A* remain difficult to interpret. We sought to determine whether physicochemical properties (PCPs) would further discriminate between case mutations and control variants.

METHODS: Type, frequency and location of missense mutations across *KCNQ1* (LQT1), *KCNH2* (LQT2) and *SCN5A* (LQT3) were compared between 388 definite (Schwartz score ≥ 4 and/or QTc > 480 ms) cases and 1380 unrelated controls. Mutations were localized using SwissProt analysis and PCPs, including hydrophobicity (HP), isoelectric point (IP), side chain flexibility (SCF), polarity (P), hydrogen bonding (HB) and charge (C), were recorded. Grantham values (G-values), which measure overall difference in PCPs, were computed and mutations were classified as conservative (C, G-value <50), moderately conservative (MC, G-value 51-100), moderately radical (MR, G-value 101-150) or radical (R, G-value >150).

RESULTS: There were no significant differences in HP, IP, SCF, P, HB or C in any gene regions between 128 distinct case mutations and 81 control variants. However, G-values were greater in the transmembrane and non-subunit assembly portion of the C-term of *KCNQ1* ($p < 0.001$) and overall across both *KCNQ1* ($p < 0.001$) and *KCNH2* ($p < 0.05$) in cases than controls. There was an overall trend towards higher G-values in all other gene regions in cases except for the *SCN5A* linker. Analyses were also performed using parametric scores of gene conservation as C, MC, MR and R, demonstrating a trend towards the case mutation being more radical than the control in all domains except the *SCN5A* linker.

CONCLUSIONS: Using PCPs to determine disease likelihood can be a powerful tool. Analysis of PCPs further discriminated between pathogenic mutations and otherwise rare, innocuous variants in most gene regions. However, interpretation of mutations localizing throughout much of the sodium channel wasn't improved.

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IS A572D-SCN5A A LQT3/SUDDEN DEATH SUSCEPTIBILITY MUTATION OR BACKGROUND GENETIC NOISE?

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INTRODUCTION: Considering that 4% of controls host rare, non-synonymous genetic variants in *SCN5A*, the gene responsible for type 3 long QT syndrome (LQT3), type 1 Brugada syndrome (BrS1), and 50% of the channelopathic cases of sudden infant death syndrome (SIDS), caution must be exercised when interpreting *SCN5A* genetic test results. A572D-SCN5A was published initially as an LQT3-causing mutation, identified recently in a postmortem investigation of sudden deaths, and functionally characterized in *Xenopus* oocytes as having a shorter recovery time from inactivation. The purpose of this investigation was to determine whether or not A572D is a pathogenic mutation or background “genetic noise”.

METHODS: The frequency of A572D was compared between 2675 mostly Caucasian referred cases of LQTS, SIDS, and sudden unexplained death syndrome (SUDS) and 2308 Caucasian controls. A572D-SCN5A was engineered into *SCN5A* using the most commonly spliced transcript (Q1077del, hH1c clone) for expression in HEK cells and functional characterization using the patch clamp technique.

RESULTS: A572D-SCN5A was detected in 14 cases (0.52%) including 14/2280 (0.61%) cases referred for LQTS genetic testing, 0/281 SIDS cases, and 0/114 SUDS cases. By comparison, 10 controls (0.43%) hosted A572D as well ($p = \text{NS}$). Among the 14 A572D-positive LQTS referrals, 6 hosted definite LQTS-causing mutations elsewhere (G269S and L273F in the pore of *KCNQ1*, a frameshift/premature truncation, V288fs/60X, in *KCNQ1*, A341V in S6 of *KCNQ1*, a C-terminal frameshift/truncation, S1057fs/60, in *KCNH2*, and a sporadic late sodium current producing mutation, N275K, in *SCN5A*). Functional studies in HEK cells showed no gating kinetic or current density differences compared to wild type channels.

CONCLUSIONS: There is insufficient evidence to conclude that A572D-SCN5A is a pathogenic mutation. Instead, A572D is present in about 0.5% of both cases and controls and has a wildtype phenotype when expressed in HEK293 cells. These findings underscore the scrutiny necessary to distinguish an LQT3-causative mutation from an otherwise innocuous, rare genetic variant in *SCN5A*.

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SHOULD A MINIMUM CORRECTED QT INTERVAL (QT_c) BE A PREREQUISITE FOR LONG QT SYNDROME GENETIC TESTING?

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BACKGROUND: Long QT Syndrome (LQTS) genetic testing has been available clinically since August 2004. In cases of bona fide LQTS, the yield of genetic testing is approximately 75%. Recently (HRS 2007), Priori and colleagues have suggested that referral for LQTS genetic testing should be based on the screening QT_c citing a yield of < 2% for cases referred to their research program with a QT_c < 440 ms. Here, we analyze the effect of QT_c on the yield in two of the largest assembled cohorts of unrelated patients (pts) referred for LQTS genetic testing.

METHODS: From August 1997 to July 2006, 1125 unrelated pts (717 females, average age at diagnosis 23.5 yrs, average QT_c 473 ms) were referred to either the Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory (N = 541) for research-based LQTS genetic testing or to PGxHealth (N = 574) for the FAMILION[®] LQTS genetic test. Comprehensive open reading frame and splice site analysis (60 exons) for LQTS-causing mutations involving *KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3), *KCNE1* (LQT5), and *KCNE2* (LQT6) was conducted by either dHPLC/DNA sequencing (Mayo), or by direct DNA sequencing (FAMILION).

RESULTS: The probability of a positive genetic test increased dramatically with increasing QT_c, ranging from 5% for the 38 pts referred for genetic testing despite a QT_c < 400 ms to 60% for the 168 pts with a QT_c > 500 ms (p < 0.00001). Akin to the Priori cohort, the yield of the genetic test was similar among the subset of pts with a QT_c > 470 ms (64% Priori, 61% Mayo cohort, and 53% FAMILION, p = NS). However, the yield for those pts with a QT_c between 440 and 470 ms was much greater in the Mayo (49%) and FAMILION (36%) cohorts compared to the Priori cohort (14%, p < 0.0005). In contrast to the 2% yield observed in Italy, 22% of pts with QT_c < 440 ms referred to Mayo Clinic and 14.5% of the FAMILION referrals had a positive genetic test (p < 0.001).

CONCLUSIONS: Whereas only 2% of patients with a QT_c < 440 ms had a positive genetic test in Italy, the yield was ten times greater among those referred to Mayo Clinic despite the same QT_c cut-off. The cumulative index of suspicion for LQTS rather than a pre-specified QT_c cut-off should guide clinical decision making as to the appropriate utilization of the genetic test.

Heart Rhythm Society 2007, Abstract ABI0-3

CLINICAL PHENOTYPE AND THE YIELD OF THE FAMILION™ GENETIC TEST FOR CONGENITAL LONG QT SYNDROME

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INTRODUCTION: Long QT syndrome (LQTS) is the most common cardiac channelopathy marked by significant genotypic and phenotypic heterogeneity. In August 2004, genetic testing for LQTS became clinically available. Here, we examine the effect of clinical phenotype including sex, age at testing, and QTc on the yield of the LQTS genetic test.

METHODS: From August 2004 to August 2006, 574 consecutive, unrelated patients (62.5% females, 86% Caucasian, average age, 23 ± 16 years, and average QTc, 464 ± 54 ms) were referred to PGxHealth for the FAMILION™ LQTS genetic test whereby comprehensive mutational analysis for LQTS-causing mutations involving *KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3), *KCNE1* (LQT5), and *KCNE2* (LQT6) was conducted using DNA sequencing.

RESULTS: Overall, 219 patients (38%) were genotype positive. The yield correlated significantly with QTc ranging from 11% when QTc < 420 ms to 54% when QTc > 480 ms ($p < 0.0001$) but was unaffected by sex or age at testing. The yield was only 22/80 (28%) among those with exertional syncope compared to 39/60 (65%) for cases with either swimming-, sleep-, auditory-, or postpartum-triggered events ($p < 0.0001$). The yield was less than the overall yield derived from either one of the largest cohorts (N = 541) studied during the 10-year era of LQTS research genetic testing (50%), or the Mayo Clinic's single institution yield for highest probability cases (N = 47, 79%, $p < 0.0001$).

CONCLUSIONS: Compared to the yields derived during the research era, the yield of the FAMILION™ LQTS genetic test is lower, likely reflecting increased use of the test for patients presenting with low-to-intermediate probability LQTS. Accepting the possible limitation of under-reporting, the low yield observed for syncope during exertion only should prompt careful consideration for mimickers of LQTS, especially in the absence of overt QT prolongation.

Heart Rhythm Society 2007, Abstract AB28-2

THE EFFECT OF MUTATION CLASS ON QTC IN UNRELATED PATIENTS REFERRED FOR THE FAMILION™ GENETIC TEST FOR LONG QT SYNDROME

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INTRODUCTION: Long QT syndrome (LQTS) genetic testing became clinically available in 2004. Given the veracity of the molecular/functional evidence, LQTS-mutations are annotated as 'probable'- (class I) or 'possible'- (class II) deleterious mutations. Class III genetic variants represent common polymorphisms occurring in >0.5% of the general population. Here, we examined the effect of genotype and mutation class on the heart rate corrected QT interval (QTc).

METHODS: From August 2004 to August 2006, 574 consecutive, unrelated patients (62% females, 86% white, average age, 23 ± 16 years, and average QTc, 464 ± 54 ms) were referred to PGxHealth for the FAMILION™ LQTS genetic test. Patients were classified as Genotype Positive (LQT1, LQT2, LQT3, LQT5, or LQT6) or Genotype Negative. Non-synonymous variants were classified as either class I, II, or III and the QTc was compared between genotypes and mutation classification.

RESULTS: Overall, 219 patients (84 LQT1, 77% class I; 82 LQT2, 65% class I; 24 LQT3, 79% class I; and 21 Multiples, 81% class I) were genotype positive. Among the genotype negative cases, 313 (88%) hosted = 1 class III variants, similar to the frequency in healthy controls. The average QTc was significantly longer among genotype positive cases (484 ± 52 ms), LQT1 (484 ± 42 ms), LQT2 (485 ± 60 ms), and multiples (512 ± 67 ms) compared to the genotype negative cases (453 ± 50 ms, p value < 0.0001). The QTc (477 ± 62 ms) for patients with a possibly deleterious LQTS mutation (class II) was similar to patients with a class I mutation (483 ± 52 ms) and significantly greater than genotype negative patients with common polymorphisms (454 ± 49 ms, p value < 0.003).

CONCLUSIONS: Over 75% of genotype positive patients have probable (class I) LQTS-susceptibility mutations. Although the remaining 25% with class II variants are vulnerable to the possibility of being a "false positive", overall there was a significant QT prolonging effect in patients with class II mutations compared to patients with a negative genetic test regardless of the number of common polymorphisms present.

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PREVALENCE OF EARLY ONSET ATRIAL FIBRILLATION IN CONGENITAL LONG QT SYNDROME

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INTRODUCTION: Recently, gain- and loss-of-function mutations in the *KCNQ1*-encoded potassium channels, gain-of-function mutations in *KCNH2*-encoded potassium channels, and loss-of-function mutations in the atrial-specific, *KCNA5*-encoded Kv1.5 potassium channel and the *SCN5A*-encoded sodium channel have been reported in atrial fibrillation (AF). A mechanism of atrial *torsades* has been suggested. The prevalence of documented AF in patients with genotype-positive congenital long QT syndrome (LQTS) is unknown.

METHODS: History of documented AF was sought from two independent cohorts including i) 252 consecutive patients (146 females, average age at diagnosis, 23 ± 16 years, QTc, 465 ± 51 ms) with genetically proven LQTS seen in Mayo's LQTS Clinic between 1998 and 2006 and ii) 205 consecutive, unrelated patients (133 females, average age at testing, 23 ± 16 years, QTc, 479 ± 51 ms) with a positive FAMILION™ genetic test for LQTS.

RESULTS: Overall, early onset AF was documented in 6/457 patients (1.3%, 279 females) including 4/252 (1.5%) seen at Mayo Clinic and 2/205 (1%) unrelated patients with a positive FAMILION™ test. From a genotype perspective, 4/211 patients (1.9%) with LQT1-susceptibility mutations had AF, compared to 0/174 LQT2 patients, 0/59 LQT3 patients, 1/1 patient with Andersen Tawil syndrome (LQT7), and 1/34 patients with multiple mutations. This patient had onset of AF at 4 years of age and had compound heterozygosity involving both *KCNH2* and *SCN5A*). For the 4 AF/LQTS positive patients evaluated in a single center, the average age at diagnosis of AF was 16.8 years (range 4 years - 31 years).

CONCLUSIONS: Prevalence of AF in the young (< 40 years of age) is estimated to be less than 1 in 1,000 in the setting of a structurally normal heart. In contrast, early onset AF was observed in over 1% of patients with genetically proven LQTS and should be viewed as an uncommon but possible LQT-related dysrhythmia. Due to under-reporting and under-detection, we may be underestimating the burden of AF in LQTS. It is anticipated that distinct pharmacotherapeutic strategies to treat channelopathic AF will be needed.

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CLINICAL GENETIC TESTING FOR CONGENITAL LONG QT SYNDROME: SPECTRUM OF MUTATIONS DISCOVERED IN THE FIRST TWO YEARS

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OBJECTIVES: The purpose of this study is to determine the spectrum of cardiac mutations among a cohort of unrelated patients referred for long QT syndrome (LQTS) commercial genetic testing and to compare the findings with reports derived from research laboratories.

BACKGROUND: Inherited LQTS is a potentially lethal arrhythmia syndrome marked by significant genetic heterogeneity. Ten LQTS susceptibility genes have been identified encompassing 600 mutations. In August 2004, LQTS testing became a commercially available genetic test.

METHODS: A comprehensive open reading frame/splice site mutational analysis of all 60 translated exons in *KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3), *KCNE1* (LQT5), and *KCNE2* (LQT6) was performed using DNA sequencing for 524 unrelated patients referred to Clinical Data (formerly Genaisance Pharmaceuticals) for LQTS testing between August 2004 and May 2006.

RESULTS: Overall, 191 (36%) index cases (126 female) had a positive test: LQT1 (84, 44%), LQT2 (70, 37%), LQT3 (21, 11%), LQT5 (5, 2.6%), LQT6 (0), and complex multiples (11, 5.8%). 159 distinct mutations were identified: 69 in *KCNQ1*, 60 in *KCNH2*, 26 in *SCN5A*, and 4 in *KCNE1*. None of these mutations were seen in over 2600 reference alleles. The majority (74%) were missense mutations. Compared with two large studies that each found a 59% prevalence of novel mutations, only 67 (42%; $p < 0.001$ vs. each) of the mutations were novel: 20 in *KCNQ1*, 28 in *KCNH2*, 17 in *SCN5A*, and 2 in *KCNE1*.

CONCLUSIONS: During the decade (1995 – 2005) of research-based genetic testing, over 500 LQTS-associated mutations were discovered. The past two years of clinical LQTS testing has increased the compendium of unique mutations by 67, and the approximate number of mutations discovered in cases of LQTS is now over 650. Our results indicate that the rate of novel mutations is on the decline. We estimate that mutation saturation within these five genes will exceed 1000 distinct mutations. The frequency of positive genetic test results (36%) is lower than observed from the largest research based cohort of unrelated patients (50%) or the expected yield of 75% when the clinical diagnosis of LQTS is high probability, probably reflecting the referral case mix.

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DISTINGUISHING CAUSATIVE AND NON-CAUSATIVE MUTATIONS IN LONG QT SYNDROME

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BACKGROUND: Congenital long QT syndrome (LQTS) affects about one in 3000 individuals. Among the most definitive LQTS cases (Schwartz score ≥ 4), about 75% are found to have at least one rare or unique protein-altering mutation in critical cardiac channel subunits encoded by *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*. More than half of the case mutations discovered today are novel. Frustrating the need to interpret these mutations in patients is our prior observation that approximately 5% of healthy individuals are also found to carry unique, seemingly causative mutations in these genes. Our objective was to identify characteristics of causative mutations that can be used to distinguish them from non-causative “background” mutations.

METHODS: We compared the patterns of mutation discovery in 1367 healthy controls and 541 unrelated cases referred for LQTS genetic testing, focusing on the nature of the changes at the protein level and relative discovery rates in cases and controls.

RESULTS: While 50% of the published cases had protein-altering mutations in one of the five LQTS-causing channel genes, 6% of controls (11% of African-Americans, and 5% of Europeans, Asians and Hispanics) also bore unique mutations. The ratio of case to control mutation rates varied greatly by gene and gene region. This ratio was effectively infinite in the pore-encoding regions of *KCNQ1* and *KCNH2*. In contrast, the ratio was 1:1 for novel non-synonymous mutations in *SCN5A* that localize outside of the transmembrane/pore regions. Amino acid substitutions were more physico-chemically radical in most regions of most genes for cases relative to controls.

CONCLUSIONS: The causality of a particular mutation discovered in a putative LQTS patient can be near certain or highly questionable. Two strong messages emerge: first, careful attention to clinical and mutational specifics must be paid to avoid over/mis-interpreting mutations found in patients; second, it is essential to scan hundreds or even thousands of healthy controls for any genetic disease with family specific mutations to identify normal variants and also to provide a frequency framework to enable an informed interpretation of patient mutations.

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THE SINGLE NUCLEOTIDE POLYMORPHISM D85N-KCNE1 IS ASSOCIATED WITH BOTH CONGENITAL AND DRUG-INDUCED LONG QT

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BACKGROUND: Rare, often unique, protein-altering mutations (defined as variants below 0.5% frequency) in the cardiac ion channel genes *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* cause approximately 75% of congenital Long QT Syndrome (LQTS). We hypothesized that one or more polymorphisms (defined as variants over 0.5% frequency), protein-altering or not, in the five genes above might contribute to LQTS. We predicted that such contributing polymorphisms would be particularly overrepresented in mutation-negative cases of congenital LQTS relative to non-diseased controls.

METHODS: The five LQTS-associated channel genes were sequenced for 98 unrelated, white cases with clinically identified LQTS but no clear pathogenic mutation (M-) and for 364 white controls. Hundreds of single nucleotide polymorphisms and several insertions/deletions were identified. We tested for correlation between case/control status and gene variants ranging from single polymorphisms up to 4-polymorphism haplotypes; permutation testing was used to control for multiple comparisons.

RESULTS: The common D85N-KCNE1 polymorphism was significantly more common among M- cases (11/98, 11%) than in controls (9/364, 2.5%) ($p = 0.0007$). No other polymorphism or haplotype was significant after multiple comparisons correction. In an effort to validate these findings, we reviewed sequence data from the FAMILION™ (Genaisance Pharmaceuticals, New Haven, CT) test for LQTS, which includes KCNE1. Thirteen of 147 M- patients carried D85N (8.8%, $p = 0.0028$ vs. controls).

CONCLUSIONS: The one significant genetic difference between controls and M- cases was overrepresentation of D85N among cases, which we have confirmed in a second case sample. Both clinical and functional studies have previously implicated D85N as a risk factor for drug-induced torsade de pointes. This congruence of findings strongly implicates D85N-KCNE1 as contributing to the phenotypic presentation of LQTS. Further studies are needed to determine whether D85N has a causative or modifying role in congenital LQTS.

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D85N-KCNE1: A COMMON SINGLE NUCLEOTIDE POLYMORPHISM ASSOCIATED WITH CONGENITAL LONG QT SYNDROME

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INTRODUCTION: Rare, often unique, protein-altering mutations in the genes *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* cause approximately 75% of congenital Long QT Syndrome (LQTS). We hypothesized that one or more polymorphisms, protein-altering or not, in the five genes above might contribute to LQTS. We predicted that such contributing polymorphisms would be particularly overrepresented in mutation-negative cases of congenital LQTS relative to non-diseased controls.

METHODS: The five LQTS-associated channel genes were sequenced for 98 unrelated, white cases with clinically identified LQTS but no clear pathogenic mutation (M-) and for 364 white controls. Hundreds of single nucleotide polymorphisms and several indels were identified. We tested for correlation between case/control status and gene variants ranging from single polymorphisms up to 4-polymorphism haplotypes; permutation testing was used to control for multiple comparisons.

RESULTS: The common *KCNE1* polymorphism D85N was significantly more common among M- cases (11/98, 11%) than in controls (9/364, 2.5%) ($p = 0.0007$). Notably, all 11 D85N+, M- cases were female ($p = 0.03$). No other polymorphism or haplotype was significant after multiple comparisons correction. In an effort to validate these findings, we reviewed data from the FAMILION genetic test for LQTS. Nine (five female) of 95 M- patients carried D85N (9.5%, $p = 0.0045$ vs. controls).

CONCLUSIONS: The one significant genetic difference between controls and M- cases was overrepresentation of D85N among cases, which we have confirmed in a second case sample. Clinical and functional research have previously implicated D85N as a risk factor for drug-induced torsade de pointes. This congruence of findings strongly implicates D85N-KCNE1 as contributing to the phenotypic presentation of LQTS in some patients. However, further studies are needed to determine whether the D85N has a causative or modifying role.

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A COMPARISON OF MUTATION PATTERNS IN SUSPECTED CASES OF CONGENITAL LONG QT SYNDROME AND CONTROLS

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Congenital long QT syndrome (LQTS) affects about one in 5000 individuals. LQTS is a heritable condition often characterized by prolonged QT intervals as seen by a 12-lead electrocardiogram. Although nearly half of susceptible hosts experience a lifelong asymptomatic course, LQTS can present with sudden cardiac death even in infancy. Among the most definitive cases, about 75% are found to have rare or unique protein-altering mutations in the cardiac ion channel genes *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*; hundreds of such mutations have been reported, few of which have been observed in healthy controls. Here, we compared the patterns of mutation discovery in 611 healthy controls with that of 541 cases published in a recent compendium. While 50% of the published cases had protein-altering mutations in one of the five LQTS causing genes, we found that almost 5% of the controls also had such mutations. In addition to the overall rates, the relative numbers of mutations among the five genes was also significantly different between cases and controls ($p = 5.9 \times 10^{-8}$), as seen in the table below. From these relative rates, it is clear that causality can be confidently assigned to case mutations found in *KCNQ1* and *KCNH2*, and less so for *SCN5A*. Further, when a case has two mutations, we can be confident that at least one of the mutations is causative, but we can estimate that for half of those cases, one of the mutations may represent a rare but clinically insignificant “background” variant.

Table 1. Percentages and counts among Cases and Controls in the 5 genes.

	KCNQ1	KCNH2	SCN5A	KCNE1	KCNE2	Multiple Mutations
Controls (611)	1.0% (6)	1.1% (7)	2.3% (14)	0.3% (2)	0.5% (3)	0.2% (1)
Cases (541)	22.2% (120)	17.2% (93)	4.8% (26)	0.6% (3)	0.2% (1)	5.4% (29)

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

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BACKGROUND: Congenital long QT syndrome (LQTS) genetic testing 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 5 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. Here, we explore the possibility of allelic drop-out as a possible mechanism underlying false negative test results.

METHODS: The published primers currently utilized by many research laboratories to conduct a comprehensive analysis of the 60 protein-encoding 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. 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 388 consecutive, unrelated index cases referred for LQTS genetic testing.

RESULTS: Common intronic single nucleotide polymorphisms (SNP) residing within the primer sequence were found in the reverse primer used for exon 15 of KCNQ1 and the reverse primer used for exon 4 for KCNH2. The allelic frequency was 5% (1794+32 g>t, KCNQ1) and 39% (916+60 c>t, KCNH2). Following primer redesign to eliminate the possibility of allelic drop-out, 4 previously genotype negative index cases were found to possess LQTS-causing mutations: R591H-KCNQ1 and R594Q-KCNQ1 for exon 15 and E229X-KCNH2 found in two unrelated cases. Repeat analysis of the amplicons in 400 reference alleles did not identify these or any additional amino acid variants.

CONCLUSIONS: Allelic drop-out secondary to intronic SNP-primer mismatch prevented the discovery of LQTS-causing mutations in 4 cases. Considering that many LQTS genetic testing research laboratories have utilized these primers, patients who are reportedly genotype negative may benefit from a re-examination of exon 15 in KCNQ1 and exon 4 in KCNH2.

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REPEAT LONG QT SYNDROME GENETIC TESTING OF PHENOTYPE POSITIVE CASES: PREVALENCE AND ETIOLOGY OF DETECTION MISSES

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BACKGROUND: Long QT syndrome (LQTS) genetic testing has been performed since 1995. Approximately 75% of patients with definite LQTS have an identifiable mutation in one of 5 LQTS-causing channel genes. Possible explanations for genotype negative cases include LQTS phenocopies, novel LQTS-causing genes, unexplored regions of the known genes, and genetic testing detection failures. Here, we sought to determine the prevalence and etiology of detection misses from our research laboratory.

METHODS: No LQTS-causing mutations were seen in 166/388 (43%) consecutive, unrelated patients who had been referred to Mayo Clinic's Sudden Death Genomics Laboratory for LQTS genetic testing that utilized denaturing high performance liquid chromatography (DHPLC). Among this genotype negative subset, 46 subjects (29 female, average age = 23 ± 15 years, average QTc = 512 ± 55 ms) having the highest clinical likelihood for LQTS were selected for repeat genetic testing using direct DNA sequencing.

RESULTS: Putative LQTS-causing mutations were identified in 7/46 (15%) phenotype positive/previously genotype negative subjects including 4 with LQT1 (S225L, G568R, R591H, and R594Q), 2 with LQT2 (H70R and G925R), and 1 with LQT3 (V411M). None of these variants were seen in over 1500 reference alleles. Analysis of the misses revealed 1) normal DHPLC detection profile in 2 (detection method failure), 2) faulty DHPLC column in 1 (equipment failure), 3) allelic drop-out in 2 (primer design miss), and failure to detect abnormal signal in 2 (user miss).

CONCLUSIONS: In this study, a LQTS-causing mutation was elucidated in 15% of subjects who had completed mutational analysis in our research laboratory and were deemed genotype negative. Extrapolation of this data suggests an approximate 95% accuracy with respect to our DHPLC-based genetic testing platform. Analysis of the "misses" revealed several different etiologies that have been addressed to improve detection of channelopathy-associated mutations. Repeat genetic testing should be considered for genotype negative individuals in whom the clinical diagnosis was strongly suspected.