Review Article

Genetic Epidemiological Concepts, Tools and Models, in understanding Primary Open Angle Glaucoma

ABSTRACT

Primary open-angle glaucoma (POAG) is a multifactorial chronic optic neuropathy with significant genetic heterogeneity. The pathogenesis of POAG involves an imbalance between the production and drainage of aqueous humor (AH). Genetic theories suggest that transgenic mice demonstrating the *GLU50LYS* mutation in optineurin (OPTN) experience retinal ganglion cell apoptosis, while mutant myocilin (MYOC) proteins induce endoplasmic reticulum (ER) stress, leading to an unfolded protein response (UPR) and subsequent apoptosis of trabecular meshwork cells (TMC). Furthermore, the interaction between MYOC and mitochondria in the trabecular meshwork (TM) and astrocytes may lead to mitochondrial membrane depolarization and calcium channel dysregulation, contributing to POAG. Overexpression of MYOC variants (P370L, Q368X) is also implicated, as are epigenetic modifications and signaling pathways such as histone and DNA modification. POAG has been associated with autosomal dominant inheritance, with mutations in MYOC and OPTN being prominent causative factors, although many cases involve multiple genetic loci. Currently, over 20 genetic loci have been linked to POAG, with 14 chromosomal loci (GLC1A to GLC1N) identified, 5 of which contribute to juvenile-onset open-angle glaucoma (JOAG). Of these loci, MYOC, OPTN, WD repeat domain 36 (WDR36), and neurotrophin-4 (NTF4) are the most studied causative genes. The ongoing study of molecular genetics offers potential pathways for future therapeutic advances in the treatment of POAG.

Keywords: Primary open-angle glaucoma, Mutation, Gene locus, Genetic mapping, Mendelian inheritance, Gene penetrance

1. INTRODUCTION

Glaucoma is a heritable and irreversible degenerative optic neuropathy. Primary open-angle glaucoma (POAG) is a genetically complex disorder with multiple risk factors and a multifactorial etiology that remains poorly understood. POAG is characterized by a chronic, progressive loss of retinal ganglion cells (RGCs), leading to structural damage in the optic nerve head (ONH) and retinal nerve fiber layer (RNFL), ultimately causing visual field defects [1]. The dysfunction of the trabecular meshwork (TM), which leads to delayed aqueous humor (AH) outflow, contributes to the imbalance in AH production and drainage, and elevated intraocular pressure (IOP) [2].

POAG is typically an insidious, adult-onset disease with variable age at onset and slow progression [1]. It presents asymptomatically and bilaterally with asymmetrical features. It is a significant cause of chronic disability, with diagnosis and disease progression often based on functional and structural abnormalities of the ONH and RNFL[1].

Epidemiological studies have revealed that POAG is partly heritable, with classic risk factors such as advanced age, ethnicity, elevated IOP, family history, and ancestry[1].. The significant ethnic predisposition and familial aggregation suggest that genetic factors play an important role in the disease's pathogenesis [3].

Recent advancements in molecular genetics have identified multiple gene mutations across various chromosomal loci that contribute to POAG[4]. POAG, identified as phenotype OMIM137760, is the most common form of glaucoma and is characterized by an open anterior chamber angle [4]. Genetic studies have confirmed that POAG is genetically heterogeneous and follows a multifactorial inheritance pattern, resulting from interactions between susceptibility genes and environmental factors [5]. Single-gene forms of POAG with autosomal dominant inheritance, such as those associated with MYOC and OPTN mutations, are rare but highly penetrant [6].

While Mendelian linkage approaches have identified several loci responsible for a small percentage of POAG cases, most cases exhibit a complex genetic basis involving multiple genetic and environmental risk factors. Over 30 chromosomal loci have been implicated in POAG, although many have not been consistently replicated across populations [6]. Mapping of gene loci suggests that POAG is either polygenic or represents a heterogeneous group of diseases with similar phenotypes [7].Four major genes—MYOC, OPTN, WDR36, and NTF4—have been well-characterized, and new genes continue to be identified[7] [9] [10].

2. EPIDEMIOLOGY OF POAG: THE GENETIC PERSPECTIVE

Glaucoma is a complex disease affecting over 70 million people worldwide, making it the second leading cause of blindness. Despite this, the genetic contributions to POAG remain largely unknown in more than 95% of cases [13].It is the third most prevalent visual impairment among White and Black Americans, with undiagnosed glaucoma affecting approximately 50% of cases in developed countries and up to 90% in developing regions [14].

The prevalence of glaucoma varies significantly by race, ethnicity, and ancestry. For instance, prevalence rates in Europe are around 2.2%, 2.7% in China, 3.7% in Japan, 3.4% in Latin America, and 4.3% in Africa [15].In the U.S., more than 2.25 million Americans aged 40 and older suffer from POAG, which is the most common form of glaucoma, affecting over 33 million people globally [1]. Heritability estimates vary widely, from 13% to 93%, with first-degree relatives showing a ninefold increased risk [16].

Less than 10% of POAG cases follow a Mendelian inheritance pattern, and most cases are caused by multiple genetic and environmental risk factors[17]. Gene variants in MYOC are linked to early-onset POAG, affecting up to 20% of early-onset cases and 3%-

5% of adult-onset cases. Although these variants may explain around 5% of POAG cases, the genetic causes remain unknown for the vast majority of cases[18]. Ethnic disparities exist, with individuals of African descent five times more likely to develop POAG than individuals of European descent, and with Black populations more susceptible to severe optic nerve damage" [14].

3. BIOLOGICAL PATHWAYS AND MECHANISMS IN POAG

Although the exact mechanism of primary open-angle glaucoma (POAG) has not been fully elucidated, several theories have been proposed regarding its pathogenesis. These mechanisms explain the interplay of genetic, environmental, and molecular factors in the progression of POAG.

3.1 Pathological Theories and Mechanisms

3.1.1 The Vascular Theory

The vascular theory suggests that vascular dysfunction may trigger ischemia, leading to optic nerve cell death. This may be due to elevated intraocular pressure (IOP) or a primary vascular insult, which disrupts blood flow and triggers apoptosis in the retinal ganglion cells (RGCs) REFERENCE.

3.1.2 Mechanical Compression Theory

The mechanical compression theory posits that elevated IOP leads to axon dysfunction by causing compression of the cribriform plate. This compression results in backward bowing of the lamina cribrosa, potentially leading to focal ischemia, neurotrophin deprivation, or interference with axoplasmic flow, which in turn triggers RGC death" [19].

3.1.3 RGC Pressure

This mechanism focuses on the hypothesis that elevated pressure on the RGCs, combined with ischemia and hypoxia, induces cell death through glutamate-induced excitotoxicity, increased inflammatory mediators, and disruption of trophic factor flow. These events block anterograde and retrograde axonal transport, leading to axotomy and eventual blindness " [20].

3.1.4 Elevated IOP

Elevated IOP has been found to stimulate the expression of heat shock proteins, which activate immune cells such as memory T cells. These immune cells attack retinal neurons, contributing to optic nerve degeneration and vision loss. This autoimmune response has been hypothesized to play a role in glaucoma, resembling the immune response to bacterial infections " [21].

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3.1.5 Prostaglandin Biosynthesis

Prostaglandin (PG) biosynthesis is influenced by Cyclooxygenase-2 (COX-2), a rate-limiting enzyme in the process. Loss of COX-2 expression in aqueous humor-secreting cells has been linked to POAG, suggesting that reduced prostaglandin levels may contribute to disease " [22].

3.1.6 Pressure-Induced Injury of the ONH

Increased pressure in the optic nerve head (ONH) leads to alterations in retinal gene expression, astrocyte responses, oxidative stress, mitochondrial dysfunction, neurotrophic factor changes, and autoimmunity. These factors collectively contribute to RGC death, the final common pathway in POAG pathogenesis [23].

3.2 Genetic Mechanisms

3.2.1 Genetic Predisposition

Genetic predisposition plays a key role in POAG development. Mutations in specific genes can trigger cell death, releasing neurotransmitters such as glutamate, which leads to excitotoxicity. The subsequent release of calcium, free radicals, and nitric oxide induces secondary apoptosis in neighboring cells [19].

3.2.2 GLU50LYS Mutation in Optineurin

Transgenic mouse models have demonstrated that the GLU50LYS mutation in optineurin (OPTN) results in apoptosis of RGCs. This suggests that optineurin-mediated glaucoma may arise from disrupted interactions between OPTN and GTP-binding protein Rab8, affecting protein trafficking and RGC viability [24].

3.2.3 Unfolded Protein Response (UPR)

Mutations in the MYOC gene can induce endoplasmic reticulum (ER) stress, triggering the unfolded protein response (UPR) in trabecular meshwork cells. This stress response leads to increased resistance to aqueous humor outflow, elevated IOP, and ultimately, glaucoma [25].

3.2.4 Interaction of MYOC with Mitochondria

The interaction between mutant MYOC and mitochondria in the trabecular meshwork (TM) and astrocytes is cell-specific. TM cells expressing the Pro370Leu MYOC mutant exhibit mitochondrial dysfunction, which increases their vulnerability to cellular damage, impairing function and leading to cell death (Ms_OR_125244) [26].

3.2.5 Calcium Channel Dysregulation

MYOC mutations cause dysregulation of calcium channels, leading to mitochondrial

membrane depolarization in TM cells, reduced outflow of aqueous humor, and elevated IOP[26].

3.2.6 Overexpression of Wild-Type and Mutant MYOC

Overexpression of wild-type MYOC, as well as mutant variants P370L and Q368X, has been shown to inhibit neurite outgrowth in neuronal cells, potentially contributing to neurodegenerative changes in glaucoma [27].

3.3 Epigenetics and Signaling Pathways

Epigenetics refers to heritable changes in gene expression that do not involve alterations to the DNA sequence itself. These modifications can affect how cells interpret genetic information, influencing disease susceptibility. Factors such as age, environment, lifestyle, and disease state can all influence epigenetic changes [28].

Epigenetic mechanisms are thought to contribute to POAG risk by influencing several key pathways, including transforming growth factor-beta (TGF-β), Rho kinase, and calcium-calpain signaling pathways. These pathways promote the upregulation of pro-apoptotic genes, downregulation of neuroprotective factors, and fibrosis of the trabecular meshwork, potentially obstructing aqueous humor outflow [12].

3.3.1 Histone and DNA Modification

Epigenetic modifications, such as histone acetylation and DNA methylation, regulate gene activation or suppression. Additionally, non-coding RNAs like microRNAs and long non-coding RNAs (lncRNAs) can modulate gene expression by altering cellular signaling pathways, which can impact disease progression [31]. In glaucomatous eyes, hypoxic conditions activate the hypoxia-inducible factor 1-alpha (HIF1-α), which travels to the nucleus to regulate gene expression and promote fibrosis, contributing to increased IOP [32].

3.3.2 Brain-Derived Neurotrophic Factor (BDNF) and Other Neurotrophic Factors

Brain-derived neurotrophic factor (BDNF) supports the survival of RGCs. In glaucoma, elevated IOP blocks the axonal transport of BDNF from the brain to the RGCs, resulting in cell death through the activation of c-Jun N-terminal kinase (JNK) and caspase pathways [34].

3.3.3 Epigenetic Pathways and Predisposing Factors

3.3.3.1 TGF-β Signaling Pathway

TGF-β plays a crucial role in POAG pathogenesis by promoting structural changes in the extracellular matrix and fibrosis of the trabecular meshwork, which obstructs aqueous humor outflow. Elevated levels of TGF-β2 have been found in the aqueous humor of POAG patients [35].

3.3.3.2 Calcium-Calpain Pathway

Disruptions in calcium homeostasis in RGCs, triggered by elevated IOP, can activate calpain, a cysteine protease, which induces apoptosis by cleaving calcineurin, leading to the release of cytochrome C and the activation of cell death pathways [36].

3.3.3.3 Rho Signaling Pathway

The Rho GTPase/Rho kinase pathway regulates the contractile properties and permeability of the trabecular meshwork and Schlemm's canal. The Rho family, including 'Rho, Rac, and Cdc42 subfamilies', is involved in cell migration, adhesion, proliferation, and 'actin cytoskeletal organization'. Cross-linked actin networks (CLANs) may cause glaucoma by 'decreasing cell elasticity and impairing aqueous humor outflow'Alterations in these properties increase IOP and contribute to POAG progression [37].

4. CONCEPT OF GENE ALTERATIONS IN POAG GENETICS

The inheritance of primary open-angle glaucoma (POAG) is often transmitted as a Mendelian trait, typically following an autosomal dominant pattern. Single-gene mutations are responsible for certain forms of glaucoma, and these mutations almost always lead to POAG and are rarely observed in individuals with normal vision. The primary genes implicated in POAG are *MYOC* and *OPTN*, which are considered causative genes. Another class of mutations, known as risk alleles, can promote the development of POAG when combined with other risk alleles and environmental factors [6].

4.1 Mutation

Mutations are genetic alterations that can cause a gene to malfunction, resulting in disease. In POAG, mutations are classified into two distinct categories. The first category includes mutations that are sufficient to cause POAG on their own, with little influence from other genes or environmental factors. These mutations typically follow an autosomal dominant inheritance pattern and are almost always associated with POAG [6]. Several genes capable of causing POAG with minimal influence from other genes or environmental factors have been identified. Nonetheless, these known genes account for less than 5% of POAG cases.

The genetic landscape of the majority of POAG cases is 'complex', with up to 95% of instances resulting from a combination of numerous genetic and possibly environmental risk factors. [38]. Conducting genome-wide association studies (GWAS) have been used to search genetic risk factors that contribute to the development of glaucoma, and comparing the genomes of POAG patients with normal eyes, to find 'gene sequences' that are 'statistically more common' in patients with glaucoma [8].

4.2 Gene Isolation

The identification of disease-causing genes provides insights into the pathogenesis of heritable eye diseases. By identifying key genes, researchers can elucidate the biological pathways involved in disease development, which can aid in the creation of DNA-based diagnostic tests. These tests may help clinicians assess an individual's risk for POAG and differentiate between similar clinical conditions. For example, mutations in *MYOC* can lead to protein misfolding in trabecular meshwork cells, disrupting aqueous outflow and raising intraocular pressure.

Several genes capable of causing POAG with minimal influence from other genes or environmental factors have been identified. Nonetheless, these known genes account for less than 5% of POAG cases. The genetic landscape of the majority of POAG cases is 'complex', with up to 95% of instances resulting from a combination of numerous genetic and possibly environmental risk factors. [38]. Conducting genome-wide association studies (GWAS) have been used to search genetic risk factors that contribute to the development of glaucoma, and comparing the genomes of POAG patients with normal eyes, to find 'gene sequences' that are 'statistically more common' in patients with glaucoma [39].

4.3 Glaucoma-Causing Genes

The genes implicated in POAG, including *MYOC*, *OPTN*, and *TANK-binding kinase 1* (TBK1), provide critical insights into the disease's pathogenesis. Mutations in *MYOC* account for 3-4% of POAG cases with intraocular pressure (IOP) greater than 21 mmHg. Mutations in *OPTN*, *TBK1*, and *MYOC* also contribute to 1% of POAG cases where IOP remains less than or equal to ≤21 mmHg, i.e. normal tension glaucoma. Mutations in *MYOC* cause a cascade of abnormalities in the trabecular meshwork including intracellular retention of MYOC protein, stimulation of endoplasmic reticular (ER) stress, decreased aqueous outflow, higher intraocular pressure, and glaucoma. Mutations in *OPTN* and *TBK1* cause a 'dysregulation of autophagy' which may directly cause retinal ganglion cell damage and normal tension glaucoma" [38].

5. MENDELIAN GENETICS IN POAG

5.1 Mendelian Autosomal Dominant and Recessive Patterns of Inheritance

"Mendelian autosomal dominant and recessive forms of glaucoma are caused by single gene defects, with extreme phenotypes like high IOP or severe optic nerve degeneration." However, most patients with POAG do not have 'extreme phenotype', Their underlying genetic etiologies are not from single gene defects, but from multiple genetic factors which independently contribute to moderate IOP alterations, optic nerve degeneration, and collectively more severe disease [40]. Glaucoma genetics is characterized by inherited "Mendelian-dominant or recessive traits, typically early-onset forms (before age 40), or a heritable susceptibility consistent with complex trait inheritance, typically adult-onset forms (after age 40)" (Table 1) [41]. Common age-related ocular disorders like adult-onset glaucoma, including POAG, exhibit Mendelian inheritance patterns with

‘significant heritability’, affecting individuals of all ages. Genetic factors contribute to complex disorders, which are susceptible to environmental exposure and complex inheritance, making it challenging to identify genes contributing to these conditions [10].

“Rare forms of POAG, affecting children and young adults, are inherited as Mendelian disorders with either recessive or dominant inheritance of a single gene”. Early onset glaucoma genes are rare with greater biological effects, while adult-onset variants have smaller effects. Genetic approaches define molecular events and identify chromosome locations [10]. Linkage studies of POAG families have identified a role for the MYOC gene in POAG.

In contrast, common forms of POAG affecting adults older than 50 years are inherited as non-Mendelian or complex traits. “The classification of POAG into inherited, familial, and sporadic categories is suggested. These categories differ in inheritance pattern, familial aggregation, methodology, and gene mapping outcomes. Inherited POAG involves three relatives, ‘proband/index case’ inclusive, documented with POAG in two consecutive generations, one person being a first-degree relative of the other two. Familial POAG involves two first-and/or second-degree relatives and does not meet the criteria for inherited POAG. Sporadic POAG involves a single patient without affected first or second-degree relatives”. This classification can guide clinical practice and genetic studies [9].

5.2 Single Genetic Mutations in Mendelian Inheritance

“Mendelian disorders are conceptually the simplest demonstration of how genes can be responsible for disease. A single genetic defect alone causes a disease and if this is passed on by parents, their children will potentially inherit the disease. Common forms of inheritance of Mendelian disorders include ‘autosomal dominant’, ‘autosomal recessive’ and ‘X-linked recessive’. Such genetic alterations, sufficient to cause a gene to malfunction and result in disease are termed ‘mutations’. This is applicable to Mendelian disease caused by a single genetic alteration, usually rare, acts alone; and caused only a fraction of defects” [7].

Classical Mendelian autosomal dominant and recessive pattern of glaucoma inheritance are caused by single gene defects, resulting in extreme phenotypes like high intraocular pressure or severe optic nerve degeneration. A genotype at one locus is both necessary and sufficient for the phenotype to be expressed” [40] [42]. ”Typically, early-onset forms of glaucoma are inherited as Mendelian dominant or Mendelian-recessive traits, including early-onset open angle glaucoma” [43]. ‘Adult-onset glaucoma’, including 5% of primary open angle glaucoma, is attributed to ‘single-gene’ or Mendelian forms caused by mutations in *MYOC* or *OPTN*, with high likelihood of causing glaucoma and significant heritability in normal subjects (Table 1) [10] [8]. Mendelian inheritance pattern shows that some glaucoma cases have genetic basis, with ‘twin and familial clustering’ suggesting heredity. Glaucoma classification is tailored to each individual based on family history, or family glaucoma genotype [44].

5.3 Complex Genetic Traits and Inheritance

“Glaucoma, is characterized as a ‘complex' disease, with phenotype exhibiting ‘heterogeneity’, ‘polygenic inheritance’, ‘phenocopies’, and ‘incomplete penetrance’” [45]. POAG patients typically do not exhibit extreme phenotypes due to its genetic complexities, and the underlying genetic causes are not believed to stem from single gene

defects. Multiple genetic factors contribute to moderate IOP and optic nerve disease, independently causing alterations and collectively causing more severe disease [40].

POAG is typically caused by 'oligogenic', 'polygenic', or 'multifactorial' mechanisms. Most genes follow a Mendelian inheritance pattern, with transmission mostly in 'monogenic' form in juvenile-onset and complex forms in adults. 72% of POAG cases have an inherited component [46]. POAG have a complex genetic basis, influenced by multiple genetic and environmental risk factors. These factors, while more common in patients with POAG, are also observed in normal subjects [8]. Complex disease is not caused by a single genetic defect, but rather by the combined effects of multiple factors. Each risk factor is insufficient to cause disease on its own, and may not be present in all cases of glaucoma [7] [9]. This suggested classification may serve as a useful guide in clinical practice and genetic studies where ethnic background and familial aggregation must be taken into consideration [9].

5.4 Complex Inheritance Supported by Linkage Studies

"Discovering genes that contribute to disorders with complex inheritance is more difficult" [10]. Glaucoma is characterized as a 'complex' disease, with phenotype exhibiting heterogeneity, polygenic inheritance, phenocopies, and incomplete penetrance" [45]. Traditional family linkage analysis has identified many genes, particularly those encoding simple Mendelian disease phenotypes.

Inherited POAG has been linked to seven chromosomal loci. Traditional linkage analyses have been used to identify glaucoma linkage to specific loci in families with multiple affected members. However, complex human diseases require novel genetic strategies [47]. Familial POAG lacks a clear Mendelian inheritance pattern and is typically studied using sib-pair analysis and family-based association analysis. Sporadic POAG cases often carry known POAG-causing mutations, suggesting genetic predisposition [9].

Recent tools like sib-pair analysis, 'transmission/disequilibrium test', and 'homozygosity mapping' have made gene identification more efficient [48]. "To be statistically significant, a three-generation family with over 10 affected individuals needs a logarithm of odds score of >3, resulting in a P-value of 0.05". The results identify a large chromosomal region with a causative gene. 'Positional cloning' was used to identify the MYOC gene, which segregates with the family phenotype and is found mutated in other patients [47].

6. CONCEPTS, TOOLS, AND MODELS IN UNDERSTANDING MOLECULAR GENETICS OF POAG

The study of molecular genetics has provided significant insights into the mechanisms that contribute to primary open-angle glaucoma (POAG). Advances in genetic research have led to the identification of mutations associated with the disease and the development of models and tools to understand its pathogenesis.

6.1 Concepts of Molecular Genetics

While most of the molecular mechanisms that lead to POAG remain unknown, genetic studies have identified specific gene mutations linked to the disease. These mutations result in retinal ganglion cell (RGC) death, which is a key pathological feature in glaucoma. The discovery of different genetic causes of POAG may lead to improved classification of the disease, based on the precise genetic mutation rather than the clinical presentation(Ms_OR_125244). Genetic studies also present opportunities to explore the heritability of the disease using various tools and models, which can help in identifying new mutations associated with POAG REFERENCE

6.2 Tools, Models, and Approaches in Understanding POAG

6.2.1 Mendelian Autosomal-Dominant and Autosomal-Recessive Inheritance

“Mendelian autosomal-dominant or autosomal recessive trait, or as a complex multifactorial trait, is associated with glaucoma inheritance’ (Table 2). This is a viable tool in molecular genetics. While minority of POAG pedigrees demonstrates a Mendelian pattern of inheritance, a large number have pedigrees with autosomal recessive inheritance. “Several autosomal dominant pedigrees have also been described, with a degree of penetrance varying from 60% to 100%, especially in early glaucoma” [41].

6.2.2 Epigenetics

“Epigenetics is the study of changes in gene function that are ‘mitotically’ and/or ‘meiotically’ heritable and do not entail a change in DNA sequence. It is the heritable alterations in the gene expression profile of a cell that do not involve, or caused by changes in the DNA sequence” [60. “‘Epi’ means on or above in Greek, and ‘epigenetics’ describes factors beyond the genetic code. Epigenetic changes are modifications to DNA that regulate whether genes are turned on or off. These modifications are attached to DNA and do not alter the sequence of DNA building blocks.

These changes may remain through cell divisions for the remainder of the cell’s life and may also last for multiple generations. Within the complete set of DNA in a cell (genome), all of the modifications that regulate the activity (expression) of the genes is known as the epigenome” [61]. It refers to the changes in phenotype without a change in genotype, which affect how cells read genes. It applies to 'characteristics passed from a cell to its daughter cells and to traits of an organism'. It deals with changes in organisms caused by modification of gene expression, which can alter cellular signaling pathways and affect individual susceptibility to diseases.

'Epigenetic inheritance refers to the transmission of certain epigenetic marks to offspring, which are reversible and do not change the DNA sequence". It occurs regularly, naturally and is influenced by age, environment, lifestyle, and disease state. These factors can interact with the genome, affecting gene expression at various stages throughout a person's life and even in later generations. Physical and social environment features can

affect gene expression rather than the DNA sequence itself. “Epigenetic inheritance mechanisms include regulation of transcription sustained by at least three systems including ‘DNA methylation’, ‘histone modification’ and ‘non-coding RNA’ (ncRNA)-associated gene silencing” [62]. Modifications in epigenetics can modulate gene expression and/or alter cellular signaling pathways affecting individual susceptibility to various diseases. Some findings suggests that the glaucomatous eye is associated with a hypoxic environment. Epigenetics regulates retinal development, and disturbances due to this regulation, may lead to ophthalmologic diseases like glaucoma, optic neuritis, and hereditary RGC degeneration. The effect of Epigenetic forces contributing to glaucoma disease also manifest in lamina cribrosa cells [63].

6.2.3 Sib-Pair Analysis

Sib-pair analysis is a method used to study the genetic basis of diseases like POAG by examining genetic similarities between siblings. This approach identifies genetic loci associated with the disease and helps uncover the mechanisms underlying its development REFERENCE. Sib-pair analysis is particularly useful for understanding complex diseases, as it allows researchers to detect shared genetic segments that contribute to disease risk REFERENCE .

6.2.4 Family-Based Association Analysis

6.2.4 Family-based association analysis

“The family-based design is a crucial strategy in genetic association analysis, utilizing a statistical method to examine the relationship between genetic variants and phenotypic traits within families” [65]. This method involves dividing genotypes into ‘between-family’ and ‘within-family’ components, ‘permuting them separately’, and performing association analysis on each component. “This allows for enrichment of rare variants, control for ‘heterogeneity’, ‘population stratification’, direct estimation of genetic contribution, examination of variant transmission, and revealing allele parental origin effects” [66]. Family-based association tests (FBATs) are a cost-effective and less powerful study design in human genetic research, allowing for any type of pedigree structure. They can apply missing parental data, multiple siblings, and extended pedigrees. Standard FBATs only use within-family information, resulting in a significant portion of genetic data being utilized. “Family-based data is robust to ‘population stratification’, minimizing concerns about the control group's representativeness” [67]. The Challenge is that many disease phenotypes results from complex interactions among multiple genes, while a single genotype can influence multiple phenotypes. Family-based association analysis is crucial for understanding the genetic basis of complex traits within families. [66].

6.2.5 Candidate Gene Approach

“The candidate gene approach identifies disease-causing genes, especially when a known gene is a strong suspect”. Research aims to uncover additional genes and variants associated with POAG, providing insights into disease mechanisms and potential therapeutic targets [68]. The candidate gene approach involves researchers identifying potential genes-causing glaucoma by altering normal functions.

They test a large group of unrelated glaucoma patients for defects in these genes, potentially identifying a disease-causing gene. “This method is useful when a disease is believed to be caused by a limited number of known genes.

The technique's limitation lies in its insufficient value in studying the genetics of glaucoma due to the vast number of potential causative genes".

A study examining over '25 candidate genes, including 'collagen', 'fibrillin', and 'elastin', for A-POAG linkage' found no connection, while another failed to show linkage between 'angiotensin' and 'glucokinase' and A-POAG.[69]. "Positional cloning is a method used to identify a gene's causative gene within a large chromosomal region.

This involves selecting candidate genes and assessing if a plausible mutation is detected, which segregates with the family phenotype and is found in other patients with the same disease". The MYOC gene was identified via this approach of 'positional cloning' [70]. Identifying molecules and genes in sick RGCs helps understand glaucoma death and identify key targets for genetic therapy. Focusing on genes involved in RGC function, neuroprotection, or apoptosis can help identify key targets. However, traditional candidate gene approaches may not be suitable for complex diseases. Genome-wide association is another method for identifying genes contributing to complex diseases [71].

6.2.6 Linkage Analysis and Recombination Mapping

"Linkage analysis is a genetic method that searches for chromosomal segments that 'co-segregate' with the ailment phenotype through families. It may be either parametric (if we know the relationship between phenotypic and genetic similarity) or non-parametric" [72]. This method identifies linkage of different forms of glaucoma to particular loci, maps genes for both binary and quantitative traits [71], studies the genetic basis of POAG and identifies glaucoma-causing genes within large families.

"The DNA within the linked regions of DNA segments is always passed down through the family, along with glaucoma. This method helps identify disease genes without prior knowledge of the underlying pathology of the condition. It uses the concept that genes close to each other, are less likely to be separated by the process of 'recombination' during 'meiosis', than those which lie far apart, resulting in a close-linked inheritance". This technique involves tracking the co-segregation of markers with a disease gene in affected families to establish the disease gene's approximate location [73].

New POAG gene identification requires a methodology examining association across the entire genome, requiring around 400 markers to cover the whole genome, allowing for hypothesis-independent examination. "Until recently, it was not feasible to examine all independently inherited SNPs genome-wide. Linkage studies have identified genes associated with glaucoma, such as myocilin, optineurin, and WDR36" [74]. Mutations in these genes cause "autosomal dominant Mendelian POAG" in studied families (Table 2) [50]. J-POAG, A-POAG, and ocular hypertension are all linked to to the region 1q21-q31, the same glaucoma gene as showed by Linkage analysis and recombination mapping, suggesting a "clinical continuum artificially divided at 40 years". [75].

Recent identification of TIGR supports GLC1A involvement in primary open angle glaucomas, with J-POAG and early onset A-POAG individuals linked to the GLC1A interval (Table 2) [70].

Table 2: “Glaucoma loci defined by linkage studies”

”Chromosome location	Locus	Gene	Condition	Inheritance pattern
1q24.3-q25.2	GLC1A	MYOC	JOAG and adult-onset POAG	JOAG, AD Adult-onset POAG, complex
1p36.2-p36.1	GLC3B		Congenital glaucoma	AR
2p15-p16	GLC1H		Adult-onset POAG	AD
2p22-p21	GLC3A	CYP1B1	Congenital glaucoma	AR
2cen-q13	GLC1B		Adult-onset POAG	AD
2q11-q14	NNO3		Nanophthalmos	AD
3p22-p21	GLC1L		Adult-onset POAG; NTG	AD
3q21-q24	GLC1C		Adult-onset POAG	AD
4q25-q26	RIEG1; IRID2	PITX2	Axenfeld-Rieger syndrome	AD
			Iridogoniodysgenesis	
5q21.3-q22.1	GLC1G	WDR36	Adult-onset POAG	AD; complex
5q22.1-q32	GLC1M		JOAG	AD
6p25	RIEG3; IRID1	FOXC1	Axenfeld-Rieger syndrome;	AD
			Iridogoniodysgenesis	
7q35-q36	GLC1F		Adult-onset POAG	AD
7q35-q36	GPDS1		Pigment dispersion syndrome	AD
8q23	GLC1D		Adult-onset POAG	AD
9q22	GLC1J		JOAG	AD
9q34.1	NPS	LMX1B	Nail-patella syndrome	AD
10p15-p14	GLC1E	OPTN	Adult-onset POAG; NTG	AD
11p	NNO1		Nanophthalmos	AD
11p13	AN	PAX6	Aniridia	AD
11q23	NNO2	MFRP	Nanophthalmos	AR
13q14	RIEG2		Axenfeld-Rieger syndrome	AD
14q24.3	GLC3C		Congenital glaucoma	AR
14q24	GLC3D	LTBP2	Congenital glaucoma	AR
15q11-q13	GLC1I		Adult-onset POAG	Complex
15q22	XFS	LOXL1	Exfoliation glaucoma	Complex
15q22-q24	GLC1N		JOAG	AD
19q13.3	GLC1O	NTF4	Adult-onset POAG; NTG	Complex
20p12	GLC1K		JOAG	AD”
AD: ‘Autosomal dominant’ AR: ‘Autosomal recessive’.				

Source: "Fan BJ, Wiggs JL. Glaucoma: genes, phenotypes, and new directions for therapy. J Clin Invest. 2010;120(9):3064-3072. https://doi.org/10.1172/JCI43085"

6.2.7 Linkage Disequilibrium and Admixture Mapping

"Linkage disequilibrium (LD) is a non-random association of alleles or genetic markers at different loci (two or more loci), acting as a sensitive indicator of the population genetic forces, that structure a genome" [76]. "Linkage disequilibrium between alleles affects haplotype frequencies and is linked to mutation time, genetic distance, and population history. It is crucial for association studies and understanding past evolutionary events, inherited diseases, and gene mapping. Factors influencing population LD include genomic region selection, genetic drift, recombination rate, mating system, and genetic linkage. The pattern of linkage disequilibrium signals population genetic processes, with the likelihood of association relying on controlling bias and poor phenotyping. The discovery of disease-related genes through mapping by admixture linkage disequilibrium (MALD) necessitates a map of polymorphic markers that distinguish between founding populations and the variances in allele frequencies of the disease gene". However, 'Admixture mapping' is a new approach to whole genome association mapping that uses long-range LD generated by admixture between genetically distinct ancestral populations. This could be a practical genetic approach in POAG [25]. "It is more robust to allelic heterogeneity and requires fewer markers than case-control association designs. Admixture mapping can be more powerful and achieve higher mapping resolution than traditional linkage studies, provided that the underlying trait variants occur at sufficiently different frequencies in the ancestral populations. However, it has significant cost implications and may not be suitable for all genetic variations" [77].

6.2.8 Genome-Wide Association Studies (GWAS)

Genome-wide genetic approaches, such as linkage analyses and GWAS, have identified loci contributing to POAG disease, including "chromosomal regions and genetic variants associated with POAG and related endophenotypes" [78]. Linkage analysis and association studies are powerful genetic approaches for studying the genetic basis of POAG. GWAS, a case-control study, identifies genes contributing to complex diseases in a population.They are more powerful compared to linkage analysis in discovering genes of small effect that might contribute to the development of POAG [45]. "The study aims to link common genetic variations in the human genome with disease using single-nucleotide polymorphism (SNP) arrays. SNPs, the most common type of genetic variation, are found at a frequency of 1 DNA base in every 1000". They can affect gene function and act as 'biological markers', helping scientists locate genes associated with disease [79] [80]. Although genome-wide association studies have identified more than ten genes associated with POAG on an individual basis, variants in these genes do not predict POAG in populations [7]. GWAS is a method of scanning the entire human genome for SNPs linked to specific genetic traits or diseases. "It has been performed on thousands of human genomes, with results stored in the NIH database" [81]. Association mapping offers high-resolution quantitative traits mapping, but requires extensive knowledge of SNPs. "Careful phenotyping and large numbers of cases are crucial for minimizing errors" [77]. Recent GWAS have identified sequence variants and genetic

loci associated with POAG susceptibility in European and East Asian populations. “These GWAS can identify areas of previously unsuspected pathogenesis, and suggest that most cases of glaucoma may be due to contributions from multiple polymorphisms. GWAS has identified genes causing complex forms of POAG, including ‘CAV1/2, CDKN2B-AS1, ATOH7, SIX1, TMCO1, TLR4, SRBD1, and ELOVL5’, highlighting the genetic complexity of glaucoma. Mendelian genes account for 3-5% of glaucoma, with the rest likely resulting from a combination of risk factors [82]. Recent advancements in linkage analysis and GWAS have identified rare variants in MYOC and OPTN, while common variants in genomic regions have smaller effects”. Despite this, the heritability of POAG remains largely unexplained, accounting for only 5-10% of cases [83].

6.2.9 Modifier Genes

“Modifier genes are genes that affect the phenotypic and/or molecular expression of other genes. Their studies seek to identify genes that can be manipulated, to increase RGC survival, which is critical due to their connections between the eye and the brain” [84]. Modifier genes, act as ‘susceptibility factors that allow other genes or environmental influences, define molecular processes causing glaucoma, providing insights for disease biomarkers and innovative treatments [85]. “Glaucoma is a heterogeneous disorder with Mendelian and ‘multifactorial traits’, requiring ‘multifactorial’ etiologies including ‘polygenic’ and environmental factors”. WDR36, a gene of unknown function, was recently identified as causative for POAG, but its role remains unclear since its abnormalities alone are not sufficient to cause POAG. Studies suggest defects in the WDR36 gene may contribute to POAG as a modifier gene [86]. Current genetic data suggests WDR36 may modify POAG or cause it in certain populations. Variants may mark disease ‘haplotypes’ and influence disease severity. Identifying glaucoma susceptibility and modifying genes is crucial for molecular definition of POAG, as they influence phenotypic variation [40].

6.2.10 Epistasis

Epistasis refers to the interactions between multiple genes that contribute to the development of complex diseases like POAG. These interactions may affect the severity of the disease, with certain combinations of genes increasing or decreasing the likelihood of disease manifestation(Ms_OR_125244). Understanding epistatic interactions is essential for studying the genetic complexity of POAG [40].

6.2.11 Transgenic Mouse Models

Transgenic mouse models have been used to study the role of specific genes in glaucoma development. For instance, transgenic mice expressing the *MYOC* mutation demonstrate retinal ganglion cell loss and increased intraocular pressure, mimicking the clinical features of POAG(Ms_OR_125244). These models are valuable for exploring the underlying mechanisms of POAG and for testing potential therapeutic interventions [89].

6.2.12 RNA Interference and Gene Silencing

POAG is linked to disease-causing gene MYOC mutations, which causes mutants MYOC to misfold and accumulate in ER. This leads to unfolded protein response (UPR), an adaptive mechanism to restore the ER to normal state. If UPR fails, apoptosis is initiated to eliminate unhealthy cells [25] [90]. "RNA interference (RNAi) is a strategy that can reverse the pathological process of trabecular meshwork cells and treat POAG caused by MYOC gene mutation. It can be applied to protein-misfolding diseases and suppress the expression of a single protein. RNAi therapies can be effective at lower concentrations and may be valuable in modeling diseases and studying silencing-specific genes' effects in vitro and in vivo. RNAi is a gene silencing therapy that effectively "eliminates mutant myocilin proteins in trabecular meshwork cells, either mutation-dependent or mutation-independent", through the engineering of small interfering (si) RNA" [25]. RNAi is a conserved 'post-transcriptional' gene silencing phenomenon triggered by the presence of 'short interfering RNAs (siRNAs)', a 'double-stranded RNA molecules'. "siRNA can be effectively delivered to the human trabecular meshwork through 'intracameral perfusion'. This functional delivery can inhibit targeted genes and downstream effectors, potentially enhancing therapeutic applications" [91]. 'Mutation dependent RNAi' involves synthesizing a customized siRNA to target a mutant MYOC sequence, suppressing mutant alleles with a single nucleotide. 'Mutation-independent RNAi' involves siRNA complementary to target mRNA, suppressing both wild type (WT) and mutant alleles. A replacement WT MYOC gene with modified untranslated regions (UTRs) can be generated, allowing WT expression while suppressing mutant alleles simultaneously [90].

7. GENE PENETRANCE, LOCATIONS, AND GENETIC MAPPING IN POAG

Glaucoma is characterized as a complex disease with phenotypic heterogeneity, polygenic inheritance, phenocopies, and incomplete penetrance. The genes associated with glaucoma exhibit various inheritance patterns, including autosomal dominant and autosomal recessive, which can be mapped to specific loci in the human genome using affected pedigrees and standard linkage analysis(Ms_OR_125244).

7.1 Trait and Genotype in POAG Genetics

Genes are segments of DNA that contribute to specific traits or functions by coding for proteins that influence physiological processes. Alleles represent different versions of a gene, with variations at specific nucleotide positions in the genome. The combination of alleles in an individual, known as their genotype, determines their traits (Ms_OR_125244). Several causative genes have been identified in POAG, including *MYOC* (associated primarily with juvenile-onset glaucoma), *OPTN* (linked to low-pressure glaucoma), and others, such as *WDR36*, *NTF4*, *TBK1*, and *ASB10* (Ms_OR_125244).

7.2 Penetrance in POAG

Penetrance refers to the proportion of individuals with a particular genetic variant (genotype) who exhibit the associated trait (phenotype). In genetics, the penetrance of a disease-causing mutation indicates the likelihood that individuals with the mutation will show clinical symptoms(Ms_OR_125244). For example, a mutation with 75% penetrance means that 75% of individuals with the mutation will develop the disease, while the remaining 25% will not show symptoms. In POAG, penetrance can be

influenced by factors such as environment, interactions with other genes, age, and ethnicity(Ms_OR_125244).

7.2.1 Complete Penetrance POAG-Causing Genes

'Complete penetrance' means a trait's genes are expressed in all individuals with the genotype, while highly penetrant alleles produce a trait almost always present in those carrying the allele, indicating disease-causing mutation [96]. "Glaucoma is characterized as a 'complex' disease. 'High penetrance' signifies that most or all carriers of a gene variant will develop POAG. However, some POAG exhibit a classical Mendelian inheritance pattern in which a genotype at one locus is both necessary and sufficient for the phenotype to be expressed" [42]. Recent genetic research links the GLC1A (TIGR) gene MYOC, to some forms of open-angle glaucoma. The MYOC gene, responsible for 3%-4% of POAG cases, was first identified in JOAG their most common mutation, and Gln368Stop mutation highly associated with late-onset POAG. [71]. "The penetrance of MYOC mutations is influenced by factors such as mutation sites, continuous growth, age increase, and ethnic difference". Understanding these rules is crucial for assessing POAG risk in carriers. Previous studies identified "heterozygous MYOC mutations in familial and sporadic POAG patients" [97]. Over 70 MYOC gene mutations have been identified in various racial/ethnic populations and cell cultures, contributing to POAG pathogenesis and numerous SNPs causing or not causing glaucoma [98]. "MYOC glaucoma is the most common form of inherited glaucoma, with about 2–4% of glaucoma worldwide" [6]. POAG is linked to high IOP in early and later onset forms, with MYOC mutations affecting protein trafficking and IOP regulation through "intracellular misfolded MYOC protein formation, leading to decreased outflow through an unclear mechanism" [71]. "One study found that the penetrance of MYOC gene mutation ranged from 16.7% to 100% in different populations" [95]. OPTN, identified in 2002 by Rezaie et al, is linked to POAG and may be responsible for 16.7% of hereditary forms of NTG, with an additional risk factor of 13.6% in familial and sporadic cases [99]. Transgenic mice show '*OPTN*-mediated' glaucoma may result from the GLU50LYS mutation, disrupting the interaction between *OPTN* and Rab8, affecting protein trafficking and retinal ganglion cell apoptosis [24].

7.2.2 Controversial POAG-Causing Genes with High Penetrance

While *MYOC* and *OPTN* are well-established as high-penetrance POAG-causing genes, other genes, such as *WDR36* and *NTF4*, have been controversial. Studies on *WDR36* and *NTF4* have produced conflicting results regarding their role in POAG pathogenesis. These genes have not been consistently associated with glaucoma across populations, making them a controversial group with no established role in POAG " [77].

7.2.3 Incomplete Penetrance POAG-Causing Genes

"Glaucoma is characterized as a 'complex' disease, with a phenotype that exhibits 'heterogeneity', 'polygenic inheritance', 'photocopies', and 'incomplete penetrance'". 'Incomplete/Reduced/Low penetrance allele' signifies that only some or few carriers will develop POAG. This manifests "when some individuals who do not or fail to express the trait, even though they carry the allele" [45]. It is a condition where an allele only

occasionally produces the associated trait, resulting from a combination of genetic, environmental, and lifestyle factors. This makes it difficult for "genetics professionals to interpret a person's family medical history and predict the risk of passing a genetic condition to future generations". Incomplete penetrance occurs when less than 100% of individuals with a particular genotype express the corresponding phenotype [100]. Incomplete penetrance, a condition where environmental factors are difficult to distinguish from genetic factors, can also occur in autosomal dominant individuals. ’Phenocopies’ are individuals with identical phenotypes due to environmental factors, while genotype determines disease probability but does not fully determine outcome [45]. “‘Genetic heterogeneity' indicates that different genes or different genetic mechanisms are involved in different pedigrees. Clinically, ‘genetic heterogeneity’ refers to the presence of a variety of genetic defects causing the same disease” [101]. Incomplete-penetrance genes and risk alleles have been identified in NTG genes such as APOE, TNF, TLR4, OPA1, and TP53, as demonstrated in association studies [102].

7.2.4 Incomplete Penetrance Genes and Risk Alleles in POAG

Certain genes and risk alleles increase the likelihood of developing POAG but do not cause the disease on their own. Examples include variants in *CAV1/2*, *CDKN2B-AS1*, *ATOH7*, *SIX1*, *TMCO1*, *TLR4*, *SRBD1*, and *ELOVL5*. These genetic variants increase susceptibility to POAG, particularly in individuals who carry multiple risk alleles[82]. Genome-wide association studies (GWAS) have identified several of these loci as contributing to POAG risk, though their individual effects are generally small [103].

7.3 Genetic Loci in POAG Genes

Gene responsible for a specific form of POAG has been identified for the first time, “linking to at least 20 genetic loci through human genetic screening” [104]. “Among them, 14 chromosomal loci have been designated from GLC1A to GLC1N by the HUGO Genome Nomenclature Committee (http://www.genenames.org/; ‘GLC': glaucoma, ‘1': primary open angle, ‘A to N': chronological order of genes discovered; 5 of them (GLC1A, GLC1J, GLC1K, GLC1M, and GLC1N) contributed to JOAG, whereas the others contributed only to adult-onset POAG” (Table 3) [74]. Only three to four main genes, each with a locus, are causal genes identified. They are ‘MYOC, OPTN, WDR36 and NTF4’ [104] [11]. MYOC and OPTN are genetically linked to POAG, with “MYOC at locus GLC1A causing high IOP and OPTN at locus GLC1E causing normal-tension POAG. WDR36 at locus (GLC1G) is associated with adult-onset POAG but may modify the disease. Recently, rare mutations in NTF4 have been identified.The NTF4 gene is on locus GLC1O. In addition, four other glaucoma gene loci (GLC1B, GLC1C, GLC1D, GLC1F) have been identified using large, affected pedigrees and Mendelian linkage approaches” (Table 3) [11].

7.3.1 Mapping Gene Loci on Chromosomes in POAG

Nowadays, the positions of genes responsible for various forms of glaucoma have been localised, not just to individual chromosomes, but to specific small regions on those chromosomes [73]. More than 30 chromosomal loci have so far been implicated in POAG, but many of them have failed to be replicated across populations [71]. The

mapping of the gene locus on a chromosome, further confirms that primary open-angle glaucoma may be 'polygenic' [105]. or 'heterogeneous' group of disease, with at least 6 different loci resulting in a similar phenotype. The classification of major POAG gene in affected person carriers, could have ramifications for selecting the most effective treatment regimen for that person [106]. "In particular, 4 genes have been characterized: MYOC on locus GLC1A to chromosome (1q32), OPTN on locus GLC1E to chromosome (10p25), WDR36 on locus GLC1G which might cause some cases of POAG, to chromosome (5q22.3), but sometimes, may not be the causative gene for POAG, but may act as a modifier of the disease and NTF4 on locus GLC1O to chromosome (19q13.3)" [11]. "Thus locus, of the gene on locus GLC1B, associated with cases of A-POAG is mapped within the 2cen-q13 region, but none have been suggested as obvious candidate genes. The A-POAG gene locus, termed (GLC1C), has been described on chromosome 3, 3q21-q24 [107]. A sixth gene for POAG termed (GLC1F) has been described and mapped to chromosome 7q35-q36" (Table 3) [106].

New NTG gene, TANK-binding kinase-1 (TBK1), has been mapped to chromosome 12q14. Copy number variations (CNV)s associated with NTG development have been identified. Linkage studies of autosomal dominant glaucoma families have identified multiple genes responsible for glaucoma "(GLC1A-P., with three new chromosome locations of genes identified for POAG on chromosomes 9q22 (GLC1J) and 20p12 (GLC1K), and on chromosome 5q" (Table 3) [108.

The latest identified loci associated with POAG include the new POAG locus, GLC1Q, located on chromosome 4 at 4q35.1–q35.2. Association studies have revealed several low-penetrance genes and risk alleles in NTG genes, such as apolipoprotein E (APOE) at 19q13.2, TNF at 6p21.3, toll-like receptor 4 (TLR4) at 9q32–q33, optic atrophy 1 (OPA1) at 3q28–q29, and tumor protein p53 (TP53) at 17p13.1. Additionally, CYP1B1 at 2p22-p21 has been linked to JOAG (Table 3) [109].

Table 3: "The review of the genes associated with different forms of glaucoma"

"Genes and Loci Associated with Glaucoma"

"Loci	Chromosome	Gene	Phenotype
GLC1A	1q23-24	MYOC	JOAG, POA
GLC1B	2cen-q13		POAG
GLC1C	3q21-24		POAG
GLC1D	8p23		POAG
GLC1E	10p14-15	OPTN	LTG, POAG
GLC1F	7q35-36		POAG
GLC1G	5q22.1	WDR36	POAG
GLC1H	2p16.3-p15		POAG
GLC1I	15q11-q13		POAG
GLC1J	9q22		JOAG
GLC1K	20p12		JOAG
GLC3A	2p21	CYP1B1	PCG, Peters
GLC3B	1p36		PCG PCG
GLC3C	14q24.3		PCG
RIEG1(IRID2)	4q25-27	PITX2	Axenfeld-Reiger, iridogoniodysgenesis

RIEG2	13q14		Axenfeld-Reiger
IRID1	6p25	FOXC1 (FKHL7)	Axenfeld-Reiger, PCG
PAX6	11p13	PAX6	Anirida, Peters, Axenfeld-Reiger
	15q24	LOXL1	Pseudoexfoliation glaucoma"

"JOAG indicates juvenile open angle glaucoma; LTG, low tension glaucoma; PCG, primary congenital glaucoma; POAG, primary open angle glaucoma".
Source: "Challa P. Glaucoma genetics. Int Ophthalmol Clin. 2008;48(4): 73–94. doi:10.1097/IIO.0b013e318187e71a"

8. MOLECULAR GENETICS AND ADVANCES IN POAG INTERVENTION

The intervention in POAG disease is shifting from a conventional to a genetic approach, offering accurate diagnostic tests for presymptomatic detection of individuals at risk. This allows for the screening of offspring to determine their risk and potentially preventive action. Advances in molecular biology and delivery systems have allowed for targeting genes at cells and tissues, leading to the development of novel interventions and a paradigm shift in glaucoma treatment. "Further advances in the interventions in POAG disease include: 'Neuroprotection', 'neuroenhacement', 'nanotechnology', 'routine screening', RNAi, gene mapping, 'human genome project', 'bioinformatics', 'gene identification technologies', 'gene array technology'" [12].

8.1 Genetic-Based Interventions in POAG

Genetic research has enabled the development of diagnostic tests that can identify individuals at risk of POAG before clinical symptoms appear. Genetic screening allows for the identification of mutations in key genes, such as *MYOC* and *OPTN*, which may indicate an increased risk for POAG. Early detection provides an opportunity for preventive interventions, including lifestyle changes, pharmacological treatments, or surgical options that may delay or prevent disease onset NEW REFERENCE

8.2 Targeted Therapeutic Approaches

As our understanding of POAG genetics deepens, targeted therapies are being developed to address the specific molecular defects that cause the disease. Advances in gene editing technologies, such as CRISPR-Cas9, offer the potential to correct genetic mutations in POAG patients, preventing the misfolding of proteins like myocilin (MYOC) that disrupt aqueous humor outflow(Ms_OR_125244). Gene silencing techniques, such as RNA interference (RNAi), are also being explored as a way to inhibit the expression of disease-causing genes REFERENCE

8.3 Neuroprotection and Neuroenhancement

In addition to targeting the genetic causes of POAG, researchers are exploring neuroprotective therapies that aim to preserve the function of retinal ganglion cells (RGCs) and optic nerve fibers. Neuroenhancement strategies seek to restore the function of damaged cells and promote the regeneration of neural pathways affected by elevated intraocular pressure(Ms_OR_125244). These approaches may offer new hope for slowing or reversing the progression of POAG REFERENCE

8.4 Gene Mapping and Bioinformatics

Gene mapping and bioinformatics tools have become critical in the study of POAG. These technologies enable researchers to analyze large datasets from genome-wide association studies (GWAS) and other genetic screening efforts to identify new loci and mutations associated with POAG(Ms_OR_125244). Bioinformatics also allows for the integration of genetic, environmental, and clinical data, leading to a more personalized approach to glaucoma treatment(Ms_OR_125244).

9. DISCUSSION

The causes of POAG is attributed to an inefficiency of the TM, which disrupts aqueous outflow and increases intraocular pressure (IOP). Genetic researchers corroborated this by suggesting the following mechanisms: Mutant MYOC protein inducing endoplasm reticulum stress, MYOC interaction with mitochondria, TM cells overexpressing Pro370Leu mutant MYOC, and MYOC disregulating calcium channels, leading to reduced outflow and IOP elevation [26].

MYOC and OPTN are linked as mutation-causing genes, which is the genetic basis of POAG. Molecular geneticists have identified several mutation-specific phenotypes, providing information about the pathogenesis and genetic pathways of heritable POAG disease including TGF-β pathway and the transgenic mice model. They further explained these genetic dynamics using other tools and models of molecular genetics, like: "Mendelian autosomal-dominant and autosomal-recessive inheritance, epigenetic theory, linkage analysis, sib-pair analysis, family-based association analysis, and GWAS" [12].

"Glaucoma is a complex disease with 'heterogeneity', 'polygenic inheritance', and 'incomplete penetrance'". This findings are consistent with the previous studies which identified POAG as displaying a "strong heritability but genetically heterogeneous". However, MYOC mutations with a heterozygous genotype in both familial and sporadic POAG patients, has a high penetrance. It is the first to be linked to some forms of open angle glaucoma (OAG). Other high penetrance POAG-causing genes, such as WDR36 and NTF4, have been controversially linked to POAG, raising uncertainty among experts [77].

Genetic studies have identified gene mutations in various populations and established a genetic basis for glaucoma pathogenesis. Several chromosomal regions and genetic variants have been linked to POAG and related "endophenotypes". Linkage analyses and GWAS have been used to identify loci contributing to the disease. "Six causal genes have been associated with POAG at six loci: MYOC, OPTN, WDR36, NTF4, TBK1, and ASB10. Over 30 chromosomal loci have been implicated in POAG, but many have failed to be replicated across populations".

The mapping of gene loci on chromosomes confirms that POAG may be a "polygenic or heterogeneous" group of disease [105], with at least 6 different loci resulting in a similar phenotype. Findings on the gene identifications is sustained by the "Linkage studies of large families with autosomal dominant glaucoma inheritance, mapping the chromosomal locations of multiple genes responsible for glaucoma (GLC1A-P)" [17].

10. CONCLUSION

This review discusses the understanding of the latest advancement in molecular genetics of POAG using the necessary concepts, tools and models. Significant advances have been made in identifying "glaucoma-associated genes" and their associated pathways. It highlights the importance of understanding the role of these genes in POAG and the potential for discovering additional disease-causing genes through linkage analysis. The highlights of the roles of the four genes characterized by MYOC, OPTN, WDR36, and NTF4 in POAG cannot be overemphasized. The review suggests that POAG can be caused by numerous gene mutations in various chromosomal loci, and that it has an autosomal dominant inheritance with incomplete penetrance. The genetic mechanisms and pathways makes the understanding of POAG better, in developing new genetic research interventions, therapy and management which has shifted from the conventional approach to genetic approach.

Future research will likely focus on refining our understanding of POAG's complex genetic landscape, exploring epigenetic influences, and developing more effective interventions. As we continue to uncover the molecular drivers of POAG, there is hope that new treatments will emerge to improve patient outcomes and preserve visión.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

It was not applicable.

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