Arab American University
Faculty of Graduate Studies
Department of Health Sciences
Master Program in Molecular Genetics and Genetic Toxicology

Screening for Gene Mutations Associated with Primary Congenital Glaucoma in a Cohort of Palestinian Patients

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Thesis Approval

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This thesis was defended successfully on June 5, 2025 and approved by:

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Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis is substantially my own work and has not been submitted for any other degree at the Arab American University or any other institution.

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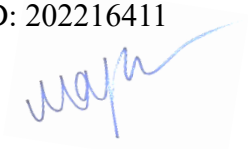
Signature: 

Date of Submitting the Final Version of the Thesis: July 21, 2025

Dedication

I dedicate this thesis to glaucoma patients, whose stories and experiences have profoundly influenced my commitment to this research. May this study contribute to advancing knowledge and improving the quality of life of glaucoma patients everywhere.

I also dedicate this work to my parents and brother for their unconditional love and support. I love you and I appreciate everything you have done for me. Thank you for teaching me the value of hard work and perseverance, which guided me throughout this journey. This accomplishment is as much yours as it is mine.

Mariam Hussam Abdelhafeth Alqam

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Screening for Gene Mutations Associated with Primary Congenital Glaucoma in a Cohort of Palestinian Patients

Mariam Hussam Abdelhafeth Alqam

Supervision committee: Prof. Hisham Darwish, Dr. Ibrahim Taha, Dr. Sanaa' Mushen

Abstract

Background: Primary congenital glaucoma (PCG) is a severe childhood ocular disease that can lead to irreversible blindness if not detected and treated early. In Palestine, PCG is more prevalent than global averages, likely due to high consanguinity rates. Currently, diagnosis is only possible after birth, and many affected Palestinian children are diagnosed too late for optimal intervention. The only available treatment in Palestine is surgical. Understanding the genetic basis of PCG in this population is critical for improving early diagnosis and clinical management.

Objective: This study aims to investigate the molecular genetic basis of PCG in a Palestinian cohort by screening the *CYP1B1* and *MYOC* genes associated with PCG.

Methods: A total of 46 Palestinian PCG patients were recruited from the West Bank between November 1, 2023, and November 1, 2024, with the assistance of ophthalmologists from the German Eye Center and St. John of Jerusalem Eye Hospital and local specialists. Sanger Sequencing was performed to identify *CYP1B1* and *MYOC* variants.

Results: Genetic analysis revealed that 22 patients harbored homozygous pathogenic *CYP1B1* variants in exons 2 and 3. Nine patients carried heterozygous pathogenic *CYP1B1* variants, including one in compound heterozygous form, while 15 were *CYP1B1*-negative. In total, seven pathogenic variants were identified, one novel and six previously reported. The identified genetic profile closely resembles that of other Arab populations.

Conclusion: This study provides the first comprehensive molecular analysis of PCG in the Palestinian population, identifying both novel and previously reported *CYP1B1* variants. These findings contribute to a better understanding of PCG genetics and emphasize the need for genetic screening programs to facilitate early diagnosis, targeted interventions, and potential future therapeutic developments.

Keywords: Primary Congenital Glaucoma, CYP1B1, MYOC, Palestinian population, Genetic Screening

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List of Definitions of Abbreviations

Abbreviations	Title
AAUP	Arab American University Palestine
AAVs	Adeno-Associated Viruses
AIPL1	Aryl Hydrocarbon Receptor Interacting Protein-Like 1 (associated with retinal dystrophy)
ASD	Anterior Segment Dysgenesis
BDNF	Brain-Derived Neurotrophic Factor
BDRR	BigDye Terminator Ready Reaction
COL4A1	Collagen Type IV Alpha 1
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CYP1B1	Cytochrome P450 Family 1 Subfamily B Member 1
DMSO	Dimethyl Sulfoxide
ECM	Extracellular Matrix
E1	Estrone
E2	17β-Estradiol
ER	Endoplasmic Reticulum
ERAD	Endoplasmic Reticulum-Associated Degradation
FOXC1	Forkhead Box C1
GDDs	Glaucoma Drainage Devices
GDNF	Glial Cell-Derived Neurotrophic Factor
GPATCH3	G Patch Domain Containing 3
Grp94 chaperone	Glucose-Regulated Protein 94 Chaperone
iPSCs	Induced Pluripotent Stem Cells
IOP	Intraocular Pressure
IRB	Institutional Review Board
JOAG	Juvenile Open Angle Glaucoma
LTBP2	Latent Transforming Growth Factor Beta Binding Protein 2
MMP9	Matrix Metalloproteinase 9
MSCs	Mesenchymal Stem Cells
MYOC	Myocilin
NAD+(H)	Nicotinamide Adenine Dinucleotide (oxidized/reduced form)
NTC	No-Template Control
NTG	Normal-Tension Glaucoma
OS	Oxidative Stress
PBA	Sodium 4-Phenylbutyrate
PACG	Primary Angle-Closure Glaucoma
PCG	Primary Congenital Glaucoma
PCR	Polymerase Chain Reaction
PGAs	Prostaglandin Analogs
PI3K	Phosphatidylinositol 3-Kinase
PITX2	Paired Like Homeodomain 2
POAG	Primary Open-Angle Glaucoma
PTK2	Protein Tyrosine Kinase 2

RGC	Retinal Ganglion Cell
ROCK inhibitors	Rho-Associated Protein Kinase Inhibitors
SPARC	Secreted Protein Acidic and Rich in Cysteine
SR	Sustained Release (e.g., Bimatoprost SR)
TEK	TEK Receptor Tyrosine Kinase
TIGR	Trabecular Meshwork Glucocorticoid-Inducible Response Protein
TM	Trabecular Meshwork
UPR	Unfolded Protein Response

Chapter One: Introduction

1.1 Overview of Glaucoma and its Types

Glaucoma is a worldwide leading cause of irreversible blindness, with more than 70 million affected individuals (World Health Organization, 2023). It is a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells (RGCs). These cells are central nervous system neurons that have their cell bodies in the inner retina and axons in the optic nerve, as shown in Figure 1.1. Degeneration of these nerves results in *cupping* of the optic nerve head (Figure 1.2), thus damaging the visual-field. RGCs death is related to elevated intraocular pressure (IOP) as a result of impaired drainage of the aqueous humor (Chiarugi, 2023).

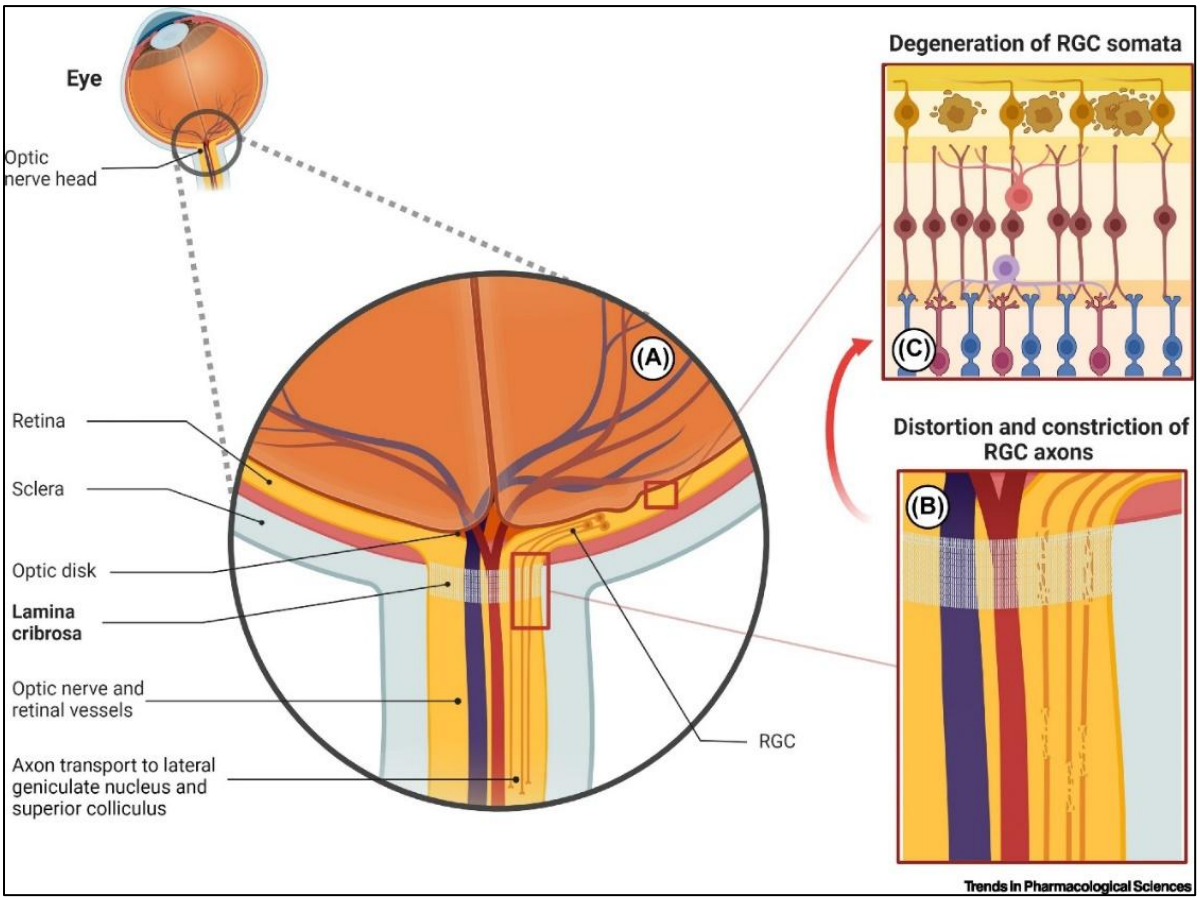

Figure 1.1 Visualization of the anatomical structures involved in neurodegeneration during glaucoma. Source: (Chiarugi, 2023)

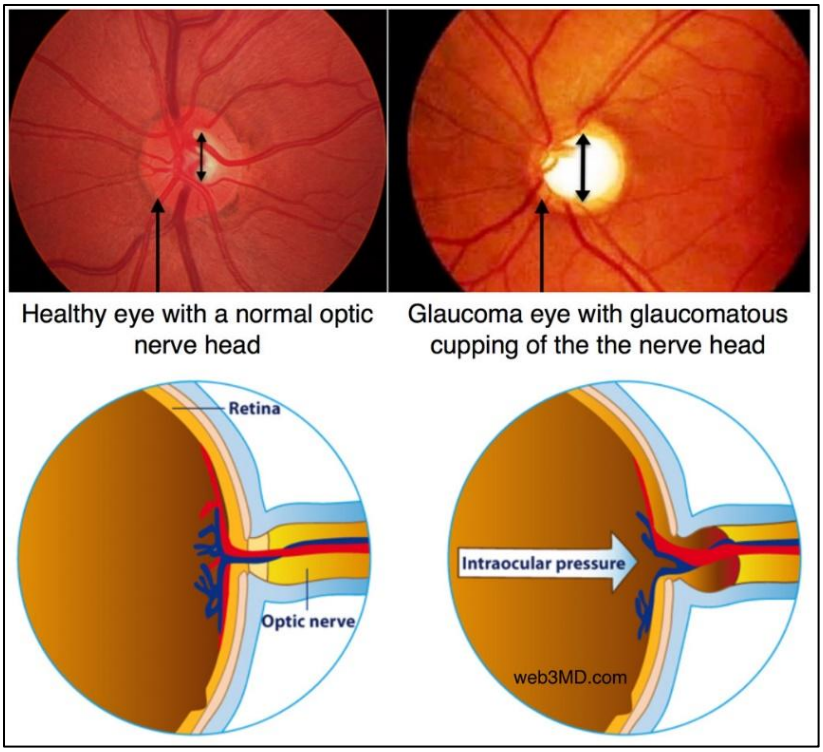

Figure 1.2 Cupping of optic nerve in normal and glaucoma. Source: (https://www.drseemabehl.com/glaucoma.php)

1.2 Types of Glaucoma

Glaucoma is classified into two main categories: primary glaucoma and secondary glaucoma. Primary glaucoma occurs without an identifiable cause, often due to genetic factors or ageing. Secondary glaucoma results from an identifiable cause, such as eye injury, eye disease, eye surgery, or medication (Jamie Dietze et al., 2024).

1.3 Types of Primary Glaucoma

Primary glaucoma is typically classified into 3 types: Primary Open-Angle Glaucoma (POAG), Primary Angle-Closure Glaucoma (PACG), and Developmental Glaucoma. POAG is further divided into High-Tension Glaucoma (HTG) and Normal-Tension Glaucoma (NTG) based on intraocular pressure levels. PACG can present in either an acute or chronic form, depending on the severity and progression of angle closure. On the other hand, developmental glaucoma occurs in infants and young children due to developmental abnormalities in the drainage system of the eye. Its subtypes are primary

congenital glaucoma (PCG) and juvenile open-angle glaucoma. (JOAG) Figure 1.3 illustrates this classification.

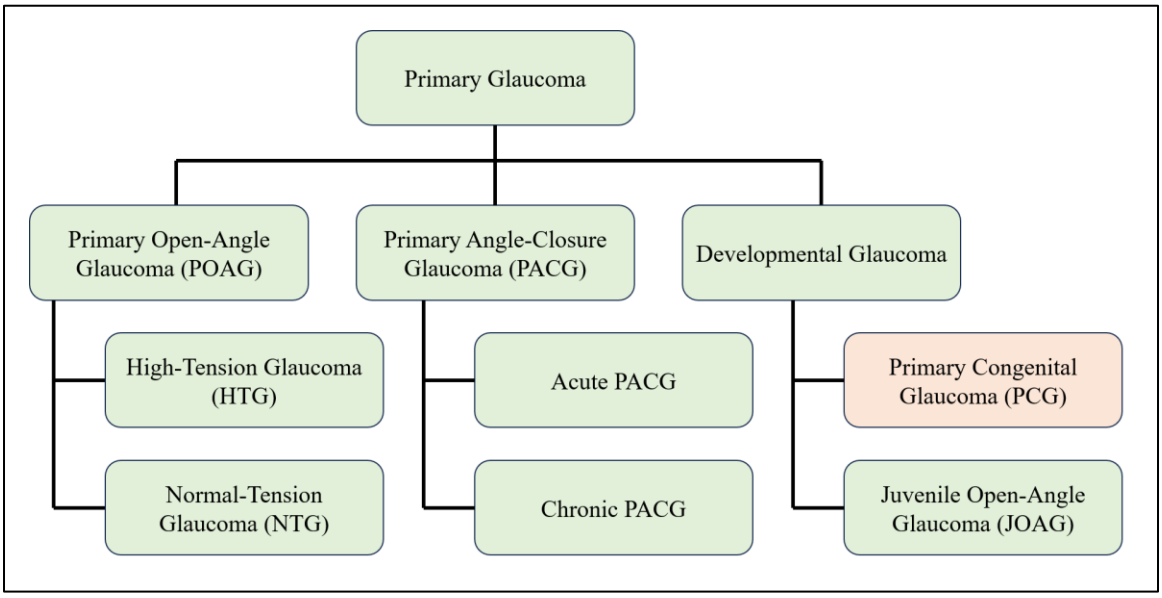

Figure 1.3 Classification of Primary Glaucoma A hierarchical representation of primary glaucoma types, including Primary Open-Angle Glaucoma (POAG), Primary Angle-Closure Glaucoma (PACG), and Developmental Glaucoma, along with their respective subtypes.

1.3.1 Primary Open-Angle Glaucoma (POAG)

Primary Open-Angle Glaucoma (POAG, OMIM # 137760) is a chronic, progressive, and irreversible multifactorial optic neuropathy. The angle of the anterior chamber is open, resulting in increased IOP, optic nerve head changes, retinal nerve fiber layer thinning, and progressive loss of peripheral vision (Ibinson & Ferguson, 2024). In advanced stages, central visual field loss and blindness can occur. Figure 1.4 shows the types of visual defects in glaucoma, and Figure 1.5 shows an example of what a POAG patient's point of view would look like.

Age, genetic predisposition, ethnicity, and myopia are well-documented risk factors for POAG. It disproportionately affects individuals of African descent, with a higher prevalence and more severe disease progression (Charlson et al., 2015).

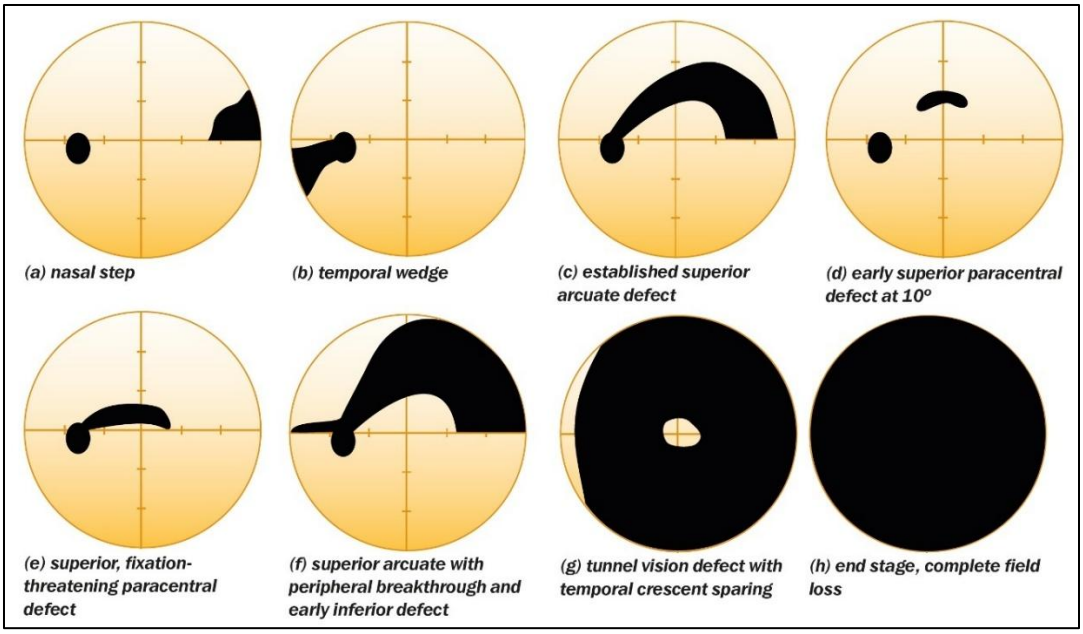

Figure 1.4 Glaucomatous visual field defects. (a) nasal step (b) temporal wedge (c) established superior arcuate defect (d) early superior paracentral defect at 10º (e) superior, fixation threatening paracentral defect (f) superior arcuate with peripheral breakthrough and early inferior defect (g) tunnel vision defect with temporal crescent sparing (h) end stage, complete field loss. Image retrieved from: (https://geekymedics.com/primary-open-angle-glaucoma/)

Figure 1.5 Point of view of a vision of a patient with glaucoma. Image retrieved from: (https://www.privateophthalmologist.co.uk/glaucoma/open-angle-glaucoma/)

1.3.1.1 High-Tension Glaucoma (HTG)

High-Tension Glaucoma (HTG) is the most common form of POAG and is characterized by elevated IOP above the normal range (≥21 mmHg). One of the most well-established genes associated with HTG-POAG is *MYOC* (Myocilin), where mutations lead to protein misfolding and accumulation in the trabecular meshwork, impairing aqueous humor drainage and increasing IOP (Yadav et al., 2023).

In addition to genetic factors, environmental influences, such as corticosteroid use and vascular dysregulation, may contribute to disease progression (Gao et al., 2018). Treatment strategies for HTG primarily focus on reducing IOP through medications (prostaglandin analogs, beta-blockers), laser therapy, or surgical interventions (Gao et al., 2018).

1.3.1.2 Normal-Tension Glaucoma (NTG)

Normal-Tension Glaucoma (NTG) is a subtype of POAG in which patients develop glaucomatous optic nerve damage and visual field loss despite having IOP within the normal range (<21 mmHg). NTG is thought to result from vascular dysregulation, oxidative stress, and impaired neuroprotection (Ibinson & Ferguson, 2024).

Key genetic factors associated with NTG include *OPTN* (Optineurin), which plays a role in apoptosis and inflammation in retinal ganglion cells, and *TBK1* (Tank-Binding Kinase 1), where copy number variations may contribute to cell stress responses and neurodegeneration. Other risk factors include hypotension, migraine, sleep apnea, and vascular diseases, which may compromise blood flow to the optic nerve. Since IOP is not significantly elevated in NTG, treatment approaches include neuroprotective therapies and optimizing ocular perfusion(Gao et al., 2018).

1.3.2 Primary Angle-Closure Glaucoma (PACG)

Primary Angle-Closure Glaucoma (PACG # 618880) is caused by a mechanical obstruction of aqueous humor outflow, often due to pupillary block or plateau iris, as shown in Figure 1.6. The angle between the iris and cornea becomes increasingly narrow, predisposing the patient to intermittent or complete angle closure. Hyperopia, older age, female sex, and Asian ethnicity are significant risk factors (Inooka et al., 2024; Y. Zhang et al., 2022).

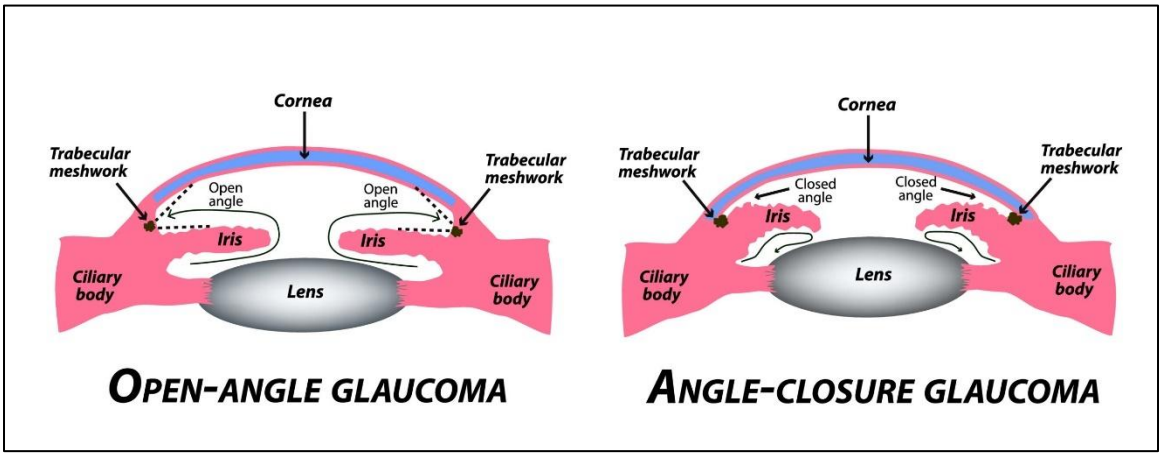

Figure 1.6 Comparison of Open-Angle and Angle-Closure Glaucoma. In open-angle glaucoma, the angle between the iris and cornea remains open, allowing for slow drainage of aqueous humor through the trabecular meshwork, which can become inefficient over time, leading to increased intraocular pressure. In contrast, angle-closure glaucoma is characterized by a narrowed or blocked drainage angle, preventing aqueous humor outflow and causing a rapid increase in intraocular pressure. Image retrieved from: (https://www.eyenm.com/glaucoma-center-albuquerque/types-of-glaucoma/)

1.3.2.1 Acute Primary Angle-Closure Glaucoma (Acute PACG)

Acute PACG is a sight-threatening emergency characterized by a sudden and complete blockage of the drainage angle in the eye. This leads to a rapid rise in IOP, causing symptoms such as severe eye pain, headache, nausea, vomiting, blurred vision, and halos around lights (Khazaeni et al., 2023). Genetically, *PLEKHA7* has been strongly associated with angle-closure glaucoma, as it plays a role in cell adhesion within the trabecular meshwork(Kondkar, 2021). (Shi et al., 2021) Acute PACG requires urgent intervention to lower IOP and reopen the drainage angle.

1.3.2.2 Chronic Primary Angle-Closure Glaucoma (Chronic PACG)

Chronic PACG develops gradually over time due to progressive closure of the drainage angle, leading to silent and sustained elevation of IOP. Unlike acute PACG, chronic PACG is asymptomatic in the early stages and often remains undiagnosed until significant optic nerve damage and visual field loss occur. The gradual narrowing of the angle may result from repeated subacute episodes of angle closure, progressive thickening of the lens, or changes in the ciliary body that push the iris forward. Genetic studies have identified *HGF* (Hepatocyte Growth Factor) as a key gene associated with chronic PACG, influencing anterior segment structure and trabecular meshwork function (Ong et al., 2021).

1.3.3 Primary Congenital Glaucoma (PCG)

Primary congenital glaucoma (PCG, OMIM #231300) is a rare disease that affects children. It used to be considered an untreatable inevitable vision loss. However, advances in biochemical and genetic studies, introduction of medications that lower IOP, and improved surgical techniques have led to better understanding of this devastating disease and preserving the vision of affected children (A. H. Badawi et al., 2019).

PCG manifests at birth or shortly after, with different subtypes based on the age of onset. True congenital glaucoma is present at birth or within the first month of life (25% of cases). Infantile glaucoma typically presents between 1 - 36 months of age (65%), while JOAG manifests after three years but before adulthood (10%) (Kaur et al., 2024). The disease is characterized by defects in the trabecular meshwork and anterior chamber angle. This leads to impaired drainage of the aqueous humor resulting in elevated IOP. Figure 1.7 visualizes the aqueous humor production and flow.

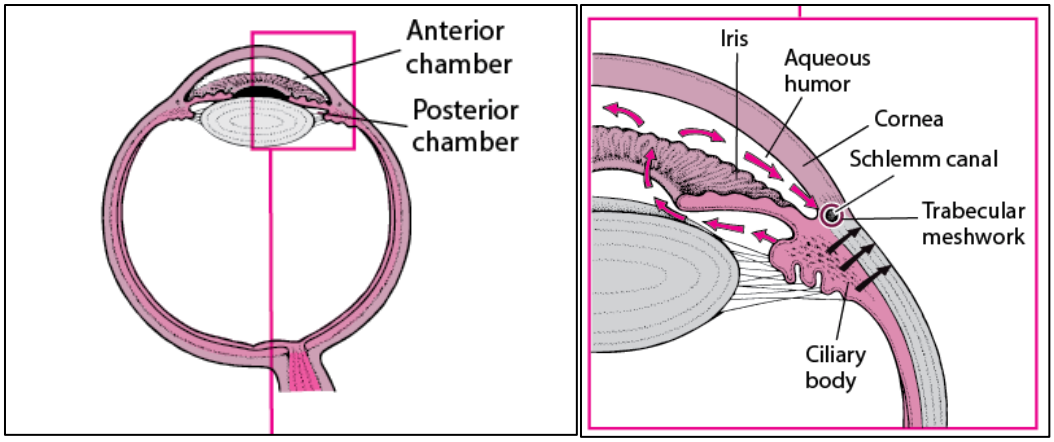

Figure 1.7 Aqueous Humor Production and Flow. Aqueous humor is produced in the ciliary body behind the iris (in the posterior chamber), passes into the front of the eye (anterior chamber), and then exits through the drainage canals or the uveoscleral pathway (black arrows). Image retrieved from: (https://www.msdmanuals.com/home/children-s-health-issues/eye-disorders-in-children/primary-infantile-glaucoma)

PCG typically presents in infancy with a triade of symptoms: photophobia, epiphora, and blepharospasm, along with signs such as corneal clouding, megalocornea, and buphthalmos (Kaur et al., 2024), as shown in Figure 1.4. Parents notice bluish discoloration of the eyes, abnormally enlarged eyeballs, or a sudden whitening of the cornea. If left untreated, it may lead to blindness.

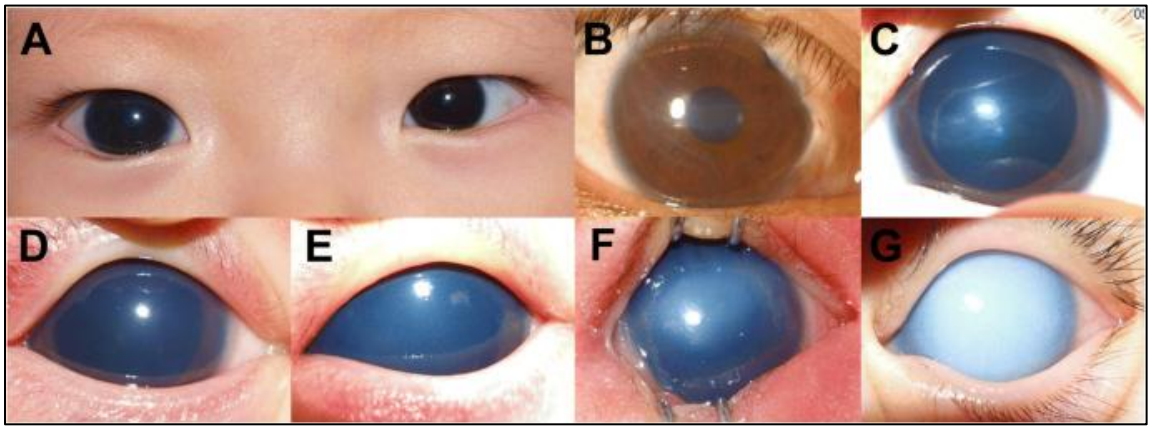

Figure 1.8 Representative photographs of corneal abnormalities associated with congenital glaucoma. (A) Enlarged cornea (buphthalmos) in the right eye with congenital glaucoma. (B, C) Horizontal lines of Haab striae are present in the cornea. (D) Grade 1 corneal opacity. Minimal and superficial opacity is observed. € Grade 2 corneal stromal opacity. Both anterior chamber and iris are well-visible despite the opacity. (F) Grade 3 corneal stromal opacity. The pupil is still visible but iris details difficult to see through the opacity. (G) Grade 4 corneal stromal opacity. Pupil is invisible due to total stromal opacity of the cornea. Image retrieved from https://bmcophthalmol.biomedcentral.com/articles/10.1186/s12886-018-0865-4 .

The prevalence of PCG varies depending on ethnicity and consanguinity. In Western countries like Ireland, the United Kingdom, and the United States, PCG is relatively rare, with occurrence rates ranging from one in 20,000 to 45,000 live births(Aponte et al., 2010). In contrast, this number is much higher in consanguineous communities. For examples, it is reported to be as high as one in 1,000 in Senegal, one in 1,250 among Slovakian Roms (Gypsies), one in 2,500 in Saudi Arabia (MacKinnon et al., 2004), and one in 8200 among Palestinians (Elder, 1993). There is no available data on the incidence of PCG in Palestine after 1993. Lastly, the Republic of Zaire presents with the highest incidence, with the reported primary infantile glaucoma around one in 500 (MacKinnon et al., 2004).

1.4 Significance of the study

The incidence of PCG in Palestine was reported to be one in 8200 live births in 1993, which is relatively high for a rare condition. Since then, no reports have been published on the incidence rate of congenital glaucoma, nor is this information available from the Palestinian Central Bureau of Statistics. This study represents a comprehensive investigation of the molecular genetics of PCG among a Palestinian cohort, in collaboration with leading physicians in the field. Like all congenital diseases, it is important to identify the specific spectrum of associated variants that describe the disease in every population which could have major impact on improving clinical diagnoses, allow early intervention, provide a tool for families inflicted with these variants to ensure the birth of healthy children, and may provide insights in therapy research.

1.5 Problem Statement

The available literature on PCG in Palestine is extremely limited, with only one published study (Elder, 1993). This study presented the status of patients with PCG treated at St John of Jerusalem Eye Hospital between 1981 and 1990. To date, no studies have investigated the genetic profile and relevant variants associated with PCG among the

Palestinian population. In other words, for more than 20 years, no research has been done on primary congenital glaucoma in Palestine.

1.6 Aim of Study

This study aims to identify the associated genetic variants in selected genes with PCG among Palestinian primary Glaucoma patients to enhance global understanding of the molecular genetics underlying PCG development.

1.7 Study Questions

- What are the pathogenic or likely pathogenic variants in the CYP1B1 and MYOC genes among Palestinian patients diagnosed with primary congenital glaucoma?
- Are there any novel variants in these genes not previously reported in the literature?
- How does the spectrum and frequency of detected variants among Palestinian patients compare to those reported in neighboring Arab countries and globally?
- Can the identified genetic variants help improve diagnostic and counseling strategies for families affected by PCG in Palestine?

1.8 Hypothesis

Several known and novel Variants in the *CYP1B1* and *MYOC* genes are associated with PCG among Palestinian Glaucoma patients with relatively close resemblance to variants identified in other Arab populations.

1.9 Study Boundaries and Delimitations

This study focuses exclusively on Palestinian patients clinically diagnosed with primary congenital glaucoma who presented to St John Eye Hospital and Dr. Amer Muhsen and agreed to participate in this study. The research is limited to the analysis of variants in the *CYP1B1* and *MYOC* genes only; other genes associated with glaucoma are beyond the scope of this investigation due to limited funding. The sample size is limited to patients who consented to participate and provided clinical and genetic material for analysis. The study findings are specific to the Palestinian population and may not be fully generalizable to other ethnic or geographic groups. The analysis does not include functional validation of variants (e.g., in vitro assays), and interpretation is based on bioinformatic prediction tools and literature review.

1.10 Terminological and Procedural Definitions

- Primary Congenital Glaucoma (PCG): A severe form of glaucoma that presents within the first few years of life, characterized by elevated intraocular pressure, buphthalmos, corneal edema, and optic nerve damage.
- *CYP1B1* gene: Cytochrome P450 Family 1 Subfamily B Member 1 gene, frequently implicated in autosomal recessive PCG.
- *MYOC* gene: Myocilin gene, more commonly associated with juvenile and adult-onset glaucoma but occasionally reported in congenital cases.
- Variant: A change in the DNA sequence compared to the reference genome. Variants are classified as pathogenic, likely pathogenic, benign, likely benign, or of uncertain significance based on ACMG guidelines.
- Sanger Sequencing: A method of DNA sequencing used to detect genetic variants in the study samples.
- In-silico Analysis: Use of bioinformatics tools to predict the pathogenicity and functional consequences of genetic variants.
- Pathogenic Variant: A genetic alteration causally related to disease development, supported by multiple lines of evidence.

Chapter Two: Literature Review

Primary congenital glaucoma is caused by genetic variants associated with key molecular pathways involved in aqueous humor regulation. Over the past two decades, advances in molecular genetics have identified several causative genes where *CYP1B1* and *MYOC* being the most studied in PCG and JOAG, respectively. These genes play critical roles in trabecular meshwork function, aqueous humor outflow, and extracellular matrix maintenance (Cascella et al., 2015). This section reviews of the molecular aspects of congenital glaucoma, with an emphasis on genetic variants in *CYP1B1* and *MYOC*, their molecular mechanisms, and population-specific variations. Given the high consanguinity rates in the Middle East, definitely understanding the genetic basis of PCG is critical for developing early diagnostic tools and potential targeted therapies.

2.1 Genetics of PCG

Globally, 90% of PCG cases are sporadic, while 10% of cases are familial with autosomal recessive mode of inheritance (Kaur et al., 2024). On the contrary, nearly 90% of the PCG cases in Saudi Arabia are hereditary, with autosomal recessive inheritance and high penetrance (Malik et al., 2017). This suggests that in Palestine, PCG cases are more likely to be familial than sporadic due to high consanguinity, similar to that in Saudi Arabia, and opposite to the global trend.

Several genes have been identified in PCG pathogenesis, including: *CYP1B1, MYOC, LTBP2, TEK, FOXC1* and *PITX2*. Mutations in these genes disrupt the development and function of the trabecular meshwork, leading to impaired aqueous humor drainage and elevated IOP (Lewis et al., 2017). A recent systematic review studied the genetic epidemiology of PCG among 10 Arab populations including Saudi Arabia, Kuwait, Oman, Egypt, Morocco, Lebanon, Tunisia, Iraq, Algeria, and Mauritania. A total of 77 disease-causing variants from 361 patients and 88 families were identified. Thirty-three (33) of these variants were unique to Arabs and 69 variants were identified in the *CYP1B1* gene, 5 in the *MYOC* gene, and single variants were reported in *NTF4*, *FOXC1*, and *WDR36* genes. The most common identified variant was the c.182G>A in the *CYP1B1* gene.

Consanguinity represented the majority of Arab PCG patients' families, ranging from 45% to 100% (Jemmeih et al., 2022a).

Since *CYP1B1* and *MYOC* mutant variants are the most commonly associated genes with PCG among the Arab populations, this study was initiated to study the prevalence of these variants among Palestinian PCG patients.

2.1.1 *CYP1B1* gene.

This gene is locted on the short arm of chromosome 2 (2p22.2) (Figure 2.1). It consists of 3 exons and 2 introns, where the majority of identified pathogenic variants are concentrated in exon 2 and the begining of exon 3, as shown in Figure 2.2. Exon 1 includes is a non-coding sequence (Fuse et al., 2024).

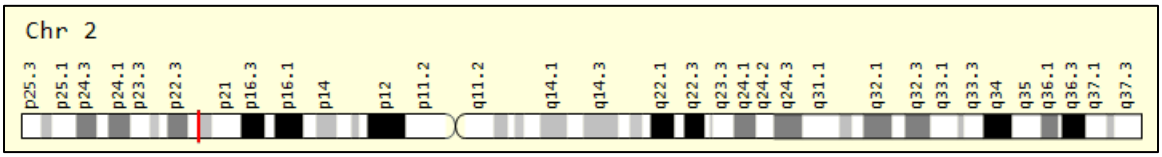

Figure 2.1 Location of *CYP1B1* gene in the human genome. Image retrieved from GeneCards (https://www.genecards.org/)

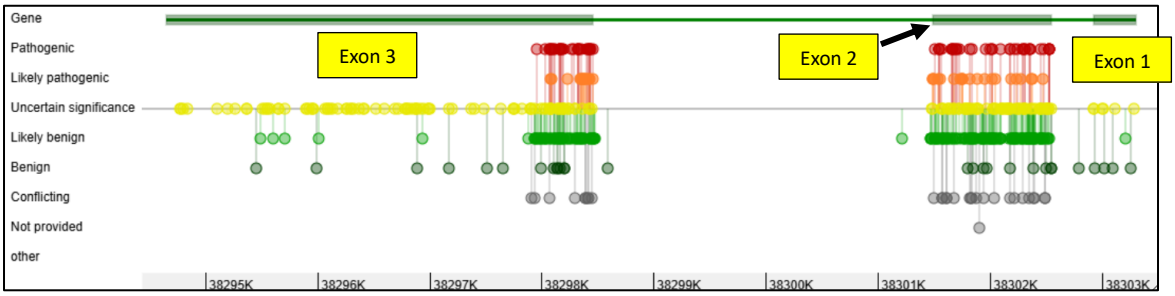

Figure 2.2 Graphical Representation of *CYP1B1* gene, including classified variants submitted to ClinVar. Image Retrieved from ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

CYP1B1 is a member of the cytochrome P450 superfamily of enzymes. These monooxygenases are involved in the metabolism of various substances including drugs, vitamins, cholesterol, steroids, and other lipids. The CYP1B1 localizes to the endoplasmic reticulum. It inserts one oxygen atom into a substrate, and reduces the second into a water molecule, with two electrons provided by NADPH via cytochrome P450 reductase

(NADPH--hemoprotein reductase) (Lee et al., 2003). CYP1B1 is involved in the metabolism of hormones and vitamins. It converts estrone (E1) and 17β-estradiol (E2) into hydroxy-estrogens (A. F. Badawi et al., 2001). It also metabolizes testosterone and progesterone into hydroxylated derivatives (Shimada et al., 1999), and plays a role in all-trans retinoic acid biosynthesis by converting all-trans retinol to retinal and then to all-trans retinoic acid (H. Chen et al., 2000). Moreover, CYP1B1 plays an important role in retinal vascular development. It promotes retinal angiogenesis and capillary morphogenesis under hypertoxic conditions, and helps maintain oxidative homeostasis and trabecular meshwork organization (Elmergreen et al., 2011).

In summary, CYP1B1 is vital for steroid hormone metabolism, retinoic acid biosynthesis, oxidative stress management, and xenobiotic detoxification, and is involved in eye health and vascular function.

2.1.2 *MYOC* gene.

The gene is located on the long arm of chromosome 1 (1q24.3) (Figure 2.3). It consists of 3 exons and 2 introns as well, with limited number of reported pathogenic variants (Figure 2.4).

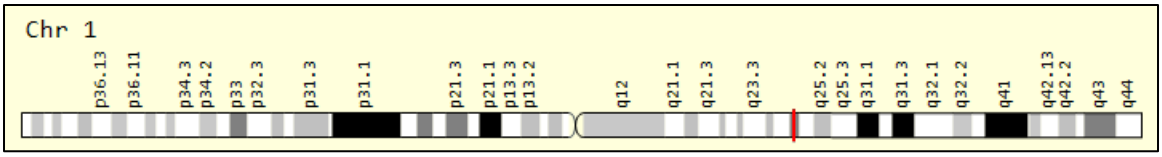

Figure 2.3 Location of *MYOC* gene in the human genome. Image retrieved from GeneCards (https://www.genecards.org/)

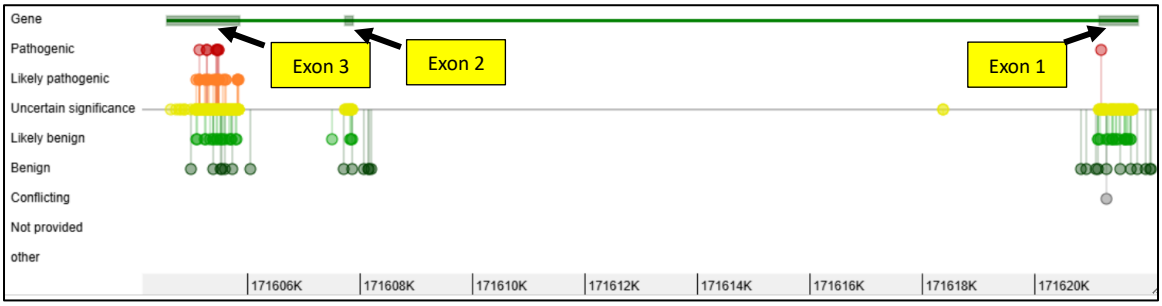

Figure 2.4 Graphical Representation of *MYOC* gene, including classified variants submitted to ClinVar. Image Retrieved from ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

MYOC encodes the protein myocilin, which is involved in cytoskeletal function and is expressed in many ocular tissues, including the trabecular meshwork (TM). It was revealed to be the TM glucocorticoid-inducible response protein (TIGR). It interacts with extracellular matrix (ECM) components such as laminin, fibronectin, SPARC, and hevin, which help maintain TM structure and aqueous humor outflow (Kanagavalli et al., 2004).

MYOC glycoprotein plays a role in cellular processes such cell adhesion, spreading, and formation of focal contacts. It modulates actin cytoskeleton, influencing stress fiber assembly via Rho protein signaling and Wnt pathways interactions. Furthermore, MYOC enhances cell migration by activating PTK2 and downstream PI3K (phosphatidylinositol 3-kinase) signaling (Kanagavalli et al., 2004).

In summary, MYOC is crucial for TM function, ECM interactions, and cytoskeletal regulation, affecting cell adhesion, migration, and signaling pathways essential for ocular homeostasis.

2.1.3 Other Genes Associated with Primary Congenital Glaucoma (PCG)

While *CYP1B1* and *MYOC* are well-established PCG-associated genes, studies have identified additional genes implicated in trabecular meshwork function, Schlemm's canal development, and aqueous humor outflow regulation. Figure 2.5 demonstrates where TM and SC are located in the eye.

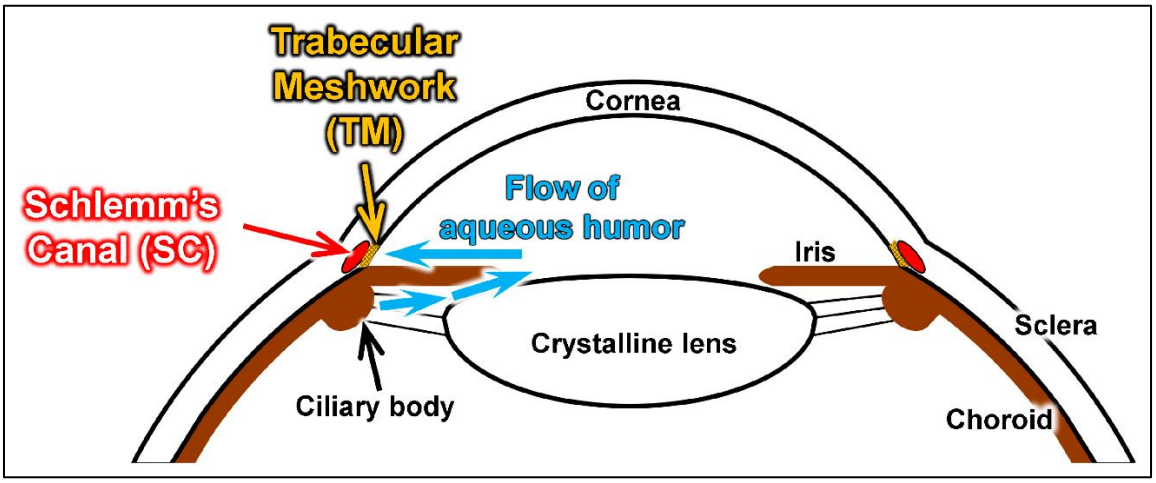

Figure 2.5 Schematic image of angle structure at corneal limbus and flow of aqueous humor in a human eye. Image retrieved from (Gallab et al., 2019).

2.1.3.1 Genes Involved in Trabecular Meshwork and Aqueous Humor Drainage

Dysfunction in aqueous humor drainage is a major contributor to the dysregulation of IOP and PCG development. Key genes involved in the TM integrity and aqueous humor regulation include *LTBP2, COL4A1*, and *GPATCH3.*

LTBP2 (Latent Transforming Growth Factor Beta Binding Protein 2) is located on chromosome 14q24.3 and plays a significant role in trabecular meshwork development and extracellular matrix maintenance. It is particularly relevant in populations where *CYP1B1* mutations are absent. Mutations in *LTBP2* disrupt ECM function, impair aqueous humor drainage, and elevate IOP, contributing to PCG pathogenesis (Narooie-Nejad et al., 2009).

COL4A1 (Collagen Type IV Alpha 1) on chromosome 13q34, encodes a collagen protein essential for basement membrane integrity in ocular structures. Mutations can weaken trabecular meshwork architecture, hindering aqueous humor outflow (Coupry et al., 2010).

Lastly, *GPATCH3* (G Patch Domain Containing 3), located on chromosome 1p34.2, has been recently identified in a whole exome sequencing study as a new gene involved in ocular development (Ferre-Fernández et al., 2017).

2.1.3.2 Genes Involved in Schlemm's Canal Development and Angiopoietin-TIE Signaling

The Schlemm's canal is an essential structure for aqueous humor outflow pathway, and the angiopoietin-TIE signaling pathway regulates its formation and function. Mutations in *TEK* and *ANGPT1* genes can impair this pathway and increase susceptibility to PCG (Thomson et al., 2017).

TEK (TEK Receptor Tyrosine Kinase), located on chromosome 9p21.2, encodes a receptor tyrosine kinase that interacts with angiopoietins to regulate vascular and lymphatic vessel development. Mutations in TEK disrupt the angiopoietin-TIE signaling pathway, leading to malformation or dysfunction of Schlemm's canal. This increases resistance to aqueous humor outflow, elevating IOP, contributing to PCG (Choi et al., 2024).

ANGPT1 (Angiopoietin 1), on chromosome 8q23.1, encodes a ligand that activates the TEK receptor. It plays a key role in stabilizing Schlemm's canal endothelium and facilitating aqueous humor drainage. Mutations in *ANGPT1* reduce TEK receptor activation, thereby increasing PCG risk (Thomson et al., 2017).

2.1.3.3 Genes Associated with Anterior Segment Dysgenesis and PCG

Anterior segment dysgenesis (ASD) refers to developmental abnormalities in the structures of the anterior chamber, including the trabecular meshwork and Schlemm's canal. Many ASD-related genes, such as *FOXC1* and *PITX2*, are also linked to PCG (Kaushik et al., 2022).

FOXC1 (Forkhead Box C1), located on chromosome 6p25, encodes a transcription factor crucial for developing the cornea, iris, and trabecular meshwork. Mutations disrupt normal anterior segment formation and are frequently associated with ASD disorders, which often present with congenital glaucoma. This genetic overlap underscores FOXC1's role in both ASD and PCG (Kaushik et al., 2022).

Similarly, *PITX2* (Paired Like Homeodomain 2), on chromosome 4q25, is essential for trabecular meshwork and anterior segment development. Mutations are strongly linked to Axenfeld-Rieger syndrome, a disorder characterized by ocular and systemic anomalies, with affected individuals often developing early-onset glaucoma (Kaushik et al., 2022).

2.2 Molecular Pathophysiology of *CYP1B1* and *MYOC* in PCG

2.2.1 Molecular Pathophysiology of *CYP1B1* in PCG

CYP1B1 is expressed in the anterior uveal tract, which regulates aqueous humor secretion and outflow. A study demonstrated the functional interaction between CYP1B1 and MYOC (myocilin) where 17β estradiol acts as a mediator. Normally, CYP1B1 metabolizes 17β-estradiol into less active forms, preventing excessive binding to estrogen receptors (ERs) (Mookherjee et al., 2012).

Mutations in *CYP1B1* results in impaired metabolism of 17β-estradiol. Accumulation of 17β-estradiol increases binding to estrogen receptors (ERα and ERβ) in the TM cells. The overabundance of 17β-estradiol leads to excessive formation of 17β-estradiol-estrogen receptor complex. The resulting overproduction of the 17β-estradiol-ER complex upregulates MYOC expression (Figure 2.5). Excess myocilin accumulates in the endoplasmic reticulum (ER), causing protein misfolding, ER stress, and TM cell apoptosis. This disrupts ECM remodeling, impairs aqueous humor drainage, and increases resistance to outflow, leading to elevated IOP and glaucoma. Persistent IOP elevation damages the optic nerve, resulting in the characteristic features of PCG (Mookherjee et al., 2012).

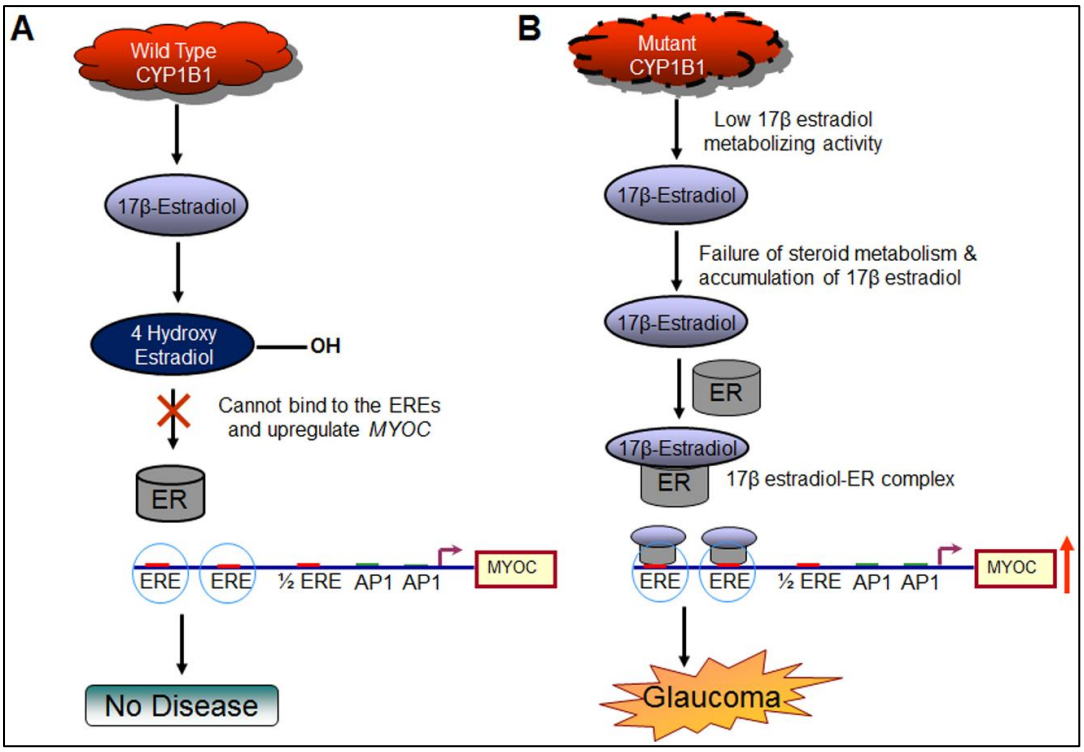

Figure 2.6 Schematic diagram showing potential influence of CYP1B1 mutants on MYOC expression. In Panel **A** fully functional wild-type CYP1B1 metabolizes 17β-Estradiol; thus limiting the steroid to form the hormone-receptor complex (17β-Estradiol-ER) whereas in Panel B restricted CYP1B1 enzymatic activity results in higher levels of the steroid available for formation of 17β-Estradiol-ER complex which in turn leads to MYOC upregulation through estrogen response elements (EREs) in MYOC promoter. The latter condition might have a potential implication in glaucoma pathogenesis. Image retrieved from (Mookherjee et al., 2012).

2.2.2 Molecular Pathophysiology of *MYOC* in PCG

Some glaucoma patients carry pathogenic variants in *MYOC* without mutations in *CYP1B1*. Wang et. al (2018) described multiple mechanisms by which *MYOC* mutations contribute to PCG. Mutant myocilin misfolds in the ER and fails to be secreted properly. This accumulation induces ER stress, which triggers the unfolded protein response (UPR). Prolonged ER stress results in TM cell apoptosis.

Additionally, secreted mutant myocilin aggregates abnormally and traps wild-type myocilin. This increases ECM stiffness, thereby reducing aqueous humor outflow and raising IOP (H. Wang et al., 2018). Glucose-regulated protein 94 (Grp94) chaperone is recruited by misfolded mutant myocilin and accelerates its aggregation via the ER-associated degradation (ERAD) system. Inhibiting Grp94 has been explored as a therapeutic strategy to prevent myocilin aggregation and promote alternative clearance pathways, reducing cytotoxicity (Huard et al., 2019; Stothert et al., 2014) (Figure 2.6).

Additionally, mutant myocilin aggregation in TM cells activates the NF-κB signaling pathway, leading to chronic inflammation. Increased levels of IL-1β and oxidative stress (OS) contribute to TM cell damage and further elevation of IOP (H. Wang et al., 2018).

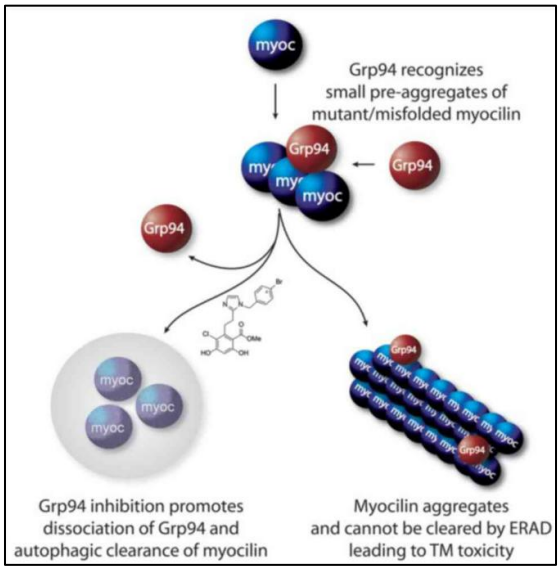

Figure 2.7 Co-aggregation of Grp94 with mutant/misfolded myocilin (80) (H. Wang et al., 2018).

2.3 Therapeutic Strategies for Primary Congenital Glaucoma (PCG)

PCG is primarily managed through surgical intervention, as medical therapy alone is often insufficient in controlling the high IOP due to structural abnormalities in the trabecular meshwork. However, recent advances in molecular genetics have paved the way for potential gene-based therapies.

2.3.1 Surgical Approaches

Surgery remains the gold standard for PCG treatment. The primary goal of surgery is to enhance aqueous humor outflow by modifying or bypassing the defective trabecular meshwork. Goniotomy, a minimally invasive procedure, involves incising the trabecular meshwork and is most effective in early-diagnosed PCG with clear corneas. Trabeculotomy, a similar procedure performed externally using a probe to disrupt Schlemm's canal, is preferred for cases with hazy corneas where goniotomy is not feasible. In severe cases or when IOP remains uncontrolled, a combined trabeculotomy-trabeculectomy is performed to increase outflow through both Schlemm's canal and a newly created drainage channel (Kaur et al., 2024).

For refractory cases, glaucoma drainage devices (GDDs), such as Ahmed valves and Baerveldt implants, offer an alternative drainage pathway to lower IOP. In advanced or resistant cases, cyclodestructive procedures like cyclophotocoagulation use laser energy to reduce aqueous humor production by targeting the ciliary body. This approach is typically reserved as a last resort for refractory PCG or blind, painful eyes (Kaur et al., 2024).

2.3.2 Molecular Genetics Approaches and Emerging Therapies

With the identification of key PCG-associated genes (*CYP1B1, TEK, LTBP2, FOXC1*), researchers are exploring gene-targeted and cell-based therapies as potential alternatives or adjuncts to surgery.

2.3.2.1 Gene Therapy

Gene therapy offers a promising approach for treating PCG by targeting its genetic causes. Gene supplementation therapy, which introduces a functional copy of a defective gene, has shown success in inherited retinal diseases. A recent study on AIPL1-associated retinal dystrophy demonstrated improved visual function and structural preservation with early intervention (Michaelides et al., 2025) Building on these findings, similar strategies could be applied to *CYP1B1, TEK, LTBP2,* and *FOXC1* mutations in PCG.

Viral vectors like AAVs and gene-editing tools such as CRISPR hold potential for restoring trabecular meshwork function and preventing optic nerve damage. While still experimental, gene therapy could offer a long-term alternative to surgery and medication in PCG treatment (Kharisova et al., 2025).

2.3.2.2 Pharmacogenomics and Personalized Medicine

Understanding the genetic basis of PCG allows for the development of targeted drug therapies. Personalized medicine aims to optimize glaucoma treatment by tailoring drug selection and dosage based on genetic variations, improving efficacy while minimizing side effects. Ongoing research focuses on refining Rho kinase (ROCK) inhibitors and exploring new pharmacogenomic strategies for PCG management (Wang et al., 2023).

ROCK inhibitors, such as netarsudil (0.02%) and ripasudil (0.4%), lower intraocular pressure (IOP) by enhancing aqueous humor outflow and relaxing the trabecular meshwork. These drugs show promising neuroprotective effects but may cause temporary conjunctival congestion or bleeding. Administering netarsudil at night can reduce congestion (Wang et al., 2023).

Small molecule therapies are being explored to modulate ECM remodeling and neuroprotection in PCG. Matrix metalloproteinases (MMP9) inhibitors aim to prevent excessive ECM buildup in the trabecular meshwork, reducing outflow resistance and lowering IOP. Prostaglandin analogs (PGAs) enhance ECM turnover by increasing MMP expression, facilitating aqueous humor outflow. Sustained-release PGA implants, such as

Bimatoprost SR, offer prolonged IOP reduction by inducing long-term ECM remodeling (Weinreb et al., 2020).

Moreover, neuroprotective agents like brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) aim to preserve retinal ganglion cells (RGCs) and prevent apoptosis. While preclinical studies show promising neuroprotective effects, clinical applications remain under investigation (Lambuk et al., 2022).

2.3.2.3 Stem Cell Therapy

Stem cell-based approaches hold promise for trabecular meshwork regeneration and optic nerve repair in PCG. Induced pluripotent stem cells (iPSCs) are being explored for restoring trabecular function, potentially reversing structural defects (Zhu et al., 2023). Mesenchymal stem cells (MSCs) may enhance neuroprotection and promote optic nerve regeneration, preserving vision in advanced cases (B. Y. Hu et al., 2024).

Despite progress in stem cell therapies for optic nerve damage, challenges remain, including efficient retinal ganglion cell (RGC) integration and axon regeneration. Advances in imaging and stem cell engineering will improve the ability to track cell migration, survival, and function in vivo (B. Y. Hu et al., 2024).

2.3.2.4 Conclusion

While surgical interventions remain the mainstay of PCG treatment, advances in molecular genetics offer promising future therapies. Gene therapy, stem cell-based regeneration, and personalized pharmacogenomic approaches could potentially transform PCG management, and reduce the need for repeated surgeries as well as improving long-term visual outcomes. Continued research into targeted molecular therapies is important in complementing existing surgical techniques and providing more effective and sustainable treatments for PCG patients.

2.3.3 Targeted Molecular Therapies for *MYOC*-Associated Glaucoma

A growing body of research has investigated potential therapeutic strategies for mitigating the pathogenic effects of *MYOC* mutations in glaucoma. These approaches aim to prevent disease progression by targeting the clearance of misfolded myocilin, which reduces ER stress, leading to neuroprotection of RGCs.

2.3.3.1 PBA

One promising therapeutic approach involves sodium 4-phenylbutyrate (PBA). PBA is a chemical chaperone that enhances proper folding and secretion of myocilin. A study by Zode et al. demonstrated that topical ocular administration of PBA significantly improved myocilin secretion and reduced its intracellular accumulation in mutant *MYOC* transgenic mouse model of POAG. This PBA treatment reduced ER stress in TM and lowered IOP (Zode et al., 2012).

2.3.3.2 Grp94

Another molecular target under investigation is the previously mentioned Grp94, which is involved in myocilin aggregation. Studies have shown that Grp94 inhibitors can facilitate the degradation of toxic myocilin aggregates, thus prevent TM dysfunction and lower IOP (Huard et al., 2019). Since Grp94 promotes the persistence of misfolded myocilin, its inhibition is considered a promising strategy for rescuing TM cells from cytotoxic stress and restoring normal aqueous humor outflow.

2.3.3.3 NAD+(H)

Aside from targeting the TM and IOP regulation, neuroprotective strategies to manage glaucoma-related damage have been considered as a therapeutic approach. The NAD+/NADH redox state has been proposed both as a biomarker and therapeutic target for glaucoma. NAD+(H) plays a central role in ATP production and mitochondrial function. Disruptions in NAD+(H) homeostasis have been associated with RGC degeneration(Petriti et al., 2021). Therefore, modulating NAD+ levels could be used for protecting RGCs against glaucomatous damage.

Chapter Three: Methodology

3.1 Study Subjects:

The study received approval from the Arab American University Palestine (AAUP) Institutional Review Board (IRB) and adhered to the principles of the Helsinki Declaration. Patients diagnosed and confirmed as primary congenital glaucoma cases were recruited. Recruitment was conducted with the assistance of ophthalmologists, who contacted the patients. Patients were referred from glaucoma specialist ophthalmologists Dr. Amer Muhsen and Dr. Sana Muhsen, as well as St John of Jerusalem Eye Hospital. An informed consent (Appendix 1) was obtained from all participants or their guardians.

Patients were recruited during the period from November 1, 2023 to November 1, 2024 in the West Bank. The study included a total of 46 patients, 15 of which were familial cases involving at least two affected siblings or family members. Blood samples were collected from patients, and their families if available. Figure 3.1 and Table 3.1 show the distribution of patients in the West Bank. Further demographic characteristics are described in Table 3.2. Patients received their results in a report in Arabic Language (Appendix 2).

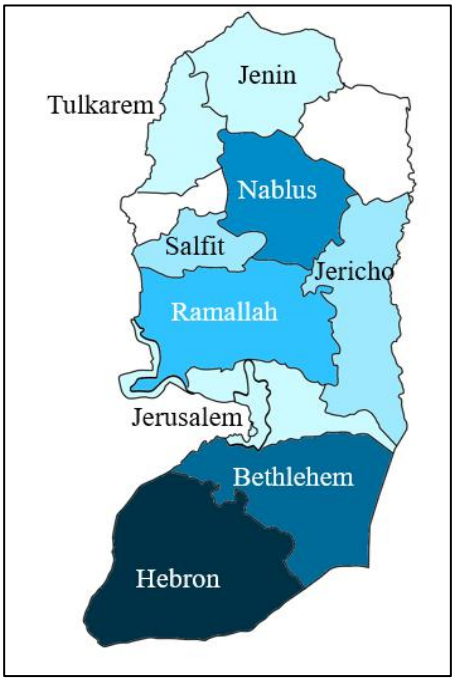

Figure 3.1 Heatmap of the distribution of PCG patients in the West Bank. Darker colors indicate higher prevalence.

Table 3.1 Patients Places of Residence

Place of Residence	n (%)
Bethany	1 (2.2%)
Bethlehem	9 (19.6%)
Hebron	12 (26.1%)
Jenin	1 (2.2%)
Jericho	3 (6.5%)
Jerusalem	2 (4.3%)
Nablus	8 (17.4%)
Ramallah	6 (13.0%)
Salfit	3 (6.5%)
Tulkarem	1 (2.2%)

Table 3.2 Demographic characteristics of the patients participating in the study

Characteristics		**n (%)**
Total Patients		46
Age at diagnosis	Since Birth	32 (69.6%)
	1 - 3 years	11 (23.9%)
	At 10 years old[1]	3 (6.5%)
Gender	Male	27 (58.7%)
	Female	19 (42.3%)
Consanguinity		30 (65.2%)
Family history		16 (34.7%)
Consanguinity & Family History		15 (93.8%)
Affected eyes	Unilateral	6 (13.0%)
	Bilateral	40 (87.0%)
Blindness as perceived by patients	OD	4 (8.7%)
	OS	2 (4.3%)
	OU	5 (10.9%)

3.2 Genomic DNA Preparation

3.2.1 DNA Extraction

Blood samples (3 mL) were collected and centrifuged at x3,000 rpm for 15 minutes. Buffy coat (200 µL) was transferred to an Eppendorf tube. DNA was extracted using the Promega Wizard® Genomic DNA Purification Kit (USA). The manufacturer extraction guidelines were followed with some modifications.

Cell lysis solution (900 µL) was added to the Eppendorf tube containing the buffy coat, and the sample was mixed by inversion and incubated at room temperature for 10 minutes followed by centrifugation at x14,000 rpm for 1 minute. The supernatant was discarded. The pellet was vortexed and 300 µL of nuclei lysis buffer was added to the tube. The sample was pipetted 5–7 times, followed by the addition of 100 µL protein

[1] There is a screening program in Palestine to check on eye health of 5th grade students

precipitation solution, then vortexed for 20 seconds. The sample was centrifuged at x14,000 rpm for 3 minutes. The supernatant was transferred to a tube containing 700 µL isopropanol and mixed until a white thread appeared. After centrifugation for 10 minutes, the supernatant was discarded, and the pellet air-dried for 10 minutes. DNA was rehydrated with 100 µL DNA rehydration solution and incubated at 56°C for 1 hour or left at room temperature overnight. Finally, DNA was stored at -30°C until use.

3.2.2 DNA Quantification

The concentration and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). DNA purity was evaluated based on the A260/A280 and A260/A230 ratios. Samples with an A260/A280 ratio of 1.8–2.0 were considered of acceptable purity for downstream applications. The measurements were performed by pipetting 1 µL of the DNA sample onto the NanoDrop pedestal, and results were recorded for further analysis.

2.2.3 DNA Qualification

The integrity and size of the extracted DNA were assessed using agarose gel electrophoresis. A 0.8% agarose gel was prepared by dissolving 0.8 g of agarose in 100 mL of 1X TAE buffer. Mixture was boiled until fully dissolved. After cooling slightly, Ethidium bromide was added to the mixture to enable DNA visualization. The gel was poured into a casting tray with a comb and left to solidify.

DNA samples were mixed with a loading dye and loaded into the gel wells, alongside a molecular weight ladder for size reference. The gel was run at 100V for 30 minutes, and the DNA bands were visualized under UV light. The results were documented to confirm the quality and integrity of the DNA (Figure 3.2).

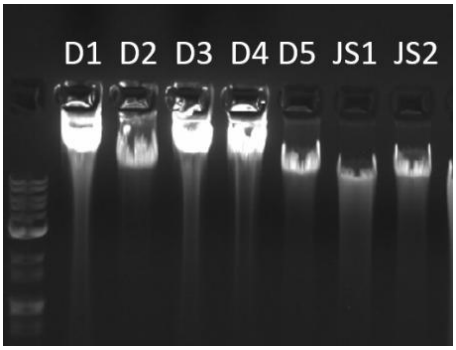

Figure 3.2 Results of an agarose gel electrophoresis run to evaluate the DNA quality of samples. A DNA ladder is included in the first lane as a molecular weight reference. The presence of bright, high-molecular-weight bands in all lanes suggests intact, high-quality genomic DNA. Smearing or degraded bands in certain lanes indicate possible fragmentation or degradation.

3.3 Primers Preparation

3.3.1 Primers Design

Primers were initially selected for *CYP1B1* and *MYOC* based on a literature review (Afzal et al., 2019). However, four *CYP1B1* primers failed to perform during optimization. They worked after adding Dimethyl sulfoxide (DMSO) and testing higher annealing temperatures. The list of primers is provided in Table 3.3 and Table 3.4.

Table 3.3 Primer pairs used for amplification of the *CYP1B1* gene

Exon	Primer ID	5' - 3' Sequence	Melting Temperature	Product Size (bp)
2	2.1 F	ACCCAACGGCACTCAGTC	59.67	517
	2.1 R	CCGAGTAGTGGCCGAAAG	59.39	
2	2.2 F	CCCCATAGTGGTGCTGAATG	61.32	512
	2.2 R	CTCGAATTCGCGGAAAAC	59.35	
2	2.3 F	TCAGCCACAACGAAGAGTT	56.92	531
	2.3 R	CACTGTGAGTCCCTTTACCG	58.22	
3	3.1 F	GCAAGGCCTATTACAGGAAA	57.02	463
	3.1 R	TTCACAGACCACTGGTTGAC	56.98	
3	3.2 F	TATGTCCTGGCCTTCCTTTA	57.31	512
	3.2 R	AGCTTGCCTCTTGCTTCTTA	57.15	
3	3.3 F	AATGAGCCTGCGAAAATG	57.35	513
	3.3 R	ATGGCCTGGTTACCAAAATA	56.99	

Table 3.4 Primer pairs used for amplification of the *MYOC* gene

Exon	Primer ID	5' - 3' Sequence	Product Size (bp)
1	1.1 F	CTCTGTCTTCCCCCATGAAG	462
	1.1 R	AGCCTGGTCCAAGGTCAAT	
1	1.2 F	AGGCCATGTCAGTCATCCAT	478
	1.2 R	GCGCCTGTAGCAGGTCACTA	
2	2.1 F	GCAGCCTATTTAAATGTCATCCT	310
	2.1 R	TGGGTGGGCATTTACCCTAT	
3	3.1 F	TCCGCATGATCATTGTCTGT	467
	3.1 R	ACCCCAAGAATACGGGAACT	
3	3.2 F	ACTCGGGGAGCCTCTATTTC	461
	3.2 R	CTCCAGGGGGTTGTAGTCAA	
3	3.3 F	CCCAGAGAATCTGGAACTCG	478
	3.3 R	CGCCCTCAGACTACAATTCC	

3.3.2 Primer Optimization

Primers were optimized to ensure efficient and specific amplification. Gradient PCR was used to find the best annealing temperature, testing a range of 55–65°C with a standard DNA template. PCR products were checked on a 2.0% agarose gel to confirm proper amplification.

3.4 PCR Amplification

All 46 samples were amplified using PCR for each primer set. A primer mix was prepared by combining 0.5 picomoles of forward and reverse primers (1 µL each) with 80 µL of nuclease-free water. The reagents for the PCR reaction are detailed in Tabe 3.5.

Tabe 3.5 Reagents and volumes used for PCR reaction.

Reagent	Volume
PCR Master Mix (1X)	10 µL
Primer Mix	10 µL
DNA Sample	1 µL
Nuclease Free Water	8 µL
Total Volume	**20 µL**

The prepared reaction mix was placed in a FlexCycler2 thermocycler, and the following program was used (**Table 3.6**):

Table 3.6 PCR program used to amplify DNA fragments housing the expected variants.

PCR Step	Temp	time	Cycle number
Initial Denaturation	95 °C	5 minutes	x1
Denaturation	95 °C	30 seconds	
Annealing	60 °C	30 seconds	x35
Extension	72 °C	30 seconds	
Final Extension	72 °C	5 minutes	x1
Cooling	4 °C	Pause	∞

The PCR products were visualized on a 2% agarose gel alongside a molecular weight ladder and a no-template control (NTC) (Figure 3.3).

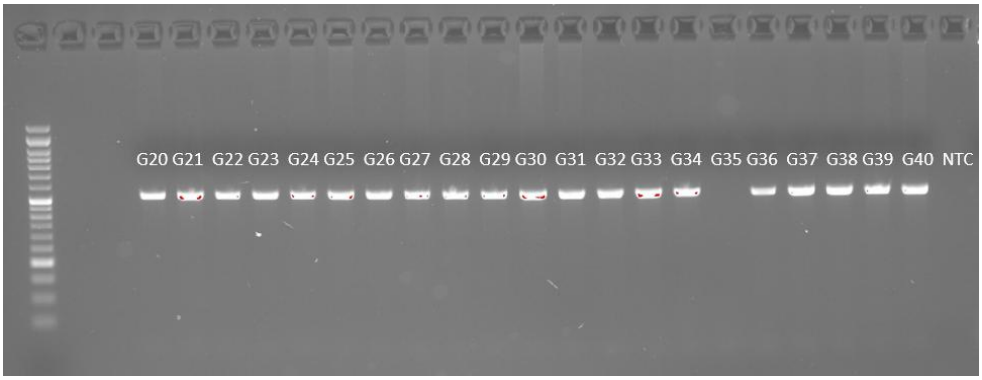

Figure 3.3 PCR amplification of *CYP1B1* 3.1 primers, with an expected product size of 463 bp. The DNA ladder shows the samples bands are around 500 bp. All samples exhibit distinct, bright bands around the expected size, except for G35. A no-template control (NTC) is included to check for contamination, and no visible bands in this lane confirm the absence of non-specific amplification.

3.5 Sanger Sequencing

Samples were sequenced using the BigDye™ Terminator Cycle Sequencing Kit and an Applied Biosystems Genetic Analyzer, following the manufacturer's instructions.

3.5.1 PCR Product Cleaning

PCR products were cleaned using EPPIC-FAST reagent (Catalog #1021-100F, A&A Biotechnology), a mix of thermolabile nucleotide hydrolase and recombinant exonuclease I. For each reaction, 1 µL of EPPIC-FAST was added to 5 µL of PCR product. The mixture was incubated on a thermocycler at 37°C for 10 minutes, followed by 80°C for 1 minute to inactivate the enzymes.

3.5.2 Sequencing

Cycle sequencing was performed by adding 18 µL of BigDye Terminator (BDRR) mix to 2 µL of cleaned PCR product. The reaction was run on a thermocycler under conditions specified in Table 3.7. The reagents used to prepare the BDRR mix are detailed in Table 3.8.

Sequenced products were then purified using EDTA-ethanol precipitation. 60 µL of 100% cold ethanol. 5 µL of 12.5 mM EDTA were added to the sequenced sample, followed by centrifugation at x2,200 rpm for 30 minutes. The supernatant was discarded. The pellet was washed with 80 µL of 80% ethanol. The mixture was centrifuged at x1,600 rpm for 15 minutes, after which the supernatant was removed. The pellet was air-dried. High Dye was added to the pellet, and the sample was heated at 95°C for 5 minutes, followed immediately by chilling on ice for another 5 minutes.

Table 3.7 BDRR PCR program used

PCR Step	Temp	time	Cycle number
Initial Denaturation	95 °C	20 seconds	x1
Denaturation	95 °C	10 seconds	
Annealing	50 °C	50 seconds	x25
Extension	6 °C	4 minutes	
Cooling	4 °C	Pause	∞

Tabe 3.8 Reagents and volumes used for PCR reaction.

Reagent	Volume
Sequencing Buffer (5X)	3.5 µL
Nuclease Free Water	11.5 µL
Sequencing Primer	2.0 µL
BDRR	1.0 µL
Total Volume	**18 µL**

Initially, the *CYP1B1* gene was sequenced. Samples that did not exhibit pathogenic or likely pathogenic variants in *CYP1B1*, sequencing of the *MYOC* gene was subsequently performed.

3.6 *In-Silico* Analysis

Resulting sequences were analyzed using publicly available bioinformatics tools. Sanger sequencing reads were examined using the UCSC Genome Browser. Variants were assessed using MutationTaster, SIFT, and PolyPhen to predict their potential pathogenicity. Known clinical variants were cross-referenced using ClinVar, Franklin, and Ensembl Variant Effect Predictor (VEP). Intronic variants were assessed via SpliceAI for pathogenicity. Multiple sequence alignments were performed using PhyloP on UCSC Genome Browser to evaluate evolutionary conservation of the identified variants. The observed *CYP1B1* and *MYOC* variants were analyzed for population frequency using the Genome Aggregation Database (gnomAD). Protein structures were plotted via AlphaFold.

- UCSC Genome Browser: https://genome.ucsc.edu/
- MutationTaster: https://www.mutationtaster.org/
- SIFT: https://sift.bii.a-star.edu.sg/
- PolyPhen: http://genetics.bwh.harvard.edu/pph2/
- ClinVar; https://www.ncbi.nlm.nih.gov/clinvar/
- Franklin: https://franklin.genoox.com/clinical-db/home
- Ensembl VEP: https://asia.ensembl.org/Tools/VEP
- gnomAD: https://gnomad.broadinstitute.org/
- SpliceAI: https://spliceailookup.broadinstitute.org/
- AlphaFold Protein Structure Database: https://alphafold.ebi.ac.uk/

Chapter Four: Results

4.1 Identification and Distribution of *MYOC* and *CYP1B1* Variants

Sequencing was initially performed on exon 3 of *MYOC* gene, with no variants identified. Therefore, subsequent analysis focused on CYP1B1gene. Variants with pathogenic scores were recorded, while samples without identified variants underwent further screening of potential variants in exons 1 and 2 of the *MYOC* gene. Four variants were identified in *MYOC* gene, three in exon 1 and one in intron 2, and none detected in exon 3. A summary of the identified *MYOC* genes variants is presented in Figure 4.1.1.

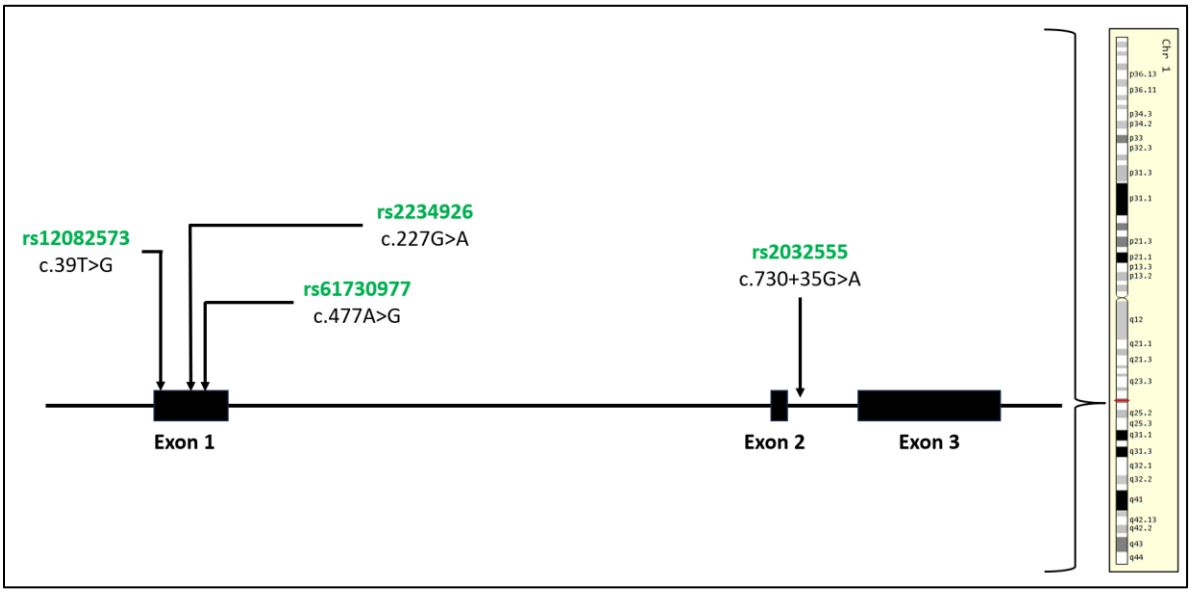

Figure 4.1.1 Schematic representation of the identified *MYOC* variants

On the other hand, a total of 17 distinct variants in the *CYP1B1* gene were identified. These variants were distributed across intronic, exonic, and untranslated regions (UTRs) of the gene and exhibited diverse mutation types, frequencies, and pathogenicity profiles, as shown in Figure 4.1.2.

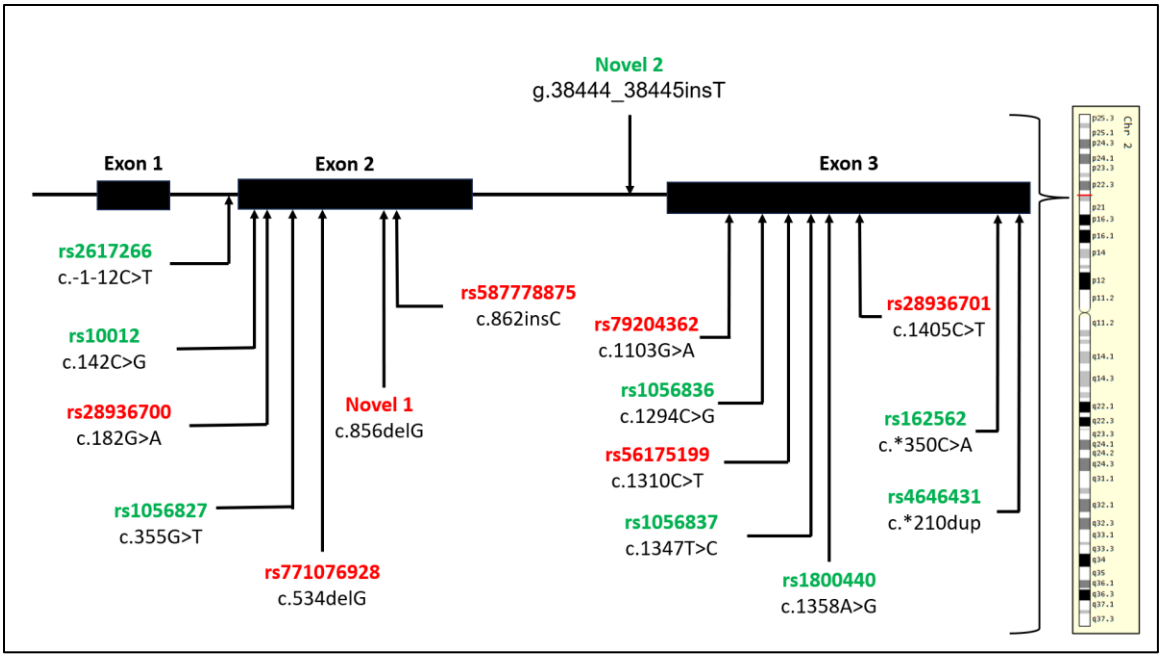

Figure 4.1.2 Schematic representation of identified *CYP1B1* variant.

Two intronic variants were detected: chr2:38302544G>A and a novel insertion (g.38444_38445insT), both classified as benign. Exon 2 harbored six variants, including three SNPs, two deletions, and one insertion. Among these, c.182G>A (p.Gly61Glu), c.534_534delG (p.Ala179Argfs18), and c.862_863insC (p.Arg290Profs37) were pathogenic, while c.856_856delG (p.Ala287Profs6)* represented a novel pathogenic deletion. Notably, one patient was compound heterozygous for c.182G>A and c.1103G>A (p.Arg368His), a variant of uncertain significance (VUS) in exon 3.

Exon 3 contained eight variants, including p.Arg368His, p.Leu432Val, p.Pro437Leu, and p.Arg469Trp. The c.1310C>T (p.Pro437Leu) substitution showed an exceptionally low gnomAD frequency (0.000021) and was classified as pathogenic. Two variants: p.Arg368His and p.Asn453Ser were VUS, while others such as p.Leu432Val were benign. The 3′UTR included two benign variants: a SNP (*c.350C>A) and an insertion (*c.210dup).

Allele frequencies ranged from rare to relatively common. Several pathogenic variants, including c.182G>A and c.534_534delG, were present in the homozygous state, supporting their disease association. In contrast, benign variants like c.1294C>G

(p.Leu432Val) appeared frequently in both homozygous and heterozygous forms, suggesting they are polymorphic in this population.

Interestingly, a recurrent combination of three variants: c.-1-12C>T (rs2617266), c.142C>G (p.Arg48Gly; rs10012), and c.355G>T (p.Ala119Ser; rs1056827) was observed in eight patients, always co-inherited and never found in isolation. Four of these individuals were homozygous for the combination, while four were heterozygous. This consistent co-occurrence suggests the presence of a haplotype block in linkage disequilibrium. Each variant was classified as a tolerated or benign polymorphism by *in-silico* tools.

Two novel variants were identified: c.856_856delG (p.Ala287Profs6)* and g.38444_38445insT, classified as pathogenic and benign, respectively. The presence of rare, homozygous, and compound heterozygous pathogenic variants reflects the genetic heterogeneity of CYP1B1-associated PCG in this Palestinian cohort, likely shaped by consanguinity and founder effects.

A summary of all identified variants in the MYOC and CYP1B1 genes, including their location, type, frequency in the study cohort, presence in families, and reported pathogenicity are reported on pages 62-63.

4.2 *MYOC* Variants Assessment.

4.2.1 Patient G12 – Compound heterozygous

rs12082573: c.39T>G (p.Pro13=)

rs61730977: c.477A>G (p.Leu159=)

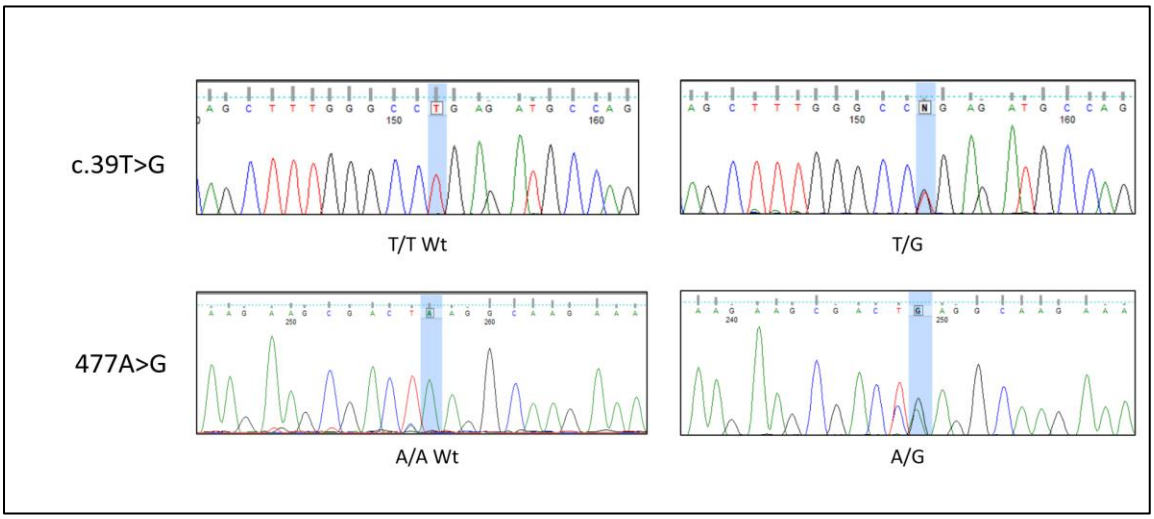

Figure 4.3 Nucleotide sequencing results for Patient G12. Patient has 2 heterozygous variants in exon 1, c.39T>G and c.477A>G. Right: shows the heterozygous variants.

Two heterozygous variants were identified in exon 1 of *MYOC* in a single patient. Both are synonymous changes with no predicted functional impact, as indicated by multiple tools (**Table 4.1**). The c.39T>G (p.Pro13=) variant has been reported in POAG individuals from Morocco, African American, and South African populations (Liu et al., 2012; Melki et al., 2003; Williams et al., 2015) and is considered a benign polymorphism with no significant association with POAG, consistent with ClinVar submissions. Similarly, the c.477A>G (p.Leu159=) has been widely reported in South African, Moroccan, and African American patients (Liu et al., 2012; Whigham et al., 2011) and is classified as a neutral polymorphism with no known contribution to glaucoma or other ocular diseases. Their gnomAD allele frequencies (0.004048 and 0.004044, respectively) indicate rarity in the general population, though not absent.

Table 4.1 Prediction Tools Assessing *MYOC*:c.39T>G and c.477A>G Variants

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
c.39T>G	**Polymorphism**	**TOLERATED**	**0.018**	**4.36**	**0.00**	**0.608**
c.477A>G	**Polymorphism**	**TOLERATED**	**-**	**8.31**	**0.00**	**3.22**

4.2.2 rs2234926: c.227G>A (p.Arg76Lys)

The c.227G>A variant was identified in 30% of patients. Five patients in a homozygous form and nine heterozygous. Multiple prediction tools (Table 4.2) and a functional analysis study (Nakahara & Hulleman, 2022) classify it as likely benign. ClinVar also list it as Benign. With a gnomAD allele frequency of 0.1383, this variant is relatively common, making pathogenicity less likely. Additionally, Figure 4.3 shows that R76 is conserved except in one species.

Table 4.2 Prediction Tools Assessing *MYOC*:c.227G>A Variant

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
Score	Polymorphysim	TOLERATED	0.018	0.00900	0.0200	-4.20

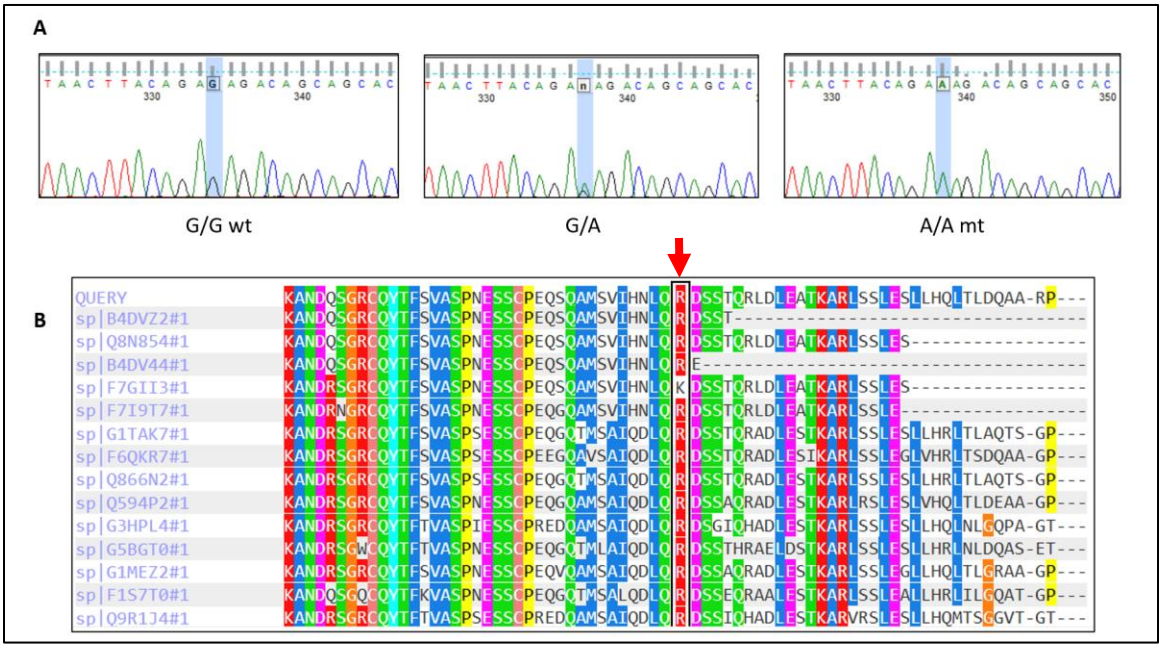

Figure 4.4 Nucleotide sequencing results and Multiple Sequence Alignment showing c.227G>A. (A) Sequencing chromatogram demonstrating the c.227G>A variant; left (G/G) homozygous wild-type, middle (G/A) heterozygous, right (A/A) homozygous mutant. (B) Multiple Sequence Alignment of the Protein Sequence on PolyPhen. Alignment shows a somewhat conservation of the region surrounding the variant across different species.

4.2.3 rs2032555: c.730+35G>A

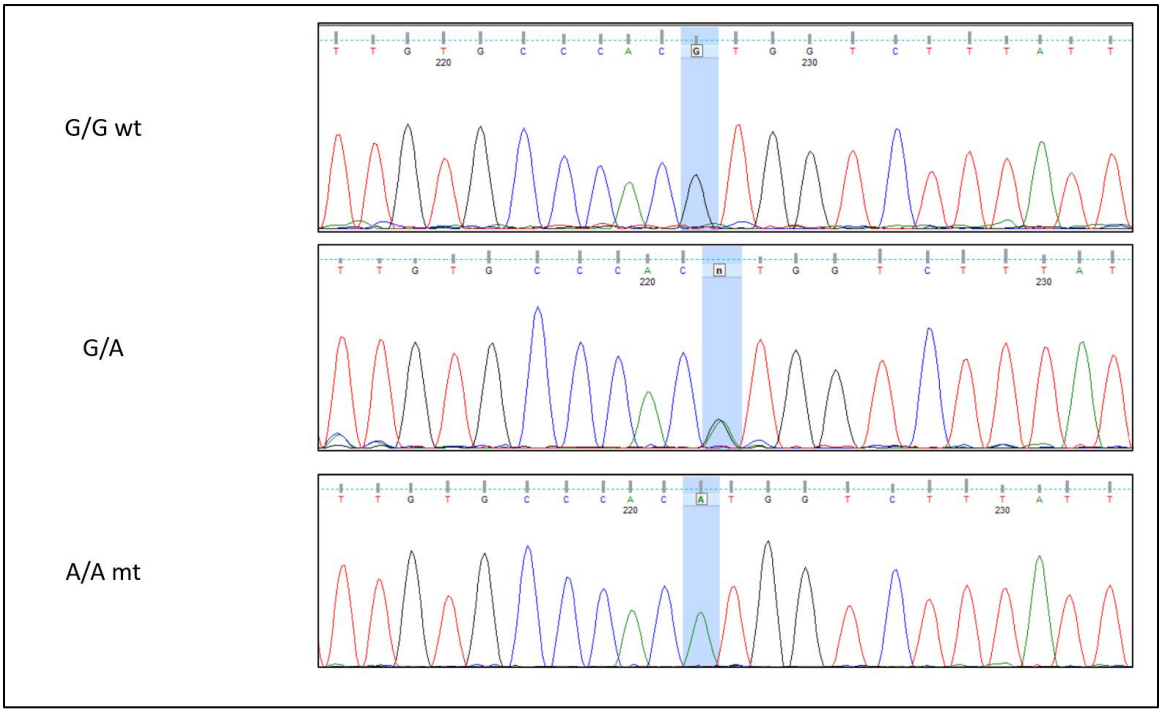

Figure 4.5 Nucleotide sequencing results showing c.730+35G>A: Top (G/G) homozygous wild-type, middle (G/A) heterozygous, bottom (A/A) mutant.

The c.730+35G>A variant was identified in 84% of 19 investigated patients in this study. ClinVar classifies it as benign. A Malay study suggested this polymorphism increases susceptibility to JOAG, showing a significant difference between affected and non-affected individuals. However, other reports noted no significant association of this variant with glaucoma in Indian and Chinese populations (Mimivati et al., 2014). Further research is needed to determine whether this variant represent real risk for PCG or is a population-specific polymorphism. Bioinformatics prediction tools indicate it is likely benign (Table 4.3).

Table 4.3 Prediction Tools Assessing *MYOC*:c.730+35G>A Variant

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
Score	Polymorphism	-	-	0.009	0.02	-4.20

4.3 *CYP1B1* Variants Assessment

4.3.1 Exon 2

4.3.1.1 Novel 1: c.856_856delG (p.Ala287Profs*6)

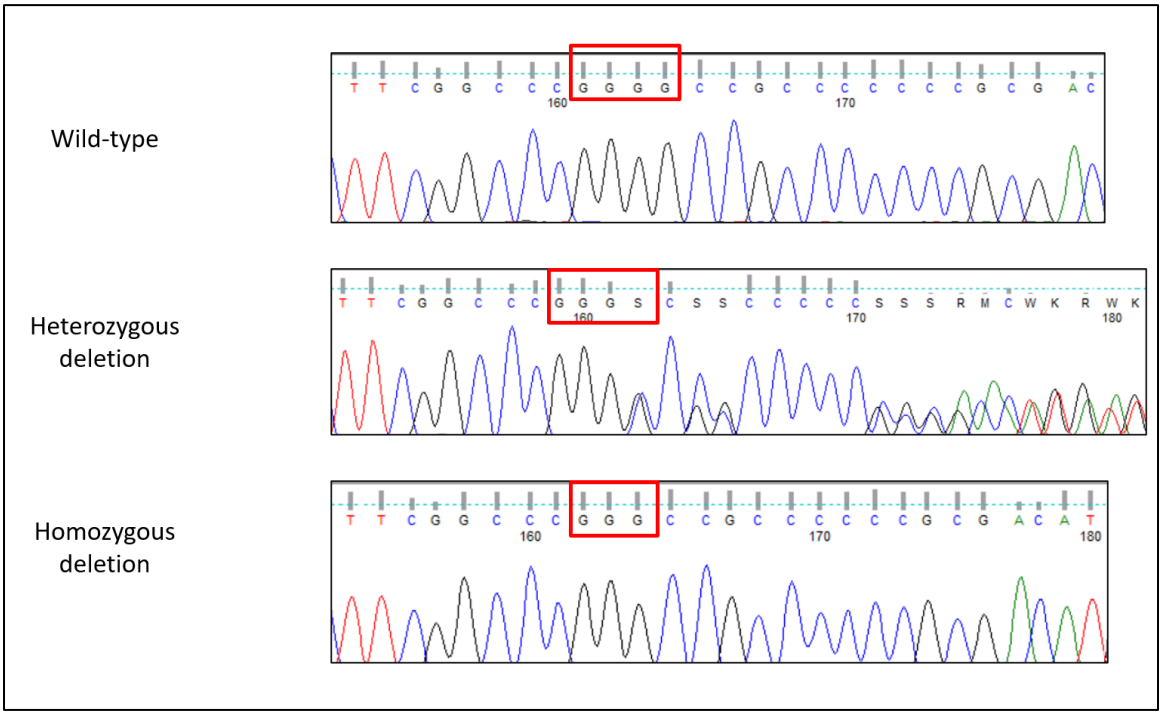

Figure 4.6.1 Nucleotide sequencing results showing the c.856_856delG mutant: top homozygous wild-type, middle (G/-) heterozygous deletion, bottom (G/-) homozygous deletion.

A novel frameshift deletion, c.856_856delG (p.Ala287Profs6)*, was identified in four patients from the study cohort. Among them, three were homozygous and one was heterozygous for the mutation. Two of the homozygous cases were first cousins from the same extended family. However, due to ethical considerations and mutual privacy concerns, family segregation analysis was not conducted, as each individual participated under the condition that the other remained unaware. Figure 4.6.2 illustrates the familial relationship. Figures 4.6.3 demonstrates segregation patterns in a different family carrying the mutation in a heterozygous state.

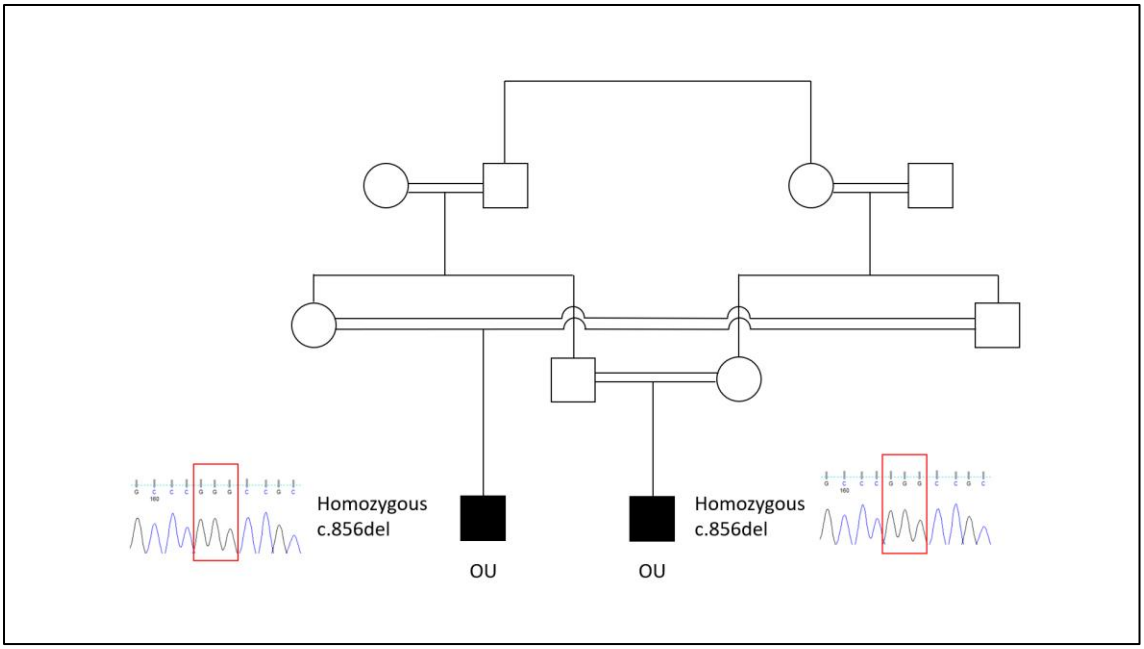

Figure 4.6.2 Pedigree of a family with 2 affected cousins

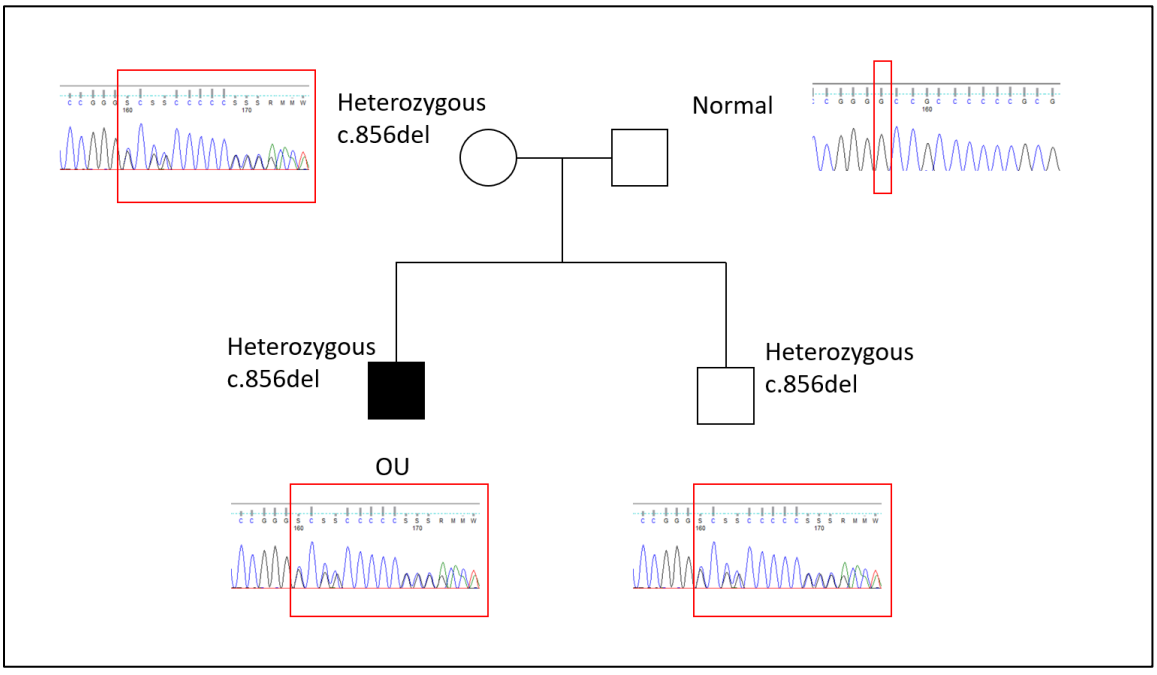

Figure 4.6.3 Pedigree of a family with a child affected with PCG, harboring c.856del mutation in a heterozygous form.

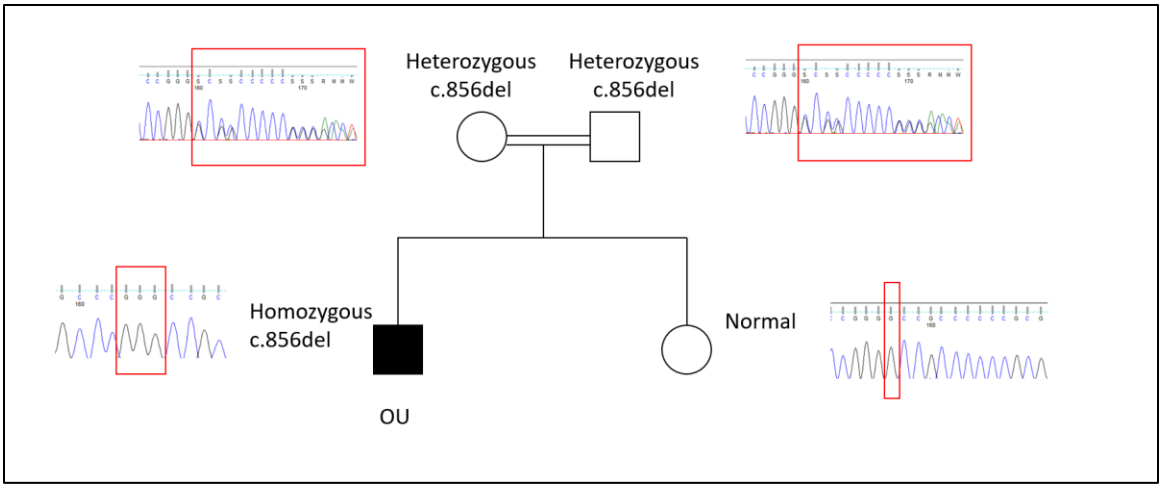

Figure 4.6.4 Pedigree of a family with a child affected with PCG, harboring c.856del mutation in a homozygous form.

This variant introduces a single nucleotide deletion at position 856 of the coding sequence, resulting in a frameshift and the creation of a premature stop codon six amino acids downstream. The resulting truncated protein lacks essential enzymatic domains required for normal *CYP1B1* function. As shown in Figure 4.6.5, the deletion leads to significant loss of amino acid sequence. The predicted protein model comparison using AlphaFold (Figure 4.6.6) illustrates the disruption in the tertiary structure between the wild-type and mutant forms.

Functionally, this mutation is predicted to be pathogenic due to its likely consequence on protein integrity and function. It may trigger nonsense-mediated mRNA decay (NMD) or produce a non-functional, truncated protein incapable of normal enzymatic activity. This variant has not been reported in ClinVar or the gnomAD database, suggesting it is previously undocumented and population-specific.

Further supporting its potential pathogenicity, the mutation site exhibits strong evolutionary conservation, with a phyloP score of 3.594, indicating that the affected nucleotide is highly conserved across species. *In-silic*o prediction using MutationTaster classified the variant as "disease causing." The full summary of in-silico predictions is presented in Table 4.4.

Table 4.4 Prediction Tools Assessing *CYP1B1*:c.856_856delG Variant

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
Score	Disease Causing	-	-	-	-	3.594

wildtype AA sequence	MGTSLSPNDP WPLNPLSIQQ TTLLLLLSVL ATVHVGQRLL RQRRRQLRSA PPGPFAWPLI GNAAAVGQAA HLSFARLARR YGDVFQIRLG SCPIVVLNGE RAIHQALVQQ GSAFADRPAF ASFRVVSGGR SMAFGHYSEH WKVQRRAAHS MMRNFFTRQP RSRQVLEGHV LSEARELVAL LVRGSADGAF LDPRPLTVVA VANVMSAVCF GCRYSHDDPE FRELLSHNEE FGRTVGAGSL VDVMPWLQYF PNPVRTVFRE FEQLNRNFSN FILDKFLRHC ESLRPGAAPR DMMDAFILSA EKKAAGDSHG GGARLDLENV PATITDIFGA SQDTLSTALQ WLLLLFTRYP DVQTRVQAEL DQVVGRDRLP CMGDQPNLPY VLAFLYEAMR FSSFVPVTIP HATTANTSVL GYHIPKDTVV FVNQWSVNHD PVKWPNPENF DPARFLDKDG LINKDLTSRV MIFSVGKRRC IGEELSKMQL FLFISILAHQ CDFRANPNEP AKMNFSYGLT IKPKSFKVNV TLRESMELLD SAVQNLQAKE TCQ*
mutated AA sequence	MGTSLSPNDP WPLNPLSIQQ TTLLLLLSVL ATVHVGQRLL RQRRRQLRSA PPGPFAWPLI GNAAAVGQAA HLSFARLARR YGDVFQIRLG SCPIVVLNGE RAIHQALVQQ GSAFADRPAF ASFRVVSGGR SMAFGHYSEH WKVQRRAAHS MMRNFFTRQP RSRQVLEGHV LSEARELVAL LVRGSADGAF LDPRPLTVVA VANVMSAVCF GCRYSHDDPE FRELLSHNEE FGRTVGAGSL VDVMPWLQYF PNPVRTVFRE FEQLNRNFSN FILDKFLRHC ESLRPGPPPA T*

Figure 4.6.5 Amino acid sequence comparison between the wild-type and truncated c.856_856delG mutant. The novel deletion mutation results in a premature stop codon, leading to a truncated protein. Image retrieved from MutationTaster.

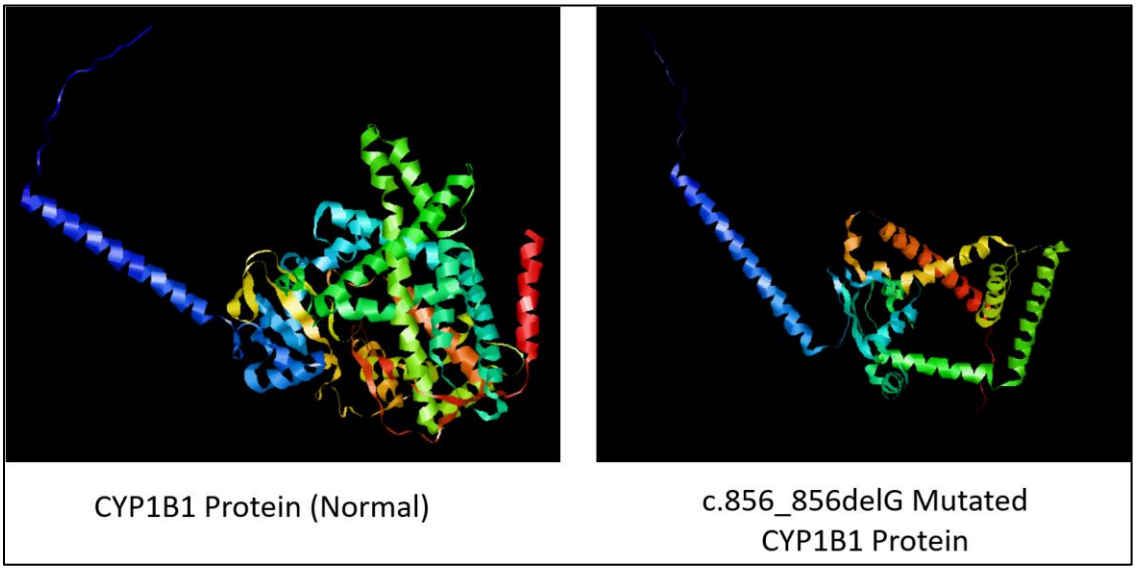

Figure 4.6.6 Protein sequence comparison between the wild-type and mutated amino acid sequences disruption in the tertiary structure between the wild-type and mutant forms of CYP1B1.

4.3.1.2 rs28936700: c.182G>A (p.Gly61Glu)

The variant results in the substitution of glycine (Gly) with glutamic acid (Glu) at position 61 of the protein. This missense variant has been reported in ClinVar, with six submissions classifying it as pathogenic. In this study, it was identified in 10 patients, nine homozygous and one heterozygous.

Structurally, Gly61 is near the substrate entry channel to the active site of CYP1B1, a region crucial for enzymatic function. Substituting glycine with glutamic acid introduces a charged residue, potentially disrupting substrate access and impairing catalytic activity. This suggests a potential mechanistic basis for its pathogenicity.

Population frequency data from gnomAD indicate that this variant is rare (allele frequency: 0.0003077), supporting its classification as a disease-associated mutation rather than a common polymorphism.

Computational predictions further support its pathogenic nature (Table 4.5). MutationTaster classifies it as disease-causing, SIFT predicts it to be deleterious, and PolyPhen labels it as probably damaging. The high CADD score (26.0) suggests significant functional impact, while the phyloP score (8.65) indicates strong evolutionary conservation.

These findings collectively support the pathogenicity of c.182G>A (p.Gly61Glu) and its potential role in CYP1B1-related disease.

Table 4.5 Prediction Tools Assessing *CYP1B1*:c.182G>A Variant

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
Score	Disease Causing	DELETERIOUS	PROBABLY DAMAGING	26.0	0.00	8.65

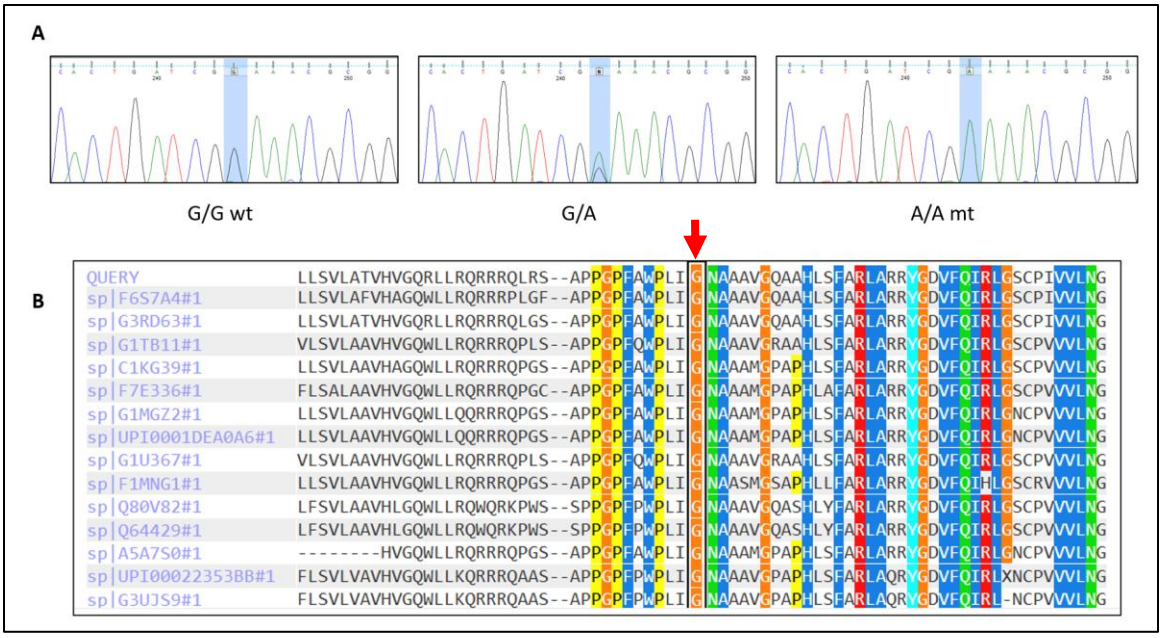

Figure 4.7 Nucleotide sequence results and Multiple Sequence Alignment for c.182G>A. A. Sequencing chromatogram demonstrating the c.182G>A variant; left (G/G) homozygous wild-type, middle (G/A) heterozygous, and right (A/A) homozygous mutant. B. Multiple Sequence Alignment of the Protein Sequence on PolyPhen. Alignment shows the variant is highly conserved across different species.

4.3.1.3 Possible Haplotype Block Inheritance

A distinct pattern of co-occurrence was observed among three *CYP1B1* variants:

- **rs2617266: c.-1-12C>T**
- **rs1056827: c.355G>T (p.Ala119Ser)**
- **rs10012: c.142C>G (p.Arg48Gly)**

Eight patients carry the indicated three variants (c.-1-12C>T, c.355G>T, and c.142C>G) together, four were homozygous, while the other four were heterozygous as shown in Figure 4.8.1. Notably, none of these three variants were found in isolation among other patients. This is striking and suggests strong linkage disequilibrium and potential inheritance as a haplotype block. Despite their co-occurrence, prediction tools classify them as polymorphisms. They are likely of low individual pathogenic significance; however, their consistent linkage raises the possibility of synergistic effect influencing gene function. Table 4.6 summarizes the in-silico predictions for the three variants. MutationTaster classifies all as polymorphisms, while SIFT and PolyPhen predict them to be tolerated and benign. CADD scores for these variants range from 6.90 to 10.4, indicating low to moderate predicted impact. The phyloP conservation scores suggest minimal conservation in all three variants.

Nevertheless, the inheritance pattern of this variant trio is striking. Figures 4.8.2 and 4.8.3 present pedigrees showing segregation of the haplotype in heterozygous and homozygous form, further supporting the hypothesis of a conserved, inherited haplotype block within affected families. While their individual pathogenic potential appears minimal, the tight linkage and consistent inheritance pattern warrant further functional investigation to assess any collective impact on *CYP1B1* function or disease susceptibility.

Table 4.6 Prediction Tools Assessing the Four Variants.

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
c.-1-12C>T	**Polymorphism**	**-**	**-**	**10.4**	**0.00**	**0.105**
c.355G>T	**Polymorphism**	**TOLERATED**	**Benign**	**6.90**	**0.00**	**-0.0750**
c.142C>G	**Polymorphism**	**TOLERATED**	**Benign**	**8.19**	**0.00**	**-0.0690**

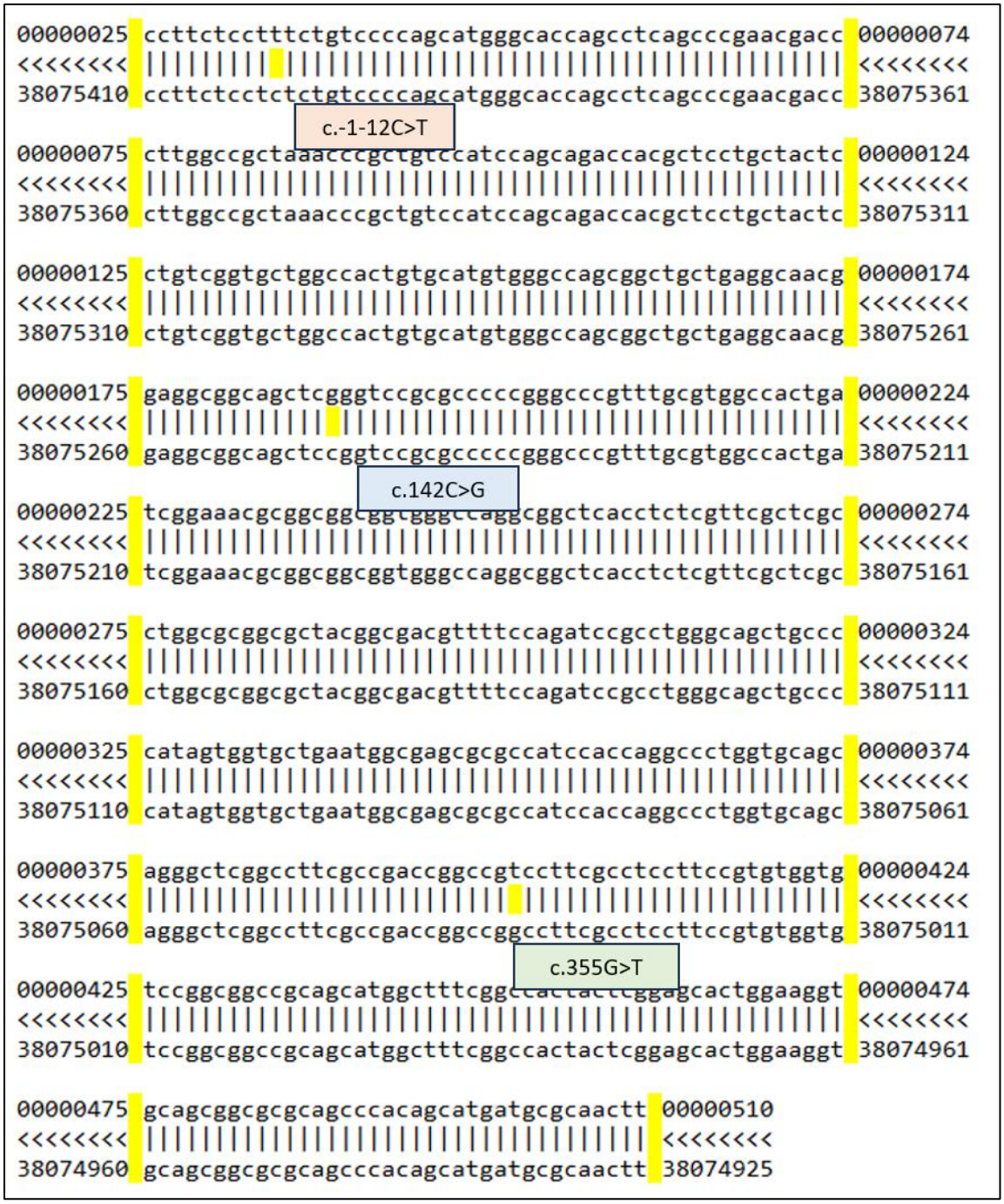

Figure 4.8.1 The three indicated variants (c.-1-12C>T, c.355G>T, and c.142C>G) are linked together as shown in their respected locations. This image was retrieved from UCSC Genome Browser.

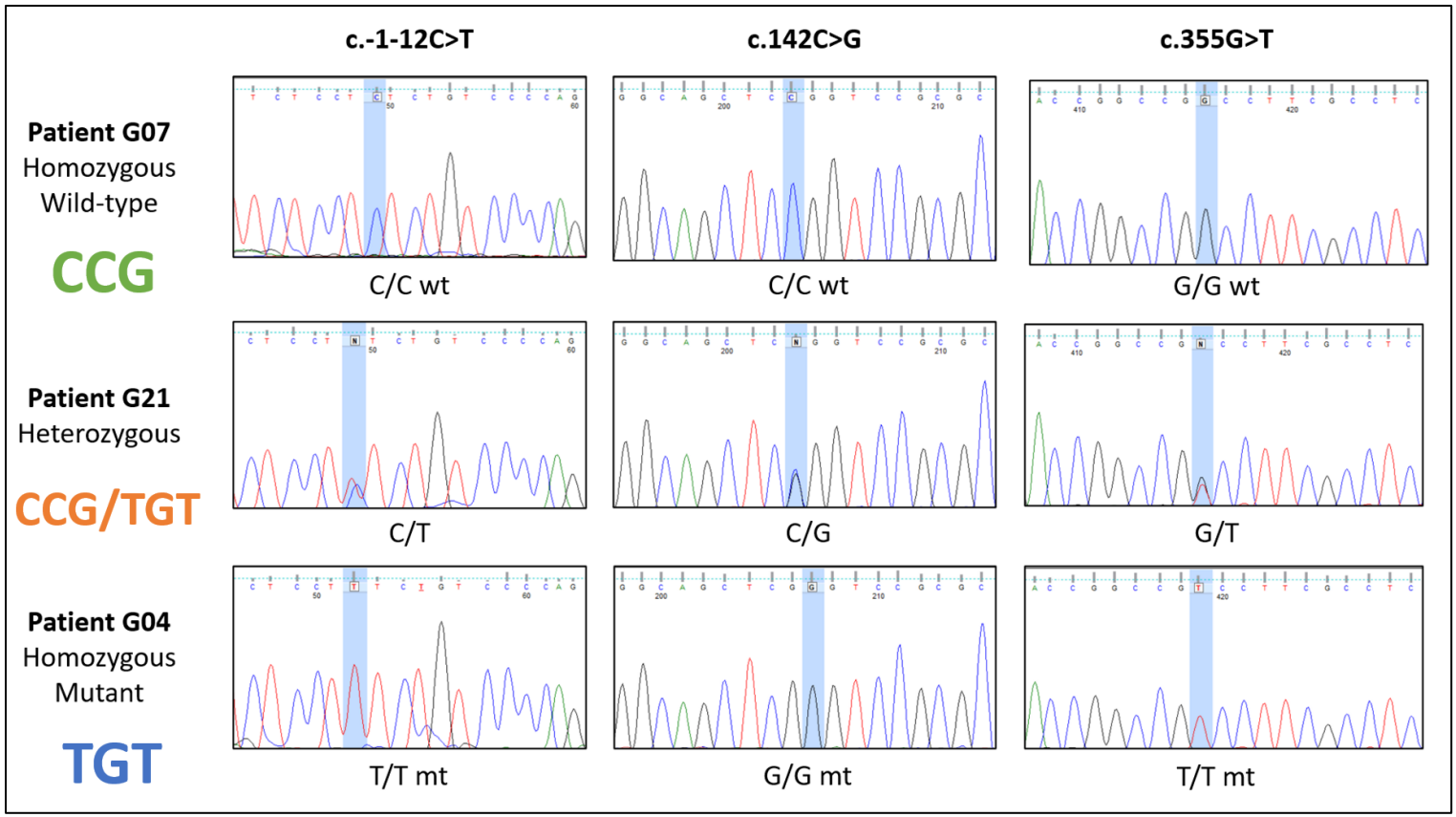

Figure 4.8.2 Sanger Sequencing Validation of CYP1B1 Variants (c.-1-12C>T, c.142C>G, c.355G>T) in three patients (G07, G21, and G04) demonstrating the genotypic variations at the three loci in the *CYP1B1* gene: c.-1-12C>T (rs2617266), c.142C>G (p.Arg48Gly, rs10012), and c.355G>T (p.Ala119Ser, rs1056827). The highlighted regions indicate the position of each variant. Patient G07 (Wild-type Homozygous) carries the wild-type allele (C/C, C/C, G/G) at the three loci. No mutations were detected. Patient G21 (Heterozygous for the three variants): This patient carries a heterozygous genotype (C/T, C/G, G/T) at the three loci. Patient G04 (Mutant Homozygous) is homozygous for the mutant alleles (T/T, G/G, T/T) at all three loci, indicating a complete alteration of the wild-type sequence. The consistent co-occurrence of these variants supports their potential inheritance as a haplotype block.

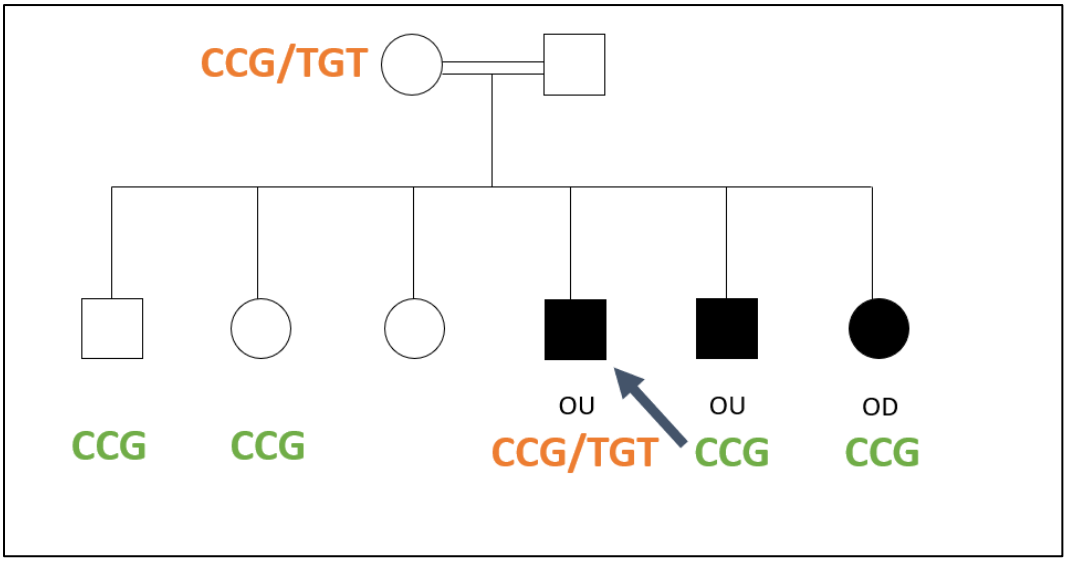

Figure 4.8.3 Pedigree of Family A showing heterozygous inheritance of the *CYP1B1* haplotype block.

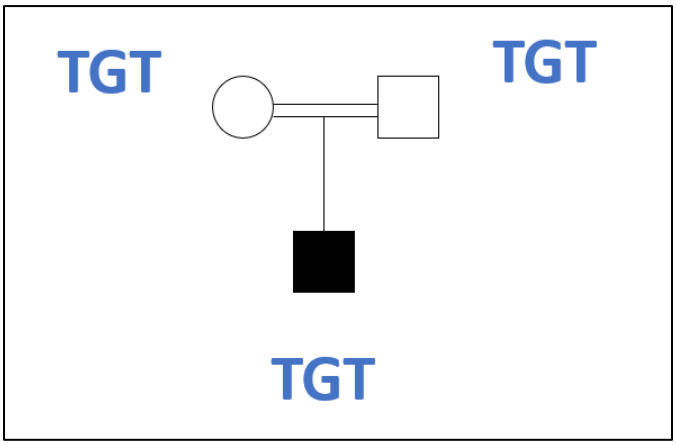

Figure 4.8.4 Pedigree of Family B showing homozygous inheritance of the *CYP1B1* haplotype block.

4.3.1.4 rs587778875: c.862_863insC (p.Arg290Profs*37)

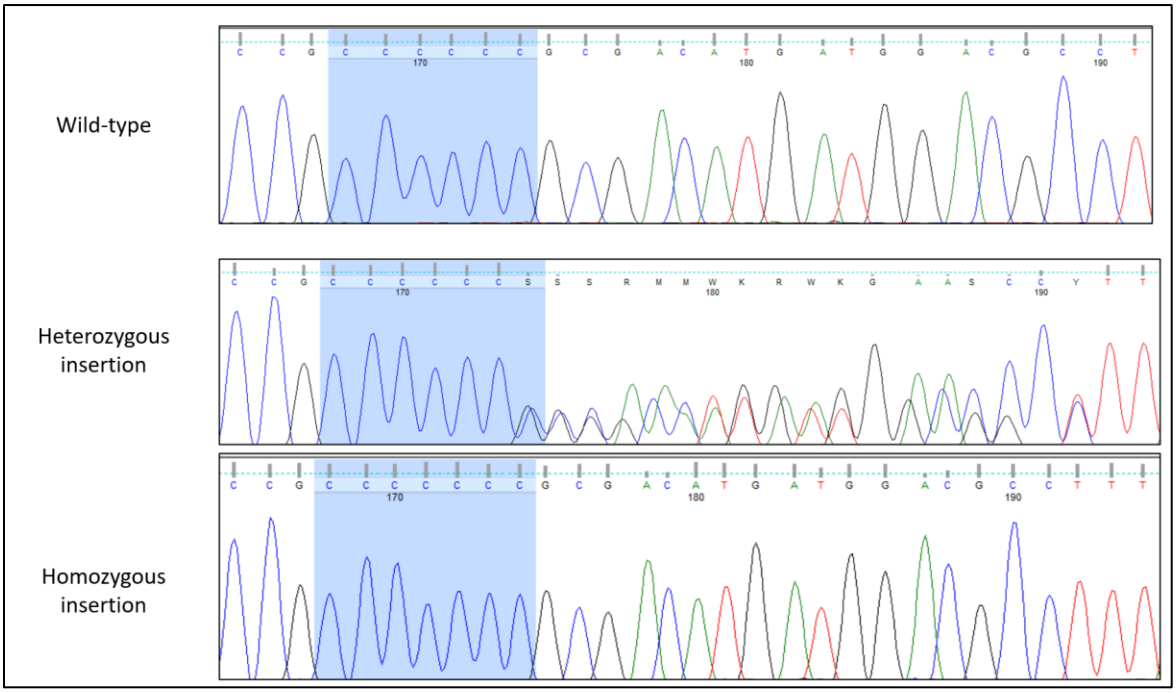

Figure 4.9.1 Nucleotide sequencing results showing the c.862_863insC mutant variant; top homozygous wild-type, middle (-/C) heterozygous insertion, bottom (-/C) homozygous insertion.

This variant results in a frameshift mutation. Arginine residue at position 290 is altered to proline, followed by a premature stop at codon 37 downstream. This leads to the production of a truncated CYP1B1 protein (Figure 4.9.2). This likely results in a non-functional CYP1B1 protein due to the loss of essential enzymatic regions.

This variant was detected in four patients (two homozygous, two heterozygous) and is extremely rare in gnomAD (allele frequency: 0.00001990). Computational predictions strongly support its pathogenicity. MutationTaster classifies it as disease-causing, while a high CADD score (31.0) suggests significant functional impact. The phyloP score (8.89) indicates strong evolutionary conservation, reinforcing its importance.

Table 4.7 Prediction Tools Assessing *CYP1B1*: c.862_863insC Variant

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
Score	Disease Causing	-	-	31.0	0.00	8.89

wildtype AA sequence	MGTSLSPNDP WPLNPLSIQQ TTLLLLLSVL ATVHVGQRLL RQRRRQLRSA PPGPFAWPLI GNAAAVGQAA HLSFARLARR YGDVFQIRLG SCPIVVLNGE RAIHQALVQQ GSAFADRPAF ASFRVVSGGR SMAFGHYSEH WKVQRRAAHS MMRNFFTRQP RSRQVLEGHV LSEARELVAL LVRGSADGAF LDPRPLTVVA VANVMSAVCF GCRYSHDDPE FRELLSHNEE FGRTVGAGSL VDVMPWLQYF PNPVRTVFRE FEQLNRNFSN FILDKFLRHC ESLRPGAAPR DMMDAFILSA EKKAAGDSHG GGARLDLENV PATITDIFGA SQDTLSTALQ WLLLLFTRYP DVQTRVQAEL DQVVGRDRLP CMGDQPNLPY VLAFLYEAMR FSSFVPVTIP HATTANTSVL GYHIPKDTVV FVNQWSVNHD PVKWPNPENF DPARFLDKDG LINKDLTSRV MIFSVGKRRC IGEELSKMQL FLFISILAHQ CDFRANPNEP AKMNFSYGLT IKPKSFKVNV TLRESMELLD SAVQNLQAKE TCQ*
mutated AA sequence	MGTSLSPNDP WPLNPLSIQQ TTLLLLLSVL ATVHVGQRLL RQRRRQLRSA PPGPFAWPLI GNAAAVGQAA HLSFARLARR YGDVFQIRLG SCPIVVLNGE RAIHQALVQQ GSAFADRPAF ASFRVVSGGR SMAFGHYSEH WKVQRRAAHS MMRNFFTRQP RSRQVLEGHV LSEARELVAL LVRGSADGAF LDPRPLTVVA VANVMSAVCF GCRYSHDDPE FRELLSHNEE FGRTVGAGSL VDVMPWLQYF PNPVRTVFRE FEQLNRNFSN FILDKFLRHC ESLRPGAAPP RHDGRLYPLC GKEGGRGLAR WWRAAGFGER TGHYH*

Figure 4.9.2 Amino acid sequence comparison between the wild-type and truncated c.862_863insC mutant. The insertion mutation results in a premature stop codon, leading to a truncated protein. Image retrieved from MutationTaster.

4.3.1.5 rs771076928: c.534_534delG (p.Ala179Argfs*18)

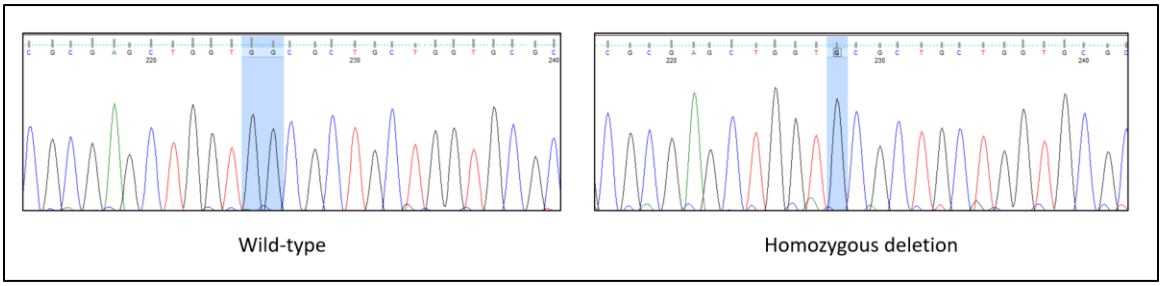

Figure 4.10.1 Nucleotide sequencing results showing the c.534_534delG mutant variant; left homozygous wild-type, right (G/-) homozygous deletion.

This variant is a frameshift mutation that introduces a premature termination codon at position 196, significantly truncating the protein compared to the wild-type length of 544 amino acids. This loss of essential functional domains likely results in a non-functional protein.

The mutation was identified in a single affected family within the cohort. Segregation analysis confirmed that both parents were heterozygous carriers of the variant. The affected child was homozygous for the mutation, while an unaffected sibling was homozygous for the wild-type allele, providing strong evidence for autosomal recessive inheritance consistent with *CYP1B1*-associated primary congenital glaucoma (Figure 4.10.3)

Multiple submissions on ClinVar classify this variant as pathogenic. Population data from gnomAD indicates that it is extremely rare, with an allele frequency of 0.00005118. Computational predictions also support its pathogenicity; MutationTaster classifies it as disease-causing, and the CADD score of 23.5 suggests a significant functional impact. However, the phyloP conservation score of 0.536 indicates moderate evolutionary conservation at this site.

Table 4.8 Prediction Tools Assessing *CYP1B1*: c.534_534delG Variant

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
Score	Disease Causing	-	-	23.5	0.00	0.536

wildtype AA sequence	MGTSLSPNDP WPLNPLSIQQ TTLLLLLSVL ATVHVGQRLL RQRRRQLRSA PPGPFAWPLI GNAAAVGQAA HLSFARLARR YGDVFQIRLG SCPIVVLNGE RAIHQALVQQ GSAFADRPAF ASFRVVSGGR SMAFGHYSEH WKVQRRAAHS MMRNFFTRQP RSRQVLEGHV LSEARELVAL LVRGSADGAF LDPRPLTVVA VANVMSAVCF GCRYSHDDPE FRELLSHNEE FGRTVGAGSL VDVMPWLQYF PNPVRTVFRE FEQLNRNFSN FILDKFLRHC ESLRPGAAPR DMMDAFILSA EKKAAGDSHG GGARLDLENV PATITDIFGA SQDTLSTALQ WLLLLFTRYP DVQTRVQAEL DQVVGRDRLP CMGDQPNLPY VLAFLYEAMR FSSFVPVTIP HATTANTSVL GYHIPKDTVV FVNQWSVNHD PVKWPNPENF DPARFLDKDG LINKDLTSRV MIFSVGKRRC IGEELSKMQL FLFISILAHQ CDFRANPNEP AKMNFSYGLT IKPKSFKVNV TLRESMELLD SAVQNLQAKE TCQ*
mutated AA sequence	MGTSLSPNDP WPLNPLSIQQ TTLLLLLSVL ATVHVGQRLL RQRRRQLRSA PPGPFAWPLI GNAAAVGQAA HLSFARLARR YGDVFQIRLG SCPIVVLNGE RAIHQALVQQ GSAFADRPAF ASFRVVSGGR SMAFGHYSEH WKVQRRAAHS MMRNFFTRQP RSRQVLEGHV LSEARELVRC WCAAARTAPS STRGR*

Figure 4.10.2 Amino Acid sequence comparison between the wild-type and truncated mutant. The insertion mutation results in a premature stop codon, leading to a truncated protein. Image retrieved from MutationTaster.

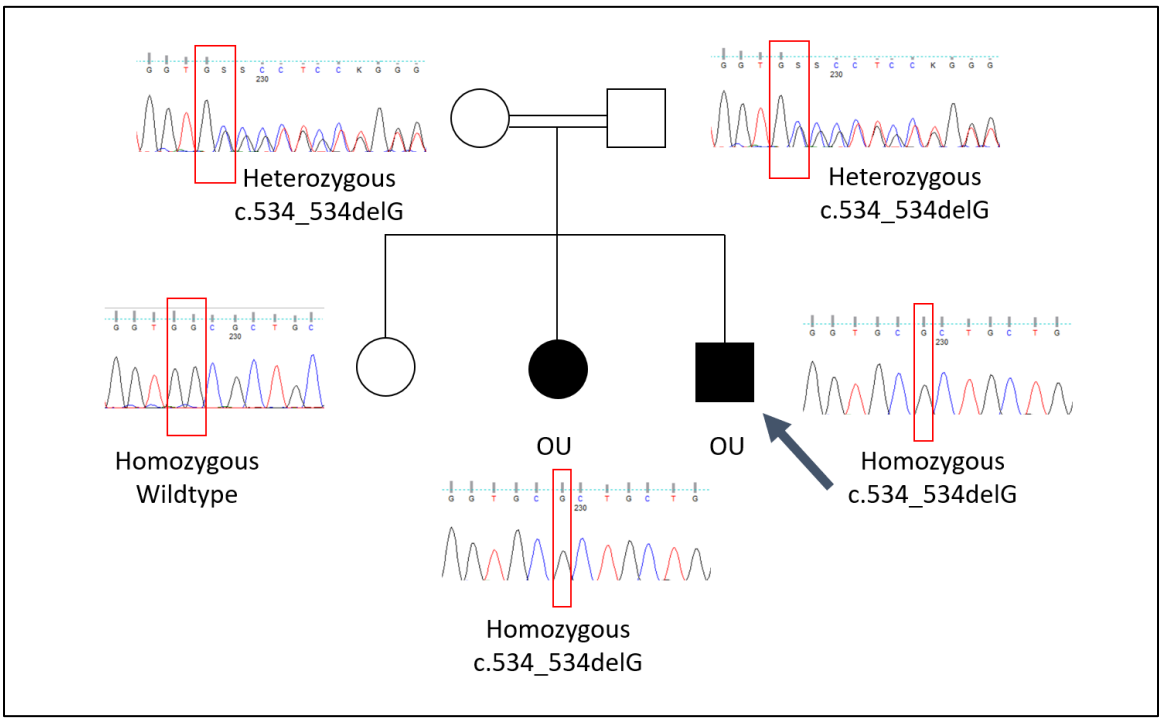

Figure 4.10.3 Pedigree of a family segregating the CYP1B1 frameshift mutation leading to a premature stop codon at position 196.Both parents are heterozygous carriers of the variant.

4.3.2 Exon 3

Six variants identified in exon 3 of the *CYP1B1* gene.

- **rs79204362: c.1103G>A (p.Arg368His)**
- **rs1056836: c.1294C>G (p.Leu432Val)**
- **rs56175199: c.1310C>T (p.Pro437Leu)**
- **rs1056837: c.1347T>C (p.Asp449=)**
- **rs1800440: c.1358A>G (p.Asn453Ser)**
- **rs28936701: c.1405C>T (p.Arg469Trp)**

4.3.2.1 Sanger Sequencing Results

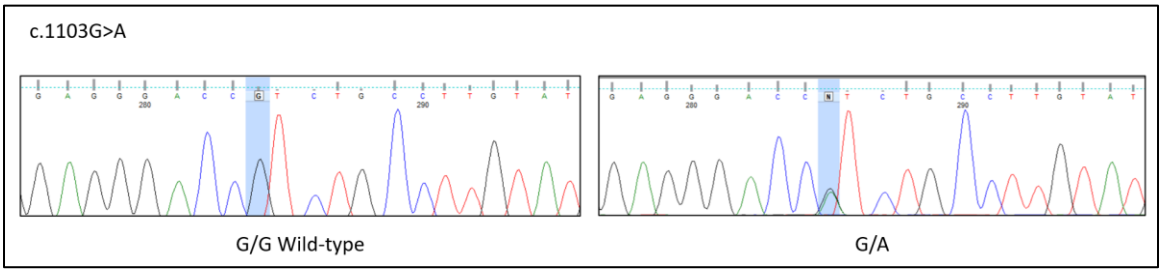

Figure 4.11.1 Nucleotide sequencing results showing the c.1103G>A mutant variant; left homozygous wild-type (G/G), right heterozygous (G/A)

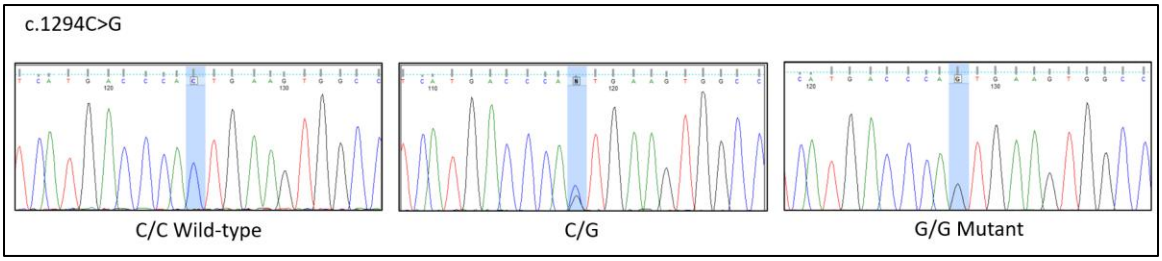

Figure 4.11.2 Nucleotide sequencing results showing the c.1294C>G mutant variant; left homozygous wild-type (C/C), middle heterozygous (C/G), right homozygous mutant (G/G)

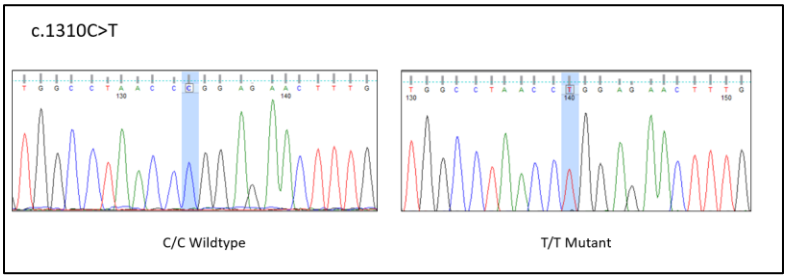

Figure 4.11.3 Nucleotide sequencing results showing the c.1310C>T mutant variant; left homozygous wild-type (C/C), right homozygous mutant (T/T)

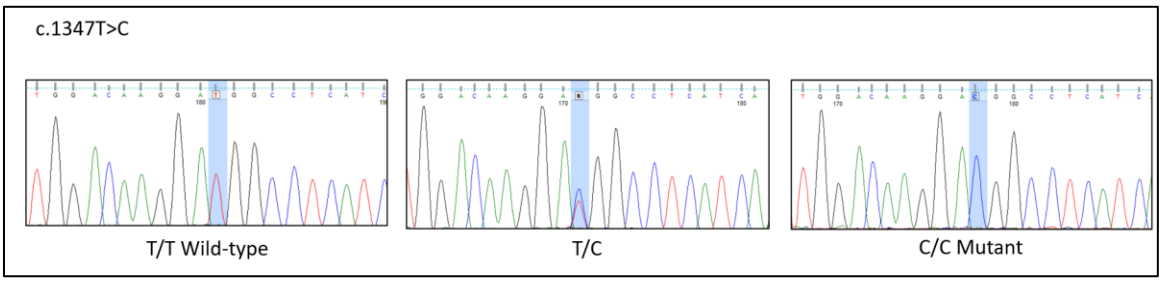

Figure 4.11.4 Nucleotide sequencing results showing the c.1347T>C mutant variant; left homozygous wild-type (T/T), middle heterozygous (T/C), right homozygous mutant (C/C)

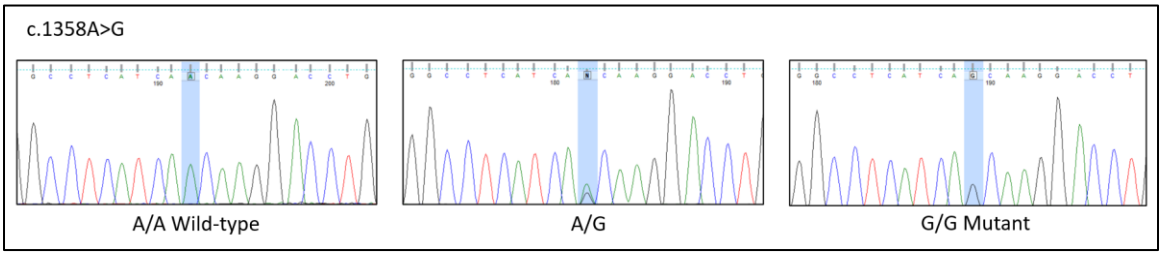

Figure 4.11.5 Nucleotide sequencing results showing c.1358A>G mutant variant; left homozygous wild-type (A/A), middle heterozygous (A/G), right homozygous mutant (G/G)

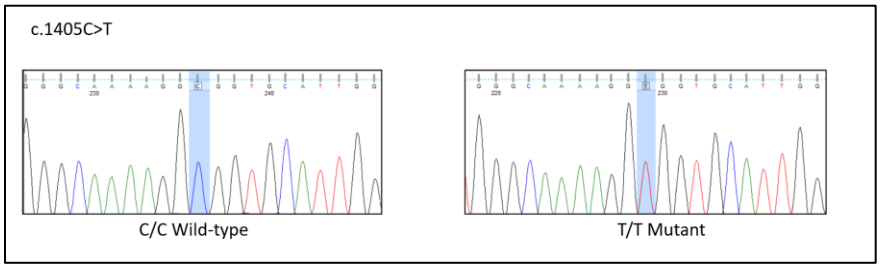

Figure 4.11.6 Nucleotide sequencing results showing the c.1405C>T mutant variant; left homozygous wild-type (C/C), right homozygous mutant (T/T)

4.3.2.2 Assessment of variants via prediction tools

The computational tools predictions suggest that c.1103G>A, c.1310C>T and c.1405C>T are likely deleterious, while c.1294C>G and c.1347T>C appear benign. The c.1358A>G variant shows mixed predictions, indicating potential significance as shown in Table 4.12.

4.3.2.2.1 rs79204362: c.1103G>A (p.Arg368His)

This missense variant results in the substitution of arginine with histidine at position 368, occurring in a functionally important region of the enzyme, impacting its structural integrity and catalytic activity. It was identified in three heterozygous individuals out of 30 in this study. Its gnomAD allele frequency (0.005272) suggests it is rare but not exclusive to disease populations. ClinVar submissions show conflicting interpretations ranging from pathogenic to uncertain significance (VUS). Computational tools strongly suggest a deleterious effect, with MutationTaster predicting it as disease-causing, SIFT classifying it as deleterious, and PolyPhen giving a high damaging probability score (0.992). The CADD score of 28.9 and phyloP score of 8.82 indicate high conservation and a strong likelihood of pathogenicity.

4.3.2.2.2 rs1056836: c.1294C>G (p.Leu432Val)

This missense variant results in a leucine-to-valine substitution at codon 432. It was found in 14 homozygous and 7 heterozygous individuals out of 30 patients. Its gnomAD allele frequency (0.4247) indicates it is highly prevalent in the general population. Computational tools classify it as benign, with MutationTaster identifying it as a polymorphism, SIFT labeling it tolerated, and PolyPhen categorizing it as benign. The CADD score of 10.1 suggests minimal functional impact.

4.3.2.2.3 rs56175199: c.1310C>T (p.Pro437Leu)

This missense mutation was identified in one patient in this study in a homozygous form. It has been reported in ClinVar with five likely pathogenic submissions and has a very low gnomAD allele frequency of 0.00002107. Computational tools strongly suggest a deleterious effect, with MutationTaster predicting it as disease-causing, SIFT classifying it as deleterious, and PolyPhen giving a high damaging probability score (1.00). The CADD score of 25.9 and phyloP score of 8.71 indicate high conservation and a strong likelihood of pathogenicity.

4.3.2.2.4 rs1056837: c.1347T>C (p.Asp449=)

This is a synonymous substitution. It does not alter the amino acid sequence. It was found in 8 homozygous and 5 heterozygous individuals out of 30 patients. Its gnomAD allele frequency (0.5771) suggests it is a common polymorphism rather than a rare disease-associated mutation. ClinVar classifies it as benign, supported by six submissions. Computational tools confirm its neutral impact, with MutationTaster identifying it as a polymorphism, SIFT and PolyPhen predicting it as tolerated/benign, and a low CADD score (3.60). The phyloP score of -3.94 indicates poor evolutionary conservation.

4.3.2.2.5 rs1800440: c.1358A>G (p.Asn453Ser)

This missense mutation was identified in four heterozygous and three homozygous individuals in this study. It has been reported in ClinVar with six benign submissions and has a gnomAD allele frequency of 0.1711. Prediction tools provide mixed assessments; MutationTaster classifies it as a polymorphism, SIFT as deleterious, and PolyPhen as possibly damaging. The CADD score of 24.8 suggests potential functional impact, while the phyloP score of 4.69 indicates moderate evolutionary conservation.

4.3.2.2.6 rs28936701: c.1405C>T (p.Arg469Trp)

This missense variant results in the substitution of arginine with tryptophan at position 469, located in a critical functional domain of CYP1B1. It was identified in three heterozygous individuals in this study and has a gnomAD allele frequency of 0.00004151, indicating extreme rarity. Computational tools consistently predict it as deleterious, with SIFT (deleterious), PolyPhen (probably damaging), and a high CADD score (27.7). The phyloP score of 4.07 supports evolutionary conservation. ClinVar classifies it as pathogenic, with five supporting submissions.

4.3.2.3 Variant co-occurrence among patients

Analysis of variant co-occurrence among patients revealed distinct patterns of inheritance. Table 4.13 summarizes the distribution of these variants across the patient cohort. Among the identified variants, c.1294C>G (p.Leu432Val) and c.1347T>C (p.Asp449=) were the most frequent, appearing in both homozygous and heterozygous states. Their high prevalence suggests that they are common polymorphisms within the population. In contrast, c.1103G>A (p.Arg368His) was rare, detected only in heterozygous form in three patients. c.1405C>T (p.Arg469Trp) was observed in three different individuals as well.

Patterns of co-occurrence were also evident among certain variants. Specifically, patients G05, G07, and G23 carried both c.1294C>G and c.1347T>C, while G19, G20, and G29 shared c.1103G>A and c.1294C>G.

These findings indicate that some variants segregate together more frequently than expected, suggesting possible haplotype blocks.

Table 4.9 Prediction Tools Assessing the six *CYP1B1* Variants in Exon 3

Tool	MutationTaster	SIFT	PolyPhen	CADD	SpliceAI	phyloP
c.1103G>A	Disease causing	DELETERIOUS	POSSIBLY DAMAGING	28.9	0.00	8.82
c.1294C>G	Polymorphism	TOLERATED	BENIGN	10.1	0.00	2.04
c.1310C>T	Disease causing	DELETERIOUS	POSSIBLY DAMAGING	25.9	0.00	8.71
c.1347T>C	Polymorphism	TOLERATED	BENIGN	3.60	0.00	-3.94
c.1358A>G	Polymorphism	DELETERIOUS	POSSIBLY DAMAGING	24.8	0.00	4.69
c.1405C>T	Disease causing	DELETERIOUS	POSSIBLY DAMAGING	27.7	0.02	4.07

4.3.3 Intronic Variants

4.3.3.1 Intron 1 - Novel 2: g.38444_38445insT, c.1044-148dup

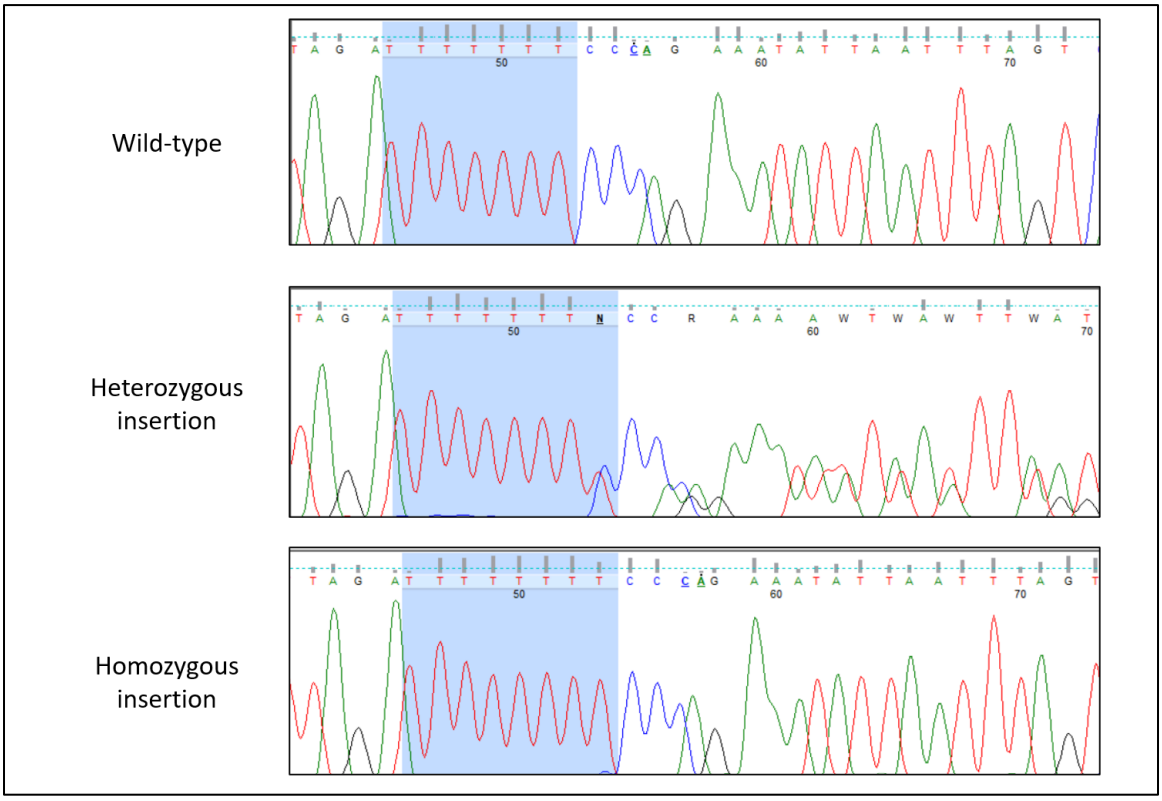

Figure 4.12 Nucleotide sequencing results showing the g.38444_38445insT mutant variant; top homozygous wild-type, middle (-/T) heterozygous insertion, bottom (-/T) homozygous insertion.

This intronic variant was identified in 16 homozygous and 8 heterozygous individuals out of a total of 46 screened subjects. The variant is located within intron 2 of CYP1B1 and does not directly alter the amino acid sequence of the encoded protein. However, its presence in the intronic region may influence splicing efficiency or gene regulation. Notably, there are four AGG sequences before exon 2, which could potentially affect splice site recognition or introduce a cryptic splice site. Given its location 148 bp from the exon-intron boundary, it may disrupt splicing enhancers or silencers, leading to aberrant mRNA transcripts. Further functional validation, such as RNA analysis (RT-PCR) or minigene splicing assays, would be necessary to determine the precise impact of this variant on CYP1B1 mRNA processing and expression.

4.3.3.2 Intron 2

4.3.3.2.1 rs162562: c.*350C>A

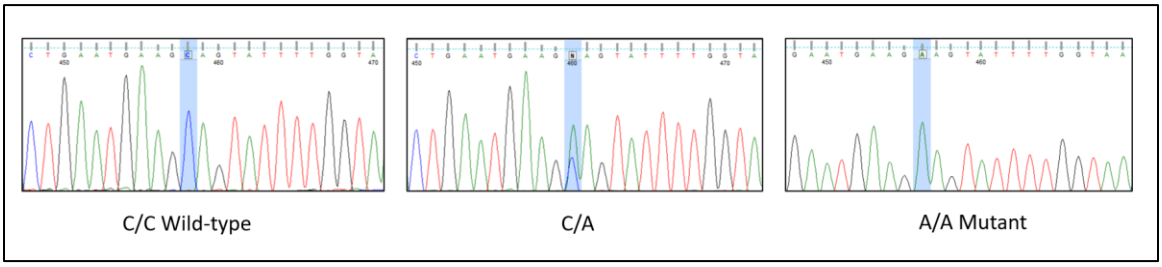

Figure 4.13 Nucleotide sequencing results showing the c.*350C>A mutant variant; left homozygous wild-type (C/C), middle heterozygous (C/A), right homozygous mutant (A/A)

The variant *c.350C>A is located in the 3' untranslated region (3' UTR) of the CYP1B1 gene. This variant was observed in 14 homozygous and 8 heterozygous individuals in this study. According to gnomAD, it has a relatively high allele frequency of 0.7362, indicating that it is a common variant in the general population.

Two submissions on ClinVar classify it as benign. Given its location in the 3' UTR, this variant may have potential effects on gene regulation, mRNA stability, or protein translation efficiency, though no direct functional consequences have been established.

4.3.3.2.2 rs4646431: c.*210dup

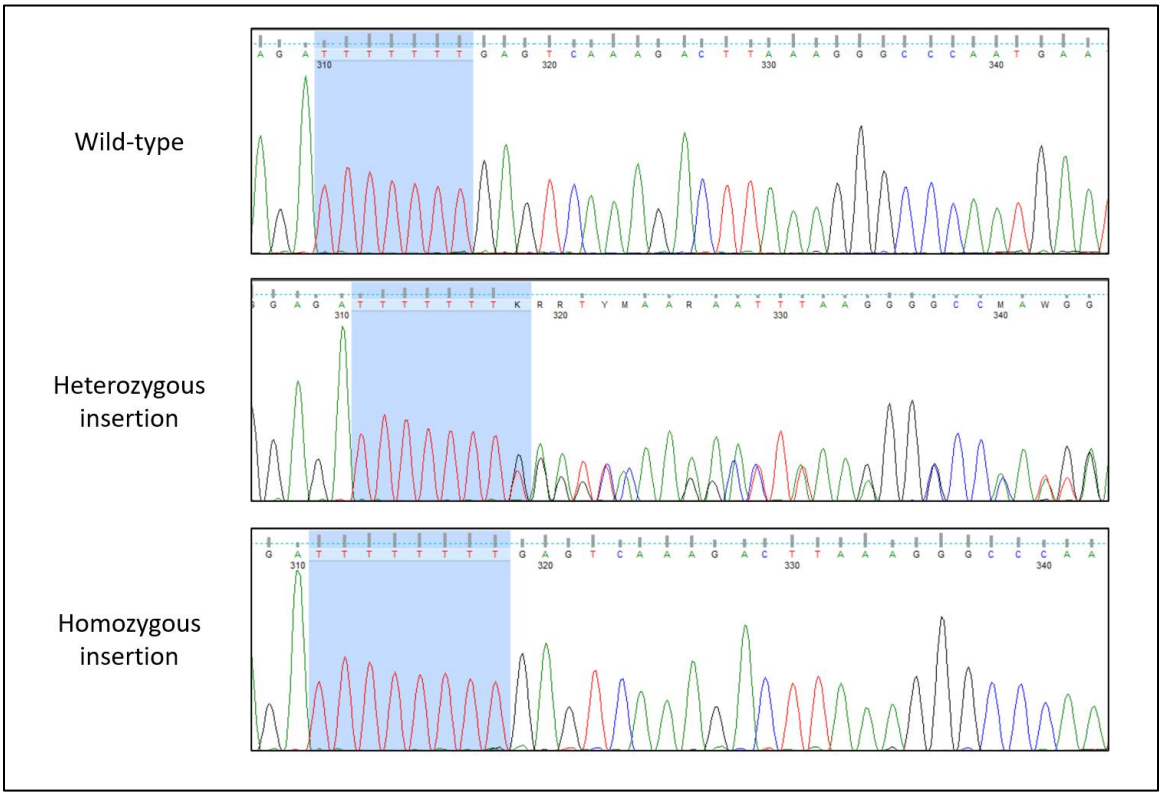

Figure 4.14 Nucleotide sequencing results showing the c.*210dup mutant variant; top homozygous wild-type, middle (-/T) heterozygous insertion, bottom (-/T) homozygous insertion.

The variant *c.210dup represents a duplication of a nucleotide within the 3' untranslated region (3' UTR), located 210 nucleotides downstream from the stop codon. This variant has the potential to influence post-transcriptional regulation of the gene, as the 3' UTR contains elements responsible for mRNA stability, localization, and translation efficiency. Alterations in this region could affect these regulatory mechanisms.

This variant was identified in 7 homozygous and 3 heterozygous individuals in this study. According to gnomAD, the allele frequency of this variant is 0.5218, suggesting it is relatively common. ClinVar contains three independent submissions classifying it as benign, indicating that it is unlikely to have a significant impact on gene function or disease susceptibility.

Table 4.10 Summary of variants identified in the *MYOC* gene, including their location, type, frequency in the study cohort, presence in families, and reported pathogenicity.

Location	Genomic Position	cDNA	Amino Acid	Mutation Type	Variant ID	gnomAD	Number in Cohort			Pathogenicity
							Frequency	Homozygous	Heterozygous	
Exon 1	chr1:171621713A>C	c.39T>G	p.Pro13=	SNP	rs12082573	**0.004048**	46	-	1	**Benign**
Exon 1	chr1:171621525C>T	c.227G>A	p.Arg76Lys	SNP	rs2234926	0.1383	46	5	9	**Benign**
Exon 1	chr1:171621275T>C	c.477A>G	p.Leu159=	SNP	rs61730977	**0.004044**	46	-	1	**Benign**
Intron 1	chr1:171649526 G>A	c.730+35G>A	-	SNP	rs2032555	0.7220	19	12	4	**Benign**

Table 4.11 Summary of variants identified in the *CYP1B1* gene, including their location, type, frequency in the study cohort, presence in families, and reported pathogenicity.

Location	Genomic Position	cDNA	Amino Acid	Mutation Type	Variant ID	gnomAD	Number in Cohort			Pathogenicity
							Frequency	Homozygous	Heterozygous	
Intron 1	chr2:38302544G>A	c.-1-12C>T	-	SNP	rs2617266	0.2923	46	4	4	**Benign**
Exon 2	chr2:38302390G>C	c.142C>G	p.Arg48Gly	SNP	rs10012	0.3189	46	4	4	**Benign**
Exon 2	chr2:38302177C>A	c.355G>T	p.Ala119Ser	SNP	rs1056827	0.3235	46	4	4	**Benign**
Exon 2	chr2:38302350C>T	c.182G>A	p.Gly61Glu	SNP	rs28936700	**0.00029**	46	9	1	**Pathogenic**
Exon 2	chr2:38301998_38301998delC	c.534_534delG	p.Ala179Argfs*18	Deletion	rs771076928	**0.000048**	46	1	-	**Pathogenic**
Exon 2	chr2:38301676_38301676delC	c.856_856delG	p.Ala287Profs*6	Deletion	**Novel 1**	NA	46	3	1	**Pathogenic**
Exon 2	chr2:38301669_38301670insG	c.862_863insC	p.Arg290Profs*37	Insertion	rs587778875	**0.00002**	46	2	2	**Pathogenic**
Intron 2	chr2:38298600_38298601insA	g.38444_38445insT	-	Insertion	**Novel 2**	NA	46	16	8	**Benign**
Exon 3	chr2:38298394C>T	c.1103G>A	p.Arg368His	SNP	rs79204362	**0.005272**	46	-	3	**VUS**
Exon 3	chr2:38298203C>G	c.1294C>G	p.Leu432Val	SNP	rs1056836	0.6172	46	22	8	**Benign**
Exon 3	chr2:38071044G>A	c.1310C>T	p.Pro437Leu	SNP	rs56175199	**0.000021**	46	1	-	**Pathogenic**
Exon 3	chr2:38298150A>G	c.1347T>C	p.Asp449Asp	SNP	rs1056837	0.5771	46	9	6	**Benign**
Exon 3	chr2:38298139T>C	c.1358A>G	p.Asn453Ser	SNP	rs1800440	0.1495	46	6	3	**VUS**
Exon 3	chr2:38298092G>A	c.1405C>T	p.Arg469Trp	SNP	rs28936701	**0.000047**	46	3	-	**Pathogenic**
3'UTR	chr2:38297515G>T	c.*350C>A	-	SNP	rs162562	0.6787	46	14	8	**Benign**
3'UTR	chr2:38297661_38297662insA	c.*210dup	-	Insertion	rs4646431	0.5218	46	7	3	**Benign**

Table 4.12 Detected CYP1B1 and MYOC gene variants across all patient samples. Genotypes for each variant are shown per patient. Insertions (ins), deletions (del), and heterozygous deletions (del (het)) are indicated where applicable. Pathogenic and known variants are highlighted in color for clarity.

Sample		CYP1B1															MYOC			
	c.-1-12C>T	c.355G>T	c.142C>G	c.182G>A	c.534_534delG	c.856_856delG	c.862_863insC	g.38444_38445insT	c.1103G>A	c.1294C>G	c.1310C>T	c.1347T>C	c.1358A>G	c.1405C>T	c.*350C>A	c.*210dup	c.227G>A	c.39T>G	c.477A>G	c.730+35G>A
G01	C/C	G/G	C/C	**A/A**				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		**G/A**	T/T	A/A	A/A
G02	**T/T**	**T/T**	**G/G**	G/G		**del**			G/G	C/C	C/C	**C/C**	A/A	C/C	X	X	G/G	T/T	A/A	**G/G**
G03	C/C	G/G	C/C	G/G			**ins**		G/G	**G/G**	C/C	T/T	A/A	C/C	X	X	G/G	T/T	A/A	A/A
G04	**T/T**	**T/T**	**G/G**	G/G		**del (het)**			G/G	C/C	C/C	T/T	**G/G**	C/C	X	X	G/G	T/T	A/A	**G/A**
G05	C/C	G/G	C/C	G/G				**ins**	G/G	**C/G**	C/C	**T/C**	**A/G**	C/C	C/C	**Ins (het)**	G/G	T/T	A/A	**G/A**
G06	C/C	G/G	C/C	G/G				**ins**	G/G	**C/G**	C/C	**T/C**	A/A	C/C	C/C	**Ins (het)**	G/G	T/T	A/A	A/A
G07	C/C	G/G	C/C	G/G				**ins**	G/G	**C/G**	C/C	**T/C**	**A/G**	C/C	C/C	**Ins (het)**	G/G	T/T	A/A	**G/A**
G08	C/C	G/G	C/C	G/G				**ins**	G/G	C/C	C/C	**C/C**	**G/G**	C/C	**A/A**	**ins**	G/G	T/T	A/A	A/A
G09	C/C	G/G	C/C	G/G					G/G	**G/G**	C/C	T/T	A/A	**T/T**	**A/A**		G/G	T/T	A/A	A/A
G10	C/C	G/G	C/C	**A/A**				**ins**	G/G	C/C	C/C	T/T	A/A	C/C	**A/A**		**A/A**	T/T	A/A	A/A
G11	**T/T**	**T/T**	**G/G**	G/G		**del**		**ins**	G/G	C/C	C/C	T/T	**G/G**	C/C	X	X	**G/A**	T/T	A/A	**G/G**
G12	C/C	G/G	C/C	G/G				**ins**	G/G	C/C	C/C	T/T	A/A	C/C	X	X	G/G	**G/G**	**G/G**	**G/G**
G13	C/C	G/G	C/C	G/G			**ins**		G/G	**G/G**	C/C	T/T	A/A	C/C	C/C		**G/A**	T/T	A/A	A/A
G14	C/C	G/G	C/C	**A/A**					G/G	**G/G**	C/C	T/T	A/A	C/C	X	X	G/G	T/T	A/A	A/A
G15	C/C	G/G	C/C	G/G					G/G	**G/G**	C/C	T/T	A/A	C/C	X	X	**G/A**	T/T	A/A	A/A
G16	C/C	G/G	C/C	**A/A**				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		**A/A**	T/T	A/A	A/A

G17	C/C	G/G	C/C	**A/A**					G/G	**G/G**	C/C	T/T	A/A	C/C	X	X	**A/A**	T/T	A/A	A/A
G18	C/C	G/G	C/C	G/G				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	X	X	**A/A**	T/T	A/A	**G/A**
G19	C/C	G/G	C/C	G/G				**ins**	**G/A**	**G/G**	C/C	T/T	A/A	C/C	**C/A**		G/G	T/T	A/A	A/A
G20	C/C	G/G	C/C	**G/A**				**ins**	**G/A**	**C/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
G21	**C/T**	**G/T**	**C/G**	G/G			**ins (het)**		G/G	**C/G**	C/C	**T/C**	A/A	C/C	X	X	G/G	T/T	A/A	X
G22	C/C	G/G	C/C	G/G					G/G	C/C	C/C	**C/C**	A/A	C/C	**A/A**	**ins**	G/G	T/T	A/A	X
G23	C/C	G/G	C/C	G/G					G/G	**C/G**	C/C	**T/C**	**A/G**	C/C	C/C		**G/A**	T/T	A/A	X
G24	C/C	G/G	C/C	G/G					G/G	C/C	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
G25	C/C	G/G	C/C	G/G				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		**A/A**	T/T	A/A	X
G26	C/C	G/G	C/C	**A/A**				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
G27	C/C	G/G	C/C	G/G					G/G	**C/G**	C/C	**C/C**	A/A	C/C	**A/A**	**ins**	G/G	T/T	A/A	X
G28	C/C	G/G	C/C	G/G			**ins (het)**	**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	X	X	G/G	T/T	A/A	X
G29	C/C	G/G	C/C	G/G				**ins**	**G/A**	**G/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
G30	**C/T**	**G/T**	**C/G**	G/G					G/G	C/C	C/C	**C/C**	A/A	C/C	**A/A**	**ins**	G/G	T/T	A/A	X
G31	C/C	G/G	C/C	G/G				**ins**	G/G	**G/G**	**T/T**	T/T	A/A	C/C	X	X	G/G	T/T	A/A	X
G32	C/C	G/G	C/C	G/G				**ins**	G/G	**G/G**	C/C	T/T	A/A	**T/T**	**A/A**		**G/A**	T/T	A/A	X
G33	**C/T**	**G/T**	**C/G**	G/G				**ins**	G/G	**C/G**	C/C	**T/C**	A/A	C/C	**A/A**		G/G	T/T	A/A	X
G34	C/C	G/G	C/C	**A/A**				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
G35	C/C	G/G	C/C	G/G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
G36	C/C	G/G	C/C	G/G				**ins**	G/G	C/C	C/C	T/T	A/A	C/C	X	X	G/G	T/T	A/A	X
G37	C/C	G/G	C/C	**A/A**				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
G38	C/C	G/G	C/C	G/G				**ins**	G/G	C/C	C/C	T/T	A/A	**T/T**	**A/A**		G/G	T/T	A/A	X
G39	C/C	G/G	C/C	G/G					G/G	C/C	C/C	**C/C**	**G/G**	C/C	**A/A**	**ins**	G/G	T/T	A/A	X
G40	C/C	G/G	C/C	G/G					G/G	C/C	C/C	**C/C**	**G/G**	C/C	**A/A**	**ins**	G/G	T/T	A/A	X
D1	C/C	G/G	C/C	G/G				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
JS1	C/C	G/G	C/C	G/G	**del**				G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
N6	C/C	G/G	C/C	**A/A**				**ins**	G/G	**G/G**	C/C	T/T	A/A	C/C	**A/A**		G/G	T/T	A/A	X
R2	**C/T**	**G/T**	**C/G**	G/G					G/G	C/C	C/C	**C/C**	**G/G**	C/C	**A/A**	**ins**	G/G	T/T	A/A	X
OB1	**T/T**	**T/T**	**G/G**	G/G					G/G	C/C	C/C	**C/C**	A/A	C/C	**A/A**		**G/A**	T/T	A/A	X

Chapter Five: Discussion

Primary congenital glaucoma (PCG) is a serious eye condition that manifests at birth and can cause permanent irreversible blindness if not treated in time. In Palestine, PCG is more common than in many other countries, mainly due to the high rate of consanguineous marriages. Unfortunately, PCG in Palestine can only be diagnosed after birth, and in some cases, children are not diagnosed until it is too late for effective treatment. Moreover, in Palestine, surgery is currently the only available treatment, and it is most beneficial when performed early. Thus, early diagnosis is extremely important, which could be aided by understanding the molecular genetics of PCG.

PCG not only affects the child's vision and quality of life but also places a significant emotional and financial burden on their families. This pressure is especially heavy in low-income and resource-limited settings. Understanding the causes of PCG through research is therefore essential as it offers hope for affected children and helps prevent future cases. So far, the exact genetic causes of PCG in Palestine remain unclear, and this research sets a foot into unraveling the genetic causes of this rare disease. Genetic testing could play a vital role in identifying the disease earlier, guiding treatment and lifestyle decisions, and providing families with important information through genetic counseling.

The purpose of this study was to investigate the *MYOC* and *CYP1B1* gene variants that contribute to PCG phenotype in a cohort of Palestinian patients. Patient recruitment was a critical component of this study and was achieved through close collaboration with glaucoma-specialist ophthalmologists.

In total, forty-six PCG patients, both sporadic and familial cases, were recruited for this study. However, recruitment was limited to patients referred by Dr. Amer Mohsen and Dr. Sana Muhsen, which may have introduced selection bias or excluded undiagnosed cases, particularly in remote or underserved areas. For example, one familial case was identified through outreach to a school for the blind, where two brothers and a sister diagnosed with PCG at birth had never sought medical treatment, surgical intervention, or regular check-ups. Additionally, logistical and social challenges, especially in reaching

rural areas and contacting extended family members, restricted the collection of samples in some familial cases.

Despite these limitations, the study successfully assembled the largest known PCG cohort in Palestine, providing a strong foundation for future genetic and clinical investigations.

5.1 *MYOC* Gene Variants in Palestinian Patients

Several studies in various populations showed that more than 100 pathogenic variants in the *MYOC* gene have been identified and associated with glaucoma, particularly POAG and JOAG. Several pathogenic *MYOC* variants have been previously reported in Iraqi patients (Jubair et al., 2019).

In this study, four *MYOC* variants were detected and all are classified as non-pathogenic. One patient exhibited a compound heterozygous state with the c.39T>G (p.Pro13=) and c.477A>G (p.Leu159=) variants, both are synonymous that do not alter the amino acid sequence. These variants have low gnomAD allele frequencies, indicating their rarity. Interestingly, a previous study in Morocco patients reported the two variants were detected together in six patients but found no evidence of forming defined haplotypes. These variants were reported to be more frequent in Moroccan and African American populations compared to Caucasians and Asians (Melki et al., 2003). Subsequent studies confirmed their prevalence as benign polymorphisms among African American and Black South African populations (Liu et al., 2012; Williams et al., 2015). Therefore, these two variants do not appear to be the cause of the disease, and their presense may indicate that the patient has African decents.

The c.227G>A (p.Arg76Lys) variant was identified in five patients in a homozygous form and nine patients in a heterozygous form. It is one of the most frequently reported *MYOC* variants in glaucoma patients, and mostly classified as a benign polymorphism (Bhattacharjee et al., 2007; Faucher et al., 2002; Narooie-Nejad et al., 2017). Functional analysis studies further support its benign nature. A study investigating 14 *MYOC* variants with varying degrees of predicted pathogenicity showed the R76K

(p.Arg76Lys) mutant was readily secreted and soluble, suggesting it does not disrupt *MYOC* protein function and is therefore likely benign (Nakahara & Hulleman, 2022).

Finally, the c.730+35G>A intronic variant was detected in 84% of the 19 patients analyzed. and predicted as benign. A study in a Malay population suggested its association with increased susceptibility to JOAG, showing a significant difference between affected and non-affected individuals (Mimivati et al., 2014), however, studies in Korean and African populations found no significant correlation between this variant with glaucoma (Kim et al., 2011; Whigham et al., 2011), suggesting its role in glaucoma susceptibility remains uncertain.

Overall, the *MYOC* variants identified in this study were all classified as non-pathogenic. The presence of c.39T>G (p.Pro13=) and c.477A>G (p.Leu159=) likely reflects African ancestry rather than contributing to disease. While c.227G>A (p.Arg76Lys) is evolutionarily conserved, its benign classification suggests minimal functional impact. The intronic variant c.730+35G>A lacks conclusive evidence of pathogenicity, with conflicting findings across different populations.

5.2 *CYP1B1* Gene Variants in Palestinian Patients

All the patients with primary congenital glaucoma were eventually screened for existence of *CYP1B1* gene variants. Twenty-two patients had homozygous pathogenic variants in exons 2 and 3, including a novel mutation. Nine patients had heterozygous *CYP1B1* pathogenic variants, whereas 15 patients showed wild type *CYP1B1* gene. Seven pathogenic variants could be identified: including one Novel, and six previously reported mutants.

One novel variant, the c.856_856delG (p.Ala287Profs*6), results in a frameshift mutation that introduces a premature stop codon six nucleotides after codon 287. This leads to a truncated protein and loss of function of the H-helix. The H-helix helps in maintaining the overall stability of the protein, and is located near the active site of the enzyme and plays a role in substrate positioning, potentially affecting ligand access and binding involved in metabolic processes (Bart et al., 2020).

Six previously reported variants including c.182G>A (p.Gly61Glu), c.868_869insC (p.Arg290Profs*37), c.534_534delG (p.Ala179Argfs*18), c.1103G>A (p.Arg368His), c.1310C>T (p.Pro437Leu) and c.1405C>T (p.Arg469Trp) were detected. Among them, the c.182G>A (p.Gly61Glu) was the most prevalent. This variant was detected in 10 patients, nine in a homozygous genotype and one heterozygous. The patient with heterozygous c.182G>A also presented with the variant c.1103G>A in heterozygous form, indicating a compound heterozygous form of inheritance. Two patients represent to familial cases with other affected members. This variant has been frequently reported in several Arab populations (Jemmeih et al., 2022), including Morocco, Egypt, Saudi Arabia, Iraq, and Iran (Badeeb et al., 2014; Belmouden et al., 2002; Chitsazian et al., 2007; Fassad et al., 2017; Jubair et al., 2020; Qashqai et al., 2018). Additionally, it has been identified in Spanish (Campos-Mollo et al., 2009), Pakistani (Bashir et al., 2015), Turkish (Ava et al., 2021), and Israeli Arab Bedouin populations (Bar-Yosef et al., 2010). The c.182G>A variant is located in the hinge region of the CYP1B1 protein, where the substitution of glycine (a non-polar amino acid) with glutamine (a polar amino acid) alters the protein structure. Functional studies have demonstrated that this change significantly reduces estradiol- and retinoic acid-metabolizing activity, leading to an unstable mutant protein and impaired enzyme function (Banerjee et al., 2016; Jansson et al., 2001; López-Garrido et al., 2010). Moreover, a recent study linked this variant to decreased brain metabolism, progressive inflammation, and microglial dysfunction, potentially exacerbating PCG complications and influencing post-surgical recovery (Alghamdi et al., 2020)

The c.868_869insC (p.Arg290Profs*37) variant was detected in homozygous genotype in one patient and heterozygous genotype in two patients. This mutation results in a premature stop codon just before the H-helix, leading to protein truncation. One heterozygous patient has multiple affected family members, indicating the need for family segregation to understand the mode of inheritance of this variant, including the identification of the other potential mutation for the expected compound heterozygous genotype. This mutation has been reported in Pakistan, Australia (JOAG patients), Spain, and Turkey (Bagiyeva et al., 2007; López-Garrido et al., 2013a; Souzeau et al., 2015; Tehreem et al., 2022a).

The c.534_534delG (p.Ala179Argfs*18) variant, which was detected in a single familial case in this study, is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. It has been reported in Portuguese patients (Cardoso et al., 2015), a Portuguese patient residing in Switzerland (Lang et al., 2020), in Tunisia (Bouyacoub et al., 2014), Morocco (Belmouden et al., 2002), and Moroccans residing in Denmark (Grønskov et al., 2016). In Morocco, this variant is considered a founder mutation.

The c.1103G>A (p.Arg368His) variant was identified in three heterozygous patients in this study, none of which had familial history of glaucoma. This variant has been reported with high frequency in Gilan, Iran (Qashqai et al., 2018), and was observed in a compound heterozygous form in the Korean population, in combination with c.55C>T in the same gene, suggesting a potential digenic effect (Kim et al., 2011). Beyond its association with glaucoma, this polymorphism has been significantly observed in females with uterine leiomyoma, suggesting potential broader biological implications beyond ocular disease (SALIMI et al., 2014).

Structurally, the p.Arg368His substitution occurs between helices J and K in an exposed loop of the CYP1B1 protein structure. The wild-type arginine at position 368 interacts with G-365, D-367, V-363, and D-374, stabilizing the local structure. The replacement of arginine with histidine alters the electrostatic interactions in this region, specifically weakening the interactions between D-367 and D-374 (Tanwar et al., 2009). Although the precise functional consequences remain unclear, such changes could impact protein stability and enzyme activity.

The c.1310C>T (p.Pro437Leu) variant was identified in a single patient in a homozygous form. This variant was reported in China, Pakistan, Brazil and Spain (CAI et al., 2021; El-Ashry et al., 2007; López-Garrido et al., 2013b; Tehreem et al., 2022b). The c.1405C>T (p.Arg469Trp) variant was identified in three homozygous patients in this study. This variant has been reported in multiple populations, including Saudi Arabia (Badeeb et al., 2014b; Bejjani et al., 1998), Spain (Millá et al., 2013), Pakistan (Rauf et al., 2016), and Iran (Chitsazian et al., 2007b; Qashqai et al., 2018b). In the Spanish population, it has been observed in a compound heterozygous state with p.Thr404fsX30, suggesting a

potential synergistic pathogenic effect. Clinical observations have demonstrated that p.Arg469Trp may contribute to severe ocular phenotypes beyond glaucoma. A patient harboring this mutation was also affected by microphthalmia, corneal opacification in the right eye, anterior staphyloma in the left eye, bilateral type II Peters anomaly, and congenital glaucoma (Reis et al., 2016). This variant is located in the heme-binding region of the CYP1B1 protein (Y. Chen et al., 2008).

Functional studies have shown that p.Arg469Trp significantly reduces enzymatic activity, with reported reductions ranging from complete loss of function (null alleles) to approximately 70% of wild-type activity (Banerjee et al., 2016). This is further supported by a study that used western blot analysis showing reduced protein levels for the p.Arg469Trp mutant and decreased enzymatic activity by 30% toward steroid hormone substrates (Campos-Mollo et al., 2009). Given its functional significance and widespread reporting in various populations, the p.Arg469Trp variant is a strong candidate for pathogenicity, likely contributing to CYP1B1-related glaucoma through enzymatic dysfunction and structural instability in the heme-binding region.

In addition to the pathogenic variants, several polymorphisms and intronic variants were identified in this study. The c.1294C>G (p.Leu432Val) variant results in a leucine-to-valine substitution at codon 432. This variant has been extensively studied in the context of cancer susceptibility, particularly in hepatocellular carcinoma (F. Liu et al., 2015), squamous cell carcinoma (Moghadam et al., 2018), and ovarian carcinoma (L. Zhang et al., 2021). It was also detected in prostate cancer patients, where cells with longer telomere length showed significantly higher proportion of the CYP1B1 c.1294C>G CG and GG genotypes, suggesting it may confer genetic susceptibility to prostate cancer by altering telomere length (Gu et al., 2018).

This variant may have a role in male and female infertility. Hu et al. (2011) correlated p.Leu432Val with idiopathic male infertility (W. Hu et al., 2011). A study by Zou et al. (2013) predicted this variant significantly increases the risk of polycystic ovary syndrome susceptibility (Zou et al., 2013), however, its role in *CYP1B1*- related glaucoma remains unclear, and studies have shown no association between this variant and PCG susceptibility(Z. Wang et al., 2015).

The detected c.1358A>G (p.Asn453Ser) - rs1800440 variant has been implicated in lung cancer risk (Xu et al., 2012), but studies have yielded conflicting results regarding its clinical significance. This variant may have a protective role against the development of frontal fibrosing alopecia. A study by Saceda-Corralo et al. (2023) identified the majority of frontal fibrosing alopecia patients lack the protective role of rs1800440 polymorphism (Saceda-Corralo et al., 2023). It is worth noting that this variant is usually reported in literature along with other *CYP1B1* variants including rs10012 (c.142C>G), rs1056827 (c.355G>T) and rs1056836 (c.1294C>G). All these variants were detected in this study as well.

A potential haplotype block inheritance pattern was also observed, with three polymorphisms, rs2617266 (c.-1-12C>T), rs1056827 (c.355G>T, p.Ala119Ser), and rs10012 (c.142C>G, p.Arg48Gly) found in eight patients. They were all either in mutant homozygous form (in four patients) or all in heterozygous form (in four patients), while the remaining 38 patients did not have any of the three polymorphisms. Previous studies have suggested that certain *CYP1B1* haplotypes may be associated with colorectal cancer (Szuman et al., 2024). Notably, the variants in this haplotype been observed in Saudi Arabian populations, suggesting a shared genetic background for *CYP1B1* mutations between the Palestinian and Saudi ethnic groups.

Furthermore, this study identified intronic variants, including a novel g.38444_38445insT in Intron 2, and two previously reported polymorphisms, *c.350C>A (rs162562) and *c.210dup (rs4646431) in Intron 3. While these variants do not directly alter protein coding regions, they may influence gene expression or splicing and require further functional studies to determine their impact on CYP1B1 activity and PCG susceptibility.

5.3 Study Limitations

Several limitations should be acknowledged. First, the sample size is not representative entirely of the Palestinian populations. We were not able to collect samples from many affected families living in closed-community rural areas due to logistics complications. Additionally, a larger number of primary congenital glaucoma patients resided in the Gaza Strip. This limits the generalizability of the findings to the broader Palestinian population. A larger cohort would be beneficial to confirm the observed genetic patterns.

Secondly, during data collection from other cities, several blood samples were retrieved hemolyzed due to logistical challenges on the road and prolonged time spent on checkpoints. This is also true for Saliva samples.

Thirdly, the study primarily focused on sequencing *MYOC* and *CYP1B1* genes, yet primary congenital glaucoma and juvenile open-angle glaucoma are genetically heterogeneous disorders. Other candidate genes, such as *FOXC1, PITX2, LTBP2,* and *TEK*, have been shown to be implicated in glaucoma pathogenesis. Sequencing and investigating these genes may provide better insights into the genetic characterization of PCG among Palestinian patients, especially that 15 patients did not harbor *CYP1B1* pathogenic variants. This can be done via extended whole exome sequencing.

Fourthly, functional validation of the identified variants was not performed. While bioinformatics tools and previously published studies provided insights into the pathogenic potential of specific mutations, experimental analyses, such as protein expression studies, enzyme activity assays, and structural modeling, are needed to definitively assess their impact on MYOC and CYP1B1 protein function.

Finally, the study did not incorporate clinical data such as intraocular pressure, optic nerve assessment, and treatment outcomes. The inclusion of phenotypic data would allow for genotype-phenotype correlation analysis, which could further elucidate the role of these genetic variants in disease severity and progression.

5.4 Future Directions

Future research should address the limitations outlined above and explore additional avenues to expand our understanding of glaucoma genetics in the Palestinian population. First, increasing the sample size by recruiting additional wide spread Palestinian primary congenital glaucoma patients in the West Bank.

Second, whole exome sequencing should be utilized to uncover additional genetic factors that may contribute to PCG in our population. This approach would allow the identification of novel genes and variants beyond *MYOC* and *CYP1B1*, providing more comprehensive view of the genetic landscape of glaucoma in this population.

Third, functional studies should be conducted to validate the impact of the novel and rare variant identified in this study.

Fourth, a longitudinal clinical study incorporating genetic and phenotypic data should be established to investigate the clinical consequences of identified mutations. Such studies could provide insights into disease prognosis, treatment response, and potential personalized therapeutic strategies based on genetic findings.

Finally, investigating potential environmental and epigenetic factors may modulate the penetrance and expressivity of *CYP1B1* and *MYOC* mutations in PCG patients would be valuable. This could include studying DNA methylation, histone modifications, and gene-environment interactions.

5.5 Conclusion

In conclusion, this study represents the first genetic analysis of *MYOC* and *CYP1B1* variants in Palestinian patients with PCG. The findings confirm the presence of known pathogenic *CYP1B1* mutations in this population and reveal a novel frameshift mutation (c.856_856delG) that likely disrupts enzymatic function. Additionally, all *MYOC* variants identified were classified as non-pathogenic, with no clear contribution to glaucoma pathogenesis.

The study reinforces the importance of *CYP1B1* as a major contributor to PCG in Palestinian patients while highlighting the need for expanded genetic screening to identify additional risk factors. Population-specific studies remain crucial for understanding the genetic architecture of glaucoma and developing targeted diagnostic and therapeutic strategies. While this study advances knowledge of glaucoma genetics in Palestine, further research utilizing larger cohorts, expanded gene panels, and functional validation is necessary to fully understand the development of PCG. A multidisciplinary approach integrating genetics, clinical ophthalmology, and molecular biology is key in shaping future diagnostic and treatment or congenital glaucoma.

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Appendices

Appendix 1

موافقة للمشاركة في دراسة بحثية

تهدف هذه الدراسة لتحديد انواع الطفرات الوراثية في مورثتي (جينات) CYP1B1 و MYOC المرتبطة بالإصابة بمرض الزرق الخلقي الاولي (Glaucoma) لدى المرضى الفلسطينيين
هذه الدراسة يُجريها فريق البحث تحت إشراف أ.د. هشام درويش في الجامعة العربية الأمريكية كجزء من رسالة الماجستير للطالبة مريم علقم في برنامج الوراثة الجزيئية والسمية الجينية.

ما الهدف من هذه الدراسة؟
الهدف من هذا المشروع هو تجميع عينات الدم من المرضى لمساعدة الباحثين في تقييم هذا التغير وعمل التوصيات اللازمة للتعامل معه.

كم عدد الأشخاص المشاركين في هذه الدراسة؟
من المتوقع مشاركة 30 مصاب بالمرض المذكور .

ما المطلوب مني في هذه الدراسة؟
نحن نطلب إذنك بأخذ عينة دم (3-5 مل) من أحد أوردة ذراعك. لذا قد نضطر لوخزك بإبرة صغيرة لأخذ هذه العينة, كما ونطلب إذنك للحصول على بعض المعلومات التي ستساعدنا في تقييم نتائج البحث. وسيتم ذلك مع المحافظة على سرية وخصوصية هذه المعلومات.

حول استعمال عينة الدم لأغراض بحثية
البحث الذي يستند على عينة دم مهم لفهم الوضع الصحي وأمراض البشر. بعض الباحثين يستخدمون هذه العينات لمحاولة فهم سبب الأمراض ويقومون بتطوير فحوصات جديدة لكشف ومراقبة الأمراض، بينما قد يقوم آخرون بتطوير طرق جديدة للوقاية وعلاج بعضها او كلها في المستقبل بينما يقوم بعض الباحثين يقومون بتطوير منتجات جديدة وأدوية جديدة. جميع عينات الدم الذي سيتم جمعه سيحفظ في مختبر الوراثة الجزيئية للأبحاث في حرم الجامعة الأمريكية في حي الريحان قرب رام الله.

كم المدة التي سأبقى فيها في الدراسة؟
إذا اخترت المشاركة، تتم ترتيب عملية سحب الدم في وقت مناسب لك علما بأنه يمكنك اختيار التوقف عن المشاركة في أي وقت مستقبلا بعد إعلام الباحثين شخصيا بذلك.

ما هي المخاطر المترتبة على المشاركة بهذه الدراسة؟
المخاطر المترتبة على سحب عينة دم من ذراعك تتضمن انزعاج لحظي من دون تكوّن كدمة.
قيامنا بسحب عينة الدم مشابه تماما عندما يسحب لك عينه دم لإجراء فحوصات طبيه كجزء من الإجراء الروتيني لرعايتك الصحية لذلك فإن الازعاج المترتب على مشاركتك في البحث تكون ضئيلة.

الخصوصية والسرية

ستحفظ المعلومات البحثية والطبية الخاصة بهذه الدراسة تحديداً في ملف خاص بالبحث منفصل عن الملفات الطبية الاخرى. للمحافظة على الخصوصية، لذلك فإن اسمك أو أية معلومات قد تقوم بالتعريف بك ستزال من عينة الدم ومن كل المعلومات عن وضعك الطبي وسيرتك المرضية التي ترافق عينتك بك ستحفظ عن طريق إعطاء رمز خاص. وهذا الرمز سيكون معروفا فقط لدى المسؤولين عن جمع العينات.

هل هناك فوائد للمشاركة في هذه الدراسة؟
هناك فوائد أساسية للمجتمع نتيجة هذه الأبحاث، منها معرفة مسببات الأمراض، كيفية الوقاية منهم، وكيفية علاجهم والشفاء منهم. تحديداً، نحن نطمح لمعرفة كيفية الوقاية وعلاج الخلل في المادة الوراثية.

هل هناك بدائل عن المشاركة في هذه الدراسة؟
يمكنك اختيار عدم إعطائنا عينة من دمك لهذه الدراسة.

ما هي التكاليف؟
لن يكون هناك أية تكاليف عليك من خلال مشاركتك بهذه الدراسة

ماذا عن التعويضات؟
لن نقوم بالدفع لك لمشاركتك بهذه الدراسة.

ماذا عن حقي في رفض المشاركة بهذه الدراسة أو الانسحاب منها؟
المشاركة بهذه الدراسة هي مشاركة طوعية بحتة. يمكنك رفض المشاركة في هذه الدراسة أو في حال وافقت، يمكنك الانسحاب منها إن رغبت في أي وقت. قرارك بعدم المشاركة أو الانسحاب من المشاركة بهذه الدراسة لن يترتب عليه أية عقوبة أو خسارة لأية امتيازات، ولن يؤثر ذلك على مقدرتك على الحصول على الرعاية الصحية.

بمن أتصل في حال كانت لدي أسئلة أو واجهتني مشاكل؟
للسؤال عن الدراسة أو عن أو إذا كانت لديك مشاكل، مخاوف، أسئلة أو اقتراحات حول البحث، الاتصال ب أ.د. هشام درويش أو الطالبة مريم علقم على العنوان الالكتروني التالي: hisham.darwish@aaup.edu
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نص الموافقة
تم شرح لي الهدف من هذه الدراسة، الخطوات التي سيتم اتباعها، المخاطر والفوائد المترتبة على المشاركة بها. لقد تم السماح لي بسؤال أسئلة، وتمت الإجابة عن تساؤلاتي لحد يرضيني. لقد تم إخباري بمن أتصل إذا كانت لدي تساؤلات، أو لمناقشة مشاكل، أو مخاوف، أو اقتراحات متعلقة بالبحث، أو للحصول على معلومات أو إعطاء أية إضافات حول البحث. لقد قمت بقراءة وثيقة الموافقة هذه وأوافق على المشاركة بهذه الدراسة، مع العمل أنه بإمكاني الانسحاب أنّى شئت.

☐ أوافق على أن يتم أخذ عينة دم مني لفحص المادة الوراثية.

الاسم الثلاثي للمشارك _____
توقيع المشارك _____ التاريخ _____

الاسم الثلاثي للباحث/ة _____
توقيع الباحث/ة _____ التاريخ _____

Appendix 2

التاريخ: 2025/6/10

حضرة السيد/ السيدة ولي أمر الطفل [----------]،

يتقدم الفريق البحثي في مختبر أبحاث الوراثة الجزيئية في الجامعة العربية الأمريكية بجزيل الشكر والتقدير لمساهمتكم ومشاركتكم في هذه الدراسة العلمية الهامة التي تهدف إلى فهم الأسباب الوراثية لمرض الجلوكوما الخلقية في المجتمع الفلسطيني، والمساعدة في تحسين سُبل التشخيص والعلاج المبكر لهذا المرض.

نتائج الفحص الجيني:

بعد تحليل العينة الجينية الخاصة بطفلكم/طفلتكم، تبيّن وجود طفرة جينية في أحد الجينات المرتبطة بمرض الجلوكوما الخلقية، وتفاصيلها كما يلي:

الجين	نوع الطفرة	تفسير الطفرة
CYP1B1	c.182C>T Homozygous	**طفرة مرضية (Pathogenic)** معروفة تؤثر بشكل مباشر في زيادة احتمالية الإصابة بمرض الجلوكوما الخلقية

ماذا تعني هذه النتيجة؟

- **طفرة CYP1B1: c.182C>T**

هي طفرة مَرَضية معروفة تؤثر على وظيفة جين CYP1B1، هو أحد الجينات المسؤولة عن تنظيم نمو وتطور العين. هذه الطفرة تُضعف وظيفة البروتين الناتج عن الجين، مما قد يؤدي إلى تراكم السوائل داخل العين وزيادة الضغط، وهي من الأسباب الشائعة لظهور أعراض الجلوكوما في وقت مبكر من حياة الطفل. هذه الطفرة ظهرت عند طفلكم بشكل متماثل (Homozygous)، أي أنه ورث نفس التغيّر من كلا الوالدين، وهذا يزيد من احتمالية ظهور المرض.

هذه الطفرة تم توثيقها في بعض العائلات الفلسطينية.

توصياتنا لكم:

- متابعة طبية منتظمة مع طبيب العيون المختص في أمراض الأطفال، لمراقبة ضغط العين ونمو العصب البصري.
- الحصول على استشارة وراثية لتفسير أوسع حول الطفرة وتاريخ العائلة، ولمساعدتكم في اتخاذ قرارات صحية مستقبلية.
- عدم استخدام نتائج التقرير بشكل فردي في اتخاذ قرارات علاجية دون الرجوع إلى الطبيب المختص.
- الاحتفاظ بنسخة من هذا التقرير في ملف الطفل الطبي لاستخدامها في حال زيارة مراكز طبية أخرى أو التحويل للعلاج بالخارج.

ملاحظة هامة:

هذا التقرير مخصص فقط لإعلامكم بنتائج التحليل الجيني ولا يغني عن الاستشارة الطبية المباشرة مع الطبيب المختص . والهدف من المعلومات الواردة في هذا التقرير هي لإعلام المريض أو ولي الأمر بنوع الطفرة الوراثية باصابته بمرض الجلوكوما الخلقية، وقد تكون هي المسبب الأساس للمرض، أو أحد المسببات. لذلك، هذا التقرير ليس للاستخدام في أي علاجات أو أي أغراض أخرى، إلا بعد استشارة الطبيب المختص. علما بأنه تم الحصول على عينة الدم من المريض بعد توقيع ولي الأمر على نموذج الموافقة على المشاركة.

للمزيد من الاستفسارات يمكنكم التواصل معنا:

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كلمة أخيرة من فريق البحث:

نفهم مشاعركم كأهالي وحرصكم على صحة أطفالكم، وندرك مشاعر القلق والخوف. نود أن نؤكد أن هدفنا من هذه الدراسة هو تقديم المعرفة والمساعدة في التشخيص المبكر، مع الأمل أن تساهم هذه الجهود مستقبلا في تطوير علاجات أفضل لأطفالنا.

نتمنى لطفلكم الصحة والعافية والسلامة الدائمة، ونسعد دوماً بالإجابة عن أي تساؤلات أو تقديم أي دعم تحتاجونه.

مع خالص الشكر والاحترام،

فريق مشروع بحث الجلوكوما الخلقية
الجامعة العربية الأمريكية – حرم رام الله

فحص الطفرات الجينية المرتبطة بمرض الجلوكوما الخلقية الأولية لدى مجموعة من المرضى الفلسطينيين

مريم حسام عبد الحافظ علقم

لجنة الاشراف: د. هشام درويش، د. ابراهيم طه، د. سناء محسن

ملخص

الجلوكوما الخلقية هي حدى الأمراض الخطيرة التي تصيب عيون الطفل عند الولادة، وقد تؤدي إلى فقدان البصر بشكل دائم إذا لم يتم النشخيص والمعالجة في الوقت المناسب. في فلسطين، معدلات إصابة أعلى من المعدل العالمي، ويُعزى ذلك غالبا إلى انتشار زواج الأقارب. حاليا، لا يمكن تشخيص المرض إلا بعد الولادة، والجراحة هي الخيار العلاجي الوحيد المتوفر، مما يجعل فهم الأسس الجينية للمرض ضروريا لتحسين فرص الكشف المبكر والرعاية السريرية.

تهدف هذه الدراسة إلى تحليل الطفرات في الجينين CYP1B1 و MYOCلدى مجموعة من المرضى الفلسطينيين الذين تم جمع عيناتهم من مناطق مختلفة في الضفة الغربية، وذلك بالتعاون مع عدد من أطباء العيون والمراكز المتخصصة. وقد تم إجراء التحليل الجيني باستخدام تقنية سانجر للتسلسل.

أظهرت النتائج وجود طفرات متماثلة الزيجوت في CYP1B1 لدى 22 مريضا، من بينها طفرة جديدة لم تُسجل سابقًا. كما وُجدت طفرات متغايرة لدى 9 مرضى، بينما لم تُكتشف أي طفرات في هذا الجين لدى 15 مريضًا. في المجمل، تم تحديد سبع طفرات مرضية، منها طفرة جديدة وست طفرات معروفة، وقد أظهر التوزيع الجيني لهذه الطفرات نمطًا مشابهًا لما هو موثق في مجتمعات عربية أخرى.

تُعد هذه الدراسة أول تحليل جيني شامل للجلوكوما الخلقي في فلسطين، وتسلّط الضوء على أهمية الفحص الجيني كوسيلة للكشف المبكر، مما يساهم في تحسين التدخلات العلاجية ويعزز فرص تطوير علاجات أكثر فاعلية في المستقبل.

كلمات مفتاحية: الجلوكوما الخلقية الأولية، المجتمع الفلسطيني، فحص جيني، CYP1B1, MYOC,