Breakpoint Analysis Method for Structural Variant Calling Improves Diagnostic Yield of Ophthalmology Genetic Testing

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Purpose

- Structural variants (SVs) are a diverse group of large (>50 bp) genomic alterations, including deletions, duplications, insertions (including retrotransposable elements [RTEs]), inversions, translocations, and other complex rearrangements.
- Clinically relevant SVs have been identified in genes causing Mendelian disorders; however, they may be undetected by standard gene panel testing as short-read sequencing is inadequate to detect SVs.
- We investigated the additional positivity rate of an internally developed read breakpoint analysis method for SV detection in ophthalmology gene panel testing.

Methods

- DNA samples from consecutive patients referred for ophthalmology panels were target-sequenced with the Illumina platform (short reads).
- An internally developed read breakpoint analysis method combining read-pair orientation–based and split-read/clipped-read–based methods was used for SV calling.
- This method can detect deletions, tandem duplications, and inversions >50 bp in size, as well as RTE insertions such as Alu, SVA, and L1 with resolutions of up to a few base pairs.
- Only variants classified as pathogenic (P), likely pathogenic (LP), or of uncertain significance (VUS) that were not detected by standard variant calling methods were included in the study.
- Reported SVs were confirmed using quantitative PCR, digital PCR, long-range PCR, Sanger sequencing, or additional bioinformatic analyses.
- Variant interpretation aligns with ACMG/ClinGen guidelines. A positive result was defined as a P/LP/VUS-high variant(s).[1,2]

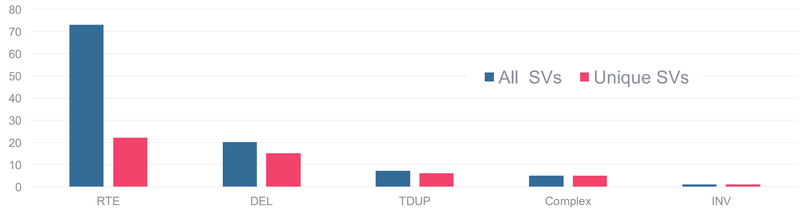

Figure 1. The number of different SVs detected by the breakpoint analysis method but not detected by other variant calling methods.
Complex, complex event; DEL, deletion; INV, inversion; RTE, retrotransposon element; SV, structural variant; TDUP, tandem duplication.

Almost 4/5 of the clinically relevant SVs detected with this method supported a genetic diagnosis.

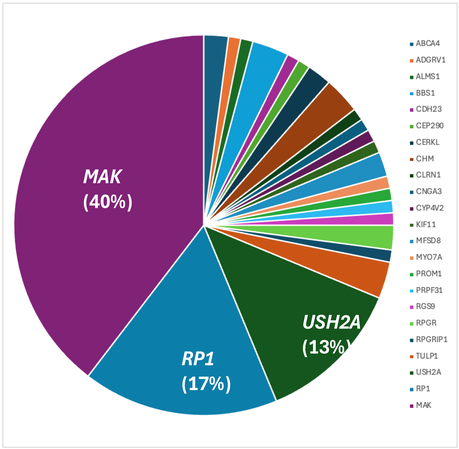

Figure 2. Distribution of P/LP SVs (n=96) in different genes.

Results

- Of the 22,280 individuals tested, a total of 106 (49 unique) SVs were detected by the breakpoint analysis method (**Figure 1**).
- All SVs were identified in genes related to inherited retinal dystrophy (IRD). Of the 106 SVs, 81 (76%) contributed to positive findings and 25 (24%) were reported as additional findings (phenotype discordance or no second variant in AR gene).
- A positive result was reported for 54% of patients tested, and SVs that were additionally detected by the breakpoint analysis method contributed to 0.7% of positive findings.
- Of the 49 unique SVs, 39 (80%) were classified as P or LP, and Alu insertions were the most common type of SV, followed by deletions involving almost exclusively partial exons.
- SVs were identified in a total of 31 different genes and P/LP SVs were distributed in 23 genes (**Figure 2**).
- The most common recurrent SVs were Alu insertions in *MAK* c.1297_1298insAlu and *RP1* c.2321_2322insAlu.

Conclusions

- Our study indicates that SVs additionally detected by the breakpoint analysis method contributed to 0.7% of positive findings in patients tested with an ophthalmology panel.
- These results suggest that incorporating additional SV calling methods into standard IRD gene panel testing is useful, as multiple clinically relevant SVs were identified in IRD genes.

References:
1.Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015 May;17(5):405-24.
2.Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med*. 2020 Feb;22(2):245-257.

Conflict of interest statement: All authors are employed by Blueprint Genetics