

Isabella Y. Bartholomew
LSU Health New Orleans School of Medicine

Mentors: Minghao Jin, Ph.D. and Songhua Li, Pharm.D.
LSUHSC, Neuroscience Center of Excellence

**Distinct effects of three disease-causing mutations on secretion and stability of
Interphotoreceptor Retinoid-Binding Protein**

Interphotoreceptor Retinoid-Binding Protein (IRBP) is an extracellular glycoprotein secreted by photoreceptors in the retina that is essential to the visual cycle, a biochemical pathway that regenerates the 11-*cis*-retinal chromophore of the opsin visual pigments and confers light sensitivity to photoreceptors (Zeng et al., 2020). IRBP localizes to the interphotoreceptor matrix and facilitates bidirectional trafficking of all-*trans* and *cis*-retinoids between photoreceptors and the retinal pigment epithelium, in addition to protecting retinal photoreceptor cells from photooxidative damage by preventing photooxidation of retinoids (Li et al., 2026). More than 40 mutations in the IRBP gene have been found in patients with retinitis pigmentosa (RP)—an inherited, degenerative disease that degrades the retina and eventually results in loss of vision—as well as childhood-onset retinal dystrophy with high myopia (CRDHM) and cone-rod dystrophy (Li et al., 2026). Recently, three nonsense mutations have been identified in patients with RP (R388X and W465X) or CRDHM (Y510X) (Vargas et al., 2022) and (Georgiou et al., 2024). However, pathogenicity and the underlying pathogenic mechanisms of these mutations are unknown.

In the present study, we investigated the effects of these three mutations on secretion, stability, and subcellular localization of IRBP in order to identify the potential pathogenicity and the disease mechanisms of the mutations. Through site-directed mutagenesis, we introduced R388X, W465X, and Y510X mutations individually into the human IRBP cDNA cloned in the pRK5 mammalian expression vector. These three mutant clones, as well as the wild-type human IRBP (positive control) and pRK5 mock (negative control) plasmids were then transfected into 293T cells. Through Western blot analysis of the cell media and cell lysates, we determined which, if any, of the IRBP mutants were secreted into the media. A rabbit anti-IRBP antibody and Horseradish Peroxidase (HRP)-conjugated anti-rabbit antibody were used as the primary and the secondary antibodies, respectively. We detected wild-type IRBP (133 kDa) and R388X mutant (~45 kDa) in the media. However, both W465X and Y510X mutants were undetectable in the media. Analysis of cell lysate samples showed that all mutants including W465X (~55 kDa) and Y510X (~60 kDa) were contained within the cells. This data indicates that R388X, but not W465X and Y510X, mutant IRBP was secreted. In the absence of dithiothreitol (DTT) treatment—which cleaves the disulfide bond between cysteines—the mutant proteins migrated slowly and formed high molecular ladders in the gel. This result suggests that the mutant IRBP proteins formed high molecular weight complexes via disulfide bonds.

We are now analyzing the subcellular localization of the mutant proteins in the transfected cells and identifying physical and chemical factors that influence secretion of the mutant proteins. We hope these experiments will provide new insight into the pathogenic mechanisms and the development of rescue strategies for the mutations.

References:

1. Zeng S, Zhang T, Madigan MC, Fernando N, Aggio-Bruce R, Zhou F, Pierce M, Chen Y, Huang L, Natoli R, Gillies MC, Zhu L. Interphotoreceptor Retinoid-Binding Protein (IRBP) in Retinal Health and Disease. *Front Cell Neurosci.* 2020 Nov 19;14:577935. doi: 10.3389/fncel.2020.577935. PMID: 33328889; PMCID: PMC7710524.
2. Li S, Sato K, Jin M. RGR-Mediated Photopic Visual Cycle and Oxidative Stress: Potential Mechanisms for Cone Vision Impairment and Retinal Degeneration in Retinitis Pigmentosa Linked to D1080N-IRBP. *J Neurosci.* 2026 Apr 1;46(13):e1924252026. doi: 10.1523/JNEUROSCI.1924-25.2026. PMID: 41775632; PMCID: PMC13064807.
3. Vargas J, Izquierdo N, Oliver A, Muns S, Garcia-Rodriguez O, Villegas V, Emanuelli A. Genetic Analysis of Patients With Nonsyndromic and Syndromic Retinitis Pigmentosa in Puerto Rico: A Genetic Legacy. *Ophthalmic Genetics.* 2022;43:4, 454-461, doi: 10.1080/13816810.2022.2050764.
4. Georgiou M, Fujinami K, Robson AG, Fujinami-Yokokawa Y, Shakarchi AF, Ji MH, Uwaydat SH, Kim A, Kolesnikova M, Arno G, Pontikos N, Mahroo OA, Tsang SH, Webster AR, Michaelides M. RBP3-Retinopathy-Inherited High Myopia and Retinal Dystrophy: Genetic Characterization, Natural History, and Deep Phenotyping. *Am J Ophthalmol.* 2024 Feb;258:119-129. doi: 10.1016/j.ajo.2023.09.025. Epub 2023 Oct 7. PMID: 37806543; PMCID: PMC11139644.