Current challenges in the classification of large genetic events affecting *TTN*

INVITAE

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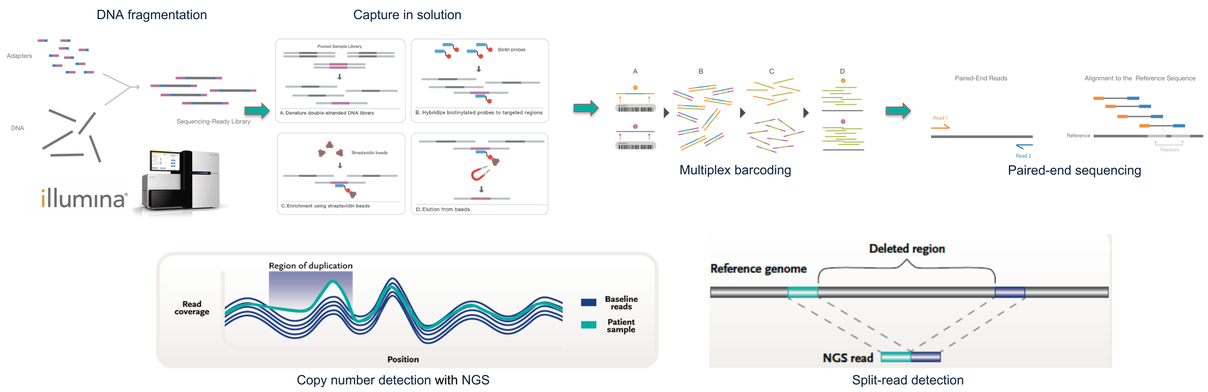
INTRODUCTION

The advent of next-generation sequencing (NGS) in clinical diagnostics has allowed for the unprecedented capability to test large genes, which previously represented a challenge by Sanger sequencing. Although the *TTN*-encoded protein titin was associated with dilated cardiomyopathy (DCM) by linkage analysis in 1999, it was only in 2012 that the first series of deleterious variants were identified in DCM patients. Since then, many pathogenic and likely pathogenic variants have been identified (mostly nonsense, frameshifts, and splice site-affecting variants). Here, we present the use of simultaneous sequence and exonic copy number variant (CNV) detection for *TTN* and discuss the challenges in interpreting CNVs. Thus far, no clinically actionable CNV have been described in the *TTN* gene.

METHODS

Samples were 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 Variant Call Format (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

In 5,688 unrelated individuals tested by a multi-gene panel for cardiac and neuromuscular diseases, we observed 101 total CNVs including three unique CNVs (one deletion and two duplications), which spanned multiple bands in three individuals (0.05%). Exons of *TTN* were numbered according to the metatranscript (NM_001267550.2).

A CNV involving the duplication of exons 197 to 199 highlights the first challenging issue with *TTN* variant interpretation. Many CNVs occur in the non-biologically functional isoform and add to the background noise of the gene instead of offering a cause for disease (supported by internal exome data). Due to the lack of evidence for CNVs in these non-functional isoforms, this variant was classified as a variant of uncertain significance (VUS).

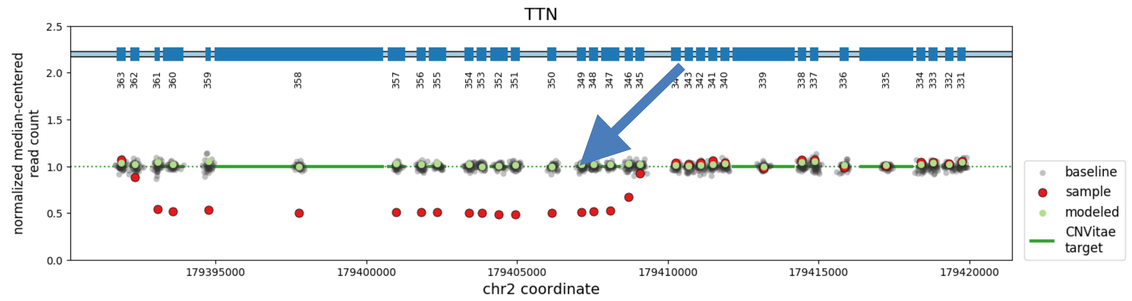
Further, we detected a duplication involving exons 243 to 295, coding for part of the I/A bands. We could not definitely determine whether the event represented a tandem duplication and thus classified this variant as uncertain. A similar duplication variant, affecting exons 244-295 has been previously reported (Ceyhan-Birsoy O *et al*., 2015) and also classified as VUS.

Finally, we detected a partial deletion of exon 346 (formerly 347, based on 364 exons count) with the full deletion of all subsequent exons through exon 362 (formerly 363, based on 364 exons count) by our CNV pipeline (**Figure 2**) and split-reads analysis (**Figure 3**). This variant abolished the last part of the A-band and almost the entire M-band and was classified as likely pathogenic.

These last two rearrangements affect exons present in both long (N2BA) and short (N2B) cardiac isoforms and also cross the boundaries between the I/A-bands and A/M-bands, respectively.

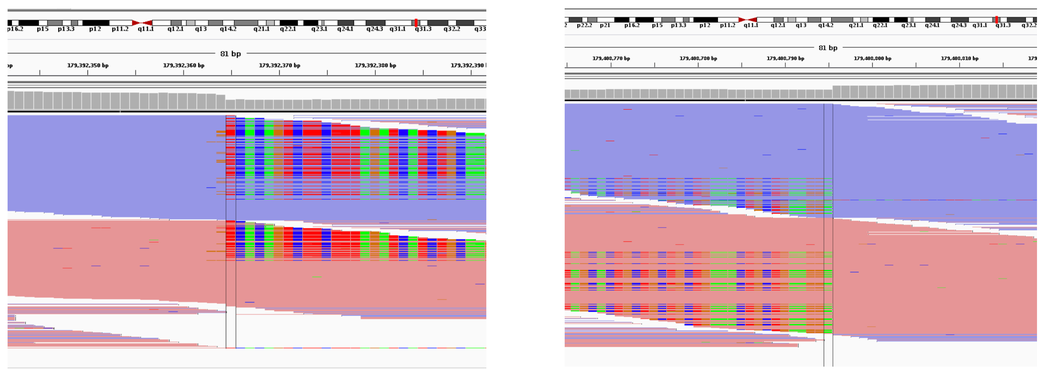
RESULTS (CONT.)

Figure 2. CNV analysis detected a multiple-exon deletion in *TTN* with NGS.



Arrow: Loss of read count over exon 346-362 indicative of the comparative loss of properly mapping reads.

Figure 3. Split-read analysis precisely characterized the ex346-362 *TTN* event.



Exon 362 split-read signal

Exon 346 split-read signal

Table 1. Summary of detected *CNV in TTN NM_001267550.2* in 5688 tested individuals using multi-gene panel for cardiac and neuromuscular diseases.

Event Type	Patients harboring events (%)	Events affecting single band	Classification	Events affecting multiple bands	Classification
Deletion	57 (1.0)	56 (I-Band)	VUS	1 (A/M Bands)	Likely Pathogenic
Duplication	44 (0.77)	43 (I-Band)	VUS	1 (I/A Bands)	VUS

CONCLUSIONS

- Our findings illustrate the novel challenge of classifying variants that span functional boundaries, especially within specific bands of titin known to be associated with neuromuscular or cardiac phenotypes. An additional challenge in properly interpreting these cross-band variants is that no good functional assay exists to help elucidate the effects of these rearrangements.
- Further evidence will be necessary to support accurate CNV interpretation with *TTN*, but these initial observations emphasize that a multi-gene panel can be effectively employed to reliably detect a broad range of variant types, including both sequence and copy number variants in *TTN*.

References: Ceyhan-Birsoy O, Pugh TJ, Bowser MJ, Hynes E, Frisella AL, Mahanta LM, Lebo MS, Amr SS, Funke BH. Next-generation sequencing-based copy number analysis reveals low prevalence of deletions and duplications in 46 genes associated with genetic cardiomyopathies. *Mol Genet Genomic Med*. 2015 Dec;4(2):143-51. [PMID: 27066507]

Disclosures: *MV, DB, RT, CT, HW, RH, RL, PT, JW, and TW are employees and shareholders of Invitae.*