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ABSTRACT SUBMISSION FORM

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ABSTRACT

Abstract title: Molecular Characterization of a Novel Mucopolysaccharidosis (MPS) type VI-causing Mutation – Indirect Proof of Principle on its Pathogenicity

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Text body: Introduction: With its unprecedented throughput, scalability and speed, next-generation sequencing (NGS) is revolutionizing clinical research. Targeted sequencing in particular is now available in many labs. Still, 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 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: After both Sanger and NGS, the novel c.1213+5G>T ARSB homozygous mutation was detected as the most probable cause for disease, with several bioinformatic predictors supporting its pathogenecity and segregation studies confirming its presence in heretozygozity in both parents. Still, we only had access to an extremely degraded cDNA sample obtained from blood of one of his parents and after ARSB amplification, its splicing pattern observed was surprisingly normal. 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 SNP in the surroundings of the mutation, the same individual seemed wild-type homozygous for that exact same SNP at cDNA level. This observation provided indirect proof of the mutation's effect on splicing, suggesting that the mutant transcript is degraded by nonsense-mediated mRNA decay (NMD). We are currently performing in vitro analysis using a minigene assay to further confirm its pathogenic mechanism. 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). Acknowledgements This work was partially supported by Fundação Millennium bcp and N2020 (bcp/LIM/DGH/2014;NORTE2020/DESVENDAR/DGH/jn2016). MFC and JIS are grantees from the FCT (SFRH/BPD/101965/2014;SFRH/BD/124372/2016).

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