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Determining the pathogenicity of genetic variants affecting splicing in Mendelian disorders

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Motivation and Aim: Despite the success of whole-exome sequencing (WES) in the diagnosis of Mendelian disorders, ~50–75 % of the patients still do not receive a genetic diagnosis [1]. One of the reasons for this is the limitation of bioinformatic approaches to detect pathogenic variants of the nucleotide sequence, especially in noncoding regions. It is now known that mutations affecting splicing can cause the Mendelian disorder. But because of the complexity of splicing regulation, it is not always possible to predict accurately the effect of nucleotide variants on splicing events and RNA structure. In this work, we focused on functional analysis of genomic variants affecting splicing in a variety of Mendelian disorders.
Methods and Algorithms: To determine the effect of mutations we used two experimental approaches: (1) RT-PCR from available patient's samples and (2) *in vitro* minigene assay. For different cases, we performed one or both methods. Human Splicing Finder and IntSplice on-line tools were used to predict the effect of different nucleotide variants on pre-mRNA splicing.
Results: We analyzed >27 previously uncharacterized genetic variants in >12 genes, associated with different Mendelian disorders. These variants are located in both exons and introns and mostly were classified as variant of unknown significance (VUS). We determined the effect of these variants on mRNA structure; it allowed us to classify most of them as pathogenic and to make assumption of the mechanisms involved in the molecular pathogenesis of diseases (e. g. RNA degradation by NMD, disruption of functional domain of protein). Additionally, we compared our experimental data with prediction tools for splicing events and revealed that it is not always possible to predict accurately the effect of mutation on splicing.
Conclusion: Although it is now known that mutations affecting splicing can cause the Mendelian diseases, however their contribution may be underrepresented due to limitation of diagnostic procedures. To prove the pathogenicity of these mutations, additional functional analysis is often required.

References

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