

Abstract

Introduction: The clinical significance of exon-level copy number variation (CNV) in inherited cardiovascular conditions has not been widely studied to date. Sequence changes make up the vast majority of reported pathogenic variants in genes associated with inherited cardiomyopathies and arrhythmias.

Methods: We considered a sequential series of 287 patient samples submitted for an arrhythmia panel, 370 for a cardiomyopathy panel, and 36 for both panels. Germline DNA from blood was tested, and the gene panels were selected by ordering physicians based on each patient's clinical indication. Sequence and CNV analyses were performed with validated Next Generation Sequencing (NGS) methods, and identified CNVs were confirmed with microarray analysis.

Results: Six single- or multi-exon deletions were identified in patient samples: two deletions in MYBPC3 in two unrelated patients presenting with hypertrophic cardiomyopathy, two RYR2 deletions in two unrelated patients presenting with arrhythmia, a PKP2 deletion in a patient presenting with arrhythmogenic cardiomyopathy, and a CTNNA3 deletion in a patient presenting with cardiomyopathy. With the exception of CTNNA3, all deletions were classified as pathogenic, and no other pathogenic variants were identified in these individuals.

Conclusions: Previously, CNV analysis for these genes was unavailable clinically or was offered as a reflex test. In this case series, CNVs accounted for approximately 4% of the positive findings, which suggested that CNV analysis may be a key component of genetic testing for inherited cardiomyopathies and arrhythmias in the future.

Introduction

The implementation of NGS panels has significantly improved the ability to identify molecular causes of disease in genetically heterogeneous conditions such as cardiomyopathies and arrhythmias. Previously, CNV analysis for genes associated with cardiac conditions was unavailable clinically or was offered as a reflex test. As a result, the actual frequency of these types of events has been relatively unknown.

Two studies recently analyzed the frequency of CNVs in cardiomyopathy genes and revealed a detection rate of <1% (1, 2).

The purpose of this study was to evaluate the frequency of CNVs in patients presenting with cardiomyopathy, arrhythmia, or both, in an effort to clarify the contribution of CNV variants in these cardiac conditions.

Methods

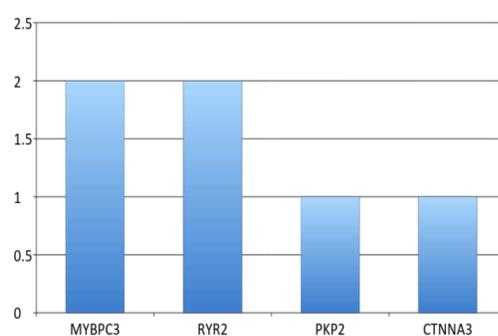
A sequential series of 693 patient samples were considered for sequence and deletion/duplication analysis of genes associated with inherited cardiomyopathies, arrhythmias, or both. Within this cohort, 287 patient samples were submitted for an arrhythmia panel, 370 for a cardiomyopathy panel, and 36 for both.

Germline DNA from blood was tested, and the gene panels were selected by ordering physicians based on each patient's clinical indication and the clinician's discretion. Sequence and CNV analysis with validated NGS methods allows for concurrent analysis of sequence variants and exonic CNVs, thereby providing a method for evaluating the contribution of CNVs in inherited cardiomyopathies and arrhythmias. All reported CNVs were confirmed with array CGH or an alternative method.

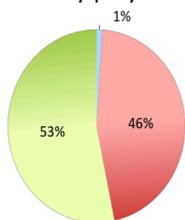
Results

Six single- or multi-exon deletions were identified in the patient samples:

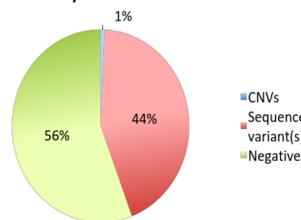
- two deletions in MYBPC3 in two unrelated patients presenting with hypertrophic cardiomyopathy
- two RYR2 deletions in two unrelated patients presenting with arrhythmia
- one PKP2 deletion in a patient presenting with arrhythmogenic cardiomyopathy
- one CTNNA3 deletion in a patient presenting with cardiomyopathy



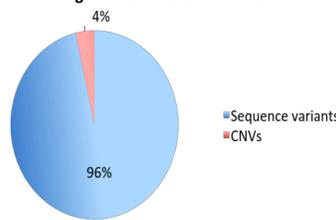
Cardiomyopathy cohort variants



Arrhythmia cohort variants



Distribution of Pathogenic or Likely Pathogenic Variants within cohort



With the exception of CTNNA3, all deletions were classified as pathogenic, and no other pathogenic variants were identified in these individuals.

Of note, a recurrent partial gene deletion in the RYR2 gene was identified in two unrelated individuals in our cohort. This deletion is a key contributor to disease in patients presenting with arrhythmia, arrhythmogenic cardiomyopathy, or both. This event results in an in-frame deletion, which follows the gain-of-function mechanism for RYR2 pathogenic variants (3).

Variant description of the pathogenic RYR2 exon 3 deletion:

- This variant is a gross deletion of the genomic region encompassing exon 3 of the RYR2 gene and leads to an in-frame deletion, preserving the integrity of the reading frame.
- Deletions that encompass exon 3 of the RYR2 gene have been reported in individuals affected with noncompaction cardiomyopathy and catecholaminergic polymorphic ventricular tachycardia and have been shown to segregate with the disease in families (3–6).
- Experimental studies have shown that this in-frame deletion changes the channel properties of RYR2 by markedly reducing the luminal Ca²⁺ threshold at which Ca²⁺ release terminates (7).
- For these reasons, this variant has been classified as pathogenic.

CNV analysis via NGS

Invitae uses a proprietary, validated algorithm to detect deletions and duplications with NGS. The algorithm calls exonic deletions and duplications by calculating the statistical likelihood of each copy number state through comparison of the depth of sequence coverage at targeted exons to depth measured from a set of baseline samples (8).

All reported deletions or duplications are confirmed with alternative methods of analysis.

Figure 1: Read-depth approach to deletion/duplication analysis by NGS. Example of a duplication.

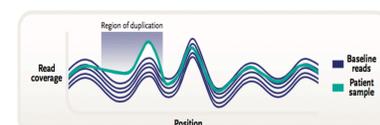
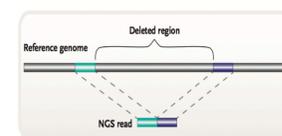


Figure 2: Split-read approach to deletion/duplication analysis by NGS.



Split-read: NGS reads that span the breakpoint of a deletion or duplication will show distinct patterns when mapped to the reference genome, and these patterns are detected by a process called split-read analysis (Figure 2). Some events detected by split-read analysis are exactly those that are harder for read-depth approaches, such as events that start or end in the middle of an exon. Not only do the two approaches complement each other, but used together they can resolve novel complex events that can be difficult for probe-based methods like qPCR, MLPA, or microarrays.

Conclusion

In this case series, CNVs accounted for approximately 4% of the positive findings. Overall, CNVs were observed in 6 of 693 (0.86%) patient samples sent for a cardiomyopathy panel, an arrhythmia panel, or both. These results are consistent with the findings of previous studies. Although CNVs do not account for a majority of causal variants for these indications, their contributions are of value when pursuing a molecular diagnosis.

These results suggest that CNV analysis is an important component of genetic testing for inherited cardiomyopathies and arrhythmias. Because improvements in technology have rapidly changed the genetic testing process, the cost of CNV analysis should no longer be considered a significant barrier to testing.

References

1. Lopes, LR, et al. *Eur J Med Genet.* 2015; 58(11):611-6.
2. Ceyhan-Birsoy, O, et al. *Mol Genet Genomic Med.* 2015; 4(2):143-51.
3. Medeiros-Domingo, A, et al. *J Am Coll Cardiol.* 2009; 54(22):2065-74.
4. Bhuiyan, ZA, et al. *Circulation.* 2007; 116(14):1569-76.
5. Marjamaa A, et al. *BMC Med Genet.* 2009; 10:12.
6. Ohno, S, et al. *Europace.* 2014; 16(11):1646-54.
7. Tang, Y, et al. *Circ Res.* 2012; 110(7):968-77.
8. Invitae White Paper. Detecting deletions and duplications using next-generation sequencing (NGS) (WP100-2). <https://resources.invitae.com/h/i/235702135-invita-white-paper-deletions-duplications-wp100-2>