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Molecular Characterization of a Novel Mucopolysaccharidosis type VI-causing Mutation - Indirect Proof of Principle on its Pathogenicity

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Introduction: With its unprecedented throughput, scalability and speed, next-generation sequencing (NGS) is revolutionizing clinical research. Targeted sequencing in particular is now implemented in many labs. While being well known that this unparalleled capacity is speeding up molecular diagnostics, it is also true that whenever a novel variant is detected, its pathogenicity must be carefully assessed and every now and again, a case pops up to highlight how tricky and delicate this process can be. Here we present a case of a molecular diagnosis of a patient with a clinical suspicion of Mucopolysaccharidosis (MPS) type VI, where even though the causal mutation was easy to detect by both Sanger and NGS, only through indirect studies could we present proof of principle on its pathogenicity. Methods: Initial studies were performed in gDNA, by classical sequencing of the ARSB gene, encoding arylsulfatase B, the enzyme deficient in MPS VI. Additional analyses included segregation studies, cDNA sequencing and NGS with in a custom gene for Lysosomal Storage Diseases (LSD) that includes 86 genes implicated in lysosomal function. Results and Discussion: Sanger sequencing of all ARSB exons and their intronic flanking regions unveiled the presence of a novel c.1213+5G>T [IVS6(+5)] homozygous mutation, with several bioinformatic predictors supporting its pathogenecity. Moreover, segregation studies confirmed its presence in heretozygozity in both parents. Still, only after a proper cDNA analysis could we confirm its effect in splicing. Unfortunately, we only had access to an extremely degraded cDNA sample obtained from blood of one of his parents. Surprisingly however, the splicing pattern observed after cDNA amplification of that sample was absolutely normal.
Thus, the case was included in a set of samples subjected to a NGS-based workflow for the identification of LSD-causing variants. After variant calling, it became clear that the IVS6(+5) ARSB mutation was the most probable cause for disease. Still, its pathogenicity had yet to be proven. We then conducted a classical sequencing approach of the ARSB gDNA and cDNA on the proband's father and ended up demonstrating that, while being heterozygous for a few SNPs in the surroundings of the mutation, the same individual seemed to be wild-type homozygous for those exact same SNPs at cDNA level. This observation provided indirect proof of the mutation's effect on splicing, further suggesting that the mutant transcript is degraded by nonsense-mediated mRNA decay (NMD). Overall, this case reminds us that, whatever the technology we use, genetic testing still needs much perseverance and cunning strategies to identify the causative mutation(s).
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