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 and genetically heterogeneous. The pathogenesis of POAG is the imbalance between the production and drainage of the aqueous humour (AH)". The genetic theories propose the "transgenic mice, demonstrating 'GLU50LYS mutation in optineurin (OPTN)' leading to apoptosis of retinal ganglion cells; mutant myocilin (MYOC) protein inducing 'endoplasmic reticulum (ER) stress' with resultant unfolded protein response (UPR) inducing apoptosis in the trabecular meshwork cells (TMC); the interaction of MYOC with the mitochondria in the trabecular meshwork (TM) and in astrocytes; MYOC causing 'disregulation of calcium channels' leading to 'mitochondrial membrane depolarization in TMC'; overexpression of wild-type MYOC or P370L and Q368X mutants; 'the epigenetics and signaling pathways'; as well as 'histone and deoxyribonucleic acid (DNA) modification'". The pattern of inheritance transmitted as Mendelian trait, often with an 'autosomal dominant inheritance pattern', involves the single-gene forms of glaucoma. These types of mutations 'almost always leads to POAG' and are hardly found in normal eyes. MYOC and OPTN genes are at the fore front as mutations causing genes of POAG while risk alleles is the other class of mutations. POAG links to at least '20 genetic loci through human genetic screening'. Among them, '14 chromosomal loci have been designated from GLC1A to GLC1N, where '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'. Causative genes only reported for three to four major genes, characterized with their loci. They are 'MYOC, OPTN, WD repeat domain 36 (WDR36) and Neurotrophin-4 (NTF4)'. A better understanding of molecular genetics is a great window for future research advances in POAG cure.

Key Words: Primary open angle glaucoma gene; Mutation; Gene locus; Genetic Mapping; Mendelian Inheritance; Gene Penetrance

1. INTRODUCTION

"Glaucoma is a heritable and irreversible degenerative optic neuropathy; while POAG is a genetically complex disorder with multiple risk factors, associated with a 'multifactorial etiology' with unclear mechanisms. POAG is a chronic optic neuropathy, characterized by progressive loss of retinal ganglion cell (RGC), structural damage to the optic nerve head (ONH) and retinal nerve fibre layer (RNFL), with subsequent visual field defects" [1]. POAG is attributed to TM inefficiency, causing aqueous outflow delay, imbalance in AH production and drainage, with a rising IOP [2]. "It is insidious, adult-onset, variable-age, and slowly progressive; with asymptomatic, bilateral, asymmetrical features. It is associated with chronic disabilities, with detection and progression based on 'functional or structural abnormalities in the ONH or the RNFL'" [1]. "Epidemiological studies have shown that POAG is caused, at least in part, by heritable factors and classic risk factors including: Advanced age, ethnicity,

elevated intraocular pressure, family history and ancestry" [1]. Ethnic predisposition and familial patterns suggest that genetic factors significantly contribute to POAG, a complex hereditary disease influenced by mutations in susceptibility genes and environmental factors [3]. Recent advances in molecular genetics have shown that POAG can be caused by multiple gene mutations across different chromosomal loci. POAG of "phenotype OMIM137760" is the most prevalent type of glaucoma characterized by an open anterior chamber angle [4]. "However, genetic studies have established that POAG is genetically 'heterogeneous', and has an inheritance pattern that seems to be 'multifactorial', resulting from the interaction of one or several susceptibility gene and perhaps also environmental factors" [5]. Mendelian trait with 'autosomal dominant' inheritance causes single-gene POAG, with MYOC and OPTN mutations contributing, while risk alleles promote POAG when combined with other risk alleles and environmental factors [6]. Mendelian disorders are caused by a single genetic defect, 'often rare and alone', which can be passed on to children through common inheritance forms like "'autosomal dominant', 'autosomal recessive', and 'X-linked recessive'". Genetic alterations that are significant enough to impair suficiently, as to cause gene malfunction and disease are known as 'mutations' [7]. Mendelian 'autosomal dominant' and recessive glaucoma is caused by single gene defects, while other cases have a 'complex' genetic basis due to multiple genetic and environmental risk factors, both are found in patients and normal subjects [8]. Identifying genes contributing to complex inheritance disorders is challenging, as each individual risk factor may not cause disease independently and may not be present in all cases [7] [9] [10]. Mendelian linkage approaches and small numbers of large pedigrees affected by POAG, identified 7 genetic loci for POAG, with glaucoma predisposing genes in GLC1A-G. These genes account for a small fraction of cases, reflecting the Mendelian trait. Mutations in genes like MYOC, OPTN, and WDR36 cause 'autosomal dominant' Mendelian POAG in families, contributing to only 6% of POAG cases in the general population [6]. Over 30 chromosomal loci have been implicated in POAG, but many have not been replicated across populations. "The mapping of gene loci confirms that POAG may be 'polygenic' or 'heterogeneous group of diseases', with at least 6 different loci resulting in a similar phenotype. Four genes have been characterized: MYOC, OPTN, WDR36, and NTF4. A new NTG gene, TKBK, has been mapped to chromosome 12q14. Linkage studies have identified three new chromosomal locations of POAG genes" [11]. The understanding of molecular genetics for POAG has been enhanced through various tools like "'Mendelian autosomal-dominant inheritance', 'epigenetics', 'linkage analysis', 'sib-pair analysis', 'family-based association analysis', 'candidate gene', 'genome-wide association studies', 'modifier gene', 'transgenic mouse model', 'gene silencing', and 'RNA interference'" [12].

2. EPIDEMIOLOGY OF POAG: THE GENETIC PERSPECTIVE

"Glaucoma, a complex disease affecting 70 million people globally, is the second leading cause of blindness, with genetic contributions still unknown in over 95% of the cases". It is the third most prevalent visual impairment among white Americans and black Americans [13]. Undiagnosed glaucoma affects 50% of cases in the western world, with higher rates in specific ethnicities, and up to 90% in developing countries, according to various studies [14]. Glaucoma prevalence varies by race/ethnicity/ancestry, with Europe having the highest prevalence at 2.2%, China at 2.7%, Japan at 3.7%, Latin America at 3.4%, and Africa at 4.3% [15].

Over 2.25 million Americans aged 40 and older suffer from POAG, the most prevalent form of glaucoma, affecting over 33 million people globally [1]. "Population-based studies suggest a genetic basis for POAG, with inherited and familial cases accounting for 72% of cases. Heritability varies from 13% to 93%, with first-degree relatives having a 9-fold increased risk and carrying a lifetime glaucoma risk of 22%, compared to 2–3% for normal controls" [16]. POAG is complex, with less than 10% following a Mendelian pattern, and 95% caused by multiple genetic and environmental risk factors [17]. Defects in the POAG gene MYOC are linked to 'early-onset' POAG, affecting up to 20% of patients and 3%-5% of patients with 'adult-onset'. Gene variants may contribute 5% of POAG and NTG cases, with 95% genetic contribution unknown [18].

“Prevalence of glaucoma varies among ethnic groups, with black Africans being five times more likely to develop the disease early compared to Europeans. Blacks have a more aggressive form, and up to six times more susceptible to optic nerve damage” [14].

3. BIOLOGICAL PATHWAYS AND MECHANISMS IN POAG

Although the exact mechanism of POAG has not been completely elucidated, there are several theories regarding to the events.

3.1 Pathological Theories and Mechanisms

3.1.1 The vascular theory

“The vascular theory proposes vascular dysfunction with the onset of ischaemia which triggers cell death to the optic nerve, whether induced by elevated IOP or as a primary insult”.

3.1.2 Mechanical compression theory

The mechanical compression theory suggests axon dysfunction due to cribriform plate compression, caused by elevated IOP. This causes backward bowing of lamina cribrosa, potentially leading to focal ischaemia, neurotrophin deprivation, or ‘axoplasmic flow’ interference, ‘triggering cell death’ [19].

3.1.3 RGC pressure

This mechanism suggests that RGC pressure, followed by ischaemia and hypoxia, leads to death due to ‘glutamate-induced excitotoxicity’, increased inflammatory mediators, and trophic factor flow alteration. The events result in the obstruction of both ‘anterograde and retrograde’ axonal transport, resulting in ‘axotomy’ and blindness [20].

3.1.4 Elevated IOP

Elevated IOP triggers the expression of ‘heat shock proteins’, triggering immune cells like ‘memory T cells’ to attack retinal neurons, leading to optic nerve degeneration and vision loss. This autoimmune response, similar to bacterial infections, is hypothesized to contribute to glaucoma. [21].

3.1.5 Biosynthesis of prostaglandin

“In the biosynthesis of prostaglandin (PG), Cyclooxygenase-2 (COX-2) is considered a rate-limiting enzyme. There is this association between POAG with loss of COX-2 expression in aqueous humor–secreting cells In the eye” [22].

3.1.6 Pressure-induced injury of the ONH

RGC death is the final outcome of all pathogenic mechanisms causing POAG. The ‘putative mechanisms’ of how this occurs are legion. They include ‘pressure-induced injury’ of the ONH, leading to retinal gene expression alterations, ’astrocyte response’ to changes in IOP, oxidative stress, mitochondrial dysfunction, neurotrophic factors and autoimmunity” [23].

3.2 Genetic mechanisms

3.2.1 Genetic predisposition

Genetic theories suggest that genetic predisposition triggers axon cell death, releasing neurotransmitters like glutamate which causes ‘excitotoxicity’. Then the environmentally released calcium, free radicals, and nitric oxide, triggering ‘secondary apoptosis’ in neighboring cells [19].

3.2.2 GLU50LYS mutation in optineurin

“Transgenic mice have demonstrated that the ‘GLU50LYS mutation in optineurin’ leads to apoptosis of retinal ganglion cells. They have suggested that ‘optineurin-mediated’ glaucoma may result from a disruption of an interaction between OPTN and a GTP-binding protein Rab8, and its effects on protein trafficking” [24].

3.2.3 Unfolded protein response

Mutant MYOC protein induces endoplasmic reticulum (ER) stress, with resultant unfolded protein response (UPR) inducing apoptosis in the trabecular meshwork cells, leading to increase in resistance to aqueous humor outflow, elevated IOP and ultimately glaucoma [25].

3.2.4 Interaction of MYOC with mitochondria

"The interaction of MYOC with the mitochondria in the TM and in astrocytes appears to be cell specific. TM cells overexpressing Pro370Leu mutant MYOC, demonstrate features of mitochondrial dysfunction, which may increase vulnerability of TM cells to cellular insults causing impaired function and even cell death" [26].

3.2.5 Disregulation of calcium channels

MYOC causes 'disregulation of calcium channels' causing 'mitochondrial membrane depolarization' in TM cells, TM contraction, and subsequently reduced outflow and IOP elevation [26].

3.2.6 Overexpression of wild-type mutant MYOC

"Overexpression of wild-type MYOC or P370L and Q368X mutants, caused an inhibition of neurite outgrowth in neuronal cells and may contribute to the development of neurodegenerative glaucoma" [27].

3.3 Epigenetics and Signaling Pathways

"Epigenetics is the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself". It involves heritable changes in gene expression that do not entail "alterations to the underlying DNA sequence (a change in phenotype without a change in genotype) which influences how cells interpret genes". These changes are natural and regular, but can also be affected by factors like age, disease state, environment and lifestyle. [28]. Epigenetics, in collaboration with predisposing genetic and environmental factors contribute to increased risk of POAG. These factors work through several pathways, including 'TGF-β, Rho kinase, Calcium-Calpain signalling' and others [12]. These pathways collectively result in the 'upregulation of proapoptotic' gene expression, the 'downregulation of neuroprotective' and 'prosurvival factors', and the generation of fibrosis at the trabecular meshwork, which may block aqueous humor drainage.

3.2.1 Histone and DNA modification

Epigenetics refers to heritable genetic changes that activate or suppress genes, such as 'histone acetylation' and 'DNA demethylation'. It can also involve repressive changes like 'histone deacetylation' and 'DNA methylation' with modifications induced by 'noncoding RNAs' such as 'microRNA' and 'long noncoding RNA (lncRNA)', which modulate gene expression and/or alter cellular signaling pathways, potentially affecting individual disease susceptibility. Glaucomatous eyes are linked to 'hypoxic environments', where 'hypoxia-Inducible Factor 1-α (HIF1-α)' travels to the nucleus to regulate gene expression. This is 'facilitated by the HIF1-α promoter', which has a 'methylated HIF Response Element' leading to 'epithelial-to-mesenchymal transition', causing fibrosis and increased IOP [31]. "Disturbances in retinal development regulation can cause glaucoma, optic neuritis, and hereditary RGC degeneration. 'Histone lysine methyltransferases' promote RGC survival by 'methylating histones', while acute optic nerve injury increases nuclear histone deacetylase 3 activity in dying RGCs" [32]. Glaucoma may be influenced by epigenetic forces in lamina cribrosa cells. Studies show increased global DNA 'methylation' in glaucomatous eyes and 'upregulating genes involved in extracellular matrix production'. However, 'unmethylated DNA in the transforming growth factor-β 1 promotor region causes increased transcription of TGF-β'. This may allow uninhibited genes promoting fibrosis [33].

3.2.2 Brain derived neurotrophic factor [BDNF] and other neurotrohpic factors.

The brain and retina among other organs that supports the growth, differentiation, and survival of neurons produce a protein called BDNF, which is very important for RGC survival. Ordinarily, BDNF and other neurotrophic factors are transported from the brain to the RGCs. In glaucoma however, the raised IOP blocks axonal transport at the optic nerve head, decreasing neurotrophic levels in the RGCs. The loss of BDNF in these cells contributes to cell death and thus glaucoma through JNK activation and c-Jun phosphorylation, which

eventually leads to caspase activation [34].

3.2.3 Epigenetics pathways in collaboration with other predisposing factors

3.2.3.1 TGF-β Signaling pathway

"Corticosteroids can increase IOP in glaucoma patients, suggesting that TIGR may be a gene responsible for outflow obstruction. TGF, a cytokine involved in signaling cascades, is particularly relevant to the eye due to its role in POAG pathogenesis. Increased TGF-2 in glaucomatous eyes contributes to structural changes in the extracellular matrix, causing fibrosis and blocking aqueous humor outflow. Patients with POAG have significantly higher levels of TGF-2 in the aqueous humor" [35].

3.2.3.2 Calcium-calpain pathway

"Disruptions in calcium homeostasis occur in many 'neurodegenerative diseases', including glaucoma. The increased IOP in this disorder intensifies the influx of extracellular calcium into RGCs. Calcium activates calpain, a cysteine protease that cleaves calcineurin. 'Calcineurin' goes on to trigger apoptosis in RGCs via dephosphorylation of BAD and the release of Cytochrome C" [36].

3.2.3.3 Rho signalling pathway

"Rho GTPase/Rho kinase signaling in the trabecular outflow pathway increases IOP by altering the contractile cell adhesive, and permeability barrier characteristics of trabecular meshwork and Schlemmer's canal tissue. 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'. [37].

4. CONCEPT OF GENE ALTERATIONS IN POAG GENETICS

"The pattern of inheritance transmitted as Mendelian trait, often with an 'autosomal dominant inheritance pattern' involves the 'single-gene forms of glaucoma'. These types of mutations 'almost always' leads to POAG and are rarely observed in normal eyes. MYOC and OPTN genes are at the fore front as mutations causing genes of POAG while 'risk alleles' is the other class of mutations". They promote the development of POAG when combined with other glaucoma 'risk alleles' and environmental factors but do not cause disease on their own [6].

4.1 Mutation

"Mutation is genetic alteration, sufficient to cause a gene to malfunction and result in disease" [7]. Gene mutation is the genetic basis of POAG, and POAG causing mutations are grouped into two distinct classes with very unique characteristics. "The first class of mutations are capable of 'causing' POAG on their own with little influence from other genes or the environment. These single-gene forms of glaucoma, follows the pattern of inheritance transmitted as Mendelian trait, often with an autosomal dominant inheritance pattern. These types of mutations almost always leads to POAG and are rarely observed in normal eyes. Examples of mutations that 'cause' POAG Mutations are in the MYOC and OPTN genes. 'Risk alleles', is the other class of mutations. This other class of mutations may promote the development of POAG when combined with other glaucoma risk alleles and environmental factors but do not cause disease on their own" [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 information about the pathogenesis of heritable eye diseases at the most basic level. For example, disease-causing genes may be part of important biological pathways, that once identified, may help clarify the mechanisms that lead to disease. This will also enable the design of DNA-based tests that may help physicians assess their patient's risk for disease and may also differentiate between clinically similar disorders. Specific mutations responsible for a patient's disease solidifies the diagnosis, and may also help predict likely clinical course. Several mutation-specific phenotypes of hereditary eye diseases including glaucoma, have already been reported [8]. The disease-causing mutation in Mendelian POAG offers the potential for targeted therapy to fix the specific molecular defect caused by the mutation. Myocilin mutations may result in misfolded MYOC protein, accumulating in trabecular meshwork cells resulting in an adverse effect [39].

4.3 Glaucoma-causing Genes

“The POAG disease-causing genes, namely: MYOC, OPTN, and TANK binding kinase 1 (TBK1) have given insights into glaucoma pathogenesis. Mutations in each of these three genes, may cause POAG inherited as a Mendelian trait. MYOC mutations cause 3–4% of POAG cases with IOP >21 mmHg, while mutations in ‘OPTN, TBK1, and MYOC’ each cause about 1% of POAG with IOP ≤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 Pattern 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 Simple/Single genetic mutation 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 trait 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].

5.4.1 Adult-Onset POAG

Model free linkage analysis genomic studies identified adult-onset POAG susceptibility genes in white US sibling pairs, identifying 7 genomic regions. Recent follow-up showed additional POAG-susceptibility loci on '14q11 and 15q (GLC1I)'. A study in Barbados identified two chromosome regions on chromosomes 2q and 10p as significant risk factors for POAG in sibling pairs affected by POAG. The study also found loci on '5q and 14q' in West Africans. These findings suggest that 'SNP-based technologies' are effective for adult-onset POAG, highlighting the importance of understanding complex diseases [49]. "Mendelian linkage approaches and small numbers of large pedigrees affected by POAG described 7 genetic loci

for POAG (GLC1A-G), and identified glaucoma predisposing genes in three loci: GLC1A, myocilin7, GLC1E, optineurin9, and GLC1G, WDR36. These genes are responsible for a small fraction of POAG cases, reflecting the small percentage of POAG inherited as a Mendelian trait rather than as a complex trait. Mutations in genes associated with glaucoma, such as MYOC, OPTN and WDR36, have been reported to cause autosomal dominant Mendelian POAG in the studied families" (Table 1) [50]. While a mutation in one of these genes may completely explain the development of POAG in some families collectively, mutations in these genes contribute to only around 6% of POAG cases in the general population [6]. "In a recent family study, TANK binding kinase 1 (TBK1) was identified as another cause of Mendelian POAG" [16]. It is the duplication of this gene and the resultant increase in function that appears to be causing the glaucomatous process, rather than a mutation within the gene.

Table 1: Summary of glaucoma genes, characteristics, inheritance patterns and age at onset

Gene	Locus	Location	Phenotype	Inheritance Pattern	Age at Onset
MYOC	1q24.3	Chromosome 1	Primary open-angle glaucoma (POAG)	Autosomal dominant	Adult
CYP1B1	2p22.2	Chromosome 2	Primary congenital glaucoma (PCG)	Autosomal recessive	Infant
OPTN	10p13	Chromosome 10	Normal-tension glaucoma (NTG)	Autosomal dominant	Adult
FOXC1	6p25.3	Chromosome 6	Axenfeld-Rieger syndrome	Autosomal dominant	Variable
PITX2	4q25	Chromosome 4	Axenfeld-Rieger syndrome	Autosomal dominant	Variable
LTBP2	14q24.3	Chromosome 14	Primary congenital glaucoma (PCG)	Autosomal recessive	Infant
TBK1	12q14.2	Chromosome 12	Normal-tension glaucoma (NTG)	Autosomal dominant	Adult

Sources: "Gemenetzi M, Yang Y, Lotery AJ. Current concepts on primary open-angle glaucoma genetics: a contribution to disease pathophysiology and future treatment. Eye. 2012;26:355–369". "Wiggs JL. Genetic Etiologies of Glaucoma. Arch Ophthalmol. 2007;125(1):30-37"'. Courtesy: As Amended.

5.5 Risk alleles in POAG mutation

"The risk factors for POAG include elevated IOP, advancing age, genetic disposition, environment, family history of POAG, African ancestry, myopia, and perhaps the presence of certain systemic diseases such as diabetes and hypertension. Other risk factors are: race, refractive error and central corneal thickness". "The etiology of POAG is 'multifactorial', and it was first proposed in 1967, as it demonstrates a variable age of onset and severity. More than 25 of these POAG risk factor genes have been discovered to date and more remain to be identified". IOP is considered the most important risk factor in POAG. However, these risk factors alone do not cause glaucoma [51].

5.5.1 Multiple genetic influence, familiarity and family history

Several genes associated with POAG have been identified, confirming the genetic influence and key roles in the development of POAG, "though accounting for less than 5% of all POAG in the general population". The hereditary aspect of POAG is likely to be 'polygenic' and that 'gene-environment interactions' are important**.** "Various disease-causing mutations in OPTN, MYOC, and WDR36 genes have been long identified as the cause of familial forms of POAG" [52]. "The US surgeon general has affirmed the importance of the awareness of the medical value of family history and that familial aggregation cannot be over emphasized. The genetic contribution to POAG has long been recognized, with a strong genetic component having a large proportion of inherited and familial cases" [9]. Studies reveal that 16-20% of POAG risk is due to genetic factors, affecting 'first and second-degree relatives', governed by a complex inheritance pattern with evidence of 'gene-gene interaction' [29]. Studies like the Baltimore Survey show that 50% of patients with POAG have positive familiarity, indicating a significant genetic defect in the pathology. Siblings of glaucoma patients have a 10-fold increased risk of developing the disease, with European-derived sibling having a 10% chance and African American sibling having a 20% chance. This highlights the importance of genetics and familiarity in the development of glaucoma [1]. Family history is a significant risk factor for POAG, with first-degree relatives of POAG patients having a 9-fold increased risk of developing glaucoma in their lifetime in The Rotterdam Study. Population-based studies show familial clustering of POAG cases, suggesting a genetic basis. Early twin studies reported a 13% heritability, but recent studies estimate it to be 70% and more recently, 93%. The Rotterdam Study and other population-based studies have shown a genetic basis for POAG [38]. The risk of developing glaucoma in relatives of patients with POAG is 22%, compared to 2–3% for normal controls. A first-degree relative with POAG is a risk factor, with odds ratios ranging from 3 to 13. The risk is higher still if the affected relative is a sibling. Siblings of affected patients are at the highest risk of developing POAG compared to parents or children. With 56% of probands having familial or inherited POAG, approximately 72% of all POAG cases are predicted to have this condition [53].

5.5.2 Environment

Significant advances have been made in identifying glaucoma-associated genes and their associated pathways. "Glaucoma is a heterogeneous group of disorders with both Mendelian and multifactorial traits" [54]. Several studies suggested an 'autosomal dominant inheritance with incomplete penetrance. However, the inheritance pattern of this disorder seems to be multifactorial resulting from the interaction of one or more genes and/or environmental stimuli". Even within individual families, there could be large variations in the phenotypic presentation of gene mutations. Therefore, other 'multifactorial etiologies' must be involved in glaucoma development. This can include 'polygenic and environmental factors'. Some genes may act as 'susceptibility factors' that allow other genes or environmental influences to produce glaucoma. "There are certain mutations necessary, but not sufficient to cause the disease. Such mutations requires other additional genetic defects or environmental factors to be fully manifest" [10]. "There are certain environmental factors that could raise IOP, like high wind instruments, coffee, certain yoga positions, tight neckties, and weightlifting, that seem to contribute to glaucoma" [55]. "To date, there have been over 20 genetic loci and 3 genes, MYOC, OPTN, and WDR36 that have been linked to POAG" [29] [54].

5.5.3 Age

"Glaucoma prevalence increases with age, with 1% in white patients under 40 and 2% to 5% in those over 75, and 1% and 11% respectively in patients of African-descendant. In the US, 3-6 million people, including 4- 10% of those over 40, are at risk due to elevated IOP of 21

mm Hg or higher, with 0.5-1% per year of those with elevated IOP developing glaucoma over 5-10 years" [1]. POAG is an open-angle glaucoma caused by genetic predisposition and age, with some mutations being more common in adult-onset patients. Defects in the MYOC gene, which codes for myocilin protein, were initially associated with early-onset POAG. However, certain MYOC mutations are more common in older patients. Adult-onset POAG typically occurs after age 50 and is associated with elevated IOP. "Adult-onset glaucoma often occurs in multiple family members (familial aggregation) , but does not follow a clear Mendelian inheritance pattern". Multiple risk factors and/or environmental factors may be responsible for this disease in older individuals [56].

5.5.4 Race and ethnicity

Ethnic background is crucial in clinical practice and genetic research. It helps estimate disease prevalence and mutation frequency, ethnicity and familial aggregation on given considerations for better results. "POAG heritability is determined by significant variation between races and ethnicities, with African Americans and Hispanic Americans being five-fold more common than Caucasians" [38]. The MYOC gene mutation frequency in 'probands' with POAG is similar among Caucasians, African Americans, and Asians. A recent study found a higher frequency of MYOC mutations in the African-American general population due to their higher prevalence of POAG than Caucasians. "However, the higher prevalence of POAG in African Americans was not due to a higher frequency of MYOC mutations in their general population" [6]. "People of African descent are disproportionately affected by POAG. It develops earlier, presents with greater severity, and progresses more rapidly in them. Blacks with POAG also reach adverse end points more frequently, including worse visual fields and optic disc cupping, blindness, vision-related decrease in quality of life, and increased mortality" [57].

5.5.5 Other associated risk factors in POAG

POAG is influenced by, in addition to IOP, other factors such as biology, environment, socioeconomic status, lifestyle, and eye health. It is prevalent among U.S. blacks and has been observed in diabetic patients. However, there is debate over whether diabetes is a genuine risk factor for POAG, as extensive population studies have reported no link. These factors contribute to the high prevalence of glaucoma among U.S. blacks. Blood pressure plays a complex role in POAG development, with hypertension potentially causing glaucomatous damage and low blood pressure reducing optic disc perfusion pressure. There is limited data on lifestyle and nutritional epidemiology of POAG. Despite this, a diet high in green collards and omega 6 to omega 3 fatty acids may be protective against POAG, suggesting exercise and green collard-rich diets [58]. The prevalence of POAG shows sex-disparity, with women accounting for 59% of glaucoma cases. Systemic diseases like hyperlipidemia, obstructive sleep apnea, and thyroid disease are considered risk factors. Exercise, antioxidants, and a diet rich in omega-6 and omega-3 fat may decrease risk [55].

5.5.6 Epigenetic regulation

Epigenetic regulation is an important causal factor for glaucoma, as implicated by emerging research. Epigenetics, genetics and environmental factors, influences the signaling pathways that are ultimately responsible for disease progression [29].

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

6.1 The Concepts of Molecular Genetics.

"While most of the molecular mechanisms leading to the development of POAG remain unknown, genetic studies identified gene mutations to be associated with the disease". These mutations result in retinal ganglion cell death, a common outcome in glaucoma pathogenesis. Discovery of the differing genetic causes of POAG, may lead to an improved classfication of the condition based on the precise causative genetic mutation rather than the current vague clinical classfication [59]. The fact that glaucoma could be inherited, presents the possibility of studying the disease using several tools, models and approaches to molecular genetics.

These provide the opportunity for further identification specific mutations causing genes, associated with the disease.

6.2 Tools, Models and Approaches in understanding POAG

6.2.1 Mendelian autosomal-dominant and autosomal-recessive

"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 helps understand the genetic basis of diseases like glaucoma by studying siblings' genetic segments. It identifies susceptibility loci and disease mechanisms, allowing for the identification of shared genomic segments. Sib-pair analysis uses genetic markers to identify genetic loci in siblings. It requires high penetrance to identify specific phenotypes. Common test statistics include the 'Concordant-only' and 'Concordant-discordant' tests. Efficiency is influenced by factors like "affected siblings, genotyped sibling pairs, risk attributable to linked and unlinked loci, genotyping unaffected siblings, and multipoint analysis for fine mapping" [64]. This method can be used by researchers to determine the genetic basis of complex features.

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 gene

"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

It is becoming increasingly apparent that human complex disorders arise because of 'multiple genetic interactions (epistasis)' and gene environment interactions. Epistatic interactions

caused by modifier genes may make the disease more severe or less severe. Genes that contribute to POAG may not cause clinical evidence of the disease unless they are coupled with other genes or environmental factors. If disease features are dependent on the combined effects of multiple factors then the identification and characterization of any one disease-predisposing factor can be difficult when using traditional linkage approaches [40].

6.2.11 Transgenic mouse model

Myocilin, a human gene responsible for POAG, has been studied extensively, with a mouse strain expressing the Tyr423His MYOC point mutation being developed for further research. [87]. The MYOC model at 18 months showed "retinal loss, axonal degeneration, and increased IOP. A transgenic mouse strain with collagen type I mutation showed open angles, progressive ON axonal loss, and IOP elevation, suggesting an association with collagen turnover" [87] [88]. Transgenic mouse models using 'TGF-b2 and CTGF' can induce elevated IOP and glaucoma development, providing new insights into POAG pathogenesis, as 'TGF-b2 levels are elevated in AH'. On the other hand, "aged, overexpressing mutated OPTN transgenic mice show retinal changes without increased IOP", making them a valuable model for studying normal-tension glaucoma and identifying new drug targets to regulate outflow pathways effectively [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 a phenotype that exhibits 'heterogeneity', 'polygenic inheritance', 'photocopies', and incomplete penetrance. The Genes associated with glaucoma types exhibit 'autosomal-dominant', 'autosomal-recessive', and other 'mendelian inheritance patterns', can be in the human genome using large, affected pedigrees (typically at least 11 members) and standard linkage analysis" [10].

7.1 Trait and gynotype in POAG Genetics

"Genes are chunks of DNA that contribute to particular traits or functions by coding for protein that influence physiology. Alleles are different versions of a gene, which vary according to the nucleotide base present at a particular genome location. An individual's combination of alleles is known as their genotype". Genes determine individual traits, like the organism's genotype and it is one in number per genus locus, while alleles contribute the diversity in phenotype expression, like the organism's phenotype, and they are two in number per genus locus. An organism's genotype consists of its entire set of genes [92]. Causative genes identified as

having association with POAG at six loci have been reported. “MYOC, primarily mutated in juvenile-onset subjects, whereas OPTN, is mainly mutated in low pressure POAG individuals. Others are WDR36, NTF4, TANK binding kinase 1 (TBK1), and Ankyrin repeat and SOCS-box containing 10 (ASB10)” [93].

7.2 Penetrance in POAG

Penetrance is the likelihood of a gene or trait being expressed, and despite a dominant allele, not all humans with the allele will display the condition. “In genetics it is the proportion of individuals carrying a particular variant (or allele) of a gene (genotype) that also expresses an associated trait (phenotype)”. It focuses on the degree of trait displayed, the correlation between genotype and phenotype, and the relationship between observable physical characteristics and specific genes. “In medical genetics, the penetrance of a disease-causing mutation is the proportion of individuals with the mutation that exhibit clinical symptoms among all individuals with such mutation. For example: If a mutation in the gene responsible for a particular autosomal dominant disorder has 75% penetrance, then 75% of those with the mutation will go on to develop the disease, showing its phenotype, whereas 25% will not” [94] [12]. Penetrance only refers to an individual's ‘phenotypic signs or symptoms’, not variable ‘expressivity’, which varies depending on the extent or degree of the disease-causing mutation [94]. Their likelihood of POAG disease is influenced by factors such as environmental factors, interactions with other genes,age and ethnicity [95]. The literature on glaucoma genetics categorizes penetrance into “established glaucoma-causing genes, controversial genes, and low-penetrance risk alleles” which contribute to disease likelihood, don't cause disease on their own but depends on other factors [77].

7.2.1 Complete penetrance POAG-causing genes

MYOC and OPTN

‘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 Complete penetrance POAG-causing genes with controversy

WDR36 and NTF4

“Primary open-angle glaucoma displays a strong heritability but is genetically heterogeneous”. WDR36 and NTF4 are high penetrance POAG-causing genes with controversy, with rare mutations in a novel gene neurotrophin-4 (NTF4), linked to POAG. This has led to uncertainty about their role in glaucoma pathogenesis. Many studies have failed to substantiate WDR36

and NTF4 as ‘glaucoma-causing genes’, making them a “controversial group with no established role in glaucoma pathogenicity” [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 POAG-causing genes, and risk alleles

Genes like CAV1/2, CDKN2B-AS1, ATOH7, SIX 1, TMCO1, TLR4, SRBD1, and ELOVL5 [82] increase the risk of POAG or NTG. “A GWAS in Iceland found one variant, rs4236601[A], near CAV1 and CAV2. GWAS in Australians of European descent identified two susceptibility loci for advanced POAG” [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 chromosome locations (regions) 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’”, etc [12].

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.

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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