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Xin Yee Ooi  
*The University of Texas Rio Grande Valley*

Rujman Khan  
*The University of Texas Rio Grande Valley*

Anjalee Choudhury  
*The University of Texas Rio Grande Valley*

Francisco Xavier Elisarraras  
*The University of Texas Rio Grande Valley*

Jeff Grigsby

*See next page for additional authors*

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Authors
Xin Yee Ooi, Rujman Khan, Anjalee Choudhury, Francisco Xavier Elisarraras, Jeff Grigsby, Brandi Obregon, and Andrew T C Tsin

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Recent advances on visual cycle protein research and progress on clinical translation

Xin Yee Ooi¹, Rujman Khan¹,², Anjalee Choudhury¹, Francisco Xavier Elisarraras¹,², Jeff Grigsby¹,³,⁴, Brandi Obregon¹, Andrew Tsin*¹

Abstract
Since the publication of our previous paper, Visual cycle proteins: Structure, function, and roles in human retinal disease (Tsin, et.al, JBC 293:13016, 2018) there has been significant progress on multiple topics discussed in this paper. In the present communication, we further explore research advances on two visual cycle proteins: DES1 and IRBP. In addition, we emphasize the progress of clinical translation of other visual cycle protein research, including the breakthrough of FDA-approved gene therapy for Leber’s congenital amaurosis, and additional gene therapies at different stages of clinical trials for various retinal diseases such as retinitis pigmentosa, diabetic retinopathy, and Stargardt’s disease.

Keywords: Visual Cycle, DES1, IRBP, RPE 65, Gene Therapy, LCA, RP, DR, STGD

Introduction
Since the publication of Visual cycle proteins: Structure, function, and roles in human retinal disease, there have been significant advances in research involving visual cycle proteins and the translation of basic science discoveries into treatment of retinal diseases, especially in terms of gene therapy. In our prior review article, specific visual cycle proteins, their gene mutations, and novel gene therapy treatments were summarized. Additional investigations, many of which involve clinical trials, have provided further information on the translational potential of visual cycle protein research to treat retinal diseases.

Recent Advances in Visual Cycle Proteins Research

Interphotoreceptor Retinoid Binding Protein (IRBP)

As the most abundant soluble protein in the interphotoreceptor matrix (IPM), IRBP has been linked as a major contributor in maintaining the IPM’s integrity [1]. However, the effects of its presence and absence to visual structures requires further examination. Recently, researchers demonstrated the absence of IRBP through CRISPR knockout irbp-/- mice and the presence of IRBP in wildtype mice. In irbp-/- mice, the structure of the outer segments were abnormally altered, with thinning and change in space between the structures observed [2]. Because of the role of IRBP as a significant anti-oxidant in the retina, these results are consistent with others on IRBP-induced oxidative stress affecting the thickness of subretinal space [3,4].

A recent publication highlighted the significance of IRBP levels to combat detrimental effects of diabetes to vision. When examining vitreous and retina samples from healthy non-diabetic and diabetic patients, researchers discovered that there was a five-fold decrease in the expression of IRBP in patients with severe diabetic retinopathy (DR) in comparison to patients that did not have diabetes [5]. These researchers also established that there was a negative correlation between IRBP expression and severity of DR. Furthermore, a negative correlation between IRBP levels and levels of inflammatory cytokines (VEGF and IL-6) was also established. Thus, decreased IRBP levels may indicate a lack of resistance to the development of DR [5]. These novel findings reveal the opportunity to use IRBP as a biomarker when evaluating and treating DR.

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IRBP is an important visual cycle protein to support visual function. In a recent study, HMG CoA reductase inhibitor (statin) simvastatin was found to reduce degeneration of rod photoreceptors and upregulate levels of IRBP under oxidative stress conditions induced by all-trans retinal [6]. Specifically, the study discovered that rod photoreceptors treated with simvastatin first had improved mitochondrial function when induced with oxidative stress. Further experiments are needed to investigate the detailed mechanism of simvastatin's use in protecting photoreceptor degeneration through the increase of IRBP expression.

Isomerase enzyme of the intra-retinal visual cycle - DES1 and RGR

Dihydroceramide desaturase 1 (DES1) was considered the main enzyme involved in the photoisomerization reaction in the intraretinal/cone visual cycle, as it was thought to convert the retinoid chromophore back to cis configuration after the chromophore was converted to trans configuration in the photoisomerization reaction. It has been shown to convert all-trans-retinol to 11-cis-retinol [7]. However, recent studies using knockout DES1 retinas of mice and zebrafish show that the cone visual cycle has no interruptions, suggesting the DES1 does not have a significant role in supplying visual chromophore for cone pigment regeneration [8,9]. It has been reported that RGR (Retinal G-protein couple Receptor) opsin, working with retinol dehydrogenase 10, can provide 11-cis retinal for cone pigment regeneration in a light activated mechanism in Müller cells [10].

Clinical Translation of Visual Cycle Protein Mutations - Gene Therapy for Retinal Disease

In addition to genetic research performed on mice models, human gene therapy has allowed for improved prognoses of previously irreversible blindness diseases such as retinitis pigmentosa, Leber's congenital amaurosis, diabetic retinopathy, and Stargardt's disease. The progression of gene therapy now exceeds the advancement of RPE65 gene treatment, with results promising a brighter future in vision.

Leber's congenital amaurosis (LCA)

While there are several mutations of visual cycle proteins associated with Leber's congenital amaurosis (LCA), LCA caused by mutations of RPE65 is observed in nearly 6% of cases [11]. Focus on this pathology mechanism has grown rapidly because of the United States Food and Drug Administration's 2017 approval of Luxturna (voretigene neparvovec-ryl), a form of one-time gene therapy, to treat RPE65 mutations in LCA patients. Functional assays can be utilized to decide which specific RPE65 gene mutations are best suited for gene therapy in different individuals [12]. In one study, investigators used functional assays to determine the association between variants of uncertain significance (VUS) and their impact on RPE65 protein function as an indicator of gene therapy success. Measuring isomerase activity of RPE65 VUS using functional assays revealed some patients do not exhibit isomerase activity or proper protein function. Thus, making certain VUS unsuitable for gene therapy. In comparison, patients whose RPE65 VUS exhibited isomerase activity went on to receive successful gene therapy procedures [12]. Usage of functional assays allows for accurate estimation of VUS pathogenicity to determine gene therapy compatibility.

One of the markers of LCA is impairment of photoreceptors due to 11-cis-retinal deficiency and degradation of photoreceptor cells, often caused by proteasome stress [13]. Knockout mice models rpe65-/- (LCA2) and Irat -/- (LCA14) with deletion of M-opsins were used to determine the effects of opsin absence on photoreceptors. Mice with the deleted M-opsin gene experienced lower levels of proteasome stress and subsequent preservation of cone photoreceptors. Since cone opsins are found in higher quantities than other proteins, they have the largest impact on photoreceptor viability. However, investigators confirmed that cone photoreceptors were able to survive without cone opsins [13]. Through closer examination of this mechanism, there is potential in analyzing other options in addressing photoreceptor degeneration beyond 11-cis-retinal deficiency compensation. Further investigations may be conducted to pharmaceutically promote proteasome activity and photoreceptor preservation to supplement gene therapy.

The long term efficacy of gene therapy has been a looming yet present concern. Investigators recognized that RPE65 gene therapy was capable of retaining photoreceptors during primary onset of disease, before any degeneration of photoreceptors. However, gene therapy in later stages of the disease, when photoreceptor degeneration transpired, was substantially less effective. A group of researchers tested gene therapy effects at different stages of RPE65 gene therapy using animal models [14]. After initiating gene therapy in mutant canines with varying severity of photoreceptor loss and ages, researchers found that some regions in canine ocular tissue showed well-preserved photoreceptor nuclei in retinal regions with high expression of RPE65. Interestingly, the same research group found retinal regions where photoreceptors were viable with no RPE65 expression. Researchers noted that it was not the age of subjects, but the progression of the disease, primarily the extent of photoreceptor degeneration, that may determine long-term success of gene therapy [14]. With the current data in canines available, to translate the study to humans, it is important to consider that retinal degeneration may still occur even if disease conditions may be ameliorated.

There have also been new studies that investigate CRISPR gene-editing application for inherited retinal diseases. A subtype of LCA, LCA10, has had some recent gene editing advancements using in-vitro models. LCA10 involves a specific mutation in the lipoprotein gene CEP290 [15,16]. Most LCA10 patients have the IVS26 mutation, which creates an erroneous splice in an intron sequence and results in the non-functional CEP290 protein. A potential candidate for LCA-type 10 treatment is EDIT-101 [16]. It uses an adeno-associated virus type 5 vector to transmit S. Aureus Cas9 and CEP290-specific guide RNAs (gRNAs) to photoreceptor cells via subretinal injection. In this study, researchers recognized two particular gRNAs that are specific to the CEP290 gene locus, allowing for the gRNA to efficiently edit the mutated CEP290 in human cells and retinal tissue. The pairing of SaCas9 and gRNAs to remove the inaccurate insertion of the intron sequence is shown to restore the CEP290 gene for proper RNA splicing and consequent protein expression [16]. Other options in genetic alterations increase the chances of reversing this inherited retinal disease.

Retinitis pigmentosa (RP)

Previously, we indicated that retinitis pigmentosa (RP) presents as rod-cone dystrophy, meaning that there is a loss of rod
photoceptors before cone photoceptors. As a result, recent studies focused on decreasing photoceptor loss to improve visual function. The use of gene therapies, using adeno-associated vectors (AAV), AAV2/5-HPDE6β vector to target rod cGMP phosphodiesterase 6β are currently in phase 1 of clinical trials, with a high possibility to move on to the final stage of testing (phase 2) [17]. Other gene therapies currently in phase 1 of clinical trials are the AAV2/5-hRKP vector and the AAV vector RST-001 (Chop2), of which the latter is used to increase the photosensitivity gene to treat underlying conditions found in RP [17,18]. Additionally, current treatments under investigation include the same voretigene neapavroc-r asyl that was FDA-approved for LCA treatment, AAV serotype 8 (AAV8)-sCX3CL1, and ciliary neurotrophic factor (CNTF)-secreting neural stem cells (NSC) that have provided potential protection to photoceptor cells in RP models [17-19]. The gene therapies currently under clinical trial demonstrate the promise of possible cures for RP.

Although RPE65 mutations account for a small percentage of RP cases, one of the major causes of X-linked RP includes a mutation on the RP GTPase (RPGR) gene that induces the thinning of the ONL. Since this gene is not as large as other genes that affect RP, RPGR has been the target of current research and development using AAV8 [20,21]. The idea of using AAV8 to transfect a codon-optimized RPGR coding sequence (AAV8-coRPGR) to treat RPGR-associated RP cases is currently being tested on human subjects [21]. Researchers established that patients receiving a higher dosage of the AAV8-coRPGR treatment showed a greater retention of improved visual field. Considerable progress has been made in establishing an alternative gene therapy to correct RP-related visual impairment, but more testing is needed to verify this prospective treatment.

Diabetic retinopathy (DR)

Previously, we mentioned some of the challenges in treatment of DR. Diabetic vascular complications remain one of the leading causes of blindness worldwide, and, although there have been significant improvements with the advent of anti-VEGF injections, it is still unclear what factors allow for preventing disease progression. It has been suggested that anti-VEGF therapies provide protection to Müller cells thus preventing further neural degradation [22]. Furthermore, researchers discovered that IRBP could block the damaging effects of VEGF and inflammatory cytokines by inhibiting tyrosine phosphorylation and by blocking GLUT1 to halt transportation of sugar into endothelial cells like Müller cells [5]. With this groundbreaking data, the study demonstrated that restoring IRBP levels up to physiologic baseline concentrations in DM rat models could potentially prevent or even reverse retinal abnormalities like vascular permeability [5]. In another study, diabetes-induced oxidative stress was attributable to superoxide from photoceptors in early phase in the development of diabetic retinopathy [23]. Nonetheless, gene therapy investigations are underway to better understand the dynamic pathway leading to retinal neovascularization and vascular hyperpermeability.

Mesenchymal stem cell therapy has also demonstrated potential as immunomodulatory agents in DR, illustrating neuroprotective effects in light- and ischemia-induced retinal degeneration of animal models [17]. It is thought that stem cells exhibit neuroprotection by secreting neurotrophic factors, which promote tissue regeneration, inhibit fibrosis and apoptosis, and modulate the immune system to reduce inflammation [24]. In particular, endothelial progenitor cells (EPCs) and endothelial colony forming cells (ECFCs), a subtype of EPCs, have displayed promising capabilities of incorporating themselves into host retinal tissue and preventing dysfunctional angiogenesis [17,25,26]. Clinical trials have reported adequate safety and tolerance when evaluating stem cell treatment for photoreceptor degenerative diseases. However, future studies are needed to enhance knowledge of this type of therapy and to reinforce its efficacy [24,26].

Stargardt's disease (STGD)

Stargardt's disease (STGD) is caused by a number of mutations to the ABCA4 gene [27]. Improper functioning of this transporter protein results in all-trans-retinaldehyde buildup, collapsing the visual cycle and leading to photoreceptor cell death. There is currently no effective therapy for the disease, but there is hope for a solution soon, as major progress has been in possible therapies. Direct gene replacement, AAV gene delivery, and lentiviral gene delivery have all been researched extensively, but lentiviral has been demonstrated to be most effective due to its capacity to securely carry ABCA4 cDNA, which is nearly 7kb [28]. There are also non-viral gene therapies, mainly through nanoparticles, being researched.

Phase I/IIa clinical trials are being completed with lentiviral vectors and compacted DNA nanoparticles to treat patients [29]. A very recent publication using (1-aminoethyl)iminobis[N-(oleoylcysteinyl-1-amino-ethyl)propio-namide (ECO) to transduce wild-type ABCA4 into mice shows great promise [30]. ECO is a pH-sensitive amino lipid that forms stable nanoparticles with nucleic acids; with pABCA4 plasmids, ECO easily forms stable nanoparticles. The ECO/pABCA4 nanoparticles self-assemble and have in vitro transfection efficiency. This system needs to be further modified to increase efficiency and enhance gene expression, but if successful, a therapy with little to no side-effects due to the nature of the nanoparticles could be used to treat STGD [30]. As ABCA4 malfunction applies to STGD, types of RP, cone-rod dystrophy, and age-related macular degeneration, gene therapies to deliver functional ABCA4 may also be useful in treating several other retinal diseases.

Conclusion

The publication of Visual cycle proteins: Structure, function, and roles in human retinal disease highlighted the need to further explore pathways in the visual cycle and related clinical investigations including gene therapy. Recent discoveries about the role of IRBP, DES1, and RGR have further clarified the understanding of visual cycle pathway. Clinical trials are presently ongoing to test the effectiveness of gene therapy treatments. As additional research is conducted, our understanding of blinding diseases will be continual to expand leading to the likelihood of future breakthroughs to novel treatment of many ocular ailments.

Conflicts of interest

There are no conflicts of interest.

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References

1. Gonzalez-Fernandez F. Interphotoreceptor retinoid-binding protein—an old gene for new eyes. Vision Research. 2003 Dec 1;43(28):3021-36.

2. Gonzalez-Fernandez F, Lippincott JK, Powell AM, Coleman T. Rat CRISPR/Cas9 knockout of interphotoreceptor retinoid-binding protein (IRBP, RBP3) suggests role in maintaining lamellar disc outer segment stability. Investigative Ophthalmology & Visual Science. 2020 Jun 10;61(7):1528.

3. Berkowitz BA, Podolsky RH, Lins-Childers KM, Li Y, Qian H. Outer Retinal Oxidative Stress Measured In Vivo Using QUEnch-assiSTed (QUEST) OCT. Investigative Ophthalmology & Visual Science. 2019 Apr 1;60(3):1566-70.

4. Berkowitz BA. Preventing diabetic retinopathy by mitigating subretinal space oxidative stress in vivo. Visual Neuroscience. 2020;37(E002):1-10.

5. Yokomizo H, Maeda Y, Park K, Clermont AC, Hernandez SL, Fickweiler W, et al. Retinol binding protein 3 is increased in the retina of patients with diabetes resistant to diabetic retinopathy. Science Translational Medicine. 2019 Jul 3;11(499):eaau6627.

6. Zhang T, Gillies M, Wang Y, Shen W, Bahrami B, Zeng S, et al. Simvastatin protects photoreceptors from oxidative stress induced by all-trans-retinal, through the up-regulation of interphotoreceptor retinoid binding protein. British Journal of Pharmacology. 2019 Jun;176(12):2063-78.

7. Tsin A, Betts-Obregon B, Grigsby J. Visual cycle proteins: structure, function, and roles in human retinal disease. Journal of Biological Chemistry. 2018 Aug 24;293(34):13016-21.

8. Kiser PD, Kolesnikov AV, Kiser JZ, Dong Z, Chaurasia B, Wang L, et al. Conditional deletion of Des1 in the mouse retina does not impair the visual cycle in cones. The FASEB Journal. 2019 Apr;33(4):5782-92.

9. Ward R, Kaylor JJ, Cobice DF, Pepe DA, McGarrigle EM, Brockhoff SE, et al. Non-photopic and photopic visual cycles differentially regulate immediate, early, and late phases of cone photoreceptor-mediated vision. Journal of Biological Chemistry. 2020 May 8;295(19):6482-97.

10. Morshedian A, Kaylor JJ, Ng SY, Tsin A, Frederiksen R, Xu T, et al. Light-driven regeneration of cone visual pigments through a mechanism involving RGP opsin in Müller glial cells. Neuron. 2019 Jun 19;102(6):1172-83.

11. Sweeney MO, McGee TL, Berson EL, Dryja TP. Low prevalence of LRAT mutations in patients with Leber congenital amaurosis and autosomal recessive retinitis pigmentosa. Molecular Vision. 2007;13:5388.

12. Yang U, Gentleman S, Gai X, Gorin MB, Borchert MS, Lee TC, et al. Utility of in vitro mutagenesis of RPE65 protein for verification of mutational pathogenicity before gene therapy. JAMA Ophthalmology. 2019 Dec 1;137(12):1381-8.

13. Xu H, Enemchukwu N, Zhong X, Zhang O, Fu Y. Deletion of M-opsin prevents “M cone” degeneration in a mouse model of Leber congenital amaurosis. The American Journal of Pathology. 2020 Feb 18;190(5):1059-1067.

14. Gardiner KL, Cideciyan AV, Swider M, Dufour VL, Sumaroka A, Komáromy AM, et al. Long-term structural outcomes of late-stage RPE65 gene therapy. Molecular Therapy. 2020 Jan 8;28(1):266-78.

15. Cideciyan AV, Jacobson SG, Drack AV, Ho AC, Charrng J, Garafalo AV, et al. Effect of an intravitreal antisense oligonucleotide on vision in Leber congenital amaurosis due to a photoreceptor cilium defect.

Nature Medicine. 2019 Feb;25(2):225-8.

16. Maeder ML, Stefanidakis M, Wilson CJ, Baral R, Barrera LA, Bounoutas GS, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nature Medicine. 2019 Feb;25(2):229-33.

17. Ludwig PE, Freeman SC, Janot AC. Novel stem cell and gene therapy in diabetic retinopathy, age related macular degeneration, and retinitis pigmentosa. International Journal of Retina and Vitreous. 2019 Dec;5(1):1-4.

18. Ong T, Pennessi ME, Birch DG, Lam BL, Tsang SH. Adeno-associated viral gene therapy for inherited retinal disease. Pharmaceutical Research. 2019 Feb;36(2):34.

19. Wang SK, Xue Y, Rana P, Hong CM, Cepko CL. Soluble CX3CL1 gene therapy improves cone survival and function in mouse models of retinitis pigmentosa. Proceedings of the National Academy of Sciences. 2019 May 14;116(20):10140-9.

20. Giacalone JC, Andorf JL, Zhang Q, Burnight ER, Ochoa D, Reutzel AJ, et al. Development of a molecularly stable gene therapy vector for the treatment of RPGR-associated X-linked retinitis pigmentosa. Human Gene Therapy. 2019 Aug 1;30(8):967-74.

21. Cehajic-Kapetanovic J, Xue K, de la Camara CM, Nanda A, Davies A, Wood LJ, et al. Initial results from a first-in-human gene therapy trial on X-linked retinitis pigmentosa caused by mutations in RPGR. Nature medicine. 2020 Mar;26(3):354-9.

22. Xu B, Zhang H, Zhu M, Le YZ. Critical Role of Trophic Factors in Protecting Müller Glia: Implications for Neuroprotection in Age-Related Macular Degeneration, Diabetic Retinopathy, and Anti-VEGF Therapies. InRetinal Degenerative Diseases 2019; Pp. 469-473.

23. Du Y, Veenstra A, Palczewski K, Kern TS. Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. Proceedings of the National Academy of Sciences. 2013 Oct 8;110(41):16586-91.

24. Puertas-Neyra K, Usategui-Martin R, Coco RM, Fernandez-Bueno I. Intravitreal stem cell paracrine properties as a potential neuroprotective therapy for retinal photoreceptor neurodegenerative diseases. Neural Regeneration Research. 2020 Sep 1;15(9):1631.

25. Li Y, Chen T, Wan C, Cang H. circRNA_0084034 contributes to the progression of diabetic retinopathy via sponging miR-140-3p and inducing TGFA gene expression in retinal pigment epithelial cells. Gene. 2020 Apr 4;144653.

26. Kramero AA, Ljubimov AV. Stem cell therapies in the treatment of diabetic retinopathy and keratopathy. Experimental Biology and Medicine. 2016 Mar;241(6):559-68.

27. ABC4A gene: National Library of Medicine (US). 2020 June 23, 2020; Available from: https://ghr.nlm.nih.gov/gene/ABCA4A#:~:text=More%20than%20640%20mutations%20in,afflicting%20central%20and%20night%20vision.

28. Auricchio A, Trapani I, Allikmets R. Gene therapy of ABCA4-associated diseases. Cold Spring Harbor Perspectives in Medicine. 2015 May 15;5(5):a017301.

29. Han Z, Conley SM, Naash MI. Gene therapy for Stargardt disease associated with ABCA4 gene. InRetinal Degenerative Diseases 2014; pp. 719-724.

30. Sun D, Schur RM, Sears AE, Gao SQ, Vaidya A, Sun W, et al. Non-viral gene therapy for stargardt disease with ECO/pRHO-ABCA4 self-assembled nanoparticles. Molecular Therapy. 2020 Jan 8;28(1):293-303.