Editorial

LQT genotype–phenotype relationships: patients and patches

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See article by Huang et al. [14] (pages 670–680) in this issue.

The electrophysiological basis of the congenital long QT syndrome (LQTS), including both the Romano–Ward (RW) and the Jervell and Lange–Nielsen syndrome (JLN), relates to dysfunctioning ion channels, secondary to genetic aberrancies in their encoding genes (for review see Roden and Spooner [1]). Because these ion channels have different time and voltage characteristics gene-specific elements in clinical presentation have been searched for. Indeed, a gene-differentiating potential has been demonstrated, initially in small series and later confirmed in larger patient populations, for the morphology of ST-T segments [2–4] and for symptoms-related triggers [5–7]. To some extent also the clinical course including prognosis relies on the underlying gene defect [8]. Based on these data and other, more subtle differences [9], correct prediction of the genotype of clinically affected patients is feasible with reasonable accuracy. At present this prediction only refers to the causally related gene, i.e. KCNQ1, KCNH2 (HERG) or SCN5A and not to the site of a mutation within a specific gene. Recent data suggest, however, that, for example in LQT2/KCNH2 specific ST-T segment characteristics exist for the type and localization of mutations [4].

In LQT1 and LQT2 a reduction in respectively the slow and rapid component of the delayed rectifier (i.e. $I_{K_s}$ and $I_{Kr}$) are believed to underlie the prolongation of the action potential and the QT interval. The voltage- and time-dependent characteristics of $I_{K_s}$ are dependent on the interaction of at least two transmembrane proteins, the pore-forming alpha subunit, encoded by KCNQ1 and a regulatory subunit IsK, encoded by KCNE1. Mutations in both genes have been demonstrated to be causally involved in RW and JLN syndrome. Homozygous mutations in either gene cause JLN syndrome and heterozygous mutations RW syndrome, LQT type 1 and 5, respectively [1].

In an autosomal dominant inherited disorder, like RW syndrome, the normal allele will produce normal proteins and the abnormal allele, containing the mutated gene, will produce abnormal proteins. Functional potassium channels are the result of a coassembly of four alpha subunits (and regulatory subunits) in a tetrameric structure. In the presence of both normal and altered subunits, these tetramers may contain both and consequently display altered channel function. The inheritance pattern predicts that in JLN patients only abnormal $I_{K_s}$ alpha subunits are present (or only abnormal KCNQ1 gene products), and that in LQT1 patients and in the parents of JLN patients both normal and abnormal gene products are present. Based on the fact that the same genes are causally involved it is not surprising that JLN patients, homozygous for the aberrant gene products, are generally more seriously affected than heterozygously affected RW patients and that similar clinical features, among which the symptom-related triggers, are present in both syndromes [10]. Reasoning along the same lines would predict a similar phenotype of RW LQT1 patients and of heterozygously affected JLN parents, but clinical observations indicate a significantly milder LQT phenotype, with only marginally prolonged QT-intervals and rarely LQT-related symptoms in the latter category [11–13]. Based on these differences in clinical phenotype one would expect more or less diminished $I_{K_s}$ channel function. But is the step from bed to bench that straightforward?

The contribution of Huang et al. [14] published in the present issue of this journal is one of the attempts to correlate the basic electrophysiological ‘channel phenotype’ to the clinical phenotype. The electrophysiological effects of seven JLN KCNQ1 mutations and one RW mutation are studied and the results are compared to the
available clinical data. cRNA injections of JLN-related K⁺ channel mutants (572–576del, R243H, E261D, 1008delC, R518X, Q530X, R594Q) in Xenopus oocytes in the presence of WT KCNE1 revealed no K⁺ current. This confirms results observed for other JLN mutants (W305S, R243H, 544) [12,15], but is also observed for the majority of RW-related KCNQ1 mutants indicating that the clinical phenotypical differences are not explained by this behaviour.

The presence of both normal and abnormal proteins enables the possibility that mutated subunits alter the function of normal subunits (dominant negative effect). A strong dominant negative effect is expected if one abnormal subunit is sufficient to completely inactivate channel function. In that case only 6.25% (1/16th) of the channels will contain four normally functioning proteins and function normally. An alternative mechanism of reduced K⁺ current is the inability of mutated subunits to assemble with normal gene products, putatively leading to a 50% reduction in channel function. The latter effect is probably pertinent for truncating mutations leading to incomplete proteins (see below).

When wild-type and mutant cRNA are injected in a 1:1 ratio, thus more closely mimicking the clinical condition of a heterozygous carrier, RW mutations generally produce a strong dominant negative effect [13–16]. In contrast, for all but two JLN mutations (R243H, E261D) no dominant negative effect was observed (Table 1) [13,14]. Similar effects were observed for W305S and Δ544 (JLN mutants), when expressed with WT (1:1) in Xenopus oocytes [17] or COS cells [15]. Indeed, no (or less) dominant negative effect of JLN mutations compared to RW mutations might contribute to a milder phenotype.

Studying the function of WT Iₖ, and ‘mutated’ Iₖ, in heterologous cell systems is a complex issue. The functional impact of Iₖ, the regulatory protein, is large, but is usually taken into account by cotransfecting with KCNE1. The expression system is also of importance, seemingly in particular for the effects of R243H, which does induce a small K⁺ current in COS cells [12,13] and no current in oocytes [14]. Further complexity is added by the presence in the human heart of an N-terminus truncated KCNQ1 splice variant (isoform 2) with a strong dominant negative effect on isoform 1 (the full length KCNQ1 protein) [18]. The potential importance of isoform 2 in explaining the difference between heterozygous RW and JLN mutations carriers has recently been highlighted [12]. In contrast to the RW mutations it could be demonstrated that the R243H, W305S and the Δ544 JLN mutations suppressed the dominant negative effect of mutated isoform 2 on WT isoform 1. This leaves the K⁺ current carried by the isoform 1 from the normal allele only subject to the dominant negative effect of isoform 2 from the same allele [12]. In contrast, in RW patients both isoforms 1 and 2 from the mutated allele and isoform 2 from the WT allele would exert a dominant negative effect [12]. Whether this mechanism is pertinent to the JLN mutations studied by Huang et al. remains to be studied but it certainly could contribute to the milder clinical phenotype as also observed in this study for JLN parents and other heterozygous family members [14].

From the evidence discussed above one may conclude that patch data provide a reasonable explanation for the clinical difference between RW patients and JLN parents. Hence, the lack of a dominant negative effect and/or inhibition of the dominant negative effect of isoform 2 will lead to JLN syndrome in homozygous carriers and a mild phenotype in heterozygous carriers (among which their parents). It is likely that mutant proteins which are unable to coassemble with WT proteins will be devoid of a dominant negative effect. Indeed, the far majority of JLN mutations are truncating mutations, unlikely to coassemble, or mutations that directly affect subunit assembly (Table 1) [19]. Experimental evidence for interference with subunit assembly and an inability to coassemble is available for one of them, Δ544 [17,20]. On the other hand, RW-related mutations are almost all missense mutations [21] and exert a dominant negative effect when expressed in heterologous systems.

Table 1
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Protein domain</th>
<th>Amino acid change</th>
<th>Predicted protein</th>
<th>Current (±KCNE1)</th>
<th>DNE</th>
<th>Both JLN+RW and</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del572–576TGGCC</td>
<td>S2–S3 loop</td>
<td>fs codon 191</td>
<td>Truncated</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>[12]</td>
</tr>
<tr>
<td>728G→A</td>
<td>S4</td>
<td>R243H</td>
<td>Full length</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>[13,14]</td>
</tr>
<tr>
<td>783G→C</td>
<td>S4–S5 loop</td>
<td>E261D</td>
<td>Full length</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>[14]</td>
</tr>
<tr>
<td>914G→C</td>
<td>Pore</td>
<td>W305S</td>
<td>Full length</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>[12,15]</td>
</tr>
<tr>
<td>1008 del C</td>
<td>S6</td>
<td>fs codon 337</td>
<td>Truncated</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>[14]</td>
</tr>
<tr>
<td>1552C→T</td>
<td>C-terminus</td>
<td>R518X</td>
<td>Truncated</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>[14]</td>
</tr>
<tr>
<td>1588C→T</td>
<td>C-terminus</td>
<td>Q530 X</td>
<td>Truncated</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>[14]</td>
</tr>
<tr>
<td>1630 del 7/ins 8</td>
<td>C-terminus</td>
<td>fs codon 544</td>
<td>Truncated</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>[12,15,17,20]</td>
</tr>
<tr>
<td>1781G→A</td>
<td>C-terminus</td>
<td>R594Q</td>
<td>Full length</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>[14]</td>
</tr>
</tbody>
</table>

fs, frame shift from codon (numbered); DNE, dominant negative effect.

a Implicated in JLN and RW syndromes.
b Small when expressed in COS cells [12].
c No dominant negative effect in COS cells [12].
cell systems (see above). Yet, similar KCNQ1 mutations are implicated in both RW and JLN syndrome [21], indicating the complexity of the genotype–phenotype relationship. These include the truncating mutations (insG567–568, 572–576, R518X, Q530X) and two mutations in the C-terminal end implicated in subunit assembly (T587M, R594Q). For all these mutations one may assume a 50% reduction in \( I_{\text{k_s}} \) amplitude (based on a lack of coassembly) and thus, for homozygous carriers clearly a JLN phenotype is anticipated. Apparently, heterozygous carriers are also diagnosed as RWS, pointing to the fact that other factors, among which putative modifier genes, contribute to the phenotype.

It is to be concluded that within KCNQ1 LQTS the basic electrophysiological phenotype does not completely predict the clinical phenotype. The general picture that emerges is that mutations without a (strong) dominant effect, i.e. truncating mutations and mutations in the C-terminal end, are associated with a mild clinical phenotype and lead to JLN syndrome when homozygously present. On the other hand, missense mutations exert a strong dominant negative effect and are associated with RW syndrome. Several notable exceptions exist, including the above-mentioned mutations leading to either syndrome and the autosomal recessive RW mutations (A300T, notably associated with mild electrophysiological alterations and no dominant negative effect [17,22]) and for example the JLN E261D mutation, characterized by a strong dominant negative effect [14]. These observations indicate that many steps between bench and bedside are still insufficiently explained and merit studies in which the basic electrophysiology is correlated to the clinical characteristics of affected patients.

References