LINKAGE AND MUTATION ANALYSIS IN TWO TAIWANESE FAMILIES WITH LONG QT SYNDROME

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Abstract: Long QT syndrome (LQT) is a cardiovascular disorder causing syncope and sudden death from arrhythmias. Mutations in KCNQ1, KCNH2, KCNE1, KCNE2, and SCN5A genes encoding cardiac potassium and sodium ion channels cause LQT. Two Taiwanese LQT families were screened for mutations in these ion channel genes. In family H87, the diagnosis was made in the 25-year-old female proband and six family members based on recurrent syncope and/or a prolonged QT interval. Genotyping revealed a novel nonsense mutation, R744X (C to T transition in codon 744), in the KCNH2 potassium channel gene, resulting in truncation of the putative cyclic nucleotide-binding domain and C-terminal region of the HERG K+-channel in all affected family members. The mutation was confirmed by Ddel endonuclease digestion of the DNA from each family member. The 26-year-old female proband in family L89 developed repeated syncope with QTc of 0.61 seconds. After linkage and mutation analysis, the syndrome in this family was associated with a novel KCNQ1 missense mutation, T309I, causing the substitution of a threonine residue at position 309, in the pore region of the KvLQT1 K+-channel, with an isoleucine. By Tsp45I restriction analysis, the mutation was noted in the proband and the proband’s asymptomatic brother, but was not detected in 100 unrelated normal individuals. Identification of a mutation has clinical implications for presymptomatic diagnosis and therapy.

Long QT syndrome (LQT) is a cardiac disorder characterized by abnormal ventricular repolarization, prolonged QT interval on electrocardiogram (ECG), arrhythmia (torsade de pointes), syncope, and sudden death [1]. Although the syndrome may occur sporadically, familial clustering is recorded in the majority of cases [2]. Therapy with β-adrenergic blockers has dramatically improved the long-term survival of patients with the disorder [3]. However, the recurrence of syncopal attacks and risk of sudden death are still serious clinical problems in many patients.

The inherited basis of LQT is heterogeneous. Mutations in five genes, all coding for subunits of ion channels involved in the cardiac action potential, have been associated with LQT. The KCNQ1 (chromosome 11p15.5) gene encodes a KvLQT1 channel, which associates with IšK protein (encoded by the KCNE1 gene on chromosome 21q22.1–q22.2) to underlie the cardiac slow delayed-rectifier Iks current [4, 5]. The KCNH2 (chromosome 7q35–q36) and KCNE2 (chromosome 21q22.1–q22.2) genes encode an HERG protein and an MiRP1 protein, respectively. Through

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heteromultimerization of HERG with MiRP1, the ion channel responsible for the cardiac rapidly activating current $I_{Kr}$ is assembled [6, 7]. Mutations in the four $K^+$-channel subunit genes cause functional defects/cessation of function, leading to a prolongation of the repolarization of the action potential. The SCN5A gene (chromosome 3p24–p21) encodes the $\alpha$-subunit of the Na$^+$ ion channels responsible for the initial upstroke of the action potential, and mutations lead to a gain of channel function [8]. The described mutations in the five genes causing LQT are generally distributed all over the coding segments of the respective genes [9].

In the present study, clinical and genetic analyses were performed to examine the molecular basis of LQT in two Taiwanese families. A novel nonsense mutation distal to the S6 region of HERG and a novel missense mutation in the pore region of KvLQT1 were identified.

Case Reports

Family H87 was followed at Chang Gung Memorial Hospital. The diagnosis of LQT was made in seven family members who had a QTc (QT interval corrected for heart rate) of at least 0.47 seconds. The proband (Fig. 1, upper panel, II-4) had a past history of recurrent syncope. Her younger brother (II-6) died suddenly at age 22 years during a military hike. As the first step to identify the molecular lesion, the entire coding regions of the KCNE1 [10] and KCNE2 [7] genes were amplified by polymerase chain reaction (PCR) and sequenced. No mutation was found. The family was then genotyped with polymorphic markers linked to the KCNQ1, KCNH2, and SCN5A genes [11]. After haplotype analysis, the KCNQ1 and SCN5A mutations were excluded (data not shown). The disease phenotype was linked to markers flanking the KCNH2 gene (Fig. 1, upper panel). To test the hypothesis that a KCNH2 mutation was responsible for LQT in this family, the coding sequences and exon-intron borders of KCNH2 were amplified from the proband as previously described [6, 12], and screened for mutation by means of single strand conformation polymorphism (SSCP). SSCP, using the primer pair spanning the sequences encoding the region distal to the S6 region of HERG, identified an aberrant pattern in the proband and affected family members (Fig. 2A). Sequencing of the aberrant band revealed a C to T transition at the first nucleotide of codon 744, which resulted in the substitution of an arginine for a termination codon (R744X) (Fig. 2B). The mutation creates a DdeI restriction site: if the mutation is present, after PCR amplification and DdeI restriction, the 272-base pair (bp) fragment is cut into two fragments of 205 and 67 bp (Fig. 2C). All affected family members were heterozygous for the mutation. No R744X mutation was found in unaffected family members.

Family L89 was identified through the diagnostic registry at Wei Gong Memorial Hospital. The proband (Fig. 1, lower panel, II-3) was a 26-year-old woman who developed characteristic features of LQT, including prolongation of the QT interval (QTc = 0.61 sec) and repeated syncope (Fig. 3A). Three days after treatment with propranolol 40 mg tid and mexilet 100 mg q8h, 12-lead ECG showed QT prolongation with a QTc of 0.51 seconds. When the KCNE1 and KCNE2 genes were examined, no mutation was found. The family was then genotyped as described and only the SCN5A mutation was excluded (data not shown). The coding sequences and exon-intron borders of KCNQ1 [4, 10] and KCNH2 genes were amplified and screened for mutation. An abnormal SSCP pattern was observed in the fragment containing the pore region of KvLQT1 (data not shown). A novel T309I mutation was found after sequence analysis (Fig. 3B). The missense mutation caused the substitution of a threonine
Potassium Channel Gene Mutations and Long QT

Discussion

The inherited basis of LQT is heterogeneous. Through clinical and genetic analyses, we identified a novel R744X mutation distal to the S6 region of HERG and a novel missense mutation in the pore region of KvLQT1 in two Taiwanese families. Together with the previously described A614V mutation in the pore region of HERG [13], a total of three Taiwanese cases of K+ channel mutations have now been reported.

The novel R744X mutation in family H87 resulted from a C to T transition at HERG codon 744 (CGA to TGA). The methylation-mediated deamination of 5-methylcytosine in hypermutable CpG dinucleotide is frequently associated with point mutations in various genes [14]. The mutation is predicted to result in truncation of the putative cyclic nucleotide-binding domain (NBD) and C-terminal region of the protein. Since cardiac $I_K$ was unaffected by protein kinase A-dependent intracellular signaling [15], and the activity of normal HERG was insensitive to cyclic nucleotides by in vitro expression study [16], the functional role of NBD is not clear. Recently, a 104-amino acid domain from the C-terminus of HERG was identified and appears essential for recapitulation of $I_K$ in a heterogeneous expression system [17]. This finding indicates a similar mechanism for R744X and other uncharacterized mutations that disrupt the C-terminal.

The novel T309I mutation in family L89 resulted from a C to T transition at KvLQT1 codon 309 (ACA to ATA). This mutation would be predicted to change a conserved threonine to an isoleucine in the pore region of the KvLQT1 channel. This threonine is located at position 1 of the K+ channel signature sequence, a stretch of eight amino acids (TTTVGYG) that is shared by the Shaker K+ channels [18]. In heterologous expression using Xenopus oocytes, T309A and T309V mutant channels did not express current, whereas the T309G mutation rendered the channel nonselective among monovalent cations [18]. Therefore, based on these studies, T309I mutation would be expected to encode for a K+ channel without function or with altered conductance properties. The reported T309R, T311I, and T312I mutations in the K+ channel signature sequence caused susceptibility to ventricular arrhythmias and sudden death [4, 19, 20] suggesting that T309I is likely to be responsible for the LQT in this family.

In family L89, the T309I mutation was also noted in the proband’s asymptomatic brother (Fig. 3C). This finding suggests that measurements of QTc intervals in ECG recordings of members of an LQT family do not permit an accurate diagnosis of LQT in all cases. This is in accordance with previous observation that some

residue (ACA) at position 309, in the pore region of the KvLQT1 K+ channel, with an isoleucine (ATA). The mutation abolishes a Tsp45I restriction site in the PCR product so that, on digestion, a 35-bp fragment appeared in addition to the wild type 29- and 6-bp fragments (Fig. 3C). On Tsp45I restriction analysis, the mutation was also present in the proband’s asymptomatic brother (Fig. 3C). The mutation was not detected in 100 unrelated normal individuals. To exclude other mutations causing LQT in this family, the entire coding sequences and exon-intron borders of the KCNQ1 and KCNH2 genes were sequenced and no mutation was found (data not shown).

Fig. 2. A) Single-strand conformation polymorphism (SSCP) analysis of polymerase chain reaction products containing the region distal to S6 of HERG. The arrowhead marks the band representing the major conformer of the mutant allele. B) DNA sequence analysis of the aberrant SSCP conformer. A C to T transition resulting in an arginine (CGA) to termination (TGA) substitution at codon 744 (R744X) is noted. C) Restriction enzyme analysis of the mutant allele. The primer pair 9F-16R-amplified products were digested with DdeI and resolved on a 10% polyacrylamide gel. Lane M (Hinfl digestion of pGEM4 DNA) = size markers.
LQT patients might have a normal QTc interval [21]. The importance of this test result as an indication of appropriate care of the asymptomatic brother clearly underscores the vital role of molecular genetic testing in the diagnosis of LQT syndrome.

In summary, we examined the molecular basis of disease in two Taiwanese LQT families. The identification of a mutation has important implications for treatment of affected members and reduction of susceptibility to ventricular arrhythmias and sudden death in asymptomatic carriers. Future ion channel studies may provide insight into the mechanisms of LQT.

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