Novel mutations in KvLQT1 that affect $I_{Ks}$ activation through interactions with Isk

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Abstract

Objectives: We report the functional expression of four KCNQ1 mutations affecting arginine residues and resulting in Romano–Ward (RW) and the Jervell and Lange–Nielsen (JLN) congenital long QT syndromes. Results: The R539W and R190Q mutations were found in typical RW families with an autosomal dominant transmission. The R243H mutation was found in a compound heterozygous JLN patient who presents with deafness and cardiac symptoms. The fourth mutation, R533W, was a new case of recessive form of the RW syndrome since homozygous carriers experienced syncopes but showed no deafness, whereas the heterozygous carriers were asymptomatic. The R190Q mutation failed to produce functional homomeric channels. The R243H, R533W and R539W mutations induced a positive voltage shift of the channel activation but only when co-expressed with IsK, pointing out the critical role of these positively charged residues in the modulation of the gating properties of KvLQT1 by IsK. The positive shift induced by R533W was merely 15%. This small effect was compatible with the recessive character of the RW phenotype transmission. The average QTc was significantly longer ($P<0.01$) in patients carrying mutations inducing a total loss of channel function and those patients were also prone to cardiac adverse symptoms (whether syncopes or sudden death) to a greater extent (62 vs. 21%, $P<0.001$). Conclusions: Novel mutations are described that induce a voltage shift of the channel activation only in the presence of IsK. They appear associated with a milder cardiac phenotype. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Congenital defects; Ion channels; K-channel; Long QT syndrome; Ventricular arrhythmias

1. Introduction

Long QT syndromes (LQTSs) are characterized by an abnormally prolonged ventricular repolarization and a propensity to polymorphic ventricular arrhythmias known as torsades de pointes [1–3]. These arrhythmias may lead to syncopes and sudden death. In their congenital form, LQTSs include two clinically specific syndromes that share similar cardiac abnormalities. The most frequent, called the Romano–Ward (RW) syndrome, is autosomal dominant and with few clinical features but cardiac [4,5]. Conversely, the Jervell and Lange–Nielsen (JLN) syndrome is rare, transmitted as a recessive trait and associates a profound bilateral deafness with the cardiac phenotype [6]. All the genes identified so far that are responsible for congenital LQTSs encode ion channels involved in the control of the cardiac action potential duration. They include the Na$^+$ channel gene SCN5A [7] and four K$^+$ channel genes, HERG [8], KCNE2 [9], KCNQ1 (previously designed KvLQT1 [10]) and KCNE1 [11]. The latter two encode KvLQT1 and IsK proteins, two subunits which recapitulate the slow component, $I_{Ks}$, of the delayed rectifier K$^+$
current, \( I_K \) [12,13]. Interestingly, \( I_K \) current has also been implicated in the \( K^+ \) endolymph secretion within the inner ear [14,15] and mutations in \( KCNQ1 \) and \( KCNE1 \) genes were found to be the cause of both the RW and the JLN syndromes [16–21]. The resulting phenotype depends on the extent of the \( K^+ \) current suppression. A partial loss of current, generally achieved in the heterozygous state, only affects the heart whereas a more severe loss of function, as present in the homozygous state, affects both the heart and inner ear functions [22].

Mutations in the \( KCNQ1 \) gene account for the vast majority of congenital LQTSs. Most are missense mutations that are located in the conserved core domain of the protein and in the cytoplasmic C-terminal domain [23,24]. Functional expression studies of mutated KvLQT1 and IsK proteins have been previously reported [11,23,25–27]. Mutations within the core domain of the KvLQT1 protein seem to cause loss of function and dominant-negative effect is often observed when the mutated proteins are co-expressed with the wild type (WT) protein (to mimic the heterozygous state). However, a P-domain mutation with a very mild functional effect has recently been reported [27]. One C-terminal mutation, R555C, has been associated with a ‘forme fruste’ of LQTS [24], that leads to a functional channel with an altered voltage-dependence of channel opening [23]. We report in this study the functional consequences of three novel missense mutations in \( KCNQ1 \). One of these mutations is located in the S4 segment and the two others in the C-terminal cytoplasmic tail. All three mutations lead to modifications of the gating properties of the \( I_K \) that require the presence of IsK in the channel complex to manifest.

The fact that the identity of the mutated gene in the RW LQTS influences the clinical outcome of the patients has been previously reported [28]. LQT1 patients, affected by mutations in the \( KCNQ1 \) gene, are more prone to adverse cardiac events than those affected by the \( HERG \) (LQT2) or the \( SCN5A \) (LQT3) gene mutations. Significant genotype–phenotype relationships within \( KCNQ1 \) gene can be deduced from this present study. When considering previously expressed \( KCNQ1 \) mutations [23] it appears that mutations leading to a voltage-shift of the current activation instead of a total loss of function are associated with less severe phenotypic cardiac manifestations.

2. Methods

2.1. Subjects

Clinical evaluation and blood samples from five LQTS families were collected after written informed consent in accordance with the guidelines set down by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale du Groupe Hospitalier de la Pitié-Salpêtrière (Paris, France). The investigation conforms with the principles outlined in the Declaration of Helsinki. Family RW10021 originated from Algeria, and the others from France. All subjects underwent clinical and cardiovascular examinations. Probands had at least one syncope and a QTc interval (QT interval measured in lead II and corrected for heart rate by the Bazett formula) longer than 440 ms.

In the RW family 2811, two children (III-3 and III-5) died suddenly while sleeping at the age of 3 and 2 months, respectively, without knowledge of their genotype, possibly due to sudden infant death syndrome. The proband (III-4) experienced an episode of palor, hypotonia and unconsciousness at 17 days of age. She was hospitalized and a prolonged QT interval was evidenced on ECG. She was treated by propranolol but remained symptomatic. A left stelllectomy was performed and the propranolol treatment was switched to nadolol. She is now asymptomatic with a follow-up of 10 years. Other heterozygous carriers were asymptomatic. In the RW Family 10021 (consanguineous parents), patients II-3 and II-5 experienced syncopes since the age of 12 and 5, respectively, and patient II-5 is unconscious since an aborted sudden death while swimming. The rest of the family was completely asymptomatic. In RW family 1654, patient II-2 died suddenly at 16 years while riding a bicycle without knowledge of her genotype. Her sister (II-4) experienced one syncope under physical stress. The patient III-4 died suddenly at 16 years while swimming and her two brothers (III-1 and III-2) experienced syncopes at the age of 10. Other heterozygous carriers were asymptomatic.

In JLN family 11418, the compound heterozygous JLN patient (II-2) is a 44-year-old deaf woman who experienced several syncopes since the age of 2. None of her heterozygous relatives were symptomatic.

2.2. Identification of the mutations

Genomic DNA was extracted from blood samples taken from family members by a standard procedure and all \( KCNQ1 \) exons and of flanking intron sequences were amplified [29]. Single strand conformational polymorphism (SSCP) analysis was performed at two temperatures (7 and 25°C) as previously described [16]. For each abnormal SSCP pattern, the cosegregation with the disease was studied in the family. The PCR products were sequenced by the dideoxynucleotide chain termination method on an ABI-Prism 377 DNA sequencer (Perkin/Elmer/Applied Biosystems). The nucleotide mutations were G679A, G838A, C1708T and C1725T for R190Q, R243H, R533W and R539W, respectively. The positions of the nucleotides are those corresponding to the sequence GenBank AF000571.

2.3. Mutagenesis and electrophysiology in transfected \( COS-7 \) cells

Site directed mutagenesis of human KvLQT1 and IsK cDNA were performed as described [23]. Cells were
transfected using the DEAE–dextran precipitate method with 0.5 μg of pCI–KvLQT1 (or 1 μg in co-transfection WT plus mutant) and 0.5 μg of pCMV–hIsK per dish. Membrane currents were measured at room temperature (22±2°C) by the whole-cell variant of the patch-clamp technique. The internal pipette solution contained (in mmol/l): 150 KCl, 0.5 MgCl₂, 5 EGTA and 10 Hepes±KOH at pH 7.4. After the whole-cell configuration was established, the optimal current response was obtained for a series resistance compensation of about 80%. The current density–voltage relationships were normalized to cell capacitance. The relative activation curves were determined for individual experiments by fitting peak values of tail currents (Iₜₐ𝑖𝑙) vs. test potential (Vₜ) to a Boltzmann function: \( I_{tail} = I_{tail-max} / [1 + \exp \left( \frac{V_{0.5} - V_{i}}{k} \right)] \). The voltage at which the current was half-activated \( V_{0.5} \) and the slope factor \( k \) were calculated from these data and averaged values for these parameters are reported in Table 1.

3. Results

3.1. Identification of KCNQ1 mutations

The availability of the complete KCNQ1 gene sequence [29] led us to identify three novel missense mutations responsible for LQTSs. All three mutations resulted in the change of a positively charged arginine by an uncharged amino acid at physiological pH values.

The R243H mutation, located in the S4 segment (Fig. 2A), was identified in family JLN11418 in a compound heterozygous proband who displayed a typical JLN syndrome. This proband also bore another KCNQ1 mutation, 477+5 (G to A), located in intron 1 which induces the skipping of exon 1 (according to the nomenclature of Lee et al. [30], Itoh et al. [31] and Neyroud et al. [29]), resulting in a frameshift and a premature stop codon at the end of S4 transmembrane domain of KvLQT1. Amplifying and sequencing the cDNA from lymphoblastoid cells of the affected individual has effectively confirmed the skipping of exon 1 (data not shown). This splice mutation was also found at a heterozygous state in four relatives of the affected individual has effectively confirmed the skipping of exon 1 (data not shown). This splice mutation was also found at a heterozygous state in four relatives of

<table>
<thead>
<tr>
<th>KvLQT1 channel type</th>
<th>Current density at +30 mV (pA/pF)</th>
<th>Activation curve</th>
<th>Time constant of deactivation (ms)</th>
<th>( E_{rev} ) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>9.6±0.7 (14)</td>
<td>−23.4±1.1 (10)</td>
<td>10.5±0.3 (10)</td>
<td>626.2±30.2 (7)</td>
</tr>
<tr>
<td>R190Q</td>
<td>− (9)</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>R243H</td>
<td>8.8±0.7 (7)</td>
<td>−21.7±0.7 (7)</td>
<td>10.9±0.4 (7)</td>
<td>680.6±40.9 (7)</td>
</tr>
<tr>
<td>R533W</td>
<td>9.6±1.4 (11)</td>
<td>−21.0±0.5 (7)</td>
<td>11.6±0.6 (7)</td>
<td>177.4±19.4 (7)</td>
</tr>
<tr>
<td>R539W</td>
<td>16.7±1.8 (26)**</td>
<td>−21.1±1.0 (12)</td>
<td>11.3±0.4 (12)</td>
<td>623.2±45.8 (10)</td>
</tr>
<tr>
<td>WT+IsK</td>
<td>40.9±3.2 (16)</td>
<td>21.7±1.3 (10)</td>
<td>22.3±1.0 (10)</td>
<td>666.5±23.3 (9)</td>
</tr>
<tr>
<td>R190Q+IsK</td>
<td>− (8)</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>R243H+IsK</td>
<td>1.4±0.2 (10)***</td>
<td>87.9±2.0 (9)***</td>
<td>16.0±0.8 (9)***</td>
<td>73.1±7.5 (9)***</td>
</tr>
<tr>
<td>R533W+IsK</td>
<td>29.3±3.9 (11)*</td>
<td>35.6±3.9 (9)***</td>
<td>20.1±0.9 (19)</td>
<td>497.3±18.0 (9)***</td>
</tr>
<tr>
<td>R539W+IsK</td>
<td>7.0±1.2 (23)***</td>
<td>55.6±2.7 (14)***</td>
<td>21.9±1.6 (14)</td>
<td>273.0±17.4 (11)***</td>
</tr>
<tr>
<td>WT+D76N</td>
<td>2.3±0.5 (3)***</td>
<td>68.7±1.8 (3)***</td>
<td>17.1±0.6 (3)***</td>
<td>134.5±5.9 (3)***</td>
</tr>
<tr>
<td>WT+WT+IsK</td>
<td>39.3±4.8 (15)</td>
<td>23.6±2.0 (8)</td>
<td>23.8±0.8 (4)</td>
<td>650.6±48.1 (7)</td>
</tr>
<tr>
<td>WT+R190Q+IsK</td>
<td>21.3±3.7 (12)**</td>
<td>26.3±2.4 (9)</td>
<td>23.8±0.5 (9)</td>
<td>717.1±45.2 (9)</td>
</tr>
<tr>
<td>WT+R243H+IsK</td>
<td>19.8±2.0 (13)*</td>
<td>46.1±4.3 (10)***</td>
<td>24.9±0.5 (10)</td>
<td>301.7±26.5 (10)***</td>
</tr>
<tr>
<td>WT+R533W+IsK</td>
<td>24.8±3.5 (9)*</td>
<td>26.2±5.1 (6)</td>
<td>24.9±2.4 (6)</td>
<td>616.4±30.2 (6)</td>
</tr>
<tr>
<td>WT+R539W+IsK</td>
<td>20.9±2.5 (19)**</td>
<td>45.8±4.8 (15)**</td>
<td>23.9±0.6 (15)</td>
<td>443.4±22.3 (15)***</td>
</tr>
</tbody>
</table>

*Data are expressed as means±S.E.M. (number of cells). \( E_{rev} \) corresponds to the reversal potential of the tail current. The line WT + D76N refers to results obtained in the present study of the co-expression of WT KvLQT1 with mutated D76N IsK [11,19]. *P<0.05, **P<0.01 and ***P<0.001 statistically significant differences (Student t-test) compared to WT, WT+IsK and WT+WT+IsK for homomeric associations in the absence and in the presence of IsK and for heteromeric associations in the presence of IsK, respectively.

Table 1: Properties of currents expressing WT and mutant KvLQT1 in the absence and in the presence of IsK and co-expressing WT plus each of mutant KvLQT1 in the presence of IsK.
three children, heterozygous for the R533W mutation, were asymptomatic with normal QTc values (380–431 ms). An audiogram (250–11,000 Hz) of the oldest symptomatic brother (II-3) showed a flat profile for the two ears between 250 and 3000 Hz, and a slight decline for sound frequencies over 3000 Hz especially for the left ear. However, this might have resulted from the loudness of a pop concert he attended the preceding day. The other C-term mutation, R539W, was identified in a RW family (1654, Fig. 1) in which two females died suddenly at the respective ages of 16 and 18.

3.2. Functional expression of mutated KvLQT1 channels

The three missense mutations have been individually introduced by site-directed mutagenesis into the human KvLQT1 cDNA. The mutant cDNAs were transiently transfected into COS cells in the absence and in the presence of the IsK regulatory subunit [12,13]. In the absence of IsK, the three mutations, R243H, R533W and R539W only induced minor changes of KvLQT1 currents (Fig. 2B, Table 1) as compared to wild-type (WT). The current–voltage relationship of the mutated channels was only slightly modified for R243H and R533W, as compared to WT (Fig. 3A). It is noticeable that the R539W mutation induced a significant increase of the current density at all voltages (Figs. 2B and 3A) (which may be attributed to a better efficacy of the cDNA for expression, though). The relative activation curves of all mutated homomeric channels were identical to WT (Fig. 3C).

The co-expression of IsK with the mutated proteins led to important changes in channel properties. The steady state currents recorded at the end of a 4 s depolarizing pulse to +30 mV, were decreased by 97, 28 and 83% of that of WT, respectively for R243H, R533W and R539W (Table 1). A positive shift of the isochronal activation–voltage curves accounted for the observed reduction in the current density (Fig. 3, and Table 1). Hence, in the presence of IsK, the steady state activation curves of mutated channels were shifted to the right, the largest
Fig. 2. WT and mutant human KvLQT1 homomeric channels expressed in transfected COS cells in the absence and in the presence of IsK. (A) Schematic location of LQT-associated mutations in KvLQT1 investigated in this study. (B and C) WT and mutants R190Q, R243H, R533W and R539W KvLQT1 currents in the absence (B) and in the presence (C) of IsK in response to depolarizing voltage steps. K⁺ currents were measured at the end of 4-s pulses applied in 20 mV from −80 mV to +60 mV in the absence of IsK and from −80 mV to +120 mV in the presence of IsK. Tail currents were elicited upon repolarization to −40 mV.

shifts corresponding to the largest current reductions (Fig. 3B and D). The R243H mutation was the most severe, inducing a 66-mV shift with $V_{1/2}$ of activation of 88 mV whereas the $V_{1/2}$ of WT KvLQT1/IsK channels was of 22 mV (Table 1). The mildest shift was observed for the R539W mutation ($V_{1/2}$ of 36 mV). The R539W mutation produced a 34-mV intermediary shift with a $V_{1/2}$ of 56 mV. Independently of the amplitude of the gating properties modifications induced by the different mutations, none of them induced any alteration in the channel ion selectivity, as indicated by the absence of changes in the reversal potential.

As compared to WT, the mutations did not influence the rate of activation of KvLQT1 and KvLQT1/IsK currents (Fig. 2B and C). In contrast, all three mutations accelerated the deactivation kinetics. As shown in Fig. 3F, deactivation time constants were strongly decreased at all voltages between −120 and −40 mV for the R243H and R539W mutations in the presence of IsK. However, this is not the case in the absence of IsK, and little change if any occurs (Fig. 3E and F and Table 1). The R533W mutation behaved differently. The acceleration of the deactivation process was larger in the absence of IsK (Fig. 3E and F and Table 1). The R243H and R539W mutations also induced a slight current inactivation in the absence of IsK, but only for highly depolarizing pulses (Fig. 2B).

In order to provide informative elements concerning the R190Q mutation [24], its effects were studied by functional expression. The R190Q mutation, located in the S2–S3 cytoplasmic loop, was identified in a RW family (RW2811, Fig. 1). This mutation led to a non-functional channel, independently of the presence of IsK (Fig. 2). Whether or not the R190Q mutated channel was addressed to the membrane was not investigated. Upon co-expression with the WT subunits, $I_{Ks}$ decreased by 50% without any significant change of the gating properties (Fig. 4A, Table 1).

The functional expression of the splicing mutation 477 + 5 (G to A) found in family JLN11418 (Fig. 1) has not been performed, as it leads to a truncated protein.

3.3. Co-expression of mutated and wild-type KvLQT1 subunits

In most cases, the RW syndrome is dominant with both WT and mutated alleles coexisting in the same cell. Thus,
Fig. 3. Electrophysiological properties of WT and mutant KvLQT1 homomeric channels in the absence and in the presence of IsK. (A and B) Mean±S.E.M. current density–voltage relationships for WT and mutant R243H, R533W and R539W KvLQT1 channels in the absence (A) and in the presence (B) of IsK (n=7–14 cells in each curve) using the same voltage protocols as in Fig. 2. (C and D) Relative activation curves of WT and mutant R243H, R533W and R539W KvLQT1 channels determined at the peak of the tail current in the absence (C) and in the presence (D) of IsK and fitted to a Boltzmann distribution. Data points are mean±S.E.M. (same symbols as in A and B). The averaged half-point activation values (V_{1/2}), the corresponding slope factors (k) and the number of cells recorded in each case were indicated in Table 1. (E and F) Time constant (τ) of deactivation versus membrane potential for WT and mutant KvLQT1 channels in the absence (E) and in the presence (F) of IsK (same symbols as in A and B) using the voltage protocol shown in the inset. Decay of tail current was fitted with a single exponential. Data points are mean±S.E.M., n=5–7 cells in each case.
co-expression experiments were performed, in which equal amounts of WT and mutated DNAs were transfected together with IsK. A dominant-negative effect that resulted in a decrease of the current density was observed with all mutated subunits (Fig. 4B). The R243H and R539W mutations, inducing a large voltage shift of the activation in the homomeric state, produced a smaller but still significant shift in the heteromeric state (Fig. 4C, and Table 1). The acceleration of the deactivation process upon repolarization was also present in these co-expression experiments (Fig. 4D, Table 1).

3.4. Genotype–phenotype correlation

In our experience, six missense mutations in the core domain, R174C, R190Q (S2–S3), G269D (S4–S5), G314S, Y315S (P-domain), L342F (S6), identified in 52 patients belonging to 12 different families have had their functional consequences evaluated after expression in COS cells (present data and [23]). They all display a dominant-negative effect upon co-expression with the WT subunit. These mutations appear to be associated with a high proportion of symptomatic carriers (32/52, ~62%) and sudden deaths (14/52, ~27%) before the age of 40 (Table 2). Four other missense mutations, R243H (S4), R533W, R539W, R555C (C-term) led to a positive voltage shift of the activation (present data and [23]). In this group, the QTc intervals were shorter by 22 ms ($P<0.01$, $n=95$) and the incidence of cardiac related symptoms were reduced by two third ($P<0.001$, $n=95$), as compared with ‘loss of
Table 2
Phenotypic characterization of patients carrying KCNQ1 mutations leading to known functional consequences upon K⁺ current expression in mammalian cells

<table>
<thead>
<tr>
<th>K⁺ channel activity</th>
<th>KCNQ1 domain</th>
<th>Total carriers (families)</th>
<th>QTc (ms) (mean ± S.D.)</th>
<th>Symptomatic carriers (%)</th>
<th>Asymptomatic carriers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Syncoes before 40 years</td>
<td>Sudden deaths before 40 years</td>
</tr>
<tr>
<td>Non funct.</td>
<td>S2–S3</td>
<td>13 (5)</td>
<td>463 ± 34</td>
<td>23.1</td>
<td>115.4</td>
</tr>
<tr>
<td>Non funct.</td>
<td>S4–S5</td>
<td>8 (1)</td>
<td>473 ± 4</td>
<td>0</td>
<td>62.5</td>
</tr>
<tr>
<td>Non funct.</td>
<td>P-domain</td>
<td>27 (5)</td>
<td>487 ± 34</td>
<td>53.6</td>
<td>25.0</td>
</tr>
<tr>
<td>Non funct.</td>
<td>S6</td>
<td>4 (1)</td>
<td>459 ± 32</td>
<td>20.0</td>
<td>0</td>
</tr>
<tr>
<td>Non funct.</td>
<td>Core</td>
<td>57 (13)</td>
<td>476 ± 34**</td>
<td>38.6***</td>
<td>24.6***</td>
</tr>
<tr>
<td>Volt. shift</td>
<td>S4</td>
<td>2 (1)</td>
<td>451 ± 23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Volt. shift</td>
<td>C-term</td>
<td>55 (5)</td>
<td>458 ± 31</td>
<td>14.5</td>
<td>7.3</td>
</tr>
<tr>
<td>Volt. shift</td>
<td>Positive domain</td>
<td>57 (6)</td>
<td>454 ± 31 (n = 55)</td>
<td>14.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Missense mutations which are referred to are as follow: R174C, R190Q for S2–S3, G269D for S4–S5, G314S, Y315S for the P-domain, L342F for S6, R243H for S4, and R533W, R539W, R555C for C-term. The positive domain refers to a domain including a cluster of positively charged Arg and Lys residues located in the C-terminal tail of KvLQT1 and the last Arg residue of the S4 segment. QTc: QT corrected according to the Bazett formula. There is a highly significant difference in the phenotype of mutation carriers, according to the functional consequences and hence the localization of the mutation within the channel protein. (***P < 0.001; for independent variables, vs. voltage shift).

function’ mutations (Table 2). The moderate effect of the R533W mutation corresponded to a milder phenotype, since patients bearing this mutation are recessive variants of the RW LQTS. They do manifest cardiac symptoms, but with no deafness associated, as would be expected in the homozygous state. This is the second case reported of such a peculiar genotype–phenotype relationship. The first example, described by Priori et al. [27], concerned homozygous carriers of a mutation (A300T) located at the beginning of the P-domain. Like the R533W, this mutation induced a weak disturbance in the channel function. In those conditions the level of current appears sufficient to fulfil the transport of K⁺ ion in the endolymph of the inner ear [15], but not the tuning of the cardiac action potential.

4. Discussion

Three novel missense mutations in KCNQ1 have been identified that all induced a change of a positively charged arginine by an uncharged amino acid. Their functional consequences mainly rely on a voltage shift of the activation process that result in a smaller Ikᵢ. Since most of the previous mutations led to non functional channels, these results enable us to relate phenotypic manifestations to genotypic characteristics in term of voltage shift modification versus loss of function. The preeminent role of IsK in revealing the effects of the mutations as well as their functional similarities despite their different locations bring new insight in the comprehension of the KvLQT1–IsK interactions.

4.1. Clinical symptomatology associated with channel dysfunctions

The milder phenotypic consequences of mutations inducing a voltage shift are noticeable. In addition to the R243H, R533W and R539W presently studied, we have previously characterized the R555C mutation that also led to a voltage shift [23] and was associated with a ‘forme fruste’ phenotype [24]. These four missense mutations were associated with a significantly smaller prolongation of the QT interval and less cardiac symptoms including sudden deaths than ‘full loss of function’ mutations (see Table 2). It seems therefore that the functional consequences of KCNQ1 mutations, in term of either lack of function or voltage shift influence the cardiac condition. Wang et al. [32] has recently pointed out the discrepancy within ‘loss of function mutations’. In our study, ‘voltage shift mutations’ also result in highly variable phenotypes. For example the greatest shift in current–voltage is associated with the R243H mutation whereas the heterozygous carriers of that mutation are asymptomatic with no major QTc prolongation. Conversely, several carriers of the R539W are symptomatic and two of them died suddenly. However, in this group of mutations, R533W clearly has the lowest functional impact and has even been associated with a recessive form of RW LQTS. Epigenetic factors must be taken into account to explain part of the variability within each group. Genetic factors are also likely to play a role. That may be due to the impact of the mutation on the N-terminus-truncated KvLQT1 isoform (isoform 2), endogenously expressed in the human heart, which exerts a strong dominant-negative effect on the full-length
KvLQT1 protein [33,34]. Mohammad-Panah et al. have recently proposed that some mutations can abolish this dominant-negative effect of the isoform 2, thus adding a further step to the complexity of the expression of the $I_{KS}$ current in vivo [35]. Even though a high variability burdens any genotype–phenotype extrapolation within each group, mutations that only modify the gating properties of the channel are clearly associated with a milder phenotype than that inducing a loss of function.

4.2. Functional effects of KCNQ1 mutations inducing a voltage shift

The majority of the KCNQ1 mutations is located in the central core of the protein, whether in the loops S2–S3, S4–S5, the hydrophobic S6-, or in the P-domain. Most of the previously characterized mutations resulted in a total loss of function in the homomeric state and shared a dominant-negative effect upon co-expression with the WT subunit. The R190Q mutation in the S2–S3 loop is no exception. However, the three novel mutations R243H, R533W and R539W all decrease $I_{KS}$ as a result of a voltage shift of the activation, thus inducing only a partial decrease of the current. The fact that these mutations cause LQT syndrome and that the channel dysfunction strictly relies on the presence of IsK further emphasizes the role of this auxiliary subunit in vivo. These functional features are similar to the effects of the IsK D76N mutation that was described in both a dominant RW [11,19] and a recessive JLN [19,20] syndrome. The functional consequences of this latter have been reported by Splawski et al. after expression in Xenopus oocytes [11]. In order to compare IsK D76N with our other results, we expressed it in a mammalian COS cell line and the results (listed in Table 1) are very close to those of the R243H mutation. All these data support the view that, even if the location of the mutations differ, i.e. S4 segment for R243H, the C-terminal tail for R533W and R539W, and the cytoplasmic portion of IsK for D76N, they all involve amino acids that participate in a same functional domain. Such a domain modulates the voltage dependent activation of the KvLQT1–IsK complex, but not that of the KvLQT1 channel on its own. This observation may be an interesting lead for studying the interacting domains between the two proteins. Recently, two LQTSs-associated mutations in the KvLQT1 S4 segment and the S4–S5 loop were reported to induce a positive voltage shift of the activation [36]. Interestingly, one of these mutations, R243C, was similar to the R243H reported in the present study. Homomeric R243C KvLQT1 channels expressed in Xenopus oocytes displayed a +16 mV shift of activation, while co-expression with IsK suppressed the current. However, the voltage range used in this study peaked at +60 mV. One can therefore speculate that the IsK-dependent abolition of the current is due to a major voltage shift of the activation that would bring the activation threshold beyond +60 mV. In any case, the presence of the IsK subunit in the channel complex dramatically increases the deleterious effects of the mutation, as in our study, suggesting an interaction of the S4–S5 loop with IsK.

In conclusion: if the degree of $I_{KS}$ dysfunction poorly predicts the severity of the LQT1 patient phenotype, the functional expression of the mutations can be of help. It appears that mutations inducing a voltage-shift in the activation process less severely affect the patient’s phenotype. The determining role that has been previously ascribed to IsK in the $I_{KS}$ function gets preeminent in revealing KCNQ1 mutations, otherwise silent.

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