Review Article

The Genetics of PrimaryOpen Angle GlaucomaInterventions: Therapeutic Directions, and FuturePredictions.

ABSTRACT

Primary open angle glaucoma (POAG) is a multifactorial chronic optic neuropathy, characterized by progressive loss of retinal ganglion cells (RGC), structural damage to the optic nerve head (ONH) and retinal nerve fiber layer (RNFL), with visual field defects. The major risk factors are high intraocular pressure (IOP), age, genetics, family history, race, etc. The pathogenesis of POAG includes, pressure induced injury of the ONH, leading to retinal gene expression alterations. The resultant fluid back-up increases the IOP with consequent optic nerve damage, causing POAG.Genetic studies shows that POAG is genetically heterogeneous, caused by several susceptibility genes and environmental factors. A total of 12 chromosomal loci, designated as GLC1A to GLC1L, have been mapped for POAG, with three genes, myocilin (MYOC), optic neuropathy-inducing protein (Optineurin (OPTN)),) and WD repeat domain 36 (WDR36), being identified, as the GLC1A, GLC1E and GLC1G genes respectively. A better understanding of the molecular genetic pathways and the pathological mechanisms involving the disease-causing genes, may help clarify thepathophysiology that leads to the disease, and a targeted treatment. The role of genetics in POAG highlights the importance of gene in recent research advances, its future directions, applicationsand therapy. The advent of modern geneticdiscoveries and future directions in vector engineering,makesthe cure forPOAG possible. The paradigm shift in glaucoma treatment has moved from direct RGC and ONH therapy to targeting associated brain centers.

Key Words: Primary open angle g*laucoma:* g*laucomagene; Mutation; Gene locus;Gene therapy; Genetic Mapping; Mendelian Inheritance; Gene transfer vector transduction,Vector Engineering.*

1.0. INTRODUCTION

POAG is a form of glaucoma, with a complex disorder associated with a multifactorial etiology. It is a chronic optic neuropathy, characterized by progressive loss of RGC, leading to structural damage to the ONH and RNFL, with visual field defects [1]. Though the cause is unknown, POAG is highly attributed to an “inefficiency” of the trabecular meshwork (TM), retarding the aqueous outflow facility, creating an imbalance in the production and drainage of the aqueous humour (AH), which raises the IOP [2].

Gene mutation is the genetic basis of POAG,and these POAG mutationcausing genes 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. Thegenes that cause POAG mutationsare the MYOC and OPTN genes. Risk alleles, is the other class of mutations that may promote the development of POAG when combined with other glaucoma risk alleles and environmental factors; but do not cause disease on their own [3].

POAG has about a total of 12 chromosomal loci, mapped and designated as GLC1A to GLC1L. Three genes, MYOC, OPTN and WDR36, have been identified respectively, as the GLC1A gene on 1q23-q25, GLC1E gene on 10p15-p14 and GLC1G gene on 5q22.1. Among them, OPTN is linked particularly to NPG cases. Mutations in OPTN have been found in 16.7% of families with hereditary POAG. Approximately 80% of those families had the most prevalent E50K mutation. Of the E50K-affected subjects, 18% had high and the remaining had normal IOP values [4].

There are wide range genetic techniques and approaches to treatment of POG and future cure; including genetic screening,gene manipulations and the use of various models towards future therapeutic directions. Recently, novel therapeutic approaches including promising strategies based on gene therapy are part of the search for an efficient way to overcome neurodegeneration, focusing directly on preventing cell death and ensuring axonal integrity. With routine geneticscreening for disease susceptibility, appropriatediagnosis of glaucoma in patients could be highly beneficial.The establishment of clinical features that define glaucoma phenotypes associated with specific mutations (genotypes), before a useful clinical information can be acquired from DNA-based diagnostic testing becomes useful. The genetic testing for MYOC and OPTN mutations makes the understanding of the underlying pathophysiology clearer, and leads to the development of new DNA-based diagnostic tests and therapeutic approaches [5]. Advances in the field of vector engineering during the past few years have yielded safe delivery systems that do not produce an inflammatory response, are able to act locally and have negligible distribution to organs other than the targeted eye [6].

2.0. THE GENETIC PREVALENCE

Glaucoma is the most common cause of preventable, irreversible blindness worldwide. It is the second leading cause of blindness worldwide, affecting around 70 million people, with over 2.5 million people in the USA alone. It is the third most prevalent cause of visual impairment and blindness among white Americans, and the leading cause of blindness among black Americans [7]. POAG on the other hand, is responsible for 12.3% of blindness worldwide. In people older than 40 years old, the prevalence of POAG is 1.86%. POAGprevalence varies greatly among ethnic groups, and is up to five times more frequent in black African populations compared to Europeans. Prevalence of POAG is 3-4 times higher in blacks than in Caucasians; in addition, blacks are up to 6 times more susceptible to optic nerve damage than Caucasians [8]. In particular, persons of African ancestry have three to five times increased risk of POAG, and have a more severe course of disease with a higher risk of blindness [9]. Published data estimated that inherited and familial POAG cases may account for approximately 72% of all POAG cases.Population-based studies have also shown familial clustering of POAG cases that suggest a genetic basis;first degree relatives have a 9-fold increased risk, carrying a lifetime glaucoma risk of 22%;while early twins studies reported the heritability of POAG to be approximately 13%. Recent and much larger studies have estimated POAG heritability to be 70%; and more recently, 93%. Relatives of POAG patients have a 22% risk of developing glaucoma at some point in their lives, whereas the risk for the relatives of the normal controls is 2–3% [10]. Genetics and familiarity in some studies, among them the Baltimore Survey; 50% of the patients suffering from POAG had a positive familiarity, suggesting the genetic defect as important, in the development of the pathology [1].

3.0 GENETICMECHANISMS AND PATHWAYSIN POAG

3.1. Genetic mechanisms

(i). Genetic theories propose that genetic predisposition triggers cell death of axons. This releases substances like glutamate a neurotransmitter that causes excitotoxicity. Other substances such as the calcium, nitric oxide, and free radicals are also released into the environment and cause a secondary triggering of apoptosis in neighboring cells [11].

(ii). Transgenic mice have demonstrated that the GLU50LYS mutation in OPTN, leads to apoptosis of retinal ganglion cells. They have suggested that OPTN-mediated glaucoma may result from a disruption of an interaction between OPTN and a GTP-binding protein Rab8, and its effects on protein trafficking[12].

(iii). Mutant MYOC protein induces endoplasmic reticulum (ER) stress and the resultant unfolded protein response (UPR) induces apoptosis in the trabecular meshwork cell (TMC), which leads to an increase in resistance to aqueous humor outflow, elevated intraocular pressure (IOP), and, ultimately, glaucoma [13].

(iv). The interaction of MYOC with the mitochondria in the TM and in astrocytes appears to be cell specific. TMC overexpressing Pro370Leu mutant MYOC, demonstrate features of mitochondrial dysfunction, which may increase vulnerability of TM cells to cellular insults, which causes impaired function and even cell death [[14]..

(v). MYOC causes disregulation of calcium channels, leading to mitochondrial membrane depolarization in TMC, TM contraction, and subsequent reduced outflow and IOP elevation [14].
(vi)**.** 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 [15].

(vii). 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 [16].

3.2. Epigenetics and signaling pathways

Epigenetics studies changes in organism, due to expression of gene modifications, rather than alteration of the genetic code itself. In other words, it is a study of the heritable changes in gene expression that do not involve changes to the underlying DNA sequence (a change in phenotype without a change in genotype), which in turn affects how cells read the genes. It is a naturally occurring and regular changes, but can also be influenced by factors like age, environment, life style and disease state. Epigenetics, in collaboration with predisposing genetic and environmental factors contribute to increased risk of POAG. It may also be involved in providing potential new therapeutic targets. These factors work through several pathways, including TGF-β, Rho kinase, Calcium-Calpain signaling and others [17].

3.2.1. Histone and DNA modification

Epigenetics involves heritable nonencoded genetic changes that turn genes on or off, including activating changes such as histone acetylation and DNA demethylation. It also involves repressive changes like histone deacetylation and DNA methylation, as well as modifications induced by noncoding RNAs, like the MicroRNA and long noncoding RNA (lncRNA). Epigenetic modifications can modulate gene expression and/or alter cellular signaling pathways, which may affect individual susceptibility to various diseases like POAG [18].

3.2.2. Brain derived neurotrophic factor [BDNF]

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 [19].

3.2.3. Epigenetic Pathwaysin collaboration with other predisposing factors

1. TGF-β signalingpathway:
Since corticosteroids can increase IOP in a high percentage of glaucoma patients, some authors have hypothesised that TIGR could be a gene responsible for outflow obstruction in glaucoma. TGF is a cytokine involved in many signaling cascades that cause differentiation, proliferation, chemotaxis, or fibrosis. There are three isoforms of TGF (TGF-1, TGF-2, and TGF-3), but TGF-2 has the most relevance to the eye, as it has been implicated in the pathogenesis of POAG. [20].

2. Calcium-calpainpathway:
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 [21].

3. Rho signalingpathway:
The activation of Rho GTPase/Rho kinase signaling in the trabecular outflow pathway increases IOP by altering the contractile, cell adhesive and permeability barrier characteristic of TM and schlemme's canal tissue, and by influencing extracellular matrix production and fibrotic activity. The Rho family is composed of the Rho, Rac, and Cdc42 subfamilies, which are involved in cell migration, adhesion, proliferation, and actin cytoskeletal organization [22].

4.0 GENETIC BASIS OF POAG

4.1. Mutation of POAG Genes

Mutation is genetic alteration, sufficient to cause a gene to malfunction and result in disease. Gene mutation is the genetic basis of POAG. 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, like 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 [3]. 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[23].

4.2. Gene Isolation in POAG

With the advent of affordable high-throughput DNA genotyping, there have now been multiple genes identified as contributing to susceptibility for POAG. The identification of disease-causing genes provides information about the pathogenesis of heritable eye diseases at the most basic level. Disease-causing genes may be part of important biological pathways, that once identified, may help clarify the mechanisms that lead to disease. 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 [24]. The POAG disease-causing genes, namely: myocilin (*MYOC*), optineurin (*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. The disease-causing mutation in Mendelian POAG offers the potential for targeted therapy to fix the specific molecular defect caused by the mutation[25].

5.0. PATTERN OF INHERITANCE IN POAG

"Glaucoma may be inherited as Mendelian-dominant or Mendelian recessive traits (usually early-onset forms of the disease) or may exhibit a heritable susceptibility consistent with complex trait inheritance (typically adult-onset forms of the disease)". In other words, it can occur at all ages, with early onset disease (before the age of 40 exhibiting Mendelian inheritance and adult-onset forms (developing after age 40) inherited as complex traits. These common age-related ocular disorders like the common forms of adult-onset glaucoma, including POAG, exhibit Mendelian inheritance patterns, with a significant heritability.
However, the genetic contributions to these disorders are complex, resulting from interactions of multiple genetic factors and are susceptible to the influence of environmental exposures. Discovering genes that contribute to disorders with complex inheritance is more difficult.

Rare forms of POAG affecting children and young adults usually are inherited as Mendelian disorders with either recessive or dominant inheritance of a single gene. By implication, the early onset glaucoma genes are rare with greater biological effects, while variants contributing to the adult-onset glaucoma are common with smaller effects. [5].

5.1. Simple/Single form of genetic trait and Mendelian inheritance in POAG

While glaucoma genetics is overall complex, a fraction of it is caused primarily by single genes defects or mutations. Typically, early-onset forms of glaucoma are inherited as Mendelian dominant or Mendelian-recessive traits, including early-onset OAG. 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. A classical Mendelian inheritance pattern is one in which a genotype at one locus is both necessary and sufficient for the phenotype to be expressed [26].

5.2. Complex form of genetic trait and inheritance in POAG

Other cases of POAG have a more complex genetic basis and are caused by the combined effects of many genetic and environmental risk factors, each of which do not act alone to cause glaucoma. These factors are more frequently detected in patients with POAG but are also commonly observed in normal subjects. Discovering genes that contribute to disorders with complex inheritance is more difficult**.** Conceptually, it can be considered that each individual risk factor is insufficient to cause disease on its own and that each risk factor may not be present in all cases of disease. 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 [27].

5.3.Complex form of POAG inheritance supported by linkage Studies.

Glaucoma is characterized as a 'complex' disease, with phenotype exhibiting heterogeneity, polygenic inheritance, phenocopies, and incomplete penetrance [28]. With these features, traditional linkage analyses have been widely used to identify linkage of different forms of glaucoma to particular loci, utilizing one or more families with multiple members affected. The analysis of complex human diseases requires novel genetic strategies and approaches as we enter the known genomic sequence era. Approaches involving traditional family linkage analysis have yielded the locations of many genes, especially those that are highly penetrant and encode simple Mendelian disease phenotypes [29].

5.4. Risk alleles in POAG mutation.

The etiology of POAG is multifactorial, as it demonstrates a variable age of onset and severity. The risk factors for POAG include elevated IOP, advancing age, genetic disposition, environment, family historyof POAG, African ancestry, myopia, and perhaps the presence of certain systemic diseases such as diabetes and hypertension. Other risk factors are: race, refractive errorand central corneal thickness.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[30].

5.4.1. Multiple genetic influence, familiarity and family history

Genetic influence play key roles in the development of POAG and several genes associated with POAG have been identified, though these account for less than 5% of all POAG in the general population. It is therefore thought that the hereditary aspect of POAG is likely to be polygenic and that gene-environment interactions are important**.** Various disease-causing mutations in MYOC, OPTN and WDR36 genes have long been identified as the cause of familial forms of POAG [31]. The US surgeon general has affirmed the importance of the family history and familial aggregation. The genetic contribution to POAG has long been recognized,with a strong genetic component having a large proportion of inherited and familial cases[27].

From studies, approximately 16–20% of the risk of POAG is attributable to genetic factors, which places first and second degree relatives of affected patients at risk. The process is

governed by a complex inheritance pattern with evidence of gene-gene interaction [17]. Family history is one of the strongest risk factors for POAG. It is of clinical importance because the risk for POAG among, first-degree relatives of POAG patients, have been shown to have a 9-fold increased risk of developing glaucoma in their lifetime, compared to relatives of controls in the population-based Rotterdam Study.A first-degree relative with POAG is a risk factor for the development of POAG. This has been reported in several studies with the odds ratio varying from 3 to 13. The risk is thought to be higher still if the affected relative is a sibling. Siblings of an affected patient were at the highest risk of developing POAG compared to parents or children [32].

6.0.TOOLS FOR MOLECULAR GENETICS IN POAG

Discovery of the genetic causes of POAGmay lead to an improved classification of the condition based on the precise causative genetic mutation, rather than the current vague clinical classification. Heritability of glaucoma presents the possibility of its study, using the tools of molecular genetics. The toolsprovide the opportunity to identify specific genes necessary for maintaining normal IOP and stabilizing the optic nerve, as well as identifying gene mutations associated with the disease[33].

6.1. Mendelian autosomal-dominant and autosomal-recessive trait in POAG inheritance.

Mendelian autosomal-dominant or autosomal recessive trait, or as a complex multifactorial trait, is associated with glaucoma inheritance. Mendelian autosomal dominant and recessive forms of glaucoma are caused by single gene defects that are associated with extreme phenotypes: either highly elevated intraocular pressure or severe optic nerve degeneration. However, most patients with POAG do not have extreme phenotypes, and the underlying genetic etiologies are not thought to result from single gene defects, but from contributions of multiple genetic factors that independently cause moderate alterations in intraocular pressure and optic nerve disease, and collectively cause more severe disease [34].

6.2. 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. 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 [35]. It is characterized by a change in phenotype without a change in genotype, which in turn affects how cells read the genes. It can apply to characteristics passed from a cell to its daughter cells in cell division and to traits of a whole organism. It deals with the changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself. This modification can modulate gene expression and/or alter cellular signaling pathways, which may affect individual susceptibility to various diseases. Epigenetic inheritance thus refers to the transmission of certain epigenetic marks to offspring. Unlike genetic changes, epigenetic changes are reversible and do not change the DNA sequence, but can change how the body reads a DNA sequence. Epigenetic change occurs regularly and naturally, but influenced by age, environment, lifestyle and disease state, which can interact with genome to influence epigenetic change, which may be reflected at various stages throughout a person’s life and even in later generations. The effect of Epigenetic forces contributing to glaucoma disease also manifest in lamina cribrosa cells [36].

6.3. Linkage Analysis and technique

The linkage technique is the most valuable tool in attempts to unravel the genetics of POAG. This approach investigates the genetic basis of POAG by analyzing familial inheritance patterns. It has a valuable usage in localizing a disease gene without any prior knowledge of the underlying pathology of the condition. The linkage analysis relies on the fact that genes which lie close to one another on a chromosome are less likely to be separated by the

process of recombination during meiosis than those which lie far apart. Such genes will therefore tend to be inherited together and are described as 'closely linked' [37].

6.4. Genome wide association study (GWAS)

GWAS are another method of identifying genes contributing to complex diseases and POAG. They are powerful tools for investigating the genetic architecture of human diseases. and are more powerful compared to linkage analysis in discovering genes of small effect that might contribute to the development of the disease. To date, thousands of GWAS have been performed on the human genome in attempt to identify Sops associated with a wide variety of complex human diseases. The results of all such published GWAS are maintained in an NIH data base [38].

6.5. Sib-pair analysis

Sib-pair analysis provides valuable insights into the genetic architecture of glaucoma diseases. This approach can help identify susceptibility loci and shed light on disease mechanisms. It involves studying pairs of siblings, to understand the genetic basis of a trait or disease. The primary goal is to determine whether siblings who share a common genomic segment (measured by genetic markers) tend to express the same disease phenotype (or similar quantitative trait values. Sib-pair analysis does not rely on specific assumptions about the underlying genetic model. It requires high penetrance to identify genetic loci. Siblings are genotyped for specific genetic markers. The analysis focuses on identical by descent (IBD) segments, which are regions of the genome inherited from a common ancestor [39]. Researchers can leverage this approach to unravel the genetic basis of complex traits.

6.6. Family - based association analysis

The family-based design has been considered to be an important strategy in genetic association analysis. In this, genotypes are divided into, between-family and within-family components. The components are permuted separately, and then association analysis is performed on the within-family component, between-family component, or their sum. This offers the ability to enrich the genetic loci containing rare variants; the methods to control for heterogeneity and population stratification; the direct estimates of the genetic contribution of different loci; the opportunity to examine the transmission of variants with phenotypes; and the ability to reveal the effects of parental origin of alleles.However, Family-based association tests (FBAT)s can apply any type of pedigree structure including missing parental data, multiple siblings, and extended pedigrees. The standard FBAT only uses the within-family information and therefore if a basic FBAT analysis is performed, a sizeable portion of genetic data will remain utilized [40].

7.0. PENETRANCE ANDPOAG GENETICS

7.1 POAG Genes

'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. There are causative genes identified as having association with POAG. These causative genes at six loci have been reported as: MYOC, primarily mutated in juvenile-onset subjects, on locus GLC1A mapped to chromosome (1q32), and OPTN mainly mutated in low pressure POAG individuals, on locus GLC1E to chromosome (10p25). Also reported was chromosomal mapping of a new POAG locus on 5q22.1, designated as GLC1G, and identification of its causative gene, WDR36, from within this region [41].

7.2. Gene penetrance in POAG

Penetrance in genetics is the proportion of individuals carrying a particular variant (or allele) of a gene (genotype) that also expresses an associated trait (phenotype). 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 [42]. The penetrance of a gene may depend on factors, such as environmental influences or interactions with other genes. Several genes have been associated with POAG, and they are MYOC**,** OPTN**,** WDR36**,** and others. These genes may have different levels of penetrance, which means the proportion of individuals who carry a gene variant and express its related trait [43].

7.2.1. High penetrance POAG-causing genes.

High penetrance signifies that most or all carriers of a gene variant will develop POAG. With complete penetrance, genes for a trait are expressed in all the population who have the genes. In other words, all of the individuals in a population who carry a specific genotype express the corresponding phenotype. An allele is highly penetrant, when the trait produced will almost always be apparent in an individual carrying the allele, indicating that individuals who have the disease–causing mutation, have clinical symptoms of the disease [44]. The MYOC gene was the first gene identified as causative of POAG, and accounts 3%–4% for most cases among different populations. OPTN was the second gene associated with POAG. They suggested that mutations in OPTN may be responsible for 16.7% of the hereditary forms of NTG, with an additional risk factor of 13.6% in both familial and sporadic cases. Primary open-angle glaucoma displays a strong heritability but is genetically heterogeneous. There are other high penetrance POAG-causing genes with controversy, which includes WDR36 and NTF4.Rare mutations associated with POAG in a novel gene, neurotrophin-4 (NTF4), have been recently identified in a European, as well as in a Chinese population. The controversial role of WDR36, NTF4 and their variants in POAG has created great uncertainty among experts about their contribution in the pathogenesis of glaucoma [45].

7.2.3. Low penetrance POAG-causing genes.

“Glaucoma is characterized as a ‘complex' disease, with a phenotype that exhibits heterogeneity, polygenic inheritance, phenocopies, and incomplete penetrance”. Low penetrance signifies that only some or few carriers will develop POAG. ’Incomplete/ Reduced/ Low penetrance allele manifests “when some individuals who do not or fail to express the trait, even though they carry the allele”. It indicates that this allele will only sometimes produce the symptoms at a detectable level. However, some genes do not show complete penetrance, and less than 100% of the individuals who bear a particular genotype express the corresponding phenotype, in cases of incomplete penetrance. Reduced penetrance is a term used exclusively for autosomal dominant disorders, until recently. A number of chromosomal regions and genetic variants have been identified as being linked to or associated with POAG and related endophenotypes. Linkage analysis identifies linkage of different forms of glaucoma to particular loci and association studies identifies genes contributing to complex diseases in genome-wide association. They are genetic approaches in the investigation of the genetic basis of POAG. Genome-wide association studies (GWAS) are more powerful compared with linkage analysis in discovering genes of small effect that might contribute to the development of the disease [45].

8.0. INSIGHT INTO CURRENT DIAGNOSIS OF POAG AND THE PARADIGM SHIFT TO MOLECULAR GENETICS

The present diagnosis of POAG falls within the comprehensive assessment of the ONH, RNF and visual field, for assault resulting from elevated IOP. This is achieved with ophthalmic instrument and computer-based diagnostic imaging. However the emphasis is gradually shifting to molecular genetics, through genetic screening, diagnosis and gene therapy for POAG.

8.1The paradigm shift to genetic diagnostic approach.

Genetic approach for glaucoma intervention and therapy is a paradigm shift from the conventional diagnosis to seeking genetic therapy and permanent cure.Molecular geneticswould clearlybecome beneficial in some specific situations like screening of family

members for autosomal dominant POAG of early onset. This approach isequally indicated for diagnosis,treatment,prognosis, counseling, and research purposes.

8.1.1. Genetic testing /screening and benefits

Glaucoma being a leading cause of blindness requires a development of an accurate diagnostic test for presymptomatic detection of individuals at risk. Ocugene is a non invasive in-office test, where the DNA sample of the patient is collected using cheek brushes. With this, the clinician identifies people at risk, particularly genetically predisposed nonglaucomatous family members whose diagnosis cannot be established with the current glaucoma testing. With Ocugene, 15–20% of POAG patients test is positive and 99% sensitive. It is useful for both diagnostic and prognostic purposes. If the genetic defect responsible for the disease in the family is identified, it is possible to screen offspring to determine their risk of disease and potentially take preventative action [46]. The ordering of diagnostic tests that will aid genetic discoveries, like identifying specific genetic mutations by clinicians, provides detailed understanding of the natural history of the patient's glaucoma, as well as information for genetic counseling. Diagnostic test identifies Individuals at risk in the family, and offers the opportunity for closer follow-up and immediate institution of glaucoma therapy.This however offers hope for actualization of primary prevention for various glaucoma. For instance, if testing for MYOC mutation which is the mutation causing POAG in a family is identified, then the individual concerned can be tested for that mutation [47].

8.1.2. Paradigm in neurodegeneration research.

There is also a paradigm shift in glaucoma treatment from applying therapy directly on RGC and ONH to targeting associated brain centers. Research has shown that glaucomatous nerve damage in the eye may spread to major visual centers of the brain. This is associated with well-known process in other neurodegenerative diseases. When the damage spreads, nerve cells in the brain related to visual function begin to shrink and die. Treatments could be targeted at these brain centers. Recent research suggests that cells in the retina other than RGCs are equally affected or equally contribute to the rate of decline of the ganglion cells. This means looking at the occurrence of neurodegeneration in a new light, with research underway to identify connections in the brain, and how these connections may be strengthened [48].

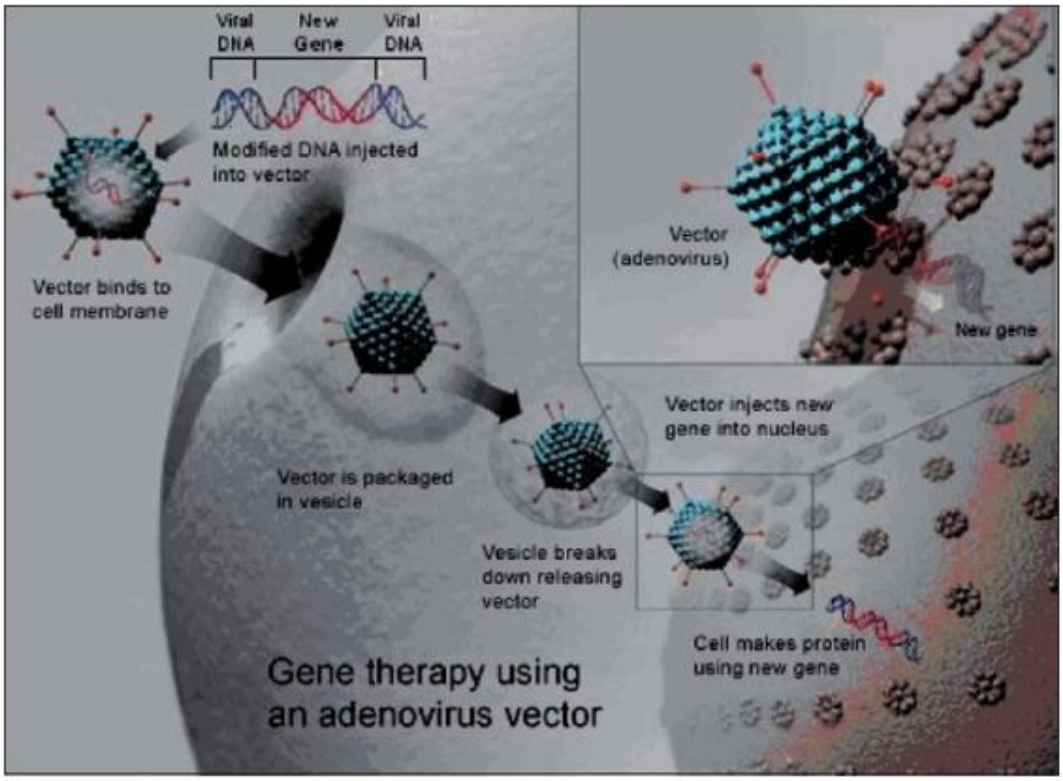
9.0.VECTOR ENGINEERING STRATEGIESIN POAG THERAPEUTICS

The genetic therapy in POAG requires the understanding of the molecular and cellular mechanisms leading to treatment, and vector-associated setbacks; which has resulted in the development of highly sophisticated gene transfer tools, with improved safety and therapeutic efficacy.The four basic prerequisites for any genetic therapy targeted at an ocular disease, are: (1) An efficient and nontoxic gene delivery technique. (2) Sufficient characterization of the genetic basis of the disease to select an appropriately matched therapeutic approach. (3) Proper control of the expression of the therapeutic gene. (4) The availability of an animal model of the disease for preclinical testing.[49].

9.1 Gene transfertechniqueandviralvectors

A successful gene therapy depends on safe and effective gene delivery as prerequisite. Gene transfer technology relies on the first step of replication and, at the same time, builds blocks to prevent production of infectious virus. Transduction is the key principle in gene transfer therapy. It is a non-replicative or dead-end infection that allows heterologous (i.e. non viral) genetic information to be delivered to a precise cell. To do so, the viral genome is radically rearranged to eliminate genes essential for replication and pathogenicity whilst making space for the heterologous genes.The working of Gene therapy requires the delivering of the therapeutic gene to the patients' target cellsthrough the carrier molecule called a vectorFigure 1. A virus that has been genetically altered to carry normal human DNA, is currently the most common vector.Viruses have evolved exquisite strategies to reach and penetrate specific target cells where they hijack and take over the cellular machinery to express viral genes and produce progeny particles irrespective of their origin, strain and family.[50].

Figure 1:Modified virus vector in which a new gene is incorporated and delivered to the diseased cell.



Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2992156/figure/F0001/

9.2. Routes of administrationanddeliveryof genes

Gene transfer therapy could be delivered or administered to the target organs through the following routes: Intravitreal delivery, Intracameral delivery and transcorneal administration. Based on evidence, it has been suggestedthat the BDNF is a potential neuroprotective agent in glaucoma. As such, RGC are trophically dependent on BDNF retrogradely transported from target areas in the brain, to ganglion cell bodies in the retina. [51].

9.3. Gene deliveryvectors, and targets

Current gene delivery vehicles, namely vectors, are categorized into two classes: DNA (non-viral) vectors and viral vectors. Both types of vectors can directly deliver genes into human body. However, Viral vectors take advantage of the infectious nature and gene-shuttling capability of certain viruses, but are deliberately engineered to minimize harm by removing as many viral genes as possible. On the other hand, non-viral vectors which include plasmids, nanoparticles, and liposomes are safer but less efficient than viral vectors. The recent advances in gene delivery techniques proved to be much improved in both safety and efficacy. Viral vectors are more commonly used than non-viral delivery due to their superior gene transfer efficiency. Viral vectors inherit many intrinsic features from their parental viruses. In many cases these features are double-edged[52].

9.3.1.Target genes and tissues

The appropriate target structures or cell types for glaucoma gene therapy are TM, ciliary epithelium, ciliary muscle, RGCs, and Muller cells.This is as a result of their role in aqueous production, drainage, and pathogenesis of glaucomatous damage. There are aboutsix delivery systems (vector virus) with the ability to deliver genes to the relevant tissues or cells. They include: Adenoviruses, adeno associated viruses, herpes simplex viruses (HSVs),

lentiviruses, feline immunodeficiency virus, human immunodeficiency virus (HIV), lithesome, and naked DNA [53].

9.3.2.Vectors and selected target areas

Many viruses that infect mammals have been explored as gene delivery vectors, and are naturally evolved gene delivery vehicles for gene therapy. With the surface proteins on viral particles, it can interact with their corresponding receptors on target cells, which triggers the cellular uptake process known as endocytosis. Once inside a target cell, viruses eventually deliver their genetic information in the form of DNA into the nucleus for viral gene expression. [54].

The Anterior segment.

Several studies have established that adenovirus vectors delivers transgenes very efficiently to the TM after intracameral injection as shown in Table 1. Due to the natural flow of aqueous humor, intracameral delivery of vectors carries the viruses directly to the TM.[55]. Other delivery vectors to the structures within the anterior segment of the eye are: HSV, lentiviruses, lithesomeetc,Table 1.

The Posterior segment

Intravitreal delivery has now been established as the preferred route to genes delivery to the RGCs. Intravitreal injection of Adenovirus vectors results in efficient delivery to MullerCells. Adenovirus gene transfer to the RGCs is very limited[51].

Table 1: Glaucoma Relevant Tissues and Available Vector Systems[55].

Tissue Type	*Vector*	*Route*
Trabecular Meshwork	Adenovirus	Intracameral
		Intracameral
	Adeno-associated virus serotypes 2, 3, 4	Intracameral
		Tissue culture
	Herpes simplex virus	Intracameral
	Lentivirus	Intracameral
	Liposomes	Intracameral
Ciliary Epithelium	Adenovirus	Intracameral
		Lens culture
	Adeno-associated virus	
	Herpes simplex virus	Intracameral
	Lentivirus	
	Liposomes	
Ciliary Muscle	Adenovirus	
	Adeno-associated virus	
	Herpes simplex virus	Tissue culture
	Lentivirus	
	Liposomes	
Retinal Ganglion Cells	Adenovirus	Intravitreal
	Adeno-associated virus	Intravitreal
	Herpes simplex virus	Intravitreal
		Retrograde
	Lentivirus	
	Liposomes	

10.0.ADVANCES IN THERAPY AND FUTURE PREDICTIONS IN GENETIC INTERVENTIONS IN POAG

10.1. Advances in genetic Interventions in POAGtherapy

10.1.1 Gene therapy

Gene therapy is a technique for correcting defective genes responsible for disease development. In theory, a normal copy of the gene can physically take the place of the flawed gene and restore the gene function of the cell. The aim of gene therapy presently, is to add a useful gene to the cell or tissue that suffers the consequences of the flawed gene. In some cases, the new gene may code for an entirely different protein whose function compensates for the protein encoded by the flawed gene. This requires the use of DNA or RNA for the treatment, cure or prevention of human disorders. Depending on the type of disease, this can be achieved either by delivery of a functional therapeutic gene, as a substitute for the defective or missing endogenous counterpart or by reducing the levels of a harmful defective gene product using various sophisticated tools; including naked oligonucleotides, modified viral and non-viral vectors, in which a new gene is incorporated and delivered to the diseased cell.A general approach to gene therapy is to use an altered (recombinant) virus to carry the gene of interest to the desired tissue. Using genetic engineering techniques, the viral DNA is

modified so that the viral genes required for virus proliferation are removed and the therapeutic gene is put in their place. Such a virus may invade the diseased tissue, become incorporated into the host DNA, and express the desired gene. Because the modified virus does not have the viral genes required for viral replication, the virus cannot proliferate, and the host cell does not die [56].

10.1.2. Neuro-Protection

Neuroprotection is the ability for a therapy to prevent neuronal cell death by intervening in, and inhibiting the pathogenetic cascade that results in cell dysfunction and eventual death. It is a non IOP-related intervention that can prevent or delay glaucomatous neurodegeneration. Neuroprotection relatively preserves the neuronal integrity, structure and function in case of an insult, with implied reduction in the rate of neuronal loss over time. This novel therapeutic strategies to glaucoma are aimed at promoting the neuroprotection of both the cell soma of retinal ganglion cells and the axons of the optic nerve. Genes selected to protect RGCs have traditionally been genes encoding neurotrophins,antiapoptotic, and defense genes.[57].

10.1.3. Rho-associated protein kinase (ROCK) inhibitors

Rho/ROCK signaling pathway plays an important role in the pathogenesis of glaucoma.
The significance of inhibiting Rho kinase for lowering IOP was identified. and has been demonstrated to be a promising target for therapeutics. Rho kinase is a downstream effector of Rho GTPase signaling that regulates actomyosin dynamics in numerous cell types. Its inhibitors decreased fibronectin and smooth muscle actin.This suggests that TM rigidity and acellular matrix production mediated by the Rho pathway may be involved in decreasing aqueous humor outflow, raising IOP. Rho kinase inhibitors reduce cell rigidity, increasing outflow and lowers IOP via relaxation of the TM, which enhances AH outflow. [58].

10.1.4. Combinatorial gene therapy

Pathways to promote RGC survival and axonal regeneration are not usually overlapping. For either promoting neuroprotection or inducing axonal regeneration, different signaling pathways and regulatory molecules are critical. The combination of both strategies in a single-gene therapy approach would likely be highly beneficial for glaucoma. With an efficient neuroprotective approach, more RGCs will survive the injury and, thus, be available to successfully regenerate their axons in response to a proregenerative stimulus. On the other hand, an effective regenerative approach will guarantee the integrity of the axons of RGCs that have been already partially or completely lost, with the potential to recover neuronal function and favour cell survival at a long term, inclusive of retrograde neurotrophic support from the axonal targets. With non usually overlappingneuroprotective and regenerative pathways,the genetic manipulation of apoptosis-related genes BAX and Bcl-2, can even have opposite consequences in each one. The gene knockout of the proapoptotic protein BAX and the constitutive overexpression of the antiapoptotic protein Bcl-2 are very efficient strategies to prevent the neurodegeneration of RGCs, with survival of almost all cells in the ganglion cell layer of the retina but cannot efficiently regenerate their axons [59].

10.1.5. Nanomedicine in glaucoma

Nanotechnology introduces a potential paradigm shift in the approach towards reduction of intraocular pressure. The improved ability to manipulate matter at the molecular level opens up a new vista and unprecedented opportunities in the approach to treating glaucoma. Recent advancements may offer novel drug delivery vectors to enhance bioavailability and uptake, as well as instruments that could improve intraocular pressure-reducing surgical outcomes. As drug delivery vectors, nanomaterials exhibit unique chemical and physical properties that, in conjunction with drug molecules, may potentially increase the availability of a pharmaceutical compound to a target site. These properties include increased rates of permeability through barriers, protection of nanoparticle-loaded drug molecules from degradation, and prolonged contact time between the drug and target tissue [60].

10.1.6. Poly-Therapeutic (Combination)Strategy

Poly-Therapeutic or combination treatment combines two or more medications or other therapies or interventionto treat a single condition with one or multiple symptoms.Since It is now widely recognized that lowering IOP in the treatment of glaucoma is not enough, the combination treatments such as IOP-lowering drugs with neurotrophic factors and/or antioxidants and/or anti-apoptotic agents may be necessary. In addition to neuroprotective and neuroregenerative approaches, poly-therapeutic strategies may be the future. [61].

10.1.7. Calcium channel blockers(CCBs)

CCBs have been implicated in glaucoma neuroprotection, by preventing calcium-mediated apoptosis and improving ocular blood flow and delayed progression of visual field defects. Topical addition of calcium channel blockers onto the optic nerve has been shown to block the rise in intracellular calcium, preventing acute superficial axonal destruction. In particular, brovincamine and nilvadipine are 2 CCB's that permeate the blood-brain barrier and, selectively influence the optic nerve circulation without appreciably affecting systemic circulation. However, this process is speed up by Calcium ionophores.[62].

10.1.8. Neuro-Enhancement

Neuroenhancement is the concept of supporting injured RGCs and enhancing their function before they die. They are currently in early-phase of human trials. One promising potential therapy is an implant that provides sustained delivery of ciliary neurotrophic factor (CNTF), a growth factor known to promote the growth and survival of nerve cells. This concept is being considered for future glaucoma therapy. Further research is on the development of possible drugs delivered slowly in biodegradable form, through sub-conjunctival injection near the cornea, potentially providing pressure reduction for up to 3–6 months [48].

10.2. Predictionsand Perspectives in POAGtherapies and cure

10.2.1. Gene silencing technique using small interfering (si) RNA

RNA interference (RNAi) is a gene silencing therapy that can completely eliminate the mutant myocilin proteins in the TM. Depending on the engineering technique of the small interfering (si) RNA, it could be applied either in a mutation-dependent or mutation-independent way. This strategy can reverse the pathological process of TMC, thereby treating the POAG caused by MYOC gene mutation. This strategy can also be applicable to many protein-misfolding diseases caused by gain-of-function mutant proteins. The readily available siRNA can be delivered to the intact human TM by intracameral perfusion. The delivered naked siRNA is functional, inhibiting not only the targeted gene but also their downstream effectors. This functional intracameral delivery might be of use to protect the TM from unwanted insults and could have important therapeutic applications [13].

10.2.2. Routine screening for disease susceptibility

With the current genetic research in primary open angle glaucoma, there is a clear indication of high hopes of better therapeutic directions and cure. In the near future, screening of individuals routinely for disease susceptibility may become possible with the functional interaction of GAS7 and TMCO1 genes with known glaucoma disease genes. This depends on the variation between the increase in sensitivity and specificity of genotyping and its decrease in cost. However, these genes are highly expressed in the ciliary body and TM as well as in the lamina cribrosa, optic nerve and retina [63].

10.2.3. Identification of genetic mutations

In POAG, the identification of validated and replicated genetic mutations, offers hope of correct identification of the most important pathways, to RGC death. Since insufficiently folded mutant MYOC accumulates in the ER, causing the unfolded protein response with subsequent activation of cell apoptosis; the use of recently proposed RNAi as a gene silencing therapy for complete elimination of mutant MYOC from human trabecular meshwork (HTM) cells [64]. will in turn identified as novel treatment strategies.

10.2.4. RNA interference (RNAi)

RNA interference (RNAi) is a useful strategy, thatwhen suppressing the expression of a single protein, addresses the symptoms or pathology of the disease. In RNAi therapies, lower concentrations or doses are required with fewer adverse effects[65]. RNAi may be as

valuable in modeling diseases, studying the effects of silencing-specific genes in vitro and in vivo, as it is in treating them,and as we can see in some other novel strategies.

11.0. CONCLUSION

Modern genetic approaches and techniques have challenged the current believe that glaucoma is just a treatable (but not yet curable) disease, with IOP currently being the only modifiable risk factor, where therapeutic lowering of intraocular pressure reduces the risk of developing glaucoma and retardation of disease progression.
The understanding of the pathophysiology and genetic mechanisms of POAG, advances the sophistry of the evolvingnovel tools like, gene transfer technique, gene silencing model, nanotechnology, neuroenhancement etc, in leveraging the therapeutic efficacyin POAG intervention.
Molecular genetics offers great promise that would continue to clarify the role of genes in POAG disease, and reshape its diagnosis and therapy. Ultimately,gene therapy has considerable promise asthe future therapy of POAG byreplacing the current conventional drops.
The sensitivity and specificity of genotyping, the current neurodegeneration research targeting associated brain centers, the gene mapping unraveling their defects through specific disease classification, the screening of individuals routinely for disease susceptibility and the important diagnostic precision of POAG disease-specifics to individuals,are the paradigm shift to a new frontier and insight into the future predictions of POAG permanent cure.

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