Unaltered myocilin expression in the blood of primary open angle glaucoma patients.

Khaled K Abu-Amero  
*College of Medicine, King Saud University*

Taif Anwar Azad  
*College of Medicine, King Saud University*

George L Spaeth  
*Wills Eye Institute, Thomas Jefferson University*

Jonathan Myers  
*Wills Eye Hospital, Thomas Jefferson University*

L Jay Katz  
*Wills Eye Hospital, Thomas Jefferson University*

Follow this and additional works at: [https://jdc.jefferson.edu/willsfp](https://jdc.jefferson.edu/willsfp)

Recommended Citation  
Abu-Amero, Khaled K; Azad, Taif Anwar; Spaeth, George L; Myers, Jonathan; Katz, L Jay; Moster, Marlene; and Bosley, Thomas M, "Unaltered myocilin expression in the blood of primary open angle glaucoma patients." (2012). *Wills Eye Hospital Papers*. Paper 18.  
https://jdc.jefferson.edu/willsfp/18
Unaltered myocilin expression in the blood of primary open angle glaucoma patients

Khaled K. Abu-Amero,1,2 Taif Anwar Azad,1 George L. Spaeth,3 Jonathan Myers,3 L. Jay Katz,3 Marlene Moster,3 Thomas M. Bosley3

1Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia; 2Department of Ophthalmology, College of Medicine, University of Florida, Jacksonville, FL; 3William and Anna Goldberg Glaucoma Service, Wills Eye Hospital, Thomas Jefferson University, Philadelphia, PA

Purpose: To investigate the expression of the myocilin gene (MYOC) in the blood of primary open angle glaucoma (POAG) patients to determine if altered systemic expression is playing a role.

Methods: Patients (n=47) were eligible for inclusion if they met standard clinical criteria for POAG. Control subjects (n=27) were recruited who were free from glaucoma by examination. RNA was extracted from leukocytes of patients and controls and converted to cDNA by reverse transcriptase enzyme, and quantitative PCR was used to assess expression levels of MYOC and the housekeeping gene β-globulin (HBB). The ratio of MYOC expression to HBB expression for POAG patients was compared to that of controls and to clinical characteristics of POAG patients.

Results: Mean gene expression values were statistically similar in POAG patients and controls for both MYOC (p≤0.55) and HBB (p≤0.48). MYOC/HBB ratios were also statistically indistinguishable between POAG patients and controls (p≤0.90). MYOC/HBB ratios were not significantly associated with age, sex, or ethnicity of patients within the POAG group. Similarly, MYOC/HBB ratios were not significantly associated with clinical parameters related to POAG severity, including maximum intraocular pressure, vertical cup-to-disk ratio, static perimetry mean deviation, or static perimetry pattern standard deviation.

Conclusions: MYOC expression is not altered in the blood of POAG patients, unlike MYOC expression in trabecular meshwork (TM) cultures. These results suggest that MYOC expression is not altered systemically but rather that MYOC expression may contribute to POAG pathogenesis in specific tissues such as TM.

Glucoma is one of the leading causes of blindness worldwide [1,2], characterized by chronic degeneration of axons in the optic nerve head. Primary open angle glaucoma (POAG) is the most prevalent type of glaucoma in western countries and has risk factors that include elevated intraocular pressure (IOP) and age [3]. Elevated IOP is associated with increased aqueous humor outflow resistance in the trabecular meshwork (TM) of the eye [2], although the exact mechanism and causative factors for this increase is unclear. Up to half of all patients with POAG have a positive family history [4,5], and these and other observations suggest that genetic factors may contribute to POAG [1,6,7].

Myocilin (MYOC) was the first gene linked to POAG [8] and is the one most studied [9]. It is located in chromosome 1, contains three exons, and codes for a largely extracellular matrix protein. This protein has an NH2-terminal coiled region and a COOH-terminal olfactomedin domain [10], but its function is still not well understood. To date, mutations in MYOC seem most likely to have their pathogenic effect largely because of inability of the protein to fold properly [11]. This may result in an unfolded protein response in TM cells, activating a mitochondria-independent apoptosis pathway which ultimately leads to cell death, breakdown of TM cell structure, obstruction of aqueous humor outflow pathway, ocular hypertension, and ultimately the optic nerve damage of glaucoma [12-14]. MYOC may directly impair optic nerve function when mutated [15]; however, direct evidence for this hypothesis is still lacking [11,15].

Most studies investigating MYOC expression in POAG have employed human cultured TM cells [16,17]. However, whole blood gene expression studies have been used to investigate POAG [18], other hereditary optic neuropathies [19], and diseases affecting brain anatomy and function [20, 21] because the target tissues for these diseases is not readily available. Therefore, the current study investigated MYOC expression in whole blood from POAG patients in hope that this approach will add to our knowledge of whether altered systemic expression of this gene contributes to POAG pathogenesis.

METHODS

Patients and controls: Patients (n=47) were evaluated in the Glaucoma Service at the Wills Eye Institute, Philadelphia, PA, and enrolled after examination by a glaucoma specialist.
Patients were eligible for inclusion if they met the following clinical criteria for POAG [22-25]: age greater than 40 years; intraocular pressure (IOP) \( \geq 21 \) mmHg in one or both eyes before initiation of glaucoma treatment; normal-appearing, open anterior chamber angles bilaterally by gonioscopy; optic nerve appearance characteristic of the optic discs typically observed in primary open-angle glaucoma (with localized narrowing or absence of the neuro-retinal rim, with the amount of cupping exceeding the amount of paller of the rim, and with asymmetric cupping of the optic discs in the two eyes); and static visual field (using a full threshold 24–2 program; Humphrey Field Analyzer II; Carl Zeiss Meditec, Inc., Dublin, CA) showing abnormalities typical of glaucoma (as per Advanced Glaucoma Intervention Study criteria) [26]. Good agreement was required between the appearance of the optic disc and the visual field. Exclusion criteria included historical, neuroimaging, or biochemical evidence of another possible optic neuropathic process affecting either eye, significant visual loss in both eyes not associated with glaucoma, or choosing not to participate. This research adhered to the tenets of the Declaration of Helsinki, and all patients and controls signed an informed consent approved by the Wills Eye Institute institutional review board.

All control subjects (n=27), frequently spouses of patients, had full ophthalmologic examinations documenting IOPs that were <21 mmHg and symmetric in the two eyes, normal anterior chambers, optic discs that were normal and symmetric in appearance, entirely normal static perimetry OU, and no prior history of glaucoma. All controls had static symmetric in appearance, entirely normal static perimetry normal anterior chambers, optic discs that were normal and IOPs that were <21 mmHg and symmetric in the two eyes, patients, had full ophthalmologic examinations documenting patients and controls signed an informed consent approved by the tenets of the Declaration of Helsinki, and all

### Table 1. Primer sequences, PCR annealing temperature and amplicon size for the myocilin gene.

| Exon | Primer sequence | Annealing temp (°C) | Amplicon size (bp) |
|------|-----------------|---------------------|-------------------|
| Pro-F | TGTAAAACGACGGCCAGT | 55 | 592 |
| Pro-R | CAGGAAACAGCTATGACC | 57 | 775 |
| 1F | TGTAAAACGAGGGCCAGT | 57 | 725 |
| 1R | CAGGAAACAGCTATGACC | 57 | 325 |
| 2F | TGTAAAACGAGGGCCAGT | 57 | 850 |
| 2R | CAGGAAACAGCTATGACC | 57 | 700 |
| 3AF | TGTAAAACGAGGGCCAGT | 57 | |
| 3AR | CAGGAAACAGCTATGACC | 57 | |
| 3BF | TGTAAAACGAGGGCCAGT | 57 | |
| 3BR | CAGGAAACAGCTATGACC | 57 | |

F=forward; R=reverse; Pro – promoter. Bold and underlined sequences are those of the M13.

Fragments were then run on the 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer protocol. All sequenced fragments were then analyzed using SeqScape software v2.6 (Applied Biosystems). Table 1 details the cycle sequencing kit (Applied Biosystems, Foster city, CA). Fragments were then run on the 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer protocol. All sequenced fragments were then analyzed using SeqScape software v2.6 (Applied Biosystems). Table 1 details the sequence of the primers used the PCR annealing temperature and the expected amplicon size.

**Quantitative RT–PCR:** A two-step semi-quantitative reverse transcriptase polymerase chain reaction (RT–PCR) method was used to measure gene expression levels of MYOC and \( \beta \)-globulin (HBB) in POAG patients and controls. Random hexamers were used as primers in the first step of cDNA synthesis. Total RNA (1 \( \mu \)g) was combined with 0.5 \( \mu \)g primers, 200 \( \mu \)M dNTPs, and sterile Milli-Q water (Millipore, Billerica, MA) and preheated at 65 °C for 2 min to denature secondary structures. The mixture was then cooled rapidly to 20 °C and then 10 \( \mu \)l 5× RT Buffer, 10 mM dithiothreitol, and 200 U Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen Life Sciences, Grand Island, NY) were added for a total volume of 50 \( \mu \)l. The RT mix was incubated at 37 °C for 90 min then stopped by heating at 95 °C for 5 min. The cDNA stock was stored at −20 °C.

Relative RT–PCR was performed to measure gene expression of MYOC and HBB according to standard guidelines [27]. Primer sequences and optimal PCR annealing temperatures (ta) are listed in Table 2. Primer sequences were designed to span intron regions to insure that no false positive PCR fragments would be generated from pseudogenes and contaminate genomic DNA. In addition, all forward PCR primers were labeled with fluorescein (6-FAM), making quantitation more accurate. Polymerase chain reactions were performed using 100 ng of cDNA, 5 pmoles of each oligonucleotide primer, 200 \( \mu \)M of each dNTP, 1 unit of HotStar Taq-polymerase (Qiagen, Valencia, CA) and 1× PCR buffer in a 20 \( \mu \)l volume. The PCR program initially started with a 95 °C denaturation for 5 min, followed by 25 cycles of 95 °C for 1 min, 57 °C for 45 s, and 72 °C for 1 min. Linear amplification range for each gene was tested on the adjusted cDNA, and 25 cycles were found to be optimal for both

### Table 2. Primer sequences, PCR annealing temperature and amplicon size for the myocilin gene.

| Exon | Primer sequence | Annealing temp (°C) | Amplicon size (bp) |
|------|-----------------|---------------------|-------------------|
| Pro-F | TGTAAAACGACGGCCAGT | 55 | 592 |
| Pro-R | CAGGAAACAGCTATGACC | 57 | 775 |
| 1F | TGTAAAACGAGGGCCAGT | 57 | 725 |
| 1R | CAGGAAACAGCTATGACC | 57 | 325 |
| 2F | TGTAAAACGAGGGCCAGT | 57 | 850 |
| 2R | CAGGAAACAGCTATGACC | 57 | 700 |
| 3AF | TGTAAAACGAGGGCCAGT | 57 | |
| 3AR | CAGGAAACAGCTATGACC | 57 | |
| 3BF | TGTAAAACGAGGGCCAGT | 57 | |
| 3BR | CAGGAAACAGCTATGACC | 57 | |

F=forward; R=reverse; Pro – promoter. Bold and underlined sequences are those of the M13.
**RESULTS**

Age (POAG patients 67.3 years; controls 63.6 years; p≤0.17) and sex (POAG 26 males/21 females; controls 12/15; p≤0.18) of the 47 unrelated POAG patients were similar to the 27 control individuals, but ethnicity differed between the POAG group (25 Caucasian/22 African American) and the control group (23 Caucasian/4 African American; p≤0.003).

After aligning and reading all sequences neither POAG patients nor controls were found to have any significant mutation or polymorphism in the coding or promoter regions of MYOC.

Mean gene expression values for both MYOC and HBB (p≤0.48) were statistically similar in POAG patients and controls (Table 3). MYOC/HBB ratios (p≤0.90) were also indistinguishable between POAG patients and controls. Because of ethnic differences between the POAG group and controls, gene expression values and ratios were also compared between Caucasian POAG patients and Caucasian controls. Mean MYOC (p≤0.90) gene expression and MYOC/HBB (p≤0.54) ratios also did not differ between these groups.

**DISCUSSION**

The 47 patients reported here met rigorous clinical criteria for POAG [22-25] with elevated IOP, normal anterior chamber, and evidence on funduscopy and visual fields of glaucomatous optic nerve damage. They did not have evidence of other types of glaucoma or alternative causes of optic nerve injury by clinical criteria, and none had dysmorphism or an obvious genetic syndrome. They were compared to 27 control individuals in whom POAG and other evidence of optic nerve damage were carefully excluded.

Screening the full MYOC gene and its promoter region revealed no mutations or significant polymorphisms in POAG patients or controls. These results are not surprising, since the prevalence of MYOC mutations is generally less than 5% in adult POAG populations [28]. Currently, there are 85 glaucoma causing mutations listed in the comprehensive myocilin database. They were classified as a glaucoma causing mutations based on the following criteria: i) predicted disruption of protein translation (e.g., frame-shift mutations and premature stop codons); ii) sequence variant frequency in control (unaffected) populations (those with a frequency >1% were classified as polymorphisms); iii) variant location (i.e., protein homology domain; cross species conservation of coding sequence); iv) evidence for partial segregation with the phenotype within a family and v) results of solubility studies. Interestingly, several sequence changes have been reported in the MYOC promoter region, but they were defined as neutral polymorphisms based on the pathologic characteristics.

| Parameter                        | Number POAG:control | POAG          | Control | p≤  |
|----------------------------------|---------------------|---------------|---------|-----|
| MYOC expression; mean (SD)       | 47:26               | 7476 (4325)   | 6839 (4271) | 0.55 |
| β-globulin expression; mean (SD) | 44:24               | 109533 (31355)| 103885 (31047)| 0.48 |
| MYOC/β-globulin; mean (SD)       | 42:23               | 0.0685 (0.0520)| 0.0667 (0.0597)| 0.90 |
| MYOC expression in Caucasians; mean (SD) | 26:22               | 7132 (4526)   | 7268 (4121)   | 0.90 |
| β-globulin expression in Caucasians; mean (SD) | 22:20               | 104941 (31552)| 99392 (32171) | 0.58 |
| MYOC/β-globulin in Caucasians; mean (SD) | 21:19               | 0.0652 (0.0487)| 0.0730 (0.0619)| 0.54 |

POAG=primary open angle glaucoma; MYOC=myocilin gene expression; SD=standard deviation.

Statistical analysis: Absolute RT–PCR values were used to calculate a ratio of the MYOC peak area in the selected linear amplification cycle divided by that of HBB, creating an MYOC/HBB ratio. All clinical and genetic data were analyzed using SPSS v17 (IBM, Chicago, IL).

The significance of RNA expression and ratios was calculated using the Mann-Whitney test. A p-value of ≤0.05 was considered statistically significant.

**TABLE 2. PRIMER SEQUENCES AND ANNEALING TEMPERATURE β-GLOBULIN AND MYOCILIN FLUORESCENT LABELED PRIMERS.**

| Primer name | Primer sequence | Annealing temp (°C) |
|-------------|-----------------|---------------------|
| β-globulin-F | (6-FAM)AGCCTGCTTGGCCGA | 57 |
| β-globulin-R | CTGGTGCCCTGGGCGC | |
| MYOC-LAB-F  | (6-FAM)TTTCTACGGAATTGGACA | 59 |
| MYOC-R      | GTAGGTGGGGCTTGGGGCTT | |

F=forward; R=reverse. The forward primers were labeled with 6-FAM.

| Primer name | Primer sequence | Annealing temp (°C) |
|-------------|-----------------|---------------------|
| β-globulin-F | (6-FAM)AGCCTGCTTGGCCGA | 57 |
| β-globulin-R | CTGGTGCCCTGGGCGC | |
| MYOC-LAB-F  | (6-FAM)TTTCTACGGAATTGGACA | 59 |
| MYOC-R      | GTAGGTGGGGCTTGGGGCTT | |

F=forward; R=reverse. The forward primers were labeled with 6-FAM.
A potential limitation of this study is that the number of individuals studied was relatively small, bringing up the possibility that the lack of a statistical difference in MYOC expression and MYOC/HBB ratio between POAG patients and controls might be due to a type II statistical error because of inadequate power. This same patient group was adequate to confirm statistically significant differences in optic atrophy type 1 (OPA1) expression and the OPA1/HBB ratio between POAG patients and controls [18], but it is possible that differences in MYOC expression between POAG patients and controls are smaller, although still present. Similarly, the lack of correlation between the MYOC/HBB ratio and various clinical parameters within the POAG group may be subject to type II statistical errors. The population studied was predominantly Caucasian and African-American, and different results might be obtained in other ethnicities.

We found that systemic MYOC expression was unchanged in these POAG patients compared to controls. One interpretation of these results is that the MYOC protein plays a particularly important role in the globe and that regulation of MYOC expression that might be pertinent to POAG, congenital glaucoma, and/or steroid-induced glaucoma is relatively specific to the TM and may not be reflected to a significant extent in bone marrow or other non-ocular tissues. It is also possible that POAG is not altered by wild-type MYOC expression in any tissue [37]. A gain-of-function disease model was suggested after identification of mutant, misfolded forms of the MYOC protein were found aggregated in the endoplasmic reticulum of TM cells [38]. TM cells are essential for homeostatic regulation of aqueous humor, and their disruption may cause elevated intraocular pressure. A mutation-dependent, gain-of-function association between human MYOC and the peroxisomal targeting signal type 1 receptor (PTS1R) led to the [39] hypothesis that specific MYOC mutations may cause different amounts of MYOC

| Clinical parameter | MYOC/β-globulin | p≤ |
|-------------------|----------------|----|
| Age in years      | 0.204          | 0.21|
| Sex               | 0.040          | 0.81|
| Ethnicity         | 0.122          | 0.45|
| Visual acuity OD  | 0.016          | 0.92|
| Visual acuity OS  | 0.075          | 0.64|
| Maximum IOP OD    | 0.114          | 0.48|
| Maximum IOP OS    | 0.237          | 0.14|
| Vertical c/d ratio OD | 0.129 | 0.43 |
| Vertical c/d ratio OS | 0.171 | 0.29 |
| MD OD             | −0.042         | 0.80|
| MD OS             | −0.025         | 0.88|
| PSD OD            | 0.211          | 0.19|
| PSD OS            | 0.024          | 0.89|

MYOC/β-globulin column contains correlation coefficients; OD=right eye; OS=left eye; IOP=intracocular pressure; c/d=cup to disk; MD=Humphrey visual field mean deviation; PSD=Humphrey visual field pattern standard deviation.
misfolding, with corresponding varying degrees of recognition by the ubiquitin degradation pathway. A greater opportunity for mutant MYOC to interact with PTS1R may allow for poorer clearance from the TM endoplasmic reticulum and greater trabecular cell dysfunction, culminating in a higher IOP phenotype [39].

REFERENCES

1. Quigley HA. Number of people with glaucoma worldwide. Br J Ophthalmol 1996; 80:389-93. [PMID: 8695555]
2. Quigley HA. Glaucoma. Lancet 2011; 377:1367-77. [PMID: 21453963]
3. Gherghel D, Hosking SL, Orgul S. Autonomic nervous system, circadian rhythms, and primary open-angle glaucoma. Surv Ophthalmol 2004; 49:491-508. [PMID: 15325194]
4. Tielsch JM, Katz J, Sommer A, Quigley HA, Javitt JC. Family history and risk of primary open angle glaucoma. The Baltimore Eye Survey. Arch Ophthalmol 1994; 112:69-73. [PMID: 8285897]
5. Tielsch JM, Sommer A, Katz J, Royall RM, Quigley HA, Javitt J. Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. JAMA 1991; 266:369-74. [PMID: 2056646]
6. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90:262-7. [PMID: 16488940]
7. Abu-Amero KK, Morales J, Bosley TM. Mitochondrial abnormalities in patients with primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2006; 47:2533-41. [PMID: 16723467]
8. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polanksy JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC. Identification of a gene that causes primary open angle glaucoma. Science 1997; 275:668-70. [PMID: 9005853]
9. Gong G, Kosoko-Lasaki O, Haynatzki GR, Wilson MR. Genetic dissection of myocilin glaucoma. Hum Mol Genet 2004; 13:R91-102. [PMID: 14764620]
10. Fingert JH, Ying L, Swiderski RE, Nystuen AM, Arbour NC, Alward WL, Sheffield VC, Stone EM. Characterization and comparison of the human and mouse GLC1A glaucoma genes. Genome Res 1998; 8:377-84. [PMID: 9548973]
11. Sheffield VC, Stone EM. Genomics and the eye. N Engl J Med 2011; 364:1932-42. [PMID: 21591945]
12. 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]
13. Zhou Z, Vollrath D. A cellular assay distinguishes normal and mutant TIGR/myocilin protein. Hum Mol Genet 1999; 8:2221-8. [PMID: 10545602]
14. Jia LY, Gong B, Pang CP, Huang Y, Lam DS, Wang N, Yam GH. Correction of the disease phenotype of myocilin-causing glaucoma by a natural osmolyte. Invest Ophthalmol Vis Sci 2009; 50:3743-9. [PMID: 19234343]
15. Ray K, Mookherjee S. Molecular complexity of primary open angle glaucoma: current concepts. J Genet 2009; 88:451-67. [PMID: 20090207]
16. Liton PB, Luna C, Chall P, Epstein DL, Gonzalez P. Genome-wide expression profile of human trabecular meshwork cultured cells, nonglaucomatous and primary open angle glaucoma tissue. Mol Vis 2006; 12:774-90. [PMID: 16862071]
17. Wentz-Hunter K, Shen X, Okazaki K, Tanihara H, Yue BY. Overexpression of myocilin in cultured human trabecular meshwork cells. Exp Cell Res 2004; 297:39-48. [PMID: 15194423]
18. Bosley TM, Hellani A, Spaeth GL, Myers J, Katz LJ, Moster MR, Milcarek B, Abu-Amero KK. Down-regulation of OPT1 in patients with primary open angle glaucoma. Mol Vis 2011; 17:1074-9. [PMID: 21552501]
19. Abu-Amero KK, Jaber M, Hellani A, Bosley TM. Genome-wide expression profile of LHON patients with the 11778 mutation. Br J Ophthalmol 2010; 94:256-9. [PMID: 19726426]
20. Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet 2006; 141B:261-8. [PMID: 16526044]
21. Tang Y, Gilbert DL, Glauser TA, Hershey AD, Sharp FR. Blood gene expression profiling of neurologic diseases: a pilot microarray study. Arch Neurol 2005; 62:210-5. [PMID: 15710849]
22. Spaeth GL. Prognostic factors for progression of visual field damage in patients with normal-tension glaucoma. Japanese Journal of Ophthalmology 2007; 51:156. [PMID: 17401633]
23. Eid TM, Spaeth GL, Bitterman A, Steinmann WC. Rate and amount of visual loss in 102 patients with open-angle glaucoma followed up for at least 15 years. Ophthalmology 2003; 110:900-7. [PMID: 12750087]
24. Bayer A, Harasymowycz P, Henderer JD, Steinmann WG, Spaeth GL. Validity of a new disk grading scale for estimating glaucomatous damage: correlation with visual field damage. Am J Ophthalmol 2002; 133:758-63. [PMID: 12036666]
25. Read RM, Spaeth G. The natural history of cup progression and some specific disc field correlations. Trans Am Acad Ophthalmol Otolaryngol 1974; 78:OP255-74. [PMID: 4825055]
26. Kim J, Dally LG, Ederer F, Gaasterland DE, VanVeldhuisen PC, Blackwell B, Sullivan EK, Prum B, Shafranov G, Beck A, Spaeth GL, Investigators A. The Advanced Glaucoma Intervention Study (AGIS): 14. Distinguishing progression of glaucoma from visual field fluctuations. Ophthalmology 2004; 111:2109-16. [PMID: 15522379]
27. Bustin SA, Beaulieu JF, Huggett J, Jaggi R, Kibenge FS, Olsvik PA, Penning LC, Toegel S. MIQE precis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. BMC Mol Biol 2010; 11:74. [PMID: 20858237]
28. Allingham RR, Liu Y, Rhee DJ. The genetics of primary open-angle glaucoma: a review. Exp Eye Res 2009; 88:837-44. [PMID: 19061886]
29. Lenhard B, Sandelin A, Mendoza L, Engstrom P, Jareborg N, Wasserman WW. Identification of conserved regulatory elements by comparative genome analysis. J Biol 2003; 2:13. [PMID: 12760745]
30. Hu VW, Nguyen A, Kim KS, Steinberg ME, Sarachana T, Scully MA, Soldin SJ, Luu T, Lee NH. Gene expression profiling of lymphoblasts from autistic and nonaffected sib
pairs: altered pathways in neuronal development and steroid biosynthesis. PLoS ONE 2009; 4:e5775. [PMID: 19492049]

31. Saris CG, Horvath S, van Vught PW, van Es MA, Blauw HM, Fuller TF, Langfelder P, DeYoung J, Wokke JH, Veldink JH, van den Berg LH, Ophoff RA. Weighted gene co-expression network analysis of the peripheral blood from Amyotrophic Lateral Sclerosis patients. BMC Genomics 2009; 10:405. [PMID: 19712483]

32. Takahashi M, Hayashi H, Watanabe Y, Sawamura K, Fukui N, Watanabe J, Kitajima T, Yamanouchi Y, Iwata N, Mizukami K, Hori T, Shimoda K, Ujike H, Ozaki N, Iijima K, Takemura K, Aoshima H, Someya T. Diagnostic classification of schizophrenia by neural network analysis of blood-based gene expression signatures. Schizophr Res 2010; 119:210-8. [PMID: 20083392]

33. Kurian SM, Le-Niculescu H, Patel SD, Bertram D, Davis J, Dike C, Yehyawi N, Lysaker P, Dustin J, Caligiuri M, Lohr J, Lahiri DK, Nurnberger JI Jr, Faraone SV, Geyer MA, Tsuang MT, Schork NJ, Salomon DR, Niculescu AB. Identification of blood biomarkers for psychosis using convergent functional genomics. Mol Psychiatry 2011; 16:37-58. [PMID: 19935739]

34. Knaupp C, Flugel-Koch C, Goldwich A, Ohlmann A, Tamm ER. The expression of myocilin during murine eye development. Graefes Arch Clin Exp Ophthalmol 2004; 242:339-45. [PMID: 14749932]

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. Clark AF. Basic sciences in clinical glaucoma: steroids, ocular hypertension, and glaucoma. J Glaucoma 1995; 4:354-69. [PMID: 19920699]

37. 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]

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

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