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Heterozygous mutation in the pore of potassium channel gene *KvLQT1* causes an apparently normal phenotype in long QT syndrome

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Mutations in *KvLQT1*, a gene encoding a potassium channel, cause both the recessive Jervell and Lange-Nielsen (JLN) syndrome and the dominant Romano-Ward (RW) syndrome. These diseases are characterised by a prolonged QT interval on the ECG, syncope and sudden death due to cardiac arrhythmias. The JLN syndrome is also associated with a congenital bilateral deafness. We report here a novel missense mutation, W305S, in the pore region of *KvLQT1* identified by PCR-SSCP analysis in two consanguineous JLN families. In contrast to several missense mutations found in the same region of *KvLQT1* in RW patients which are associated with severe cardiac phenotypes, the W305S mutation is responsible for an apparently normal phenotype in heterozygous JLN carriers.

Keywords: Jervell and Lange-Nielsen syndrome; *KvLQT1*; potassium channel; long QT syndrome; QTc interval

Introduction

Mutations in *KvLQT1* cause the autosomal recessive form of the long QT syndrome, the Jervell and Lange-Nielsen (JLN) syndrome.^{1,2} This disease is characterised

by a congenital bilateral deafness, a prolonged QTc interval on the resting ECG, syncopal attacks due to ventricular tachyarrhythmias and a high risk of sudden death.^{3,4} *KvLQT1* encodes a potassium channel that produces in association with IsK the I_{Ks} cardiac current involved in ventricular repolarization,⁵ and plays an important role in the control of endolymph homeostasis in the inner ear.^{1,6} This gene is also responsible for the LQT1 type of Romano-Ward (RW) syndrome,⁷ the dominant form of the long QT syndrome without deafness. Two JLN mutations have been reported so

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far, an insertion-deletion in the C-terminal domain of the protein and a 1-bp insertion, both leading to putative truncated proteins.^{1,2}

We evidenced a novel missense mutation in two consanguineous JLN families. It is the first mutation in the pore region of *KvLQT1* which is the cause of an apparently normal phenotype at the heterozygous state, but a life-threatening JLN syndrome at the homozygous state.

Subjects and Methods

Subjects

Clinical evaluation and blood samples from two consanguineous families (JLN-1 and JLN-5) were collected after written informed consent. All subjects underwent clinical and cardiovascular examination, including a 12-lead ECG. QT intervals were measured in lead II and corrected for heart rate (QTc) by the Bazett formula. Subjects were considered as affected in cases of a QTc > 460 ms and syncopes associated with a congenital bilateral deafness.

Family 1 (JLN-1) has already been described.¹ The pedigree of family 5 (JLN-5) is shown in Figure 1. Two of the three children were affected. Subject II-1 was deaf-mute and had his first syncope at the age of 6 years. Subject II-2 was diagnosed at birth and was immediately treated by beta-blocking agents. He never experienced any syncopal episode.

Clinical data of JLN individuals was compared to a group of RW patients with mutations in the pore region of *KvLQT1* and to a control group which consisted of non-affected subjects of family JLN-1 and RW families with mutations in the pore region of *KvLQT1*.

Genotyping

DNA was prepared from 10 ml of blood using standard protocols (Applied 341 Biosystem Nucleic Acid Purification). (CA)_n microsatellite markers were provided by GENE-THON human genetic linkage maps⁸ and genotyping was performed as described.¹

SSCP Analysis and Direct Sequencing of PCR Products

Primers designed by Wang *et al*⁷ were used to amplify the *KvLQT1* region between S2 and S6 transmembrane segments from genomic DNA. LIS-SSCP analysis and sequencing of the PCR products were performed as described.^{1,9}

Statistical Analysis

Results are expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was used to compare mean QTc intervals between the four following groups: controls, heterozygous JLN subjects, RW patients and homozygous JLN patients. *Post hoc* pairwise *t* test comparisons were therefore performed to identify which group differences account for the significant overall ANOVA. A *P*-value less than 0.05 was considered as significant for the ANOVA. The significance level was adjusted for the number of comparisons using the Bonferroni correction. Pairwise comparisons were

then considered not significant unless the corresponding *P*-values were less than 0.008.

Results

As the parents were first degree cousins, linkage of the disease to chromosome 11p15.5 markers was established by homozygosity mapping. All affected children were homozygous for the markers linked to the *KvLQT1* locus (Figure 1a).

In a first attempt to find a mutation in the S2 to S6 transmembrane domains of *KvLQT1* in family JLN-1, we used standard SSCP conditions but failed to detect any abnormal conformer.¹ The use of the LIS solution to generate stable single-stranded DNA⁹ allowed us to detect abnormal conformers corresponding to the heterozygous and the homozygous disease-linked haplotypes in families JLN-1 and JLN-5 in the fragment corresponding to the primer-set 7L-8R (Figure 1a). Direct sequencing of the two strands demonstrated a homozygous G-to-C transition at position 305 in the three affected children (Figure 1b). This missense mutation induced a change of a tryptophan (W305S) in a sequence motif which is conserved throughout evolution. Since no restriction site changes were induced by the mutation, SSCP was used to screen 100 healthy individuals for whom the W305S mutation was not found.

In addition, members of families JLN-1 and JLN-5 showed a C-to-T DNA sequence variation in intron 2 (at cDNA position 605-24) which was not detected in 100 individuals and is carried by the affected chromosome. Both the JLN-1 and JLN-5 families originate from Morocco and display the same mutation and the same rare variant in *KvLQT1* which suggests a founder effect. Nevertheless, we could not evidence common microsatellite alleles linked to the mutation.

Table 1 shows symptoms and mean QTc intervals of JLN and RW^{10,11} patients with mutations in the pore region of *KvLQT1*. A highly significant difference between the mean QTc intervals of control, heterozygous JLN, homozygous JLN and RW individuals was evidenced by the ANOVA ($F_{(3;82)} = 67.6$, $P < 0.0001$). The pairwise *t* test comparisons (Table 1) showed that the mean QTc interval of homozygous JLN patients was higher than those of RW patients ($P = 0.001$), heterozygous JLN subjects ($P < 0.0001$) and controls ($P < 0.0001$). RW patients displayed higher QTc values than heterozygous JLN subjects and controls (P values < 0.0001). There was no significant difference between

Figure 1 Cosegregation of the W305S mutation with the disease-linked haplotypes in families JLN-1 and JLN-5. In Panel A, affected individuals are represented by shaded symbols and unaffected individuals by unshaded symbols. Haplotypes of chromosome 11p15.5 microsatellites are shown below pedigrees. The SSCP analysis of PCR-products on 10% polyacrylamide gel shows normal patterns for the two control individuals (C) and the non-affected child of family JLN-1 and two different patterns for homozygous and heterozygous carriers of the mutation. Panel B shows sequencing results from a non-affected, an affected and a heterozygous individual. Numbering of nucleotides is given according to Yang *et al* [¹³]

Table 1 Mean QTc intervals and symptoms of JLN and RW patients with mutations in the pore region of *KvLQT1*

	KvLQT1 mutations	QTc interval (ms)	QTc interval range	Individuals (n)	Gender	Symptoms	First report
Non affected subjects	None	395±27 ^{b,c}	335–437	44	23M/21F	None	This study
Heterozygous JLN subjects	W305S	420±31 ^{b,c}	375–458	7	3M/4F	None	This study
RW patients	T309R, G314S Y315S, P320A	482±31 ^a	429–569	30	16M/14F	16 syncope ^d	Russell <i>et al</i> ¹⁰ Donger <i>et al</i> ¹¹
Homozygous JLN patients	W305S	532±52	480–610	5	2M/3F	3 syncope 1 sudden death	This study

Data are expressed as mean±standard deviation.

^a, ^b $P=0.001$ and $P<0.0001$ respectively when compared with homozygous JLN patients.

^c $P<0.0001$ when compared with RW patients.

^dWe had no information concerning the symptoms of the three RW patients in the Russell *et al* study¹⁰. Moreover, the 30 RW patients of this table do not include 7 individuals who died suddenly without knowledge of their ECG¹¹. Additional RW mutations have been reported in the pore of *KvLQT1* without knowledge of the corresponding QTc values: G306R, T312I and I313M^{7, 14}.

QTc intervals of heterozygous JLN individuals and controls ($P = 0.05$).

Discussion

This study reports the first missense mutation in the highly conserved pore region of *KvLQT1* in the Jervell and Lange-Nielsen syndrome. A mutation in the pore was unexpected in our population of JLN patients. Indeed, several mutations in this region of *KvLQT1* have been found in RW patients with severe clinical presentation, such as exercise-induced syncope, sudden death and major QTc prolongation.^{7,10,11} As shown in Table 1, the mean QTc value of RW patients was higher than 470 ms. In contrast, only two of the seven heterozygous JLN carriers (two females) displayed a QTc value higher than 440 ms and none of them were symptomatic.

The effects of the W305S mutation have been studied by producing and expressing the mutated protein in COS cells in the presence of IsK.¹² The mutated subunit was totally inactive and no K⁺ current could be obtained. When wild type and mutated subunits were coexpressed in a 1:1 ratio to reproduce the situation occurring in a heterozygous cardiac cell, the mutated subunit behaved as a weak dominant-negative subunit and partially inhibited the expression of the wild type channel subunit. This dominant-negative effect was clearly less pronounced than dominant-negative effects of RW mutations found in the same region of the channel (G314S and Y315S).¹² This is in agreement with the clinical observation that heterozygous JLN carriers have QTc intervals either in the normal range or slightly increased and are asymptomatic (Table 1). We

can hypothesise that the W305S mutation produces subunits which associate only weakly with wild type subunits or which inhibit wild type subunits only when two or three mutated subunits are present in a tetramer. This could explain the weak dominant-negative effect of this JLN mutation when compared to RW mutations located in the pore region of *KvLQT1*.

In conclusion, this study describes the first missense mutation affecting a conserved aromatic amino acid of the pore of the K⁺ channel *KvLQT1* which produces an apparently normal phenotype in heterozygous carriers, but a life-threatening disease associated with deafness in homozygous carriers. Nevertheless, the JLN heterozygous carriers may risk drug-induced arrhythmias and have to be clinically followed up.

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