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Genetically Increasing *Myoc* Expression Supports a Necessary Pathologic Role of Abnormal Proteins in Glaucoma

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Despite the importance of MYOC for glaucoma, the protein's normal function(s) and the pathogenic mechanism(s) of *MYOC* mutations are not clear. Elevated intraocular pressure (IOP) and glaucoma are sometimes induced by corticosteroids, and corticosteroid use can result in substantially increased *MYOC* expression. It has been suggested, therefore, that steroid-induced MYOC protein levels cause steroid-induced glaucoma and that protein level-increasing mutations in *MYOC* contribute to glaucoma not associated with steroid use. A causative role of elevated MYOC levels in steroid-induced glaucoma is controversial, however, and it is not clear if elevated MYOC levels can result in IOP elevation. To directly test if increased levels of MYOC can cause IOP elevation and glaucoma, we generated bacterial artificial chromosome transgenic mice that overexpress *Myoc* at a level similar to that induced by corticosteroid use. These mice do not develop elevated IOP or glaucoma. Our present findings, along with the absence of glaucoma in mice completely lacking MYOC, show that changing the level of MYOC is not pathogenic (from absent to approximately 15 times normal). These findings suggest that noncoding sequence variants are unlikely to influence glaucoma and that disease pathogenesis in primary open-angle glaucoma patients is dependent upon the expression of abnormal mutant proteins. This work does not support a causative role for increased MYOC levels or the *MYOC* gene in steroid-induced glaucoma.

Glaucoma is a leading cause of preventable blindness in the world (30, 46). Elevated intraocular pressure (IOP) is one of the strongest known risk factors (41) and can cause glaucoma in animal models (6). IOP elevation in human glaucoma results from increased resistance to aqueous-humor drainage (36). Although the etiology of glaucoma is complex, the result is death of retinal ganglion cells and loss of vision (31). Primary open-angle glaucoma (POAG) is the most common form of glaucoma in the United States and affects up to 1 to 2% of people over 40 years of age (47).

Multiple genetic loci are reported to contribute to POAG (32, 34, 42, 49, 51, 52). Disease-associated mutations have been identified in the myocilin gene, *MYOC* (14, 43). Studies from a broad range of ethnic backgrounds generally agree that *MYOC* mutations are responsible for approximately 3% of adult-onset POAG and a greater proportion of juvenile-onset open-angle glaucoma (2, 14). Despite this, neither the normal function(s) of MYOC nor how *MYOC* mutations result in IOP elevation and glaucoma has been defined.

MYOC has also been implicated in steroid-induced ocular hypertension with glaucoma. Steroid-induced ocular hypertension in response to glucocorticoid treatment occurs in as many as 40% of people treated with glucocorticoids (37, 53). MYOC is up regulated in cultured trabecular meshwork (TM; an ocular drainage structure) cells treated with glucocorticoids. Thus, *MYOC* was identified as a candidate to mediate glucocorticoid-induced glaucoma (26, 28). Supporting this, IOP elevation (assessed as an increase in drainage structure resistance) is glucocorticoid inducible in some but not all human anterior segment perfusion cultured eyes. In these cultures, IOP elevation correlates with *MYOC* induction (8) and ultrastructural changes (9) in the TM. Cultured anterior segments that developed elevated IOP had *MYOC* induction, while those that did not develop elevated IOP had no *MYOC* induction (8). Similarly, monkeys treated with glucocorticoids develop ocular hypertension (13), and in at least some eyes glucocorticoids induce MYOC and cause ultrastructural changes in the TM (8).

Further studies, which do not involve steroid use, also can support a role for elevated MYOC levels in IOP elevation and glaucoma. In some glaucoma patients, the TM has elevated MYOC levels and broadened MYOC distribution (24). Cultured human anterior segments perfused with recombinant MYOC are reported to develop elevated IOP, whereas those perfused with an equal amount of other proteins or denatured MYOC do not (12). Finally, in the albino Wistar rat strain, experimental IOP elevation did not induce ocular *MYOC*, suggesting that *MYOC* induction does not occur secondary to IOP elevation (1).

Despite these circumstantial data, there is no direct in vivo evidence that elevated MYOC levels cause IOP elevation and glaucoma. The rat study mentioned above (1) does not exclude MYOC elevation as a secondary response to increased IOP in different settings (either in different genetic contexts or in response to more chronic IOP elevation, as occurs in human

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patients). In fact, in some cultured human anterior segments, *MYOC* expression is elevated in response to IOP elevation (3). Thus, it is not clear if MYOC elevation causes IOP elevation and glaucoma or if MYOC is simply induced because of the stress of elevated IOP, secondary tissue damage, or glaucoma treatments. Whether or not increased MYOC levels cause IOP elevation and glaucoma needs to be experimentally tested in an in vivo model. This knowledge is important for understanding pathogenic mechanisms associated with MYOC.

Determining whether or not increased MYOC levels cause glaucoma also is important for assessing the risk of disease associated with noncoding mutations. Some investigators suggest that *MYOC* promoter variants may influence the progression of glaucoma (10, 11, 29), while others disagree with this conclusion (2). Since human patients are currently offered genetic testing based on the presence of noncoding variants, the issue is very important. In the present study, we used transgenic mice to directly test if genetically elevating MYOC is sufficient to cause elevated IOP and glaucoma. Mice with increased MYOC levels did not develop elevated IOP or glaucoma. This clearly indicates that MYOC elevation alone is not sufficient to cause glaucoma. These results question the hypotheses that MYOC is involved in steroid-induced glaucoma and that promoter sequence variants alone are involved in POAG.

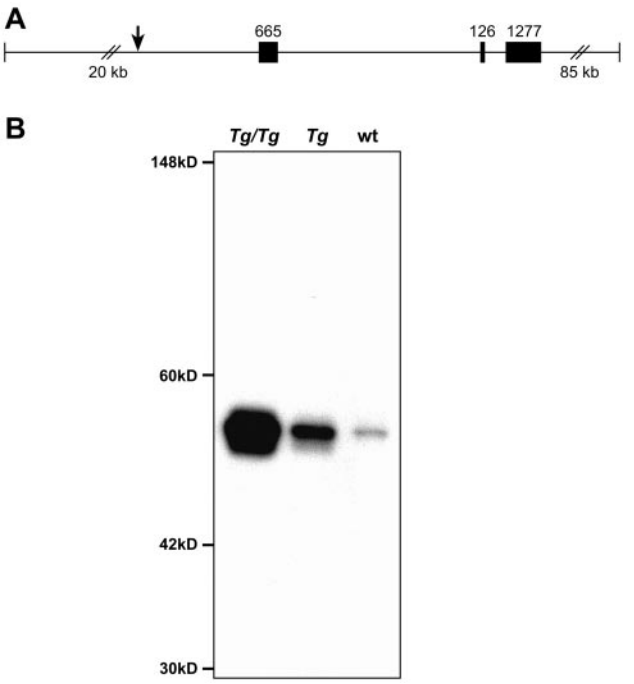

FIG. 1. BAC transgene and protein levels. (A) Schematic of the BAC containing the entire *Myoc* gene and flanking genomic DNA. The BAC contains an approximately 20-kb region upstream of exon 1 and an 85-kb region downstream of exon 3. The upstream sequence includes a strain-specific sequence length polymorphism (arrow) that is approximately 4 kb from exon 1. The length of each exon is indicated in nucleotides. (B) Substantial increase in MYOC protein in transgenic mice compared to that in control mice. Total protein was isolated from ocular tissue enriched in drainage structures and analyzed by Western blotting (see Materials and Methods). Twenty-five micrograms of total protein was loaded in each lane. Lanes are homozygous transgenic (*Tg*/*Tg*), hemizygous (*Tg*), and control (wt [wild type]). Molecular masses are indicated on the left.

MATERIALS AND METHODS

Animal husbandry and stocks. All experiments were conducted in compliance with institutional guidelines and the Association for Research in Vision and Ophthalmology statement on the use of animals in ophthalmic and vision research. Mice were housed under previously defined conditions (39). We created transgenic mice by pronuclear injection of a bacterial artificial chromosome (BAC; no. 16652; Genome Systems, St. Louis, Mo.) (48) containing the mouse *Myoc* gene and flanking genomic sequence (Fig. 1A) into C57BL/6J zygotes. The mouse strain used to generate the BAC was a 129-derived strain and has a repeat length polymorphism compared to C57BL/6J that is located approximately 4 kb upstream from the start codon. The strain-specific alleles were resolved by electrophoresis of the repeat amplicon on a 6% polyacrylamide gel (Repeat-forward, 5′ACTGCAGCTGACCTGACACA3′; Repeat-reverse, 5′CACAGAG ACTCTTTTCCCACT3′). Founder pups were defined as being positive if they carried the 129 allele of *Myoc* and both insert-BAC junctions (T7-forward, 5′TGTAATACGACTCACTATAG3′; 16652-T7-reverse, 5′TGGTGCAGACA AACAATATG3′; 16652-Sp-forward, 5′TTATAGCCGTGCTTTCATC3′; Sp6Belo-reverse, 5′ACCATGATTACGCCAAGC3′).

Transgenic progeny from each of the founders were tested for the level of *Myoc* expression. Transgenic progeny from a line [C57BL/6J-Tg(*Myoc*)1Sj] with consistent overexpression of *Myoc* were intercrossed to produce mice homozygous for the transgene (*Tg*/*Tg*). Potential homozygous mice were confirmed by the observation that they only produce pups hemizygous for the transgene when crossed to nontransgenic C57BL/6J mice. The *Tg*/*Tg* line was then maintained by intercrossing homozygotes.

Quantitative PCR. Relative RNA quantification was performed by using standard TaqMan protocols (TaqMan; PE Biosystems, Foster City, Calif.). Total RNA was extracted from eyes of transgenic and nontransgenic control mice that were18 to 23 days old (RNeasy; QIAGEN, Valencia, Calif.). RNA was fluorescently quantified (Ribogreen; Molecular Probes, Eugene, Oreg.), and 1.8 ng was used to make cDNA (Retroscript; Ambion, Austin, Tex.). Generation of cDNA was performed in triplicate for each mouse. The cDNA was used for a fluorescent quantitative PCR assay (TaqMan; PE Biosystems). Briefly, each of the three samples from each transgenic line was assayed for the number of PCR cycles required to cross the threshold of the linear range of the reaction (C_t). The three C_t values for each mouse were averaged. Samples were normalized for the total template amount by subtracting the average *GAPDH* C_t from the average *Myoc* C_t (delta C_t). The normalized *Myoc* expression (delta C_t) for the control mice was then subtracted from the normalized expression from the transgenic mice (delta delta C_t). The relative level of expression for transgenic mice compared to control mice equals $2^{(\text{delta delta Ct})}$. The expression value reported is the average of all of the mice of that genotype tested. The oligonucleotides (Sigma-Aldrich, St. Louis, Mo.) used for quantitative PCR were *Myoc* F (5′GAATTTGGACA CGTTGGCCT3′), *Myoc* R (5′CCAGTGTCCTTCCACCCAGTA3′), *GAPDH* F (5′TTCACCACCATGGAGAAGGC3′), and *GAPDH* R (5′GGCATGGACTG TGGTCATGA3′. TaqMan probe sequences were as follows: *Myoc* probe, 5′T GCTGCCAGGCTCCAGGGAAGT3′; *GAPDH* probe, 5′TGCATCCTGCACC ACCAACTGCTTAG3′.

Western analysis. Polyclonal rabbit antiserum was raised against a glutathione *S*-transferase fusion protein containing amino acids 100 to 187 of the first exon of mouse MYOC (21). To enrich for the drainage structures, a narrow ring of tissue centered over the iridocorneal-angle drainage structures was dissected from freshly harvested eyes (ring includes iris root and TM). The tissue was homogenized in 50 mM Tris (pH 7.5)–5 mM EDTA–20 mM dithiothreitol–1 mM phenylmethylsulfonyl fluoride–10 μg of leupeptin per ml–1 μg of pepstatin per ml–1% NP-40–1% Triton X-100–0.2% sodium dodecyl sulfate and sonicated for 1 min.

Tissue was harvested from each eye of eight animals of each genotype (control, hemizygous transgenic, and homozygous transgenic). For each genotype, two pools of protein extracts (four animals per pool) were analyzed. Twenty-five micrograms of total protein was loaded onto the gel and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Equivalent protein loading was confirmed by Coomassie staining of additional gels (data not shown). Following separation, proteins were transferred to a nitrocellulose membrane. Membranes were incubated with Western blocking reagent (Roche Applied Science, Indianapolis, Ind.) and 5% skim milk in 0.05% Tween 20–Tris-buffered saline (pH 7.4) overnight at 4°C. They were next incubated for 1 h at room temperature with the affinity-purified antibody at a 1:3,000 dilution made in 0.05% Tween 20–Tris-buffered saline (pH 7.4) containing 1% skim milk. Anti-rabbit horseradish peroxidase-linked secondary antibody (Amersham Biosciences, Piscataway, N.J.) was used at a 1:25,000 dilution. The SuperSignal

chemiluminescent detection system (Pierce Biotechnology, Rockford, Ill.) was used in accordance with the manufacturer's instructions.

IOP measurement and clinical examination. IOPs were measured as previously described (18, 33). Statistical significance was tested with a two-tailed Student *t* test. For the C57BL/6J mouse strain, an IOP of >20 mmHg is greater than 3 standard deviations above the mean and is considered an elevated IOP in this study (33). Anterior segment clinical examinations were performed with a slit lamp biomicroscope (Haag-Streit USA, Mason, Ohio) (38). Retinal and optic nerve head examinations were performed with an indirect ophthalmoscope and a 60- or 90-diopter lens on eyes with pupils dilated with a drop of 1% cyclopentolate (15).

Histological analysis. Enucleated eyes were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2), dehydrated in graded ethanol, and embedded in fresh Historesin (Leica, Heidelberg, Germany). Twenty-four 1.5-μm sections were analyzed from each of three different ocular regions, with the lens as a landmark (region A, periphery of lens; region B, halfway between lens center and periphery; region C, center of lens and optic nerve head), and stained with hematoxylin and eosin (H&E) (40).

Optic nerves were harvested as follows. Immediately after death, the top of the skull and most of the brain were removed, leaving approximately 1 mm of brain overlying the intact optic nerves. The nerves were fixed in the head overnight in 0.8% paraformaldehyde and 1.2% glutaraldehyde in 0.1 M phosphate buffer and removed on the following day. The postorbital-prechiasmal sections of nerves were then embedded in EMbed 812 resin (Electron Microscopy Sciences, Ft. Washington, Pa.), and 1-μm sections were stained with *p*-phenylenediamine.

Electron microscopy. Electron microscopy procedures were performed as described previously (21, 39). Briefly, eyes were fixed for 1.5 h with 0.8% paraformaldehyde and 1.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4°C. The anterior segment was cut into multiple wedge-shaped blocks and fixed further at 4°C for 12 h. Tissues were washed in phosphate-buffered saline, postfixed with 1% osmium tetroxide, and embedded. Sections were cut and stained with uranyl acetate and lead citrate.

RESULTS

Transgenic mice and MYOC levels. We generated transgenic C57BL/6J mice with a BAC containing the mouse *Myoc* gene with endogenous regulatory sequences (Fig. 1A). Seven independent founder lines were identified out of 112 potential transgenic mice. We harvested brains and whole eyes from hemizygous progeny of each founder and generated cDNA from total RNA. The relative expression level of *Myoc* in hemizygous mice compared to that in nontransgenic mice was determined by TaqMan quantitative PCR. The line with the greatest expression level was maintained and used for further study. The founder stock with the strongest *Myoc* expression in the brain had an average increase of 3.8-fold ($n = 12$, standard deviation [SD] = 2.4) over the average of nontransgenic control mice ($n = 5$) and an average increase of 3.15-fold over the control mouse with the highest expression. In whole eyes, hemizygous mice from the same line had an average increase of 8.24-fold ($n = 10$, SD = 6.9) over the average of control mice ($n = 5$) and 4.2-fold over the control mouse with the highest expression. Although the level of *Myoc* RNA was variable, all transgenic mice had higher *Myoc* RNA levels than all of the control mice.

To determine how the increased *Myoc* expression affects the protein level, an investigator (unaware of the results of the quantitative PCR) performed a Western analysis with protein extracts from tissue enriched for ocular drainage structures (see Materials and Methods). The relative amounts of MYOC in control, hemizygous, and *Tg*/*Tg* mice were determined by densitometric analysis of Western blots normalized to the control sample. The results of the Western analysis are in agreement with those obtained by quantitative PCR. *Tg*/*Tg* mice had robust overexpression (15-fold, SD = 2.8) compared to that of control mice, and hemizygous mice had an intermediate level of expression (6-fold, SD = 2.2) (Fig. 1B). The level of overexpression in *Tg*/*Tg* mice was confirmed by comparison of dilutions of *Tg*/*Tg* protein to control protein.

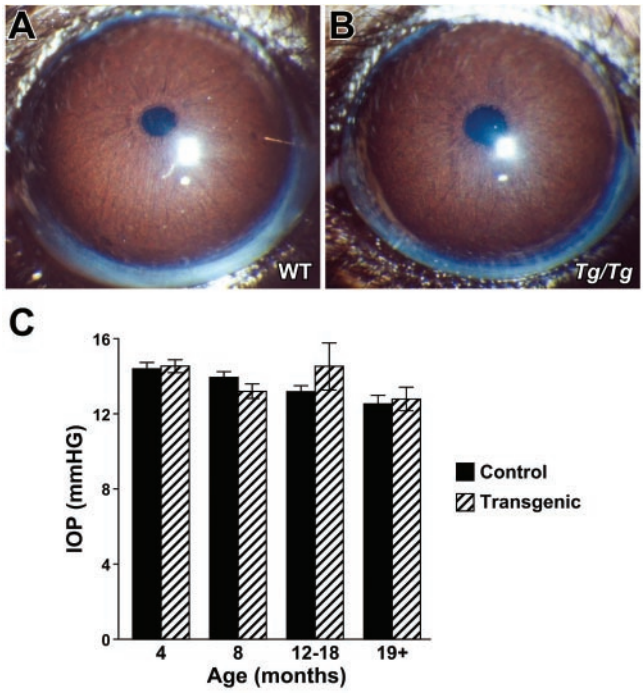

FIG. 2. Slit lamp examination and IOP measurement. Slit lamp photographs of a normal control (wild type [WT]) eye (A) and a *Tg*/*Tg* eye (B). Visible structures, i.e., the cornea, iris, and lens, all appear normal in the *Tg*/*Tg* mouse. Slit lamp examinations were conducted on 16 *Tg*/*Tg* mice between 14.5 and 27 months of age. (C) IOPs of *Tg*/*Tg* and control mice were determined at a variety of ages. For each group, the mean and standard error of the mean are shown. Sex had no effect on the IOP of either genotype, and so the data were analyzed irrespective of sex. There were no significant differences in IOP between mice of each genotype at any age ($P = 0.9$, 0.1, 0.3, and 0.8 comparing each genotype at 4, 8, 12 to 18, and >19 months, respectively). Starting with 4 months and ending with >19 months, the numbers of control (C) and transgenic (*Tg*/*Tg*) mice were as follows: C, 20 and *Tg*/*Tg*, 20; C, 17 and *Tg*/*Tg*, 11; C, 9 and *Tg*/*Tg*, 11; and C, 14 and *Tg*/*Tg*, 11.

Anterior segment and IOP. Anterior and posterior segments of *Tg*/*Tg* mice between 14.5 and 27 months of age were examined ($n = 16$). Structures of the ocular anterior segment (pupil, iris, cornea, and lens) in *Tg*/*Tg* mice were indistinguishable from those of controls (Fig. 2A and B). To test if MYOC overexpression altered IOP, we assessed IOP in control and *Tg*/*Tg* mice at approximately 4, 8, 12 to 18, and >19 months of age (Fig. 2C). There was no significant difference in IOP between control and *Tg*/*Tg* mice at any of the ages (the oldest mice were approximately 2 years of age) (Fig. 2C).

Drainage structures of the iridocorneal angle. To determine if MYOC overexpression had any morphological consequences in the ocular drainage structures, we histologically analyzed the eyes of mice more than 2 years old. Sections through eyes of *Tg*/*Tg* mice ($n = 8$) showed normal iridocorneal angles with drainage structures (Schlemm's canal and TM) indistinguishable from those of control mice (Fig. 3A and B).

Some patients with POAG or steroid-induced glaucoma

have ultrastructural changes in the extracellular matrix of the TM, including amorphous plaque material and fingerprint-like structures (8, 19, 25). To evaluate the fine structure, we performed transmission electron microscopy on the drainage structures of control and *Tg*/*Tg* mice. Both control and transgenic mice have normal drainage structures including trabecular beams containing collagen and elastic tissue surrounded by endothelium-like trabecular cells and intertrabecular spaces (Fig. 3C and D). No plaque material or fingerprint-like structures were identified, and there were no differences between control and *Tg*/*Tg* mice.

Optic nerve and optic nerve head. *MYOC* is expressed in the optic nerve. Thus, in addition to altering IOP, *MYOC* mutations may have a direct detrimental effect in the optic nerve (7, 20, 44). To assess the possibility of glaucomatous damage in the absence of IOP elevation, we examined *Tg*/*Tg* mice for hallmarks of glaucoma including cupping of the optic nerve head and unhealthy or dying retinal ganglion cell axons. Fundus examinations did not reveal optic nerve head cupping or any other abnormalities. Histologically, there were no signs of nerve fiber layer loss (retinal ganglion cell axons in the eye) or optic nerve cupping in eight *Tg*/*Tg* mice between 18 and 30 months of age (Fig. 4A and B). *p*-Phenylenediamine darkly stains unhealthy retinal ganglion cell axons in optic nerve cross sections. Mice with glaucoma have darkly stained dying axons and/or gliosis and scarring indicating previous or ongoing damage. In advanced cases there is substantial axon loss. Aged *Tg*/*Tg* mice have optic nerves that are as healthy as those of age-matched controls (Fig. 4C and D).

DISCUSSION

Here we show that mice overexpressing MYOC do not develop glaucoma. They do not develop ultrastructural defects in the TM, elevated IOP, death of retinal ganglion cells, or optic nerve cupping (hallmarks of glaucoma). Primate models of glucocorticoid-induced glaucoma have ultrastructural changes in the TM associated with a threefold increase in MYOC (8). The level of MYOC elevation in our transgenic mice (approximately 15-fold) is well within the range of MYOC induction (2- to 20-fold) by glucocorticoid treatment of cultured human TM cells, by glucocorticoid perfusion of human anterior segment cultures, or by oral glucocorticoid administration in monkeys (8, 35). The magnitude of the MYOC increase in these transgenic mice is therefore appropriate for testing the effect of increased MYOC levels on IOP and glaucoma. Although we have not yet tested whether elevated MYOC can act together with other effects of steroids to raise IOP, we conclude that MYOC level elevation is not by itself pathogenic.

Despite the association between increased MYOC levels and high IOP (in steroid-induced glaucoma and other glaucomas), our data argue that increased MYOC by itself is not the cause of IOP elevation. One possible explanation for the association of elevated MYOC with glaucoma is that MYOC is turned on in response to stress (24, 28). This could be the stress of elevated IOP, tissue damage, or glaucoma treatments. Data from experiments with cultured cells or organ-cultured anterior segments, where MYOC is up regulated in response to oxidative stress, mechanical stress, or IOP elevation, agree with this interpretation (3, 28, 45).

Previous studies have argued against a pathogenic role of elevated MYOC levels in steroid-induced glaucoma. Mutations in *MYOC* were not identified in patients with steroid-induced ocular hypertension (2, 13). These previous studies do not rule out the possibility that mutations in genes responsible for the regulation of *MYOC* result in abnormally high MYOC levels after steroid use and that this causes glaucoma. Our study confirms and extends these previous studies. It suggests not only that it is unlikely that noncoding *MYOC* mutations cause steroid-induced glaucoma but that mutations in genes that increase MYOC levels are also unlikely to cause either steroid-induced glaucoma or other glaucomas. Therefore, we suggest that other genes that are found to be differentially regulated in response to glucocorticoid treatment (8, 23, 28) be considered as candidates for causing steroid-induced ocular hypertension. The corticosteroid receptor genes that mediate glucocorticoid effects are interesting candidates. Since the steroid response may involve extracellular matrix accumulation in the iridocorneal angle (8, 25) and the ocular hypertension is reversible (13), matrix metalloproteinases (responsible for extracellular matrix turnover) and matrix metalloproteinase inhibitor genes are also good candidates.

This study shows that increasing the amount of normal MYOC protein by itself is unlikely to cause glaucoma. A previous mouse study shows that the absence of MYOC has no phenotypic consequences (21). Similarly for humans, individuals who are hemizygous for *MYOC* (50) or who are either homozygous (22) or heterozygous (27) for a very early truncating mutation (Arg46Stop, which is presumed to be a null) are not affected. Together, these studies suggest that altering the level of MYOC by itself is not important and that disease manifestation is dependent on the presence of abnormal protein molecules.

The mutant proteins may inhibit specific cellular processes or may have gained some undefined detrimental properties. Mutant proteins appear to form intracellular aggregates and to inhibit the secretion of normal MYOC molecules (4, 5, 16, 17). Accumulation of MYOC aggregates in the endoplasmic reticulum of TM or other drainage structure cells may alter cellular metabolism or kill cells and cause IOP elevation and subsequent glaucoma. This model suggests that genes or other factors (including cellular stress) that affect the ability of the endoplasmic reticulum to handle misfolded proteins, or that affect the efficiency of the proteosome in clearing misfolded proteins, may modify the course of glaucoma caused by mutations in MYOC. Some of these factors may provide therapeutic targets.

Owing to the induction of *MYOC* by different stresses, a feature of pathogenic models involving detrimental effects of mutant proteins is that the magnitude of the detrimental effect may increase at times of stress as a consequence of increased production of mutant MYOC molecules. Drugs, infection, or other factors that stress drainage structure cells therefore may influence the onset, progression, and severity of POAG. It is even possible that fluctuations in stress levels may explain clinically observed fluctuations in IOP, since stress fluctuations may result in peaks and troughs in IOP related to the abundance of abnormal MYOC molecules.

Since the level of MYOC is not important by itself, promoter sequence variants are unlikely to be sufficient to cause glaucoma. Nonetheless, similar to the suggested implications of

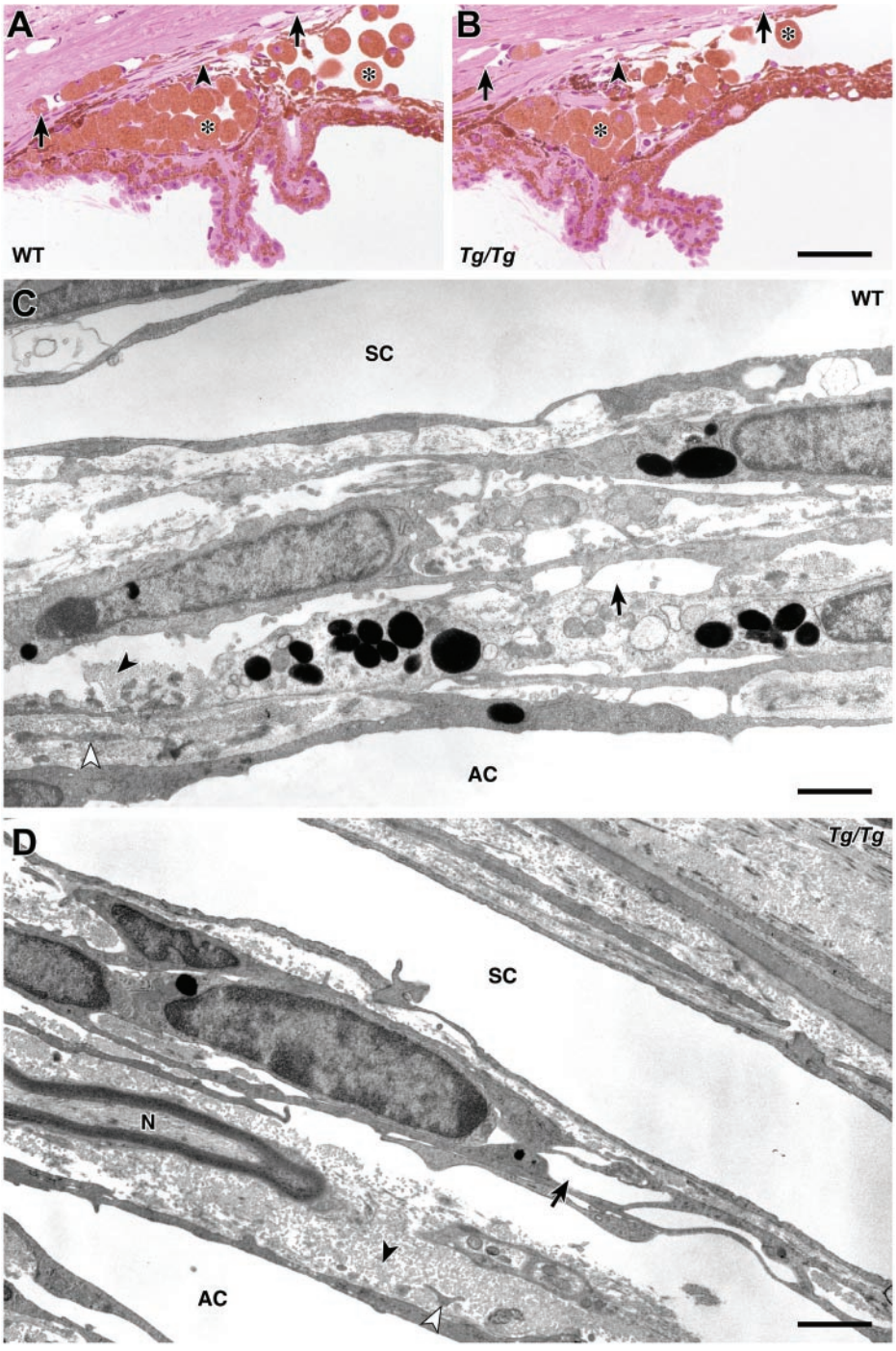

FIG. 3. Morphology and ultrastructure of the iridocorneal angle. Representative H&E-stained sections of eyes from a 25-month-old control (wild type [WT]) mouse (A) and a 27-month-old *Tg*/*Tg* mouse (B). The iridocorneal angle, TM (arrowhead), and Schlemm's canal (between arrows) are not affected by genotype and appear normal. The eyes of mice of both genotypes contain pigment-filled cells (asterisks). This is typical in C57BL/6J mice at this very old age and is not a phenotypic consequence of the transgene. There were no detectable histological differences in the iridocorneal angles of each genotype between 18 and 30 months of age. Scale bar, 40 μm. Electron micrographs of eyes from 20-month-old control (C) and *Tg*/*Tg* (D) mice. Similar results were observed in mice more than 2 years old. The TM of mice of each genotype has a normal morphology including an endothelium-lined Schlemm's canal (SC), trabecular beams with organized collagen fibrils (filled arrowhead) and elastic tissue (open arrowhead), and open intertrabecular spaces (arrow). The anterior chamber is identified as AC. A normal myelinated nerve (N) is visible in the transgenic eye. Scale bars, 1 μm.

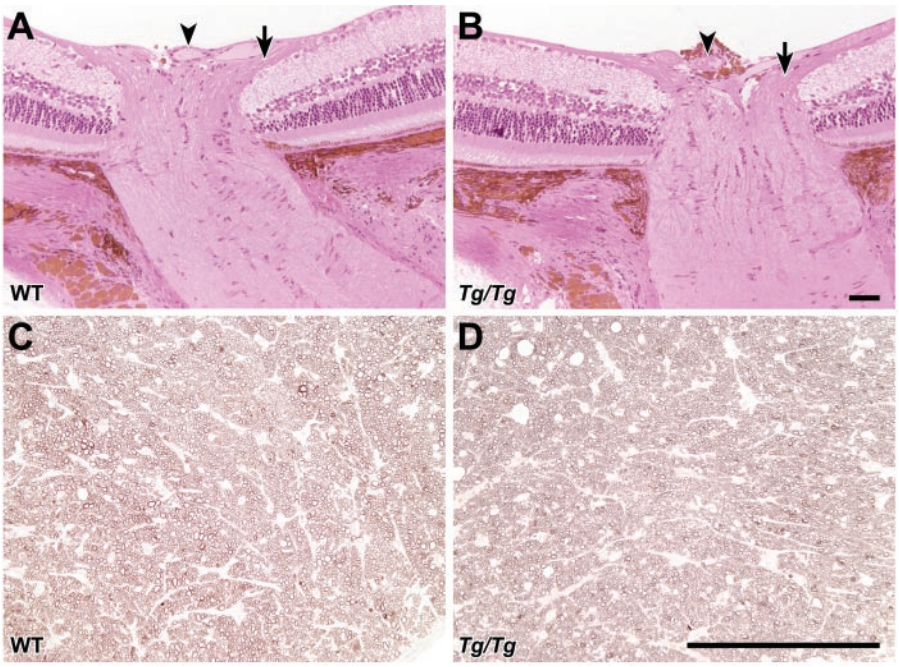

FIG. 4. The optic nerve head and retinal ganglion cell axons. Representative H&E-stained sections of eyes from a 25-month-old control (wild type [WT]) (A) and a 27-month-old *Tg*/*Tg* (B) mouse. Both have a healthy optic nerve head with a thick nerve fiber layer (arrow). The transgenic mouse shows no signs of optic nerve head cupping. A blood vessel is typically present over the central optic nerve in mice (arrowhead) and is variably associated with pigment in normal mice of this genetic background. Optic nerve cross sections of a 19-month-old control mouse (C) and a17-month-old *Tg*/*Tg* mouse (D) are indistinguishable, without significant axon damage or loss. The same result was observed in mice >2 years old. No optic nerves from aged *Tg*/*Tg* mice ($n = 6$) showed signs of glaucomatous damage. Scale bars, 100 μm.

stress, promoter variants that increase or decrease transcription of *MYOC* alleles that contain coding mutations may alter glaucoma presentation. One study addressed this possibility for the mt.1 promoter variant (a variant suggested to be a marker for worsening glaucoma over time and for which a genetic test is available). This group found no evidence that mt.1 had any impact on the course of the disease even in the presence of coding mutations (2). This suggests that these promoter variants are unlikely to contribute to glaucoma. Further studies including promoter variants that are known to alter *MYOC* transcription, however, are necessary to assess the effects on the disease of altering the transcriptional level of specific alleles that contain disease-causing coding mutations.

In summary, our data suggest that noncoding sequence variants in *MYOC* are by themselves unlikely to influence steroid-induced or non-steroid-induced glaucoma. Disease pathogenesis appears to be dependent upon the expression of abnormal mutant proteins. Further work remains to define the pathogenic mechanisms of the abnormal proteins and the potential role of intracellular aggregates. The production of mice with human disease-associated alleles will be a useful tool to address these issues.

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ADDENDUM IN PROOF

A recent important paper also supports a pathological role of abnormal misfolded MYOC molecules in glaucoma. (Y. Li and D. Vollrath, Hum. Mol. Genet. **13:**1193–1204, 2004).

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