Beyond genomics: Using RNA-seq in filter cards to unlock the clinical relevance of non-coding variation in splicing

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Background

Clinical exome and genome sequencing are first line diagnostic methods in rare diseases with diagnostic yields going up to 60%.

Still variants of unknown significance (VUS) account for up to 5% of reported variations in ClinVar database.

Here we assessed the impact of splicing variants reported in our laboratory using RNA Sequencing experiments (RNA-seq) to gain insight into their clinical relevance.

Methods

A total of 108 consecutive patients with 113 splicing variants reported after exome/genome sequencing were selected for RNA sequencing (RNAseq).

A protocol was developed to perform RNAseq using filter card (dried blood spots – DBS), library preparation and bioinformatic pipeline analysis. Relative gene expression was calculated using house keeping genes and compared against controls.

Splicing patterns in cases were inspected using IGV interactive program and adding three independent controls.

Results and Discussion

Forty-nine variants were located at canonical intronic positions 1-2 (43%), 64 (57%) were affecting other intronic regions (up to 824 bps).

Eighty-one variants were heterozygous (72%), 26 were homozygous (23%) and six were hemizygous variants (5%).

Of the 113 variants, 66 (58%) were confirmed as leading to abnormal splicing (with a minimum of 5 supporting reads), 15 (13%) did not have any evident splicing effect and 33 (29%) were inconclusive (Figure 1). The main reason for the inconclusiveness was due to insufficient coverage of the area.

Selected examples of different types of aberrant splicing variants are presented in Figures 2 and 3.

Figure 1: Results of splicing assessment of variants using RNASeq

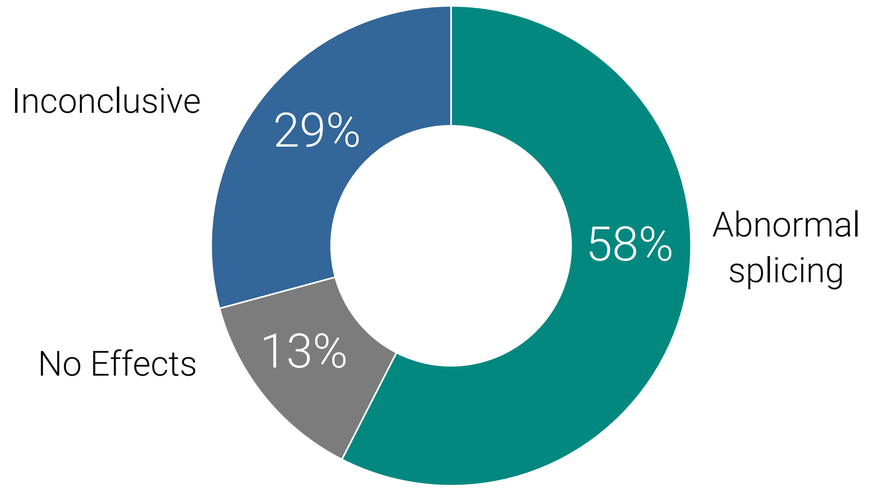
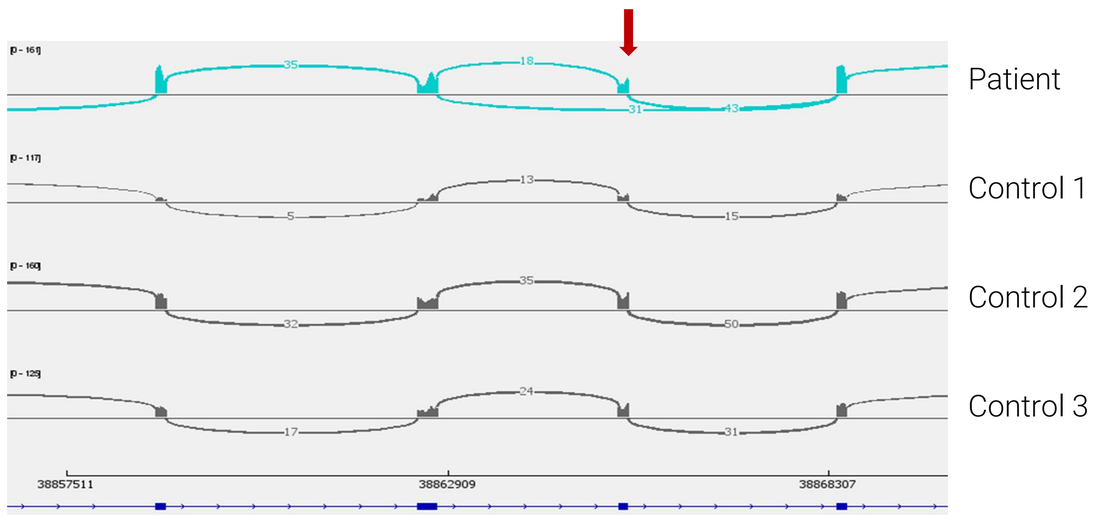


Figure 2: Splicing variant at NM_001396.3:c. 1098+2T>C, Intron 07 *DYRK1A* gene



- *DYRK1A-related* intellectual developmental disorder type 7 (autosomal dominant)
- Heterozygous splicing variant that leads to exon 7 skipping in 50% of reads

Figure 3: Splicing variant at NM_022575.3:c. 2375+1G>T, Intron 23 *VPS16* gene

- *VPS16* associated dystonia (autosomal dominant)
- Heterozygous splicing variant that leads to loss of natural splice site, intron retention and alternative splice site in exon 23

Conclusions/Outlook

We propose a method for a systematic experimental evaluation of the splicing impact of intronic variants, integrated in diagnostic exome/genome sequencing, which impact the assessment of their clinical relevance. **The approach can be implemented in the routine workflow by diagnostic laboratories, adding an additional -omics layer to the diagnosis of rare disorders.**

Disclosure of Conflict of Interest
This study was sustained in part by CENTOGENE GmbH, Rostock, Author of the presentation Dr. Bertoli-Avella, and all co-authors are employees of CENTOGENE GmbH, Rostock, Germany.