Pak. j. sci. ind. res. Ser. A: phys. sci. 2026 **69A**(1) 14-21

In-silico Analysis of Non-synonymous SNPs of CYPIBI Gene and Their Potential Effects on Cytochrome P450 Protein Associated with Glaucoma

Rimsha Zafar[a]*, Naveera Khan[b], Muhammad Awais[a] and Amina Rehman[a]
[a]Department of Biotechnology, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
[b]Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan

(received September 12, 2023; revised November 15, 2024; accepted December 12, 2024)

Abstract. Glaucoma, the leading cause of irreversible blindness worldwide, results from fluid accumulation in the eye, causing optic nerve damage. It is classified into two major types: open-angle and closed-angle glaucoma. Mutations in the CYP1B1 gene have been linked to glaucoma, as the gene encodes a protein in the cytochrome P450 enzyme family, critical for detoxifying carcinogens and metabolizing drugs, toxins and endogenous compounds. Non-synonymous single nucleotide polymorphisms in the CYP1B1 gene result in altered amino acid sequences, disrupting the enzyme's structure and function of the 612 reported mutations in CYP1B1, 113 are considered deleterious based on in silico tools. These findings provide a foundation for future in *vitro* studies on drug design and therapeutic interventions targeting glaucoma-associated CYP1B1 mutations. This research enhances our understanding of the molecular mechanisms underlying glaucoma and opens new avenues for targeted treatment strategies.

Keywords: glaucoma, CYP1B1 gene, non-synonymous SNPs, computational analysis, open-angle glaucoma, web base software

Introduction

Glaucoma is an open-angle optic nerve disorder that can lead to blindness, ranking as the second leading cause of blindness in individuals over 60. The condition is primarily characterized by elevated intraocular pressure (IOP), which results from the accumulation or overflow of aqueous humor. This fluid circulates through specific channels within the eye (Allison *et al*., 2020). Defects in the pathways of aqueous humor can lead to obstruction or excessive fluid accumulation, resulting in elevated intraocular pressure (IOP) and subsequent vision loss. If not promptly managed, this increased pressure can cause irreversible optic nerve damage and permanent visual impairment. Several physical factors may contribute to the pathogenesis of glaucoma, including ocular trauma. At the same time, in pediatric populations, the condition may exhibit a hereditary component, suggesting a genetic predisposition to glaucoma development (Lee *et al*., 2011).

Glaucoma is classified into two main types: primary open-angle glaucoma (POAG) and angle-closure glaucoma. In the United States, more than 80% of glaucoma cases are classified as primary open-angle glaucoma, characterized by obstructing aqueous drainage between the iris and cornea. This obstruction leads to elevated intraocular pressure (IOP), often occurring insidiously, which may result in the absence of early warning symptoms (Sheybani *et al*., 2020). This condition, known as primary congenital glaucoma, primarily affects infants and children under the age of 5. It is classified as an open-angle glaucoma. In contrast, angle-closure glaucoma or acute angle-closure glaucoma, presents with distinctive symptoms such as headache, blurred vision, conjunctival infection and ocular pain. Prompt recognition of symptoms associated with glaucoma necessitates immediate medical intervention; failure to do so may result in irreversible vision loss (Allison *et al*., 2020).

By reviewing the genetics of glaucoma, that several mutations in myocilin, optineurin and CYP1B1 genes are involved in the deterioration of the optic nerve, ultimately resulting in glaucoma. Other genetic factors that can give rise to glaucoma include the change in the sequences of DNA or complete loss of the original DNA. POAG is the most common type of glaucoma that occurs in babies from birth to the age of 3 and the most common reason for blindness in young people in central Europe and the Middle East. Mutation in the CYP1B1 gene has a high weightage of causing the

*Author for correspondence; E-mail: misha.143@ucp.edu.pk; Rimshazafar2200@gmail.com

POAG type of glaucoma. The genes involved in this type of glaucoma include *PITX2, PITX3, FOXC1, FOXE3, PAX6, LMX1B* and *MAF(*Awais *et al*., 2023; Lee *et al*., 2011). A total of 16 loci are involved in primary open-angle glaucoma observed by Genome-wide association studies (GWAS) . In the case of close angle glaucoma that is the second type of glaucoma, which affects more than 16 million people all over the world, is caused by the mutation or complete change in the sequence of the DNA and can be found in *PLEKHA7, PCMTD1/ST18* and *COL11A1*(Tan *et al*., 2016) There are a total of eight genes that are involved in this type of glaucoma, which include *PLEKHA7*, *COL11A1*, *PCMTD1-ST18*, *EPDR1*, *CHAT*, *GLIS3* and *FERMT2* (Wan *et al*., 2019).

Mutations in the cytochrome P450 1B1 (CYP1B1) gene primarily cause congenital glaucoma, predominantly affecting children and inherited as an autosomal recessive disorder. The mutations associated with this condition include missense mutations, frameshift mutations, deletions and nonsense mutations all of which compromise the functionality of the CYP1B1 gene (Kumar *et al*., 2024; Alsubait *et al*., 2020). Located on chromosome 2p21-22, the CYP1B1 gene comprises three exons, with research indicating that deleterious mutations primarily occur in the second and third exons. These mutations are critical for regulating aqueous humor production in the eye's anterior chamber (Tang *et al*., 1996). Furthermore, alterations in the CYP1B1 gene adversely affect the stability of the trabecular meshwork (TM), which is essential for effective fluid drainage from the eye, ultimately leading to elevated intraocular pressure (IOP) (Chouiter and Nadifi, 2017). The primary objective of this study is to identify genotypic mutations within the CYP1B1 gene using in silico analysis and protein modeling. These mutations may hinder the proper functioning of enzymes involved in the efficient drainage of aqueous humor from the anterior chamber of the eye, potentially leading to increased intraocular pressure and subsequent blindness.

Materials and Methods

Retrieval of sequence. The gene sequence is obtained from the NCBI (National centre of biotechnology information) website (https://www.ncbi.nlm.nih.gov/). The coding form of sequence for the analysis of mutation in the CYP1B1 gene, ENSEMBL is used to obtain single nucleotide polymorphisms in the coding region of the CYP1B1.

Single nucleotide polymorphism annotation PolyPhen-2. An online tool used to determine the potential effects of nsSNPs on the function and the structure of the protein is Polyphen-2 (https://www.ncbi.nlm.nih.gov/). Software scores range between 0 and 1 and SNP results are benign, possibly damaging, or probably damaging. (Adzhubei *et al*., 2010). We used this software to test the potential effects of nsSNPs on protein structure and function.

Provean. Provean is a protein variation affect analyzer (http://genetics.bwh.harvard.edu/pph2/). It is also an online web tool used to evaluate the likely consequence of amino acid substitution on protein functions that involve primarily biological functions (Awais *et al*., 2021; Choi and Chan, 2015). The default value of Provean is -2.5 and a score of SNPs less than -2.5 is considered harmful. The utilization of this tool to assess the potential impacts of amino acid substitutions on the biological functions of our protein.

Sift. Sift (https://sift.bii.a-star.edu.sg/www/SIFT_related_seqs_submit.html) is sorting intolerant from tolerant is the appropriate tool used to calculate the effect of substituted amino acids. (Kumar *et al*., 2009) Based on the chemical and physical properties of amino acids, it produces a correlation between substituted amino acids and protein function variation. Its estimated score is 0.05.SNP having a score of less than 0.05 is considered not tolerated and considerably affects protein function (Awais *et al*., 2021).

PhD-SNP. It is a web-based server that predicts the toxic effect of SNP on protein function. It depends on the support vector machine SVM classifier, which gives neutral or diseased results. (https://snps.biofold.org/phd-snp/phd-snp.html) (Capriotti and Fariselli, 2017). This tool is used to evaluate whether the amino acid substitution is likely disease-causing.

Prediction evaluation of SNP on stability of protein. ***MUpro*.** It (http://mupro.proteomics.ics.uci. edu/) is an online tool that predicts the changes in the stability of proteins that result from the single site mutation. Its score ranges from -1 to 1 and results less than zero will downregulate protein stability (Awais *et al*., 2021). This tool is used in our study to evaluate protein stability after the mutation.

***I-Mutant 3.0 site*.** It is also an SVM-based online web server and gives the result of the effect of SNP on protein stability by investigating the amino acid sequence

and protein configuration (Capriotti *et al*., 2005). This tool (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi) is used in this study to estimate the possibility of SNP effect on human health; its score is based on DDG value, which is a metric used to know how an SNP can affect the stability of the protein.

Protein structure prediction. To visualize the effect of mutant residue and compression between wild-type and mutant residue, the HOPE webserver (https://www3.cmbi.umcn. nl/hope/) was used (Venselaar *et al*., 2010). It also predicts the possible effect of modified residues on protein function and adjacent residues.

PSIPRED is also an online web software used to get a secondary structure of proteins. It uses a built-in logarithm on the amino acids to produce an accurate secondary structure (Buchan and Jones, 2019). I-TASSER is the tool used for structure prediction and evaluation. It gives five possible 3D structures of protein (Zafar and Awais, 2023; Yang *et al*., 2015). Mutated models are generated for each SNP by using the Mutation 3D tool.

Refinement and verification of 3D structure. For the refinement and verification of the protein structure, we used an online tool called Galaxy Refine (https://galaxy.seoklab.org/cgi-bin/submit_REFINE.cgi). was used It constantly applies molecular dynamic simulation to refine the 3D structure of proteins (Zafar and Awais, 2023; Ko *et al*., 2012). Consequently, the 3D structure is verified by using Verify3D because it relates the compatibility of the 3D model with the sequence of amino acids of the gene.

Results and Discussions

SNPs annotation by using different software. Using the ENSEMBL database, an online platform for gene retrieval, obtained all known variations within the CYP1B1 gene. For subsequent analysis, only non-synonymous single nucleotide polymorphisms (SNPs) were selected, as these variants alter the amino acid sequence of the encoded protein. A total of 612 non-synonymous SNPs were identified in the CYP1B1 gene. To assess their impact on protein structure and function, various in silico prediction tools was employed of the 612 variants, 113 were consistently classified as deleterious across all tools, as detailed in supplementary Table 1.

The first tool, polyphen-2, predicted that 36.9% of non-synonymous single nucleotide polymorphisms (nsSNPs) were benign, while 47.52% were classified as potentially damaging. PhD-SNP analysis identified 37.92% of the mutations as disease-causing and 62.09% as neutral. Similarly, provean results indicated that 46.73% of SNPs were deleterious, with 53.26% being neutral. SIFT analysis revealed that 59.47% of the mutations had a deleterious impact on protein function, whereas 40.52% were classified as benign shown in (Fig. 1). Using MUpro, 577 out of 612 mutations were predicted to decrease protein stability. I-Mutant analysis further confirmed that 87.091% of the mutations reduced protein stabilityas indicated in (Fig. 2).

Annotation of protein structure. HOPE produced the results depicting the consequences of mutant residues on protein morphology and function and its comparison with wild-type residues, as shown in Supplementary Table 2. 59 out of 113 nsSNPs were damaging the protein's function.

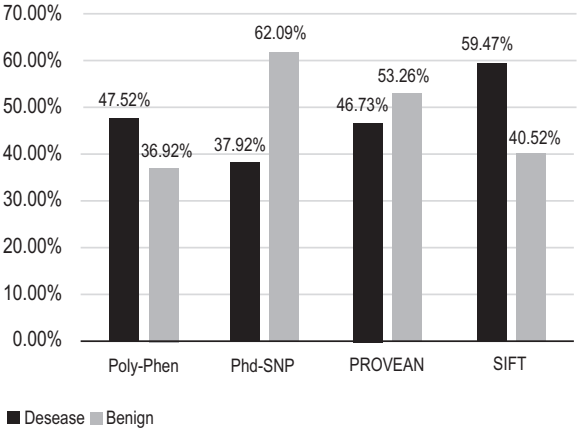

Fig. 1. Graph showing the percentage of deleterious and benign SNPs in CYP1B1 gene.

Table 1. Comparison between I-TASSER and galaxy refine

Model	GDT-HA	RMSD	Molprobity	Clash score	Poor rotamers	Rama favored
I-TASSER	1.0000	0.000	2.537	3.0	10.8	84.8
Galaxy refine	0.9581	0.399	2.039	13.9	0.6	94.3

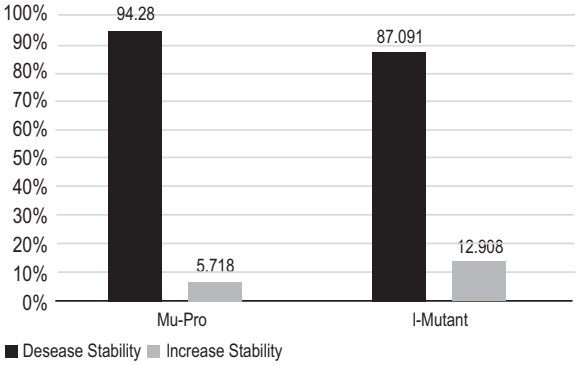

Fig. 2. Effect of SNPs on Protein stability predicted by MUpro and I-Mutant.

PSIRED is a secondary structure prediction tool for finding protein structures, including alpha helix, beta sheets and coils. As shown in Fig. 3, 543 amino acid sequences are combined to form the secondary structure of the CYP1B1 protein.

Refinement and verification of 3D structure. Following structural and functional annotation, the subsequent step involves verifying and refining the 3D protein structure. Two primary tools were employed for this process. Galaxy refine and I-TASSER. Galaxy refine was used to optimize side-chain repacking and enhance structural accuracy. A comparative analysis of the 3D models generated by Galaxy refine and I-TASSER is presented in Table 1. Structural verification was performed using the Verify 3D tool, which determined that approximately 80% of the amino acid

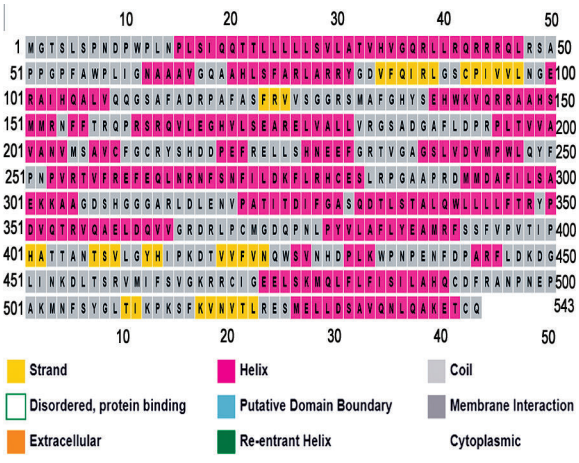

Fig. 3. Secondary structure of CYP1BI protein predicted by PSIPRED.

residues should achieve a 3D-1D profile score greater than 0.2. as per model, 82.96% of the residues exhibited a 3D-1D score =0.2, as shown in Fig. 4. The refined 3D structure of the CYP1B1 protein is illustrated in Fig. 5 and the 3D models corresponding to each single nucleotide polymorphism (SNP) are provided in the Supplementary File.

CYP1B1 gene is a member of cytochrome P450 enzyme subfamily associated with glaucoma and causes early onset of glaucoma. This gene is also associated with other diseases, such as Peters anomaly. Glaucoma is an autosomal recessive disorder and can be genetic, the second leading cause of eye blindness and the third most observed disease in white people in America (Wiggs, 2007). Mutations in CYP1B1 genes result in the abnormal functioning of its encoded protein (Pan *et al*., 2024; Song *et al*., 2019). The expression of this gene is different in normal and affected persons, making

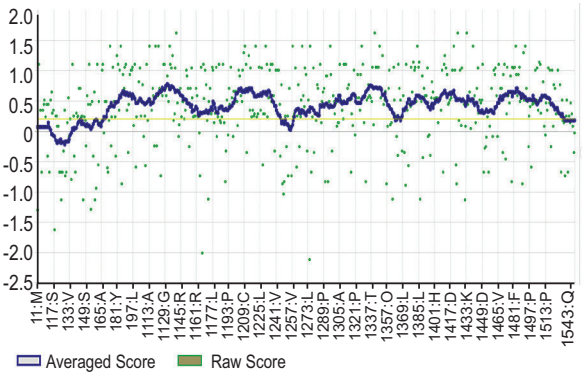

Fig. 4. Verification plot of protein tertiary structure through verification 3D.

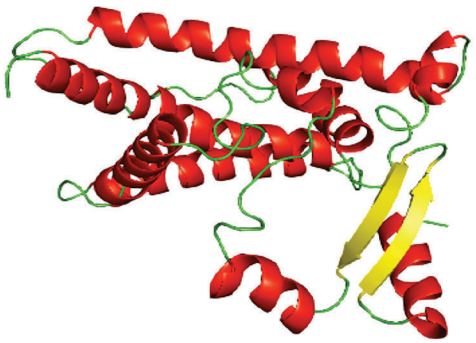

Fig. 5. 3D structure of CYP1B1 protein visualized via PyMOL. Red denotes the helix, green represents coils and yellow represents sheets.

it an important candidate for therapeutic strategies. Hence, to find out the effect of the CYP1B1 gene on the onset of glaucoma, The different tools was used like SIFT, MUpro, I-Mutant, PolyPhen, PhD-SNP and Provean, found that out of 612 SNPs present in the ENSEMBL database, 113 have deleterious effects.

SIFT prediction was used to deduce the amino acid substitutions generated by 113 nsSNPs. SIFT predicted 363 nsSNPs to be deleterious based on the degree of conservation of amino acid residues in sequence alignments produced from closely related sequences and collected by PSI-BLAST (Bhatnager and Dang, 2018). PROVEAN predicted 286 substitutions to be damaging, and the structural impact of nsSNPs was estimated by Polyphen-2, which predicted 291 substitutions were possibly damaging affecting the protein structure and function (Khalid and Almaghrabi, 2020). Additionally, PhD-SNPs identified 232 nsSNPs, which were accepted to be disease-associated (Kumar *et al*., 2015).

Manual concurrence of all the nsSNPs studied by different software was done. Total 113 substitutions, C470Y, R469W, T510P, C491S, C491R, H489R, H489Y, K477E, E473K, G472R, G466D, K454Q, G450S, F445L, R444P, P442A, F440L, F440C, F440S, P437Q, P437S, P437T, W434C, W434R, D430E, W425R, N423I, K416E, P415L, I414F, Y412C, A383D, R368H, R368C, R368G, D367Y, G365W, G365R, V364G, V364A, D361H, L360S, E359A, R355P, Q353P, L343P, S336Y, A330V, A330P, V320A, I297T, F296C, A295T, D294Y, D291G, R290C, S282R, S282R, R266C, R255H, R255C, P245S, D242E, D242H, G236R, F231Y, D218V, D218Y, R213C, G211D, N203S, N203D, P193R, P193S, D192A, G188C, R183C, L181P, E173K, L166P, R153C, R146C, R146G, W141G, S138W, G129S, G128R, S122F, P118L, R117Q, R117W, R117G, A106G, A106D, I103S, L97P, V96E, V96M, V95A, G90D, G90R, L89P, L89Q, G82S, Y81C, R80P, A78E, R76C, N62K, G61A, G61E and P58Q, deleterious in all software were selected for further verification. The effect of these nsSNP*s* on protein stability was determined through I-Mutant 3.0 and MUpro, which predicted the difference in Gibbs free energy between wild-type and mutant proteins, hence revealing changes in protein stability (Elango *et al*., 2018; Tosh *et al*., 2015). Out of 113 nsSNPs, I-Mutant showed 109 nsSNPs, and MUpro showed all 113 nsSNPs decreased the stability of CYP1B1 protein.

With these predictions the narrowed the search and focused on 113 nsSNPs predicted to be potential candidates for disease occurrence. Using a further computational algorithm, 3D models were generated by I-TASSER (Samant *et al*., 2015) and the RMSD (Root Mean Square Deviation) scores, developed by galaxy refine, have given the quantitative measurements of the similarity between two superimposed atomic coordinates of Normal and Wild-type residues (Karunakar and Pb, 2020). RMSD values of nsSNPs were highly deviated, affecting the functionality and stability of the protein. Additionally, PyMOL predicted considerable structural deviation of mutated CYP1B1 3D protein models for all 113 nsSNPs compared to the wild-type CYP1B1 3D protein model (Khalid and Almaghrabi, 2020).

Moreover, the HOPE server provides a more precise understanding of the variations in structural and functional contexts. The mutant amino acid residues (H489R, G466D, I414F, G365R, S336Y, A330V, A330P, D242E, D242H, E173K, P118L, V96M, N62K, G61A and G61E) were found to be more prominent in comparison to the wild-type, which may result in the development of bumps and incorrect protein-protein interactions. Previous studies have also shown that nsSNP, E173K, is mainly found in Iran and China and showed deleterious effects and the major driver of primary congenital glaucoma (Chen *et al*., 2015). On the contrary, F445L, P442A, F440L, F440C, W434C, V364A, L343P, V320A, F296C, L181P, L166P, R117Q, L97P, V95A, mutant amino acid residues, was found to be smaller in comparison to the wild-type, which might result in a space in protein core, leading to loss of external interaction.

The difference in nature and conservancy of mutant and wild-type amino acid residue affects the molecular interactions. HOPE results revealed nsSNP (C470Y, C491S, C491R, F440S, P437Q, P437S, P437T, W434R, W425R, A383D, V364G, I297T, A295T, S282R, S282R, P245S, F231Y, P193R, P193S, W141G, A106G, A106D, I103S, V96E, L89Q, A78E and P58Q) present in large extracellular domain showed charge shift from neutral to positive which, ultimately, leading towards repulsion with ligands or loss of hydrophobic interactions on protein surface (Zafar and Awais, 2023). Previous studies have also mentioned the deleterious effect of the nsSNPs, L89P, A106D and L89P on protein stability in Italy (Giuffre, 2011). Furthermore, based on surface

accessibility, HOPE predicted that E473K, D430E, P415L, I414F, G365R, D361H, S336Y, A330V, A330P, D242E, D242, N203D, E173K, P118L, V96M, N62K, G61A and G61E residues had affected the surface accessibility and charge, causing the loss of molecular interactions and correct protein folding which is possibly damaging to the protein structure and function. Previous studies have shown that nsSNP and G 61E missense mutations affect protein stability by affecting the enzymatic activity in glaucoma patients of Saudi Arabia (Abu-Amero *et al*., 2011). Moreover, the torsion angles for the residues G472R, G466D, G450S, G365W, G236R, G211D, G188C, G129S, G128R, G90D, G90R and G82S are changed due to glycine, causing incorrect conformation of the protein, affecting the enzymatic activity of CYP1B1.

This study was conducted to fill a gap in existing research by offering a comprehensive assessment of CYP1B1 mutations. While previous studies have highlighted specific mutations, few have cataloged various deleterious variants across different populations. Additionally, previous literature has noted associations between certain mutations and disease phenotypes but a more detailed analysis was needed to examine the functional impact of these mutations on the CYP1B1 protein's function. Our findings align with existing studies that underscore the CYP1B1 gene's significance in glaucoma and this study contributes to the literature by providing a more comprehensive mutation profile with functional predictions.

Through bioinformatics tools, 113 of the 662 SNPs are considered deleterious SNPs. These cause glaucoma by down-regulating the biological function of the protein coded by CYP1B1. The findings contribute to understanding its role in diseases like glaucoma, guiding future clinical research. The only limitation of the study is that it does not involve in vitro analysis, which can be overcome in the future for a better understanding of the genetic association of CYP1B1 and glaucoma.

Future directions of this study include validating these 113 identified deleterious mutations through functional studies, such as in *vitro* testing and protein modeling. Comparative studies across populations can confirm these findings and reveal population-specific risks. Additionally, therapeutic developments, including screening tools or genetic therapies targeting specific mutations, could be explored to improve clinical treatment strategies and patient care for conditions linked to CYP1B1 mutations.

Conclusion

In silico analysis is essential in molecular studies as it helps optimize research strategies and minimize experimental costs. By analyzing the deleterious effects of non-synonymous single nucleotide polymorphisms (nsSNPs) in the CYP1B1 gene and assessing the structural and functional alterations in proteins, this study have identified vital mutations implicated in glaucoma, the second leading cause of blindness worldwide. A total of 612 mutations in the CYP1B1 gene were analyzed, with 113 identified as deleterious, significantly impacting protein structure and function. These findings provide valuable insights that could inform future drug design strategies for glaucoma treatment.

Conflict of Interest. The authors declare they have no conflict of interest.

References

Abu-Amero, K.K., Osman, E.A., Mousa, A., Wheeler, J., Whigham, B., Allingham, R., Hauser, M.A., Al-Obeidan, S.A. 2011. Screening of CYP1B1 and LTBP2 genes in Saudi families with primary congenital glaucoma: genotype-phenotype correlation. *Molecular Vision*, **17:** 2911-2919. PMID: 22128238.

Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., Sunyaev, S.R. 2010. A method and server for predicting damaging missense mutations. *Nature Methods*, **7:** 248-249. https://doi.org/10.1038/nmeth0410-248

Allison, K., Patel, D., Alabi, O. 2020. Epidemiology of glaucoma: the past, present and Predictions for the Future. *Cureus*, **24:** e11686. https://doi.org/10.7759/cureus.11686

Alsubait, A., Aldossary, W., Rashid, M., Algamdi, A., Alrfaei, B.M. 2020. CYP1B1 gene: Implications in glaucoma and cancer. *Journal of Cancer*, **11:** 4652-4661. https://doi.org/10.7150/jca.42669

Awais, M., Wattoo, J.I., Zafar, R., Khan, N. 2021. Computational analysis of non-synonymous single nucleotide polymorphism in UROD gene linked with familial porphyria cutanea tarda. *Gene Reports*, **25:** 101413. https://doi.org/10.1016/j.genrep.2021.

101413

Awais, M., Zafar, R., Khan, N., Khan, I., Naeem, N., Bashir, M.S., Khan, Z. 2023. A review on Forkhead Box A1 (FOXA1) gene; a potent candidate for prostate and breast cancer. *Pure and Applied Biology*, **12:** 148-159. http://dx.doi.org/10.19045/bspab.2023.120016

Bhatnager, R., Dang, A.S. 2018. Comprehensive in-silico prediction of damage associated SNPs in human prolidase gene. *Scientific Reports*, **8:** 1-14. https://doi.org/10.1038/s41598-018-27789-0

Buchan, D.W.A., Jones, D.T. 2019. The PSIPRED protein analysis workbench: 20 years on. *Nucleic Acids Research*, **47:** W402–W407. https://doi.org/10.1093/nar/gkz297

Capriotti, E., Fariselli, P. 2017. PhD-SNPg: A webserver and lightweight tool for scoring single nucleotide variants. *Nucleic Acids Research*, **45:** W247-W252. https://doi.org/10.1093/nar/gkx369

Capriotti, E., Fariselli, P., Casadio, R. 2005. I-Mutant 2.0: predicting stability changes upon mutation from the protein sequence or structure. *Nucleic Acids Research*, **33:** W306-W310. https://doi.org/10.1093/nar/gki375

Chen, L., Huang, L., Zeng, A., He, J. 2015. CYP1B1 gene mutations with incomplete penetrance in a Chinese pedigree with primary congenital glaucoma: a case report and review of literatures. *International Journal of Clinical and Experimental Medicine*, **8:** 14538-14541. PMID: 26550445

Choi, Y., Chan, A.P. 2015. A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*, **31:** 2745-2747. https://doi.org/10.1093/bioinformatics/btv195

Chouiter, L., Nadifi, S. 2017. Analysis of CYP1B1 gene mutations in patients with primary congenital glaucoma. *Journal of Pediatric Genetics*, **06:** 205-214. https://doi.org/10.1055/s-0037-1602695

Elango, T., Sun, J., Zhu, C., Zhou, F., Zhang, Y., Sun, L., Yang, S., Zhang, X. 2018. Mutational analysis of epidermal and hyperproliferative type I keratins in mild and moderate psoriasis vulgaris patients: a possible role in the pathogenesis of psoriasis along with disease severity. *Human Genomics*, **12:** 1-21. https://doi.org/10.1186/s40246-018-0158-2

Giuffre, I. 2011. *Molecular Analysis of Italian Patients with Congenital Glaucoma*, 390 pp., E-Book, ISBN:978-953-51-65583, USA. https://doi.org/10.3109/13816810.2011.596891

Karunakar, P., Pb, S. 2020. In silico modelling and virtual screening for identification of inhibitors for spore wall protein-5 in Nosema bombycis. *Journal of Biomolecular Structure and Dynamics*, **40:** 1748-1763. https://doi.org/10.1080/07391102.2020.1832579

Khalid, Z., Almaghrabi, O. 2020. Mutational analysis on predicting the impact of high-risk SNPs in human secretary phospholipase A_2 receptor (PLA2R1). *Scientific Reports*, **10:** 1-11. https://doi.org/10.1038/s41598-020-68696-7

Khawaja, A.P., Cooke Bailey, J.N., Wareham, N.J., Scott, R.A., Simcoe, M., Igo, R.P., Song, Y.E., Wojciechowski, R., Cheng, C.Y., Khaw, P.T., Pasquale, L.R., Haines, J.L., Foster, P.J., Wiggs, J.L., Hammond, C.J., Hysi, P.G. 2018. Genome-wide analyses identify 68 new loci associated with intraocular pressure and improve risk prediction for primary open-angle glaucoma. *Nature Genetics*, **50:** 778-782. https://doi.org/10.1038/s41588-018-0126-8

Ko, J., Park, H., Heo, L., Seok, C. 2012. Galaxy WEB server for protein structure prediction and refinement. *Nucleic Acids Research*, **40:** W294-W297. https://doi.org/10.1093/nar/gks493

Kumar, A, Han, Y, Oatts, J.T. 2024 Genetic changes and testing associated with childhood glaucoma: A systematic review. *PLOS ONE*, **19:** e0298883. https://doi.org/10.1371/journal.pone.0298883

Kumar, P., Henikoff, S., Ng, P.C. 2009. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protocols*, **4:** 1073-1082. https://doi.org/10.1038/nprot.2009.86

Kumar, S.U., Priyanka, N., Sneha, P., Doss, C.G.P. 2015. Functional and structural characterization of missense mutations in PAX6 gene. *Frontiers in Biology*, **10:** 377-385. https://doi.org/10.1007/s11515-015-1346-2

Lee, J.H., Ki, C.S., Kim, H.J., Suh, W., Lee, S.T., Kim, J.W., Kee, C. 2011. Analysis of copy number variation using whole genome exonfocused array CGH in Korean patients with primary congenital glaucoma. *Molecular Vision*, **17:** 3583-3590. PMID: 22219654

Pan, L., Wu, J., Wang, N. 2024 Association of gene polymorphisms with normal tension glaucoma: a systematic review and meta-analysis. *Genes*, **15:** 491. https://doi.org/10.3390/genes15040491

Sheybani, A., Scott, R., Samuelson, T.W., Kahook, M.Y., Bettis, D.I., Ahmed, I.I.K., Stephens, J.D.,

Kent, D., Ferguson, T.J., Herndon, L.W. 2020. Open-angle glaucoma: burden of illness, current therapies and the management of nocturnal IOP variation. *Ophthalmology and Therapy*, **9:** 1-14. https://doi.org/10.1007/s40123-019-00222-z

Song, N., Leng, L., Yang, X.J., Zhang, Y.Q., Tang, C., Chen, W.S., Zhu, W., Yang, X. 2019. Compound heterozygous mutations in CYP1B1 gene leads to severe primary congenital glaucoma phenotype. *International Journal of Ophthalmology*, **12:** 909. https://doi.org/10.18240/ijo.2019.06.05

Tan, C., Huang, L., Yang, Z. 2016. Association of PLEKHA7, COL11A1 and PCMTD1-ST18 gene polymorphisms with primary angle closure glaucoma in ethnic Han Chinese from Sichuan. *Chinese Journal of Medical Genetics*, **33:** 545-549. https://doi.org/10.3760/cma.j.issn.1003-9406.2016.04.027

Tang, Y.M., Wo, Y.Y.P., Stewart, J., Hawkins, A.L., Griffin, C.A., Sutter, T.R., Greenlee, W.F. 1996. Isolation and characterization of the human cytochrome p450 CYP1B1 gene. *Journal of Biological Chemistry*, **271:** 28324-28330. https://doi.org/10.1074/jbc.271.45.28324

Tosh, D., Rani, H.S., Murty, U.S., Deenadayal, A., Grover, P. 2015. Mutational analysis of the FIGLA gene in women with idiopathic premature ovarian failure. *Menopause*, **22:** 520-526. https://doi.org/10.1097/GME.0000000000000340

Venselaar, H., TeBeek, T.A.H., Kuipers, R.K.P., Hekkelman, M.L., Vriend, G. 2010. Protein structure analysis of mutations causing inheritable diseases. an e-science approach with life scientist friendly interfaces. *BMC Bioinformatics*, **11:** 1-10. https://doi.org/10.1186/1471-2105-11-548

Wan, Y., Li, S., Gao, Y., Tang, L., Cao, W., Sun, X. 2019. COL11A1 polymorphisms are associated with primary angle-closure glaucoma severity. *Journal of Ophthalmology*, **2019:** 1-7. https://doi.org/10.1155/2019/2604386

Wiggs, J.L. 2007. Genetic etiologies of glaucoma. *Archives of Ophthalmology*, **125:** 30-37. https://doi.org/10.1001/archopht.125.1.30

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., Zhang, Y. 2015. The I-TASSER Suite: protein structure and function prediction. *Nature Methods*, **12:** 7-8. https://doi.org/10.1038/nmeth.3213

Zafar, R., Awais, M. 2023. Molecular identification of missense variants in SLC3A1 gene; An approach leading to computer-aided drug design for cystinuria. *Gene*, **888:** 147802. https://doi.org/ https://doi.org/10.1016/j.gene.2023.147802