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A novel splice mutation of HERG in a Chinese family with long QT syndrome*

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Abstract: Congenital long QT syndrome (LQTS) is a genetically heterogeneous disease in which six ion-channel genes have been identified. The phenotype-genotype relationships of the HERG (human ether-a-go-go-related gene) mutations are not fully understood. The objective of this study is to identify the underlying genetic basis of a Chinese family with LQTS and to characterize the clinical manifestations properties of the mutation. Single strand conformation polymorphism (SSCP) analyses were conducted on DNA fragments amplified by polymerase chain reaction from five LQT-related genes. Aberrant conformers were analyzed by DNA sequencing. A novel splice mutation in C-terminus of HERG was identified in this Chinese LQTS family, leading to the deletion of 11-bp at the acceptor splice site of Exon9 [Exon9 IVS del (−12→−2)]. The mutation might affect, through deficient splicing, the putative cyclic nucleotide binding domain (CNBD) of the HERG K^+ channel. This mutation resulted in a mildly affected phenotype. Only the proband had a history of syncopes, while the other three individuals with long QT interval had no symptoms. Two other mutation carriers displayed normal phenotype. No sudden death occurred in the family. The 4 affected individuals and the two silent mutation carriers were all heterozygous for the mutation. It is the first splice mutation of HERG reported in Chinese LQTS families. Clinical data suggest that the CNBD mutation may be less malignant than mutations occurring in the pore region and be partially dominant over wild-type function.

Key words: HERG gene, Long QT syndrome, Cardiac arrhythmia, C-terminus, Acceptor splice site mutation
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INTRODUCTION

Long QT syndrome (LQTS) is a cardiac disease characterized by prolongation of the QT interval with malignant ventricular arrhythmias leading to syncope, torsades de pointes, and sudden death (Priori *et al*., 1999a). The incidence of sudden cardiac deaths in untreated LQTS patients is close to 50% over 10 years (Roden and Spooner, 1999). Genetic analyses revealed that the congenital LQTS is genetically heterogeneous and has been linked to at least six different loci (LQT1−LQT6) (Keating and Sanguinetti, 2001; Plaster *et al*., 2001). The human ether-a-go-go-related gene (HERG) is responsible for the chromosome 7-linked LQTS form (LQT2) (Curran *et al*., 1995) and encodes the pore-forming subunit of the rapidly activating delayed rectifier potassium channel (I_{Kr}), which is the major determinant of action potential duration (Sanguinetti *et al*., 1996). Many mutations at different sites in HERG have been identified and account for 45% of the identified LQTS mutations (Splawski *et al*., 2000).

Recent reports revealed that the mechanisms for HERG channel dysfunction could be characterized by the sites at which mutations occurred (Chen *et al*., 1999; Nakajima *et al*., 1999; Lees-Miller *et al*., 2000). For example, mutations in the N-terminus accelerated HERG channel deactivation, a mutation in the S4

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voltage sensor affected the voltage dependence of activation, or mutations in the pore region affected HERG channel inactivation or ion selectivity. In contrast, little information is available for characterizing the mechanism for HERG channel dysfunction caused by mutations in the C-terminus. More recently, new LQT2 mutations have been discovered in the putative cyclic nucleotide binding domain (CNBD) in C-terminus and some studies reported that the CNBD mutants were only partially dominant over wild type function (Johnson *et al*., 2003; Splawski *et al*., 2000; Berthet *et al*., 1999). However, there are few reports describing the genotype-phenotype correlations of the CNBD mutants.

In this study, we identify a novel splice mutation in the CNBD of HERG in a Chinese family with LQTS and characterized the clinical manifestations properties of the mutant, which may provide useful information for investigating the structure-function relationships of the HERG C-terminal mutants.

METHODS

Clinical evaluation

The study was conducted in a three-generation Chinese LQTS family. Informed consent was obtained from all individuals or their guardians according to standards established by the First Affiliated Hospital of Zhejiang University. The pedigree of the family is shown in Fig.1a. Detailed medical histories were obtained from and electrocardiogram (ECG) examinations were conducted on, each study subject. They were diagnosed for LQTS on the basis of QTc (the QT interval corrected for heart rate using the Bazett's formula) and the presence of symptoms. Affected individuals were defined as those with QTc>0.46 s in lead II or QTc>0.44 s in patients with syncope, documented torsade de points, or aborted sudden death (Berthet *et al*., 1999). One hundred unrelated healthy individuals were recruited as normal controls. Peripheral blood was collected from all participants.

DNA isolation and mutation analysis

Genomic DNA was extracted from peripheral white blood cells using Puregene DNA purification kits (Genta Systems, Minneapolis, MN). All exons and their flanking intronic sequences corresponding to the five LQT-related genes (KCNQ1, HERG, SCN5A, KCNE1 and KCNE2) were amplified by polymerase chain reaction, using primers as described (Wang *et al*., 1996; Splawski *et al*., 1998). PCR fragments were subsequently screened with single strand conformation polymorphism (SSCP) analysis according to standard procedures at 4 °C and the gels were visualized with silver-staining. If abnormal SSCP patterns were found, the same PCR fragments (of all family members and 100 unrelated normal controls) were reamplified and sequenced directly or after subcloning by using ABI 377 DNA sequencers.

Splice site prediction by neural network

The normal and mutant DNA sequences between Exon8 and Exon10 in HERG were used (GenBank accession No. U04270). A neural network (http://www.fruitfly.org/seq_tools/splice.html), accessible on the Internet, was used to predict acceptor splice sites in this region (Reese *et al*., 1997).

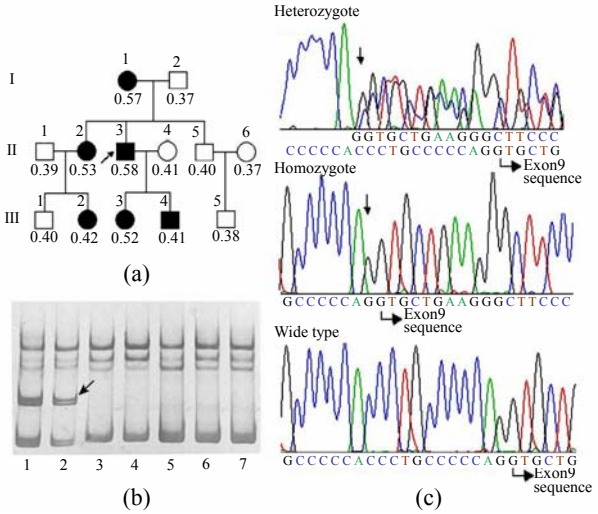


Fig.1 Genetic analysis (a) Pedigree structure of the family. Men are denoted by squares and women by circles. Darkened symbols, containing the mutation; clear symbols, no mutation. The arrow indicates the proband. The numbers below symbols show QT interval; (b) SSCP analysis of Exon9. The aberrant conformer is indicated by arrow. Lane 1~Lane 2: affected members (II-2 and II-3); Lane 3~Lane 6: unaffected members (II-1, II-4, II-5 and II-6); Lane 7: normal controls; (c) DNA sequences of the acceptor site of Exon9. The upper indicates the heterozygous mutant; the middle indicates the homozygous mutant; the down indicates the wide type. The start site of the deletion of 11-bp (ccctgccccca) is showed by arrow

RESULTS

Phenotypic characterization

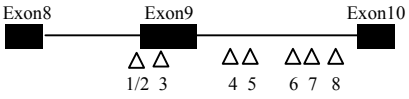
The proband II-3 was a 48-year-old man with a nearly 30-year history of recurrent syncopes. Most episodes were triggered by physical exercise or emotional stress. ECG (electrocardiograph) revealed a long QT interval (QTc=0.58 s), relative bradycardia and multiple T wave notches, which confirmed the diagnosis of LQTS (Schwartz *et al*., 1993). His audiometrics were normal, and serum K^+, Mg^+ and Ca^{2+} concentrations were also normal. Fig.1a shows the pedigree of this LQTS family. Among the mutation carriers, four individuals (I-1, II-2, II-3 and III-3) exhibited prolonged QTc (0.57, 0.53, 0.58 and 0.52 s, respectively), in which only the proband (II-3) suffered from syncopes, with no more than one time every year. The proband's son and niece (III-2 and III-4) carrying the mutation had normal QT intervals (0.42 and 0.41 s) and clinical manifestations. No family members were believed to have died as a result of LQTS. After diagnosis, the affected patients were placed on antiadrenergic treatment and no further syncopes have occurred since 1997.

Genetic analysis

SSCP analysis showed an aberrant conformer in the PCR-amplified fragment covering Exon9 in the DNA samples from the LQTS family (Fig.1b). DNA sequencing revealed a heterozygous splice mutation with a deletion of 11-bp from –2 nucleotide to –12 nucleotide at the acceptor splice site of Exon9 [Exon9 IVS del (–12→–2)] (Fig.1c). Next we screened the mutation in all members of the family by sequencing, finding it present in all affected members (I-1, II-2, II-3 and III-3) and two unaffected members (III-2 and III-4). All of them were heterozygous for the mutation. SSCP analysis of other LQTS-related gene (KCNQ1, SCN5A, KCNE1 and KCNE2) revealed no other mutations in this LQTS family.

The mutation changed the acceptor splice site of Exon9 in HERG, but did not induce any defect in the amino acid sequence. To test whether the variant was a polymorhism, we screened the mutation in 100 normal controls by SSCP analysis and found no aberrant conformer in any of these samples.

The mutation [Exon9 IVS del (–12→–2)] probably disrupted the correct splicing of the HERG mRNA by changing other nucleotide residues in the proximity of the splice sites. Using a cutoff of 0.4 giving a detection rate of 83.8% and a false-positive rate of 3.1%, we tested this hypothesis further. When the mutation was present in HERG, the neural network value of an acceptor splice site at this position changed from 0.95 to 0.30. Furthermore, the network predicted other cryptic splice sites, with neural network values above 0.4 in the sequence between Exon8 and Exon10 (Fig.2).



(a)

No.	Score	Sequence
1	0.95	c a c c c t g c c c c c a g g t g c t g a a g g g c t
2	0.30	t g a g c t g c c c c c a g g t g c t g a a g g g c t
3	0.62	c t g a g t g c c t g c a g g c t g a c a t c t g c c
4	0.99	c t c c t t c c c t t c a g g a t c c g c c a c a a a
5	0.40	c t t c c t t a a a g t a g g a t c a a a t c t a g a
6	0.64	c c t g t g t g c c t c a g g g c a g a t a t a g c a
7	0.95	a c t t t g t t t c t c a g c t c t g t a c a a a c a
8	0.77	g t a t g t t c t t t t c a g t a a a a a a a a a a a

(b)

Fig.2 Acceptor splice sites between Exon8 and Exon10 predicted by a neural network (a) Approximate location of the true acceptor splice site (1) and six pseudo splice sites (Nos. 3~8); (b) The score and sequence of the acceptor splice sites. 1: Normal acceptor splice site; 2: Mutated acceptor splice site; 3~8: pseudo acceptor splice site. The score is determined on a scale from 0~1

DISCUSSION

This paper describes a novel acceptor splice mutation [Exon9 IVS del (–12→–2)] in the CNBD of HERG K^+ channel, which is the first splice mutation of HERG reported in Chinese families with LQTS. We conclude that the mutation might affect, through deficient splicing, the CNBD of HERG K^+ channel and result in prolonged ventricular repolarization. Almost all intron sequences of eukaryotic genes are defined by a 5′ GT sequence (donor splice site) and a 3′ AG sequence (acceptor splice site). However it is likely that a change of nucleotides in the proximity of the splice sites can affect mRNA splicing and cause disease by inducing either exon skipping or synthesis of mRNA with large intron inserts, even though the sequence change does not lead to an amino acid change (Neyroud *et al*., 1997; Krawczak *et al*., 1992). The latter is the result if splicing occurring at cryptic splice sites, where the splicing enzymes skip the

normal splice site and use one of the competing pseudo splice sites. In our study, the neural network value of the mutated acceptor splice site changed from 0.95 to 0.30. Use of pseudo splice site 3 in Exon9 adjacent to the mutation will result in a deletion of 10 amino acids, which may lead to a truncation lacking the CNBD in the C-terminus. What is more, the pseudo splice sites within intron9 will result in an inframe insertion, which may also disrupt the normal function of the protein. Although it has been estimated that up to 15% of all point mutations causing human disease involve defective mRNA splicing (Krawczak *et al*., 1992), no splice site mutations of HERG have been reported in Chinese LQTS family previously.

The C-terminal CNBD is responsible for the direct activation of cyclic nucleotide-gated channels and for modulation of the HERG K^+ channel (Warmke and Ganetzky, 1994). Recent electrophysiological studies provided evidence that CNBD mutant homotetramers cannot form functional channel, but functional heterotetrameric channels with altered gating properties were detected while co-expressed with wild-type HERG (Wainger *et al*., 2001; Cui *et al*., 2001). Thus, unlike several other LQT2 mutations, the CNBD mutants showed no dominant-negative suppression of wild-type current. These findings provide new insight into the structure-function relationships of the HERG C-terminus. But the phenotype-genotype relationships of the mutations in C-terminal HERG are not fully understood.

Our clinical data revealed the novel CNBD mutation results in a mildly affected phenotype in the Chinese LQTS family. Only the proband has a history of syncopes, no more than one time every year, while the other three affected individuals have no clinical symptoms. Although genotypical mutations were found in the proband's son and niece, they display normal QT interval and clinical manifestations. The four affected individuals and the two silent mutation carriers are all heterozygous for the mutation. No family members are believed to have died as a result of LQTS. These data confirm that the mutation in CNBD of HERG may be less malignant than mutations occurring in the pore region and have dominant-negative suppression of wild-type current, which is consistent with previous reports of other C-terminal mutations. We believe that these findings might provide useful information for investigating the clinical manifestations properties of the HERG CNBD mutants. On the other hand, Priori *et al*.(1996b) reported that LQTS may appear with very low penetrance in some families, and that the family members considered to be normal may be silent gene carriers and are unexpectedly at risk of developing torsades de pointes when they are exposed to repolarization-prolonging drugs. Thus, although the cases in this LQTS family show mild or even normal phenotypes at present, we assume each of them has a potential risk, which makes strict follow-up mandatory.

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