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ARTICLE

NR2E3 PROTEIN INTERACTOME EVALUATION FOR POSSIBLE RETINITIS PIGMENTOSA DRUG DEVELOPMENT CAUSED BY MUTATIONS IN THIS GENE

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ABSTRACT:

Our study analyzes the c.481delA p.Thr161fs mutation in the NR2E3 gene, a member of the protein family that plays a crucial role in biological sensory transduction, transcription, and regulation in retinal tissue. Mutations in the NR2E3 gene can cause autosomal recessive Enhanced S-cone syndrome as well as both recessive and dominant retinitis pigmentosa in humans. The study aimed to evaluate the predicted structural alterations of the NR3E3 protein in patients with the studied mutation in the NR2E3 gene, with the potential for drug development. Protein interaction analysis in silico with STRING and Genemania instruments also showed the interaction of NR2E3 with homebox gene family inclusion. These interactions play a role in embryonic development and regulatory and signaling pathways. The modeling in DEPTH tool revealed a significant increase in depth of amino acids in the mutation coordinates, suggesting that an important binding site is no longer available for other molecules. We also provided analysis of possible sites of small and large molecules interaction in DNA binding domain of the NR2E3 protein. The mutation is located in an important region for interactions with various interaction partners including DNA. The study highlights potential of the studied mutation as a therapeutic target for retinal diseases caused by mutations in the NR2E3 gene.

KEY WORDS
Retinitis pigmentosa, NR2E3, interactome, frameshift mutation

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INTRODUCTION

Nuclear receptor subfamily 2, group member E, member 3 (NR2E3) represents a protein family of ligand-dependent transcription factors that can act as genetic modifiers for retinal embryonic development and signaling pathways [1]. Mutations in the *NR2E3* gene can cause autosomal recessive Enhanced S-cone syndrome (Goldmann-Favre syndrome) as well as both recessive and dominant retinitis pigmentosa in humans. The mutation spectrum in the *NR2E3* gene has been described in the human population. The aim of this study is to evaluate the predicted structural alterations of the *NR3E3* protein in patients with the c.481delA p.Thr161fs mutation in the *NR2E3* gene, with the potential for drug development.

NR2E3 is a transcriptional factor that functions as an activator of rod development and a repressor of cone development. It binds to the promoter region of several rod- and cone-specific genes, including rhodopsin, M- and S-opsin, and rod-specific phosphodiesterase beta subunit, ultimately enhancing rhodopsin expression while repressing M- and S-cone opsin expression. *NR2E3* plays a crucial role in biological sensory transduction, transcription, and regulation. Nuclear receptors have been shown to regulate pathways involved in embryonic development, as well as in maintaining proper cell function in adults.

MATERIALS AND METHODS

Database of inherited retinal disorders (IRDs) Russian patient registry (Oftalmic CRO) was explored and 19 patients with disease-causing mutations in *NR2E3* gene were filtered for preliminary analysis [2]. NR2E3 (NM_016346.3) c.481delA p.Thr161fs (chr15:71812086) mutation and its influence on gene function was chosen as a model for the interactome analysis, as this was the most frequent mutation in the cohort and due to attempts for human induced pluripotent stem cell gene edited retinal organoids development to rescue clinical symptoms in patient tissue donor [3]. Alamut, PyMol, JMol, Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) and FATCAT pairwise alignment tools (http://fatcat.godziklab.org/fatcat/fatcat_pair.html) were used to structure wild type (WT) and mutated protein, Genemania (genemania.org), STRING and PPI (protein-protein interaction) tools at https://www.ebi.ac.uk, dSprint and Alphafold were used to explore protein interactions.

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RESULTS

Transmission electron microscopy (TEM) of NR2E3-mutated retina [Fig. 1B] shows disarrayed disks (D) of photoreceptors and elongated microvilli (M) compared to the wild-type (WT) retina [Fig. 1A], confirming the discovered localization and function of this protein. [Fig. 3B].

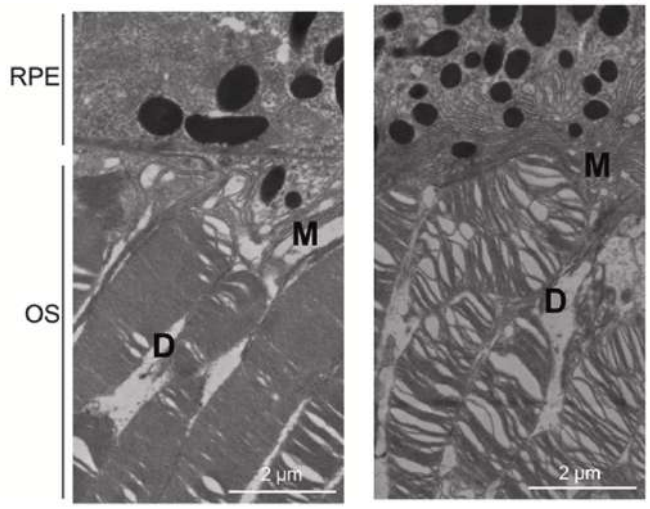

Fig. 1: A. TEM of WT retina. D – photoreceptor disks, M – microvilli. RPE – retinal pigment epithelium, OS – outer segment of photoreceptors. Scale is 2 um. **B.** *NR2E3* mutated retina shows disarrayed photoreceptor disks and elongated microvilli comparing to *WT* retina.

Other mutations in NR2E3 gene cause different malformation of NR2E3 protein [4]. Protein interaction analysis in silico with STRING and Genemania instruments shows the importance of NR2E3 in embryonic development and regulatory and signaling pathways with CRX, RAX and SIX homebox gene family inclusion [5] [Fig. 2].

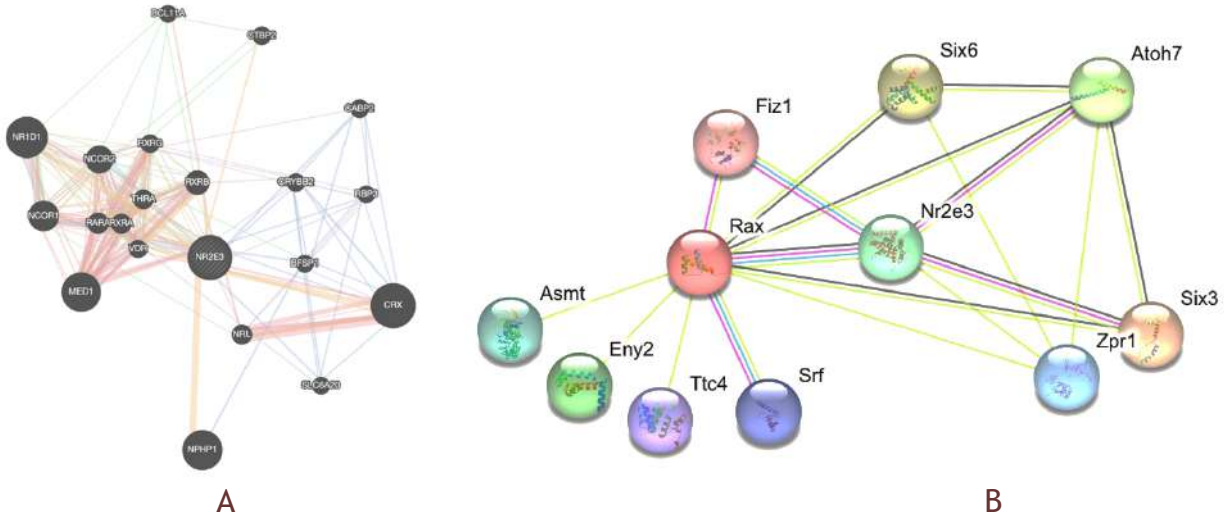

Fig. 2: Genemania (**A**) and STRING (**B**) gene interaction network of *NR2E3* gene analysis confirming involvement in signaling pathways and embryonic retinal development processes.

When attempting to model the 3D structure of the truncated protein caused by the c.481delA p.Thr161fs mutation and predict the loss of protein function, we observed that the mutation affects the DNA-binding domain which has the **Pfam** identifier PF00870. The **Alphafold** 3D prediction tool classified the model confidence as "low" in the coordinates of the mutation [Fig. 3A]. Using the **JMol** tool alignment and some other tools: transcriptome sequencing-based annotation [6], molADI [7] and preRINDS [8] we predicted severe structural abnormalities in the mutated truncated and normal NR2E3 protein [Fig. 3B]. The **PDB** 3D protein structure with highlighted blue **Thr** amino acid residues revealed possible points of altered protein-protein interaction in case of mutation [9] [Fig. 3C].

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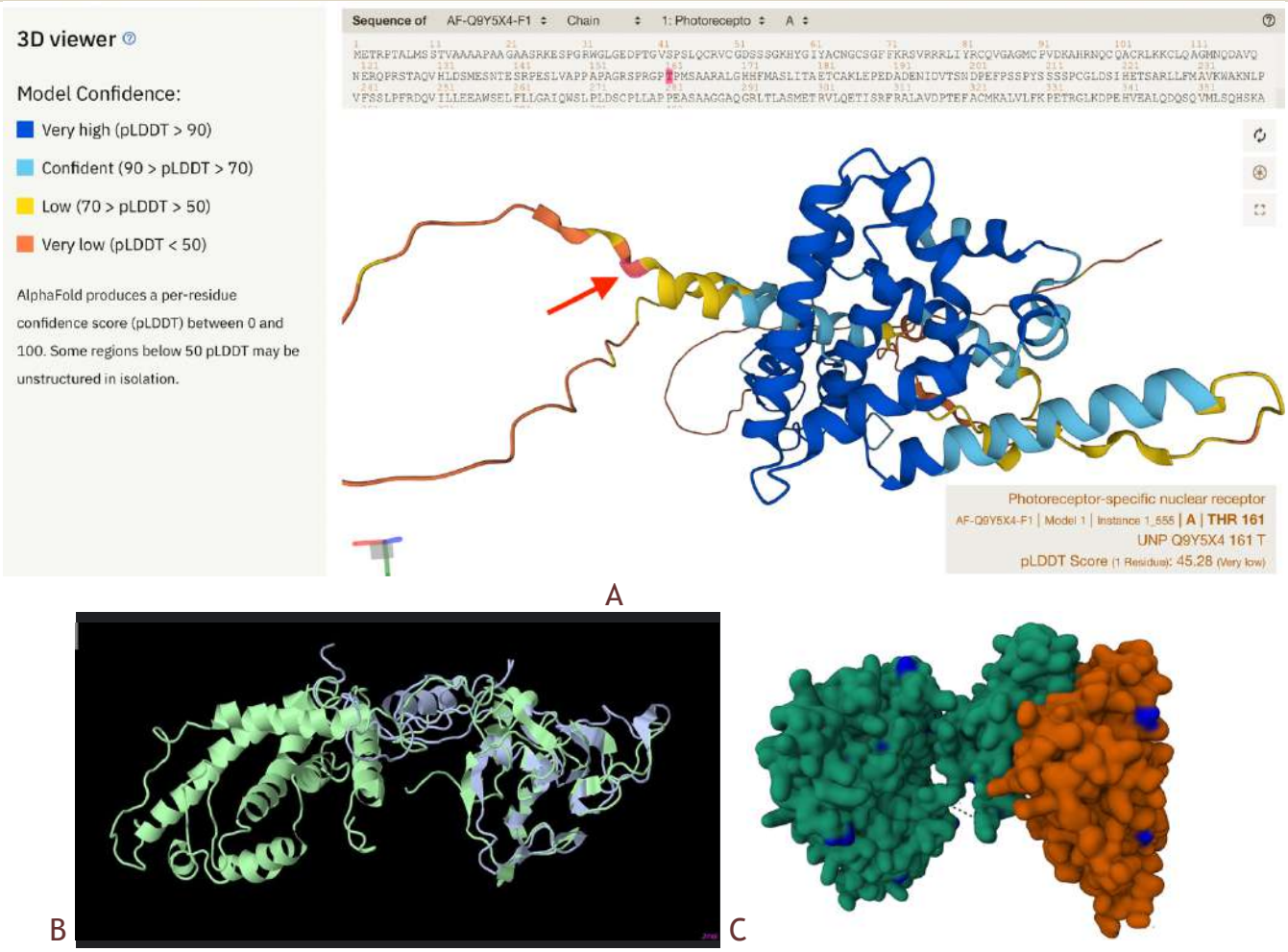

Fig. 3: NR2E3 protein structure visualization. **A.** Alphafold structure prediction. **B.** JMol visualization of WT and mutated NR2E3 protein. WT is shown in green. Mutated NR2E3 c.481delA p.Thr161fs is shown in blue and aligned with WT. **C.** PyMol PDB model of NR2E3 protein with blue highlighted – Thr amino acid residues which are possible points of altered protein-protein interaction in case of mutation.

There are certain tools like DEPTH analysis [10] for calculating the amino acid (aa) residues depth from the surface in the protein. This tool was used to calculate the aa depth in the region of the mutated DNA binding domain of the NR2E3 protein and we see from the graphic [Fig 4.] the significant increase of the depth of the residues in the coordinates of the mutation, which means that during the mutation an important binding site is no longer available for ligands.

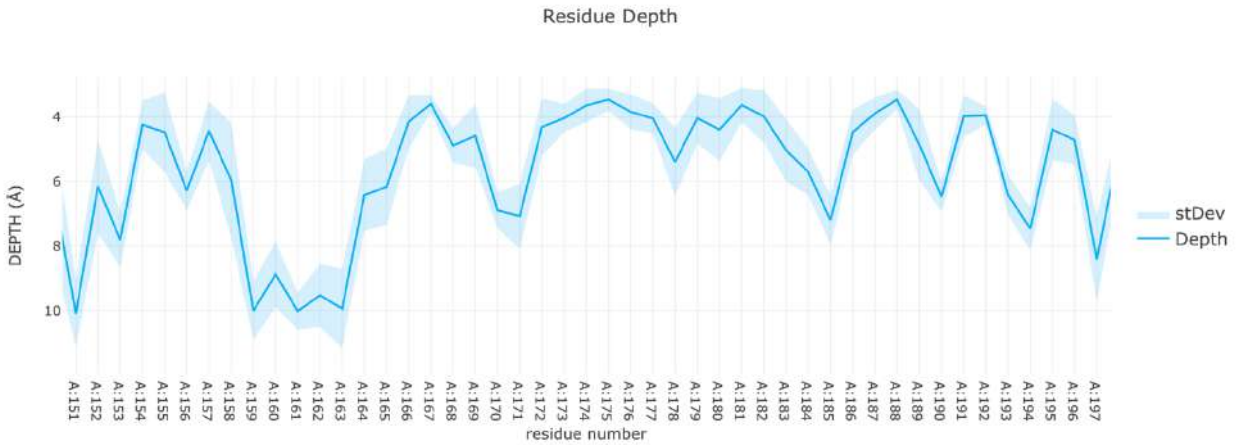

Fig. 4: Amino acid (aa) residues depth from the surface in the NR2E3 protein. DEPTH tool was used: http://cospi.iiserpune.ac.in/depth/result/20230511014946_66/1

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We also provided analysis of possible small and large molecules interaction in the research DNA binding domain of the NR2E3 protein. As we see [Fig. 5], the mutation resulting in 161 aa frameshift may lead to dysfunction in the domain which is responsible to DNA binding – the crucial role of this protein. Small molecules and ions which can show affinity in butterfly-type mode in between 140 and 180 aa residues and can bind the protein in case of mutation [11]. This can be the starting point of the drug targeting for this mutation and needs further investigation.

Fig. 5: Peptides, ions, small molecules (sm), DNA and RNA binding analysis of the NR2E3 protein investigated domain. dSPRINT tool was used https://protdomain.princeton.edu/dsprint. Phylogenetic analysis had shown that the mutation is located in the non-conservative site of the gene [Fig 6]. This position has undergone changes during evolution, despite the crucial role of this DNA binding site.

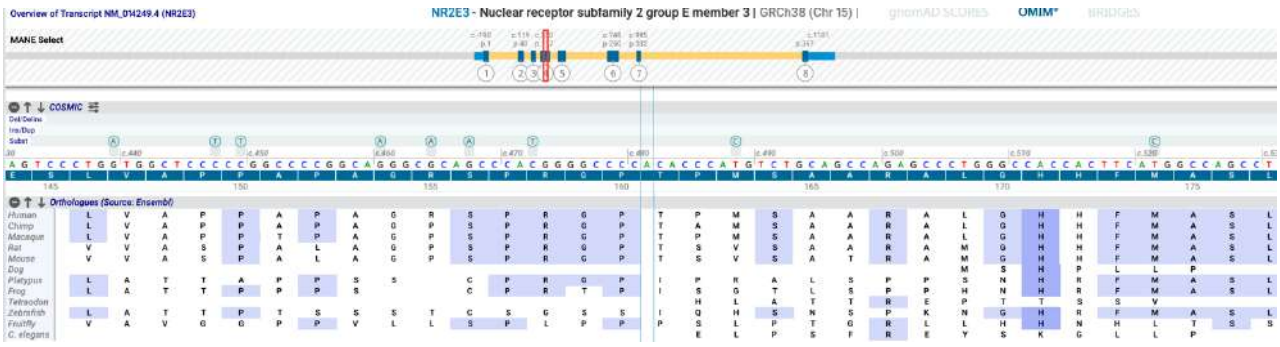

Fig. 6: Alamut phylogenetic analysis and conservativeness comparison of NR2E3 protein among species.

CONCLUSION

As 'omics' approach will continue to emerge for better understanding of disease pathogenesis of on all levels (DNA, RNA, protein, epigenetics) for searching better treatment options, modeling of specific protein and its interaction with small molecules and biologics will accelerate treatment development.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Oftalmic CRO, (protocol #02/2020). 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.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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FINANCIAL DISCLOSURE

None

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