

Functional analysis of SNVs affecting splicing in congenital aniridia

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Motivation and Aim: A congenital aniridia (OMIM 106200) is a rare autosomal dominant panocular disorder caused by mutations in the *PAX6* gene or chromosome 11p13 rearrangements. Haploinsufficiency due to LoF mutations in *PAX6* gene is thought to be the main mechanism of congenital aniridia etiopathogenesis. Mutations affecting splicing are known to be a cause of Mendelian disorders, but their pathogenic status cannot always be provided bioinformatically. This is a reason why some aniridia associated *PAX6* nucleotide variants remain outside the mutations database. Minigene splicing assay *in vitro* allowed us to analyze functional consequences of six different intronic sequence variants, one missense and one synonymous substitution in *PAX6* gene found in patients with congenital aniridia.

Methods and Algorithms: DNA samples for analysis were obtained from patients with congenital aniridia (110 patients from 84 unrelated families). The search for mutations in the *PAX6* gene was carried out by Sanger sequencing, MLPA and analysis of heterozygosity loss (LOH) in proband. Human Splicing Finder and IntSplice on-line tools were used to predict the effect of identified SNVs on *PAX6* pre-mRNA splicing. To confirm the effect of SNVs on splicing experimentally we used *in vitro* minigene assay.

Results: Molecular analysis of a large cohort of aniridia patients from Russia conducted earlier in RCMG revealed a significant proportion of *PAX6* mutations affecting splicing (17.3 %). We focused on 8 SNVs located out of canonical splicing site dinucleotides: 6 deep-intronic and 2 exonic. These variants were classified as variant of unknown significance (VUS), benign or likely pathogenic according to ACMG recommendations. On-line prediction tools analysis revealed that they could influence *PAX6* pre-mRNA splicing. *In vitro* minigene assay showed that all investigated sequence variants except one affected splicing. The variants resulted in open reading frame shifting, premature termination codon formation followed by aberrant mRNA degradation by nonsense-mediated decay. Thus, investigated SNVs produce a null allele and haploinsufficiency of the *PAX6* function. So investigated mutations were reclassified as loss of function.

Conclusion: Using functional *in vitro* analysis we confirmed the pathogenicity of 7 *PAX6* mutations affecting splicing. Our results emphasized the necessity of such an analysis and advanced search for *PAX6* mutations.

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