Physiological function of myocilin and its role in the pathogenesis of glaucoma in the trabecular meshwork (Review)

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Abstract. Myocilin is highly expressed in the trabecular meshwork (TM), which plays an important role in the regulation of intraocular pressure (IOP). Myocilin abnormalities may cause dysfunction of the TM, potentially leading to increased IOP. High IOP is a well-known primary risk factor for glaucoma. Myocilin mutations are common among glaucoma patients, and they are implicated in juvenile-onset open-angle glaucoma (JOAG) and adult-onset primary open-angle glaucoma (POAG). Aggregation of aberrant mutant myocilins is closely associated with glaucoma pathogenesis. The aim of the present review was to discuss the recent findings regarding the major physiological functions of myocilin, such as intra- and extracellular proteolytic processes. We also aimed to discuss the risk factors associated with myocilin and the development of glaucoma, such as misfolded/mutant myocilin, imbalance of myocilin and extracellular proteins, and instability of mutant myocilin associated with temperature. Finally, we further outlined certain issues that are yet to be resolved, which may represent the basis for future studies on the role of myocilin in glaucoma.

Contents

1. Introduction

Glaucoma is second leading cause of blindness after cataract, and the leading cause of irreversible blindness globally (1-4). Primary open-angle glaucoma (POAG) is the most prevalent form of glaucoma (5) and is responsible for ~90% of all cases (6). The pathogenesis of glaucoma remains unknown; however, accumulating evidence indicates that genetic factors may play a causative role (7). The myocilin gene (MYOC), which encodes the secreted protein myocilin, is the first and most extensively investigated gene associated with familial forms of POAG (8,9). MYOC is detected in the aqueous humor and MYOC mutations are the most common type of mutation in patients with glaucoma (10), accounting for 10% of juvenile-onset open-angle glaucoma (JOAG) (11) and 2-4% of adult-onset POAG (12). JOAG, unlike adult-onset POAG, has a close genotype-phenotype association with regard to myocilin mutations and glaucoma, and typical signs include high intraocular pressure (IOP) (13,14), severe optic nerve damage and earlier age at onset (usually <40 years) (15,16), which, if left untreated, results in severe visual impairment (17).

Myocilin is ubiquitously expressed in normal tissues and organs; however, myocilin-associated disease only occurs in

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Abbreviations: JOAG, juvenile-onset open-angle glaucoma; POAG, primary open-angle glaucoma; UPR, unfolded protein response; ER, endoplasmic reticulum; TGF, transforming growth factor; CHOP, c/ebp-homologous protein; Grp78, glucose-regulated protein 78; IOP, intraocular pressure; OLF, olfactomedin; LINK, linker domain; OS, oxidative stress; IL-1, interleukin-l; ROS, reactive oxygen species; ERAD, ER-associated degradation; Grp94, glucose-regulated protein 94; NF-κB, nuclear factor κB; MMP, matrix metalloproteinase; LZ, leucine zippers; PBA, sodium 4-phenylbutyrate; MYOC, myocilin; TM, trabecular meshwork; SPARC, secreted protein acidic and rich in cysteine; TIMP3, tissue inhibitor of matrix metalloproteinase 3

Key words: myocilin, function, pathogenesis, glaucoma, trabecular meshwork

the eye (18). Myocilin is widely expressed in ocular tissues and highly expressed in the trabecular meshwork (TM) (19). Wild-type myocilin is secreted in the TM (9), is located in the aqueous humor and plays an important role in the regulation of IOP (20,21). High IOP is a risk factor for glaucoma (22,23) and reduction of IOP is an approach to glaucoma treatment (24). Mutant myocilin aggregation is closely associated with glaucoma (25) and it may be involved in morphological changes in the TM that may result in cell apoptosis (7). A growing body of evidence suggests that myocilin, the TM, IOP and glaucoma are closely associated.

The present review exclusively focused on the results of the latest studies that provide an insight into the physiological functions of myocilin and its role in the pathogenesis of glaucoma in the TM. An overview of the primary findings and future research topics requiring further investigation was performed in the present review.

2. Physiological functions and characteristics of myocilin

Structure of myocilin. Despite numerous studies over the 20 years since its discovery as a glaucoma-associated gene in 1997, the physiological functions and biological activities of myocilin in the TM remain poorly understood. The first report of identified myocilin mutations in autosomal dominant POAG came from Stone *et al* (26), and myocilin was found to map to the GLC1A locus at 1q24.3-q25.2 (OMIM: 601652). Myocilin, which encodes a 504-amino acid glycoprotein and undergoes glycosylation at amino acid residues 57-59 (27), has three exons and contains two major homology regions, the N- and C-terminus (Fig. 1) (23,26,28-35). Notably, the majority of myocilin mutations are localized in exon 3 (Fig. 1). The N-terminus of myocilin contains leucine zippers (LZ) within two coil-coil domains (35). Furthermore, the N-terminus is involved in the initial myocilin oligomerization through LZ (36), and in the extracellular interactions of myocilin with other extracellular proteins through two coil-coil domains (35,37). The C-terminus contains olfactomedin (OLF), which is important for the structure and function of myocilin (35), specifically in the process of intracellular trafficking (36). Notably, N- and C-terminus functions affect the aqueous humor outflow in the TM.

Intracellular proteolytic process. Normally, myocilin is intracellularly cleaved within the endoplasmic reticulum (ER) of TM cells and secreted into the aqueous humor (33,38). C-terminal myocilin fragments have been detected in the TM and the aqueous humor (23,33). N-terminal myocilin containing LZ has also been identified (39); however, this is intracellularly retained in the ER (23,33,36,40,41). N-terminal fragments can be intracellularly degraded during proteolytic processing, or they can interact with other intracellular proteins (40). Previous studies have suggested that myocilin undergoes proteolytic cleavage, and that the location of the proteolytic cleavage site is possibly between Glu214-Leu215 (39,42) or between Arg226-Ile227 (23). It was reported that myocilin fragments containing OLF did not change the outflow capacity of the aqueous humor, suggesting that both OLF and LZ fragments must coexist for myocilin to function properly in the intracellular proteolytic process (39).

Similar to myocilin, calpain II (cysteine protease) is also present within the lumen of the Golgi apparatus and the ER (43). Calpain II is required for the intracellular proteolytic cleavage of myocilin (40). The proteolytic processing of myocilin does not require the N-terminus, and two different domains of myocilin participate in the proteolytic processing through calpain II (40): i) C-terminal OLF, which likely acts as a substrate binding site recognized by calpain II; and ii) linker domain (LINK), which acts as the cleavage site (Fig. 2) (40). These findings are supported by previous studies (23,42,44), suggesting that myocilin mutations located at OLF may inhibit the proteolytic processing of myocilin. Amino acid positions mutated in OLF likely affect the structure of the myocilin binding site to calpain II (40). Interestingly, Pro370Leu, which contributes to the most severe glaucoma phenotype (44), produces the most severe inhibition of proteolytic processing. The inhibition of proteolytic processing by Glu323Lys and Asp380Ala is less severe, causing less severe glaucoma (23). However, the association of the severity of glaucoma with the inhibition of proteolytic processing remains unclear.

Extracellular proteolytic process. Although the physiological function of the intracellular proteolytic processing of myocilin remains unknown, the amount of proteolytic myocilin may be associated with the regulation of the normal TM structure through extracellular proteins, including fibrillin-1 (45), secreted protein acidic and rich in cysteine (SPARC) (34), hevin (34,46), collagen (45), optimedin (47), decorin (45), fibronectin (48) and laminin (45,49), which contribute to the regulation of aqueous humor outflow that can affect IOP (23). The identification of proteins interacting with myocilin is a possible approach to elucidating its functions, since interacting proteins are typically involved in the same physiological and pathological processes (50).

The first report of the possible role of proteolytic processing in regulating the interactions of myocilin with itself or other extracellular proteins came from Aroca-Aguilar *et al (*33,34), who demonstrated that homoaggregates of myocilin monomers and myocilin complexes (containing oligomers, matricellular proteins and extracellular matrix proteins) can form a dynamic extracellular network (Fig. 3) (34). The proteolytic processing of myocilin occurs in the relevant elements involved in IOP homeostasis (such as the aqueous humor and the TM); thus, the proteolytic cleavage in the dynamic myocilin network possibly participates in regulating IOP (33) through controlling IOP homeostasis (aqueous humor production and drainage). Furthermore, the myocilin network may act as a link between the extracellular matrix and matricellular proteins (34). Coincidently, OLFs in the extracellular network are similar to those resulting from intracellular proteolytic processing of myocilin through calpain II (Figs. 2 and 3) (23,34,40).

Elevated IOP and overexpression of myocilin caused by inducers. Myocilin is stimulated by various inducers, including dexamethasone (DXM) (11,51-55), pentablock copolymer DXM nanoformulations (56), retinoic acid (57), transforming growth factor (TGF)-β1 (11), TGF-β2 (58), optineurin (59,60), mechanical stretch (11), rotenone (61), and hydrogen peroxide-inducible clone-5 (62). DEX is widely

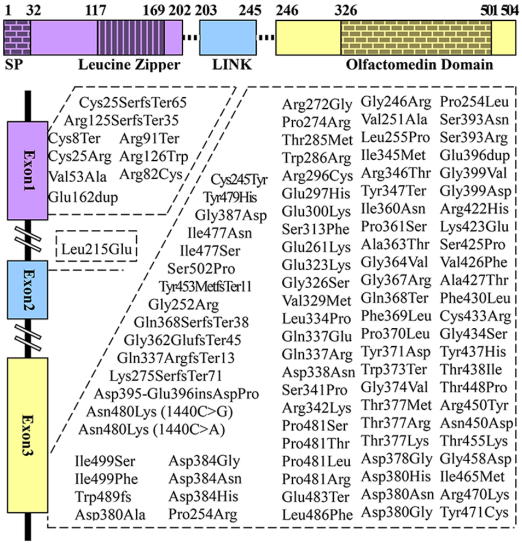

Figure 1. Structure of myocilin and pathogenic mutations localized in exons 1-3 (33). Three modules encoded by exons 1-3 approximately coincide with the N-terminus, LINK and C-terminus. SP, signal peptide; LINK, linker domain.

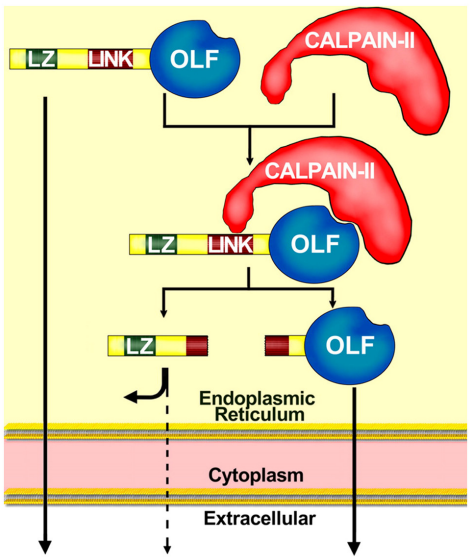

Figure 2. Proteolytic process of myocilin through calpain II (40). The proteolytic processing of myocilin is carried out by calpain II in the endoplasmic reticulum, producing two myocilin fragments: One containing LZ that is intracellularly retained and another containing OLF that is extracellularly secreted. Some full-length myocilins with LZ and OLF are also secreted. LINK contains the cleavage site. LZ, leucine zippers; OLF, olfactomedin; LINK, linker domain.

recognized as a major contributor to the induction of myocilin, which may cause high IOP.

Elevated IOP is a known primary risk factor for glaucoma (22,23); however, not all cases of glaucoma exhibit high IOP (63,64). Elevated IOP is caused by increased resistance to aqueous humor outflow through the TM (65,66) and accounts for visual field loss; however, its pathogenesis remains unknown (67). Homeostasis of aqueous humor drainage through the TM is essential for the maintenance of normal IOP (68). DXM-induced overexpression of myocilin in the TM cells (11,54,55) may increase IOP (69). Additionally, overexpression of myocilin induced by DXM possibly affects focal adhesion, stress fibers and actin reorganization in TM cells, which subsequently results in ER aggregation and TM cell stiffness, leading to increased outflow resistance (62) and elevated IOP. However, the results regarding the reversal of myocilin (52,70) and increased IOP (70,71) do not support the hypothesis that myocilin overexpression causes increased IOP or glaucoma; this is supported by the following data: i) Observation of an equivalent increase of IOP in MYOC$^{-/-}$ and wild-type mice following DXM treatment (51); and ii) observation of increased IOP and absence of myocilin overexpression following DXM treatment (71). These studies suggest that increased IOP may not be associated with myocilin overexpression.

3. Pathogenesis of mutant/misfolded myocilins

The aforementioned studies suggest that the biological functions of myocilin remain poorly understood. Similarly, the role of myocilin in the pathogenesis of glaucoma is currently unknown, although its mutations have been reported to be associated with JOAG and adult-onset POAG.

Myocilin mutations. MYOC mutations alter the myocilin protein, which affects the normal regulation of IOP and may lead to glaucoma (72). To date, 278 different myocilin mutations have been reported, among which pathogenic mutations account for 37.77% (26) (Fig. 1) (23,28-35), 9 of which have been identified in exon 1, 1 in exon 2, and 95 in exon 3. Myocilin predominantly displays two types of mutations: Missense mutations (83.8%), which are associated with JOAG and adult-onset POAG (16); and nonsense mutations (5.7%) (26). Different myocilin mutations cause POAG with varying age at onset and glaucoma phenotypes of varying severity (73). Pro370Leu is one of the most severe glaucoma phenotypes, which is involved in mitochondrial dysfunction in TM cells and may lead to apoptosis (74). Gln368Stop is the most common mutation; however, it exhibits a markedly lower penetrance for glaucoma (75).

Autosomal dominant disorders resulting from myocilin (28) may be caused by the following three pathogenic mechanisms: The dominant negative effect, gain of function, or haploinsufficiency (7,16). Some studies have demonstrated that neither haploinsufficiency (16,19,67,76) nor the dominant negative effect (77) are involved in myocilin-associated pathogenicity. However, recent findings (42,78) have suggested that the dominant negative effect may be involved in the pathogenic role of myocilin in glaucoma. Notably, the majority of experimental evidence support gain-of-function as a pathogenic mechanism involved in myocilin mutation-associated glaucoma (21,23,36,37,41,42,67,73,76,79-84).

Pathogenesis of ER stress and oxidative stress (OS) caused by mutant myocilin. Recent studies have indicated that the notable paucity of normal myocilin (16,67,76,85) or its overexpression (85) are not associated with the pathogenesis of glaucoma, and that the pathogenesis of glaucoma is dependent on the expression of mutant/misfolded myocilins (86,87). Notably, mutations may cause myocilin misfolding (88). Furthermore, the secretion of wild-type myocilin is inhibited in the presence

of co-expressed mutant myocilin (36,41). The aggregation of misfolded/wild-type myocilins in the ER may be harmful for TM cells and lead to apoptosis (19,84). Previous results have suggested that the TM is several times thicker in patients with glaucoma harboring mutations compared with that in patients without myocilin mutations (7). Therefore, myocilin mutations appear to be involved in the morphological changes in the TM, which lead to cell apoptosis (7).

Mutant myocilins aggregate intracellularly in insoluble and soluble aggresomes, interact with ER proteins and promote ER stress (19,81-84). Mutant myocilins have been suggested to induce apoptosis and may contribute to TM cell dysfunction, leading to increased IOP (86,89,90). Subsequently, ER stress may cause OS (87). During ER stress, the level of reactive oxygen species (ROS) is increased (91). Excess production and an accumulation of ROS compromise reduction-oxidation balance and cause OS (91). Notably, ER stress and OS are associated events involved in the pathogenesis of various diseases (91), including glaucoma-associated TM damage and increased IOP (87,90). However, the molecular pathways that connect ER stress and OS are poorly understood. OS is caused by the excessive production and aggregation of ROS (91). The TM, which is the most sensitive to OS among the tissues in the anterior chamber of the eye (86), comes into direct contact with the ROS-containing aqueous humor (87). Furthermore, ROS alone may cause protein misfolding (86). Misfolded myocilin can render cells more sensitive to OS (87). It was recently suggested that mutant myocilin inhibited antioxidant enzymes, such as paraoxonase 2, that efficiently decrease OS and inhibit ER stress-induced apoptosis (87). Therefore, decreased antioxidative enzymes in the TM may cause ROS elevation in the aqueous humor outflow system (87).

It has been reported that OS aggravates ER stress by inducing the overproduction of misfolded proteins (92). OS has also been considered a major factor causing damage to the TM (93). To relieve the damage from ER stress and restore homeostasis, the aggregated misfolded myocilin activates the unfolded protein response (UPR), which protects the TM cells (94-96), improves the protein folding mechanism and degrades misfolded proteins (94,95). However, if ER stress persists, UPR induces cell apoptosis (19,84). In addition, mutant myocilin cannot be cleared by ER-associated degradation, which transports degraded products of misfolded myocilin to the cytosol (19), leading to deleterious aggregation of amyloid-containing myocilin (97). These studies suggest that myocilin misfolding, UPR, ROS, OS and ER stress may be related events. Notably, ER stress and OS are risk factors for glaucoma, and together with the deleterious effect of misfolded myocilin, may cause a more severe glaucoma phenotype compared with any of these factors alone. These associated events disrupt the proteostasis of myocilin, leading to an imbalance between production and clearance of misfolded myocilins.

Pathogenesis of the co-aggregation of glucose-regulated protein (Grp) 94 with mutant myocilin. It has been reported that the inhibition of Grp94 is an effective approach to the treatment of glaucoma (79,98), supporting the co-aggregation of Grp94 with mutant myocilin and leading to retention within the ER (Fig. 4) (80). Not only does Grp94 accelerate the myocilin-OLF

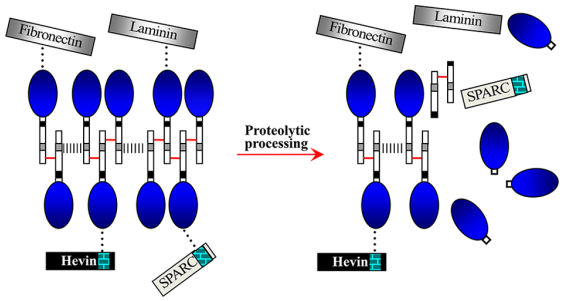

Figure 3. Interactions of myocilin with extracellular matrix proteins (laminin and fibronectin) and matricellular proteins (SPARC and hevin) (34). Homoaggregates of myocilin monomers covalently interact through disulfide bonds (short red lines) within LZ (12). Myocilin complexes interact by noncovalent bonds (grey dashed lines) in N-terminus (rectangle linked to the blue oval). Full-length myocilins non-covalently (black dots) interact with the extracellular calcium binding domains (brick pattern) of SPARC and hevin through OLF (blue oval). Interacting fashion of myocilin with laminin and fibronectin could be similar to that of SPARC and hevin. SPARC, secreted protein acidic and rich in cysteine; LZ, leucine zippers; OLF, olfactomedin.

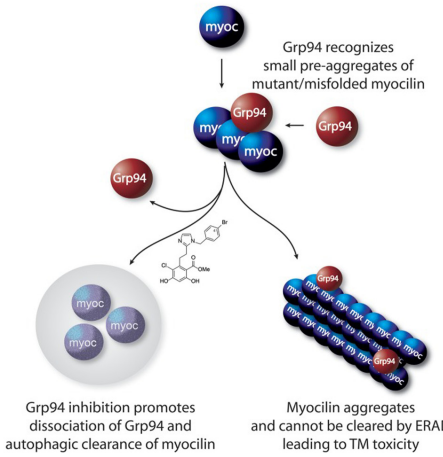

Figure 4. Co-aggregation of Grp94 with mutant/misfolded myocilin (80). Grp94, glucose-regulated protein 94.

aggregation rate, but it also enhances co-precipitation with OLF (79,80,99). Therefore, Grp94 inhibition facilitates mutant myocilin clearance as an anti-glaucoma therapy (79,97,100). 4-Br-BnIm, a Grp94 inhibitor, significantly clears aggregated myocilin caused by overexpression mutations (97), and alleviates mutant myocilin-induced toxicity against TM cells (27,79) via a secondary autophagic pathway to facilitate clearance (27). When forced to misfold and aggregate, wild-type myocilin becomes a client of Grp94 and sensitized to 4-Br-BnIm (79). Recent studies (12,101) reported that myocilin aggregates were cleared by a ubiquitin-proteasome and autophagy lysosomal pathways under normal homeostatic conditions. However, when myocilin is mutated, autophagy is activated due to dysfunction of the proteasomal degradation pathway (12), and mutant myocilin is preferably degraded by autophagy (101). Grp94 inhibitors prevent Grp94 from aggregating with mutant myocilin, which induces a secondary autophagic pathway to promote clearance of abnormal myocilin (80). Grp94 is also a regulator of UPR (79). Therefore, a reduction in Grp94 may affect UPR, which induces TM cell death under persistent ER stress due to abnormal aggregations of Grp94 and misfolded myocilin.

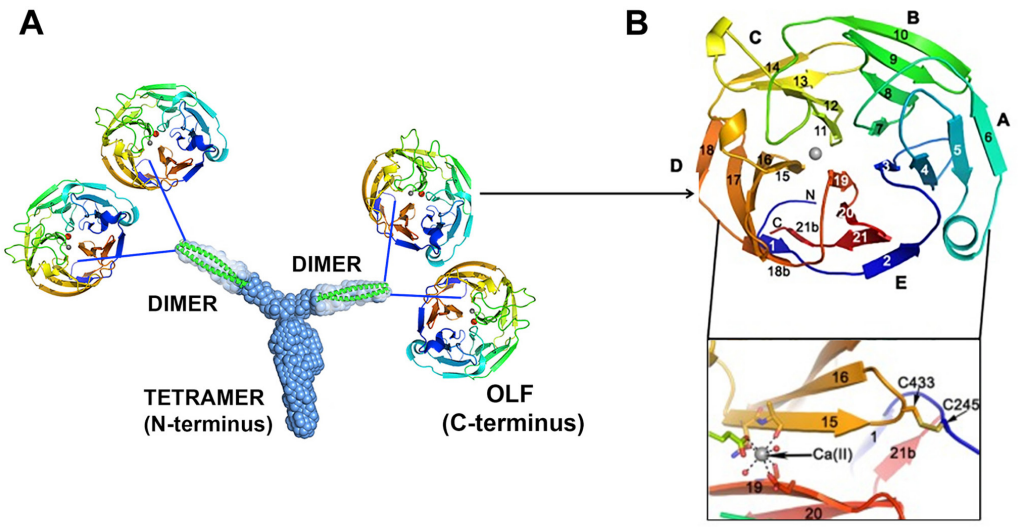

Figure 5. Structure of myocilin (108,117). (A) The N-terminus has a Y-shaped parallel (117). (B) The crystal of myocilin-OLF is a five-bladed β-propeller, and each blade is composed of four antiparallel β-strands, arranged radically around a central water-filled cavity (108). The blades in OLF are notably asymmetric. Nearly half of the toroid-shaped molecule is occupied by blades D and E. The myocilin-OLF propeller has five blades (A, B, C, D and E). A disulfide bond and calcium-binding site are located at the bottom face of the propeller, and a single disulfide bond is observed between Cys433 located within Loop D-15/D-16 and Cys245 at the N-terminus prior to the start of E-1. Tyr 437 further stabilizes the region near the single disulfide bond. Unlike other amyloid-like proteins (120), the effect of disulfide bonds on the stability of wild-type myocilin is not significant.

Myocilin and the interleukin (IL)-1/nuclear factor (NF)-κB inflammatory stress signaling pathway. Activation of IL-1/NF-κB inflammatory stress is a defining characteristic of high-tension glaucoma (102). The release of potent proinflammatory IL-1, including IL-1β, may lead to inflammation (103) through activation of the NF-κB pathway, which induces an inflammatory response (104). It has been demonstrated that chronic low-grade inflammation causes ocular hypertension (105) and is also involved in the pathogenesis of glaucoma (103). Mutant myocilins aggregate within the TM cells and activate the NF-κB signaling pathway, significantly inducing the expression of IL-1 and IL-1β; however, extracellular mutant myocilin does not activate the NF-κB signaling pathway (106). Therefore, intracellular aggregations of mutant myocilins may induce the overexpression of IL-1 via activating NF-κB, leading to chronic inflammation that can cause elevated IOP.

Interestingly, IL-1 has additional beneficial effects on glaucoma, including reducing IOP through stimulation of matrix metalloproteinase (MMP) expression (106) and inhibiting apoptosis caused by OS through NF-κB (102). IOP and OS are associated with the aggregation of misfolded/mutant myocilins (86). Notably, IL-1 was increased by 10-fold in TM cells harboring Gln368Stop; however, the increase was only 6-fold in cells harboring Try437His (106). These findings suggest that abnormal aggregation of myocilin mutations may induce OS and upregulate IL-1. However, the amount of IL-1 induced by Gln368Stop is notably higher compared with that induced by Try437His. Coincidently, Try437His causes a severe glaucoma phenotype; however, Gln368Stop only causes a moderately severe glaucoma phenotype. This may be explained by the greater extent of IOP reduction and inhibition of the apoptotic process caused by OS through IL-1 via Gln368Stop compared with Try437His in the short-term. This also explained the finding that Gln368Stop is the most common mutation; however, it revealed a markedly lower penetrance for glaucoma caused by high IOP.

4. Imbalance of pathogenic myocilin and extracellular proteins

Glaucoma-associated TM damage is involved in various biochemical and morphological changes, including aberrant extracellular matrix protein aggregation and cell apoptosis (107). Synthesis and degradation of the extracellular matrix are in a dynamic balance that is constantly remodeled by proteolysis and protein deposition (52). Certain diseases, including glaucoma, may result from disruption of this dynamic balance (107). Recently, the crystal structure of myocilin-OLF was elucidated, and it indicated that myocilin-OLF belongs to the five-bladed β-propeller family (108), which is a known hub for protein-protein interactions. To date, a number of extracellular proteins interacting with myocilin have been identified, including tissue inhibitor of matrix metalloproteinase (TIMP3) (8,50), fibronectin (107), flotillin-1 (109) and hevin (46).

Effect of pathogenic myocilin on MMPs and MMP inhibitors. MMPs have multiple physiological functions, among which cell migration and proliferation have been linked to myocilin (110,111). Changes in MMP activity are involved in the pathogenesis of glaucoma (112). Notably, MMP2 activity may be associated with the regulation of IOP, which may be predominantly associated with the TM, where myocilin and TIMP3 coexist (50). MMP2, which is abundant in the TM (8), is involved in the breakdown of extracellular matrix (52), which facilitates aqueous humor outflow (8). It has also been demonstrated that myocilin mutations or MYOC null can enhance inhibition of MMP2 by TIMP3 (8,50). An imbalance between MMPs and TIMP within the TM can

Table I. Structural location and stability of myocilin mutations.

Mutations	Melting temperature (˚C)	Structural location	Solubility	Mean age at diagnosis, years	Secretion 37˚C	Secretion 30˚C	Mean maximum IOP (mmHg)	(Refs.)
Wild-type	52.7±0.8	NA	Soluble	NA	+++	+++	NA	(37,118)
Trp286Arg	NA	β-sheet belt	NA	27.5±13.4	No	No	39.5±31.8	(26,80,97,108,118,121)
Pro370Leu	NA	Loop B-10/C-11, cation-π	Insoluble	13.3±2.4	No	Little	49±8.5	(15,19,26,82,108,118)
Lys423Glu	34.2±0.4	Loop B-10/C-11, cation-π	Insoluble	28.8±8.1	No	No	36±8.5	(26,77,108,118)
Ile477Asn	37.7±0.8	β-sheet belt	Insoluble	20.7±1.3	No	Little	44±0	(26,79,82,97,108,118)
Ile477Ser	39.7±0.2	β-sheet belt	Insoluble	33±0	No	+++	NA	(26,108,118)
Tyr437His	40.3±0.4	β-sheet belt	Insoluble	19.9±0	No	Little	43.7±0	(26,80,82,97,108,118)
Cys433Arg	40.4±0.4	β-sheet belt	Insoluble	22.8 ± 8.9	No	No	39.1 ± 12.6	(26,108,118)
Arg272Gly	41.0±0.3	β-sheet belt	NA	33±0	No	++	62±0	(26,108,118,122)
Ser502Pro	41.0±0.3	β-sheet belt	Insoluble	19±0	No	No	NA	(26,108,118)
Val426Phe	41.5±0.1	Loop B-10/C-11, cation-π	Insoluble	18.6±1.4	Little	+++	43±0	(26,108,118,122,123)
Asn480Lys	42.4±0.2	β-sheet belt	Insoluble	25.4±3.1	Little	++	37.7±12.4	(26,80,97,108,118)
Gly367Arg	42.7±0.1	Loop B-10/C-11, cation-π	Insoluble	26.6±5.5	No	+++	51±0	(26,28,108,118)
Ile499Phe	42.8±0.1	β-sheet belt	Partially insoluble	30.9±7.6	Little	+++	31±6.6	(26,82,108,118)
Gly252Arg	43.0±0.2	β-sheet belt	Insoluble	35±7.3	No	+++	62±0	(26,108,118,122)
Glu323Lys	44.0±0.5	Loop B-10/C-11, cation-π	Insoluble	19±0	Little	+++	43±0	(26,108,118,122)
Thr377Met	44.3±0.3	Loop B-10/C-11, cation-π	Partially insoluble	21.4±5.0	+	+++	44±0	(26,82,108,118,121,122)
Gly364Val	45.0±0.4	Loop B-10/C-11, cation-π	Partially insoluble	34±0	+	+++	36±0	(26,82,108,118,121)
Pro481Leu	45.5±0.4	Ca^{2+} site environs	Insoluble	33±0	No	++	46±0	(26,108,118,121)
Asp380Ala	46.6±0.3	Ca^{2+} site environs	Partially insoluble	11.9±3.5	Little	+++	34.2±17.9	(26,82,108,118)
Ala427Thr	50.5±0.2	Ca^{2+} site environs	Soluble	61±21.2	+++	NA	31.8±6.1	(26,108,118,123)
Ala445Val	54.2±0.2	NA	Soluble	51.5±16.3	+++	NA	24±0	(26,108,118,121)

IOP, intraocular pressure; NA, not available.

cause POAG (113). Of note, mutant myocilins can also reduce the active forms of MMP2 (107). Therefore, the effect of mutant myocilin on MMP2 activation disrupts the balance between MMPs and TIMPs, leading to glaucoma (96). In addition, reduction of MMP2 inhibits the decomposition of the extracellular matrix, disrupting its homeostasis, which may change the TM morphology and function, leading to increased IOP.

Effect of pathogenic myocilin on fibronectin, flotillin-1 and hevin. Fibronectin is a key component of the extracellular matrix of the TM, and is also an important mediator involved in extracellular matrix formation, which regulates aqueous humor outflow (114). Aberrant aggregation of the extracellular matrix in the TM reduces aqueous humor outflow and increases IOP in POAG (107). Overexpression of fibronectin and increased ER stress markers c/ebp-homologous protein (CHOP) and Grp78 coexist in mice harboring MYOC-Tyr437His (107). Furthermore, another study reported that mutant myocilins increased CHOP and Grp78 in the TM (96). Myocilin, fibronectin, CHOP and Grp78 expression levels were increased following DXM treatment (115), which was similar to the overexpression of myocilin and fibronectin observed following DXM-Ac treatment (51). Recent studies have suggested that a reduction of ER stress through sodium 4-phenylbutyrate decreases the DXM-induced increase of IOP (115) and fibronectin aggregation caused by mutant myocilin (107) in TM cells. Therefore, myocilin, fibronectin, CHOP, Grp78 and the extracellular matrix may be considered as elements implicated in the pathogenesis of glaucoma in the TM. Additionally, mutant myocilin induces aberrant aggregation of the extracellular matrix in the ER of TM cells, which may contribute to decreased aqueous humor outflow and increased IOP (107). Furthermore, failure of the TM to reduce ER stress caused by mutant myocilin accounts for the induction of CHOP, which may also be associated with apoptosis and increased IOP in the TM cells (96).

Flotillin-1, a structural protein of lipid rafts, interacts with myocilin (109). Myocilin mutations, including Gly364Val, Tyr437His and Lys423Glu, which are scattered in the OLF, fail to interact with flotillin-1 (109), suggesting that myocilin-OLF may be required for the interaction. Although the role of the interaction remains to be elucidated, loss of interaction with flotillin-1 due to myocilin mutations may be a pathogenic mechanism underlying the development of POAG (109). Hevin is a matricellular protein involved in the assembly of the extracellular matrix (116). Mutant myocilin causes intracellular aggregation of hevin and also affects hevin secretion (46). Hevin coexpressed with Pro370Leu, which is also known as a severe glaucoma mutation, aggravates the impairment of hevin secretion (46). These findings indicate that mutant myocilins affect the interactions with extracellular proteins, leading to disruption of extracellular matrix homeostasis, which may be involved in the pathogenesis of glaucoma.

5. Association of glaucoma pathogenesis with the crystal structure and stability of myocilin

Crystal structure of myocilin. A better understanding of the conformation of myocilin can provide a structural basis for investigating myocilin mis-/unfolding in myocilin-associated glaucoma and, in turn, result in a better understanding of myocilin-associated glaucoma pathogenesis. The myocilin N-terminus has a unique tripartite architecture, including a Y-shaped parallel dimer-of-dimers with distinct tetramer and dimer regions. Furthermore, full-length myocilin should also be branched, with two pairs of C-terminal OLFs (Fig. 5A) (117). MYOC-Glu396Asp has discontinuous D-18/D-18b and E-21/E-21b strands (Fig. 5B) (108,117) that are unlike the continuous D-18 and E-21 strands in wild-type myocilin. Accumulating evidence has revealed that pathogenic myocilin mutations are associated with intracellular aggregation propensity and thermal stability (19,32,37,41,42,82,108,118,119). Three destabilizing regions are localized in myocilin-OLF (108): i) Loop B-10/C-11 and cation-π; ii) hydrophobic β-sheet belt; and iii) Ca^{2+} site environs (Fig. 5B).

Structural location and stability of myocilin mutations. The largest number of mutations are observed in core β-sheet belts (~40%), loop B-10/C-11 and cation-π (~33%) (108), particularly the most destabilized myocilin-OLF mutations, such as Trp286Arg, Tyr437His, Lys423Glu, Pro370Leu, Ile477Asn and Ile477Ser (118,119). The myocilin-OLF mutations exhibit changes in the side chains. The most destabilizing mutations also exhibit other characteristics: i) Lower melting temperature (Table I) (108); ii) insoluble aggregates (118); and iii) earlier age at diagnosis. In contrast to the most destabilizing mutations, the most stabilizing myocilin-OLF mutations, Ala427Thr and Ala445Val (118), exhibit similar stability and structure to those of wild-type myocilin-OLF. Furthermore, the difference in their melting temperature from that of wild-type myocilin-OLF (52.7±0.8˚C) is only within 2˚C, and they are soluble (118). The trend in melting temperature follows the general stability of myocilin-OLF mutations and the severity of their aggregation. Additionally, this instability may cause structural changes in myocilin-OLF, resulting in mis-/unfolding. Notably, higher proportion of mis-/unfolded myocilin has been associated with greater exposure of interior hydrophobic regions and more severe intracellular aggregation propensity (118). Intracellular aggregation of mutant myocilin deforms TM cells (84) and changes the size of pores between the cells, through which aqueous humor outflows. Dysfunction of TM cells may contribute to the pathogenesis of glaucoma (41,84).

Myocilin mutations at a moderate level of stability (Table I) renew the secretion through shifting temperature from 37 to 30˚C (37,82,118), demonstrating their temperature-sensitive secretion. Trimethylamine N-oxide and sarcosine also stabilize myocilin mutations through shifting their melting temperatures to near that of wild-type myocilin (119). Thr377Met, Ile499Phe and Gly364Val, which are abundantly secreted at 30˚C, are associated with less severe glaucoma (82). Interestingly, Pro370Leu and Ile477Asn, which are associated with a more severe glaucoma phenotype (82,118), are not secreted at higher levels when the temperature changes (82). Thus, temperature-sensitive secretion may be a notable property of these moderate myocilin mutations (82). A temperature of 30˚C is a condition known to facilitate protein folding (37,42). Therefore, this favorable temperature promotes the correct folding of myocilin into its native form. Taken together, these

studies demonstrated that the stability of myocilin mutations may be associated with their pathogenicity. Notably, the less stable myocilin mutations were associated with the more severe glaucoma phenotype and were less sensitive to temperature.

6. Conclusion

Based on the aforementioned evidence, although myocilin mutations have been reported to be associated with JOAG and adult-onset POAG, the physiological functions and pathogenicity of myocilin remain elusive. Thus far, there are several possible points of view on the pathogenic potential of myocilin: i) Myocilin misfolding/unfolding; (ii) overexpression of myocilin; iii) co-aggregation of Grp94; iv) disruption of extracellular matrix homeostasis caused by mutant myocilin; v) OS, ER stress and IL-1/NF-κB inflammatory stress; and vi) instability resulting from conformational disorders caused by mutant myocilin. However, certain suggestions require further investigation, particularly those with contradicting conclusions. Furthermore, unsolved or partially solved problems require further research, as they could direct future studies on myocilin and contribute to novel therapeutic approaches to the treatment of myocilin-associated glaucoma.

The functions of myocilin through interactions of its binding partners should be further investigated, as interacting proteins may have similar biological functions. In addition, it is necessary to gain further insight into the proteolytic processes of myocilin, as this may contribute to an effective approach to breaking down abnormal aggregations of myocilin. Furthermore, a more detailed understanding of the structural basis of myocilin stability will be valuable for elucidating the roles of conformational disorders, such as misfolding or unfolding, in myocilin-associated glaucoma. Notably, this may help elucidate the role of myocilin mutations in the pathogenesis of glaucoma. Additionally, the association of glaucoma phenotype severity with the secretion (associated with temperature, stability and insolubility) of mutant myocilin should be emphasized, as unraveling these elusive associations may contribute to understanding the pathogenesis underlying abnormal aggregations of mutant myocilin. Future studies regarding myocilin should focus on the deleterious effect of myocilin misfolding on OS and ER stress in the TM, which are a series of associated events, each of which causes the next, leading to further injury to the TM. Furthermore, these effects are associated with increased IOP, which is a known primary risk factor for glaucoma.

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Authors' contributions

HW, ML, ZZ, HX, XC and YJ designed the article, contributed to the conception of the study and critically revised the article for important intellectual content. HW and YJ drafted the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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