Solving the puzzle of deep intronic splicing variants as missing elements in the genetic landscape of Usher syndrome

If DNA of a single human cell was placed end to end, it would span a distance of about 2 meters. However, DNA fragments encoding proteins, being the most important building and functional components of our body, encompass less than 3 centimeters of the genome. Since the impairment of protein structure and function leads to genetic diseases, for a long time genetic studies were mainly oriented at deciphering protein coding DNA fragments, bypassing the analysis of non-coding areas.

Despite the overall methodological progress in DNA sequencing, i.e. the determining the order of nucleotides within DNA, in about half of patients with genetic disorders no genetic variants causative of the disease are found. As a result, more and more importance is now attributed to the role of non-coding gene sequences – introns, and to the genetic variants located within them, referred to as deep intronic variants. These variants seem to have a much more significant contribution to genetic disorders than previously thought. The most common pathogenic mechanism associated with the occurrence of deep intronic variants is their disruptive effect on RNA splicing process, which is the mechanism of excision of introns from RNA molecules indispensable for forming functional proteins.

An example of a severe genetic disorder with many genetically unsolved cases is Usher syndrome, a progressive disease characterized by concomitant hearing and vision loss. Based on the latest reports, we believe that in the Usher syndrome patients without conclusive genetic diagnosis the cause of the disease lies within the intronic sequences of the DNA. The main objective of the proposed project is to identify new pathogenic deep intronic variants in patients with Usher syndrome. To achieve this goal, the project involves the use of the latest high-throughput DNA sequencing technologies, including sequencing of the entire genome of patients with Usher syndrome. The use of advanced bioinformatic tools will allow us to determine which of the detected genetic variants may affect the RNA splicing and contribute to the development of the disease. The results of these analyzes will be confirmed by functional *in vivo* studies focused on unraveling the effect of deep intronic variants on RNA splicing using a *minigene* model based on human cell culture. Introduction of the *in vivo* model will allow us to reveal the nature of new aberrant splicing events and to classify the severity of the newly discovered deep intronic variants.

The research project will provide a wider perspective on the genetic landscape of Usher syndrome, enable to expand the knowledge on DNA pathogenic variation and contribute to genetic diagnosis in unsolved Usher syndrome cases. Considering the comprehensive design of the project including the study of all known genes causative for different clinical types of Usher syndrome, we expect to discover a novel genetic background underlying the disease.

The approach introduced in this study has a potential to be routinely applied in the future to unravel the molecular background of unsolved cases with different genetic disorders. Results of the study will open new paths aiming at designing gene therapies that have an attainable chance of being introduced into clinical use in the future.