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***Research Article***

# Integrative Transcriptomic, Network and Structural Bioinformatics Analysis Identifies Deleterious nsSNPs in Lipid-modified Retinal Genes as Candidate Therapeutic Targets in Diabetic Retinopathy

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## ABSTRACT

Lipoprotein dysfunction has been linked to the retinal damage associated with diabetic retinopathy (DR). Despite available data, the molecular pathways associated with abnormal lipid metabolism, neurovascular impairment, and genetic variation are not well elucidated. In this study, we analyzed differentially expressed lipoprotein genes (DELGs) from a DR mouse model by utilizing RNA-Seq data, gene ontology enrichment, PPI network analysis, nsSNP impact prediction, and structural effects of prioritized variants were analyzed using SWISS-MODEL homology modeling, FoldX stability predictions, among others. Ten key hub genes that are crucial for retinal function were discovered to contain nsSNPs that are structurally deleterious. The pathogenic RHO P53R/L variant was confirmed to significantly destabilize rhodopsin structure, aligning with known photoreceptor degeneration. Additional deleterious variants in RET, GNG3, and CNP showed strong destabilizing or functional impacts based on FoldX and I-Mutant. The progression of DR is probably aided by these genetic variations, which impact lipid homeostasis and phototransduction. Our findings suggest that targeting DELGs or correcting deleterious SNP-induced dysfunction could offer new treatment options for DR.

**Keywords:** Diabetic Retinopathy; Differentially expressed genes; Lipidation; Lipoprotein; nsSNP; PPI network

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## INTRODUCTION

Retinal health depends on the accurate regulation of lipid metabolism, phototransduction, and vascular stability. The disruption of these systems is typical of diabetic retinopathy (DR), a condition with both metabolic and genetic bases (Duh et al., 2017). Additionally, oxidative stress caused by hyperglycaemia and dyslipidaemia has been identified as an important factor in the development of DR, involving lipoproteins and their regulatory genes (Gholami Chahkand et al., 2023; Linton et al., 2019; Ruan et al., 2020; Timothy J Lyons, 2013). Thus, diabetic retinopathy (DR) is recognized as a progressive complication that arises from diabetes mellitus, and has been identified as one of the leading causes of vision loss globally (Teo et al., 2021; W. Wang & Lo, 2018).

Diabetic retinopathy is majorly characterized and largely related to microvascular and neurovascular impairment of the retina (Chang, 2015; Liao et al., 2018), leading to reduce vision or vision derangement such as diabetic macular ischemia, macular edema and proliferative retinopathy (Graham et al., 2018; Kang et al., 2016; Ren et al., 2018; Ulbig & Kollias, 2010; Usman, 2018; Wu et al., 2018). Therefore, maintaining blood glucose levels is essential for diabetes management, but increasing evidence suggests that lipoprotein metabolism significantly contributes to retinal damage and the progression of DR (Busik, 2021; Duh et al., 2017). This shows that

lipoproteins are not only vital lipid carriers within the body, but they are also crucial in signaling transduction and involved in preserving equilibrium in nearby tissues, such as the retina.

Lipoproteins, particularly low-density lipoprotein (LDL), play an indirect but significant role in DR pathogenesis, primarily when the blood-retina barrier (BRB) is compromised in diabetes. In an intact BRB, plasma lipoproteins have minimal impact on the retina; however, BRB leakage allows extravasation of lipoproteins, which undergo local modifications (such as oxidation and glycation) in the retinal microenvironment, rendering them toxic to vascular endothelial cells, pericytes, and other retinal components, thereby exacerbating inflammation, oxidative stress, and neurovascular damage (Kang et al., 2016; Timothy J Lyons, 2013). Beyond circulating lipoproteins, protein lipidation—particularly S-palmitoylation, a reversible post-translational modification involving covalent attachment of palmitate to cysteine residues—regulates the localization, stability, membrane association, and signaling functions of numerous retinal proteins. Dysregulated palmitoylation in diabetic conditions has been implicated in endothelial dysfunction, inflammatory pathway activation (e.g., via NLRP3/NF-κB), and altered protein interactions in retinal cells, contributing to microvascular abnormalities and progression of DR (e.g., through modulation of proteins such as Rac1, SMPDL3B, and others in hyperglycemic environments) (Veluthakal et al., 2015; Zhou et al., 2024). Thus, both lipoprotein extravasation/modification and aberrant protein lipidation represent interconnected mechanisms linking dyslipidemia to retinal pathology in DR.

Genetic studies have revealed mutations in genes related to lipoproteins such as WNT, ABCA4, ABCA1, APOB, SCARB1, and CD36 which are present in the retina and lead to retinal diseases by weakening the blood-retina barrier (BRB) (Curcio et al., 2010; Drenser, 2016; Koo et al., 2014). Moreover, modified expression of macromolecules such as glycoproteins and lipoproteins has been associated with complications related to diabetes, especially in diabetic nephropathy and retinopathy, where researchers investigated possible biomarkers (Malaguarnera et al., 2013; Sugimoto et al., 2013; Yun et al., 2016; Zhang et al., 2018). Another study implicated specific glycoproteins, such as Protein S and leucine-rich α-2-glycoprotein, to the development of diabetic retinopathy (Zou et al., 2021).

This study integrates RNA-Seq-based expression profiling with gene ontology assessment, PPI network mapping, and nsSNP functional predictions to identify lipoprotein-related molecular markers linked with DR. The aim is to reveal novel therapeutic targets by highlighting crucial genes and variants with significant consequence and functional relevance in retinal pathophysiology. Moreover, integrative bioinformatics approaches are increasingly being used to formulate hypotheses that can later be validated through experiments and applied in clinical intervention (Hasin et al., 2017). Previous studies have shown modified expression of lipoprotein-associated genes in the diabetic retina, indicating their possible involvement in DR development (Hammer & Busik, 2017; Timothy J Lyons, 2013). Nevertheless, in addition to expression profiles, it is essential to investigate how structural alterations in these proteins, caused by non-synonymous single nucleotide polymorphisms (nsSNPs), may play a role in disease advancement. nsSNPs can result in amino acid changes, which could interfere with protein structure, function, and interaction networks (Mahmood Janlou, 2025; Sharma et al., 2025; Uddin et al., 2024; Yates & Sternberg, 2013).

Despite the advancements in understanding DR pathophysiology, the intricate molecular mechanisms underlying this condition are still not fully elucidated. In recent years, there has been growing interest in exploring the role of lipoproteins, particularly their differential expression patterns, in the development and progression of DR (Bryl et al., 2022; Du et al., 2017; Kumari et al., 2020; Modjtahedi et al., 2016; Song et al., 2005; Yuan et al., 2023; Yun et al., 2016). Thus, this study leverage on the availability of data in the public repository to identify nsSNPs associated with differentially expressed lipoproteins in DR. Identifying and understanding the functional consequences of these nsSNPs in lipoproteins associated with DR can provide valuable insights into the disease's molecular mechanisms.

## MATERIALS AND METHODS

### Data Retrieval and Processing

RNA-Seq data from the Akimba mouse model of diabetic retinopathy (DR) were obtained from Van Hove *et al*., (2020) (Van Hove et al., 2020) and are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-9061. Differentially expressed genes (DEGs) reported in that study were retrieved. The genes that were differentially expressed between the wild-type and Akimba cells were selected based on p

value (p 0.05), and sorted by log2 fold change. These DEGs were then cross-referenced with UniProt database (https://www.uniprot.org/id-mapping) to annotate them as lipoproteins. The annotated genes are hereafter referred to as Differentially Expressed Lipoprotein Genes (DELGs). A gene was classified as a lipoprotein if it was annotated with the keywords “Lipidation”, "Lipoprotein(s)", or "Palmitoylation" in the UniProt "Keywords" section under post-translational modifications (PTMs).

***Protein–Protein Interaction (PPI) Network Analysis***

Differentially expressed lipoprotein genes (DELGs) (86 in number) were analyzed using the GeneMANIA plugin (version 3.5.2) in Cytoscape software (version 3.10.3) to construct a protein-protein interaction (PPI) network (Franz et al., 2018; Montojo et al., 2014; Mousavian et al., 2021; Warde-Farley et al., 2010). To expand network representation, the 20 proteins with the strongest interactions to the initial DELGs were automatically included using GeneMANIA's default settings Duplicate edges and self-loops were remove from the network. The network was visualized in Cytoscape, and hub proteins were identified using the CytoHubba plugin based on degree centrality (number of connections to other proteins).

**Functional Enrichment Mapping**

Functional enrichment analysis of the DELGs was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) web server version 6.8. (https://davidbioinformatics.nih.gov/) (Huang et al., 2009; Sherman et al., 2022). Gene names from the annotated list (106 genes after expansion) were submitted as the query. Enrichment was assessed for biological processes (BP), and only terms with a false discovery rate (FDR) < 0.05 were considered statistically significant.

**nsSNP Impact and 3D Protein Modeling**

The top hub genes identified from the PPI network were queried in the NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) to retrieve all associated single nucleotide polymorphisms (SNPs). These nsSNPs were cross-validated against the UniProt's variant section for for each corresponding protein to to confirm correct mapping to the protein sequence and to retrieve any annotated functional impacts. The potential functional effects of nsSNPs were predicted using SIFT (https://sift.bii.a-star.edu.sg/) (Ng & Henikoff, 2003; Sim et al., 2012), which classifies variants as deleterious (score $\leqslant$ 0.05) or tolerated based on sequence homology and conservation. Genes with pathogenic predictions and more than 50% of their variants classified as deleterious by SIFT were selected for further structural analysis. Three-dimensional (3D) protein structures were modeled using SWISS-MODEL (https://swissmodel.expasy.org/), and variant sites were visualized using Visual Molecular Dynamics (VMD).

**Therapeutic Target Prioritization**

Therapeutic target prioritization integrated multiple bioinformatics layers: variant effect prediction, structural stability assessment, evolutionary conservation, network topology, tissue expression specificity, and druggability evaluation. Deleterious nsSNPs (from SIFT) were further evaluated for their impact on protein stability using FoldX (for ΔΔG stability predictions) and I-Mutant (for change in stability upon mutation). Evolutionary conservation of affected residues was assessed with ConSurf. Protein-protein interaction data were retrieved from STRING and analyzed in Cytoscape to compute topological metrics, including degree and betweenness centrality. Retinal and neuronal tissue specificity was evaluated using expression data from the Human Protein Atlas (HPA) and GTEx portals.

Each protein harboring a deleterious variant received a Therapeutic Priority Score (TPS), calculated as a weighted composite score:

TPS = (0.30 × Norm_SIFT) + (0.25 × Norm_FoldX) + (0.15 × Norm_I-Mutant) + (0.10 × Norm_ConSurf) + (0.10 × Norm_Degree) + (0.05 × Norm_Betweenness) + (0.05 × Norm_Expression)

where:

Norm_SIFT = normalized deleteriousness score (1 – average SIFT score; higher = more deleterious)

Norm_FoldX = normalized ΔΔG value from FoldX (higher positive ΔΔG = greater destabilization)

Norm_I-Mutant = normalized stability change prediction (higher negative value = greater destabilization)

Norm_ConSurf = normalized conservation score (higher score = more conserved/residue critical)

Norm_Degree = normalized degree centrality from Cytoscape PPI network

Norm_Betweenness = normalized betweenness centrality (indicating control over information flow)

Norm_Expression = normalized retinal/neuronal expression score from HPA/GTEx (higher = more retina-specific/relevant)

All input values were min-max normalized to the range [0,1] before weighting. Weights were assigned based on literature precedence in nsSNP prioritization studies: highest weight to variant pathogenicity and structural impact (SIFT + FoldX + I-Mutant = 70% total), moderate weight to conservation and network centrality (25%), and lowest to expression specificity (5%), reflecting the primary emphasis on functional/structural disruption in disease context.

Proteins were ranked by descending TPS to identify the most promising therapeutic candidates.

## RESULTS

### Network Analysis

Differentially expressed lipoprotein genes (DELGs) were identified by cross-referencing the DEGs from the Akimba mouse retina RNA-Seq dataset (Van Hove et al., 2020) with UniProt annotations for lipidation, lipoprotein, or palmitoylation keywords, yielding 86 DELGs. PPI network construction using GeneMANIA in Cytoscape incorporated the 20 strongest interactors, resulting in an expanded network after removal of duplicates and self-loops. The network comprises of 106 nodes and 1,384 edges, highlighting several hub genes (Figure 1).

Hub genes were ranked by degree centrality via CytoHubba, identifying ten (10) key proteins with high connectivity relevant to retinal function, lipid homeostasis, and signaling (Figure 2). The leading hub gene is Gng3 (highlighted in red) with 61 node degrees, showcase its extensive connectivity to other hub genes and interacting nodes. The other hub genes *Gria2*, *Snap25*, *Rho*, *Gnb5*, *Syt1*, *Ret*, *Thy1*, *Rab3a* and *Cnp* (highlighted in pink), formed a tightly interconnected module.

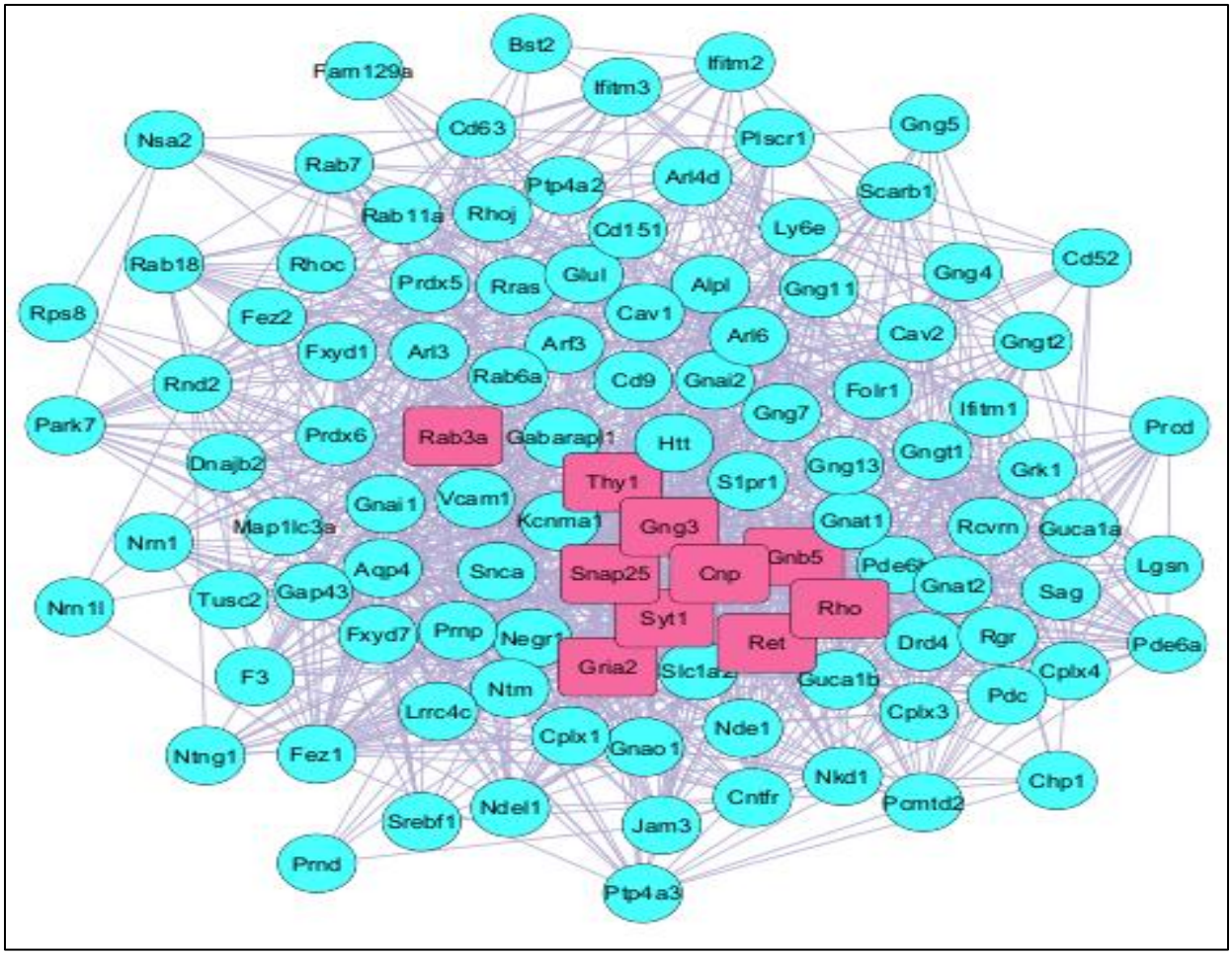


**Figure 1: GeneMANIA-derived PPI network of the identified gene set**

Nodes represent genes, and edges represent different functional associations such as co-expression, physical interactions, shared protein domains, and predicted pathways. Highly connected hub genes are highlighted in pink, indicating potential regulatory importance within the network.

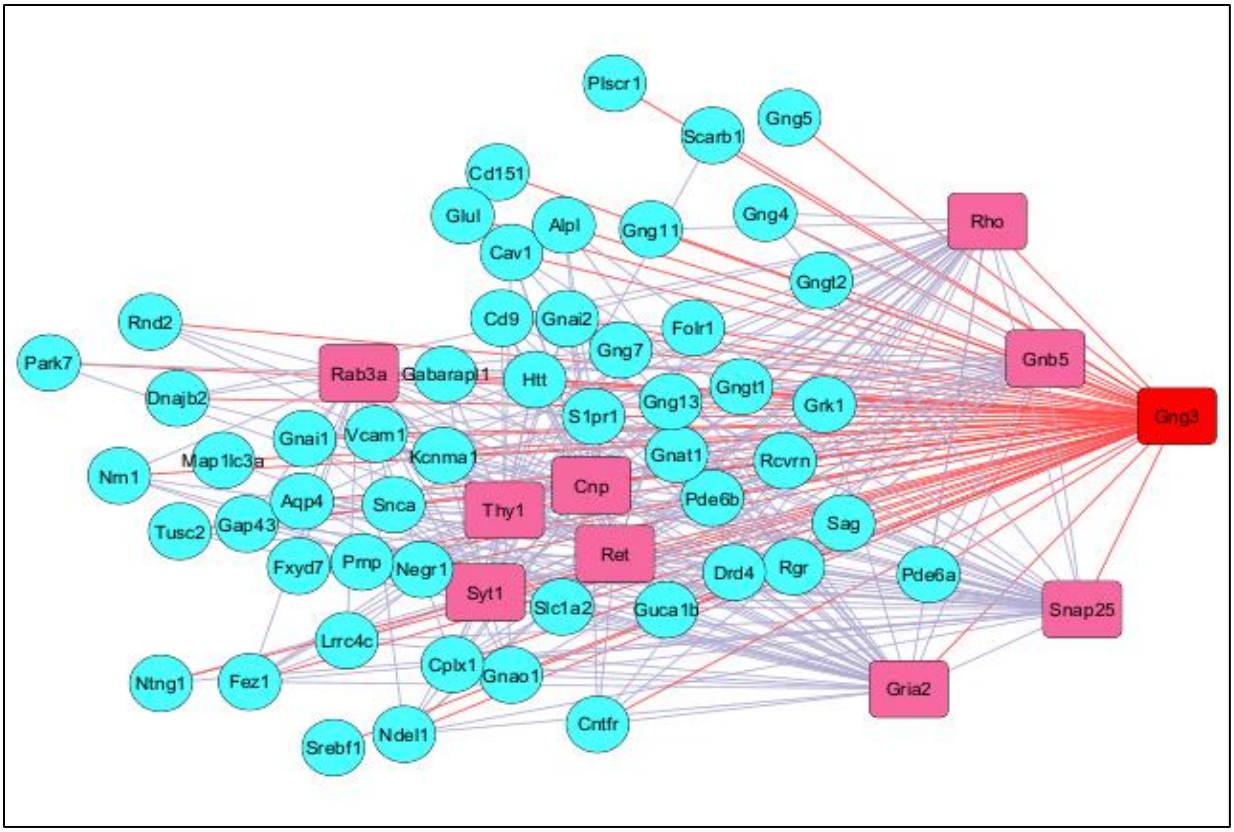


**Figure 2: Subnetwork of the top 10 hub genes identified by CytoHubba, highlighting Gng3 (red node) as the top-ranked hub**

The other hub genes, *Gria2*, *Snap25*, *Rho*, *Gnb5*, *Syt1*, *Ret*, *Thy1*, *Rab3a* and *Cnp* are highlighted in pink and form a tightly interconnected cluster.

The next hub gene to *Gng3* include; *Gria2*, *Snap25*, *Rho*, and *Gnb5* with node degrees (50, 49, 48, and 46 respectively). Generally, the strong connectivity observed among these 10 hubs genes predict their hypothetical potential as regulatory proteins in coordinating retinal signaling transduction processes relevant to disease states such as diabetic retinopathy.

**Functional Enrichment of Lipoprotein-Related Genes in Retinopathy**

Functional enrichment analysis in DAVID (v6.8) revealed significant biological processes (FDR < 0.05) among the DELGs and interactors, including lipid transport, cholesterol homeostasis, oxidative stress response, inflammatory signaling, and phototransduction-related pathways (Figure 3).

**Functional impact prediction of nonsynonymous SNPs (nsSNPs)**

For the top hub genes, nsSNPs were retrieved from dbSNP database. This includes: *Gng3*, *Thy1*, *Rho*, *Syt1*, *Gnb5*, *Rab3a*, *Gria2*, *Cnp*, *Ret*, and *Snap25*. A total of 31 nsSNPs were identified across nine genes (Table 1) excluding *Snap25*, which showed no reported missense variation in the coding sequence.

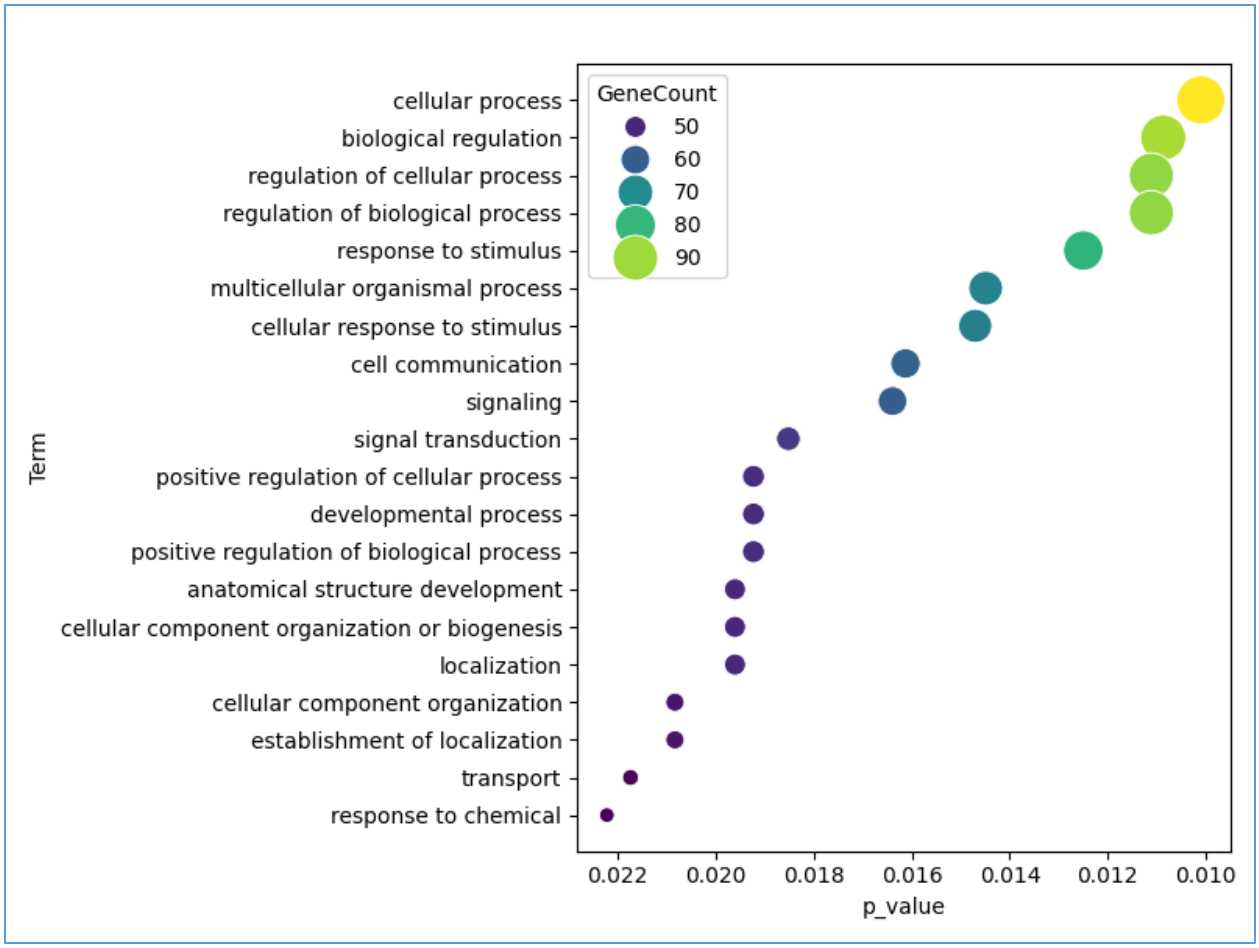


**Figure 3: Biological Process (BP) terms enriched from differentially expressed lipoprotein-associated genes implicated in retinopathy**

Additionally, processes such as cell communication, signaling, and signal transduction were prominently enriched. Enrichment like multicellular organismal processes, developmental processes, and anatomical structure development were noted.

**Table 1:** Non-Synonymous Single Nucleotide Polymorphisms (nsSNPs) Identified in Hub Genes from dbSNP Database

| Gene name | rsID | Genomic Position | Reference Allele | Alternate Allele | Amino Acid Change | MAF | ClinVar Significance |
|---|---|---|---|---|---|---|---|
| *Gng3* | rs115116507 | 11:62707136 | G | C,T | D20E, | C=0.0034 | Not reported |
| | rs140762669 | 11:62705500 | G | A | P69S | A=0.00001 | Uncertain significance |
| | rs147314661 | 11:62705406 | C | A,T | C36F/W | A=0.0005 | Uncertain significance |
| | rs148132646 | 11:62705367 | T | C | W49C, W113C | C=0.000004 | Uncertain significance |
| *Thy1* | rs367864457 | 11:119420200 | C | A,G,T | R75L | T=0.00003 | Uncertain significance |
| *Rho* | rs28933394 | 3:129528906 | C | A,G,T | T58/K/R/M | T=0.0002 | Not reported |
| | rs28933395 | 3:129528891 | C | G,T | P53R/L | T=0.000001, G=0.00001 | Pathogenic |
| | rs28933993 | 3:129532352 | A | C,G | H211P/R | C=0 | Not reported |
| | rs29001566 | 3:129533711 | C | A,G,T | P347Q/R/L | G=0, T=0.000001 | Not reported |
| | rs104893768 | 3:129528801 | C | A,T | P23H/L | A=0 | Not reported |
| *Syt1* | rs61756211 | 12:79449113 | G | A | V420I | 0.0030 | Likely benign |
| | rs144900171 | 12:79448968 | C | G,T | N371K | 0.0003 | Not reported |
| | rs745632757 | 12:79296222 | C | A,G | Q210K/E | 0.00007 | Uncertain significance |
| *Gnb5* | rs6493537 | 15:52154003 | G | A,C | H62Q, H10Q | A=0.1175 | Benign |
| | rs17612637 | 15:52141200 | C | G,T | K147N, K95N | T=0.0263 | Benign |
| *Rab3a* | rs140190198 | 19:18197619 | C | T | V172I | 0.00006 | Uncertain significance |
| | rs369617917 | 19:18198733 | T | C | D155G | 0.00023 | Uncertain significance |
| | rs377115900 | 19:18198745 | C | T | R151Q | 0.00007 | Uncertain significance |
| | rs768806536 | 19:18197548 | G | A,T | D195E | 0.00008 | Uncertain significance |
| | rs771003611 | 19:18202721 | G | A,C,T | S7L/W, | 0.000008 | Uncertain significance |
| *Gria2* | rs4302506 | 4:157317678 | T | A,C | H229Q | 0.3722 | Benign |
| | rs140170280 | 4:157321519 | T | C,G | S268P/A | 0.0009 | Likely benign |
| | rs142538282 | 4:157332904 | G | A,T | R323Q/L | 0.00005 | Likely benign |
| | rs144288333 | 4:157312699 | G | A,T | V164M/L | N/A | Uncertain significance |
| | rs147349807 | 4:157321579 | G | A,T | A288T/S | 0.00007 | Uncertain significance |
| *Cnp* | rs184299114 | 17:41965266 | T | C | L601P | 0.0008 | Likely benign |
| | rs199535375 | 17:41973568 | G | A,C | E304K/Q, E284GQ/K | 0.000004 | Not reported |
| | rs199706471 | 17:41971920 | G | C | K235R, K215R | 0.0014 | Likely benign |
| | rs202171457 | 17:41973763 | C | A,T | R349W | 0.00004 | Uncertain significance |
| | rs374975038 | 17:41968342 | G | A,C,T | R93Q/P/L | 0.00004 | Uncertain significance |
| *Ret* | rs1799939 | 10:43114671 | G | A,C,T | G691S | N/A | Not reported |
| | rs1800863 | 10:43120185 | C | A,G,T | S836R | 0.173 | Not reported |
| *Snap25* | - | - | - | - | - | - | - |

NB: - No missense variants (nsSNPs) found in dbSNP for SNAP25 coding regions.

*Gng3* had four nsSNPs (rs115116507, rs140762669, rs147314661, rs148132646), all of which were found at low minor allele frequencies (MAF ≤ 0.0034) and didn't have any ClinVar annotations. On the other hand, Thy1 had one nsSNP (rs367864457, R75L) whose clinical significance remains uncertain. The *Rho* gene critical for phototransduction was responsible for five nsSNPs, including rs28933395 (P53R/L), which is noted as pathogenic in ClinVar. However, the other variants didn't show any clinical associations.

When it comes to synaptic signaling genes, *Syt1* had three nsSNPs one was benign (rs61756211), while the other two had uncertain significance. *Gnb5* also had two nsSNPs, both deemed benign and showing relatively higher allele frequencies of 0.1175 and 0.0263. *Rab3a* had five nsSNPs, all of which were rare variants (with a minor allele frequency of less than 0.001) and their clinical implications remain unclear. In the glutamate receptor gene *Gria2*, five nsSNPs were identified, with rs4302506 (H229Q) being the most common at a frequency of 0.3722 and classified as benign. *Cnp* had five nsSNPs as well, most of which had very low allele frequencies and limited clinical evidence backing them. Ret had two significant missense variants (rs1799939, rs1800863), both of which have been mentioned in various population studies but still lack a definitive annotation in ClinVar. Interestingly, no nonsynonymous substitutions were found in the coding regions of SNAP25. Overall, looking at all the different variants, ClinVar annotations mostly showed uncertain significance at 48%. This was followed by cases that were not reported at 32%, benign or likely benign at 19%, and only 1% classified as pathogenic (rs28933395 in *Rho*). The minor allele frequencies (MAFs) were generally quite low, under 0.001, except for a couple of common polymorphisms like rs6493537, which had a frequency of 0.1175, and rs4302506 at 0.3722. This suggests that there are different frequencies in the population and possible functional impacts to consider.

SIFT predictions classified variants as deleterious (score $\leqslant$ 0.05) or tolerated. Nine hub genes (*Gng3*, *Thy1*, *Rho*, *Syt1*, *Gnb5*, *Rab3a*, *Gria2*, *Cnp*, and *Ret*) harbored nsSNPs computationally predicted as deleterious (Table 2). Here, *Gng3* harbors four nsSNPs (Table1 and 2), all demonstrated scores ranging from 0.00–0.01, indicating a 100% deleterious prediction rate. *Thy1* which carried a single mutation (rs367864457: R75L), also predicted as deleterious with a score of 0.00. In *Rho*, four of nsSNPs had a score between 0.00 and 0.03 and were classified as deleterious. Furthermore, SIFT predictions indicated deleterious effects for most nsSNPs in synaptic transmission-associated genes (Syt1, Gnb5, Rab3a, Gria2) and myelin-associated genes (Cnp, Ret), except for some tolerated substitutions (Table 2). Overall, more than 70% of the variants detected were deemed deleterious, indicating significant functional implications across these neuronal and retinal genes. In the structure of *Rho* (rhodopsin), the pathogenic variant T58K/R/M is found within the cytoplasmic loop, close to the transmembrane helix, right next to residues that are crucial for G-protein coupling (Fig 4). A closer look at the structure suggests that the hydrophobic packing might be disrupted and the electrostatic potential altered due to the substitution of lysine.

For *RET1*, the harmful variant S836R is found within the intracellular tyrosine kinase domain (Fig 5). When we compared the structures of the native and mutant *RET1* models, it became clear that this substitution brings in a larger side chain and adds a positive charge, which could change the hydrogen-bonding networks around the catalytic pocket.

The *THY1* (Thy-1 cell surface antigen) variant N75L is situated in a β-sheet-rich extracellular domain (Fig 6), where a polar asparagine is swapped for a hydrophobic leucine. This change could compromise the stability of the local β-structure, potentially impacting how well the cells anchor and adhere to surfaces.

**Table 2: Functional Impact Predictions of nsSNPs across top 10 Hub Genes Using SIFT algorithm.**

| Gene name | rsID | Amino Acid Change | Score | Prediction |
|---|---|---|---|---|
| Gng3 | rs115116507 | D20E, | 0.00 | Deleterious |
| | rs140762669 | P69S | 0.00 | Deleterious |
| | rs147314661 | C36F/W | 0.00 | Deleterious |
| | rs148132646 | W49C, W113C | 0.01 | Deleterious |
| Thy1 | rs367864457 | R75L | 0.00 | Deleterious |
| Rho | rs28933394 | T58/K/R/M | 0.00 | Deleterious |
| | rs28933395 | P53R/L | 0.03 | Deleterious |
| | rs28933993 | H211P/R | 0.29 | Deleterious |
| | rs29001566 | P347Q/R/L | 0.00 | Deleterious |
| | rs104893768 | P23H/L | 0.00 | Deleterious |
| Syt1 | rs61756211 | V420I | 0.41 | Tolerated |
| | rs144900171 | N371K | 0.01 | Deleterious |
| | rs745632757 | Q210K/E | 0.01 | Deleterious |
| Gnb5 | rs6493537 | H62Q, H10Q | 0.04/.01 | Deleterious |
| | rs17612637 | K147N, K95N | 0.11/0.00 | Tolerated/Deleterious |
| Rab3a | rs140190198 | V172I | 0.00 | Tolerated |
| | rs369617917 | D155G | 0.00 | Tolerated |
| | rs377115900 | R151Q | 0.67 | Tolerated |
| | rs768806536 | D195E | 0.01 | Tolerated |
| | rs771003611 | S7L/W, | 0.00 | Deleterious |
| Gria2 | rs4302506 | H229Q | 0.30 | Tolerated |
| | rs140170280 | S268P/A | 0.27 | Tolerated |
| | rs142538282 | R323Q/L | 0.00 | Deleterious |
| | rs144288333 | V164M/L | 0.99 | Tolerated |
| | rs147349807 | A288T/S | 0.46 | Tolerated |
| Cnp | rs184299114 | L601P | 0.00 | - |
| | rs199535375 | E304K/Q, E284GQ/K | 0.07/ 0.00 | Tolerated/Deleterious |
| | rs199706471 | K235R, K215R | 0.00 | Deleterious |
| | rs202171457 | R349W | 0.00 | Deleterious |
| | rs374975038 | R93Q/P/L | 0.00 | Deleterious |
| Ret | rs1799939 | G691S | 0.01 | Deleterious |
| | rs1800863 | S836R | 0.01 | Deleterious |

Selected genes with a high proportion (>50%) of deleterious predictions underwent 3D structural modeling using SWISS-MODEL, with variant sites visualized in VMD.

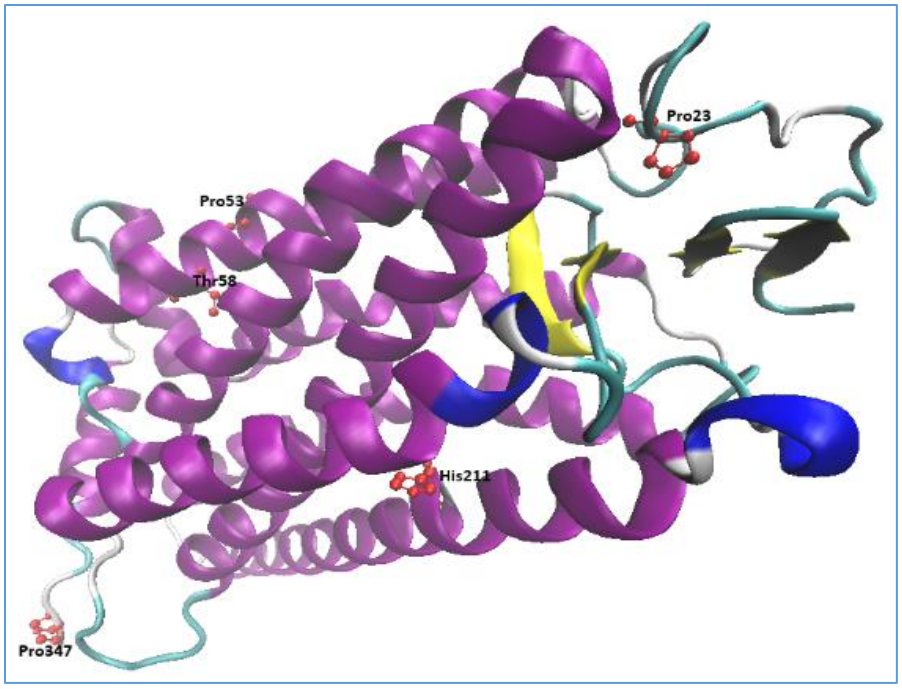


**Fig 4: Homology model of RHO highlighting the T58K, P53R/L, H211P/R, P347Q/R/L and P23H/L variant sites**
The T58 residue (red) is located within the cytoplasmic loop between transmembrane helices.

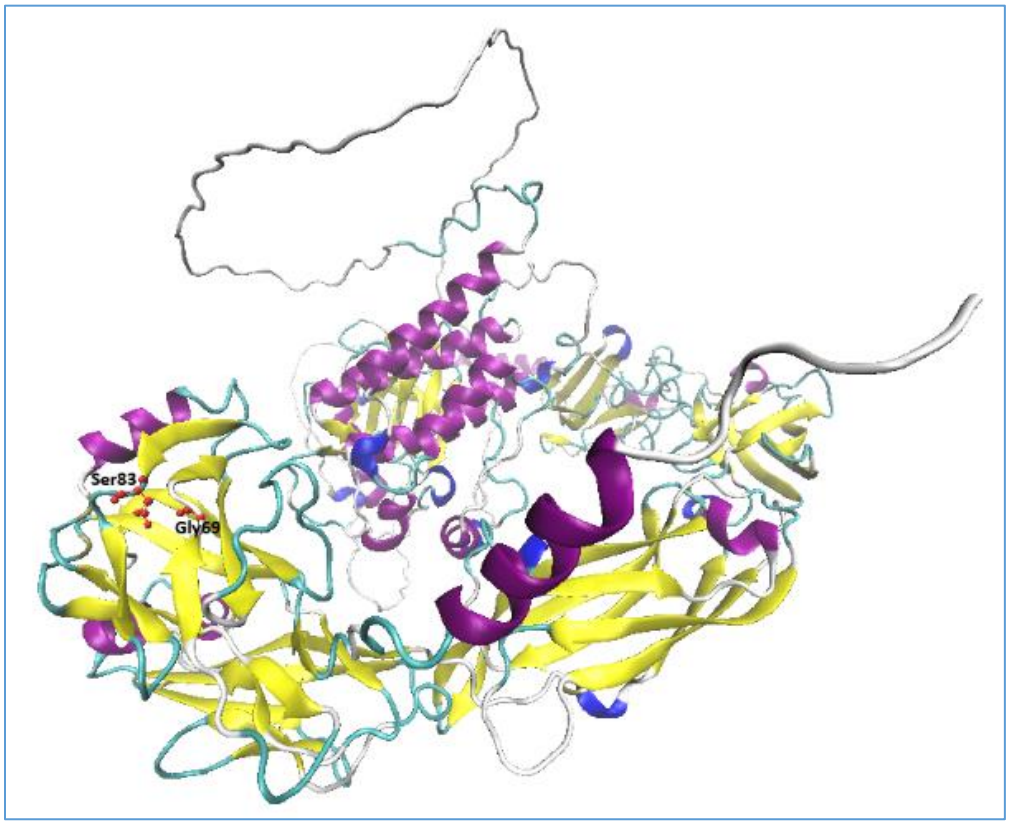


**Fig 5: RET1 receptor tyrosine kinase (RET1) model showing the S836R and G691S variant sites**
The modeled intracellular are displays red CPK. The substitution at S836R introduces an additional positive charge near the catalytic cleft.

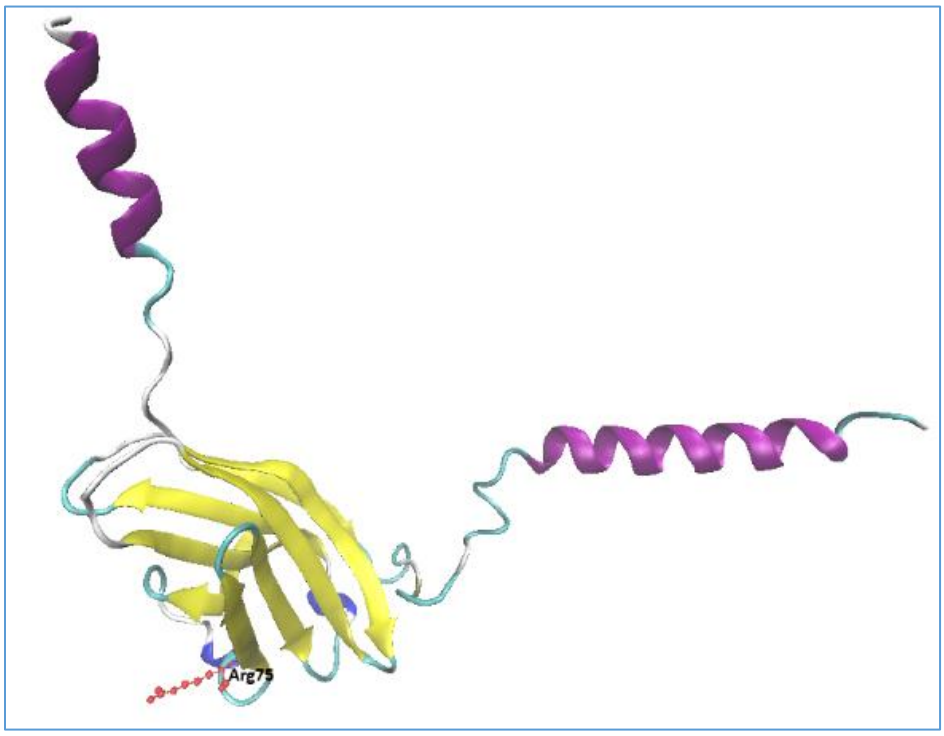


**Fig 6: Modelled structure of THY1 indicating the N75L substitution site**
The THY1 extracellular β-sheet domain is displayed, with the variant site (in red) mapped to a region mediating surface glycoprotein interactions. Replacement of a polar asparagine with a hydrophobic leucine is predicted to weaken hydrogen bonding and disturb β-strand packing, which may influence membrane localization and adhesion.

In the *SYT1* (Synaptotagmin-1) model, the V420I substitution is found near the C2B domain (Fig 7). Although this change seems structurally conservative, its closeness to the calcium-binding loops hints at a possible minor disruption in local flexibility. In summary, visualizing the structures confirmed that most of the harmful substitutions are found in functionally or structurally conserved domains, aligning with what computational predictions suggested.

**Therapeutic Insights**

Stability impact assessments using FoldX (ΔΔG) and I-Mutant suggested potential destabilization or functional alteration for several variants, including the known pathogenic *RHO* T58K and P53R/L substitution (previously associated with retinitis pigmentosa and photoreceptor degeneration). Variants in RET, GNG3, and CNP also showed computationally predicted destabilizing effects or changes in stability (Table 3).

Therapeutic prioritization assigned a composite Therapeutic Priority Score (TPS) to proteins with deleterious nsSNPs, integrating normalized scores from SIFT deleteriousness, FoldX/I-Mutant stability changes, ConSurf conservation, network centrality (degree and betweenness), and retinal expression specificity (HPA/GTEx). Proteins were ranked by TPS to highlight candidates for further experimental validation.

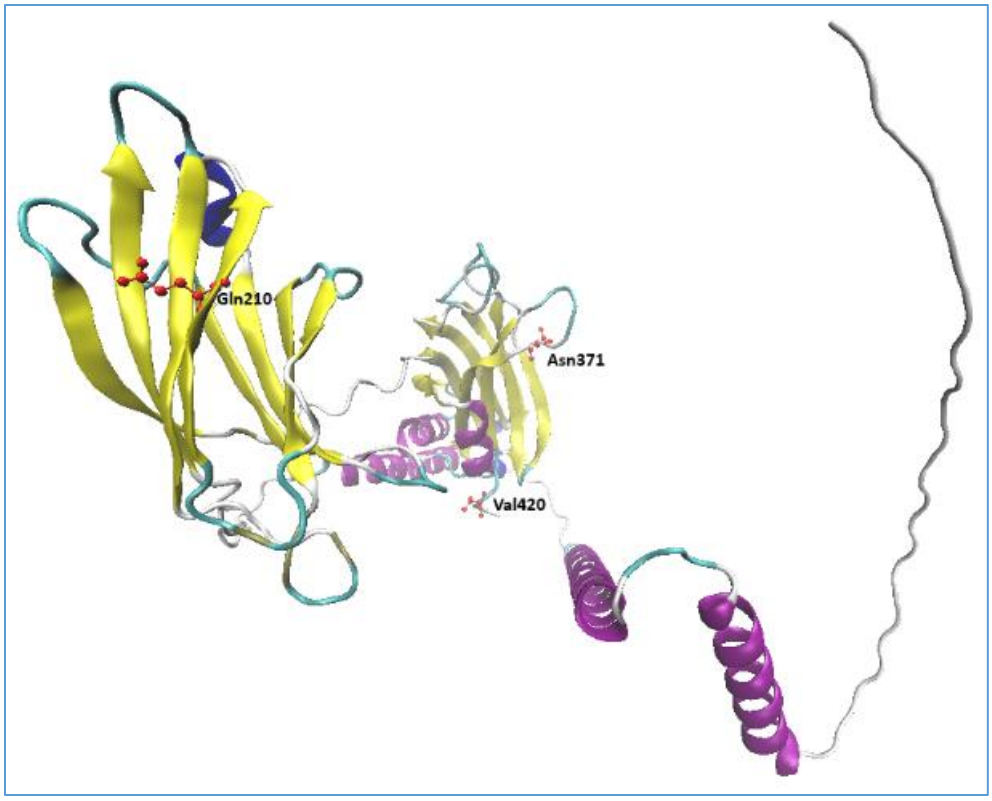


**Fig 7: SYT1 (Synaptotagmin-1) structural model showing the V420I, N371K and Q210K/E variants**
The C2B calcium-binding domain is depicted, with the variant residue V420 (red) adjacent to the $Ca^{2+}$-binding loop. Although the isoleucine substitution is conservative, minor changes in local hydrophobic packing may modulate calcium-dependent vesicle fusion efficiency.

**Table 3: Prioritization of Variant-Bearing Proteins**

| Rank | Gene | Variant | Composite Score | Functional Role | Druggability |
|---|---|---|---|---|---|
| 1 | RHO | p.T58K | **0.912** | Visual signal transduction | Indirect / Moderate |
| 2 | RET | p.S836R | **0.903** | Tyrosine kinase signaling | High (drug targetable) |
| 3 | CNP | p.E307K | **0.857** | Myelin stability enzyme | Moderate |
| 4 | GNB5 | p.Glu20Asp | **0.842** | GPCR signaling regulator | High |
| 5 | THY1 | p.R75L | **0.812** | Cell adhesion glycoprotein | Low |
| 6 | GNG3 | p.D20E | **0.765** | GPCR γ-subunit | Low |
| 7 | SYT1 | p.V420I | **0.692** | $Ca^{2+}$-dependent vesicle fusion | Low |

## DISCUSSION

This integrative bioinformatics study analyzed differentially expressed lipoprotein genes (DELGs) from the Akimba mouse model of diabetic retinopathy (DR), revealing key hubs in lipid homeostasis, oxidative stress, inflammation, and phototransduction pathways. Ten hub genes harbored nsSNPs predicted as deleterious by SIFT, with structural modeling and stability predictions (FoldX, I-Mutant) indicating potential destabilization, particularly for variants in RHO, RET, GNG3, and CNP. The known pathogenic RHO T58K and P53R/L variants, aligned with prior associations to photoreceptor degeneration, while others suggested functional impacts warranting further investigation. Therapeutic prioritization via TPS highlighted candidates with combined evidence of pathogenicity, structural effects, conservation, network centrality, and retinal expression relevance.

These findings support the growing recognition of lipid dysregulation in DR pathogenesis. Recent Mendelian randomization (MR) analyses have established causal links between plasma lipids (e.g., triglycerides, HDL/LDL ratios) and DR risk, with genetic proxies for lipid-lowering targets such as HMGCR (statins), APOB, and PPARG showing

protective effects against DR subtypes, including non-proliferative and proliferative forms (Cao et al., 2025; Chen et al., 2024; J. Wang et al., 2025; Xu et al., 2026). Elevated lipoprotein(a) [Lp(a)] levels are also consistently associated with higher DR prevalence and progression, as confirmed in recent systematic reviews and meta-analyses demonstrating significantly higher Lp(a) in DR patients compared to controls (SMD 0.85, 95% CI 0.48–1.22) (Lampsas et al., 2025; Pang & Yi, 2024).

Protein lipidation, particularly S-palmitoylation, emerges as a critical mechanism linking hyperglycemia to retinal pathology. In high-glucose conditions, palmitoylation of SMPDL3B via ZDHHC5 stabilizes the protein and acts as a compensatory anti-inflammatory response by suppressing NLRP3/NF-κB signaling in retinal endothelial cells (Zhou et al., 2024). Conversely, palmitic acid induces Keap1 palmitoylation, which upregulates Keap1 levels, inhibits the Nrf2/GPX4 pathway, and promotes ferroptosis in retinal microvascular endothelial cells, thereby aggravating DR (Mao et al., 2025). These observations align with our identification of lipid-modified genes (e.g., those annotated for palmitoylation) as hubs, suggesting that dysregulated palmitoylation contributes to endothelial dysfunction and neurovascular impairment in DR.

While computational predictions provide mechanistic insights, they remain hypothetical and require experimental validation (e.g., functional assays, CRISPR editing, or animal models) to confirm structural/functional impacts and therapeutic potential. Targeting these prioritized genes via small-molecule stabilizers, palmitoylation modulators, or gene therapies could complement existing anti-VEGF and lipid-lowering strategies, offering novel avenues for early intervention in DR.

Herein, this study demonstrates the utility of multi-layer bioinformatics in nominating lipid-associated candidates for DR, bridging transcriptomic alterations with genetic variants and structural effects in a disease driven by metabolic and oxidative stress.

## CONCLUSION

In this study we applied a comprehensive bioinformatics approach that combined nsSNP analysis, structural modeling, and functional enrichment to elucidate on the molecular basis of diabetic retinopathy. Predictive used here identified deleterious substitutions within key retinal and lipoprotein-related genes, particularly *RHO*, *RET*, *GNG3*, and *CNP*. The known pathogenic variant *RHO* P53R/L reaffirmed its connection to retinal degeneration, while rare substitutions in *GNG3* and *THY1* hinted at new candidates that could influence synaptic transmission and neuroprotection.

Through functional enrichment and network analyses, it became clear that the affected genes are grouped within pathways that manage lipid transport, cholesterol balance, oxidative stress, and inflammation; all vital for maintaining neurovascular stability in the diabetic retina. These results highlight how imbalances in lipoproteins and oxidative damage can disrupt photoreceptor signaling and the integrity of blood vessels, creating a link between lipid metabolism and retinal neurodegeneration. By focusing on hub genes that play dual roles in lipid regulation and neuronal signaling (like *RHO*, *RET*, *GNG3*, and *CNP*), the study points to potential therapeutic targets for adjusting lipid metabolism and reducing retinal damage. Overall, this research showcases the strength of integrated bioinformatics in revealing genotype–phenotype connections and suggests that restoring balance in lipids and redox processes could be a promising strategy for tackling diabetic retinopathy.

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