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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

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ARTICLE

GENE THERAPY OF *PDE6B*-RETINOPATHY AS THE PHOTOTRANSDUCTION CYCLE MOLECULE: RELEVANCE AND THE MOST PROMISING TREATMENT METHODS

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ABSTRACT:

Retinitis pigmentosa (RP) is a group of inherited retinal disorders characterized by the progressive photoreceptors and pigment epithelial cells dysfunction. It is the most common genetic retinal degeneration, responsible for loss of vision of most young people worldwide. Symptoms of RP include deterioration of vision in the dark, a decrease in peripheral vision ending up in tunnel vision. More than 50 different genes are involved in the development of RP. The products of these genes are involved in various processes of the visual and phototransduction cycles or are structural elements of the retina. In this paper we focused on one clinical example PDE6B-associated retinitis pigmentosa and analysis of the vision loss pathogenesis, gene network regulation and possible treatment approaches.

KEY WORDS
Retinitis pigmentosa, PDE6B, transcriptome analysis phosphodiesterase, small-molecule, treatment

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INTRODUCTION

Retinitis pigmentosa (RP) is a group of inherited retinal disorders characterized by the progressive photoreceptors and pigment epithelial cells dysfunction. It is the most common genetic retinal degeneration, responsible for loss of vision of most young people worldwide.[1] Symptoms of RP include deterioration of vision in the dark, a decrease in peripheral vision ending up in tunnel vision. More than 50 different genes are involved in the development of RP [2]. The products of these genes are involved in various processes of the visual and phototransduction cycles or are structural elements of the retina.

Visual processes are based on a phototransduction mechanism that converts the primary light signal in photoreceptor cells. The quantum of light is absorbed by 11-cis-retinal and isomerizes it into a fully trans-form. This is the only reaction that depends on light. The cis-trans retinal transition causes a conformational rearrangement of the protein part of the rhodopsin (opsin) molecule. As a result, rhodopsin acquires the ability to interact with the next protein in the phototransduction cycle. Transducin belongs to the family of heterotrimeric G-proteins and consists of three subunits. Active transducin activates the next protein of the visual cascade-heterotetrameric phosphodiesterase (PDE) of cyclic GMF (cGMP). This enzyme hydrolyzes cyclic guanosine monophosphate (cGMP) at a high rate. A drop in the concentration of free cGMP in the cytoplasm leads to hyperpolarization of the cell membrane. This electrical potential is a photoreceptor signal, which is then transmitted in the first synapse of the retina to the next nerve cells. Violation of any of the links in this chain can lead to incorrect operation of photoreceptor cells and, as a result, vision disorders.

MATERIALS AND METHODS

Oftalmic Russian IRD (inherited retinal disorders) clinical database was searched for the visual cycle and phototransduction gene mutations cases and out of 118 cases a male 18 y.o patient case with hemizygous mutation in PDE6B gene c.1580T>C p.Leu527Pro NM_000283.3 (chr4:654368T>C) was selected for further analysis. We used mouse animal model for transcriptome analysis. Total RNA was extracted from Pde6b$^{-/-}$ retina in our experiment using RNA Qiagen kit and the RNA quality and quantity was assessed by the absorbance at 260 nm/280 nm using a NanoDrop ultraviolet spectrophotometer; RNA integrity was verified by 1% agarose gel electrophoresis. The cDNA library was purified, and the library was sequenced on a NovaSeq 6000 platform. RNA-sequencing reads were first trimmed to remove poly(A) and unqualified reads with Cutadapt (v1.15), then aligned to the Homo sapience GRCm38 genome using Ensembl. The counts were summarized at the gene level using HTseq (0.9.1). Gene expression values were calculated from fragments per kilo basis per million fragments (FPKM). These FPKM values were used to generate a table with the Prism software. Paired differential gene expression analyses were performed with DEseq (1.30.0) with screened conditions as follows: multiple expression difference|log2fold change| > 1 and p-value < 0.05. The main biological functions associated with the differentially expressed genes were determined by GO (p-value < 0.05). The KEGG pathway enrichment analysis of the differential genes focused on the associated enriched pathways (p-value < 0.05). A protein–protein interaction (PPI) analysis of differential genes was performed using the STRING database (http://string-db.org) and genemania.org webtool to reveal the relationships between the target genes. The PPI network model was generated using Cytoscape. Statistics 28 software was used to analyze results.

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RESULTS

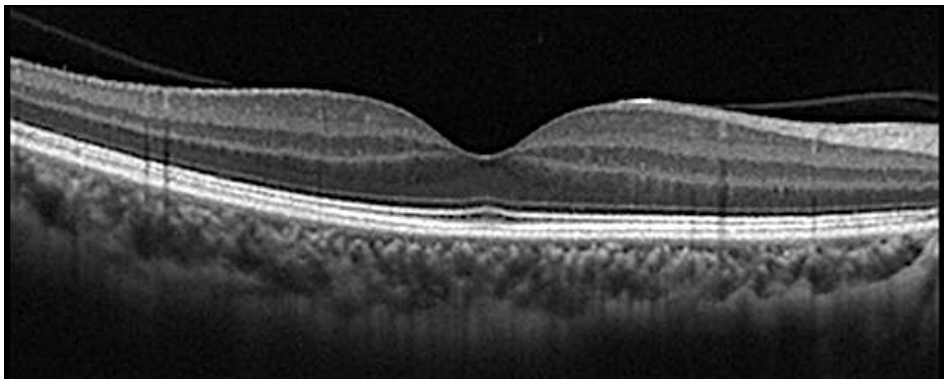
This paper discusses the PDE6B gene encoding the β-subunit of cGMP phosphodiesterase. This protein is involved in the transmission and amplification of visual signals and is necessary for the formation of a functional phosphodiesterase holoenzyme. Phosphodiesterase itself is an effector protein in the phototransduction cycle and catalyzes the conversion of cGMP to GMF, which causes the closure of ion channels and a decrease in the level of glutamate in rods.

To date, there is no single treatment for RP, but thanks to recent advances in imaging technology, DNA sequencing, gene therapy and stem cell biology, the number of clinical trials has increased,

Most mutations in the phosphodiesterase gene result in improper regulation of cGMP levels and disruption of ion channels. It is believed that this eventually causes the death of receptor cells. Gene therapy effectively restores long-term retinal function and vision and offers great prospects for human treatment. The most promising methods in the treatment of retinitis pigmentosa today are CRISPR/Cas9 and AAV gene therapy.

A 18 y.o. male patient with the hemizygous mutation in PDE6B gene c.1580T>C p.Leu527Pro NM_000283.3 (chr4:654368T>C) presented symptoms of night blindness at the age of 4 y.o, his BCVA (best corrected visual acuity) was 0.3/0.4 OD/OS at the first examination. subsequently his visual fields narrowed over the course of 5 years, his BCVA was relatively stable. His OCT (optical coherence tomography) scans are presented on Fig. 1B.

A



B

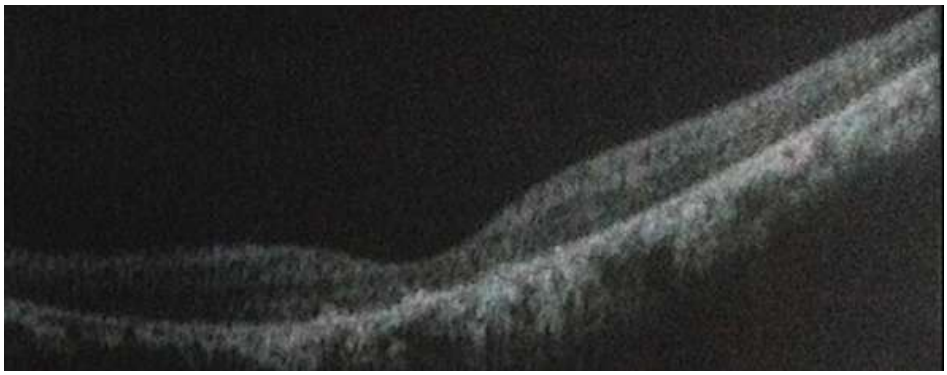


Fig. 1: OCT (optical coherence tomography) scans of a right eye healthy control retina (A) and a patient with *PDE6B* selected mutation (B).

PDB (international protein database) of P35913 UniProt identifier for PDE6B was analyzed for the proven pathogenic mutation c.1580T>C influence on protein structure in Fig. 2.

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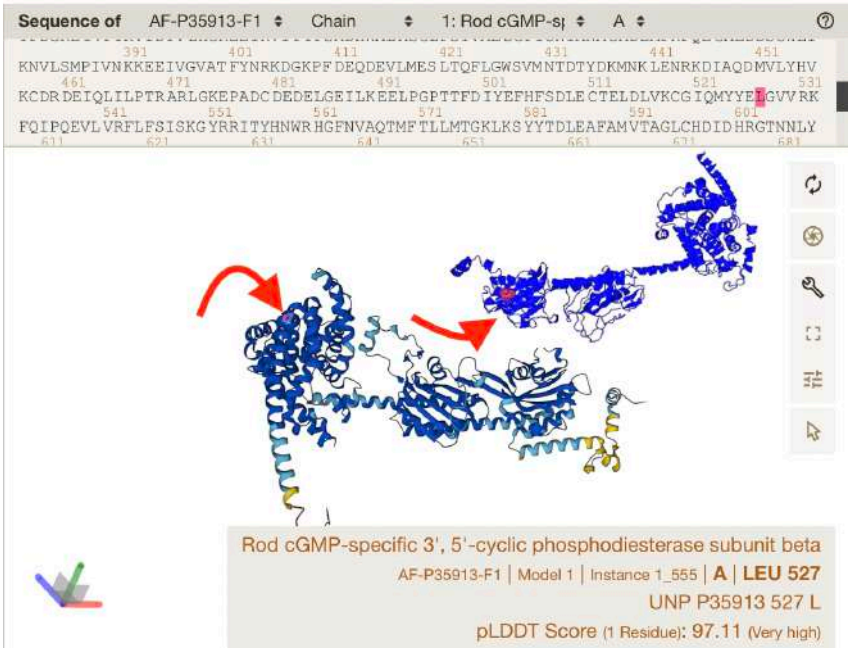

Fig. 2: The structure of phosphodiesterase 6 beta protein. The site of the mutation is marked in red PDE6B c.1580T>C p.Leu527Pro NM_000283.3 (chr4:654368T>C).

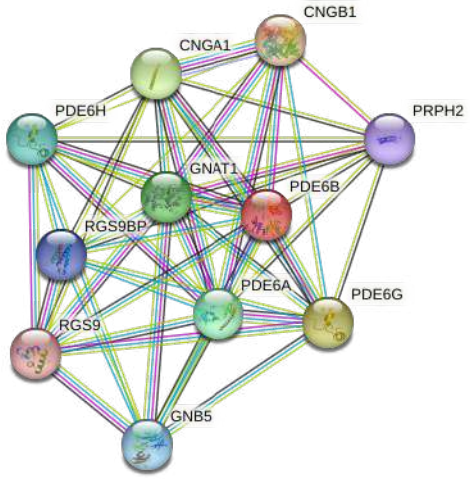

Fig. 3: *PDE6B* gene and protein interaction network involves mostly molecules of visual cycle and phototransduction.

Table 1: Transcriptome analysis of the PDE6B gene and protein network. We analyzed RNA expression in the mutated animal and compared it to normalized wild-type gene expression pattern. Expected level of expression calculated by in silico algorithms is presented in expected expression column and the experimental data analysis is presented in the experimental expression column.

Gene name	Gene function explanation	Expected expression	Experimental expression
Visual cycle			
RHO	Rhodopsin in rods	↔	↔
OPN1LW/MW/SW	Opsin 1 (cone pigments), long/medium/short-wave-sensitive	↓	↔
RBP1/2/3/4	Retinol binding protein subunits, cellular	↓	↓
RLBP1	Retinaldehyde binding protein 1	↔	↓
RPE65	Retinal pigment epithelium-specific protein 65kDa	↓	↑
LRAT	Lecithin retinol acyltransferase	↔	↔
RDH5/8/10/11/12/16	Retinol dehydrogenase different types	↓	↔
ABCA4	ATP-binding cassette, sub-family A (ABC1), member 4	↓	↑
Phototransduction			
GNAT1	Transducin, rod-specific	↓	↑
GNAT2	Transducin, cone-specific	↓	↑

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PDE6A	Phosphodiesterase 6 alpha	↓↓	↑↑
PDE6B	Phosphodiesterase 6 beta	↓↓	↓↓
PDE6G	Phosphodiesterase 6 gamma	↓↓	↑↑
CNGA1, CNGA3, CNGB1, CNGB3	Cyclic nucleotide-gated channel subunits, cone and rod specific	↔	↔
GUCA1A/B/C	Guanylate cyclase activator 1B (retina)	↑↑	↑
GUCY2D/2F	Guanylate cyclase 2D/2F, membrane (retina-specific)	↑↑	↓
Secondary			
NRL	Neural retina leucine zipper	↓	↓
NR2E3	Nuclear receptor subfamily 2 group E	↓	↓
CRX	Cone-rod homeobox	↔	↔

We analyzed PPI (protein-protein interaction) in String tool (Fig. 3) and created a table 1 of the genes for transcriptome analysis followed by the graphic (Fig.4) with the comparison of the expression levels in normal (blue) and mutated (orange) cases.

Analysis of expression shows unexpected rise of RPE65, ABCA4, GUCA1B and GUCA1C and down-regulation of GUCY2D, and GUCY2F proteins. This data needs further verification, but initially we can tell that both Phosphodiesterase 6 alpha/gamma and Guanylate cyclase activators 1B/1C were up regulated in case of mutation. RPE65 and ABCA4 levels were unexpectedly high, also both rods and cones transducin levels were higher than expected. We expected Guanylate cyclase expression to increase, and it was decreased which shows some additional mechanisms of regulation in the live cells comparing to isolated molecular analysis.

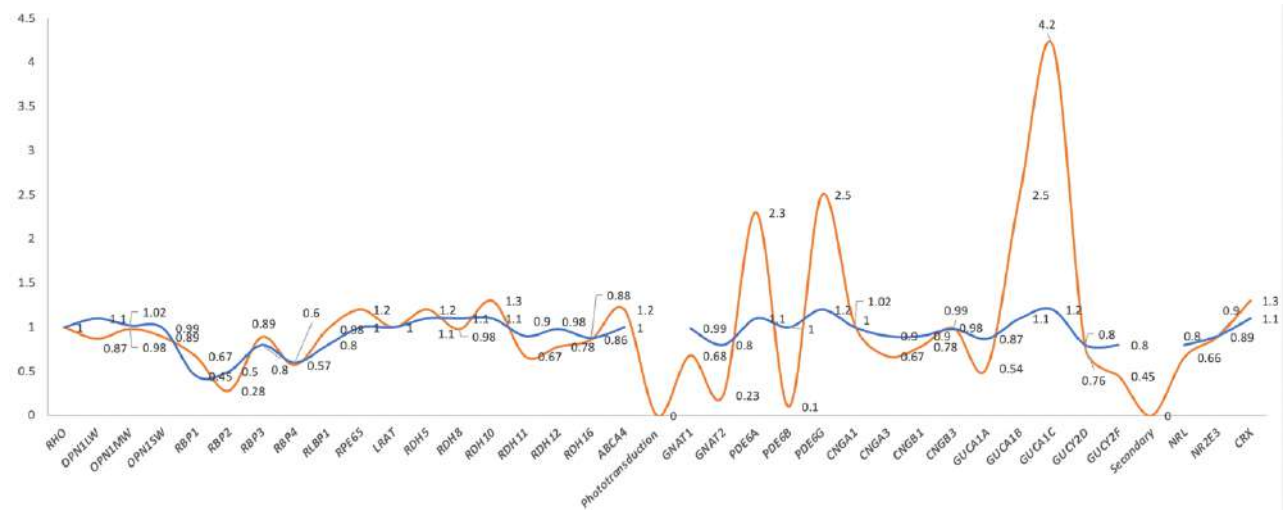

Fig. 4: *Transcriptome analysis of the PDE6B-related proteins.*

DISCUSSION

In mice, a nonsense mutation was found in the pde6b gene, which leads to incorrect operation of one of the phosphodiesterase subunits. CRISPR/Cas technology was successfully applied to mice with this mutation. This predicts excellent results for gene editing in diseased human tissues, since Pde6b, a mutated gene in mice, has orthologous intron-exon relationships comparable to the human PDE6B gene [3].

Viral vectors are a modern tool for delivering genetic material to the cell. Their main advantages: the ability to integrate the target gene into the desired location of the host genome, which prevents unwanted mutations; embedding in both dividing and resting cells; a wide transduction profile; low immune response; strong and stable expression of the transgene. The essence of the method is the penetration of the virus into the target cell, where the genome is then expressed with the necessary gene inserted into it (in our case, the phosphodiesterase gene).

Experiments have shown that the introduction of adeno-associated viral vectors can prevent retinal degeneration in mice, which is reflected in significant structural, biochemical, and electrophysiological changes. It is worth noting that clinical trials have already reached the second stage after being successfully tested on dogs. These results serve as the basis for studying the long-term rescue of the retina in humans in the future.[4][5]

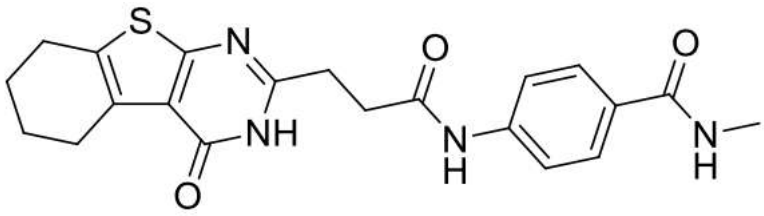

Fig. 5: *Analogue of acrylamide-azobenzene-quarternary ammonium (AAQ) molecule that can be activated by 380 nm blue light (cis-form) and deactivated by 500 nm green light (trans-form).*

Also, it is worth mentioning small molecules treatment approaches that can potentially up-regulate visual cycle in the presence of abnormal PDE6B product. In case of PDE6B mutation the signal is not amplified. What are the possible ways of signal amplification? Some researchers suggested using photo switch molecules [6], for instance, acrylamide-azobenzene-quarternary ammonium (AAQ) molecules (Fig.5) to serve as a photo activated/deactivated molecule to fire ganglion cells in the case of lack of signal activation. Such kind of small molecule can serve as a molecular channel blocker in light activated *trans*-form and can release the channel in the *cis*-form.

CONCLUSION

One clinical example PDE6B-associated retinitis pigmentosa with robust molecular investigation and transcriptome analysis of the genes and proteins involved in photocycle and phototransduction, gene network regulation and possible treatment approaches of gene therapy, gene editing and small molecules treatment were discussed in this paper.

ETHICS STATEMENT
This research adhered to the tenets of the Declaration of Helsinki. Patient signed informed consent form for the scientific analysis.

CONFLICT OF INTEREST
Authors declare no conflict of interest.

ACKNOWLEDGEMENTS
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FINANCIAL DISCLOSURE
None

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