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ARTICLE

NEW INSIGHTS FOR RHO-ASSOCIATED RETINITIS PIGMENTOSA TREATMENT APPROACHES

Ionas Abel[1*], Natalia Trankova[2]

[1]*State budgetary educational institution "Lyceum No62", Kemerovo, RUSSIA*

[2]*Skolkovo Institute of Science and Technology, Moscow, RUSSIA*

ABSTRACT:

This paper is dedicated to reveal strategies to show the role of genetic modifiers in autosomal dominant diseases, such as retinitis pigmentosa caused by mutations in RHO gene which lead to new insights for possible gene augmentation, regulation and ultimately disease phenotype rescue.

KEY WORDS
Retinitis pigmentosa, RHO, trans-regulatory variation, modifier, treatment

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***Corresponding Author**
Email: ionass.abel@gmail.com
Tel.: +7 919 036 87 45

INTRODUCTION

New approaches are being developed for RHO-associated retinitis pigmentosa treatment [1,2]. Rhodopsin is a light-sensitive protein, expressed in rods and is able to change its conformation under the influence of photons, participates in the process of hyperpolarization. In total, there are more than 250 mutations in the *RHO* gene encoding rhodopsin. There are over 150 mutations that cause the development of autosomal dominant retinitis pigmentosa. Retinitis pigmentosa is a group of rare (1 : 10,000) hereditary eye diseases characterized by damage to the retina, leading to impaired night vision and loss of peripheral vision (in rare cases, complete blindness). Retinitis pigmentosa occurs in about 1 in 4,000 people. Gene therapy can be aimed both at extending the life of the remaining rods in the event of severe damage to the retina, and at maintaining existing rods and preventing cone death.

An example of a mutation in the rhodopsin gene is P23H, one of the most common mutations (from 10-15%) in western world leading to autosomal dominant retinitis pigmentosa. According to a 2002 study [3] misfolded mutant rhodopsin was identified as a target for the ubiquitin proteosomal system. Thus, the expression of this mutation leads to disruption of the ubiquitin proteosomal system, which is the cause of photoreceptor degeneration, leading to visual impairment.

Another example of a mutation is T17M, a 2007 study [4] reported that expression of the human mutant rhodopsin transgene in mice was associated with photoreceptor apoptosis in response to moderate light exposure.

MATERIALS AND METHODS

Database of Eastern Europe inherited retinal disorders (IRDs) was explored and 45 patients with disease-causing mutations in *RHO* gene were filtered for preliminary analysis. 8 different mutations in heterozygous state were considered to be causative in pathogenesis of the disease: c.44A>G p.Asn15Ser, c.263T>C p.Leu88Pro, c.392T>C 2 p.Leu131Pro, c.403C>T p.Arg135Trp, c.620T>A p.Met207Lys, c.995_998dup p.Ser334GlyfsX21, c.1031A>C p.Gln344Pro, and c.1040C>T p.Pro347Leu. in silico analysis of *RHO* gene was made to classify mutations for being possible modifiers of the disease. Analysis of probable off-target cut in CRISPR-Cas approach was also provided.

Cis-regulatory elements, such as promoters, enhancers, and silencers, are regions of non-coding DNA, which regulate the transcription of nearby genes. In contrast, trans-regulatory factors regulate (or modify) the expression of distant genes by combining with their target sequences. Due to rapid advances in high-throughput biotechnology and international collaborative efforts, there has been a dramatic increase in public functional genomics regulators data.

These experimental data, in combination with the comparative genomics resources have been essential in the development of numerous secondary databases and algorithms that have facilitated a broader understanding of gene regulation. ENCODE project data and NIH Roadmap Epigenomics project was used in the analysis as well as

Spidex resource which contains computational predictions for ~328 million SNPs for potential impact of genetic variants on human splicing.

A linkage study, when gene-hunting technique that traces patterns of retinitis pigmentosa disease in high-risk families and association studies when approach was used to identify genomic variants that are statistically associated with a risk for retinitis pigmentosa was also applied in the analysis. Genome Wide Annotation of Variants (GVAVA), Combined Annotation Dependent Depletion (CADD) scores and a series of model predictors include FATHMM (Predicting the Functional Consequences of Non-Coding and Coding Single Nucleotide Variants), which quantifies the functional effect of variants as changes in the scores of different alleles. FATHMM in the work has been expanded by two developments: CScape and FATHMM-XF. CScape uses greedy sequential learning with leave-one-chromosome-out cross-validation (LOCO-CV) to identify the optimal features for predicting pathogenic single-point mutations in the genome. These methods helped us to reveal possible genetic modifiers of RHO-associated retinitis pigmentosa described in results section.

RESULTS

Phenotypic variability might also be due to the effect of another variant in the gene, in either *trans* or *cis* position to the primary mutation. In some dominant diseases, clinical expression may depend on the normal allele (trans effect). Good examples are erythropoietic protoporphyria, where a low expressed allelic variant of the ferrochelatase gene located in trans from the mutation explains the variability in disease expression. Alternatively, *cis* effects might be suspected when the haplotype carrying the disease mutation varies with the clinical phenotype.

A striking example is that of Creutzfeldt-Jakob and familial fatal insomnia: carriers of a single mutation in codon 128 of the prion protein gene located on chromosome 20 develop one or the other disease depending on a polymorphism at codon 129 of the same gene that codes for two different amino acids — valine or methionine.

Disease expression variability might also be explained by the effect of genes other than the primary one involved in the disease, and it is these that are usually referred to as modifier genes. Their effect on disease expression may vary from strong effects under a “monogenic-like” model to much milder effects under a “multifactorial-like” model.

Under the monogenic-like model, a single modifier gene exhibits rare fully or almost fully penetrant mutations that explain all or a very important part of the variability in disease expression. Examples of this type of modifier genes can be found among the genes involved in the splicing machinery.

Under the multifactorial-like model, disease expression depends on the effects of several genetic variants located in different modifier genes that, by themselves, only explain a small proportion of the variability but interact both with one another and with environmental factors. This is probably the most common situation and is the one on which we have chosen to focus.

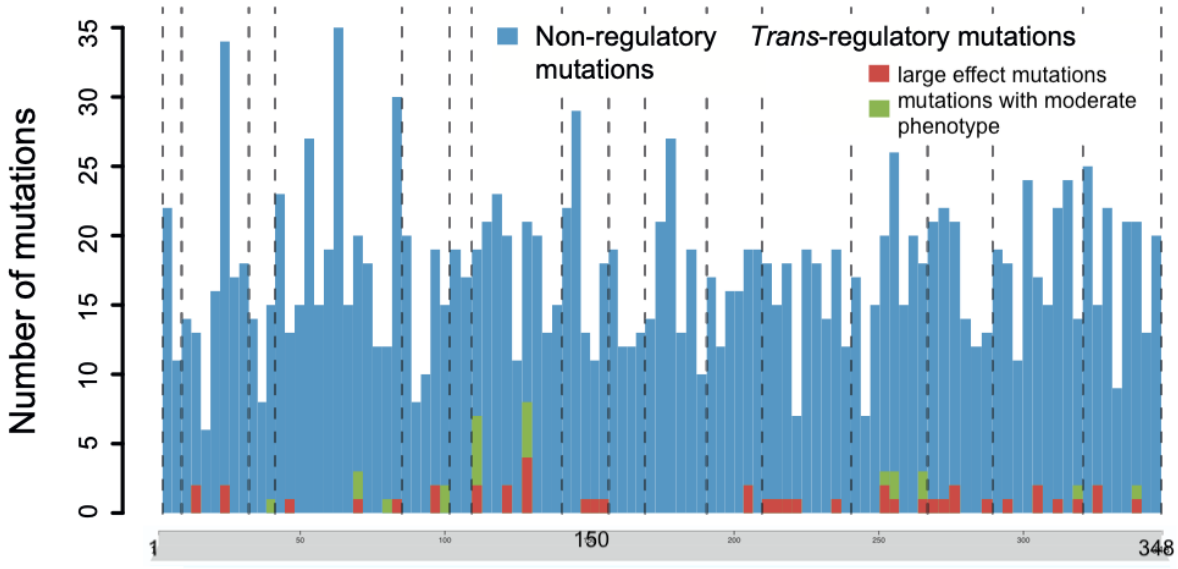

Fig. 1: Quantitative analysis of all known mutations in *RHO* gene for being possible genetic modifiers. See text for explanation.

Strategies to show the role of genetic modifiers.

Strategies used to show the role of genetic factors in phenotypic expression are often classified into two categories depending on the type of data available: linkage studies and association studies. Another distinction often made is based on the approach, which can be either a systematic approach where the whole genome is scanned or a more focused approach, where candidate genes or candidate pathways are selected.

Based on this in silico analysis was provided for RHO gene and all known mutations which were classified as non-regulatory mutations shown in blue color [Fig. 1], large effect mutations (red) and mutations which show moderate phenotype when occur in the patient (green).

All mutations c.44A>G p.Asn15Ser, c.263T>C p.Leu88Pro, c.392T>C 2 p.Leu131Pro, c.403C>T p.Arg135Trp, c.620T>A p.Met207Lys, c.995_998dup p.Ser334GlyfsX21, c.1031A>C p.Gln344Pro, and c.1040C>T p.Pro347Leu align with the in-silico prediction of the role for being genetic modifier of the phenotype severity. Frequency of all amino acid changes induced by trans-regulatory mutations were compared to non-regulatory mutations and presented in a schematic view [Fig. 2] and their impact on possible clinical signs discussed.

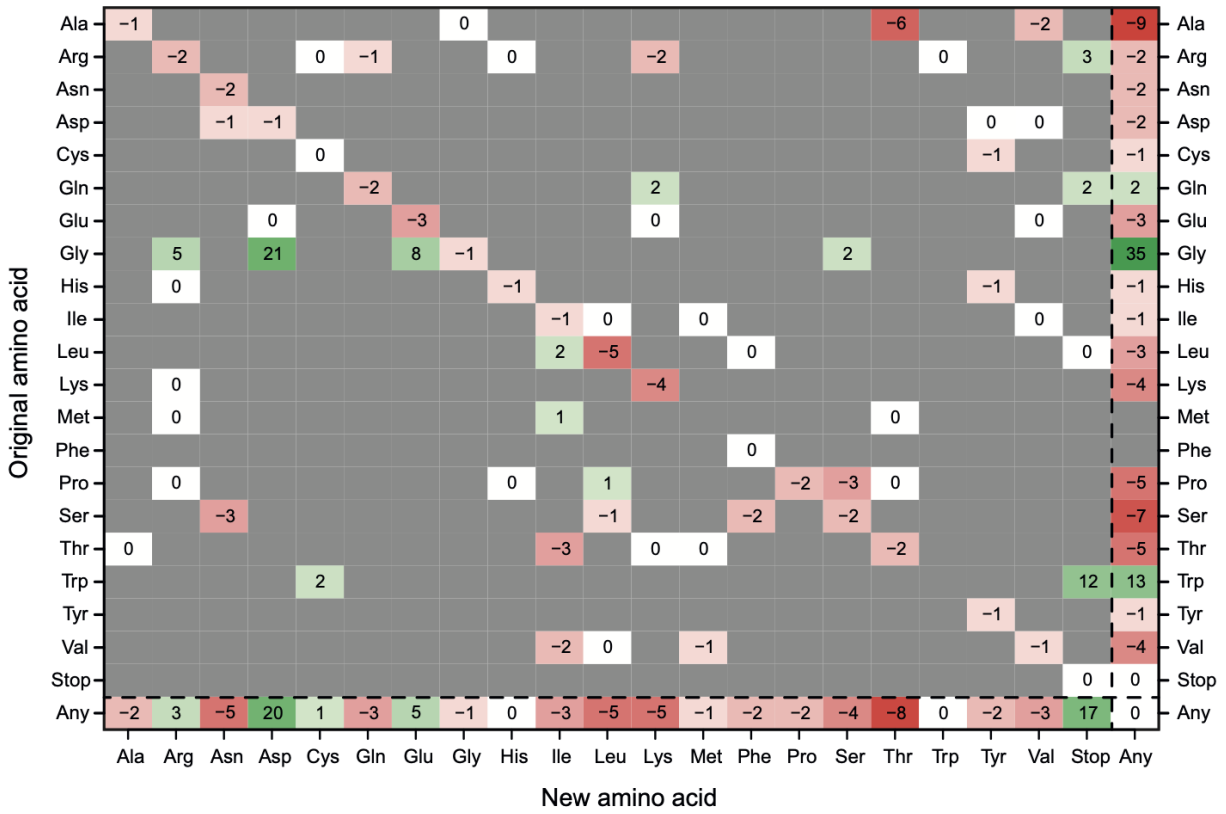

Fig. 2: Differences in the frequency of amino acid changes (%) caused by non-regulatory and *trans*-regulatory mutations. See text for explanation.

Frequency of all amino acid changes induced by *trans*-regulatory mutations as compared to non-regulatory mutations. Each entry of the table represents the difference of frequency (percentage) between non-regulatory and trans-regulatory mutations that are changing the amino acid shown on the y-axis into the amino acid shown on the x-axis. For instance, the -6 on the first row indicates that the proportion of mutations changing an Alanine into a Threonine is 6% lower among trans-regulatory mutations than among non-regulatory mutations. Shades of **red**: amino acid changes underrepresented in the set of *trans*-regulatory mutations. Shades of **green**: amino acid changes overrepresented in the set of *trans*-regulatory mutations. **White**: amino acid changes equally represented in the *trans*-regulatory and non-regulatory sets of mutations. **Gray**: amino acid changes not observed in the sets of *trans*-regulatory and non-regulatory mutations. The three aneuploidies were excluded for these plots. Non-coding mutations were excluded for these plots [Fig. 2].

DISCUSSION

Based on these mutations study, a study was conducted to suppress and replace these mutations with base editing technique. Initially, a short hairpin RNA820 (shRNA820) was selected that repressed rhodopsin proteins (both mutant and normal) by about 95%. The shRNA-resistant gene, PHO820, was modified. In tests on dogs, injections of this RNA have been shown to have a dose-dependent effect [5].

Then one vector was developed to express shRNA820 and to replace the mutant rhodopsin gene. As a result, the normal number of photoreceptors in the outer nuclear layer was preserved in the treated areas, as was the outer segment of the rods (which was not preserved when shRNA820 alone was administered). Preservation of the outer rod segments is associated with improved morphology of the inner and outer cone segments. Using the CRISPR/CAS9 genomic editing method, nucleotide changes can be made on both DNA strands with high accuracy. Therapy for retinitis pigmentosa using the Crispr/Cas9 method. The study was carried out on a model of rat retinitis pigmentosa with a dominant mutation in the rhodopsin gene, leading to the appearance of a stop codon and shortening of the protein by 15 amino acids. Since the nucleotide substitution causes the appearance of a PAM site (5'-TGG-3' instead of 5'-TGC-3'), it became possible to use Cas9-induced mutagenesis to specifically “turn off” the mutant allele and partially restore retinal function.

Therapy with AONs (Antisense Oligonucletides) which are small RNA molecules that are able to redirect normal splicing into pre-mRNA. AONs can have different effects on messenger RNA: (1) some modifications can trigger mRNA degradation (in this case, protein formation does not occur) (2) other modifications can alter some parts of the mRNA so that a modified version of the protein is formed [6].

In 2019, the first clinical trials of a drug based on AONs were launched in patients with autosomal dominant retinitis pigmentosa caused by a P23H mutation in the RHO gene which showed good clinical effect and safety profile.

CONCLUSION

It is crucially important to know all the chains in the pathogenesis of the disorder to aim the treatment approaches for beneficial clinical results. Genetic modifiers of RHO gene mutations are explored in this study and their impact on clinical manifestation is discussed.

ETHICS STATEMENT

This research adhered to the tenets of the Declaration of Helsinki. As no patients were directly involved in the research, no informed consent was needed.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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FINANCIAL DISCLOSURE

None

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