

Three N-terminal nonsense mutations associated with distinct disease phenotypes result in secretory defect of interphotoreceptor retinoid-binding protein

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The interphotoreceptor retinoid-binding protein (IRBP) is an extracellular matrix protein encoded by the retinol binding protein 3 (RBP3) gene on chromosome 10. Located within the interphotoreceptor matrix (IPM), the subretinal space surrounded by the photoreceptor neurons and the retinal pigment epithelium (RPE), IRBP mediates the bidirectional transport of all-*trans* and 11-*cis* retinoids between the RPE and photoreceptors. This transport is crucial for the visual cycle, a biochemical pathway essential for regenerating the photosensitive visual pigments that initiate vision in response to light. Using a new mouse model carrying human IRBP D1080N mutation, we demonstrated that IRBP supports color vision and protects retina from oxidative and inflammatory damage (Li et al., 2026). In humans, mutations in the RBP3 gene can lead to various retinal dystrophies. Recent studies have identified several nonsense mutations in the human RBP3 gene, including W211X (Georgiou et al., 2024), Y221X (Hanany et al., 2020), and K268X (Aguayo-Merly and Izquierdo 2025). Patients with the mutations exhibit various clinical phenotypes such as early-onset reduced visual acuity, high myopia, retinal thinning, peripheral vision loss and retinal ischemia.

The pathogenic mechanisms of these specific mutations are yet unknown. The purpose of this study is to determine the effects of these mutations on secretion, stability, cellular localization and function of IRBP. Through site-directed mutagenesis, we introduced W211X, Y221X, and K268X mutations individually into the human RBP3 cDNA cloned in the pRK5 mammalian expression vector. Following transfection of wild-type and the mutant IRBP constructs into the 293T cells, we performed immunoblot analysis of the cells and their media. Cells transfected with pRK5 mock vector were used as negative control. We observed that wild-type IRBP was abundantly secreted into the media of the transfected cells. However, none of the three mutants was present in the media. In contrast, all IRBP proteins including wild-type and the three mutants were present in the cell lysates, suggesting that the IRBP mutants are not secreted most likely due to misfolding. To test whether the mutant proteins are misfolded, we performed immunoblot analysis without treatment of the cells with dithiothreitol (DTT), the reducing reagent used to cleave disulfide bonds between cysteines. Under this condition, the mutant proteins migrated slowly and formed high molecular ladders in the gel, suggesting that they formed high molecular weight complexes. Next, we analyzed the effects of serum concentration on the secretion and stability of the IRBP mutants. We found that although serum concentrations did not influence secretion of the mutants, low serum concentration increased stability of the W211X mutant. We are now analyzing subcellular localization of the mutant proteins in the transfected cells.

In conclusion, our results indicate that W211X, Y221X and K269X mutations resulted in a severe defect in secretion of IRBP. The mutant proteins are misfolded in the cells, possibly due to abnormal inter- and intra-protein formation of the disulfide bonds. These results, together with the distinct clinical phenotypes, suggest that other genetic and/or non-genetic factors affect the disease phenotypes and underlying pathogenic mechanisms of the mutations. Future directions include testing the functionality of the IRBP mutant proteins and understanding their mechanistic role in pathogenesis of retinal dystrophy and/or myopia.

References

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