P02.10A / A - *PAX6* sequence variants affecting splicing cause congenital aniridia

Itinerary

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Authors

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Disclosures

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Abstract

Introduction: Aniridia is a rare autosomal dominant panocular disorder caused by mutations in the *PAX6* gene or chromosome 11p13 rearrangements. **Materials and Methods:** 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. To determine the effects of identified SNVs on PAX6 pre-mRNA splicing we used *in vitro* minigene assay. **Results:** Molecular analysis of a large cohort of aniridia patients from Russia conducted earlier revealed a significant proportion of *PAX6* mutations affecting splicing (14 from 81 mutations). We focused on 8 SNVs affected slicing: 6 deep-intronic and 2 exonic. These variants were classified as variant of unknown significance (VUS), benign or likely pathogenic according to ACMG recommendations. Human Splicing Finder and IntSplice on-line tools analysis predict them to disrupt PAX6 pre-mRNA splicing. To validate this hypothesis we used a minigene system and showed that all investigated sequence variants except one affect splicing. These variants result in open reading frame shifting, premature termination codon formation following by RNA degradation by nonsense-mediated decay. Thus, investigated SNVs produce a null allele and haploinsufficiency of the *PAX6*-function. So putative mutations were reclassified as loss of function. **Conclusions:** Using functional *in vitro* analysis we confirmed the pathogenicity of 7 *PAX6* mutations affecting splicing. Our results emphasized the necessity of such analysis and advanced search for *PAX6* mutations. This work is supported by RFBR grant 17-04-00475.