Partial Duplication and Poly(A) Insertion in KCNQ1 Not Detected by Next-Generation Sequencing in Jervell and Lange–Nielsen Syndrome

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Jervell and Lange–Nielsen syndrome is caused by absence of the voltage-gated potassium current $I_{Ks}$ through either homozygous recessive or compound heterozygous mutations in KCNQ1 or KCNE1. We report here a case of Jervell and Lange–Nielsen syndrome with typical clinical features in which clinical genetic testing using next-generation sequencing (NGS) revealed only a known single heterozygous KCNQ1 mutation (R518X), but failed to recognize an unusual and complex 52-bp duplication-insertion. This type of variant has not been previously reported in Long QT syndrome (LQTS) and may therefore account for a portion of genetic variant negative cases.

Case Report

A 47-year-old woman with congenital deafness, severe QT prolongation (≥800 ms on Holter monitoring; Figure 1A and 1B), and striking exercise-induced QT lability and torsades de pointes was seen in referral. She had her first episode of syncope at 6 years of age, was reported to have marked QT prolongation, started β-blocker therapy, and was then lost to follow-up. At age 40, she had an episode of syncope while working outdoors in the heat, and β-blocker (propranolol at that time) was continued although she admitted to incomplete compliance because of fatigue. At age 46, she had another syncopal episode and was switched to metoprolol 100 mg daily. An implantable cardioverter-defibrillator was recommended but she declined. During an exercise test to assess the adequacy of β-blockade, she developed marked T wave lability followed by a 40-second, self-terminating episode of torsades de pointes, during which she lost consciousness (Figure 1C). Notably, this T wave instability occurred in an unusual (nonalternans) pattern, compatible with temporal variability in repolarization with sympathetic activation, as previously suggested.

Causative Mutation Identification

Commercial testing (GeneDx Inc, Gaithersburg, Maryland) using NGS identified a single heterozygous paternally inherited, previously described nonsense variant (R518X) in exon 12 of KCNQ1, and array testing revealed no large deletions or insertions in the gene (Methods in the Data Supplement). The clinical picture strongly suggested that a second KCNQ1 variant was present but undetected by NGS. To test this hypothesis, we performed PCR amplification of each exon for Sanger sequencing (primer sets are presented in Table I in the Data Supplement). PCR amplification of exon 15 in the proband produced 2 products, one at the anticipated size of 500 bp and a second larger product of ≈550 bp (Figure 2A). The 500 bp product showed no variation compared with the reference sequence (NM_000218; Figure 2B), whereas the larger product contained a frame-shifting 52-bp insertion comprised of a partial duplication of the 5′ end of exon 15 and an insertion of 36 adenines followed by uninterrupted exon 15 sequence, consistent with a truncated Alu element (Figure 2C). The unique 52-bp insertion was a de novo event because targeted sequencing of exon 15 in parental DNA did not detect the insertion. The indel is predicted to change the coding sequence beginning at amino acid 583 with a truncation occurring after amino acid 667 (Figure I in the Data Supplement).

For the 2 identified variants to be the genetic cause of Jervell and Lange–Nielsen syndrome in this patient, they must be located in trans (on separate alleles) to one another. To determine whether this was the case, we used maternally inherited common single nucleotide variants (rs163149, rs163150, and rs1057128; Figures II and III and Table II in the Data Supplement) located upstream of exon 15 to demonstrate that the maternal common single nucleotide variants were on the same allele as the duplication/insertion (Figure IIIB in the Data Supplement; rs163149 shown as an example). Therefore, the proband inherited the variant encoding KCNQ1-R518X...
from her father, and the poly(A) insertion-partial exon 15 duplication was a de novo event that occurred on the maternally inherited allele (Figure 2D). The 2 variants were thus present in trans, establishing KCNQ1 compound heterozygosity as the pathogenesis for Jervell and Lange-Nielsen syndrome in this case.

**Reevaluation of NGS Data**

NGS uses massively parallel sequencing to generate short (50–100 bp in this case) reads that are then mapped to a reference sequence. Examination of the original NGS analysis showed a small number of reads that contained the poly-adenine sequence at the 5′ or 3′ edge of the short reads mapped to the reference KCNQ1 sequence. There was a drop-off in read depth at the insertion site, supporting the idea that the algorithm analyzing the NGS discarded reads containing the insertion near their center because they could not be aligned to the reference sequence. When the 52-bp duplication–insertion was included in a modified reference sequence, the insertion-containing variant reads mapped with high confidence, whereas reads from the paternal allele failed to map, leading to a drop-off in read depth (Figure 3).

**Summary**

We identified a novel LQTS variant that is likely composed of a severely 5′-truncated Alu element RNA intermediate insertion (hence the poly(A) tract) and a DNA target site duplication causing the partial exon 15 duplication. This unusual type of de novo insertion has not been previously reported in LQTS, either because it is rare or because it is not recognized by conventional testing. NGS has evolved as a low cost, efficient method to generate data on a large number of genes in parallel to screen a targeted panel of arrhythmia susceptibility genes in patients diagnosed with LQTS. Although current NGS algorithms detect single nucleotide variants with high confidence, insertion/deletion variants (indels) are not as sensitively detected. Genetic testing in cases with clear LQTS identifies a likely causative variant in 80% of cases. It is possible that a portion of the remaining 20% of cases without known genetic causes could result from intermediate-sized indels like the one described here. Alternatively, undiscovered LQTS-associated genes or deep intronic or regulatory variants that disrupt gene expression or transcript processing may also contribute.

**Acknowledgments**

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**Disclosures**

Dr Chung is a consultant for BioReference Laboratories. D. Macaya, Dr Konecki, and E. Venter are employed by GeneDx. The other authors report no conflicts.

**References**


**Key Words:** genetic testing □ Jervell and Lange-Nielsen syndrome □ KCNQ1 □ long QT syndrome □ next-generation sequencing
Figure 1. Electrocardiographic data. A, 12-Lead ECG showing normal sinus rhythm with broad, symmetrical T waves with corrected QT interval (QTc) 580 ms. B, Holter monitor showing postectopic QT >800 ms. C, Exercise test on metoprolol 100 mg/d. At end-exercise and early recovery, there is T wave lability in a nonalternans pattern and ventricular trigeminy. Torsades de Pointes then developed, persisting for 40 s before self-terminating. During recovery, T wave instability was once again seen.

Figure 2. Compound heterozygous variation in KCNQ1 identified by Sanger sequencing. A, Polymerase chain reaction (PCR) amplification of exon 15 visualized with ethidium bromide results in 2 distinct products in the proband that is absent in parental DNA. B, Allele-specific Sanger sequencing of smaller product, which shows normal (reference) sequence. C, Allele-specific Sanger sequencing of larger PCR product identifies a 52-bp insertion comprising a 15-bp partial duplication of exon 15 and insertion of a 36-adenine tract. D, Schematic representation of the compound heterozygous variants being located in trans to one another. JLNS indicates Jervell and Lange–Nielsen syndrome; and SNV, single nucleotide variant.
Figure 3. Next generation sequencing confirms complex 52-bp insertion by ad hoc alignment. Alignment of the next-generation sequencing reads to a reference sequence modified to include the identified insertion (modified reference sequence) confirms its presence. Gray horizontal bars indicate perfect sequence alignment. Note the decrease in read depth (top) at the insertion site, consistent with the rejection of reads that include the paternal allele.
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SUPPLEMENTAL MATERIAL

Supplemental methods

This study was reviewed and approved by the Institutional Review Board at Vanderbilt University Medical Center (Nashville, TN). All participants provided written informed consent.

Genomic DNA acquisition: DNA was extracted from peripheral blood using Puregene Chemistry on the Autopure LS robotic system according to manufacturer’s recommendations (Qiagen, product number 9001340).

GeneDx next-generation sequencing: Using genomic DNA from the submitted specimen, the coding regions and splice junctions of 12 arrhythmia-associated genes were enriched using a proprietary targeted capture system developed by GeneDx. These targeted regions were sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina platform with paired-end reads. Bi-directional sequence was assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants. Capillary sequencing was used to confirm all potentially pathogenic variants and to obtain sequence for regions where fewer than 15 reads were achieved by NextGen sequencing.

GeneDx exon array evaluation: Concurrent deletion/duplication testing was performed for the genes in the panel using exon-level oligo array CGH (ExonArrayDx). Data
analysis was performed using gene-specific filtering. Probe sequences and locations were based on human genome build GRCh37/UCSC hg19. Sequence and array CGH alterations were reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively.

*KCNQ1 Sanger sequencing evaluation:* Amplicons of each exon of *KCNQ1* were generated by PCR using the primer sets listed in supplemental table 1. The amplification was performed using Applied Biosystems Veriti 96-well thermal cycler with a melting temperature of 95°C, annealing temp listed next to each primer set in supplemental table 1, and extended at 72°C. Each reaction used 30 cycles for amplification.

*KCNQ1 allele-specific analysis:* PCR amplicons were placed in an expression vector using polyA cloning according to manufacturer’s protocol (Promega, catalog # A1360). DH5α competent E. coli were transformed and 10 individual clones were expanded for plasmid isolation and subsequent Sanger sequencing.
**Supplemental Table 1.** KCNQ1 primers used for Sanger sequencing

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<tr>
<th>Exon</th>
<th>Primer direction</th>
<th>5' -&gt; 3' sequence</th>
<th>Annealing temperature (°C)</th>
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<td>Forward</td>
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<td></td>
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<td>CTTCTGGACCATCAGACCTGA</td>
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<td>4</td>
<td>Forward</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>10</td>
<td>Forward</td>
<td>GCCTGGCAGAGGATGCTCCA</td>
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Reverse                  CAACTGCCTGAGGGGTTCT
Forward                  CTGTCCCCCACACTTTCTCCT

11
Reverse                  TGAGCTCAGTCCCCCTCCAG
Forward                  TGGCCACTCAACAATCTCCT

12
Reverse                  GCCTTGACACCCCTCCAATA
Forward                  GGCACAGGGAGGAGAAGTG

13
Reverse                  CGGCACCGCTGATCATGCA
Forward                  CGGGCAGTCAGCTGTCTGTC

14
Reverse                  CTCCCCCTTACTCCCTGGCTTTCA
Forward                  GGCCCTGATTTGGGTGTTTTA

15
Reverse                  CACAGGGAGGTCCTGCT
Forward                  CGACCGAGGGCCTTGCAGAC

16
Reverse                  CCCCCACACCGGCGCAGAGAGG

**Supplemental Table 2.** Common SNVs in KCNQ1 used to determine maternally-inherited allele. MAF = minor allele frequency.

<table>
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<td>G</td>
<td>A</td>
<td>0.200</td>
<td>G/A</td>
</tr>
</tbody>
</table>
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Supplemental Figures

Supplemental figure 1. Predicted KCNQ1 amino acid sequence resulting from frameshifting exon 15 insertion. Black text indicates the wild-type KCNQ1 amino acid sequence. Red text indicates insertion sequence. Underlined text highlights the amino acid changes resulting from the frameshifting insertion.
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Supplemental figure 2. Screening of the proband for common SNVs located between exons 12 and 15. Sanger sequencing results for SNVs with a MAF >10%. Red boxes indicate CNVs found to be heterozygous in the proband.
Supplemental figure 3. Establishing compound heterozygous variants in KCNQ1 are located on separate alleles. (A) Proband and maternal DNA possess common SNV rs163149 located 1,772 bp upstream of exon 15. PCR amplification was performed using Roche expand long template PCR system with forward primer 5’-CGTGTCTTTTTGTCCGCAG-3’ and reverse primer 5’-cacagggaggtgcctgct-3’(B)
Allele specific sequencing demonstrates the *de novo* insertion is present on the maternally inherited allele.