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Skipping of Exon 1 in the *KCNQ1* Gene Causes Jervell and Lange-Nielsen Syndrome*

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The Jervell and Lange-Nielsen syndrome (JLNS) is a rare autosomal recessive form of the long QT syndrome linked with a profound hearing loss caused by mutations affecting both alleles of either the *KCNQ1* or the *KCNE1* gene. We carried out a mutant screening of the *KCNQ1* and *KCNE1* genes in a clinical diagnosed German family with JLNS. Family members were examined by single strand conformation polymorphism analysis and PCR and amplified products were characterized by DNA sequence analysis. We identified a splice donor mutation of exon 1 in the *KCNQ1* gene (G477 + 1A). Analysis of lymphocyte RNA by RT-PCR revealed that two symptomatic patients, homozygous for the mutant allele, exclusively produce *KCNQ1* transcripts lacking exon 1 leading to a frameshift that introduced a premature termination codon at exon 4. Mutant subunits, functionally characterized in *Xenpous* oocytes, were unable to form homomeric channels but strongly reduced I_{Ks} (slowly activating delayed rectifier potassium current) *in vitro* (mutant isoforms 1 and 2 by 62 and 86%, respectively), a fact supposed to lead to severely affected heterozygous individuals. However, individuals heterozygous for the mutant allele exhibit an asymptomatic cardiac phenotype. Thus, the observed dominant-negative effect of mutant subunits *in vitro* is absent *in vivo* leaving heterozygous individuals unaffected. These data suggest mechanisms that prevent production of truncated KCNQ1 channel subunits in cardiomyocytes of individuals heterozygous for the mutant allele.

The progress of human genetics has been crucial in determining the correlation of ion channel genes and ventricular arrhythmias. Mutations in the potassium channel genes *KCNQ1* (LQT1), *KCNH2* (LQT2), *KCNE1* (LQT5), and *KCNE2* (LQT6) have been linked with different subtypes of the long QT syndrome (1–4). The *KCNQ1* gene encoded pore-forming α-subunit, and the *KCNE1* gene encoded β-subunit coassemble to form the slowly activating delayed rectifier potassium current (I_{Ks}) channel involved in repolarization of the cardiac action potential (5, 6). Mutations in both genes cause two congenital forms of the long QT syndrome, the autosomal-dominant Romano-Ward syndrome (RWS)[4] characterized by prolonged cardiac repolarization, cardiac arrhythmias, and a high risk of sudden death (7) or the JLNS characterized by a congenital bilateral deafness associated with a QT prolongation on the electrocardiogram (ECG), recurrent syncopes, seizures, and sudden death due to ventricular arrhythmias of the torsades de pointes type (8). Unlike the RWS, the JLNS is caused by mutations affecting both alleles of the *KCNQ1* or the *KCNE1* gene (8, 9). The majority of JLNS cases, however, are caused by *KCNQ1* mutations that affect the structure of the subunits by introduction of translation stop codons yielding premature subunits and by missense mutations discussed to prevent assembly of I_{Ks} channels (10–13).

We report a JLNS causing mutation of the *KCNQ1* gene and show that at the border from exon 1 to intron 1 the position of the first intronic nucleotide, nearly always a guanine followed by a thymidine (14), is replaced by adenine (G477 + 1A). This altered splice donor site is associated with a splice error. Skipping of exon 1 caused a frameshift that introduces a premature translational termination codon leading to the expression of truncated KCNQ1 potassium channel subunits. Functional analysis in *Xenopus* oocytes revealed that mutant subunits exert strong dominant-negative effects on I_{Ks} currents supposed to induce JLNS symptoms in heterozygous individuals. These results are really exciting, because a variety of clinical manifestations has been linked to the heterozygous state of mutations at the splice donor site of exon 1 (G477 + 1A; G477 + 5A; G477 + 5C; this work, Refs. 15–19). Due to the fact that most heterozygous individuals lack cardiac deficits, molecular mechanisms might exist (20, 21) that abolish suppression of I_{Ks} currents by mutant KCNQ1 subunits.

EXPERIMENTAL PROCEDURES

Subjects—Our study is based on a three-generation family of German origin comprising two symptomatic individuals affected

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[4] The abbreviations used are: RWS, Romano-Ward syndrome; aa, amino acids; ECG, electrocardiogram; JLNS, Jervell and Lange-Nielsen syndrome; RT, reverse transcribed; SSCP, single strand conformation polymorphism; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

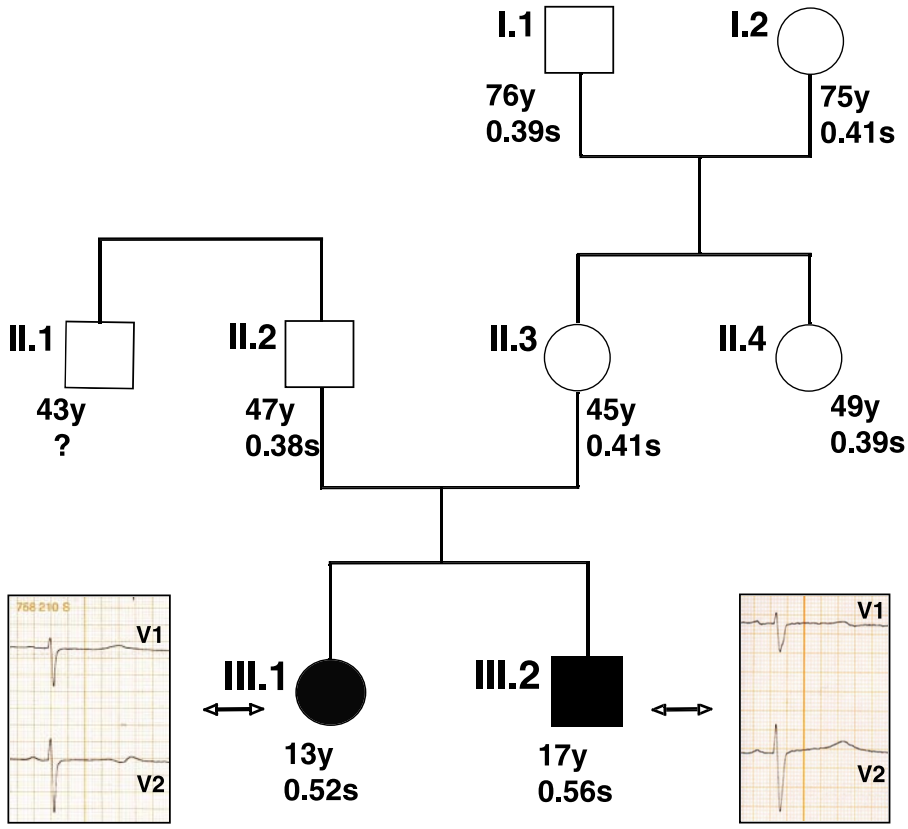


FIGURE 1. **Pedigree of the JLNS family.** The *circles* refer to women, and the *squares* indicate men. Age and QTc values of all but one family member are indicated below symbols. *Closed symbols* denote symptomatic patients. The resting ECGs (leads V1 and V2) of the index patient (III.2) and her sister (III.1) reveal a prolonged QTc interval of 0.56s and 0.52s, respectively. *Open symbols* denote inconspicuous family members. No ECG was available from the index patient's paternal uncle (II.1).

by profound sensorineural deafness and a prolonged QTc interval (Fig. 1). The subjects of this family underwent detailed clinical and cardiovascular examination, including a 12-lead ECG. The QT intervals were measured on the ECG in lead II or V5 and corrected for heart rate by the use of the Bazett formula. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Mutation Analysis—Genomic DNA was extracted from blood samples of the available family members using the genomic DNA extraction kit (Qiagen) in accordance with manufacturer's protocol. The PCR primers (22) W33 (ATGGGAGAGGCCGTGATGCTGAC) and W34 (ATCCAGCCATGCCCTCAGATGC) were used to amplify exon 1 of the human *KCNQ1* gene. The PCR reaction mix contained 50 ng DNA as template, 25 pM concentration of each primer, 200 μM 4-dNTP, 1 units of *Thermus aquaticus* DNA polymerase (Amersham Biosciences), 10 mM Tris-HCl, pH 8.3, at room temperature, 50 mM KCl, and 1.5 mM $MgCl_2$ in a volume of 50 μl. The DNA was denatured by exposing to 95 °C for 5 min and then amplified for 35 cycles in a Hybaid Omnigene Temperature Cycler. Each cycle consisted of a denaturation step at 95 °C for 15 s, an annealing step at 61 °C for 15 s, and an extension step at 72 °C for 45 s. For single strand conformation polymorphism (SSCP) analysis, PCR products were separated on a 5–15% polyacrylamide gradient gel using a Multiphor II electrophoresis system and visualized by silver staining (23).

4RT-PCR of RNA Isolated from Whole Blood Cells—Total RNA (~10 μg) was isolated out of 2.5 ml whole blood (PAXgene kit, Qiagen) and 1 μg was reverse transcribed using Superscript II reverse transcriptase (Invitrogen) and random hexamer primer. The PCR primers KCNQ1–433 (GCCGCGTCTACAACTTCCTC) and LQT201 (24) (ATGGAAATGGGCTTCCGGGCAAAGCGC) were applied to amplify a *KCNQ1*-isoform 1 wild type cDNA fragment of 277bp respectively the mutant isoform 1 derived cDNA fragment of 186bp lacking exon 1. Following denaturation at 95°C for 5 min, 35 PCR cycles were performed on a Temperature Cycler (MWG Biotech) with a 1-min denaturation step at 95 °C, a 1-min annealing step at 58 °C, and a 1-min extension step at 72 °C. Normal and aberrant PCR products were subcloned and characterized by DNA sequence analysis of both strands (23).

Heterologous Gene Expression in Xenopus laevis Oocytes—All protocols for animal use were in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institute of Health (NIH Publication number 85-23, revised 1996) and the European Community guidelines for the use of experimental animals.

cRNA of *KCNE1*, *KCNQ1*-iso1, *KCNQ1*-iso2, and their mutants, *KCNQ1*-iso1M and *KCNQ1*-iso2M, were synthesized using the mMESSAGE mMACHINE kit (Ambion, Austin, TX). Stage V–VI defolliculated *Xenopus* oocytes were injected with the same total amounts of cRNA coding for the mutant or wild type potassium channel subunits.

Electrophysiology and Statistics—Potassium channel expression was determined 2–3 days after cRNA injection by two-microelectrode voltage clamp recordings from *X. laevis* oocytes. Whole-cell recordings were performed using a Warner OC-725A amplifier (Warner Instruments, Hamden, CT) and pClamp software (Axon Instruments, Foster City, CA) for data acquisition and analysis. Data were low pass filtered at 0.2 kHz (−3 db, 4-pole Bessel filter) before digitalization at 1 kHz. Microelectrodes had a tip resistance ranging from 1 to 5 MΩ. Voltage clamp measurements were performed in a solution containing (in mM): 5 KCl, 100 NaCl, 1.5 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH 7.4 with NaOH). Current and voltage electrodes were filled with 3 M KCl solution, and the recording chamber was continually perfused. All experiments were carried out at room temperature (20–22 °C). No leak subtraction was done

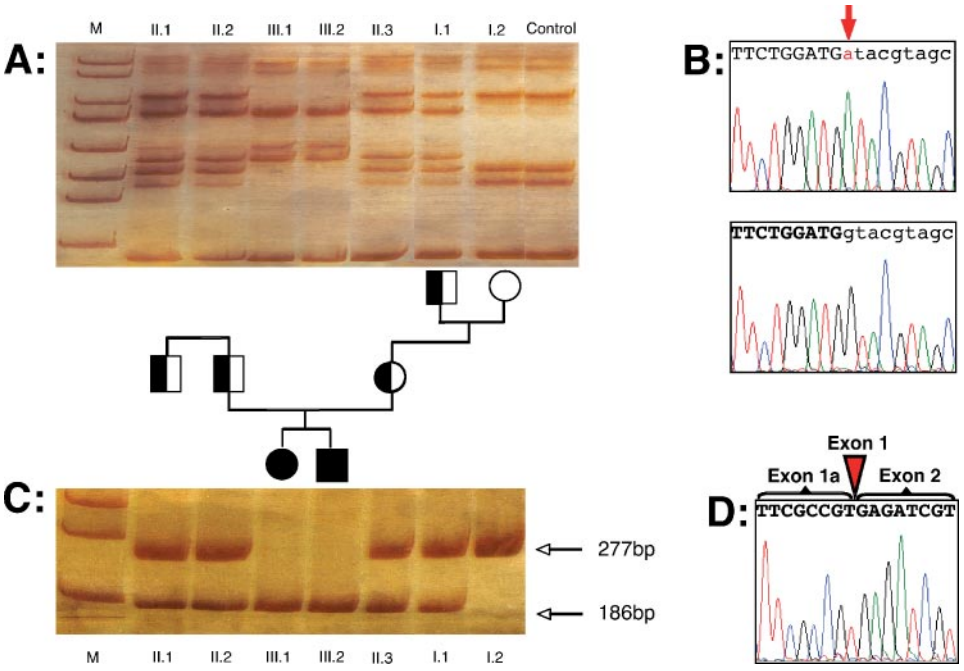


FIGURE 2. **SSCP and RT-PCR analysis.** The pedigree of the JLNS family is arranged to represent the lanes above (SSCP analysis) and below (RT-PCR analysis). No blood sample was available from family member II.4. *A*, SSCP analysis of PCR products obtained from exon 1 of the *KCNQ1* gene. *M*, marker; *C*, control samples. *B*, DNA sequence analysis of the splice donor sequence of exon 1. *C*, RT-PCR analysis of total RNA isolated from lymphocytes of family members reveal the wild type (277 bp) and the mutant (186 bp) fragments in heterozygous subjects (*lane II.1*, *II.2*, *II.3*, and *I.1*). Symptomatic patients selectively amplify the mutant fragment (*lanes III.1* and *III.2*), while inconspicuous family members (*lane I.2*) selectively amplify the wild type fragment. *D*, DNA sequence of the 186-bp mutant RT-PCR fragment indicates that exon 1 is missing in JLNS mutant transcripts. *Open symbols* denote wild type, and *filled symbols* and *split symbols* indicate homozygosity and heterozygosity, respectively.

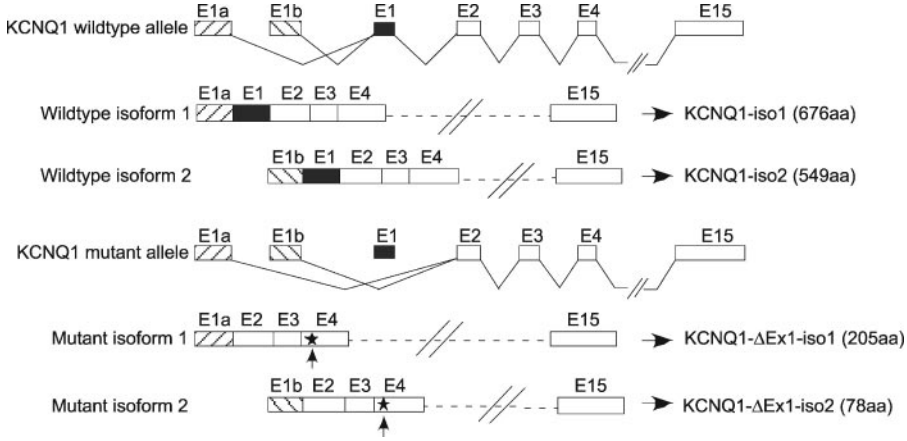


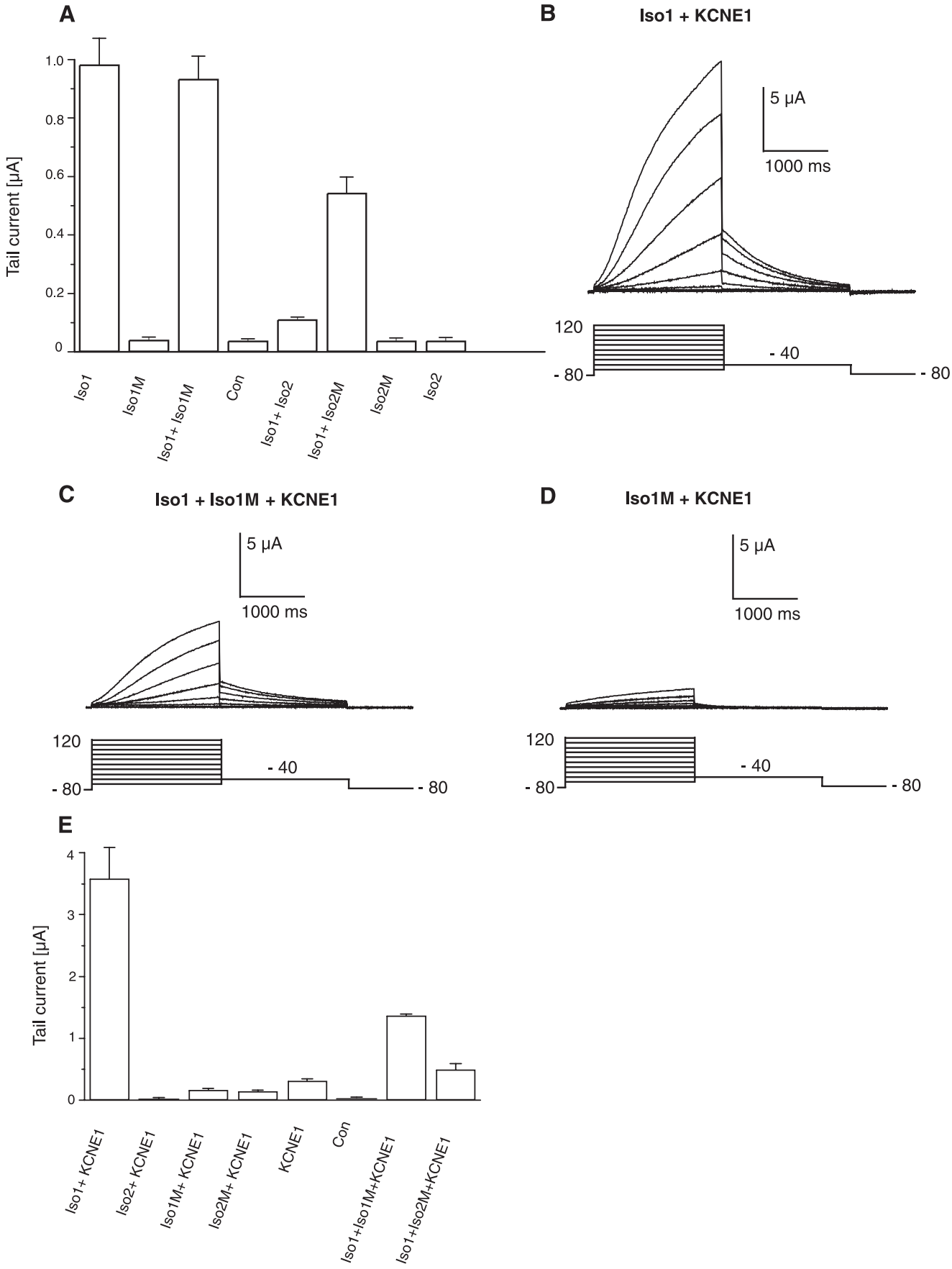
FIGURE 3. **The exon arrangement of the human *KCNQ1* gene yielding the isoforms of wild type and mutant alleles.** Wild type isoform transcripts are composed of exons 1a, 1, and 2-15 (isoform 1) encoding 676 amino acids (aa) and exons 1b, 1, 2-15 (isoform 2) encoding 549 aa, while the mutant isoforms consist of exons 1a and 2-15 (mutant isoform 1) encoding a truncated subunit of 205 aa and exons 1b and 2-15 (mutant isoform 2) encoding 78 aa. The mutant KCNQ1 isoform 1-encoded polypeptide is composed of 129 aa of exon 1a, representing the N-terminal region including one-third of the first transmembrane segment (S1) fused to 76 aa provided by the introduced frameshift. The mutant KCNQ1 isoform 2-encoded polypeptide is composed of 2 aa of exon 1b fused to the frameshift-derived 76 aa. The deleted exon 1 in mutated KCNQ1 isoforms is marked by an *asterisk*. *Arrows* indicate the frameshift introduced termination codon (*rhombus*) at exon 4 in the *KCNQ1* gene. *E* = exon.

during the experiments. All data are expressed as means ± S.E. unless otherwise specified (25).

Subunit Expression and Immunohistochemistry—COS-1 cells (2×10^4) were grown on fibronectin coated coverslips in DMEM with penicillin/streptomycin, glutamine, sodium pyruvate (Invitrogen) and fetal calf serum (Pan Biotech) at 37 °C in 10% CO_2. Cells were transiently transfected using Effectene® transfection reagent (Qiagen) and 0.2 μg of plasmid DNA encoding wild type or mutant KCNQ1 subunits FLAG-tagged at their C terminus. Plasmids were generated by cloning of the appropriate DNA fragments into the pFLAG-CMV-5.1 vector (Sigma). 24 h after transfection, cells were washed in PBS and nitrogen-fixed according to Neuhaus *et al.* (26). Anti-FLAG antibody staining was performed at 4°C over night using a 1:1000 dilution of the affinity-purified monoclonal anti-FLAG antibody (Sigma) in 2% BSA/PBS. After washing with PBS (2 × 30 min), coverslips were incubated overnight in 2% BSA/PBS at 4 °C with a 1:500 diluted secondary antibody (MFP 488 goat anti-mouse IgG, Molecular Probes) in 2% BSA/PBS. Coverslips were washed in PBS (2 × 30 min) and mounted in Citifluor AF2 (Plano).

RESULTS

Clinical and Genetic Characterization—The index patient, a now 17-year-old boy, was diagnosed with congenital deafness at the age of 10 months. Further diagnosis revealed a prolonged QTc of 0.56 s (Fig. 1, patient *III.2*, *closed square*). Treatment with propranolol was started. Because of side effects the dosage had been reduced. In the following years recurrent syncopes occurred in association with physical and emotional stress especially during swimming. Consequently, the propranolol dosage was readjusted to 3 mg/kg/day. Since the age of five no more syncopal episodes appeared under treatment with propranolol. The index patient has a 4 years younger sister who also was diagnosed with congenital deafness and prolonged QTc interval of 0.52 s (Fig. 1, patient *III.1*, *closed circle*). Treatment with propranolol at a dosage of 3 mg/kg/day was started at the age of 6 months. Recurrent syncopes occurred in the last three years in association with physical stress especially during swimming. Because of recurrent events under an adequate dosage of propranolol, implantation of a cardioverter


A
Tail current [μA]
1.0
0.8
0.6
0.4
0.2
0
Iso1
Iso1M
Iso1+ Iso1M
Con
Iso1+ Iso2
Iso1+ Iso2M
Iso2M
Iso2
B
Iso1 + KCNE1
5 μA
1000 ms
120
- 80
- 40
- 80
C
Iso1 + Iso1M + KCNE1
5 μA
1000 ms
120
- 80
- 40
- 80
D
Iso1M + KCNE1
5 μA
1000 ms
120
- 80
- 40
- 80
E
Tail current [μA]
4
3
2
1
0
Iso1+ KCNE1
Iso2+ KCNE1
Iso1M+ KCNE1
Iso2M+ KCNE1
KCNE1
Con
Iso1+Iso1M+KCNE1
Iso1+Iso2M+KCNE1

defibrillator was strongly advised but has been refused by the family. Until today no episodes of cardiac arrest occurred. The family history revealed no other family members with congenital deafness, recurrent syncope, or sudden death. ECG and clinical history of the parents, the mother's sister, and the mother's parents were inconspicuous. The uncle of the index patient (Fig. 1, *II.1*) has a history of epileptic seizures, an ECG could not be obtained. The index patient's parents are distantly related because ancestral consanguinity occurred five generations ago.

Genetic Analysis—We focused our analysis on the JLNS candidate genes encoding the potassium channel subunits KCNQ1 and KCNE1, which together form the I_{Ks} channel (5, 6). Exon sequences of both genes were assayed by PCR of genomic DNA extracted from blood samples of all family members followed by SSCP analysis (23). Evaluation of the intronless *KCNE1* gene revealed no abnormalities in the corresponding sequence and allowed us to exclude the β-subunit encoding gene as the cause of the dysfunction. Subsequently, SSCP analysis of PCR products was focused on the pore forming α-subunit encoding gene *KCNQ1*. The pattern of exon 1-derived PCR products of mutated carrier appeared disordered compared with healthy individuals and symptomatic subjects with three abnormal SSCP bands (Fig. 2*A*, *lanes III.1* and *III.2*) clearly differed from their heterozygous parents, paternal uncle, maternal grandfather (Fig. 2*A*, *lanes II.1*, *II.2*, *II.3*, and *I.1*), and inconspicuous maternal grandmother (Fig. 2*A*, *lane I.2*).

To identify the mutation underlying the altered migration pattern, DNA sequence analysis of exon 1-derived PCR products of the family members was performed on both strands and revealed a single nucleotide change. Due to a guanine (G) to adenine (A) transition at the exon 1 (*uppercase letters*) intron 1 (*lowercase letters*) border of the *KCNQ1* gene (TGGATG*g*tacgt ≫ TGGATG*a*tacgt), one of the most conserved nucleotides at the splice donor sequence (14) is altered in individuals carrying the mutated allele. Both symptomatic patients appeared homozygous for the observed replacement, whereas parents, paternal uncle, and maternal grandfather appeared heterozygous (Fig. 2*B*).

Altered Transcription in KCNQ1 Mutant Carriers—Expression analysis was performed using cDNA fragments generated by RT-PCR of total RNA from blood cells (lymphocytes) of healthy and affected individuals. As expected from the *KCNQ1* mRNA sequence (27), in healthy individuals primer KCNQ1–433 and LQT-201 exclusively amplified a 277-bp *KCNQ1* cDNA fragment (Fig. 2*C*, *lane I.2*). Corresponding to the altered splice donor site, heterozygous family members produce two different *KCNQ1* cDNA fragments, the 277-bp cDNA fragment of the wild type allele, and a shorter 186-bp cDNA fragment of the mutant allele (Fig. 2*C*, *lanes II.1*, *II.2*, *II.3*, and *I.1*). Symptomatic patients, homozygous for the mutant allele, solely amplify the shorter 186bp cDNA fragment (Fig. 2*C*, *lanes III.1* and *III.2*). DNA sequence analysis showed that heterozygous individuals produce a normal spliced wild type *KCNQ1* transcript and an alternative spliced mutant *KCNQ1* transcript lacking exon 1. The JLNS patients selectively produce transcripts missing exon 1 (Fig. 2*D*). It is important to note that the human heart expresses two different KCNQ1-isoforms, the wild type KCNQ1 subunit transcript isoform 1 (embl: AF000571) encoding 676 amino acids (27) and an alternative spliced transcript, KCNQ1-isoform 2 (embl: NM_181798), encoding a KCNQ1 subunit lacking the N-terminal 127 amino acids (24) (Fig. 3). Consequently, altered transcripts encoding both isoforms were expected to appear in lymphocytes of mutant carriers (Fig. 3). However, RT-PCR analysis of total RNA of lymphocytes detected isoform 1 but could not detect isoform 2 transcripts.

Composition of Mutant KCNQ1 Isoforms—In the heart both isoforms are transcribed (24, 27). Deletion of *KCNQ1* exon 1 yielded mutant isoform 1 transcripts (*KCNQ1*-iso1M) composed of exon 1a fused to exon 2–15 and mutant isoform 2 transcripts (*KCNQ1*-iso2M) composed of exon 1b fused to exon 2–15 (Fig. 3). Skipping of exon 1 generates a frameshift that introduced a premature termination codon at exon 4. Accordingly, mutant isoform 1 subunits consist of the exon 1a-encoded N-terminal 129 amino acids (aa) fused to 76 aa encoded by the introduced frameshift, whereas mutant isoform 2 subunits were composed of the exon 1b-encoded N-terminal 2 aa fused to the same, frameshift provided 76 aa (Fig. 3). Thus, homozygous and heterozygous carriers of the JLNS causing mutation express truncated KCNQ1 subunits of either 205 aa (KCNQ1-iso1M) or 78 aa (KCNQ1-iso2M), which have little similarity to KCNQ1 potassium channel subunits present in healthy individuals.

Functional Properties of Mutant KCNQ1 Subunits—First, we analyzed homomeric channels formed by wild type KCNQ1-iso1 subunits or mutant KCNQ1-iso1M and KCNQ1-iso2M subunits following injection of cRNA (1.8 ng each) into *Xenopus* oocytes. Currents were elicited by 2-s steps to potentials ranging from −60 to 120 mV (increment 20 mV), and tail currents were recorded at −40 mV. The holding potential was −80 mV, and pulses were applied at a frequency of 0.2 Hz during all electrophysiological measurements (25). Under these conditions, expression of KCNQ1-iso1 subunits produced channels with a mean tail current amplitude of 0.98 ± 0.092 μA ($n = 20$) (Fig. 4*A*, *column* 1), whereas oocytes expressing mutant KCNQ1-iso1M (Fig. 4*A*, *column* 2) or KCNQ1-iso2M subunits (Fig. 4*A*, *column* 7) produced no currents (KCNQ1-iso1M:

FIGURE 4. **Heterologous expression of KCNQ1 isoforms, mutant subunits, and KCNE1 in *Xenopus* oocytes.** Wild type: KCNQ1-iso1 = Iso1, KCNQ1-iso2 = Iso2. Mutant: KCNQ1-iso1M = IsoM1, KCNQ1-iso2M = IsoM2, *con* = control. *A*, mean peak tail current amplitudes obtained from two batches ($n = 20$). KCNQ1-iso1M, -iso2M, and -iso2 exhibited currents not different from (uninjected) control. Coexpression of KCNQ1-iso1 + KCNQ1-iso1M produced currents not significantly different from KCNQ1-iso1 currents, while coexpression of KCNQ1-iso1 with KCNQ1-iso2M reduced the current to 55% of KCNQ1-iso1 currents. *B*, coexpression of KCNQ1-iso1 with KCNE1 resulted in K^+ currents very similar to I_{Ks}. *C*, coexpression of KCNQ1-iso1 with KCNE1 and mutant KCNQ1-iso1M yielded I_{Ks} amplitudes reduced by ~62%, indicating a dominant-negative effect of the mutant polypeptide. *D*, expression of mutant KCNQ1-iso1M together with KCNE1 produced very low currents. Voltage protocol in *B–D*: holding potential −80 mV, test pulse −60 to 120 mV (2 s) in 20-mV increments, and return pulse constant −40 mV (2 s), respectively. *E*, expression of JLNS mutant subunits KCNQ1-iso1M and -iso2M in combination with the β-subunit KCNE1 produced very low current amplitudes. In contrast to I_{Ks} produced by channels composed of KCNQ1-iso1 and KCNE1 subunits, expression of mutant KCNQ1 subunits with KCNE1 and KCNQ1-iso1 subunits reduced I_{Ks} in a dominant-negative fashion to 38 and 14% (KCNQ1-iso1M and KCNQ1-iso2M, respectively).

0.041 ± 0.01 μA, $n = 20$; KCNQ1-iso2M: 0.037 ± 0.011 μA, $n = 20$) comparable with uninjected oocytes (Fig. 4*A*, *column* 4).

Mutant KCNQ1 Subunits Affect KCNQ1-iso1 Channels—To further investigate the functional consequences of this JLNS mutation, we determined the ability of the mutant subunits to influence wild type KCNQ1-iso1 currents. In coexpression experiments the same amount (1.8 ng) of *KCNQ1*-iso1M or *KCNQ1*-iso2M cRNA was injected with equal amounts of *KCNQ1*-iso1 cRNA, and the resulting currents were compared with KCNQ1-iso1 currents. KCNQ1-iso1M subunits caused little changes (5%) with currents similar to KCNQ1-iso1 currents (0.932 ± 0.079 μA, $n = 20$; Fig. 4*A*, *column* 3). Surprisingly, mutant KCNQ1-iso2M subunits reacted differently and caused a pronounced reduction (45%) of KCNQ1-iso1 currents to 0.543 ± 0.055 μA ($n = 20$) (Fig. 4*A*, *column* 6), an attribute basically associated to dominant-negative RWS-type, but absent from JLNS-type, mutations (12).

KCNQ1 Mutant Isoforms Cause Dominant-negative Suppression of I_{Ks}—To examine whether the truncated mutant KCNQ1 subunits interfere with I_{Ks}, we measured *Xenopus* oocytes injected with equal amounts (1.8 ng) of *KCNQ1*-iso1, *KCNQ1*-iso2, *KCNQ1*-iso1M, or *KCNQ1*-iso2M cRNA together with (1.8 ng) *KCNE1* cRNA. The combination of KCNQ1-iso1 and KCNE1 subunits induced outward potassium currents with properties nearly identical to native cardiac I_{Ks} with an average tail amplitude of 3.58 ± 0.5 μA ($n = 20$, Fig. 4*B* and *E*, *column* 1), whereas coexpression of KCNQ1-iso2 with KCNE1 displayed very low I_{Ks} amplitudes of 0.02 ± 0.02 μA (Fig. 4*E*, *column* 2) not different from uninjected control oocytes (Fig. 4*E*, *column* 6). In contrast, expression of KCNE1 β-subunits alone produced small I_{Ks} amplitudes of 0.31 ± 0.04 μA (Fig. 4*E*, *column* 5) thought to be generated by interaction with endogenous *Xenopus* KCNQ1 subunits (6). Coexpression of mutant KCNQ1-iso1M with KCNE1 exhibited tail currents of 0.17 ± 0.02 μA (Fig. 4, *D* and *E*, *column* 3) representing only 5% of the non-mutant isoform 1/KCNE1 current. Therefore the mutant protein KCNQ1-iso1M failed to produce a relevant potassium current when coinjected with KCNE1. Similarly, mutant isoform 2-encoded subunits (KCNQ1-iso2M) were not able to produce potassium currents in combination with KCNE1 subunits (0.141 ± 0.02 μA) (Fig. 4*E*, *column* 4). Currents smaller than KCNE1 produced currents implied an interaction of mutant KCNQ1 subunits with channels composed of endogenous *Xenopus* KCNQ1 and KCNE 1 subunits. To investigate the effect of mutant KCNQ1 subunits on I_{Ks}, we injected *KCNQ1* and *KCNE1* cRNA (1.8 ng each) together with 1.8 ng of cRNA encoding mutant isoform 1 or isoform 2 subunits into *Xenopus* oocytes. In the presence of KCNQ1-iso1M subunits, I_{Ks} were suppressed in a dominant-negative fashion by ~62% producing a tail current of 1.36 ± 0.28 μA (Fig. 4, *C* and *E*, *column* 7). Coexpression of KCNQ1-iso1 with mutant KCNQ1-iso2M and KCNE1 suppressed I_{Ks} even stronger (86%) and gave a significantly lower tail current of 0.49 ± 0.1 μA (Fig. 4*E*, *column* 8).

KCNQ1 Wild Type and Mutant Subunit Expression in COS-1 Cells—To study whether mutant subunits show differences in abundance, cellular distribution, and cellular localization, transient transfections of COS-1 cells were performed to express mutant and wild type KCNQ1 subunits that were FLAG-tagged at their C terminus. Antibody staining using anti-FLAG antibodies showed that mutant and wild type subunits were distributed in a similar pattern (Fig. 5, *A*–*C*), indicating that the marked truncation of the mutant subunits did neither compromise their cellular location nor their transport to the cell surface. This is in line with the functional analysis of wild type and mutant channels in *Xenopus* oocytes.

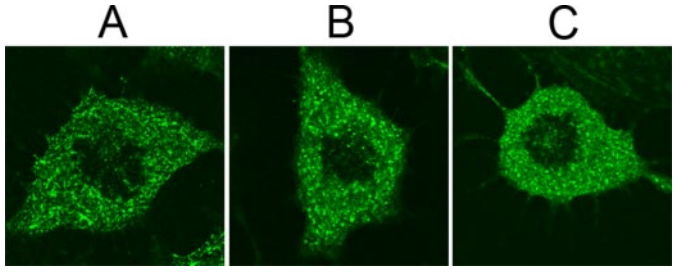


FIGURE 5. **Cellular distribution of KCNQ1 wild type and mutant subunits.** Confocal images of COS-1 cells transiently transfected with FLAG-tagged constructs encoding KCNQ1-iso1-FLAG (*A*), mutant KCNQ1-iso1-FLAG (*B*), and mutant KCNQ1-iso2-FLAG (*C*). Anti-FLAG antibody staining revealed a similar and even distribution of mutant and wild type subunits in COS-1 cells indicating that the truncation of the mutant subunits has no influence on their transport and cellular location.

DISCUSSION

The JLNS is a rare autosomal recessive inherited disease characterized by a profound hearing loss associated with QT prolongation in ECG, syncopal attacks, seizures, and sudden death from ventricular arrhythmias. JLNS-associated mutations have been identified in the pore-forming α-subunit KCNQ1 and in the auxiliary β-subunit KCNE1 (1, 2, 8–13), which coassemble to form I_{Ks} in the heart (5, 6). To date, several frameshift or truncation mutations generated by altered splicing, deletion, or insertion have been reported to be responsible for the majority of the JLNS cases caused by homozygous as well as compound heterozygous mutations of the *KCNQ1* or the *KCNE1* gene (2, 8–13, 15–19, 27–31).

With this study we characterized the structural and functional features of a mutation at the splice donor site of the *KCNQ1* geneÕs exon 1 (G477 + 1A) in a German family with JLNS. Mutant carriers produce *KCNQ1* transcripts lacking exon 1 leading to a frameshift. By this, a premature stop codon is introduced causing the expression of truncated KCNQ1 subunits. Thus, the JLNS patients in this family selectively produce altered transcripts, leading to mutant isoform 1 subunits (KCNQ1-iso1M) composed of 205 aa comprising one-third of the first transmembrane segment (129 aa encoded by exon 1a fused to 76 aa encoded by the frameshift) and mutant isoform 2-derived subunits (KCNQ1-iso2M) composed of 78 aa (2 aa encoded by exon 1b fused to the frameshift provided 76 aa) lacking any KCNQ1 transmembrane segment. In consequence, KCNQ1 wild type and mutant subunits show little structural similarities. In agreement with these findings, mutant subunits encoded by isoform 1 or 2, KCNQ1-iso1M and KCNQ1-iso2M, respectively, were unable to form homomeric potassium channels. Moreover, KCNQ1-iso1M subunits failed to affect the formation of homomeric KCNQ1-iso1 channels, whereas the much smaller KCNQ1-iso2M subunits, when coexpressed in

Xenopus oocytes, reduced KCNQ1-iso1 currents to 46%, a feature well assigned to RWS-type mutations and used to distinguish RWS from JLNS mutations (12). Of even greater importance is that in coexpression experiments with KCNE1, mutant KCNQ1 subunits showed the ability to affect I_{Ks} currents in a dominant-negative mode with current reduction to 38 and 14% (KCNQ1-iso1M and KCNQ1-iso2M), respectively.

Thus, mutant subunits confer properties more related to RWS-associated than to JLNS-associated *KCNQ1* mutations (12, 27), and these features may explain, at least in part, the variety of clinical manifestations in heterozygous carriers associated with *KCNQ1* transcripts lacking exon 1. Heterozygous individuals appear asymptomatic with normal QTc intervalls (this report and Ref. 17), asymptomatic with QTc prolongation (15, 17), as RWS patients (15, 19) and as compound JLNS (17) or compound RWS (18). In respect to such a discrepancy, expression of mutant KCNQ1 subunits and hence their ability to influence I_{Ks} in the heart might be regulated on an individual level.

A lack of I_{Ks} reduction in healthy heterozygous individuals implies mechanisms that in cardiomyocytes prevent the potentially noxious influence of the truncated subunits. In this view, the abundance and distribution of the truncated subunits in cardiaomyocytes could be affected, or alternatively, the transport of the truncated subunits to the cell surface could be impaired leading to the absence of I_{Ks} channels composed of mutant and wild type subunits. Interestingly, anti-FLAG antibody staining of COS-1 cells transiently transfected with plasmids encoding the FLAG-tagged mutant and wild type subunits revealed a similar expression pattern with mutant and wild type subunits indistinguishably distributed. In combination with their functional analysis in *Xenopus* oocytes, these data suggested that independent of their truncation, mutant subunits were transported to the cell surface, like wild type subunits. Apart from these features, we do not know whether the stability of the truncated subunits in cardiomyocytes differs from the stability of wild type subunits, yet an additional fact that could explain the presence of healthy heterozygous individuals.

On the other hand, a recently described mRNA surveillance system, the nonsense-mediated decay, which rapidly degrades mutant transcripts carrying a premature stop codon (20, 21), might offer an alternative explanation how cardiomyocytes abolish or reduce the dominant-negative effect required to establish the recessive nature of JLNS in heterozygous carriers with *KCNQ1* transcripts lacking exon 1.

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REFERENCES

1. Wang, Q., Curran, M. E., Splawski, I., Burn, T. C., Millholland, J. M., VanRaay, T. J., Shen, J., Timothy, K. W., Vincent, G. M., de Jager, T., Schwartz, P. J., Toubin, J. A., Moss, A. J., Atkinson, D. L., Landes, G. M., Connors, T. D., and Keating, M. T. (1996) *Nat. Genet.* **12,** 17–23
2. Splawski, I., Tristani-Firouzi, M., Lehmann, M. H., Sanguinetti, M. C., and Keating, M. T. (1997) *Nat. Genet.* **17,** 338–340
3. Curran, M. E., Splawski, I., Timothy, K. W., Vincent, G. M., Green, E. D., and Keating, M. T. (1995) *Cell* **80,** 795–803
4. Abbott, G. W., Sesti, F., Splawski, I., Buck, M. E., Lehmann, M. H., Timothy, K. W., Keating, M. T., and Goldstein, S. A. (1999) *Cell* **97,** 175–187
5. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) *Nature* **384,** 78–80
6. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) *Nature* **384,** 80–83
7. Ackerman, M. J. (2004) *Nat. Med.* **10,** 463–464
8. Schwartz, P. J., Spazzolini, C., Crotti, L., Bathen, J., Amlie, J. P., Timothy, K., Shkolnikova, M., Berul, C. I., Bitner-Glindzicz, M., Toivonen, L., Horie, M., Schulze-Bahr, E., and Denjoy, I. (2006) *Circulation* **113,** 783–790
9. Schulze-Bahr, E., Wang, Q., Wedekind, H., Haverkamp, W., Chen, Q., Sun, Y., Rubie, C., Hordt, M., Towbin, J. A., Borggrefe, M., Assmann, G., Qu, X., Somberg, J. C., Breithardt, G., Oberti, C., and Funke, H. (1997) *Nat. Genet.* **17,** 267–268
10. Tyson, J., Tranebjaerg, L., Bellman, S., Wren, C., Taylor, J. F., Bathen, J., Aslaksen, B., Sorland, S. J., Lund, O., Malcolm, S., Pembrey, M., Bhattacharya, S., and Bitner-Glindzicz, M. (1997) *Hum. Mol. Genet.* **6,** 2179–2185
11. Tyson, J., Tranebaerg, L., McEntagart, M., Larsen, L. A., Christiansen, M., Whiteford, M. L., Bathen, J., Aslaksen, B., Sorland, S. J., Lund, O., Pembrey, M. E., Malcolm, S., and Bitner-Glindzicz, M. (2000) *Hum. Genet.* **107,** 499–503
12. Mohammad-Panah, R., Demolombe, S., Neyroud, N., Guicheney, P., Kyndt, F., van den Hoff, M., Baro, I., and Escande, D. (1999) *Am. J. Hum. Genet.* **64,** 1015–1023
13. Schmitt, N., Schwarz, M., Peretz, A., Abitbol, I., Attali, B., and Pongs, O. (2000) *EMBO J.* **19,** 332–340
14. Shapiro, M. B., and Senapathy, P. (1987) *Nucleic Acids Res.* **15,** 7155–7174
15. Ackerman, M. J., Tester, D. J., and Porter, C. J. (1999) *Mayo Clin. Proc.* **74,** 1088–1094
16. Splawski, I., Shen, J., Timothy, K. W., Lehmann, M. H., Priori, S., Robinson, J. L., Moss, A. J., Schwartz, P. J., Towbin, J. A., Vincent, G. M., and Keating, M. T. (2002) *Circulation* **102,** 1178–1185
17. Chouabe, C., Neyroud, N., Richard, P., Denjoy, I., Hainque, B., Romey, G., Drici, M. D., Guicheney, P., and Barhanin, J. (2000) *Cardiovasc. Res.* **45,** 971–980
18. Van Langen, I. M., Birnie, E., Jongbloed, R. J., Le Marec, H., and Wilde, A. A. (2003) *J. Med. Genet.* **40,** 141–145
19. Tester, D. J., Will, M. L., Haglund, C. M., and Ackerman, M. J. (2005) *Heart Rhythm* **2,** 507–517
20. Frischmeyer, P. A., and Dietz, H. C. (1999) *Hum. Mol. Genet.* **8,** 1893–1900
21. Wagner, E., and Lykke-Andersen, J. (2002) *J. Cell Sci.* **115,** 3033–3038
22. Splawski, I., Shen, J., Timothy, K. W., Vincent, G. M., Lehmann, M. H., and Keating, M. T. (1998) *Genomics* **51,** 86–97
23. Zehelein, J., Thomas, D., Khalil, M., Wimmer, A. B., Koenen, M., Licka, M., Wu, K., Kiehn, J., Brockmeier, K., Kreye, V. A., Karle, C. A., Katus, H. A., Ulmer, H. E., and Schoels, W. (2004) *Biochim. Biophys. Acta* **1690,** 185–192
24. Lee, M. P., Hu, R. J., Johnson, L. A., and Feinberg, A. P. (1997) *Nat. Genet.* **15,** 181–185
25. Thomas, D., Wimmer, A. B., Karle, C. A., Licka, M., Alter, M., Khalil, M., Ulmer, H. E., Kathofer, S., Kiehn, J., Katus, H. A., Schoels, W., Koenen, M., and Zehelein, J. (2005) *Cardiovasc. Res.* **67,** 487–497
26. Neuhaus, E. M., Horstmann, H., Almers, W., Maniak, M., and Soldati, T. (1998) *J. Struct. Biol.* **121,** 326–342
27. Chouabe, C., Neyroud, N., Guicheney, P., Lazdunski, M., Romey, G., and Barhanin, J. (1997) *EMBO J.* **6,** 5472–5479
28. Huang, L., Bitner-Glindzicz, M., Tranebjaerg, L., and Tinker, A. (2001) *Cardiovasc. Res.* **51,** 670–680
29. Neyroud, N., Tesson, F., Denjoy, I., Leibovici, M., Donger, C., Barhanin, J., Faure, S., Gary, F., Coumel, P., Petit, C., Schwartz, K., and Guicheney, P. (1997) *Nat. Genet.* **15,** 186–189
30. Neyroud, N., Richard, P., Vignier, N., Donger, C., Denjoy, I., Demay, L., Shkolnikova, M., Pesce, R., Chevalier, P., Hainque, B., Coumel, P., Schwartz, K., and Guicheney, P. (1999) *Circ. Res.* **84,** 290–297
31. Wang, Z., Li, H., Moss, A. J., Zareba, W., Knilans, T., Bowles, N. E., and Towbin, J. A. (2002) *Mol. Genet. Metab.* **75,** 308–316