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Novel Gene and Mutation Discovery in Congenital Long QT Syndrome: Let's Keep Looking Where the Street Lamp Standeth

David J. Tester, BS and Michael J. Ackerman, MD, PhD

Departments of Medicine, Pediatrics, and Molecular Pharmacology & Experimental Therapeutics and the Divisions of Cardiovascular Diseases and Pediatric Cardiology Mayo Clinic Rochester, MN 55905

Congenital LQTS comprises a distinct group of cardiac channelopathies characterized by delayed repolarization of the myocardium, QT prolongation, and increased risk for syncope, seizures, and sudden cardiac death in the setting of a structurally normal heart and otherwise healthy individual. From a beat-to-beat perspective, this repolarization abnormality almost always is without consequence. However, rarely, when caught off guard by triggers such as exertion, swimming, emotion, auditory stimuli such as an alarm clock, or during the postpartum period, the heart can spiral electrically out of control into a potentially life threatening and sometimes lethal dysrhythmia of *torsade de pointes*.

The prevalence of LQTS may be as high as 1 in 2500 persons, with approximately 75% of clinically robust LQTS due to either “loss-of-function”- or “gain-of-function”-producing mutations in 3 genes: *KCNQ1*-encoded I_{Ks} potassium channel(1) (LQT1, loss-of-function), *KCNH2*-encoded I_{Kr} potassium channel(2) (LQT2, loss-of-function), and *SCN5A*-encoded I_{Na} sodium channel(3) (LQT3, gain-of-function) that are critically responsible for the orchestration of the cardiac action potential. Since the first discoveries of mutations in these three critical ion-channel alpha sub-units, there are now a total of 12 LQTS-susceptibility genes. However, LQT4-12 accounts for less than 5% of LQTS cases. Nonetheless, the 4 newest additions to the LQTS-susceptibility gene family have taught us to stay close to the ion channel alpha subunits that underlie the three most common subtypes of LQTS. Three of the four genes: *CAV3* (LQT9), *SCN4B* (LQT10), and *SNTA1* (LQT12) encode proteins that function in part as sodium channel interacting proteins (ChIPs). When co-expressed with an otherwise intact sodium channel alpha subunit, mutant caveolin-3, mutant sodium channel beta 4 subunit, or mutant alpha syntrophin convert the NaV1.5 sodium channel complex into a late sodium current generating one(4)(5)(6). Similarly, mutations in *AKAP9* disrupts the I_{Ks} ChIP, yotiao, causing an LQT1-like loss-of-function(7). Although it appears that no new LQTS gene is going to capture a sizable piece of the remnant of genotype negative LQTS, these discoveries remind us that the “Towbinesque” *Final Common Pathway* strategy is working and that staying close to the 3 most common LQTS-susceptibility genes and their encoded signalling pathway is worthwhile(8).

Now, Eddy and colleagues remind us that the street lamp still shineth over the two most common LQTS-susceptibility genes themselves with novel LQTS-causing mutations that

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Address for correspondence: Michael J. Ackerman, MD, PhD, Windland Smith Rice Sudden Death Genomics Laboratory, Guggenheim 501, Mayo Clinic, Rochester, MN 55905, 507-284-0101 (phone), 507-284-3757 (fax), ackerman.michael@mayo.edu.

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escaped detection by traditional strategies being caught instead by multiplex ligation-dependent probe amplification (MLPA)(9). Until recently, traditional mutational analysis relied upon the techniques of exon-specific polymerase chain reaction (PCR), an intermediate mutation analysis platform such as single stranded conformation polymorphism (SSCP) or denaturing high performance liquid chromatography (DHPLC), and subsequent direct DNA sequencing to elucidate the genetic underpinnings of this disorder. These techniques work with great precision in detecting i) single nucleotide substitutions that produce missense, nonsense, and splice site mutations and ii) small insertion/deletions. However, large whole gene, multiple exon, or single exon deletions or duplications elude detection by this approach (Figure 1a).

In contrast to the traditional approach, MLPA relies on specifically engineered probes that are designed to bind to gene sequences (typically exonic sequences) and allows for the detection of copy number changes of the target sequence. Large deletion mutations for example, will result in a loss of copy number of the target (exon) while insertions are represented as an increase in copy number (Figure 1b). In 2006, Koopmann and colleagues provided the sentinel report for any cardiovascular disease of MLPA-exposed mutations demonstrating that large gene rearrangements in the LQT2-susceptibility gene *KCNH2* may serve as a pathogenic basis for cases of seemingly genotype negative LQTS(10). The authors analyzed 21 patients that remained genotype negative following a coding (exonic) and splice site region mutational analysis of commonly involved LQTS genes, and found that 1 of the 21 (~5%) patients had a tandem duplication of 3,682 bp (involving 2.5 kb of intron 5, all of exon 6, and 121 bp of exon 7) in *KCNH2* (LQT2). This large gene rearrangement mutation in *KCNH2* co-segregated properly in this Dutch family with multiple effected individuals, including a 26-year-old female who experienced sudden unexpected nocturnal death. Consistent with LQT2 genotype, many of the cardiac events were auditory triggered. Most recently, MLPA was used to detect a 5.27 Mb deletion of chromosome 7 (7q36.1–q36.2) encompassing a whole gene deletion of *KCNH2* in a 9-year-old with significant QT prolongation (QTc of 490 msec), renal hypoplasia, and mental retardation(11). In addition, using MLPA, Bhuiyan et al, identified a 1.1 kb deletion causing an in-frame deletion of 35 amino acids (deletion of exon 3) of the *RYR2*-encoded cardiac ryanodine receptor in a family with catecholaminergic polymorphic ventricular tachycardia (CPVT), another potentially lethal cardiac channelopathic disorder(12).

In this edition of *Heart Rhythm*, Eddy and colleagues used MLPA to identify two large multiple-exon deletions in *KCNQ1* (deletion of exon 13–14) and *KCNH2* (deletion of exons 6–14), and a large intragenic duplication in *KCNH2* (duplication of exons 9–14) in 3 of 26 (11.5%) patients with a clinically robust diagnosis of LQTS. All patients were negative for traditional missense, nonsense, or small insertion/deletion coding region mutations of the three most common LQTS genes (*KCNQ1*, *KCNH2*, and *SCN5A*)(9). So far, the 3 large gene rearrangements of *KCNH2* have been identified families with a remarkable history of autopsy negative sudden unexpected death in the young, totaling 6 cases between the three families. Perhaps, large gene rearrangements in *KCNH2* confer a more “malignant” substrate, and this may have implications for postmortem genetic testing by MLPA in cases of autopsy negative sudden unexplained death (SUD) where traditional LQTS- and CPVT-associated mutations have been detected in approximately one-third of SUD cases(13)(14). Although the 6 sudden deaths involved adolescents and young adults, it is conceivable that such large gene rearrangements in these cardiac channel genes could provide a mechanism of channelopathic sudden infant death syndrome as well(15)(16). While most of the sudden deaths were inferred to be positive for each family’s respective mutation, Eddy and colleagues were able to perform a confirmatory “molecular autopsy” for the *KCNH2* exon 6–14 deletion on one of the decedents using DNA isolated from her newborn screening Guthrie card. Expectantly, no large gene rearrangements have been reported in *SCN5A* from

analysis of these LQTS cohorts as such a mutation would result almost certainly in “loss-of-function”. Perhaps, MLPA of *SCN5A* will catch additional Brugada syndrome mutations.

In terms of LQTS genetic testing, it will be interesting to see if MLPA continues to provide the answer for 5–10% of cases with an otherwise negative genetic test. If so, then additional surveillance of *KCNQ1* and *KCNH2* by MLPA might be expected to increase the maximal anticipated yield of the current clinically available genetic test by 1 – 3%. Although small in magnitude, it should be noted that this incremental increase is similar to that provided by analysis of the entire open reading frames for the nine genes responsible for LQT4–12.

Still, approximately 20% of LQTS remains genetically elusive. While targeting candidate genes that encode NaV1.5 ChIPs have yielded fruit recently, this present study reminds us that a renewed exploration of the two most common LQTS genes may be similarly fruitful. Besides traditional mutations involving the translated exons/canonical splice sites and now larger gene rearrangements, what other pathogenic mechanisms (promoter perturbations, intronic insults, and synonymous snares) within *KCNQ1* and *KCNH2* await discovery? For example, synonymous or “silent” (non-amino acid altering) single nucleotide substitutions residing within the exons could conceivably disrupt splice enhancer element sequences precipitating errant RNA splicing and subsequent exon skipping or intron inclusion. Such a mechanism has been reported for diseases like Marfan syndrome, familial adenomatous polyposis, and McArdle disease already(17)(18)(19). Although not directly illuminated by the street lamp, the shadows in its vicinity sure seem like a good place to cast more light.

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Traditional Mutational Analysis

Figure 1. Traditional Mutational Analysis by DHPLC versus MLPA Detection for Large Insertion or Deletion Mutations

Depicted is A) a traditional mutational analysis by DHPLC where exon specific PCR amplification of both the maternal (1) and paternal (2) alleles is achieved using specific primers designed to anneal to the exon flanking intronic sequences. In the case of normal patient (patient 1), PCR products representing both alleles will be produced. However, in patient 2 who hosts a large deletion, PCR primers will only anneal to the normal strand. In either patient, the resulting DHPLC and subsequent sequence will only be representative of the normal strand and the allele with the deletion will go unnoticed. B) Illustrates how

multiplex ligation probe analysis (MLPA) can be used to detect such deletions or insertions by quantifying the copy number of target sequences (exons) as determined by specifically designed probes. Here, specific probes of varying length (as a result of the variable length “stuffer” sequence-shown in yellow) are first hybridized to each patient’s gene sequence. The probes are stripped from the genomic DNA and subsequently serve as template for PCR amplification using primers that specifically anneals to all of the probes allowing them to be simultaneously amplified in a single multiplex PCR reaction. Because of the varying length of the “stuffer” sequence, the amplified probes can be separated according to size by electrophoresis and viewed on a chromatogram. The amount of probe hybridizing to the patient’s genomic DNA determines the copy number for the specific targeted sequence (exons). In this example where exon 7 and 8 are deleted, less exon 7 and 8 specific probes have been hybridized and subsequently fewer copy numbers of template are detected. Conversely, a large insertion would produce more copy numbers of the target sequence(s).