Detection of a Novel Complex Rearrangement in *KCNH2* with Next-Generation Sequencing in a Clinical Laboratory

John Garcia, PhD,[1] Amy Fuller, MS, LCGC,[1] Amy Daly, MS, LCGC,[1] Amanda Stafford, MS, ASCP(MB), CGMBS,[1] Dan Schmidt, CGMBS,[1] Michael Kennemer, MS,[1] Michelle Zeman, PhD,[1] Martin Powers,[1] Tom Callis, PhD,[1] Michael Gollob, MD, FRCPC,[2] Matteo Vatta, PhD, FACMG[1]

[1]Invitae Corporation, San Francisco, CA; [2]Toronto General Hospital, University of Toronto

Introduction

The frequency of complex rearrangements in ion channel genes associated with long QT syndrome (LQTS) has been difficult to assess. Historically, sequencing has been the standard for genetic testing in individuals with suspected LQTS, and evaluation for deletions and duplications was not performed routinely owing to cost and the need for additional technologies. Although sequence analysis for LQTS yields an approximately 75% positive detection rate, a significant proportion of affected individuals have no known explanation for their conditions. The advent of next-generation sequencing (NGS) has decreased the cost of testing and expanded its application to clinical practice, but the relative clinical contribution of copy number variations (CNVs) has not been determined conclusively.

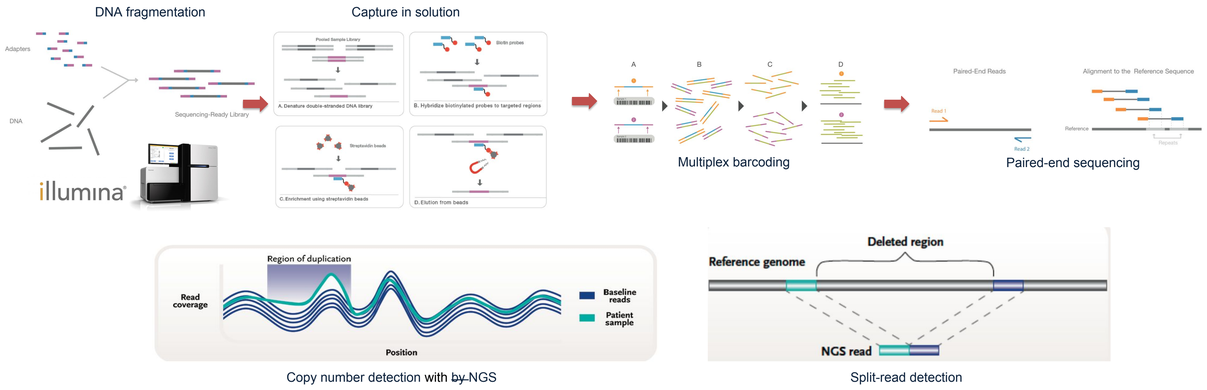
Case History

A 30-year-old Caucasian man with an incidental finding of QTc prolongation and no family history of cardiac symptoms was referred to Invitae for LQTS genetic testing. He was asymptomatic but had clear and persistent QT prolongation consistent with an LQT2 phenotype. Previous clinical testing performed by another laboratory, which included sequencing and multiplex ligation-dependent probe amplification analysis of the main ion channel genes known to cause LQTS, was negative. The clinician ordered a panel that includes 37 genes associated with common and rare forms of arrhythmia.

Methods

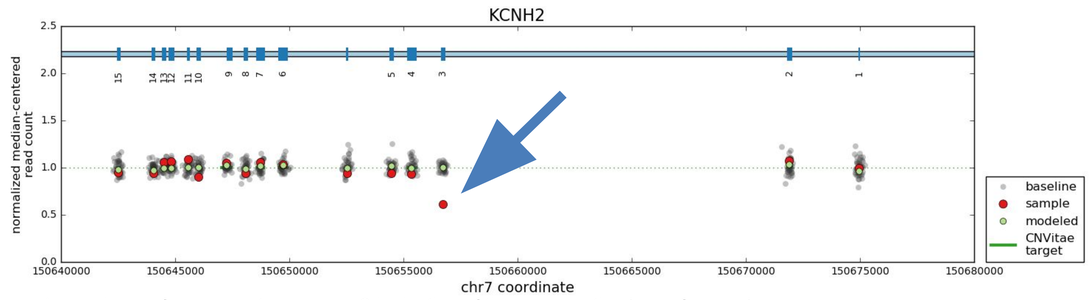
The patient was sequenced on an Illumina-based NGS platform and analyzed for sequence variants as well as deletions and duplications. Briefly, in this analysis, the DNA is sheared, ligated, and hybridized to probes, where it is then barcoded and subjected to paired-end sequencing (**Figure 1**). In addition to standard GATK-based alignments and analysis, validated coverage-based copy number detection algorithms as well as custom algorithms designed to flag possible split-read signals are applied to all samples. Once the split-read signal is manually verified by a member of the production bioinformatics team, the VCF is updated and the variants are sent to a member of the clinical genomics team for interpretation.

Figure 1. ***NGS and copy number variant methodology.***



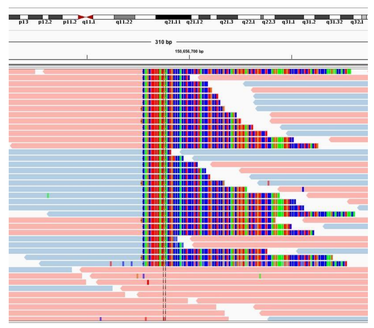
Results (cont.)

Figure 2. ***CNV analysis pipeline detected a single-exon deletion with NGS.***

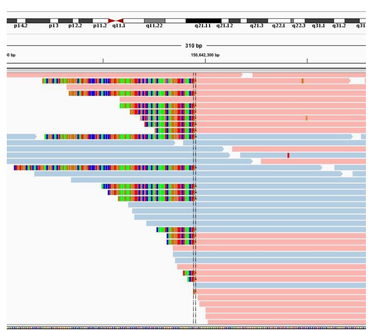


Arrow: Loss of read count over exon 3 indicative of the comparative loss of properly mapping reads.

Figure 3. ***Split-read analysis precisely characterized an inversion event.***

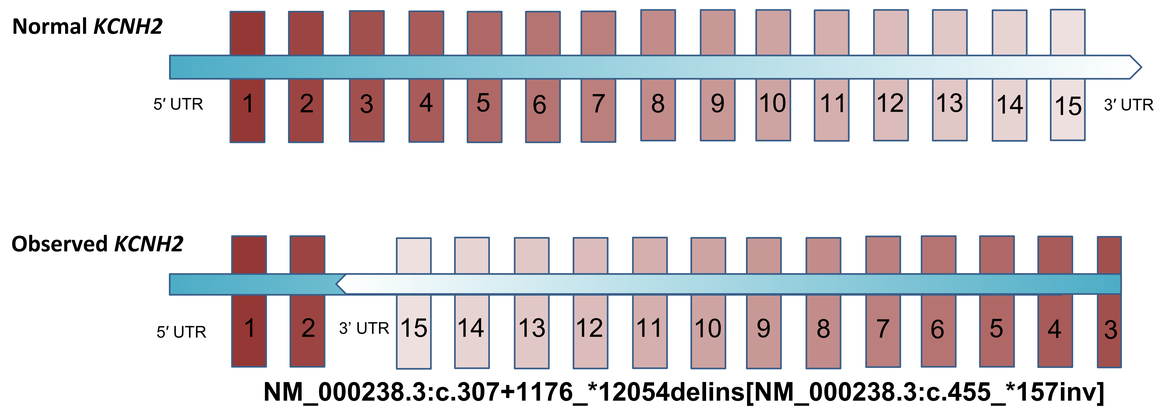


Exon 3 split-read signal



Exon 15 split-read signal

Figure 4. ***Combination of approaches revealed a novel complex rearrangement.***



Results

This routine testing identified a complex deletion and inversion in *KCNH2*, which led to the deletion of part of the intron 2–exon 3 boundary and inversion of the remainder of exon 3 through exon 17 (the entire remaining coding sequence). The variant was partially detected in our CNV analysis pipeline as a single-exon deletion (**Figure 2**). However, split-read signals and discordant mate-pair alignments allowed for more precise characterization of the mutation event, thereby enabling more accurate reporting and interpretation (**Figure 3**). This sequence change represents a complex rearrangement event leading to the heterozygous deletion of a portion of intron 2 and part of exon 3, while the remainder of exon 3 through the 3′ untranslated region (UTR) appears to be present but inverted with respect to the reference sequence. The change is expected to result in an absent or disrupted protein product (**Figure 4**). Using orthogonal Pacific Bioscience sequencing, we confirmed the exonic breakpoints and inversion identified with NGS and described the variant as NM_000238.3:c.307+1176_*12054delins[NM_000238.3:c.455_*157inv]. This variant was subsequently classified as pathogenic in accordance with the ACMG 2015 guidelines.

Conclusions

- The novel *KCNH2* inversion is consistent with a diagnosis of LQTS and is, to our knowledge, the first observation of such a complex rearrangement in an ion channel gene.
- This variant was detected using an NGS platform in a clinical laboratory as part of a routine comprehensive arrhythmia analysis. Without additional data, we are uncertain whether this event is exceedingly rare or whether such events are common but overlooked in otherwise apparently negative clinical cases.
- Increased use of high-resolution NGS in arrhythmias will eventually reveal the true frequency of complex events that likely remained undetected in the past.

Disclosures: JD, AF, AD, AS, DS, MK, MZ, MP, TC, and MV are employees and shareholders of Invitae.