A Recessive Variant of the Romano-Ward Long-QT Syndrome?

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Background—The congenital long-QT syndrome (LQTS) is a genetically heterogeneous disease characterized by prolonged ventricular repolarization and life-threatening arrhythmias. Mutations of the KVLQT1 gene, a cardiac potassium channel, generate two allelic diseases: the Romano-Ward syndrome, inherited as a dominant trait, and the Jervell and Lange-Nielsen syndrome, inherited as an autosomal recessive trait.

Methods and Results—A consanguineous family with the clinical phenotype of LQTS was screened for mutations in the KVLQT1 gene. Complementary RNAs for injection into Xenopus oocytes were prepared, and currents were recorded with the double microelectrode technique. A homozygous missense mutation, leading to an alanine-to-threonine substitution at the beginning of the pore domain of the KVLQT1 channel, was found in the proband, a 9-year-old boy with normal hearing, a prolonged QT interval, and syncopal episodes during physical exercise. The parents of the proband were heterozygous for the mutation and had a normal QT interval. The functional evaluation of the mutant channel activity showed reduction in total current, a hyperpolarizing shift in activation, and a faster activation rate consistent with a mild mutation likely to require homozygosity to manifest the phenotype.

Conclusions—These findings provide the first evidence for a recessive form of the Romano-Ward long-QT syndrome and indicate that homozygous mutations on KVLQT1 do not invariably produce the Jervell and Lange-Nielsen syndrome. The implications of this observation prompt a reconsideration of the penetrance of different mutations responsible for LQTS and suggest that mild mutations in LQTS genes may be present among the general population and may predispose to drug-induced ventricular arrhythmias. (Circulation. 1998;97:2420-2425.)

Key Words: arrhythmia — genetics — molecular biology — torsade de pointes — death, sudden

The congenital long-QT syndrome (LQTS) is a disease characterized by prolongation of ventricular repolarization and by the occurrence, usually during emotional or physical stress, of life-threatening arrhythmias that lead to sudden death in most of the symptomatic and untreated patients.1-4 Mutations in ion channel genes involved in the control of ventricular repolarization have been shown to cause LQTS.5-7 Since 1975,1 the acronym LQTS has included two variant forms of the disease with a similar cardiac phenotype: the rare Jervell and Lange-Nielsen syndrome, with congenital sensorineural deafness and ventricular repolarization abnormalities, and the more common Romano-Ward syndrome, with only cardiac manifestations. The pattern of inheritance of LQTS has always been regarded as firmly established: autosomal dominant for Romano-Ward syndrome and autosomal recessive for Jervell and Lange-Nielsen syndrome.8 Recently, concordant evidence from two laboratories9,10 demonstrated that LQT1 (the Romano-Ward syndrome form linked to chromosome 11) and Jervell and Lange-Nielsen syndrome are allelic diseases caused by mutations in the KVLQT1 gene. The KVLQT1 gene product coassembles with minK and constitutes the cardiac potassium channel conducting the IKs current, the slow component of the delayed rectifier current, IK.11,12

In 1980, contrary to current views, we hypothesized that LQTS might include patients without prolongation of the QT interval.13 This was proved correct by the evidence that cardiac arrest occurs in 4% of LQTS family members with a normal QT14 and later by the identification of KVLQT1 gene mutation carriers with a normal QT interval.15 We have now hypothesized that the spectrum of the genetic transmission of the disease might be larger than expected and might include mild mutations for the Romano-Ward syndrome that would become manifest only when a “double dose,” the homozygous state, is present. This would point to the possible presence of an extreme degree of incomplete penetrance in LQTS and would also imply the previously
unsuspected existence of a “recessive form” of Romano-Ward syndrome. Should this hypothesis be correct, there would be significant implications for establishing the frequency of LQTS mutation carriers in the general population, which could be higher than generally expected. Also, the existence of heterozygous mild mutations on KVLQT1, which would nonetheless be highly sensitive to any drug that blocks potassium currents, would be relevant to the major clinical problem of drug-induced torsade de pointes and of the acquired LQTS.4 Here, we present the evidence for the presence of a homozygous KVLQT1 mutation in a Romano-Ward syndrome family.

Methods

Mutation Analysis
DNA was extracted from peripheral blood lymphocytes by standard procedures.16 Primer pairs for LQTS5,6 were used to amplify exons of KVLQT1 gene, and [α-32P]dCTP was added to the polymerase chain reaction (PCR) mix to obtain radiolabeled fragments. Single-strand conformational polymorphism (SSCP) analysis was performed on amplified genomic DNA.17 Two to 4 mL of each PCR product was mixed with loading dye (98% formamide, 10 mmol/L EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue) in a final volume of 8 mL. The samples were then denatured for 10 minutes at 95°C, chilled on ice, and loaded on a native 6% acrylamide (62.5:1 acrylamide:bis-acrylamide) gel containing 10% glycerol. The gel was run at room temperature at 35 W for 4 hours. Samples resulting in mobility shifts were directly sequenced or subcloned into pBlueScript vector. Complementary RNAs for injection into Xenopus oocytes were prepared with the mMESSAGE mMACHINE kit (Ambion) using SP6 RNA polymerase after linearization of the plasmids with EcoRI. Each cRNA was dissolved in 0.1 mol/L KCl, and its size was verified and concentration estimated by formaldehyde-agarose gel electrophoresis. All cRNAs were diluted to the final desired concentration in a constant volume of 46 mL before oocyte injection. Currents were recorded at room temperature (≈21°C) 3 to 5 days after the injection. The conventional double-microelectrode technique was applied with an OC-772B Warner Institute amplifier. Electodes were filled with 3 mol/L KCl and had a resistance of 5 to 10 MΩ. The solution used to perfuse the oocytes contained (in mmol/L) N-methyl-D-glucamine 120, KOH 2.5, MgCl2, methanesulfonic acid 120, and HEPES 10 (pH 7.4 with Tris-OH). Data acquisition and analysis were performed with the pClamp suite of programs. Data were filtered at 0.2 kHz and digitized at 0.7 kHz.

Results

Phenotypic Characterization
A 9-year-old boy was referred to our attention in 1981 after a first syncopal episode with loss of urine during physical exercise. He was the offspring of a consanguineous marriage of second-degree cousins (see pedigree in Figure 1). A complete medical evaluation was unremarkable, the only abnormality being a prolongation of the QT interval (QTc 470 ms in lead V2) (Figure 2). Two brothers of the proband (V-1 and V-3) died suddenly at the ages of 3 and 9 years while sleeping and while swimming, respectively; no ECGs were available. A third brother had a normal QT interval (QTc 440 ms) in both at rest (Figure 2) and during sinus tachycardia. The QT shortening during increased heart rate in the affected son and in the two parents was within the limits observed for normal individuals, limits that differ only slightly from those observed among LQT1 patients.19 His mother and father had normal ECGs, negative cardiac histories, and diagnostic scores of 0.5 (<1 point=low probability of LQTS).18 Their QT, s were at the upper limits of normal values (450 and 440 ms) both at rest (Figure 2) and during sinus tachycardia. The QT shortening during increased heart rate in the affected son and in the two parents was within the limits observed for normal individuals, limits that differ only slightly from those observed among LQT1 patients.20,21; moreover, no repolarization abnormality suggestive of LQTS became evident during tachycardia in the heterozygous parents. The proband’s ECG showed the broad-based, tall T waves often encountered in LQT1 patients,22 whereas
the T wave morphologies of both parents were completely normal. The proband has been treated with β-blockers and, 16 years later, remains asymptomatic.

To exclude the possibility of a “forme fruste” of the Jervell and Lange-Nielsen syndrome, an audiogram was performed in the proband (V-4) and the unaffected brother (V-2) to verify a possible hearing difference. The audiogram assessed sound frequencies between 250 and 11,000 Hz. The curves of the proband and his brother were superimposable and bilaterally identical, showing a flat profile between 250 and 3000 Hz; the curve slightly declined for sound frequencies >3000 Hz. The response was considered optimal in both individuals. Thus, the hearing loss phenotype is absent in the proband, who shows the cardiac phenotype of LQTS. These findings are consistent with the identification of the first Romano-Ward variant of LQTS inherited as a recessive trait.

**Molecular Screening**

An abnormal migration pattern was identified in one fragment of the *KVLQT1* gene—encompassing exon. None of the 100 control individuals presented the same SSCP pattern. Sequence analysis revealed a homozygous mutation leading to a single-residue substitution that resulted in an amino acid change for alanine to threonine at position 300 (A300T). This amino acid lies at the beginning of the pore region of the predicted topology of KVLQT1.23 As shown in Figure 4, the A300 is conserved in homologous proteins deriving from such distant species as human, mouse, frog, and nematode6,11,12,24 and is positioned at the beginning of the pore, the most conserved domain. The amino acid change, replacing a nonpolar alanine with a polar hydrophilic threonine, is likely to alter this functionally important part of the molecule. At the same time, this replacement must affect the function of the protein only mildly, because the long-QT phenotype is absent in heterozygous carriers. Both consanguineous parents of the proband are heterozygous for the A300T mutation, whereas the healthy brother inherited the two wild-type alleles (Figure 1).

**Expression of the Mutant Channel cRNA**

After coinjection of wild-type *KVLQT1* and *minK* mRNAs, depolarizing voltage steps elicited slowly activating and deactivating currents typical of the *I Ks* current recorded after coinjection in *Xenopus* oocytes and CHO, SF9, COS,11,12 and HEK293 cells.23 The peak current recorded at 40 mV after coinjection of the mutant A300T-*KVLQT1* was 25% wild type and was significantly greater (P<0.05) than the current elicited in oocytes injected with *minK* alone. The activation of A300T KVLQT1* minK had a tau = 1.29 (0.02 seconds at +40 mV, n=5). The values were faster than the wt-KVLQT1* minK, 1.88 (0.05 seconds at +40 mV; n=5), and resembled the faster rate of activation observed after expression of KVLQT1 alone.11 In addition, the isochronal activation-voltage curves of A300T KVLQT1* minK differed from the wt-KVLQT1* minK in that the midpoint was shifted to more negative potentials by >20 mV (Figure 5). Therefore, these electrophysiological characteristics of the mutated isoform are compatible with the mild effect expected for a recessive mutation.

**Discussion**

The present report provides the first evidence for a Romano-Ward syndrome in which the cardiac phenotype is not manifest in heterozygous individuals, thus suggesting the existence of a recessive form of the Romano-Ward long-QT syndrome. Since its first description26,27 and until now,2 the Romano-Ward syndrome has always been considered an autosomal dominant disease. Our finding of a mutation on the *KVLQT1* gene, which produces the cardiac phenotype of Romano-Ward syndrome only in the homozygous form, is a sharp departure from previous reports.5,28 Indeed, consistent
with dominant diseases, the reported mutations on *KVLQT1* were all capable of producing the LQTS cardiac phenotype in the heterozygous carriers of the mutations. The present finding demonstrates that homozygous mutations on *KV-LQT1* do not invariably produce the Jervell and Lange-Nielsen syndrome. The implications of this observation are relevant for the definition of the variable phenotypes associated with mutations in the *KVLQT1* gene and for reconsidering the penetrance of different mutations responsible for LQTS. Finally, these observations offer new insights on the puzzling and significant problem of drug-induced long-QT syndrome.

### A Romano-Ward Family Without Dominant Inheritance

In this family, the A300T mutation was associated with the cardiac phenotype of LQTS only in the homozygous proband. Both parents are heterozygous carriers of the mutation and had a normal phenotype. The previous description of a family with QT prolongation and only partial hearing loss prompted the performance of an audiogram test on the proband that, being completely normal, ruled out even a mild form of the Jervell and Lange-Nielsen syndrome. The cardiac phenotype characterized by QT prolongation and arrhythmias of both Romano-Ward and Jervell and Lange-Nielsen LQTS is currently considered to be inherited as a dominant trait.

### Mutation-Specific Pattern of Inheritance in Ion Channel Diseases

Molecular diagnosis has revealed an unsuspected level of complexity, proving that penetrance may be variable and that different mutations produce different clinical phenotypes. Indeed, this has already been shown for other diseases, such as the Thomsen’s and Becker’s types of myotonia.

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**Figure 4.** Multiple sequence alignment analysis of KVLQT1 and homologous proteins: hKVLQT1, human; mKVLQT1, mouse; KV-LQT1, rat (GenBank PNU92655); xKVLQT1, Xenopus laevis; ce25b8 and cem60, Caenorhabditis elegans; and HNSPC, a predicted K channel cDNA from a human neuroblastoma cell line. Black line above sequence alignment denotes putative pore domain. Amino acid identities are boxed. Asterisk marks mutated A300.

**Figure 5.** Functional expression in *Xenopus* oocytes of wt-KVLQT1 and A300T. A, Examples of current traces obtained from oocytes injected with wt-KVLQT1 (500 ng/mL) or A300T (500 ng/mL) and minK (100 ng/mL). Currents were elicited by depolarizing pulses from −40 to +70 mV in 10-mV steps, starting from a holding potential of −80 mV; return potential was −60 mV. Dotted lines indicate zero current. Bottom, Mean current amplitudes elicited at +40 mV from a holding potential of −80 mV for wt-KVLQT1 (n = 7) and A300T (n = 9). A300T mutant mean current was significantly different (P < 0.05, Student’s t test) from minK mean current. B, Normalized isochronal activation curves obtained by voltage protocol described in A. Isochronal (t = 2700 ms) activation curves were averaged for wt-KVLQT1 (solid circles; n = 10) and for A300T (open circles; n = 10). Experimental data were fitted with the Maxwell-Boltzmann equation, 1/[1 + exp(V − V1/2)/k], which gave the following V1/2 and slope factor values: V1/2 = 32.8 mV, slope = 14.4 mV for wt-KVLQT1; and V1/2 = 13.9 mV, slope = 19.1 mV for A300T. Values are mean ± SEM.
former dominant and the latter recessive. They both are actually caused by different mutations on the same gene, the skeletal muscle chloride channel CLCN1. A further example is the case of Liddle’s syndrome, a dominant disease caused by mutations in the β-subunit of the epithelial sodium channel SCNN1B that result in a gain of function with increased sodium reabsorption. However, pseudohypoaldosteronism, a recessive disease, is caused by different mutations of the same SCNN1B gene leading to a loss of function of the gene product. All these examples belong to ion channel diseases in which, in remarkable analogy to what we report here for LQTS, different mutations on the same genes determine quite different phenotypes.

The evidence provided here questions the appropriateness of the traditional definition of the two forms of LQTS: Romano-Ward, with cardiac phenotype and dominant pattern of inheritance, and Jervell and Lange-Nielsen, with cardiac and auditory phenotypes associated with a recessive pattern of inheritance. Splawski et al recently proposed that the cardiac phenotype is inherited as a dominant trait and the auditory phenotype, deafness, is inherited as a recessive trait. This phenotypic difference between heterozygous and homozygous carriers of the same mutation is probably due to a different sensitivity of the affected districts: the marginal cells of the stria vascularis producing the endolymph in the inner ear are likely to be less sensitive to partial KvlQ1T inactivation than the cardiac tissue. However, the dominant pattern of inheritance of long-QT syndrome reflects the dominant negative effect of the KVLQT1 mutated subunit onto the hetero-oligomeric complexes formed by KVLQT1 and minK, which produces a loss of function of the complex. Our data indicate that the cardiac phenotype also may become manifest only in homozygous individuals.

From a strictly technical point of view, the appearance of the clinical phenotype in homozygous but not in heterozygous carriers of a mutation fits the classic definition of a “recessive” pattern of inheritance. This concept is further strengthened by the consanguinity of the parents of the proband as observed in the family we describe here. However, the situation here is more complex, and a few additional considerations are in order. The number of gene carriers not presenting the disease phenotype is clearly larger than expected as a consequence of the incomplete penetrance of some of the LQTS mutations. The one described here might represent an extreme case of incomplete penetrance of a dominant disease producing a recessive pattern of inheritance.

Our data do not allow a definitive answer. We favor the interpretation of a recessive variant because of the demonstration of the very modest in vitro electrophysiological consequence of the A300T mutation. However, at present it is not possible to estimate the risk of the proband to transmit the disease to a heterozygous offspring. Even though the two heterozygous individuals with the A300T mutation do not present the LQTS phenotype, we cannot exclude the possibility that a heterozygous individual may show some of the clinical signs of the disease.

We hypothesize that at least some of what are currently considered “sporadic cases” could be affected by a recessive form of Romano-Ward syndrome as compound heterozygotes. This possibility could explain part of the variable penetrance observed in some families.

Functional Expression of the Mutated KVLQT1 Gene

The A300T mutation clearly reduced the $I_{K_s}$ current. Two other point mutations in KVLQT1, which we found to be responsible for cases of dominantly inherited LQTS, were also tested and showed significantly greater reductions in $I_{K_s}$ current (A.M.B., unpublished data, 1997). The present mutation not only expressed a significant hyperpolarizing shift of the activation voltage curve but also had a faster activation, which is likely to attenuate the reduction in outward $K^+$ current at repolarizing potentials. These three characteristics, a less severe reduction in total current, a hyperpolarizing shift in activation, and a faster activation rate, render the A300T a mild mutation and may explain why homozygosity is required for manifestation of the cardiac phenotype.

Implications for the Acquired LQTS

The previously unsuspected evidence that the most frequent type of LQTS, the Romano-Ward syndrome, can depend on mutations that remain silent when present on only one allele suggests that the number of LQTS gene carriers may by far exceed previous considerations. The findings presented here have implications that extend beyond the congenital LQTS and involve genetically transmitted dominant diseases and the acquired long-QT syndrome. One can indeed foresee that the extension of molecular screening will demonstrate that a significant number of apparently normal individuals carry “dormant” mutations that produce a clinical phenotype either when they are present on both alleles or whenever they interact with specific external factors, eg, drugs that prolong repolarization.

It has been suspected that the drug-induced LQTS might have been a “forme fruste” of congenital LQTS, but the molecular tools to test this hypothesis were not available. We recently obtained the first evidence for typical drug-induced torsade de pointes in a 77-year-old woman who was taking cisapride, a prokinetic agent that blocks $I_{K_s}$ and who was found to have a mutation on KVLQT1. These data confirm the concurrent deleterious interaction of a genetically defective repolarization and a potassium channel–blocking agent.

Conclusions

The present report provides the first evidence of the existence of a recessive Romano-Ward syndrome. LQTS caused by KVLQT1 mutations presents substantial phenotypic heterogeneity. It may be associated with a cardiac phenotype inherited as autosomal dominant, with a cardiac phenotype inherited as autosomal recessive, and with a cardioauditory phenotype inherited as a recessive trait. It is now more accurate to define the Romano-Ward syndrome as showing the pure cardiac phenotype of LQTS, independently of its pattern of inheritance, which appears to be a mutation-dependent feature. Furthermore, the reported findings induce us to reconsider the penetrance of different mutations responsible for LQTS and also suggest that mild mutations in LQTS genes may be
present in the general population and may predispose to drug-induced life-threatening ventricular arrhythmias.

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