Clinical and genetic spectrum of hereditary cardiac arrhythmia syndromes

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An Intronic Mutation leading to Incomplete Skipping of Exon-2 in KCNQ1 Rescues Hearing in Jervell and Lange-Nielsen Syndrome

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Abstract
Romano-Ward syndrome (RWs) and Jervell and Lange-Nielsen Syndromes (JLNS) are two inherited arrhythmia disorders caused by monoallelic or bi-allelic mutations, respectively, in the \( KCNQ1 \) or \( KCNE1 \) genes. Both disorders could cause Long QT syndrome either without deafness (RWS), or with deafness (JLNS). We have performed clinical, molecular and functional investigation in two consanguineous Arabian families with history of sudden death of several children. Importantly, none of the affected individuals had (or have) any hearing impairment. Homozygosity mapping followed by molecular analysis identified a novel splice acceptor site mutation (homozygously) in intron 1 of the \( KCNQ1 \) gene (c.387-5 T>A), in these two apparently unlinked families. RNA analysis revealed that this splice site mutation causes incomplete transcriptional aberration of the \( KCNQ1 \) gene, leaving 10% of the normal allele transcript intact, which restores the hearing function. Our molecular and functional data provide the first evidence that small amount (as low as 10%) of normal \( KCNQ1 \) current can effectively maintain the hearing function but fails to maintain cardiac repolarization characteristics within normal limits.

Additionally, we have revealed four extra low frequency aberrant isoforms emphasizing the importance of intronic and other non-coding sequences in maintaining cellular homeostasis as pathologic changes in a single nucleotide can affect splicing events at distant sites. The novel \( KCNQ1 \) mutation found in this study is very likely a founder mutation in the southern province of Saudi Arabia emphasizing its screening in the LQT population in this region.

Key words: Long QT syndrome, \( KCNQ1 \), Intrinsic mutation, Hearing Rescue

Abbreviations
\( SCN5A \): Sodium channel, voltage-gated, type V, alpha subunit
\( KCNH2 \): Potassium voltage-gated channel, subfamily H (eag-related), member 2
\( KCNQ1 \): Potassium voltage-gated channel, KQT-like subfamily, member 1
\( KCNE1 \): Potassium voltage-gated channel, Isk-related family, member 1
\( KCNE2 \): Potassium voltage-gated channel, Isk-related family, member 2
\( ANK-B \): Ankyrin 2
\( KCNJ4 \): Potassium inwardly-rectifying channel, subfamily J, member 4
\( KCNJ11 \): Potassium inwardly-rectifying channel, subfamily J, member 11
\( KCNJ12 \): Potassium inwardly-rectifying channel, subfamily J, member 12
\( CALNA2 \): Calcineurin A beta subunit
\( CLCN2 \): Chloride channel 2
\( CACNA1C \): Calcium channel, voltage-dependent, L type, alpha 1C subunit
Introduction

Jervell and Lange-Nielsen syndrome (JLNs) is an autosomal recessive disorder clinically manifested by severe cardiac arrhythmias and congenital bilateral sensory neural deafness. On ECG, the cardiac phenotype is characterized by a prolonged QT interval and polymorphic ventricular arrhythmias (torsade de pointes). These cardiac arrhythmias may result in recurrent syncope, seizure, or sudden death. Homozygous or compound heterozygous mutations in the KCNQ1 or KCNE1 genes could cause JLNS. In contrast to JLNS, heterozygous mutations in either of the genes (KCNQ1, KCNE1) are responsible for autosomal dominant Romano-Ward LQT1 syndrome (RWS). Heterozygous carriers for JLNs causing mutations usually show a milder cardiac phenotype including only moderate prolongation of the QT interval. Another important distinguishing clinical feature between the homozygous and heterozygous mutation carriers (KCNQ1/KCNE1 genes) is that the heterozygous carriers don’t show any congenital hearing impairment. KCNQ1 and KCNE1 proteins co-assemble to form the cardiac K+ channel, responsible for the slowly activating delayed rectifier outward K+ current (IKs). However, IKs channel has also been detected in the inner ear as a functional channel. In the inner ear, IKs channel functions as K+ charge carrier for sensory transduction and the generation of the endocochlear potential in the endolymph required to maintain normal hearing. In a mouse model Knipper M et al. (2006) elucidated decline of functional IKs channel as the primary cause of deafness. Though, JLNS causing homozygous or compound heterozygous KCNQ1 mutation carriers in general suffer from congenital deafness and cardiac arrhythmias, yet, there are several reports where homozygous mutation carriers suffered only from cardiac arrhythmias and no deafness. Mechanism of hearing function preservation in severe arrhythmia patients (with homozygous/compound heterozygous KCNQ1/KCNE1 mutations), remained unclear. It was suggested that these recessive mutations are probably mild mutations and thus are unable to abolish the IKs channel completely like in the JLNS patients, and the residual IKs could effectively maintain hearing, but not the normal cardiac electrical potential properties. Proper and quantitative experimental data to support this hypothesis is lacking. To date only one splice site mutation in KCNQ1 has been described in connection to JLNS patients. A homozygous mutation (c.477 +1 G>A) at the splice donor site in exon 2-intron 2 junction (mentioned as exon-2 in the study of Zehelein et al. 2006) found as causal mutations in JLNS patients. At the mRNA level this donor site mutation exclusively produced KCNQ1 transcripts lacking exon 2 leading to a frameshift at the 129th amino acid (p.129fs205X), homozygous mutation carrier siblings are profoundly deaf in addition to the severe LQT cardiac phenotype. In the present study, we have performed clinical, molecular and functional studies in two consanguineous Arabian families with history of sudden cardiac death of several children.
Importantly, none of the affected individuals had any hearing impairment. Our study has elucidated a novel mutation, which is highly likely to be a founder mutation, in the \textit{KCNQ1} gene pathogenic to severe recurrent and familial arrhythmias in a Yemeni tribe from southern part of Saudi Arabia. In depth molecular, functional and clinical analysis elucidated several new findings: a) intronic mutation in our patients did affect transcriptional aberration, but 10\% of \textit{KCNQ1} gene still could escape aberrant transcription, which could effectively rescue the hearing in our patients, but not the cardiac repolarization function; b) intronic mutation detected in this study could jeopardise the homoeostasis in the exon-intron splicing leading to aberrant splicing which includes exon skipping, inclusion of introns in the mRNA and also activation of cryptic splicing. Further, this aberration affects not only the following exon/intron immediately downstream to the mutation-spot, exons/introns further downstream/ in a distant location could also be affected.

This is the first real evidence of dose effect of KCNQ1 protein on functional and clinical consequences into the heart and ear function.

**Experimental Procedures**

**Clinical Analysis**
Clinical consultation of the patients was performed at the Prince Sultan Cardiac Centre, Riyadh, Saudi Arabia. We have done our investigation in two probands from two unlinked families originating from the Southern part of Saudi Arabia. Both probands are offsprings of asymptomatic, consanguineous, Arabian parents (Figure 1A, family A and B). We have done our study on these two symptomatic and alive individuals who were affected by repeated syncope. The subjects underwent detailed cardiovascular examination and audiological examination.

**Mutation and Haplotype analyses**
Genetic investigation was performed at the Department of Clinical Genetics, Academic Medical Center, University of Amsterdam, the Netherlands.
Considering the consanguinity of the parents, and the absence of cases of sudden death among the siblings of both parents in the family-A, we have performed homozygosity mapping around several candidate genes \textit{SCN5A, KCNH2, KCNQ1, KCNE1, KCNE2, ANK-B, KCNJ4, KCNJ11, KCNJ12, CALNA2, CLCN2, CACNA1C}, and in which mutations were reported to cause electrical instability of the heart. Microsatellite repeat markers from ABI-Prism (PE Biosystems, Foster City, CA, USA) encompassing these genes were used. After PCR, the amplified fragments were separated using the ABI Prism 377 automatic
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Family A:
VI:1: Died at 10 yrs, drowning
VI:2: Died at 5 yrs, Long QT
VI:4: 7 yrs, Aborted sudden cardiac death
VI:5: Sudden cardiac death at 12 yrs

Family B:
II:1: Sudden death at 2 yrs with convulsion
II:4: 14 yrs, Aborted sudden cardiac death

Family A
Father (V:1)
Mother (V:2)
Son (VI:3)
Proband (VI:4)

Family B
Proband (II:4)
DNA sequencer (PE Biosystems), and analyzed with the Genotyper program (PE Biosystems). DNA from peripheral blood lymphocytes was isolated by PUREGENER kit (Gentra Systems, Minneapolis, MN 55447). All 16 exons of the KCNQ1 gene were amplified from genomic DNA by the PCR primers (available on request). PCR products were sequenced bi-directionally on an ABI 377 sequencer (PE Biosystems).

Thereafter, we have performed a haplotype analysis in both family-A and family-B (Figure 1A) based on the microsatellite repeat markers (D11S4046, D11S1338, D11S902) and intragenic (KCNQ1) exonic SNPs c.1638 G/A (exon 13), c. 1986 C/T (exon 16) (NCBI ref. NM_000218).

Identification of aberrantly spliced KCNQ1 mRNAs using RT-PCR
mRNA analysis for KCNQ1 has been successfully performed with the RNA from lymphocytes in previous studies.\(^{17,26}\) Total RNA was extracted from leukocytes of fresh blood using PAXgeneTM Blood RNA kit (PreAnalytiX, Qiagen & BD, Venlo, Netherlands and Franklin Lakes, NJ, USA) according to the manufacturer’s instructions. First strand cDNA synthesis was performed by SuperScript™ III Reverse Transcriptase (Invitrogen, CA, USA) according to their protocol. This first strand cDNA was used for PCR amplification, for which exon specific primers spanning exons 1 through 5 were used (information is available on request). Primers are as follows: KCNQ1-Exon 1-For-1: 5’- CGCGCGGTCTCCATCTACAGCAC-3’; KCNQ1-Exon 1-For-2: 5’-GCGTCTACAACTTCCTCGAGCGT-3’; KCNQ1-Exon 3-Rev: 5’-AGAGGCGGACCACGTACTCCGTC-3’; Transcripts were sequenced bidirectionally in an ABI-377 sequencer (PE Biosystems).

We have additionally amplified the RT-PCR product (patients and controls) with the primers KCNQ1-Exon1-For-3: 5’-ATGGGCGCGGCTCTCCCTCCCG-3’; Exon 5-Rev-1: 5’- TAG-

Figure 1A: Pedigree of the studied families and homozygosity mapping with the microsatellite repeats and intragenic SNP markers in and around the KCNQ1 gene. Affected individuals are shown as filled circles (female) and squares (male). Obligate carriers by genotyping are denoted by with black dots in symbols. Deceased individuals are indicated by slashes, proband is indicated by an arrow. Results of genotypic analysis are shown for 4-different markers D11S4046, D11S1338, D11S902, and two intragenic SNPs in exon 13 and exon-16 of the KCNQ1 gene are shown below each individual. Consanguineous marriage is indicated by =.

Figure 1B: A-D: ECG recordings in father (V:1; family A), mother (V:2;family A) and brother (VI:3;family A) and the proband (VI:4;family A) and proband II:4 (family B). Leads II, and V5 are shown for each tracing. V:1, V:2 and VI:3 showed normal sinus rhythm with QTc of 424, 456 and 381 ms, respectively. The conduction interval is normal. Proband in both families (VI:4, family A; II:4, family B) show prolonged QTc of 557 and 529, respectively.
CATCCTCAGGATCTGCAG-3’ and cloned in a TOPO cloning vector (TOPO Cloning Kit, Invitrogen, CA, USA) and were then transformed into Escherichia coli XL-1 Blue. 50 transformants containing plasmids were directly sequenced bi-directionally on an ABI 377 sequencer (PE Biosystems).

cDNA constructs for Functional Study

Wild type (WT) KCNQ1 and KCNE1 cloned in pSP64 were kindly provided by Dr. Michael C. Sanguinetti, University of Utah, Salt Lake City, Utah, USA. For mammalian cell transfection, we have subcloned the WT KCNQ1 and WT KCNE1 from the above plasmids in the eukaryotic expression vector pCGI.

Mutant KCNQ1 cDNA was generated by RT-PCR amplification of the lymphocyte RNA from the patient. For amplification and cloning the mutant KCNQ1 following PCR primers were used: KCNQ1-HindIII-For (exon1: TTAAGCTTATGGCCGCGGCCTCCTCCCCG and KCNQ1-EcoRI-Rev (exon-5): GTGAATTCTAGCATCCTCAGGATCTGCAG and the amplified RT-PCR product cloned into the eukaryotic expression vector pCGI.

Cell preparation and heterologous expression

HEK-293 cells were cultured in Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum, penicillin, streptomycin, and non-essential amino acids. To express KCNQ1 + KCNE1 channels, cells were transiently transfected with lipofectamine using 1 μg wild-type (WT) or 1 μg mutant KCNQ1 cDNA and 1 μg KCNE1 cDNA for homomeric channels, and 1 μg WT KCNQ1 cDNA, 1 μg mutant KCNQ1 cDNA and 1 μg KCNE1 cDNA for heteromeric channels. Green fluorescence protein was co-transfected to identify transfected cells under epifluorescent microscopy. After transfection, the cells were incubated in 5% CO2 at 37ºC for 48 hours.

Electrophysiology: KCNQ1+ KCNE1 currents were recorded using the whole-cell configuration of the patch-clamp technique with an Axopatch-200 amplifier (Axon Instruments). Pipettes were pulled from borosilicate glass to have tip resistances of 2-3 MΩ when filled with solution containing (in mM): 145 KCl, 1 MgCl2, 5 EGTA, 5 MgATP, and 10 HEPES (pH 7.2 with KOH). Cells were superfused with solution containing (in mM): 140 NaCl, 5.4 KCl, 1.8 CaCl2, 1 MgCl2, 5.5 glucose, and 5 HEPES (pH 7.4 with NaOH). Currents were filtered at 5 kHz and digitized at 10 kHz. Membrane capacitance and series resistance were compensated to obtain minimal contribution of the capacitive transients. Data acquisition and analysis were performed using the pCLAMP 10.0 software (Axon Instruments). All recordings had a holding potential of -80 mV and were made at room temperature.
Statistics
Values are expressed as means ± SEM. Statistical analysis for in vitro experiments was carried out using two-way repeated measures of variance (ANOVA). P<0.05 was considered as statistically significant.

Results

Clinical Analysis
Family A: Proband (VI:4, Figure 1A) is a 3 year old boy. He was referred to a Pediatric Neurology clinic due to suspicion of epilepsy. He had an episode of loss of consciousness while swimming. Neurology evaluation was normal. He was later evaluated at the Cardiology clinic and diagnosed with Long QT with QTc: 557 ms (Figure 1B and Table 1). His audiogram was normal. He was given atenolol 12.5 mg/day and he further did not develop any syncope. There was a family history of SCD of several members; one brother (VI:1, Figure 1A) died at the age of 10 year while diving, one sister (VI:2, Figure 1A) died at 5 year of age while playing in the ground. She had prior history of unconsciousness during swimming and activity, but, was without any medication. She was diagnosed with LQTS. One niece (VI:5, Figure 1A), known with a history of syncope, died at 12 year. None of the children had any history of hearing impairment. Parents are first degree relatives and without any complaints and their ECG is normal (Figure 1B), however, no provocative testing (exercise ECG) on the parents was performed.

<table>
<thead>
<tr>
<th>Individual identity</th>
<th>RR interval (ms)</th>
<th>PQ interval (ms)</th>
<th>QRS width (ms)</th>
<th>QTc (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V:1 (family A)</td>
<td>1040</td>
<td>160</td>
<td>70</td>
<td>431</td>
</tr>
<tr>
<td>V:2 (family A)</td>
<td>760</td>
<td>140</td>
<td>80</td>
<td>459</td>
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<tr>
<td>VI:3 (family A)</td>
<td>700</td>
<td>160</td>
<td>80</td>
<td>430</td>
</tr>
<tr>
<td>VI:4 (proband, family A)</td>
<td>820</td>
<td>120</td>
<td>70</td>
<td>557</td>
</tr>
<tr>
<td>II:4 (proband, family B)</td>
<td>1140</td>
<td>120</td>
<td>70</td>
<td>529</td>
</tr>
</tbody>
</table>

*Table 1: ECG parameters of the proband and the family members from family A, and from the proband in family B.*
Family B: Proband is now a 16 year old boy (II:1; Figure 1A). No complication was reported during intrauterine stage and also during birth. At age 1 he started to have generalized seizure with cyanotic lips and the seizure lasted for about 1-2 minutes. He had multiple attacks of seizure till he was 4 year, seizure was triggered by physical activity. He was given phenytoin which could not control the seizures.

At age 4, was referred to the Prince Sultan Cardiac Centre, Riyadh, for proper evaluation and his ECG showed prolonged QT. He was prescribed propranolol (adjusted for age) and after that he did not develop any seizure. Presently, he is quite active and regularly plays football at school. Now, he is receiving 20 mg of propranolol thrice daily. His present ECG showed a prolonged QTc interval of 529 ms (Figure 1B and Table 1). Audiogram was completely normal. Further family history revealed that proband’s older brother (II:4; Figure 1A) died at age 2 year with similar seizure disorder. He had also normal hearing. Parents are first degree relatives without any clinical complaints, genetic investigation was performed in the father, but the mother declined for a genetic screening. Parents and remaining siblings are devoid of any symptoms and they declined for any further test.

**Figure 2:** Screening of the KCNQ1 gene shows substitution of nucleotide “T” for an “A” at intron-1 (c.387 -5 T>A, arrow marked) (NCBI ref: NM_000218). Left panel shows homozygous changes in the probands (II:6, family A; II:4, family B). Right panel shows heterozygous changes of the same nucleotide in the carriers. Exon-intron boundary was shown by a dotted line with arrow pointing towards exons.
Genetic Analysis

Family A: Homozygosity mapping around the candidate genes (mentioned in materials and methods) showed homozygosity of the microsatellite-repeat markers encompassing the *KCNQ1* gene only in the proband (II:6, Figure 1A). Homozygosity of the microsatellite-repeat markers were not found for any other candidate genes (please see experimental procedures) in this proband, which led us to screen the *KCNQ1* gene in this family. We have identified a homozygous mutation in intron 1 of *KCNQ1* at 5-base upstream from the first nucleotide of exon-2 (c.387 -5 T>A; NCBI Ref. NM_000218) in II:6 (Figure 2). The same mutation was found heterozygously in both parents (I:1 and I:2, Figure 1A) and (Figure 2), and also in the other living brother (II:4, Figure 1A) and (Figure 2). It was not present in 200 control individuals.

Family-B: Family-B is not knowingly linked to family-A. But, due to their ancestral origin from the southern part of the Saudi Arabia, we have first decided to check for *KCNQ1* gene in the proband. We have identified the same homozygous mutation in intron 1 of *KCNQ1* at 5-base upstream from the first nucleotide of exon-2 (c.387 -5 T>A; NCBI Ref. NM_000218) in II:4 (Figure 2). This was found heterozygously in the father (I:1) (Figure

![Figure-3A](image)

**Figure-3A**: Agarose gel analysis of RT-PCR products, RNA obtained from probands with homozygous mutations (II:6, family A; II:4, family B) and non-carrier family members. Lane 1 and 3: Probands from both families show predominantly the mutant (170 bp) fragment and a faint normal sized fragment (261 bp) intensity of which is about 10% of the lower aberrant fragment. Lane 2 and 4: RNA analysis from non-carrier (WT) family members show only the wt-fragment of 261-bp. Heterozygous carriers were not analysed for this assay.

![Figure 3B](image)

**Figure 3B**: cDNA generated from the RNA was sequenced. Left panel shows cDNA sequences of part of exon-1 followed by exon-2. Right panel shows the cDNA sequences generated from homozygous carrier probands, in this panel exon-1 sequences are followed by exon-3 sequences missing the exon-2.
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Figure 4: Schematic representation of exons-introns of the KCNQ1 and the location of the intronic mutation detected in patients in this study. Exons are depicted as squares, filled square (box) represent the exon involved in the transcriptional aberration due to intronic mutation. Hatched boxed represents exons E1a and E1b, alternatively transcribed in two isoforms 1 and 2, respectively. WT isoforms (1 and 2) due to normal splicing are shown; below them the mutant transcripts are shown. Translation consequences of the mutant transcripts are shown at the right hand side of the transcripts.

Figure 5: RT-PCR amplified mRNA was cloned in E. coli and sequenced to analyse various transcripts and their ratios. Ratios of various transcripts and their putative consequence on protein translation have been mentioned.
Mother’s DNA was not available for analysis. This finding of the same intronic KCNQ1 mutation in both families prompted us to investigate whether this mutation originated from a single source (founder mutation). Figure 1A shows that mutated allele/s in both families share the same haplotype based on microsatellite repeats and KCNQ1 intragenic SNPs, which means that the mutation in these apparently unrelated families originated from a single source.

**cDNA Analysis**

Figure 3A shows RT-PCR product amplified from leukocyte mRNAs, by KCNQ1-Exon 1-For-1: 5’- CGCGCGTCTCCATCTACAGCAC-3’, KCNQ1-Exon 3-Rev: 5’-AGAGGCG-GACCACGTACTCCGTC-3’ primers. In contrast with the single WT band (261-bp) identified in normal individuals, the affected individuals had shorter bands (170-bp) as well as the normal-sized (261-bp) faint band. Intensity of this WT faint band in the patients is about 10% of the shorter band (170-bp). Direct sequencing of these both transcripts revealed skipping of exon-2 in the shorter transcript from the patients (Figure 3B).

Additionally, E. coli cloning and sequencing of RT-PCR amplified transcripts from the patients revealed more aberrant variants of KCNQ1 transcripts shown in figures 4 and 5, which were absent in the controls. As shown in the figures 4 and 5, exon-2 only skipping variant mRNA remained the major transcript (85%) in the patients, followed by WT transcript (10%).

Minor transcripts (Figure 5) are in-frame inclusion of intronic CAG (from intron 1) without skipping any exons (<2%); inclusion of intronic CAG (from intron 1) and activation of second cryptic splice site within exon-3 leading to deletion of last 56- nucleotides from exon-3 (<2%); complete skipping of exon-2, exon-3 and exon-4 (<2%). The distance between the mutation site in intron-1 and the exon-4 is 43-kb, which means that an intronic mutation could affect splicing aberration in nearby exons as well as exons in a distant site.

**Functional analysis of the major spliced mutant**

WT KCNQ1 produces two mRNA transcripts, which differs in the exon-1. We have shown in our mRNA experiments that the aberrant splicing caused by the c.387 -5 T>A mutation leads to a truncated KCNQ1 protein p.Val129fsX205 in isoform-1. Regarding the second isoform, a frameshift occurs immediately after the first methionine codon. This finding led us to make the first truncating isoform for functional study as the isoform-1 still preserves some native features of KCNQ1. We have made the mutant KCNQ1 cDNA without exon-2 from lymphocyte RNA (see methods) and checked their biophysical properties in HEK293 cells.

Cells expressing WT KCNQ1+ KCNE1 channels (1 μg each) showed a slowly activating
time-dependent outward current from membrane potentials positive to -10 mV with its amplitude increasing at more positive membrane potentials (Figure 6B, upper panel; Figure 6C). Cells expressing mutant KCNQ1 + KCNE1 channels (1 μg each) also showed an outward current with a time-dependent component. However, the currents activated faster and decayed more rapidly to a steady value. The currents were of smaller amplitude, and showed a linear relation with increasing membrane potentials (Figure 6B, middle panel; Figure 6C). These properties are characteristic for endogenous HEK-293 currents, and strongly suggest non-functional mutant KCNQ1 + KCNE1 channels.

Since heterozygote carriers have both WT and mutant KCNQ1 alleles, we co-expressed WT
and mutant KCNQ1 channel subunits to determine whether the presence of mutant KCNQ1 subunits alters WT KCNQ1+ KCNE1 currents. Similar to cells expressing WT KCNQ1 channel subunits, cells co-expressing WT and mutant KCNQ1 channel subunits showed a slowly activating time-dependent outward current from membrane potentials positive to -10 mV with its amplitude increasing at more positive membrane potentials (Figure 6B, lower panel; Figure 6C). At each tested membrane potential, the presence of mutant KCNQ1 channel subunits did not alter the amplitude of the WT KCNQ1+ KCNE1 currents. Hence, no dominant negative effect on WT currents could be demonstrated.

Discussion
In this study, we describe a novel homozygous mutation in the splice regulatory site at intron-1 of the KCNQ1 gene which causes only severe arrhythmias (without any deafness) in several children from two presumably unlinked families. In all our patients swimming and exercise were the triggers for the arrhythmia events, which is typical of arrhythmias caused by KCNQ1 mutations. A previous study from Germany described a splice regulatory site mutation involving the same exon as in our study in the KCNQ1 gene in children with JLNS (arrhythmia and deafness). As described in this study from Germany, JLNS causing mutations in KCNQ1 are mostly truncating and cause complete abolishment of IKs channel function. Intronic mutation in the Zehelein et al. study (2006) results in complete skipping of KCNQ1 exon-2 leading to transcriptional aberration with a frameshift of the protein at 129th amino acid (129fs205X). Two siblings who carried homozygous mutations suffered from severe cardiac arrhythmias with significantly prolonged QTc and profound sensorineural deafness, i.e. a true JLNS phenotype. Heterozygous carrier parents had no clinical phenotype. Functional studies with the mutant KCNQ1 channel showed that there was no discernible IKs current, which is compatible with the ear phenotype (deafness) as this channel is required for K+ cycling in the inner ear endolymph that maintains the hearing. In contrast to the findings of Zehelein et al. (2006), the analysis of leukocyte KCNQ1 transcript in our patients has identified a mutation in intron 1 (Figure 7, panel-A) that has resulted in incomplete skipping of exon-2, such that WT transcript comprised 10% of the total transcripts. Furthermore, unlike the patients of the Zehelein et al. (2006) study, our homozygous mutation carrier patients have normal hearing, but for both studies heterozygous carriers show no cardiac phenotype. Our findings have led us to conclude that this residual WT rescued the hearing in our patients who have otherwise LQT related cardiac symptoms. Cotransfection of mutants showed a dominant negative effect on the WT KCNQ1 in the
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study by Zehelein et al. (2006), which is in contrast to our findings where there was no dominant negative effect on the WT KCNQ1. In our study, we used mammalian HEK293 cells for functional studies. KCNQ1 cDNA cloned in a plasmid vector driven under a promoter effective for transcription in these cells is more reminiscent of the native condition than injecting prepared mRNA into xenopus oocytes as used by Zehelein et al. (2006). Heterozygous carriers are left with 60% normal IKs current with no cardiac or hearing phenotype. On the other hand, homozygous carriers are left with approximately 10% of the normal current which in our patients is able to maintain hearing function, but not the cardiac function. These data, for the first time, provide a real evidence of the previously suggested hypothesis of residual IKs in maintaining hearing function in the bi-allelic KCNQ1 mutation carriers. Biophysical study with the mutant protein showed that 90% of the transcripts due to c. 387 -5T>A homozygous mutation are unable to perform any IKs function and have no negative effect on the residual 10% of the WT current. This suffices to preserve hearing function whereas the cardiac phenotype is typically severe. The functions of the other transcripts were not studied, but as the quantity of these transcripts is

Figure 7: Panel-A shows the location of intronic mutation (arrow head) and their effects on splicing and clinical phenotypes of the homozygous mutation carriers found in this study and also in the study by Zehelein et al. (2006). Panel-B shows the schematic diagram of the KCNQ1 potassium channel. * mark shows that the protein is truncated after this part due to skipping of exon-2 in the studies by Zehelein et al. (2006) and in the present study. Channel consists of 6 transmembrane segments/domains (S1 to S6).
very low it is not expected that they will play an important role. Some might argue, whether the aberrant transcripts are stable. We have not studied whether the aberrant transcripts are susceptible to the Nonsense Mediated Decay (NMD) pathway as this aberration leads to a premature stop codon. But, our biophysical analysis showed that the aberrant RNA, if it escapes NMD, would make a non-functional protein without any dominant negative effect on the normal allele in the heterozygous carriers. In the homozygous carriers, patients are left only with residual $I_{Ks}$ current made from the non-skipped residual KCNQ1. The two families investigated in this study are not knowingly linked, investigation with polymorphic markers, in and outside of the causative gene, showed that the mutation in both families originated from a single origin. In a highly endogamous society, where marriages within families and within the same tribe are the norm rather than the exception, we could speculate that the reported mutation could well be a founder mutation in this tribal community from Southern Saudi Arabia. This emphasizes population screening for this mutation in children with arrhythmias from this region without any structural defect in the heart.

In conclusion, this is the first report of a homozygous mutation in any cardiac ion channel genes that causes an incomplete aberration of transcription with minor leakage of the WT transcript. In addition, it is the first clinical proof of evidence that only residual $I_{Ks}$ is sufficient enough to preserve ear function, in the presence of a severe cardiac phenotype. Another, basic finding of this study is the identification of aberrant transcripts far from the location of the mutation. This signifies that the intronic and exonic nucleotides are in homeostasis to maintain the transcriptional machineries in order.

Finally, the identification of the the regulatory components/machineries that relieve the mutant alleles found in our study from aberrant transcription might form the basis of an effective therapeutic strategy in rescuing the cardiac phenotypes in these patients. We have examined the patients on several occasions and none of them showed any deafness and there was no history of deafness among any children who died of SCD. A detailed study of hearing could be crucial to measure the level of hearing, but this was not possible as both families refused to appear in the hospital when informed and explained of the genetic findings.

Though the residual $I_{Ks}$ is sufficient to maintain the hearing in the children, it might be of clinical significance to screen patients for hearing function, if consent given, on a long term basis to check whether this function remains constant or is affected by age progression.

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References


