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Myocilin-associated Glaucoma: A Historical Perspective and Recent Research Progress

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Glaucoma a debilitating disease, is globally the second most common kind of permanent blindness. Primary open-angle glaucoma (POAG) is its most prevalent form and is often linked with alterations in the myocilin gene (*MYOC*). *MYOC* encodes the myocilin protein, which is expressed throughout the body, but primarily in trabecular meshwork (TM) tissue in the eyes. TM is principally involved in regulating intraocular pressure (IOP), and elevated IOP is the main risk factor associated with glaucoma. The myocilin protein's function remains unknown; however, mutations compromise its folding and processing inside TM cells, contributing to the glaucoma phenotype. While glaucoma is a complex disease with various molecules and factors as contributing causes, the role played by myocilin has been the most widely studied. The current review describes the present understanding of myocilin and its association with glaucoma and aims to shift the focus toward developing targeted therapies for treating glaucoma patients with variations in *MYOC*.

Introduction

Glaucoma: Glaucomas are a group of visual impairment disorders distinguished by the gradual atrophy of retinal ganglion cells (RGCs). The axons of RGCs form the optic nerve, which transmits visual stimuli from the eye to the brain. RGC degradation results in the thinning and gradual cupping of the neuroretinal rim, eventually leading to an enlarged optic disc, a hallmark for the diagnosis of glaucoma [1,2]. Most patients affected by glaucoma display no symptoms until advanced vision loss has occurred, making it the second most common cause of irreversible blindness after cataracts worldwide [3]. Currently, approximately 80 million people suffer from glaucoma [4,5]. The latest study assessing the global incidence and future prognosis of glaucoma estimates that by 2040 the number of people suffering with glaucoma will increase to almost 112 million [5].

Glaucoma is generally classified into two major subtypes, open-angle glaucoma (OAG) and closed-angle glaucoma (CAG), determined by the appearance of the iridocorneal angle formed between the iris and the cornea in the anterior compartment of the eye. Both types of glaucoma are further categorized into primary and secondary forms based on the underlying cause of the disease. The origin of primary forms of glaucoma is not discernible, while secondary forms of glaucoma are attributable to an identifiable cause, such as eye injury, cataracts, diabetes, or the prolonged use of steroids, all of which are associated with the risk of developing secondary glaucoma [6,7].

Primary OAG (POAG) is the most prevalent form of glaucoma, constituting approximately 74% of glaucoma cases globally [4,5,8]. In the US, >80% of glaucoma patients are classified as having OAG [9,10]. Several population-based studies have recognized both physiologic and genetic predisposing factors behind POAG, such as elevated intra-ocular pressure (IOP), age, race, and family history [5,11-18]. The risk of developing POAG increases with advancing age, but some subgroups of patients are diagnosed with a rather exceptional form of the disorder known as juvenile-onset OAG (JOAG). This is an early onset form of glaucoma that displays a Mendelian pattern of inheritance; this is in contrast to POAG, which is a complex genetic disease with multifactorial risk factors [19]. Genetic alterations in myocilin are responsible for nearly 4% of POAG cases and >10% of JOAG cases [20,21].

While myocilin has been studied often due to its association with inherited cases of glaucoma, its biologic function remains enigmatic. A comprehensive analysis of past and recent research efforts focusing on the physiologic role myocilin plays in causing the disease phenotype is attempted in this review. The pathophysiology of glaucoma is complex, and recent studies have revealed new insights that have helped in gaining a better understanding of this disease. A clear understanding of the structure of and physiologic role played by myocilin can help in developing targeted therapies to treat glaucoma.

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Identification of myocilin as a gene associated with OAG: IOP is instrumental in preserving the shape of the eye and allowing the precise projection of images onto the retina. IOP levels are maintained by an equilibrium between the formation and drainage of aqueous humor (AH) in the anterior region of the eye. AH is formed in the ciliary body and secreted into the anterior chamber of the eye from which it is drained into the bloodstream via two distinct routes. In humans, most of the drainage occurs through the sieve-like trabecular meshwork (TM) into the Schlemm's canal, which is the conventional route. A relatively minor amount of AH exits via the unconventional uveal sclera route [22]. The effective regulation of AH discharge across the TM is necessary to maintain normal IOP. Elevated IOP levels are observed in most OAG patients, making it the key predisposing factor behind this disease. In OAG eyes, there is increased resistance to AH outflow, which causes elevated IOP, and IOP remains the only modifiable risk factor for slowing and treating glaucoma [23-25]. However, a third of OAG patients are diagnosed without elevated IOP (<21 mm of Hg) and are referred to as having normal tension glaucoma (NTG) [26,27]. Interestingly, advancement of the disease has decreased via treatment focused on reducing IOP levels, even in NTG patients [25,28,29].

Efforts to ascertain the genes implicated in the initiation of ocular hypertension were first undertaken in the early 1990s, and genetic linkage and cellular protein expression studies were conducted in parallel by different research groups. Linkage analysis of a family with a history of JOAG led to the detection of the first locus associated with OAG. This locus was termed GLC1A and was mapped to the q arm of chromosome 1 [30]. Cell culture studies conducted by Fauss et al. in 1993 and by Polansky et al. in 1997 led to the identification of a protein progressively expressed upon long-term treatment with dexamethasone, a drug associated with steroid-induced glaucoma [31-33]. This polypeptide was formerly called the trabecular meshwork-inducible glucocorticoid response (TIGR), as its expression was induced by treatment with the glucocorticoid dexamethasone. Subsequently, the coding sequence for TIGR was mined from a cDNA library created from the mRNA of human TM cells treated with the same drug [31,34,35]. Simultaneously, in an independent study, an analogous gene was cloned from a human retina cDNA library. The protein produced by this gene displayed homology to the non-muscle protein myosin and was therefore termed myocilin [36]. Later, in 1998, the Human Genome Organization (HUGO)'s Gene Nomenclature Committee formally assigned the name myocilin to the TIGR gene and its protein product [31].

Myocilin:

The MYOC gene—The human myocilin gene *(MYOC)* is approximately 17kb in size and is comprised of three exons and two introns. *MYOC* is positioned on the long arm of chromosome 1, specifically on segment 1q24.3–1q25.2 [35,36]. Several mRNAs extending in size from 1.8–2.3 kb are transcribed from this gene. The variation in transcript length arises due to the discrepancy in the use of three polyadenylation sites present at the 3′ end of the gene [37-39]. A typical TATA-box promoter and several transcription regulatory elements, such as NF-kB and E-box, are found in the region proximately upstream of the myocilin transcription initiation site [35,37,40,41]. Myocilin is transcribed in numerous tissues within and outside the eye, but the highest mRNA level is observed in the TM tissue [31,37,38,41,42]. The cornea, iris, ciliary body, and retinal epithelium also exhibit significant expression of the *MYOC* gene [36-38]. In addition to the eyes, skeletal muscle and heart tissues display major myocilin expression levels [35,38,40]. Myocilin expression is influenced by several molecules, including steroids, transforming growth factor-β1 (TGF-β1), and the protein optineurin in cultured TM cells [41-43]. Stress, principally mechanical and oxidative stress, also induce myocilin expression [25,33,42].

The myocilin protein: The *MYOC* gene yields a secreted glycoprotein build of 504 amino acids [35,38,44]. The myocilin protein exhibits an isoelectric point (pI) of 5.2, has a predicted molecular weight of 55.3 kDa, and is visible as a doublet on denaturing PAGE flanked by 53 and 57 kDa [35,36]. The 57 kDa fragment is an outcome of N-linked glycosylation occurring at the amino acid positions 57–59 (Asn-Glu-Ser) in the polypeptide chain [41,44]. Structurally, the protein is composed of three major homology regions: the N-terminal coiled-coil (CC) domain, which includes the leucine zipper (LZ) motifs (amino acids 33–201); the intermediate linker region (amino acids 202–243); and the C-terminal olfactomedin (OLF) domain (amino acids 244–504). The protein also includes an N-terminal signal sequence (amino acids 1–32), which is partly responsible for the extracellular secretion of myocilin [35,45]. While myocilin is known to be secreted in vitro in cultured cell lines expressing this protein [44,46-49] and in vivo by its presence in AH [46,48,50-52], it has also been reported to be present inside the cells expressing it. Intracellularly, myocilin is localized within the endoplasmic reticulum (ER) [47,49,53-55], the golgi apparatus (GA) [53,55,56], and mitochondria [57-59]. It is also present cytosolically inside exosomal vesicles associated with microtubules [60-62]. Extracellular proteins are processed in the ER and GA of cells before their departure via the secretory

pathway; therefore, the presence of myocilin in the ER and GA is not surprising [41,63]. Apart from the traditional secretory pathway, some synthesized myocilin is also secreted into the extracellular milieu by means of exosomal vesicles [41,63,64].

Intracellular processing of the myocilin protein: Normally, 40% of synthesized myocilin undergoes proteolytic cleavage between arg226 and ile227 inside the ER, yielding a 35 kDa C-terminal OLF domain fragment and a 20 kDa fragment comprising the N-terminal CC domain [48,65]. The OLF domain is co-secreted with unprocessed full-length myocilin into the media of cultured TM cells, while the N-terminal domain is maintained intracellularly inside the ER [48,66]. The OLF domain is the site of more than 90% of all disease-causing myocilin variations [20,37,67].

Three-dimensional structure of myocilin: Due to the difficulty encountered in the in vitro production and recovery of native myocilin protein, elucidation of its three-dimensional (3D) structure required almost two decades of research. Even as of now, the complete crystal structure of the complete human myocilin protein remains unknown. However, the crystal structures of a part of the N-terminal CC domain and the complete C-terminal OLF domain have been determined for the mouse and human myocilin proteins, respectively [68,69]. The 3D structure of the myocilin C-terminal OLF domain was obtained first and was observed to be a five-bladed β-propeller. Propellers are widely recognized as sites mediating interactions within two or more proteins, a role that seems presumable for the OLF domain of myocilin [68]. Molecular examinations of the N-terminal CC domain of myocilin suggest it exhibits an exceptional three-way structure, a Y-shaped parallel dimer-of-dimers with a distinct tetrameric region at its N-terminal that bifurcates into dimers at its C-terminal [69]. Subsequently, the crystal structure of the C-terminal region of the mouse myocilin N-terminal CC domain was obtained, and the presence of an α-helical parallel dimer was proven [69]. Based on these recent studies, a unique structure for the full-length myocilin protein has been proposed, wherein the N-terminal of the protein emerges as a tetrameric stem that further divides at obtuse angles into two parallel dimers-of-dimers attached to paired C-terminal OLF domains via the linker regions [69].

Alterations in myocilin: Today, more than 100 disease-causing alterations in *MYOC* have been identified (myocilin) [70]. These alterations in *MYOC* can cause it to exhibit distinct phenotypes, such as having a varying age of disease onset, being prevalent among individuals of a particular race, or being influenced by environmental or epigenetic factors [20,67,71-74]. As briefly noted above, normally the wild type (WT) myocilin protein is secreted into the extracellular environment after it has been folded and processed inside the ER of TM cells. However, data from several cell culture and animal model studies have revealed that certain variant myocilin proteins are unable to undergo proper proteolytic processing and are therefore identified and retained by the cellular homeostasis machinery inside the ER of TM cells [46,47,49]. Most of the disease-associated variations are localized in the OLF domain of myocilin where they cause structural alterations in protein conformation [68]. WT myocilin is found to be cleaved by calpain II, an intracellular calcium-dependent protease localized in the ER [66]; it is suggested that the specificity of calpain action is mainly governed by the secondary and tertiary conformational determinants of its substrate. Therefore, structural changes arising in the OLF domain through the misfolding of mutants hinders the action of calpain and prevents proteolytic cleavage of the full-length protein [66]. It has also been found that alterations in the amino acid sequence of myocilin cause it to misfold and expose an otherwise cryptic carboxy terminal peroxisomal targeting sequence-1 (PTS1). The exposure of this PTS-1 site results in the aberrant transportation of myocilin variants into peroxisomes, thus interfering with the clearance of misfolded proteins by the ubiquitin–proteasome machinery [75].

Myocilin and glaucoma pathogenesis: The results of numerous empirical studies support the conclusion that a gain-of-function mechanism is involved in myocilin-associated glaucoma pathogenesis [45,46,48,75-80]. Disease-causing myocilin variants are prone to aggregate and accumulate inside the ER [47]. When both WT and mutant myocilin are present in a heterozygous state inside TM cells, proteolytic processing and the secretion of WT myocilin molecules are also impeded. This occurs due to interactions resulting in the formation of hetero-oligomers between the WT and mutant protein molecules [45,46,81].

The accumulation of overexpressed, misfolded, and aggregated protein molecules results in ER stress and the initiation of the unfolded protein response (UPR) by the ER's homeostasis machinery. When the UPR pathway is unable to remove misfolded proteins through proteolysis via the proteasome, cells are unable to recover from ER stress. This leads to apoptosis and cell death [82], followed by the degradation of TM tissue [47,83,84]. Under normal conditions, autophagic mechanisms can take over the clearance of cellular myocilin in TM cells [64], but in glaucomatous patients the autophagic machinery is dysregulated via mTOR-dependent signaling [85].

ER stress is also linked with other forms of glaucoma that are not attributable to alterations in *MYOC*, such as

steroid-induced glaucoma and complex POAG [86,87]. Steroid-induced glaucoma and POAG share similar clinical presentation features in patients, such as increased resistance to AH outflow and morphological and biochemical changes in the TM tissue [88]. ER stress in steroid-induced ocular hypertension has been attributed to the deposition of extracellular matrix (ECM) proteins in TM cells. Synthesis and processing of ECM proteins occur in the ER; treatment with glucocorticoids such as dexamethasone increases the secretory load of TM cells, exceeding their normal ER capacity and thereby inducing ER stress [89]. As dexamethasone is also known to promote the expression and accumulation of myocilin in TM cells, it was postulated that increased myocilin levels might cause obstruction of AH outflow and elevate IOP levels in steroid-induced glaucoma. However, data from recent studies suggests WT myocilin alone is not responsible for elevating IOP levels in a mouse model of steroid-induced ocular hypertension [90] and indicates the role of other ECM proteins, such as fibronectin, in pathogenesis [89].

In addition to the above factors, another contributing role of mutated myocilin in glaucoma pathogenesis has been revealed. Several ECM proteins have been found to accumulate in the glaucomatous TM tissue [91]. The elevation of ECM proteins in TM tissue is also thought to contribute to the pathogenesis of POAG by increasing resistance to AH outflow [92,93]. Recently, Kasetti et al. revealed the involvement of myocilin variants in promoting intracellular deposition of fibronectin, elastin, and type IV collagen within the ER of TM cells due to misfolded myocilin-induced ER stress [93]. Their study also pointed toward the negative effect altered myocilin has on the function of matrix metalloproteinases (MMPs); functional forms of MMP-2 and MMP-9 were found to be reduced in TM cells expressing *MYOC* variants [93]. Decreased activity of these MMPs can result in reduced turnover of ECM proteins and can lead to their enhanced deposition in TM tissues expressing mutated *MYOC*.

Protein misfolding is considered the mechanism behind the accumulation of protein molecules inside the ER and the resulting ER stress. This is because when TM cells transfected with mutant myocilin are grown at temperatures below 30 °C, a provision that favors appropriate protein folding, the misfolding of mutants is reversed and they are secreted in the same manner as WT myocilin [47]. The ER stress response and associated cell toxicity as a consequence of misfolded myocilin constitute the broadly accepted mechanism for the pathogenesis of myocilin-associated glaucoma [47,49,75,80,84,94].

Physiologic function of myocilin: Although the association between myocilin and glaucoma was established over two decades ago and continues to be widely studied, the normal physiologic function of myocilin remains elusive. Initially, researchers speculated that myocilin controls IOP levels [34]. Its abundance in TM and ciliary body tissues, which are involved in the AH drainage pathway and thus the maintenance of IOP levels, is consistent with this proposition [95]. However, in separate studies with mouse models where myocilin expression was increased [96] or completely abrogated [78], no changes in IOP levels were observed, and glaucoma was absent in these animals. The results of these studies imply that myocilin is not obligatory for normal IOP regulation [97]. Further observations, such as the non-appearance of glaucoma in an aged female homozygous for the Arg46Ter myocilin alteration [98] and in individuals hemizygous for the *MYOC* gene [99], confirmed that native myocilin is not essential for IOP regulation and that functional redundancy is provided by other proteins. Ongoing research by many groups focuses on discerning the role of myocilin in the eye and in other tissues where it is present, but its definitive function remains elusive. Because myocilin is present both extracellularly and intracellularly under physiologic conditions, in the following subsections we describe its functional activities separately based on its localization.

i. Extracellular role—Findings from recent studies support an understanding of myocilin's role as a matricellular protein [100-102]. Matricellular proteins are extracellular but are not a part of the ECM structurally; they are primarily involved in regulating cell–matrix interactions. It is well recognized that ECM turnover in the TM influences the drainage capacity of AH, and matricellular proteins are surfacing as key leads [102]. Recent studies have shown that myocilin interacts with other matricellular proteins, such as hevin and secreted protein acidic and rich in cysteine (SPARC) via its C-terminal OLF domain [102]. Hevin displays anti-adhesive properties in the ECM, which could be facilitated by opposing the adhesion mediated by fibronectin. It has also been reported to bind collagen and modulate its fibrillogenesis [102]. SPARC is a multifunctional protein participating in diverse processes, such as tissue remodeling, cellular differentiation and proliferation, cell migration, morphogenesis, and anti-angiogenesis [103]. SPARC has also been reported to influence the expression of MMPs and, like hevin, displays counter-adhesive properties [104]. Myocilin, SPARC, and hevin exhibit similar expression profiles in different ocular tissues, and this co-expression facilitates their in vivo interaction. It is suggested that the collective and coordinated activity of these three proteins regulates cell adhesion and ECM homeostasis in TM tissue [104]. Myocilin co-localizes and interacts with the ECM proteins fibronectin and laminin, which are involved in regulating several biologic

functions, including cell adhesion, cytoskeletal organization, and signal transduction; hence, it may also influence AH outflow via this pathway [100].

ii. Intracellular activity—Recently, myocilin has also been found to function in the ligand-mediated endocytosis of GPR-143, a G-protein-coupled receptor (GPCR) that functions in the retinal pigment epithelium (RPE) pigmentation pathway. This suggests that intracellular myocilin is involved in cell signaling processes [105,106]. Myocilin has also been reported to have a function in the Wnt signaling pathway through which it can regulate the actin cytoskeleton [107,108] and initiate changes in the pathway to AH outflow in the TM [108]. Functional redundancy is observable, as the Wnt proteins are capable of taking over the function of myocilin in regulating Wnt signaling [107]. Intracellular myocilin also affects mitochondrial function, and the overexpression of myocilin in TM cells decreases adenosine triphosphate (ATP) synthesis and triggers apoptotic events [57]. In addition to its role in eye tissue, in skeletal muscle tissue myocilin has been reported to interact with α1-syntrophin, a constituent of the dystrophin-associated protein complex (DAPC), via its N-terminal domain. The interaction between myocilin and α1-syntrophin results in muscle hypertrophy via the activation of regulatory pathways controlling muscle size [109].

Recent studies: A better understanding of myocilin aggregation and associated toxicity: As described previously, variations in the WT myocilin sequence contribute to the glaucoma phenotype due to the failure to clear aggregates formed as a result of protein misfolding. Recent research efforts have identified the reason for this failure in the normally efficient proteostasis machinery. It has been observed that mutant myocilin interacts with the heat-shock protein-90 (HSP-90) homolog in the ER, glucose-regulated protein 94 (Grp94), and the aberrant interaction between them prevents the clearance of toxic aggregates formed by the myocilin protein [110]. Under normal physiologic conditions, the Grp94 chaperone protein is inactive [111], but when the ER is under stress it is employed to maintain quality control by providing assistance in the proper folding of proteins [94]. The nature of misfolded, detergent-insoluble [112] aggregates formed by myocilin was discovered to be amyloidogenic [113] and is suggested to be the cause of anomalous Grp94 activity with mutant myocilin and the failure of its proteasomal clearance [94,110]. In vitro studies have further proved that Grp94 enhances the amyloid aggregation potency of WT myocilin and is incorporated into the end-stage aggregates formed [114]. When inhibition studies of Grp94 were conducted, they proved effective in clearing mutant myocilin through autophagy, as well as by lowering the cellular toxicity accompanying mutant myocilin overexpression, both in vitro and in vivo [114-117]. Another recent study found that mutant myocilin interacts with another chaperone protein, αB crystallin, leading their co-aggregation into ThT-positive amyloid aggregates. This suggests that this interaction similarly prevents the ubiquitin-mediated proteasome degradation of mutant myocilin [118].

Amyloid formation is the cause of several incapacitating ailments, such as Alzheimer's disease, Parkinson's disease, type II diabetes, and Huntington's disease, which collectively constitute a major health burden to the modern world [119]. Most of these diseases are associated with aging, in addition to being frequently allied with the accretion of misfolded and aggregated proteins, triggering oxidative stress that ultimately culminates in untimely cell death [82]. Myocilin-associated glaucoma is essentially a result of similar protein misfolding and associated cytotoxicity. However, aggregates formed by myocilin are exceptional, as no recognized disease-causing proteins are known to form amyloid fibrils inside the ER of cells and mediate toxicity [94,120,121].

Myocilin-related glaucoma is a new addition to the group of diseases arising as a result of protein misfolding and amyloid formation. This opens an avenue to explore the amyloidogenicity of myocilin as a molecular origin for both hereditary and sporadic cases of glaucoma. Prospective treatments could include drugs that impede the amyloid fibrillation of myocilin, destroy existing fibrils, or prevent the interaction between myocilin variants and the chaperone proteins in the ER that enhance its fibrillogenesis [94,113-115].

Current treatments for glaucoma and the need to target myocilin: Despite the clinical heterogeneity of glaucoma, IOP has remained the only treatable factor [7,122]. The topical administration of drugs formulated as eye drops that either diminish the production of AH or increase its drainage is the principal choice of pharmacological therapy to regulate IOP levels. In instances where such pharmacological interventions are not efficient, surgical options such as trabeculectomy and laser trabeculoplasty, which have their own risks and durability issues, are employed [122,123]. A brief overview of the different glaucoma medications currently in use, including their mechanisms of action, is provided in Table 1. Although these treatments are helpful, in most cases they are unsuccessful in completely halting disease progression [123,124] due to the complex and heterogeneous pathology of glaucoma, which is difficult to address using traditional therapies [7,123].

The present review focuses on inherited glaucoma caused by alterations in *MYOC* that accounts for 5% of all glaucoma cases and affects approximately three million people worldwide [8,19,20,125]. Variations in *MYOC* remain the most

prevalent cause of inherited glaucoma and are currently the most well understood in terms of the underlying pathogenesis; however, there are no targeted glaucoma treatments for individuals with *MYOC* variants [7]. Researchers have tried to understand the genotype–phenotype relationship between *MYOC* variants and POAG patients and have found that cases of advanced glaucoma are positively correlated with variations in the *MYOC* gene [126]. IOP levels in such patients are often not amenable to control by traditional pharmacological treatments and frequently require surgery to prevent vision loss [127-129]. The Gln368Ter variant of myocilin is the most common disease-associated version found in POAG patients, correlating with 1.6% of POAG cases that exhibit elevated IOP levels [20]. The prevalence of the Gln368Ter form of myocilin in the general population is approximately 1 in 600–700 people [130,131]. This myocilin variant has been studied for disease penetrance in several population and family studies [131-134]. It is reported to display a relatively high penetrance in patients with a family history of POAG compared to the general population, and this penetrance increases with advancing age, placing individuals carrying this variant of myocilin at a higher risk of developing glaucoma [135]. Few reports are available to compare the efficacy of traditional glaucoma therapies in treating POAG patients with or without variations in *MYOC*. However, similar studies where the clinical course has been compared in POAG patients with or without Gln368Ter variants of *MYOC* have yielded contrasting results. In the study by Craig et al., mean IOP levels, the rate of filtration surgery, and other clinical parameters were observed to be higher in glaucoma patients with Gln368Ter *MYOC* truncations than in those without them [128]. This is in contrast to the study by Graul et al., which reports that the frequency of laser trabeculoplasty and surgery was found to be similar in POAG patients with or without the Gln368Ter *MYOC* variant [136]. The Gln368Ter variant of myocilin exhibits a relatively mild form of disease phenotype. Studies with myocilin variants displaying a more severe glaucoma phenotype, such as Pro370Leu, reveal an insensitiveness to current pharmacological therapies aimed at reducing IOP levels; therefore, surgical intervention is required to prevent visual field loss [129,137]. The high prevalence and penetrance of Gln368Ter MYOC variants reported [131] along with the risk of disease advancement even after pharmacological interventions [128] indicates the need for better genetic testing to identify carriers of such *MYOC* variants and the need to develop targeted therapies to tackle the imminent health burden.

POAG is one of the most common and heritable human diseases [138] that is treatable without much loss of visual acuity, provided there is early diagnosis. However, up to 50% of POAG patients remain undiagnosed until significant vision loss has already occurred because glaucoma is usually asymptomatic in its early stages [17]. POAG features such as its chronicity, heritability, and treatability make it an ideal candidate for genetic risk profiling [124].

The clinical utility of predictive gene testing for myocilin-associated glaucoma has been investigated, and such testing is suggested to be a potent diagnostic tool in screening, enabling timely therapy, and thus preventing vision loss in high-risk patients with *MYOC* variations [139,140]. However, because variations in *MYOC* are responsible for causing only a fraction of POAG cases and population testing is likely to be cost-intensive, genetic testing for *MYOC* variants is currently limited only to familial and early-onset cases of POAG/JOAG [141,142]. Recommendations for genetic

Table 1. An overview of the different categories of drugs that are administered as ocular drops for maintaining IOP levels by the clinicians

Category	Example	Mode of Action	Observations
Cholinergic drugs	Pilocarpene	Increase TM outflow by causing the ciliary muscles to contract	Effective but causes side effects like dim vision
α-adrenergic receptor agonists	Brimonidine	Decrease AH production, and increase outflow via uveal sclera route	Allergic reaction is prominent
β-Adrenergic receptor antagonists (β-blockers)	Levobunolol, Timonol	Reduce AH inflow, by inhibiting its production in the ciliary body	Rare systemic effects such as bradycardia and fatigue
Prostaglandin analogs	Latanoprost, Tafluprost, Bimatoprost	Causes ECM morphogenesis via action of MMP's, increases outflow facility via uveal sclera pathway	First choice of clinicians, least side effects
Carbonic anhydrase inhibitors	Brinzolamide, Dorzolamide	Decrease AH production	Oral administration is more efficient butcauses side effects such as paresthesia of the hands and feet

testing in eye disorders have been prepared by the American Academy of Ophthalmology, which suggests genetic screening for *MYOC* variants only if results support disease surveillance and treatment [143]. The guidelines discourage direct-to-consumer (DCT) genetic testing and recommend consulting a genetic counsellor before and after conducting a genetic test; moreover, only certified laboratories must be engaged in carrying out such tests.

A recent study by Craig et al. used genome-wide association studies (GWAS) to identify new risk loci for glaucoma and to build a genetic risk calculation model in the form of a polygenic risk score (PRS). The study demonstrates that the developed PRS improves glaucoma risk stratification and screening remarkably across different population cohorts; however, further evaluations are required to investigate its wider applicability [144]. In future, results from such PRS evaluations can also help in delivering *MYOC* variant genetic testing to a wider population (exhibiting a high PRS score). This might help in the timely delivery of tailored medications to the identified high-risk patients (with variations in *MYOC*) in a cost-effective manner, while adequate monitoring and treatment of patients in the lower-risk group occurs.

As outlined above, patients with variations in *MYOC* have a medical need that is not adequately met by currently available remedies. Recently, with a better understanding of the mechanism by which amino acid variations in myocilin contribute to the disease phenotype, exploratory work has begun to develop better therapies for such patients. The different approaches (see Table 2) investigated thus far are as follows: (i) administering chemical chaperones to promote stability and the secretion of mutant proteins, thus relieving ER stress [145-148]; (ii) gene editing to prevent the expression of the mutated gene/protein [76]; (iii) inhibiting amyloid-like aggregation using small molecules [149]; and (iv) most recently, blocking the interaction between Grp94 and mutant myocilin by selective Grp94 inhibitors [114-117]. Promising results have been obtained, and in the future effective therapies might be available for glaucoma patients who have variations in *MYOC*.

In addition to the efforts regarding *MYOC*, recent studies have yielded drugs that likewise offer more efficiency and specificity in terms of their mode of action. Greater efficacy and specificity are a result of targeting the conventional or TM drainage pathway, which is not the case with traditional drugs used to treat glaucoma. Traditional pharmaceuticals employed as glaucoma treatments primarily modulate the non-conventional (i.e., the uveal sclera) pathway of AH drainage, which, as described previously, serves as an auxiliary route for draining AH. Netarsudil and latanoprostene bunod have recently become available to patients after successful Food and Drug Administration trials [150-153]. While netarsudil is a Rho-associated protein kinase (ROCK) inhibitor, latanoprostene bunod is a nitric oxide (NO) releaser and a soluble guanylate cyclase (sGC) modulator. ROCK inactivity is associated with the disruption of focal adhesions and the actin cytoskeleton [154-156], while the activation of GCs reduces the size of TM and Schlemm's canal cells [123,157]. Thus, both these drugs increase AH outflow facility via direct action on its conventional drainage pathway and can offer benefits to a wider range of glaucoma patients.

Concluding remarks: Due to the efforts of several research groups spanning more than two decades of intense investigation, we have a better understanding of myocilin and its role in causing glaucoma. While no definitive function has been assigned to this protein, knowledge of its importance in maintaining ECM homeostasis has become more robust. Several binding proteins with similar functions have been identified that hint at a functional redundancy and might be the reason for the normal development of people who lack this protein due to inherent changes. The past decade has been seminal in terms of elucidating the structure of myocilin. The availability of structural data holds potential for future research related to the influence different *MYOC* mutations have on the structural integrity of this protein and the manifestation of glaucoma. In addition to mutation studies, the availability of

Table 2. A summary of the different approaches directed against myocilin for treating myocilin associated glaucoma.

Method	Mode of Action	References
Treatment with chemical chaperones	Relieve ER stress by promoting folding of mutant myocilin	Yam et al. 2007 [146], Burns et al. 2016 [145], Zode et al. 2011 [147], Zode et al. 2012 [148]
CRISPR-Cas9 mediated gene editing	Cutting the expression of mutant myocilin	Jain et al. 2017 [76]
Inhibiting myocilin aggregation	Small molecules promoting stability and preventing myocilin aggregation	Orwig et al.. 2014 [149]
Inhibition of grp94-myocilin interaction	Promoting clearance of mutant myocilin via autophagy	Stothert et al. 2014 [114], Crowley et al. 2016 [115], Stothert et al. 2017 [117], Huard et al. 2018 [116],

structural data will help in expediting the search for binding partners, thus expanding the functional profile of myocilin, and in conducting drug discovery studies for POAG and JOAG patients with MYOC mutations.

REFERENCES

1. Yohannan J, Boland MV. The evolving role of the relationship between optic nerve structure and function in glaucoma. Ophthalmology 2017; 124:S66-70. [PMID: 29157364].
2. Smith C, Vianna J, Chauhan B. Assessing retinal ganglion cell damage. Eye (Lond) 2017; 31:209-17. [PMID: 28085141].
3. Leite MT, Sakata LM, Medeiros FA. Managing glaucoma in developing countries. Arq Bras Oftalmol 2011; 74:83-4. [PMID: 21779659].
4. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90:262-7. [PMID: 16488940].
5. Tham Y-C, Li X, Wong TY, Quigley HA, Aung T, Cheng C-Y. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology 2014; 121:2081-90. [PMID: 24974815].
6. Kwon YH, Fingert JH, Kuehn MH, Alward WLM. Primary Open-Angle Glaucoma. N Engl J Med 2009; 360:1113-24. [PMID: 19279343].
7. Donegan RK, Lieberman RL. Discovery of Molecular Therapeutics for Glaucoma: Challenges, Successes, and Promising Directions: Miniperspective. J Med Chem 2016; 59:788-809. [PMID: 26356532].
8. Kapetanakis VV, Chan MP, Foster PJ, Cook DG, Owen CG, Rudnicka AR. Global variations and time trends in the prevalence of primary open angle glaucoma (POAG): a systematic review and meta-analysis. Br J Ophthalmol 2016; 100:86-93. [PMID: 26286821].
9. Vajaranant TS, Wu S, Torres M, Varma R. The changing face of primary open-angle glaucoma in the United States: demographic and geographic changes from 2011 to 2050. Am J Ophthalmol 2012; 154:303-14. .
10. Sheybani A, Scott R, Samuelson TW, Kahook MY, Bettis DI, Ahmed IIK, Stephens JD, Kent D, Ferguson TJ, Herndon LW. Open-Angle Glaucoma: Burden of Illness, Current Therapies, and the Management of Nocturnal IOP Variation. Ophthalmol Ther 2020; 9:1-14. [PMID: 31732872].
11. Leske MC. Open-angle glaucoma—an epidemiologic overview. Ophthalmic Epidemiol 2007; 14:166-72. [PMID: 17896292].
12. Leske MC, Connell A, Wu S-Y, Hyman LG, Schachat AP. Risk factors for open-angle glaucoma: the Barbados Eye Study. Arch Ophthalmol 1995; 113:918-24. [PMID: 7605285].
13. Katz J, Sommer A. Risk factors for primary open angle glaucoma. Am J Prev Med 1988; 4:110-4. [PMID: 3395491].
14. Rudnicka AR, Mt-Isa S, Owen CG, Cook DG, Ashby D. Variations in primary open-angle glaucoma prevalence by age, gender, and race: a Bayesian meta-analysis. Invest Ophthalmol Vis Sci 2006; 47:4254-61. [PMID: 17003413].
15. Kim M, Kim T-W, Park KH, Kim JM. Risk factors for primary open-angle glaucoma in South Korea: the Namil study. Jpn J Ophthalmol 2012; 56:324-9. [PMID: 22661397].
16. . investigators A. The Advanced Glaucoma Intervention Study (AGIS). 12. Baseline risk factors for sustained loss of visual field and visual acuity in patients with advanced glaucoma. Am J Ophthalmol 2002; 134:499-512. [PMID: 12383806].
17. Mitchell P, Hourihan F, Sandbach J, Wang JJ. The relationship between glaucoma and myopia: the Blue Mountains Eye Study. Ophthalmology 1999; 106:2010-5. [PMID: 10519600].
18. Bahrami H. Causal inference in primary open angle glaucoma: specific discussion on intraocular pressure. Ophthalmic Epidemiol 2006; 13:283-9. [PMID: 16877288].
19. Wiggs JL, Damji KF, Haines JL, Pericak-Vance MA, Allingham RR. The distinction between juvenile and adult-onset primary open-angle glaucoma. Am J Hum Genet 1996; 58:243-[PMID: 8554064].
20. Fingert JH, Héon E, Liebmann JM, Yamamoto T, Craig JE, Rait J, Kawase K, Hoh S-T, Buys YM, Dickinson J. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. Hum Mol Genet 1999; 8:899-905. [PMID: 10196380].
21. Aldred MA, Baumber L, Hill A, Schwalbe EC, Goh K, Karwatowski W, Trembath RC. Low prevalence of MYOC mutations in UK primary open-angle glaucoma patients limits the utility of genetic testing. Hum Genet 2004; 115:428-31. [PMID: 15338275].
22. Kanagavalli J, Pandaranayaka E, Krishnadas SR, Krishnaswamy S, Sundaresan P. A review of genetic and structural understanding of the role of myocilin in primary open angle glaucoma. Indian J Ophthalmol 2004; 52:271-[PMID: 15693317].
23. Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch Ophthalmol 2002; 120:1268-79. [PMID: 12365904].
24. Maier PC, Funk J, Schwarzer G, Antes G, Falck-Ytter YT. Treatment of ocular hypertension and open angle glaucoma: meta-analysis of randomised controlled trials. BMJ 2005; 331:134-.
25. Boland MV, Ervin A-M, Friedman DS, Jampel HD, Hawkins BS, Vollenweider D, Chelladurai Y, Ward D, Suarez-Cuervo C, Robinson KA. Comparative effectiveness of treatments for open-angle glaucoma: a systematic review for the US Preventive Services Task Force. Ann Intern Med 2013; 158:271-9. [PMID: 23420235].
26. Klein BE, Klein R, Sponsel WE, Franke T, Cantor LB, Martone J, Menage MJ. Prevalence of glaucoma: the Beaver Dam eye study. Ophthalmology 1992; 99:1499-504. [PMID: 1454314].
27. Dielemans I, Vingerling JR, Wolfs RC, Hofman A, Grobbee DE, de Jong PT. The prevalence of primary open-angle glaucoma in a population-based study in the Netherlands:

the Rotterdam Study. Ophthalmology 1994; 101:1851-5. [PMID: 7800368].

28. Schulzer M, Airaksinen P, Alward W, Amyot M, Anderson D, Balazsi G, Blondeau P, Cashwell L, Cohen J, Desjardins D. Intraocular pressure reduction in normal-tension glaucoma patients. Ophthalmology 1992; 99:1468-70. [PMID: 1407981].

29. . Group CN-TGS. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Am J Ophthalmol 1998; 126:487-97. [PMID: 9780093].

30. Sheffield VC, Stone EM, Alward WL, Drack AV, Johnson AT, Streb LM, Nichols BE. Genetic linkage of familial open angle glaucoma to chromosome 1q21–q31. Nat Genet 1993; 4:47-50. [PMID: 8513321].

31. Tamm ER. Myocilin and glaucoma: facts and ideas. Prog Retin Eye Res 2002; 21:395-428. [PMID: 12150989].

32. Fauss D, Bloom E, Lui G, Kurtz R, Polansky J. Glucocorticoid (GC) effects on HTM cells: biochemical approaches and growth factor responses. Basic Aspects of Glaucoma Research III Stuttgart: Schattauer 1993:319–30.

33. Polansky JR, Fauss DJ, Chen P, Chen H, Lütjen-Drecoll E, Johnson D, Kurtz RM, Ma Z-D, Bloom E, Nguyen TD. Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product. Ophthalmologica 1997; 211:126-39. [PMID: 9176893].

34. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE. Identification of a gene that causes primary open angle glaucoma. Science 1997; 275:668-70. [PMID: 9005853].

35. Nguyen TD, Chen P, Huang WD, Chen H, Johnson D, Polansky JR. Gene structure and properties of TIGR, an olfactomedin-related glycoprotein cloned from glucocorticoid-induced trabecular meshwork cells. J Biol Chem 1998; 273:6341-50. [PMID: 9497363].

36. Kubota R, Noda S, Wang Y, Minoshima S, Asakawa S, Kudoh J, Mashima Y, Oguchi Y, Shimizu N. A novel myosin-like protein (myocilin) expressed in the connecting cilium of the photoreceptor: molecular cloning, tissue expression, and chromosomal mapping. Genomics 1997; 41:360-9. [PMID: 9169133].

37. Adam MF, Belmouden A, Binisti P, Brézin AP, Valtot F, Béchetoille A, Dascotte J-C, Copin B, Gomez L, Chaventré A. Recurrent mutations in a single exon encoding the evolutionarily conserved olfactomedin-homology domain of TIGR in familial open-angle glaucoma. Hum Mol Genet 1997; 6:2091-7. [PMID: 9328473].

38. Ortego J, Escribano J, Coca-Prados M. Cloning and characterization of subtracted cDNAs from a human ciliary body library encoding TIGR, a protein involved in juvenile open angle glaucoma with homology to myosin and olfactomedin. FEBS Lett 1997; 413:349-53. [PMID: 9280311].

39. Borrás T, Rowlette LLS, Tamm ER, Gottanka J, Epstein DL. Effects of elevated intraocular pressure on outflow facility and TIGR/MYOC expression in perfused human anterior segments. Invest Ophthalmol Vis Sci 2002; 43:33-40. [PMID: 11773009].

40. Fingert JH, Ying L, Swiderski RE, Nystuen AM, Arbour NC, Alward WL, Sheffield VC, Stone EM. Characterization and Comparison of the Human and MouseGLC1A Glaucoma Genes. Genome Res 1998; 8:377-84. [PMID: 9548973].

41. Resch ZT, Fautsch MP. Glaucoma-associated myocilin: a better understanding but much more to learn. Exp Eye Res 2009; 88:704-12. [PMID: 18804106].

42. Tamm ER, Russell P, Epstein DL, Johnson DH, Piatigorsky J. Modulation of myocilin/TIGR expression in human trabecular meshwork. Invest Ophthalmol Vis Sci 1999; 40:2577-82. [PMID: 10509652].

43. Park BC, Tibudan M, Samaraweera M, Shen X, Yue BY. Interaction between two glaucoma genes, optineurin and myocilin. Genes Cells 2007; 12:969-79. [PMID: 17663725].

44. Caballero M, Borrás T. Inefficient processing of an olfactomedin-deficient myocilin mutant: potential physiological relevance to glaucoma. Biochem Biophys Res Commun 2001; 282:662-70. [PMID: 11401512].

45. Caballero M, Rowlette LLS, Borrás T. Altered secretion of a TIGR/MYOC mutant lacking the olfactomedin domain. Biochimica et Biophysica Acta (BBA)- Molecular Basis of Disease 2000; 1502:447-60. [PMID: 11068187].

46. Jacobson N, Andrews M, Shepard AR, Nishimura D, Searby C, Fingert JH, Hageman G, Mullins R, Davidson BL, Kwon YH. Non-secretion of mutant proteins of the glaucoma gene myocilin in cultured trabecular meshwork cells and in aqueous humor. Hum Mol Genet 2001; 10:117-25. [PMID: 11152659].

47. Liu Y, Vollrath D. Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. Hum Mol Genet 2004; 13:1193-204. [PMID: 15069026].

48. Aroca-Aguilar JD, Sánchez-Sánchez F, Ghosh S, Coca-Prados M, Escribano J. Myocilin mutations causing glaucoma inhibit the intracellular endoproteolytic cleavage of myocilin between amino acids Arg226 and Ile227. J Biol Chem 2005; 280:21043-51. [PMID: 15795224].

49. Joe MK, Sohn S, Hur W, Moon Y, Choi YR, Kee C. Accumulation of mutant myocilins in ER leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. Biochem Biophys Res Commun 2003; 312:592-600. [PMID: 14680806].

50. Rao PV, Allingham RR, Epstein DL. TIGR/myocilin in human aqueous humor. Exp Eye Res 2000; 6:637-41. [PMID: 11095916].

51. Russell P, Tamm ER, Grehn FJ, Picht G, Johnson M. The presence and properties of myocilin in the aqueous humor. Invest Ophthalmol Vis Sci 2001; 42:983-6. [PMID: 11274075].

52. Fautsch MP, Johnson DH. Characterization of myocilin–myocilin interactions. Invest Ophthalmol Vis Sci 2001; 42:2324-31. [PMID: 11527946].

53. Clark AF, Kawase K, English-Wright S, Lane D. STEELY HT, YAMAMOTO T, KITAZAWA Y, KWON YH, FINGERT JH, SWIDERSKI RE. Expression of the glaucoma gene myocilin (MYOC) in the human optic nerve head. FASEB J 2001; 15:1251-3. [PMID: 11344104].

54. Sohn S, Hur W, Joe MK, Kim J-H, Lee Z-W, Ha K-S, Kee C. Expression of wild-type and truncated myocilins in trabecular meshwork cells: their subcellular localizations and cytotoxicities. Invest Ophthalmol Vis Sci 2002; 43:3680-5. [PMID: 12454036].

55. Clark AF, Steely HT, Dickerson JE, English-Wright S, Stropki K, McCartney MD, Jacobson N, Shepard AR, Clark JI, Matsushima H. Glucocorticoid induction of the glaucoma gene MYOC in human and monkey trabecular meshwork cells and tissues. Invest Ophthalmol Vis Sci 2001; 42:1769-80. [PMID: 11431441].

56. O'Brien ET, Ren X-o, Wang Y. Localization of myocilin to the Golgi apparatus in Schlemm's canal cells. Invest Ophthalmol Vis Sci 2000; 41:3842-9. [PMID: 11053284].

57. Sakai H, Shen X, Koga T, Park BC, Noskina Y, Tibudan M, Yue BY. Mitochondrial association of myocilin, product of a glaucoma gene, in human trabecular meshwork cells. J Cell Physiol 2007; 213:775-84. [PMID: 17516541].

58. Ueda J, Wentz–Hunter KK, Cheng EL, Fukuchi T, Abe H, Yue BY. Ultrastructural localization of myocilin in human trabecular meshwork cells and tissues. J Histochem Cytochem 2000; 48:1321-9. [PMID: 10990486].

59. Wentz-Hunter K, Ueda J, Shimizu N, Yue BY. Myocilin is associated with mitochondria in human trabecular meshwork cells. J Cell Physiol 2002; 190:46-53. [PMID: 11807810].

60. Hardy KM, Hoffman EA, Gonzalez P, McKay BS, Stamer WD. Extracellular trafficking of myocilin in human trabecular meshwork cells. J Biol Chem 2005; 280:28917-26. [PMID: 15944158].

61. Stamer W, Perkumas K, Hoffman E, Roberts B, Epstein D, Mckay BS. Coiled–coil targeting of myocilin to intracellular membranes. Exp Eye Res 2006; 83:1386-95. [PMID: 16973161].

62. Mertts M, Garfield S, Tanemoto K, Tomarev SI. Identification of the region in the N-terminal domain responsible for the cytoplasmic localization of MYOC/TIGR and its association with microtubules. Lab Invest 1999; 79:1237-45. [PMID: 10532587].

63. Barlowe CK, Miller EA. Secretory protein biogenesis and traffic in the early secretory pathway. Genetics 2013; 193:383-410. [PMID: 23396477].

64. Qiu Y, Shen X, Shyam R, Yue BY, Ying H. Cellular processing of myocilin. PLoS One 2014; 9:e92845[PMID: 24732711].

65. Borrás T. The effects of myocilin expression on functionally relevant trabecular meshwork genes: a mini-review. J Ocul Pharmacol Ther 2014; 30:202-12. [PMID: 24564495].

66. Sánchez-Sánchez F, Martínez-Redondo F, Aroca-Aguilar JD, Coca-Prados M, Escribano J. Characterization of the intracellular proteolytic cleavage of myocilin and identification of calpain II as a myocilin-processing protease. J Biol Chem 2007; 282:27810-24. [PMID: 17650508].

67. Gong G, Kosoko-Lasaki O, Haynatzki GR, Wilson MR. Genetic dissection of myocilin glaucoma. Hum Mol Genet 2004; 13:R91-102. .

68. Donegan RK, Hill SE, Freeman DM, Nguyen E, Orwig SD, Turnage KC, Lieberman RL. Structural basis for misfolding in myocilin-associated glaucoma. Hum Mol Genet 2015; 24:2111-24. [PMID: 25524706].

69. Hill SE, Nguyen E, Donegan RK, Patterson-Orazem AC, Hazel A, Gumbart JC, Lieberman RL. Structure and misfolding of the flexible tripartite coiled-coil domain of glaucoma-associated myocilin. Structure 2017; 25:1697-707. .

70. Hewitt AW, Mackey DA, Craig JE. Myocilin allele-specific glaucoma phenotype database. Hum Mutat 2008; 29:207-11. [PMID: 17966125].

71. Kanagavalli J, Krishnadas SR, Pandaranayaka E, Krishnaswamy S, Sundaresan P. Evaluation and understanding of myocilin mutations in Indian primary open angle glaucoma patients. Mol Vis 2003; 9:606-14. [PMID: 14627955].

72. Melki R, Idhajji A, Driouiche S, Hassani MI, Boukabboucha A, Akhayat O, Garchon H-J, Belmouden A. Mutational analysis of the Myocilin gene in patients with primary open-angle glaucoma in Morocco. Ophthalmic Genet 2003; 24:153-60. [PMID: 12868033].

73. Pang CP, Leung YF, Fan B, Baum L, Tong WC, Lee WS, Chua JK, Fan DS, Liu Y, Lam DS. TIGR/MYOC gene sequence alterations in individuals with and without primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2002; 43:3231-5. [PMID: 12356829].

74. Shimizu S, Lichter PR, Johnson AT, Zhou Z, Higashi M, Gottfredsdottir M, Othman M, Moroi SE, Rozsa FW, Schertzer RM. Age-dependent prevalence of mutations at the GLC1A locus in primary open-angle glaucoma. Am J Ophthalmol 2000; 130:165-77. [PMID: 11004290].

75. Shepard AR, Jacobson N, Millar JC, Pang I-H, Steely HT, Searby CC, Sheffield VC, Stone EM, Clark AF. Glaucoma-causing myocilin mutants require the Peroxisomal targeting signal-1 receptor (PTS1R) to elevate intraocular pressure. Hum Mol Genet 2007; 16:609-17. [PMID: 17317787].

76. Jain A, Zode G, Kasetti RB, Ran FA, Yan W, Sharma TP, Bugge K, Searby CC, Fingert JH, Zhang F. CRISPR-Cas9–based treatment of myocilin-associated glaucoma. Proc Natl Acad Sci USA 2017; 114:11199-204. [PMID: 28973933].

77. Aroca-Aguilar J-D, Sánchez-Sánchez F, Martínez-Redondo F, Coca-Prados M, Escribano J. Heterozygous expression of myocilin glaucoma mutants increases secretion of the mutant forms and reduces extracellular processed myocilin. Mol Vis 2008; 14:2097-[PMID: 19023451].

78. Kim BS, Savinova OV, Reedy MV, Martin J, Lun Y, Gan L, Smith RS, Tomarev SI, John SW, Johnson RL. Targeted

disruption of the myocilin gene (Myoc) suggests that human glaucoma-causing mutations are gain of function. Mol Cell Biol 2001; 21:7707-13. [PMID: 11604506].

79. McDowell CM, Luan T, Zhang Z, Putliwala T, Wordinger RJ, Millar JC, John SW, Pang I-H, Clark AF. Mutant human myocilin induces strain specific differences in ocular hypertension and optic nerve damage in mice. Exp Eye Res 2012; 100:65-72. [PMID: 22575566].

80. Wang H, Li M, Zhang Z, Xue H, Chen X, Ji Y. Physiological function of myocilin and its role in the pathogenesis of glaucoma in the trabecular meshwork. Int J Mol Med 2019; 43:671-81. [PMID: 30483726].

81. Gobeil S, Rodrigue M-A, Moisan S, Nguyen TD, Polansky JR, Morissette J, Raymond V. Intracellular sequestration of hetero-oligomers formed by wild-type and glaucoma-causing myocilin mutants. Invest Ophthalmol Vis Sci 2004; 45:3560-7. [PMID: 15452063].

82. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature 2016; 529:326-35. [PMID: 26791723].

83. Yam GH-F, Gaplovska-Kysela K, Zuber C, Roth J. Aggregated myocilin induces russell bodies and causes apoptosis: implications for the pathogenesis of myocilin-caused primary open-angle glaucoma. Am J Pathol 2007; 170:100-9. [PMID: 17200186].

84. Anholt RR, Carbone MA. A molecular mechanism for glaucoma: endoplasmic reticulum stress and the unfolded protein response. Trends Mol Med 2013; 19:586-93. [PMID: 23876925].

85. Porter K, Hirt J, Stamer WD, Liton PB. Autophagic dysregulation in glaucomatous trabecular meshwork cells. Biochimica et Biophysica Acta (BBA)- Molecular Basis of Disease 2015; 1852:379-85. [PMID: 25483712].

86. Zode GS, Sharma AB, Lin X, Searby CC, Bugge K, Kim GH, Clark AF, Sheffield VC. Ocular-specific ER stress reduction rescues glaucoma in murine glucocorticoid-induced glaucoma. J Clin Invest 2014; 124:1956-65. [PMID: 24691439].

87. Peters JC, Bhattacharya S, Clark AF, Zode GS. Increased endoplasmic reticulum stress in human glaucomatous trabecular meshwork cells and tissues. Invest Ophthalmol Vis Sci 2015; 56:3860-8. [PMID: 26066753].

88. Clark AF, Wordinger RJ. The role of steroids in outflow resistance. Exp Eye Res 2009; 88:752-9. [PMID: 18977348].

89. Kasetti RB, Maddineni P, Millar JC, Clark AF, Zode GS. Increased synthesis and deposition of extracellular matrix proteins leads to endoplasmic reticulum stress in the trabecular meshwork. Sci Rep 2017; 7:1-14. [PMID: 29097767].

90. Patel GC, Phan TN, Maddineni P, Kasetti RB, Millar JC, Clark AF, Zode GS. Dexamethasone-induced ocular hypertension in mice: Effects of myocilin and route of administration. Am J Pathol 2017; 187:713-23. [PMID: 28167045].

91. Belmares R, Raychaudhuri U, Maansson S, Clark AF. Histological investigation of human glaucomatous eyes: Extracellular fibrotic changes and galectin 3 expression in the trabecular meshwork and optic nerve head. Clin Anat 2018; 31:1031-49. [PMID: 30117188].

92. Vranka JA, Kelley MJ, Acott TS, Keller KE. Extracellular matrix in the trabecular meshwork: intraocular pressure regulation and dysregulation in glaucoma. Exp Eye Res 2015; 133:112-25. [PMID: 25819459].

93. Kasetti RB, Phan TN, Millar JC, Zode GS. Expression of mutant myocilin induces abnormal intracellular accumulation of selected extracellular matrix proteins in the trabecular meshwork. Invest Ophthalmol Vis Sci 2016; 57:6058-69. [PMID: 27820874].

94. Stothert AR, Fontaine SN, Sabbagh JJ, Dickey CA. Targeting the ER-autophagy system in the trabecular meshwork to treat glaucoma. Exp Eye Res 2016; 144:38-45. [PMID: 26302411].

95. Menaa F, Braghini CA, Vasconcellos JPCD, Menaa B, Costa VP, Figueiredo ESD, Melo MBD. Keeping an eye on myocilin: a complex molecule associated with primary open-angle glaucoma susceptibility. Molecules 2011; 16:5402-21. [PMID: 21709622].

96. Gould DB, Miceli-Libby L, Savinova OV, Torrado M, Tomarev SI, Smith RS, John SW. Genetically increasing Myoc expression supports a necessary pathologic role of abnormal proteins in glaucoma. Mol Cell Biol 2004; 24:9019-25. [PMID: 15456875].

97. Gould DB, Reedy M, Wilson LA, Smith RS, Johnson RL, John SW. Mutant myocilin nonsecretion in vivo is not sufficient to cause glaucoma. Mol Cell Biol 2006; 26:8427-36. [PMID: 16954374].

98. Lam DS, Leung YF, Chua JK, Baum L, Fan DS, Choy KW, Pang CP. Truncations in the TIGR gene in individuals with and without primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2000; 41:1386-91. [PMID: 10798654].

99. Wiggs JL, Vollrath D. Molecular and clinical evaluation of a patient hemizygous for TIGR/MYOC. Arch Ophthalmol 2001; 119:1674-8. [PMID: 11709019].

100. Gobeil S, Letartre L, Raymond V. Functional analysis of the glaucoma-causing TIGR/myocilin protein: integrity of N-terminal coiled-coil regions and olfactomedin homology domain is essential for extracellular adhesion and secretion. Exp Eye Res 2006; 82:1017-29. [PMID: 16466712].

101. Peters DM, Herbert K, Biddick B, Peterson JA. Myocilin binding to Hep II domain of fibronectin inhibits cell spreading and incorporation of paxillin into focal adhesions. Exp Cell Res 2005; 303:218-28. [PMID: 15652337].

102. Chatterjee A, Villarreal G Jr, Rhee DJ. Matricellular proteins in the trabecular meshwork: review and update. J Ocul Pharmacol Ther 2014; 30:447-63. [PMID: 24901502].

103. Brekken RA, Sage EH. SPARC, a matricellular protein: at the crossroads of cell–matrix. Matrix Biol 2000; 19:569-80. [PMID: 11102747].

104. Aroca-Aguilar J-D, Sánchez-Sánchez F, Ghosh S, Fernández-Navarro A, Coca-Prados M, Escribano J. Interaction of recombinant myocilin with the matricellular protein SPARC:

functional implications. Invest Ophthalmol Vis Sci 2011; 52:179-89. [PMID: 20926826].

105. McKay BS, Congrove NR, Johnson AA, Dismuke WM, Bowen TJ, Stamer WD. A role for myocilin in receptor-mediated endocytosis. PLoS One 2013; 8:e82301[PMID: 24367514].

106. Locke CJ, Congrove NR, Dismuke WM, Bowen TJ, Stamer WD, McKay BS. Controlled exosome release from the retinal pigment epithelium in situ. Exp Eye Res 2014; 129:1-4. [PMID: 25311167].

107. Kwon H-S, Lee H-S, Ji Y, Rubin JS, Tomarev SI. Myocilin is a modulator of Wnt signaling. Mol Cell Biol 2009; 29:2139-54. [PMID: 19188438].

108. Shen X, Ying H, Yue BY. Wnt activation by wild type and mutant myocilin in cultured human trabecular meshwork cells. PLoS One 2012; 7:e44902[PMID: 23028669].

109. Joe MK, Kee C, Tomarev SI. Myocilin interacts with syntrophins and is member of dystrophin-associated protein complex. J Biol Chem 2012; 287:13216-27. [PMID: 22371502].

110. Suntharalingam A, Abisambra JF, O'Leary JC, Koren J, Zhang B, Joe MK, Blair LJ, Hill SE, Jinwal UK, Cockman M. Glucose-regulated protein 94 triage of mutant myocilin through endoplasmic reticulum-associated degradation subverts a more efficient autophagic clearance mechanism. J Biol Chem 2012; 287:40661-9. [PMID: 23035116].

111. Marzec M, Eletto D, Argon Y. GRP94: An HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum. Biochimica et Biophysica Acta (BBA)- Molecular Cell Research 2012; 1823:774-87. [PMID: 22079671].

112. Zhou Z, Vollrath D. A cellular assay distinguishes normal and mutant TIGR/myocilin protein. Hum Mol Genet 1999; 8:2221-8. [PMID: 10545602].

113. Orwig SD, Perry CW, Kim LY, Turnage KC, Zhang R, Vollrath D, Schmidt-Krey I, Lieberman RL. Amyloid fibril formation by the glaucoma-associated olfactomedin domain of myocilin. J Mol Biol 2012; 421:242-55. [PMID: 22197377].

114. Stothert AR, Suntharalingam A, Huard DJ, Fontaine SN, Crowley VM, Mishra S, Blagg BS, Lieberman RL, Dickey CA. Exploiting the interaction between Grp94 and aggregated myocilin to treat glaucoma. Hum Mol Genet 2014; 23:6470-80. [PMID: 25027323].

115. Crowley VM, Khandelwal A, Mishra S, Stothert AR, Huard DJ, Zhao J, Muth A, Duerfeldt AS, Kizziah JL, Lieberman RL. Development of glucose regulated protein 94-selective inhibitors based on the BnIm and radamide scaffold. J Med Chem 2016; 59:3471-88. [PMID: 27003516].

116. Huard DJ, Crowley VM, Du Y, Cordova RA, Sun Z, Tomlin MO, Dickey CA, Koren J III, Blair L, Fu H. Trifunctional high-throughput screen identifies promising scaffold to inhibit Grp94 and treat myocilin-associated glaucoma. ACS Chem Biol 2018; 13:933-41. [PMID: 29402077].

117. Stothert AR, Suntharalingam A, Tang X, Crowley VM, Mishra SJ, Webster JM, Nordhues BA, Huard DJ, Passaglia CL, Lieberman RL. Isoform-selective Hsp90 inhibition rescues model of hereditary open-angle glaucoma. Sci Rep 2017; 7:1-9. [PMID: 29263415].

118. Lynch JM, Li B, Katoli P, Xiang C, Leehy B, Rangaswamy N, Saenz-Vash V, Wang YK, Lei H, Nicholson TB. Binding of a glaucoma-associated myocilin variant to the αB-crystallin chaperone impedes protein clearance in trabecular meshwork cells. J Biol Chem 2018; 293:20137-56. [PMID: 30389787].

119. Knowles TP, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. Nat Rev Mol Cell Biol 2014; 15:384-96. [PMID: 24854788].

120. Hill SE, Donegan RK, Lieberman RL. The glaucoma-associated olfactomedin domain of myocilin forms polymorphic fibrils that are constrained by partial unfolding and peptide sequence. J Mol Biol 2014; 426:921-35. [PMID: 24333014].

121. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 2006; 75:333-66. [PMID: 16756495].

122. Zhang K, Zhang L, Weinreb RN. Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. Nat Rev Drug Discov 2012; 11:541-59. [PMID: 22699774].

123. Adams CM, Papillon JP. Recent Developments for the Treatment of Glaucoma. Drug Delivery Challenges and Novel Therapeutic Approaches for Retinal Diseases. 2020; 189-256

124. Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L, Komaroff E. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. Arch Ophthalmol 2003; 121:48-56. [PMID: 12523884].

125. Huard DJ, Lieberman RL. Progress toward development of a proteostasis drug for myocilin-associated glaucoma. Future Science; 2018.

126. Souzeau E, Burdon KP, Dubowsky A, Grist S, Usher B, Fitzgerald JT, Crawford A, Hewitt AW, Goldberg I, Mills RA. Higher prevalence of myocilin mutations in advanced glaucoma in comparison with less advanced disease in an Australasian disease registry. Ophthalmology 2013; 120:1135-43. [PMID: 23453510].

127. Crawford A, Souzeau E, Agar A, Ridge B, Dubowsky A, Burdon KP, Craig JE. Identification of a novel MYOC mutation, p.(Trp373*), in a family with open angle glaucoma. Gene 2014; 545:271-5. [PMID: 24768183].

128. Craig JE, Baird PN, Healey DL, McNaught AI, McCartney PJ, Rait JL, Dickinson JL, Roe L, Fingert JH, Stone EM. Evidence for genetic heterogeneity within eight glaucoma families, with the GLC1A Gln368STOP mutation being an important phenotypic modifier. Ophthalmology 2001; 108:1607-20. [PMID: 11535458].

129. Zhuo Y-H, Wei Y-T, Bai Y-J, Duan S, Lin M-K, Saragovi HU, Ge J. Pro370Leu MYOC gene mutation in a large Chinese family with juvenile-onset open angle glaucoma: correlation between genotype and phenotype. Mol Vis 2008; 14:1533-[PMID: 18728751].

130. Gharahkhani P, Burdon KP, Hewitt AW, Law MH, Souzeau E, Montgomery GW, Radford-Smith G, Mackey DA, Craig JE, MacGregor S. Accurate imputation-based screening of Gln368Ter myocilin variant in primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2015; 56:5087-93. [PMID: 26237198].

131. Han X, Souzeau E, Ong J-S, An J, Siggs OM, Burdon KP, Best S, Goldberg I, Healey PR, Graham SL. Myocilin gene Gln368Ter variant penetrance and association with glaucoma in population-based and registry-based studies. JAMA Ophthalmol 2019; 137:28-35. [PMID: 30267046].

132. Allingham RR, Wiggs JL, De La Paz M, Vollrath D, Tallett DA, Broomer B, Jones KH, Del Bono EA, Kern J, Patterson K. Gln368STOP myocilin mutation in families with late-onset primary open-angle glaucoma. Invest Ophthalmol Vis Sci 1998; 39:2288-95. [PMID: 9804137].

133. Angius A, Spinelli P, Ghilotti G, Casu G, Sole G, Loi A, Totaro A, Zelante L, Gasparini P, Orzalesi N. Myocilin Gln368stop mutation and advanced age as risk factors for late-onset primary open-angle glaucoma. Arch Ophthalmol 2000; 118:674-9. [PMID: 10815160].

134. Nag A, Lu H, Arno M, Iglesias AI, Bonnemaijer P, Broer L, Uitterlinden AG, Klaver CC, van Duijn C, Hysi PG. Evaluation of the myocilin mutation Gln368Stop demonstrates reduced penetrance for glaucoma in European populations. Ophthalmology 2017; 124:547-53. [PMID: 28038983].

135. Fingert JH. Penetrance of Myocilin Mutations—Who Gets Glaucoma? JAMA Ophthalmol 2019; 137:35-7. [PMID: 30267050].

136. Graul TA, Kwon YH, Zimmerman MB, Kim C-S, Sheffield VC, Stone EM, Alward WL. A case-control comparison of the clinical characteristics of glaucoma and ocular hypertensive patients with and without the myocilin Gln368Stop mutation. Am J Ophthalmol 2002; 134:884-90. [PMID: 12470758].

137. Li C-M, Zhang Y-H, Ye R-H, Yi C-X, Zhong Y-M, Cao D, Liu X. Anticipation, anti-glaucoma drug treatment response and phenotype of a Chinese family with glaucoma caused by the Pro370Leu myocilin mutation. Int J Ophthalmol 2014; 7:44-[PMID: 24634862].

138. Wang K, Gaitsch H, Poon H, Cox NJ, Rzhetsky A. Classification of common human diseases derived from shared genetic and environmental determinants. Nat Genet 2017; 49:1319-[PMID: 28783162].

139. Souzeau E, Glading J, Keane M, Ridge B, Zhou T, Burdon KP, Craig JE. Predictive genetic testing experience for myocilin primary open-angle glaucoma using the Australian and New Zealand Registry of Advanced Glaucoma. Genet Med 2014; 16:558-63. [PMID: 24406458].

140. Souzeau E, Tram KH, Witney M, Ruddle JB, Graham SL, Healey PR, Goldberg I, Mackey DA, Hewitt AW, Burdon KP. Myocilin predictive genetic testing for primary open-angle glaucoma leads to early identification of at-risk individuals. Ophthalmology 2017; 124:303-9. [PMID: 27993484].

141. Miller MA, Fingert JH, Bettis DI. Genetics and genetic testing for glaucoma. Curr Opin Ophthalmol 2017; 28:133-8. [PMID: 27898466].

142. Khawaja AP, Viswanathan AC. Are we ready for genetic testing for primary open-angle glaucoma? Eye (Lond) 2018; 32:877-83. [PMID: 29379103].

143. Stone EM, Aldave AJ, Drack AV, MacCumber MW, Sheffield VC, Traboulsi E, Weleber RG. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. Ophthalmology 2012; 119:2408-10. [PMID: 22944025].

144. Craig JE, Han X, Qassim A, Hassall M, Bailey JNC, Kinzy TG, Khawaja AP, An J, Marshall H, Gharahkhani P. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. Nat Genet 2020; 52:160-6. [PMID: 31959993].

145. Burns JN, Orwig SD, Harris JL, Watkins JD, Vollrath D, Lieberman RL. Rescue of glaucoma-causing mutant myocilin thermal stability by chemical chaperones. ACS Chem Biol 2010; 5:477-87. [PMID: 20334347].

146. Yam GH-F, Gaplovska-Kysela K, Zuber C. Roth Jr. Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis. Invest Ophthalmol Vis Sci 2007; 48:1683-90. [PMID: 17389500].

147. Zode GS, Kuehn MH, Nishimura DY, Searby CC, Mohan K, Grozdanic SD, Bugge K, Anderson MG, Clark AF, Stone EM. Reduction of ER stress via a chemical chaperone prevents disease phenotypes in a mouse model of primary open angle glaucoma. J Clin Invest 2011; 121:3542–3553[PMID: 21821918].

148. Zode GS, Bugge KE, Mohan K, Grozdanic SD, Peters JC, Koehn DR, Anderson MG, Kardon RH, Stone EM, Sheffield VC. Topical ocular sodium 4-phenylbutyrate rescues glaucoma in a myocilin mouse model of primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2012; 53:1557-65. [PMID: 22328638].

149. Orwig SD, Chi PV, Du Y, Hill SE, Cavitt MA, Suntharalingam A, Turnage KC, Dickey CA, France S, Fu H. Ligands for glaucoma-associated myocilin discovered by a generic binding assay. ACS Chem Biol 2014; 9:517-25. [PMID: 24279319].

150. Serle JB, Katz LJ, McLaurin E, Heah T, Ramirez-Davis N, Usner DW, Novack GD, Kopczynski CC. Two phase 3 clinical trials comparing the safety and efficacy of netarsudil to timolol in patients with elevated intraocular pressure: Rho kinase elevated IOP treatment trial 1 and 2 (ROCKET-1 and ROCKET-2). Am J Ophthalmol 2018; 186:116-27. [PMID: 29199013].

151. Hoy SM. Latanoprostene bunod ophthalmic solution 0.024%: a review in open-angle glaucoma and ocular hypertension. Drugs 2018; 78:773-80. [PMID: 29761382].

152. Khouri AS, Serle JB, Bacharach J, Usner DW, Lewis RA, Braswell P, Kopczynski CC, Heah T, Benza R, Boyle JW IV. Once-daily netarsudil versus twice-daily timolol in patients

with elevated intraocular pressure: the randomized phase 3 ROCKET-4 Study. Am J Ophthalmol 2019; 204:97-104. [PMID: 30862500].

153. Fingeret M, Gaddie IB, Bloomenstein M. Latanoprostene bunod ophthalmic solution 0.024%: a new treatment option for open-angle glaucoma and ocular hypertension. Clin Exp Optom 2019; 102:541-50. [PMID: 30614563].

154. Wang SK, Chang RT. An emerging treatment option for glaucoma: Rho kinase inhibitors. Clin Ophthalmol 2014; 8:883-[PMID: 24872673].

155. Rao PV, Deng P-F, Kumar J, Epstein DL. Modulation of aqueous humor outflow facility by the Rho kinase–specific inhibitor Y-27632. Invest Ophthalmol Vis Sci 2001; 42:1029-37. [PMID: 11274082].

156. Harrison BA, Almstead ZY, Burgoon H, Gardyan M, Goodwin NC, Healy J, Liu Y, Mabon R, Marinelli B, Samala L. Discovery and Development of LX7101, a Dual LIM-Kinase and ROCK Inhibitor for the Treatment of Glaucoma. ACS Med Chem Lett 2015; 6:84-8. [PMID: 25589936].

157. Ellis DZ. Guanylate cyclase activators, cell volume changes and IOP reduction. Cell Physiol Biochem 2011; 28:1145-54. [PMID: 22179003].

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