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Poster Abstract: Diagnostic

Identification and Characterization of Aberrant Splicing in Pompe Disease Using a Generic Approach

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BACKGROUND

Identification of pathogenic variants in monogenic diseases is an important aspect of diagnosis, genetic counseling, and prediction of disease severity. Pathogenic mechanisms involved include changes in gene expression, RNA processing, and protein translation. Variants affecting pre-mRNA splicing are difficult to predict due to the complex mechanism of splicing regulation. A generic approach to systematically detect and characterize the effects of sequence variants on splicing would improve current diagnostic practice.

MATERIALS AND METHODS

By combining flanking exon RT-PCR, sequence analysis of PCR products, and exon-internal quantitative RT-PCR for all coding exons, we show that such an approach is feasible.

RESULTS

Application of this approach to six previously published and one novel variant(s) in the acid alpha-glucosidase gene causing Pompe disease enabled detection of a total of 11 novel splicing events. Aberrant splicing included cryptic splice site usage, intron retention, and exon skipping. Importantly, the extent of leaky wild-type splicing correlated with disease onset and severity.

CONCLUSION

These results indicate that this approach enables sensitive detection and in-depth characterization of variants affecting splicing, many of which are still unrecognized or poorly understood. The approach is generic and should be adaptable for application to other monogenic diseases to aid in improved diagnostics.

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