Sequence analysis of MYOC and CYP1B1 in a Chinese pedigree of primary open-angle glaucoma

Jun Chen, Su-ping Cai, Wenhan Yu, Naihong Yan, Li Tang, Xiaoming Chen, Xuyang Liu

(The first two authors contributed equally to this paper)

Ophthalmic Laboratories and Department of Ophthalmology, West China Hospital, Sichuan University, P.R. China

Purpose: To analyze two candidate genes, trabecular meshwork inducible glucocorticoid response (MYOC/TIGR) and human dioxin-inducible cytochrome P450 (CYP1B1), in a Chinese pedigree of primary open-angle glaucoma.

Methods: In a three-generation family containing 14 members, four of them were patients with primary open-angle glaucoma, one was a glaucoma suspect, and the rest were asymptomatic. All members of the family underwent complete ophthalmologic examinations. Exons of MYOC and CYP1B1 were amplified by polymerase chain reaction, sequenced, and compared with a reference database.

Results: Elevated intraocular pressure and impaired visual field were found in all patients. One MYOC heterozygous mutation G367R, in exon 3 was identified in four patients and the suspect, but not in the rest of the family members. Meanwhile, four single nucleotide polymorphisms in MYOC and CYP1B1 genes were found.

Conclusions: Although the G367R mutation of MYOC, which causes primary open-angle glaucoma in the form of autosomal dominant inheritance, has been reported in some other ethnicities, it was found in Chinese pedigree for the first time.

Glaucoma is one of the leading causes of blindness in the world and is characterized by optic disc cupping and visual field defects [1,2]. Primary open-angle glaucoma (POAG) is the most common form of glaucoma [3]. There are two forms of POAG: juvenile onset and adult onset. Usually, juvenile open angle glaucoma (JOAG) may manifest clinically between the ages of 3 and 30 [4,5], while adult POAG manifests clinically after the age of 40 [6,7]. Although the exact mechanisms of POAG remain unclear, the accumulating evidences suggest that the genetic basis plays an important role in its pathogenesis. Four genes, trabecular meshwork inducible glucocorticoid response (MYOC/TIGR), human dioxin-inducible cytochrome P450 (CYP1B1), optineurin (OPTN), and WD repeat domain 36 (WDR36), have been identified as glaucoma-causing genes [8], with MYOC being the first identified POAG gene [9]. To date, more than 70 mutations have been detected in MYOC worldwide [10]. About 90% of the mutations were located in exon 3 where the olfactomedin-like domain is located [11]. Recently, CYP1B1 has been shown to be related to POAG, especially JOAG [12-14]. Both MYOC and CYP1B1 consist of three exons, but in CYP1B1, only exon 2 and 3 encode the protein.

In this study, alterations in MYOC (three exons) and CYP1B1 (exon 2 and 3) were analyzed, and a known mutation (c.1099 G>A, G367R) in exon 3 of MYOC, which was segregated with the disorder within the family and appeared to be the disease-causing gene, was found. It is for the first time, to the best of our knowledge, that G367R mutation was found in Chinese.

METHODS

Clinical examination: This three-generation pedigree with POAG (Figure 1) was recruited from the out-patient department of Ophthalmology at West China Hospital (Sichuan University, Chengdu, P. R. China). All members of the family underwent the complete ophthalmologic examinations including slit-lamp biomicroscopy, gonioscopic examination, fundoscopic examination, fundoscopic examination, IOP measurement (Canon TX-F Non-contact tonometer; Canon Inc., Tokyo, Japan), and visual field test (Octopus 900; HAAG-STREIT International, Berne, Swiss). Diagnostic criteria for POAG included open anterior chamber angle, elevated IOP (≥22 mmHg), glaucomatous visual field defects and characteristic optic disc damage.

The study was approved by the medical ethics committee of the West China Hospital of Sichuan University. This study follows the principles of the Declaration of Helsinki. All subjects were clinically evaluated by glaucoma specialists.

Detection of MYOC and CYP1B1 mutations: Genomic DNA was extracted from 0.2 ml peripheral blood sample with Qiam Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instruction.
Intronic primers flanking the exons were designed (Table 1) based on gene sequences of MYOC (GenBank AF001620) and CYP1B1 (GenBank US6438) and synthesized by Invitrogen (Carlsbad, CA). PCR amplification was performed in a MyCycler thermocycler (Bio-Rad, Hercules, CA). The 30 μl PCR reaction mixtures included 30 ng DNA, 1× PCR buffer, 2.5 mM MgCl2, 0.3 mM of each of dNTPs, 1.5 U Pfu DNA polymerase, and 1.0 μM each of the forward and reverse primers. All reagents used in this procedure were purchased from TaKaRa (Dalian, China). The reactions were incubated at 95 °C for 4 min followed by 35 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for appropriate time (the second exon of MYOC for 30 s and the rest for 90 s), and then a final extension at 72 °C for 5 to 10 min.

PCR products were directly sequenced by an ABI 377XL automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequence data were compared pair-wise with the published MYOC and CYP1B1 sequences.

RESULTS

The proband and other patients (Table 2): The proband (II-3) was diagnosed with POAG (both eyes) at the age of 46, with elevated IOPs (26.7 mmHg in the right eye and 23.1 mmHg in the left eye), open anterior chamber angle, enlarged cup-disc ratio of 0.5/0.6 (OD/OS) and characteristic glaucomatous visual field defects (Figure 2). Other ocular abnormalities or systemic disorders were not found.

The proband’s mother (I-2) was diagnosed with POAG at the age of 38, and trabeculectomy was performed for both eyes twenty-one years ago. During this examination, a cup/disc ratio of 0.95/1.0 (OD/OS), IOPs at 16.7/19.1 mmHg (OD/OS), and late-stage glaucomatous visual field loss were noticed (Figure 2). II-1 was a JOAG patient, and bilateral trabeculectomies were performed at the age of 34, when his IOPs were as high as 50–60 mmHg (OU). Patient II-6’s onset of the glaucoma was at the age of 20, much earlier than other family members. Her maximal IOPs measured 50 mmHg in the right eye and 65 mmHg in the left eye. She underwent trabeculectomy in both eyes. The cup-disc ratio of both II-1 and II-6 was 0.9/0.95 (OD/OS). Patient III-1, 25 years old, was a glaucoma suspect, because he had a strong family history of glaucoma and his IOP measured 27.8/21.5 mmHg (OD/OS), despite no glaucomatous visual field defects and characteristic optic disc damage.

Asymptomatic family members: The proband’s father (I-1) didn’t have ocular diseases except senile cataract. No ocular...
abnormalities were found in the rest of the family numbers. Their visual acuity or corrected visual acuity was measured ≥20/20.

**MYOC**—Sequence analysis of MYOC revealed a heterozygous mutation, c.1099G>A (G367R), in exon 3 in all patients and the suspected one but not in any of the asymptomatic members of the family. The G367R MYOC mutation was cosegregated with the disorder within the family (Figure 3). One single nucleotide polymorphism (SNP, g.23096344C>T) in exon 2 of MYOC was identified.

**CYP1B1**—No mutation was found. Three CYP1B1 SNPs (g.17120037A>G, g.17120090C>G, and g.17120026T>C) were identified in exon 3.

**DISCUSSION**

MYOC was the first identified POAG gene [9]. Previous studies showed that MYOC mutations exist in nearly 3% of adult onset POAG patients and a greater proportion of JOAG patients [15,16]. In this study, a G to A transition at the first base of codon 367 (in exon 3 of MYOC), which resulted in a glycine to arginine amino acid substitution, was identified, suggesting that MYOC is the glaucoma-causing gene in this family. This mutation has been previously reported in several other ethnic groups: Japanese, Indian, Irish, Swiss, French-Canadian, Scottish, and German [17-25], however, to the best of our knowledge, this mutation was found in Chinese for the first time.

Based on the literature reviewed, the phenotype of POAG associated with the G367R mutation was summarized in Table 3: 1) there were no obvious differences between gender; 2) all carriers had open anterior chamber angle; 3) all 6 pedigrees were in autosomal dominant fashion; 4) the patients had relatively high IOP and relatively early onset age; and 5) medical control of IOP were not satisfied in most of the patients, and surgeries were usually needed. The Chinese pedigree with G367R mutation reported in this paper was in general coincidence with the characteristics mentioned.

The phenotypic variations observed here and in previous studies suggest that, in addition to G367R substitution, some as yet unidentified factors (such as the genetic and/or environmental) are responsible for the disease phenotype. Furthermore, POAG is well known to be genetically heterogeneous and several loci have been identified except MYOC. The variations in these loci may contribute to phenotypic variations. Recently, another gene CYP1B1, indeed, has been suggested to modify the glaucoma phenotype associated with the G367R mutation reported in this paper.

**TABLE 1: PRIMERS USED IN PCR FOR AMPLIFICATION OF MYOC AND CYP1B1.**

| Exons | Primer sequence (forward/reverse) | Product size (bp) |
|-------|-----------------------------------|-----------------|
| MYOC 1 | PF 5′-CCAAACAGACTTCTGGAGG-3’ | 904 |
| MYOC 1 | PR 5′-TACGAAGTCACTACGAGGC-3’ | 351 |
| MYOC 2 | PF 5′-TGTCATCCCTCAACATAGTCA-3’ | 933 |
| MYOC 2 | PR 5′-TTCTGTTCCTCTCTTTCCT-3’ | 1260 |
| MYOC 3 | PF 5′-CCAGGGCTGTGACATCTACT-3’ | 927 |
| MYOC 3 | PR 5′-CATCTCTCTTCTGGACATGTC-3’ | |
| CYP1B1 2 | PF 5′-CATTTCCTCCAGAGATGAG-3’ | |
| CYP1B1 2 | PR 5′-GCTTGCMAATCAGCATATTC-3’ | |
| CYP1B1 3 | PF 5′-ACCATTGGAAAGTCAGCC-3’ | |
| CYP1B1 3 | PR 5′-GCTTGCCTCTTTGAGTATT-3’ | |

**TABLE 2: PATIENT DATA FROM THIS POAG PEDIGREE**

| Patient | Age at study (years) | Diagnosis age (years) | Operation age (years) | Maximal IOP (mmHg) | C/D ratio | Visual field damage |
|---------|----------------------|-----------------------|-----------------------|-------------------|-----------|--------------------|
| I-2     | 68                   | 38                    | 41                    | >50 (ou)          | 0.95/1.0  | Severe             |
| II-1    | 48                   | 30                    | 34                    | 50–60 (ou)        | 0.9/0.95  | Moderate           |
| II-2    | 46                   | 46                    | —                     | 28.9 (od), 28.1 (os) | 0.5/0.6  | Moderate           |
| II-3    | 42                   | 20                    | 24                    | 50 (od), 65 (os)  | 0.9/0.95  | Severe             |
| III-1   | 25                   | 25                    | —                     | 24.4 (od), 23.6 (os) | 0.2      | Normal             |

![Figure 3. MYOC mutation in the POAG family. A: Normal individuals with homozygous G (arrow). B: The double peak of guanine (black line) and adenine (green line; B, arrow) represents a heterozygous mutation at the codon of 367th amino acid residue (Gly367Arg).](image)
TABLE 3. GLAUCOMA PHENOTYPE OF PATIENT WITH MYOCILIN G376R MUTATION.

| Ethnic origin    | Type of glaucoma | Carrier number | Maximum IOP (mmHg) | Age at diagnosis (year) | Hereditary pattern | Publication year |
|------------------|------------------|----------------|--------------------|-------------------------|--------------------|-----------------|
| Japanese         | POAG             | 1              | ND                 | 45                      | ND                 | 1997            |
| Irish            | POAG             | 5              | ND                 | 36                      | ND                 | 1998            |
| German           | JOAG             | 2              | 50                 | 36.7 (median age)        | AD                 | 1998            |
| Japanese         | POAG & suspect   | 8              | >50 (4/7)          | 34 & 21                 | AD & Sporadic      | 2002            |
| French-Canadian  | Both             | 7              | 50.6              | ND                      | ND                 | 2003            |
| UK (Scottish)    | JOAG             | 1              | 32                 | ND                      | Sporadic           | 2003            |
| Indian JOAG      | 1               | 50.6           | ND                 | ND                      | ND                 | 2008            |
| French           | ND               | 1              | ND                 | 50                      | AD                 | 2008            |
| Swiss            | Both & suspect   | 13             | 28–51             | AD                      | ND                 | 2008            |

Abbreviations are as follows: AD, Autosomal Dominant; ND, Not Described.

[8]. It may act as a modifier of MYOC expression or the two genes may interact via a common pathway [26,27]. We continued to screen CYP1B1 gene, but no mutations, except three SNPs, were detected.

It is known that patients with missense mutations such as G376R are likely to present the normal and mutant mRNA in equal amounts, and then the mRNA is translated into an equal ratio of mutant and normal proteins. Myocilin was thought to be a secreted protein [28], but mutant Myocilin formed insoluble aggregates that could not be secreted out from human trabecular meshwork (HTM) cells and accumulated intracellularly, then damaged the function of HTM cells and resulted in an increase of aqueous humor outflow resistance [29-31]. So far, the secretion of G376R mutation myocilin protein has been studied in vitro and in vivo, which revealed the same mechanism above [32].

In conclusion, the G376R mutation of MYOC in this pedigree appears to be the cause of the disease in this family. This is the first time that this mutation was found in Chinese.

ACKNOWLEDGMENTS

This work was supported by grants from the National Nature Science Foundation of China (NNSF 30772379, 30600690, and 30872830).

REFERENCES

1. Quigley HA. Number of people with glaucoma worldwide. Br J Ophthalmol 1996; 80:389-93. [PMID: 8695555]
2. Ikezoe T, Takeuchi S, Komatsu N, Okada M, Fukushima A, Ueno H, Koeffler HP, Taguchi H. Identification of a new GLC1A mutation in a sporadic, primary open-angle glaucoma in Japan. Int J Mol Med 2003; 12:259-61. [PMID: 12851728]
3. Fraser SG. Epidemiology of primary open-angle glaucoma. In: Hitchings RA, editors. Glaucoma. London: BMJ Publ Group; 2004. P.9–15.
4. Ellis OH. The etiology, symptomatology and treatment of juvenile glaucoma. Am J Ophthalmol 1948; 31:1589-96. [PMID: 1812120]
5. Goldwyn R, Waltman SR, Becker B. Primary open-angle glaucoma in adolescents and young adults. Arch Ophthalmol 1970; 84:579-82. [PMID: 5478882]
6. Wilson R, Matrone J. Epidemiology of chronic open angle glaucoma. In: Ritch R, Shields BM, Kruptin T, editors. The glaucomas. 2nd ed. St Louis: Mosby; 1996. p. 753-68.
7. Quigley HA. Open-angle glaucoma. N Engl J Med 1993; 328:1097-106. [PMID: 8455668]
8. Libby RT, Gould DB, Anderson MG, John SW. Complex Genetics of Glaucoma Susceptibility. Annu Rev Genomics Hum Genet 2005; 6:15-44. [PMID: 16124852]
9. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky 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]
10. http://www.myocilin.com/variants.php (accessed June 19, 2010).
11. Rozsa FW, Shimizu S, Lichter PR, Johnson AT, Othman MI, Scott K, Downs CA, Nguyen TD, Polansky J, Richards JE. GLC1A mutations point to regions of potential functional importance on the TIGR/MYOC protein. Mol Vis 1998; 4:20. [PMID: 9772276]
12. Vincent AL, Billingsley G, Buys Y, Levin AV, Priston M, Trope G, Williams-Lyn D, Héon E. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. Am J Hum Genet 2002; 70:448-60. [PMID: 11774072]
13. Melki R, Colomb E, Lefort N, Brézin AP, Garchon HJ. CYP1B1 mutations in French patients with early-onset primary open-angle glaucoma. J Med Genet 2004; 41:647-51. [PMID: 15342693]
14. López-Garrido MP, Sánchez-Sánchez F, López-Martínez F, Aroca-Aguilar JD, Blanco-Marchite C, Coca-Prados M, Escribano J. Heterozygous CYP1B1 gene mutations in Spanish patients with primary open-angle glaucoma. Mol Vis 2006; 12:748-55. [PMID: 16862072]
15. Alward WL, Kwon YH, Khanna CL, Johnson AT, Hayreh SS, Zimmerman MB, Narkiewicz J, Andorf JL, Moore PA, Fingert JH, Sheffield VC, Stone EM. Variations in the myocilin gene in patients with open-angle glaucoma. Arch Ophthalmol 2002; 120:1189-97. [PMID: 12215093]
16. Fingert JH, Héon E, Liebnam JM, Yamamoto T, Craig JE, Raitt J, Kawase K, Hoh ST, Buys YM, Dickinson J, Hockey RR, Donna WL, Trope G, Kitazawa Y, Ritch R, Mackey DA, Alward WL, Sheffield VC, Stoned EM. Analysis of myocilin mutations in 1703 glaucoma patients from five different...
17. Taniguchi F, Suzuki Y, Shirato S, Araie M. The Gly367Arg mutation in the myocilin gene causes adult-onset primary open-angle glaucoma. Jpn J Ophthalmol 2000; 44:443-8. [PMID: 10974305]

18. Suzuki Y, Shirato S, Taniguchi F, Ohara K, Nishimaki K, Ohta S. Mutations in the TIGR Gene in Familial Primary Open-Angle Glaucoma in Japan. Am J Hum Genet 1997; 61:1202-4. [PMID: 9345106]

19. Mansergh FC, Kenna PF, Ayuso C, Kiang AS, Humphries P, Farrar GJ. Novel Mutations in the TIGR Gene in Early and Late Onset Open Angle Glaucoma. Hum Mutat 1998; 11:244-51. [PMID: 9521427]

20. Michels-Rautenstrauss KG, Mardin CY, Budde WM, Liehr T, Polansky J, Nguyen T, Timmerman V, Van Broeckhoven C, Naumann GO, Pfeiffer RA, Rautenstrauss BW. Juvenile open angle glaucoma: fine mapping of the TIGR gene to 1q24.3–q25.2 and mutation analysis. Hum Genet 1998; 102:103-6. [PMID: 9490287]

21. Faucher M, Anciil JL, Rodrigue MA, Duchesne A, Bergeron D, Blondeau P, Côté G, Dubois S, Bergeron J, Arseneault R, Morissette J, Raymond V. Founder TIGR/myocilin mutations for glaucoma in the Quebec population. Hum Mol Genet 2002; 11:2077-90. [PMID: 12189160]

22. Cobb CJ, Scott G, Swingler RJ, Wilson S, Ellis J, MacEwen CJ, McLean WH. Rapid mutation detection by the Transgenomic wave analyser DHPLC identifies MYOC mutations in patients with ocular hypertension and/or open angle glaucoma. Br J Ophthalmol 2002; 86:191-5. [PMID: 11815346]

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

24. Melki R, Belmouden A, Brézin A, Garchon HJ. Myocilin Analysis by DHPLC in French POAG Patients: Increased Prevalence of Q368X Mutation. Hum Mutat 2003; 22:179. [PMID: 12872267]

25. Iliev ME, Bodmer S, Gallati S, Lanz R, Sturmer J, Katsoulis K, Wolf S, Tritthbach P, Sarra GM. Glaucoma phenotype in a large Swiss pedigree with the myocilin Gly367Arg mutation. Eye (Lond) 2008; 22:880-8. [PMID: 17304254]

26. Stoilov I, Akarsu AN, Alozie I, Child A, Barsoum-Homsey M, Turacli ME, Or M, Lewis RA, Ozdemir N, Brice G, Akta SG, Chevette L, Coca-Prados M, Sarrafzad M. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P450IB1. Am J Hum Genet 1998; 62:573-84. [PMID: 9497261]

27. Bagiyeva S, Marfany G, Gonzalez-Angulo O, Gonzalez-Duarte R. Mutational screening of CYP1B1 in Turkish PCG families and functional analyses of newly detected mutations. Mol Vis 2007; 13:1458-68. [PMID: 17893647]

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

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

30. 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]

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

32. Kanagavalli J, Pandaranayaka PJ, Krishnadas SR, Krishnaswamy S, Sundaresan P. In vitro and in vivo study on the secretion of the Gly367Arg mutant myocilin protein. Mol Vis 2007; 13:1161-8. [PMID: 17679945]