Original article

Contribution of a KCNH2 variant in genotyped long QT syndrome: Romano–Ward syndrome under double mutations and acquired long QT syndrome under heterozygote

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ABSTRACT

Background: Long QT syndrome (LQTS) presents two clinical phenotypes, congenital and acquired forms. This study aims to evaluate the genetic contribution of a KCNH2 variant for the two LQTS phenotypes.

Methods: From 1996 to 2014, genetic screening for LQTS probands was performed for five major genes: KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2, and 389 probands were found to be mutation carriers. We analyzed the clinical phenotypes of p.His492Tyr carriers in KCNH2.

Results: Heterozygous p.His492Tyr variant was identified in 10 LQTS families. Six probands (mean age, 26 ± 23 years) carried another mutation, and two of six had syncope associated with emotional stress or telephone ringing. The remaining four probands were significantly older at diagnosis (mean age, 42 ± 33 years) and carried no other compound mutations. All the four probands had fatal arrhythmic events in the presence of additional precipitating factors such as culprit drugs in 2, hypokalemia in 1, and bradycardia in 1. The QTc interval of carriers with p.His492Tyr alone was 445 ± 10 ms and significantly shorter than that in double mutation carriers (481 ± 40 ms, p = 0.041).

Conclusions: KCNH2 p.His492Tyr variant presented Romano–Ward syndrome in the presence of another mutation and heterozygous carriers had mild phenotypes while even heterozygous carriers should be cared for not to encounter secondary factors because incidental factors could manifest “latent” form of p.His492Tyr heterozygous carriers.

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Introduction

Long QT syndrome (LQTS) is an inherited arrhythmic disease with prolongation of QT interval on the electrocardiogram and fatal cardiac arrhythmia, called torsade de pointes (TdP) [1,2]. The typical LQTS is a congenital form with TdP of the onset induced by autonomic activity, e.g. exercise in LQTS type 1 (LQT1) or emotion and unexpected noise in LQTS type 2 (LQT2) [3]. To date, a variety of mutations were reported in 15 candidate genes responsible for congenital LQTS [2]. On the other hand, another form of LQTS, acquired LQTS, is caused by secondary factors, e.g. hypokalemia [4], drugs [5,6], or bradycardia [7]. Although acquired LQTS presents no or mild phenotypes with normal to borderline QT prolongation in the absence of secondary factors, this form may carry a mutation underlying the LQTS-related candidate genes (silent mutation carriers) [5,8–13].
In this study, we demonstrate 10 LQTS families showing unique phenotypes with a missense KCNH2 variant, p.His492Tyr. Previously, we reported a sporadic case of drug-induced LQTS, in whom we identified a heterozygous p.His492Tyr KCNH2 variant [5]. In this study, we demonstrate that carriers of the heterozygous variant alone showed a forme-fruste or acquired LQTS but symptomatic Romano–Ward syndrome in combination with the presence of another LQTS–related gene mutation [14]. We should however take special care of heterozygous carriers to avoid secondary factors, because incidental triggers could unmask them and precipitate to malignant ventricular arrhythmias. The genetic screening for this frequent variant in the family members is also of clinical importance for the prevention of fatal cardiac events.

Methods

Study population

From 1996 to 2014, 918 LQTS probands had genetic analysis in 3 centers (Shiga University of Medical Science, Kyoto University Graduate School of Medicine, and Kanazawa University Graduate School of Medical Science). Clinical information included gender, age, cardiac events such as syncope, TdP and cardiac arrest prior to β blocker therapy, family history of sudden cardiac death and LQTS, age at onset of first cardiac symptoms, and electrocardiographic (ECG) findings. Clinical categories of patients, i.e. congenital LQTS or acquired LQTS, were determined according to the phenotypes before genetic testing. Acquired LQTS was defined as those who had cardiac events (documented TdP, syncope, or cardiac arrest) in the presence of secondary triggers (drugs, bradycardia, or hypokalemia) and who had not been diagnosed as having “congenital long QT syndrome” before events.

The QT interval was manually measured as the time period between QRS onset (Q) and the point at which the isoelectric line intersected a tangent line drawn at the maximal downslope of the positive T wave or the maximal upslope of the negative T wave (Tend). Data were basically obtained from the V5 lead in the 12-lead ECG during stable sinus rhythm and corrected by Bazett’s formula [15]. In acquired LQTS, we calculated QTc interval in the absence of secondary factors.

Genetic analysis

The protocol for genetic analysis was approved by and performed under the guidelines of the Institutional Ethics Committee at each institute. Written informed consent to participate in the study including the collection and use of DNA samples for genetic analysis was obtained in each center. Genomic DNA was isolated from peripheral white blood cells using conventional methods. Genetic screening was performed for KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 corresponding to LQT1, LQT2, LQT3, LQT5, and LQT6 genes, using polymerase chain reaction/single-strand conformational polymorphism by denaturing high-performance liquid chromatography (PCR-DHPLC, WAVE system, Transgenomic Inc., Omaha, NE, USA) analysis. For the abnormal DHPLC patterns, we determined the DNA sequences on both strands with an automated sequencer (PRISM 3130 Sequencer, Perkin Elmer, Waltham, MA, USA). When mutations were detected, we checked the minor allele frequency (MAF) of the variants in three databases including Japanese, ExAC Browser (http://exac.broadinstitute.org/), 1000 Genome Project (http://browser.1000genomes.org/index.html), HGVB (http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html) and we considered variants in which MAF were <0.005 as mutations.

In silico prediction and pathogenicity

In order to examine the pathogenicity of missense variants, we checked with multiple and public prediction tools. When these showed bidirectional results, the variant was considered as “controversial”. Null variants, e.g. nonsense, frameshift, were not studied with the prediction tools because these types should have the pathogenicity [16]. The splice variants of the donor site at the 5’ end or the acceptor site at the 3’ end were respectively considered +1 and +2 or –1 and –2 as pathogenic sites [17] except IVS7 +3 previously reported [18].

Statistical analysis

The data were expressed as mean ± SD, and the statistical comparisons were made using the unpaired Student t test.

Results

p.His492Tyr in heterozygous manner vs. in the presence of another mutation

A total of 389 probands (170 LQT1, 160 LQT2, 25 LQT3, 3 LQT5, and 31 double mutations in 1 acquired LQTS and 30 congenital LQTS) were found to carry mutations in either of the above-mentioned five LQTS-related genes, and 10 of them (3%) were heterozygous for a C-to-T transition in KCNH2 leading to an amino acid substitution of tyrosine for histidine at codon 492 (p.His492Tyr). Four of the 10 probands were heterozygous carriers with p.His492Tyr alone and the remaining six carried another mutation. Table 1 lists all the variants found in these probands: 2 KCNQ1, 2 KCNH2, and 2 SCN5A mutations.

Among 389 genotyped probands, there were 355 congenital and 34 acquired LQTS patients according to the above-mentioned definition. Fig. 1 depicts frequency percentages of p.His492Tyr as bar graphs in our cohort of acquired LQTS (n = 34) and two separate groups of congenital LQTS groups: one with single and another with compound mutations (n = 325 and 30, respectively). To our interest, the p.His492Tyr variant was not detected in 325 congenital long QT syndrome with single mutation, but in 6 of 30 congenital LQTS probands with double mutations (19%) and in 4 of 34 acquired LQTS probands (12%).

Frequency in each control and pathogenicity in silico predictions

The p.His492Tyr [5] was not detected in 1000 genome project while the MAF was 0.000008 (1 of 121378 alleles) in the Exome Aggregation Consortium (ExAC) and 0.0013 (1 of 734 alleles) in the Human Genetic Variation Browser (HGVB). Another 6 mutations (Table 1) were not found in the HGVB, and the frequency of each variant was <0.005 in the 1000 genome project and ExAC. Therefore, all the variants were considered as mutations.

By using in silico tools we examined the pathogenicity of these mutations except for two mutations leading to the premature stop codon, p.Glu126Glyfs*144 and p.Arg518Ter [19–21] in KCNH2 in silico tools (Table 1). Three mutations, p.Gly269Ser [22] in KCNQ1, p.Cys441Trp in KCNH2, and p.Arg975Trp in SCN5A, were regarded as pathogenic while p.Gly1935Ser [23] in SCN5A was benign. Overall not only p.His492Tyr but also the second 5 mutations including 2 non-missense were considered as pathogenic while p.Gly1935Ser in SCN5A was controversial.

Clinical characteristics of patients with p.His492Tyr variant

Table 2 summarizes clinical characteristics of 10 probands and their 4 family member from 10 p.His492Tyr families. Four
Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation Type</th>
<th>Location</th>
<th>Minor allele change</th>
<th>Frequency in normal controls</th>
<th>Minor allele frequency in normal controls</th>
<th>In silico prediction</th>
<th>Functional analysis</th>
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<td>NA</td>
<td>Neutral</td>
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</tbody>
</table>

Fig. 1. Frequency of p.His492Tyr in acquired LQTS with the mutation, congenital LQTS with double or single mutations and Japanese normal controls in Human Genetic Variation Browser. The bars show the percentage of these variant carriers in each group. aLQTS, acquired long QT syndrome; cLQTS, congenital long QT syndrome.

probands (mean age, 42 ± 33 years) and 2 family members with heterozygous p.His492Tyr alone showed borderline QTc prolongation of 445 ± 10 ms, which was significantly shorter than that in the group with double mutations (481 ± 40 ms, p = 0.041, Fig. 2). However, these 4 probands experienced fatal arrhythmias after exposure to secondary factors such as culprit drugs in 2, hypokalemia in 1, and sick sinus syndrome in 1.

Fig. 3 shows sets of representative ECGs recorded from 3 cases with a heterozygous p.His492Tyr variant who showed acquired LQTS and TdP. ECGs in Panel A were from a 52-year-old woman with drug-induced LQTS (Table 2, family #1, proband). We previously reported this patient as one of 20 with drug-induced LQTS [5]. Although TdP appeared with QT prolongation of 585 ms during taking dysopyramide, QT interval shortened to 447 ms after withdrawing the culprit drug. Panel B of Fig. 2 shows a case of hypokalemia-induced LQTS (Table 2, family #2, proband). An 84-year-old woman had syncope under the serum potassium of 2.5 mEq/mL. QTc interval prolonged to 618 ms with repetitive TdP. The intake of potassium shortened her QTc interval to 444 ms. Panel C is a case with bradycardia-induced LQTS (Table 2, family #3, proband). A 22-year-old woman had syncope at night, and TdP frequently appeared on emergency admission. ECG showed sinus bradycardia and severe QT prolongation. Ventricular pacing at 70 bpm suppressed her TdP, and finally she received an implantable cardioverter defibrillator. Regular atrial pacing at 60 bpm shortened her QTc interval to 444 ms. Her 53-year-old father and 25-year-old brother were also carriers of His492Tyr, but remained asymptomatic. Their QTc intervals were 440 and 431 ms, respectively.

In contrast, six probands (mean age, 26 ± 23 years) and 2 family members carried additional LQTS-related mutations and carriers except family #10 showed significantly prolonged QTc interval (Fig. 4). Three probands (50%) had syncope, and 2 of them were triggered by emotional stress or telephone ringing. To consider the contribution of p.His492Tyr among carriers with p.Arg518Ter or p.Gly269Ser, we compared QTc intervals of carriers with p.His492Tyr together to single mutation carriers.

**Discussion**

The present study shows clinical and genetic aspects of p.His492Tyr carriers and demonstrated two major findings: (1) KCNH2 p.His492Tyr variant presented symptomatic Romano-Ward syndrome in the presence of another LQTS-related gene
Table 2
Clinical and genetic characteristics of subjects with p.His492Tyr variant.

<table>
<thead>
<tr>
<th>Family number</th>
<th>Age (years)</th>
<th>Gender (M/F)</th>
<th>Proband/family member</th>
<th>2nd mutation</th>
<th>Symptoms</th>
<th>Triggers</th>
<th>QTc (ms)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Gene</td>
<td>Mutation</td>
<td>Nucleotide changes</td>
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<tr>
<td>1</td>
<td>52</td>
<td>F</td>
<td>Proband</td>
<td>–</td>
<td>TdP</td>
<td>Disopyramide</td>
<td>447</td>
</tr>
<tr>
<td>2</td>
<td>84</td>
<td>F</td>
<td>Proband</td>
<td>–</td>
<td>TdP</td>
<td>hypokalemia, 2.5 mEq/L</td>
<td>444</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>F</td>
<td>Proband</td>
<td>–</td>
<td>TdP</td>
<td>SSS; 30 bpm</td>
<td>444</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>M</td>
<td>Family member</td>
<td>–</td>
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<td>–</td>
<td>440</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>M</td>
<td>Family member</td>
<td>–</td>
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<td>–</td>
<td>431</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
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<td>p.His492Tyr</td>
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<td>–</td>
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<tr>
<td>12</td>
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<td>Family member</td>
<td>p.Arg975Trp</td>
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<td>–</td>
<td>420</td>
</tr>
</tbody>
</table>

M, male; F, female; SSS, sick sinus syndrome; TdP, torsade de pointes.

mutation, in contrast, (2) heterozygous carriers had mild QT prolongation while additional triggers can be “latent” form of p.His492Tyr.

LQTS is a major cause of inherited arrhythmogenic syndromes, characterized by the prolongation of QT interval associated with fatal ventricular arrhythmias [24]. Fifteen candidate genes have been reported to cause this syndrome, and 3 genes, KCNQ1, KCNH2, and SCN5A, are the most common among these 15 LQTS-related genes [1,2]. KCNH2 gene corresponding to LQT2 is one of the major genes and encodes the cardiac rapidly activating delayed rectifier potassium channel, IC50, which plays an important role in cardiac repolarization. Electrophysiological studies have revealed that KCNH2 mutations with loss-of-function type could prolong action potential durations in rabbits [25] or human-induced pluripotency stem cells [26]. The common trigger for LQT2 is emotional stress [27] and adrenergic stimulation could modify this current [28]. IC50 current is also important for drug discovery/development because most drugs could trap to the pore formation of IC50 channel and block IC50 [29]. Some drugs were stopped during development or discontinued after the sale for the excess blocking effect of IC50.

The phenotypic severity of LQT2 depends on the severity of each mutation according to the location of KCNH2 mutations. Shimizu et al. have reported that patients with mutations in the S5–pore–S6 should be associated with severe phenotypes [30]. p.His492Tyr is located in the inner linker between the segments 2 and 3 of the transmembrane, and they reported that mutations in this region are associated with benign phenotype. The electrophysiological data were also consistent with the finding and showed non-dominant negative function [5], and thus p.His492Tyr could be a “latent” LQTS form. The accidental abuse of secondary factors manifests the “latent” LQTS form [31]. The baseline QTc interval of LQTS patients with this variant alone is around 440 ms. But we always need to take care of “mild” phenotypes in the “latent” LQTS form because various secondary factors can be triggers for the onset of acquired LQTS.

In the present study, six probands with p.His492Tyr plus another mutation showed a congenital form of LQTS. In general, LQTS patients with double or compound mutations present more severe symptoms [21] or earlier cardiac events [32,33] than those with single mutations. Indeed, the QT intervals of patients with double mutations were significantly longer than those with p.His492Tyr alone. Thus p.His492Tyr carriers with compound mutation could result in congenital LQTS form [14].

In conclusion, heterozygous KCNH2 p.His492Tyr variant showed Romano-Ward syndrome in the presence of another mutation and those with heterozygous p.His492Tyr variant alone had milder phenotypes while even heterozygous carriers should be cared for not to have secondary factors because incidental factors could manifest “latent” form of p.His492Tyr heterozygous carriers.

Limitations

We were unable to conclude whether SCN5A p.Gly1935Ser would be a pathogenic mutation. Most in silico data revealed this mutation as benign type while the consensus report from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology have not recommended that these predictions be used as the sole source of evidence to make a clinical assertion [16]. The previous electrophysiological study [23] revealed that this mutation had the functional change with loss of function but not tested the sustained current to lead QT prolongation. The contribution of p.His492Tyr variant to LQTS may be considered only in specific countries, particularly in Japan, because this variant was seldom seen in normal or LQTS cohorts based in Europe [13,34] and the USA [35]. We need to take account of the underlying genetics in each country [36].
**Fig. 3.** Electrocardiograms from representative acquired long QT syndrome cases carrying a heterozygous p.His492Tyr variant. All showed torsade de pointes (TdP) in (A) bradycardia-induced, (B) hypokalemia-induced, and (C) drug-induced conditions. Their QT intervals were shortened on remaining mild QT prolongation after atrial pacing, taking potassium, and withdrawing culprit drug, respectively.

**Fig. 4.** Electrocardiograms of lead II in p.His492Tyr carriers with other mutations.

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