Characterization of a novel mutation in the *MYOC* gene in a Chinese family with primary open-angle glaucoma

WANLIN FAN[1,2], WAN LI[1], CHAOYE DUAN[1], WENBO ZHANG[1], YONGWEI GUO[2] and FEI CHEN[1]

[1]Department of Ophthalmology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022, P.R. China; [2]Department of Ophthalmology, University of Cologne, Faculty of Medicine and University Hospital Cologne, D-50937 Cologne, Germany

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Abstract. Although primary open-angle glaucoma (POAG)-related mutations in the myocilin (*MYOC*) gene have been reported, the underlying associations remain poorly understood. In the present study, the relationship between a *MYOC* mutation and POAG was investigated using ophthalmic examination and total exon gene sequencing in a Chinese family comprised of 5 individuals with POAG and 15 unaffected individuals. Pathogenic mutations underlying POAG were identified by whole-exome sequencing and subsequently validated by Sanger sequencing. Of the family members, nine (45%) harbored heterozygous p.D208Y mutations; among these, five had POAG and four were unaffected. The mean age at diagnosis was 26.2±4.12 years and the mean intraocular pressure (IOP) was 39.7±16.58 mmHg; all affected members complained of vision loss, headaches and eye swelling. Among the five cases of POAG, two presented with blindness. Among 10 members of the family who underwent comprehensive ophthalmologic examination, 3 individuals exhibited severe visual field defects. The mean age at the time of operation was 27.2±3.54 years. In the present study, a novel *MYOC* mutation (c.G622T: p.D208Y) was identified that was associated with severe visual impairment, high IOP and the need for frequent surgical interventions. Some carriers of the mutation were young and did not show signs of glaucoma. These individuals should be followed-up to firmly establish whether the mutated gene is pathogenic for POAG.

Introduction

Globally, glaucoma is the second leading cause of vision loss after cataracts (1); it is characterized by optic neuropathy and loss of visual field. By 2020, it is estimated that 11.1 million individuals will suffer from bilateral blindness due to primary glaucoma (2). Primary open-angle glaucoma (POAG) is more common in individuals of African and European descent (3), and in China its incidence is less than that of primary closed-angle glaucoma (4). However, in recent years, its incidence rate has increased in parallel with the increased incidence of myopia (incidence of POAG: 23% higher) (5) and metabolic diseases (incidence of POAG: 49% higher) (6). The pathogenesis of POAG is usually slow, and the majority of patients will not exhibit obvious symptoms. Since patients may not be aware of the loss of vision until the advanced stages of the disease, genetic screening is important for early diagnosis, prevention and treatment (1). The primary risk factors for POAG include age, myopia, family history, high mental stress, diabetes, smoking, drinking and increased intraocular pressure (IOP) (7). Owing to familial predispositions, the prevalence of POAG in first-degree relatives is 7-10 times higher compared with the general population (8). POAG-associated gene mutations have been reported in myocilin (*MYOC*), optineurin *(OPTN)*, tANK binding kinase 1 *(TBK1)*, WD repeat domain 36 *(WDR36)* and ankyrin repeat and SOCS box containing 10 (*ASB10)* (9). *MYOC* was the first gene found to be associated with POAG. Johnson *et al* (10) and Sheffield *et al* (11) mapped the chromosome region 1q21-q31 of the POAG locus, *GLC1A*, which is associated with both juvenile- and adult-onset POAG. At present, 771 nucleotide substitutions have been reported in the *MYOC* gene. Among these, 331 substitutions are disease-causing mutations (DCM) (12). *MYOC* has been investigated for >20 years and is the most common mutated gene in patients with glaucoma (13,14). Although there are several studies on the function of WT *MYOC*, its function remains incompletely understood, and the mechanisms by which mutations in *MYOC* result in POAG, remains to be investigated in several cases. It has been reported that a possible cause of POAG in patients with a mutated *MYOC* gene is upregulated expression of the mutated troponin subtype in the endoplasmic reticulum of trabecular reticulum (TM) cells, and its retention in the endoplasmic reticulum, which leads to trabecular reticulum stress, dysfunction and apoptosis (15-18). TM cell loss is associated with the disturbance of aqueous outflow regulation and increased IOP, and ultimately leads to loss of vision (19). In this study, we characterized the clinical results of a Chinese

Correspondence to: Professor Fei Chen, Department of Ophthalmology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Avenue, Wuhan, Hubei 430022, P.R. China
E-mail: chenfei1972@hotmail.com

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POAG family and studied its molecular basis, to expand the MYOC mutation spectrum in the Chinese population.

Materials and methods

Clinical observations and diagnosis. A large 20-member family spanning four generations (Fig. 1) was enrolled in the present study at the Department of Ophthalmology, Wuhan Union Hospital (Wuhan, China), between January and February 2018. The study adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Wuhan Union Hospital. After obtaining written informed consent from all participants or their legal guardians, where required, their medical history was collected. Of the participants, 10 members of the family underwent comprehensive ophthalmologic examination, including visual acuity, IOP, slit-lamp bio-microscopy, direct fundus examination, gonioscopy, standard automated perimetry and retinal nerve fiber layer (RNFL) by optical coherence tomography. Additionally, two patients (I2, II1), with low vision and five unaffected children (III9, IV1, IV2, IV3, IV4), underwent slit-lamp bio-microscopy, IOP measurements and direct fundus examination. The three unaffected members were unable to undergo any examination. The diagnostic criteria for POAG were based on at least two of the following glaucoma characteristics, with the opening of the anterior chamber angle, excluding any secondary glaucoma: Characteristic glaucomatous changes of the optic disc, visual field defects and high IOP (>21 mmHg) (20). Ocular hypertension (OHT) was defined as IOP >22 mmHg and long-term follow-up without optic disc damage or visual field impairment (21). Unaffected individuals exhibit IOP values in the normal range (≤21 mmHg) and lack of optic nerve damage. Based on the World Health Organizations standards stated in 1992 (22), visual acuity ≤3/60 and/or visual field <10˚ in the eye with comparatively better vision was diagnosed as blindness. The University of São Paulo Glaucoma Visual Field Staging System (USP-GVFSS): Early visual field defect, visual field index (VFI) >91%; moderate visual field defect, 91%≥ VFI >78%; severe visual field defect, VFI ≤78% (23).

Genetic detection using whole-exome sequencing (WES). Peripheral blood (2 ml) was collected from each individual into EDTA-tubes (Becton, Dickinson and Company). Genomic DNA was extracted from leucocytes in the blood samples using a Blood Genome Extraction kit (Tiangen Biotech Co., Ltd.) according to the manufacturer's protocol. Genomic DNA was quantitated by Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Inc.). An NEBNext Ultra II DNA Library Prep kit (New England BioLabs, Inc.) was used for library preparation. Sample DNA (e.g., concentration of DNA were 96.2 (III8), 116 (III9) and 106 ng/ul (IV4) restpectively) was the subjected to NextSeq 500 Sequencing system (Illumina, Inc.) to perform 150 bp pair-end sequencing by Genokon Medical Laboratory (Xiamen, China).

Following sequencing, quality controls were performed to remove low-quality data by Trimmomatic (http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic/Trimmomatic-0.36.zip) (24). Clean reads were aligned to the reference human genome (GRCh37/hg19) using the Burrows-Wheeler Alignment tool (25). GATK (software. broadinstitute.org/gatk) was used to identify single-nucleotide polymorphisms and insertions or deletions (indels). Subsequently, ANNOVAR (https://annovar.openbioinformatics.org/en/latest/user-guide/download/, version number 20191024) (26) was used to annotate genetic variants with functional information. Common variants were filtered out, such as variants of intergenic, intronic, upstream, downstream, or synonymous variants and variants with minor allele frequency (MAF) >1% in the 1000 Genomes Project (27), the ExAC database (http://exac.broadinstitute.org/) and gnomAD (https://gnomad.broadinstitute.org/). SIFT (http://sift.jcvi.org), MutationAssessor (http://mutationassessor.org/r3/) PROVEAN (28), Mutation Taster2 (http://www.mutationtaster.org) and CADD (https://annovar.openbioinformatics.org/en/latest/user-guide/download/; version no. hg19_dbnsfp33a_20170221) (29) were used for pathogenicity prediction of each variant. Exomiser (30) and Phenolyzer (31) were used to perform genotype-phenotype analyses. Finally, the interpretation of variants was performed to identify the potential mutations, in accordance with the American College of Medical Genetics and Genomics Standards and Guidelines (32).

Sanger sequencing. The potential mutation of the proband and the remaining family members were validated using Sanger sequencing. DNA was extracted from peripheral blood samples from the proband and other family members. DNA sequence including the candidate mutation was amplified using the following primers: MYOC forward, 5'-TGTAGTCTCGGC TCACAG-3' and reverse, 5'-TGATAGGATAGAGGGCTT T-3'. The PCR cycle consisted of an initial denaturation step of 5 min at 98˚C followed by 35 cycles of 30 sec at 98˚C, 30 sec at 53˚C, and 45 sec at 72˚C, and a final step at 72˚C for 5 min. All PCR products were separated and then directly sequenced using BigDye Terminator v.3.1 Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and analyzed by capillary electrophoresis using an ABI Prism 3500 Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.).

Multiple sequence alignment analysis and protein structural & functional analysis. The amino acid sequence of MYOC across different species (Xenopus tropicalis: XP_002934195.3; Homo sapiens: NP_000252.1; Macaca mulatta: XP_001099905.2; Rattus norvegicus: NP_110492.1; Mus musculus: NP_034995.3) were aligned by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The structural impact of missense variants on protein was predicted and analyzed by Phyre2 (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index).

Results

Clinical findings. The pedigree of the family investigated in the present study is shown in Fig. 1. The II3 proband experienced symptoms of eye swelling, headache and nausea for the first time at 28 years of age. The proband was diagnosed with POAG by her local GP. The results of her ophthalmic examination are described in Table I. The highest IOP was 50 and 20 mmHg [oculus dexter (OD) and oculus sinister (OS)], respectively), and the cup-to-disc ratio was 0.9

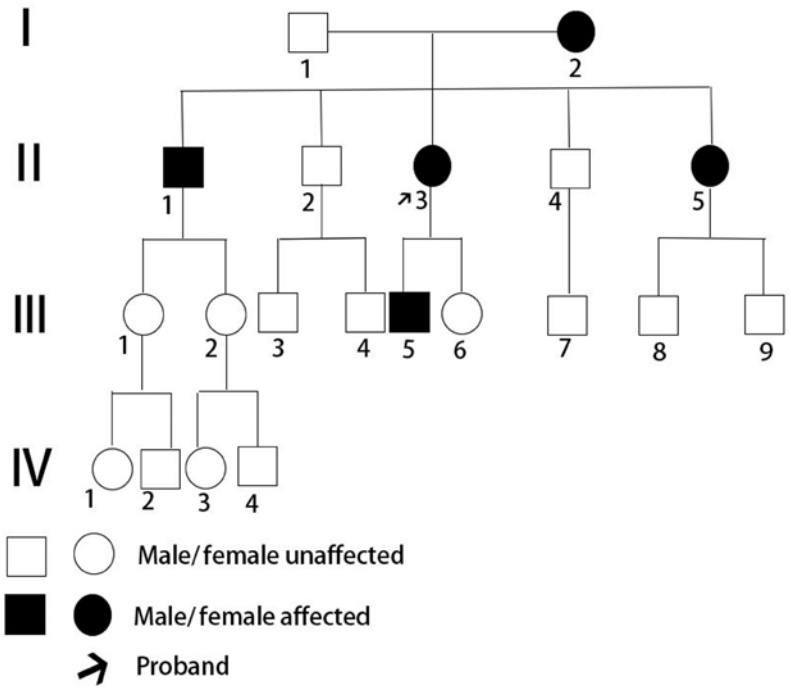
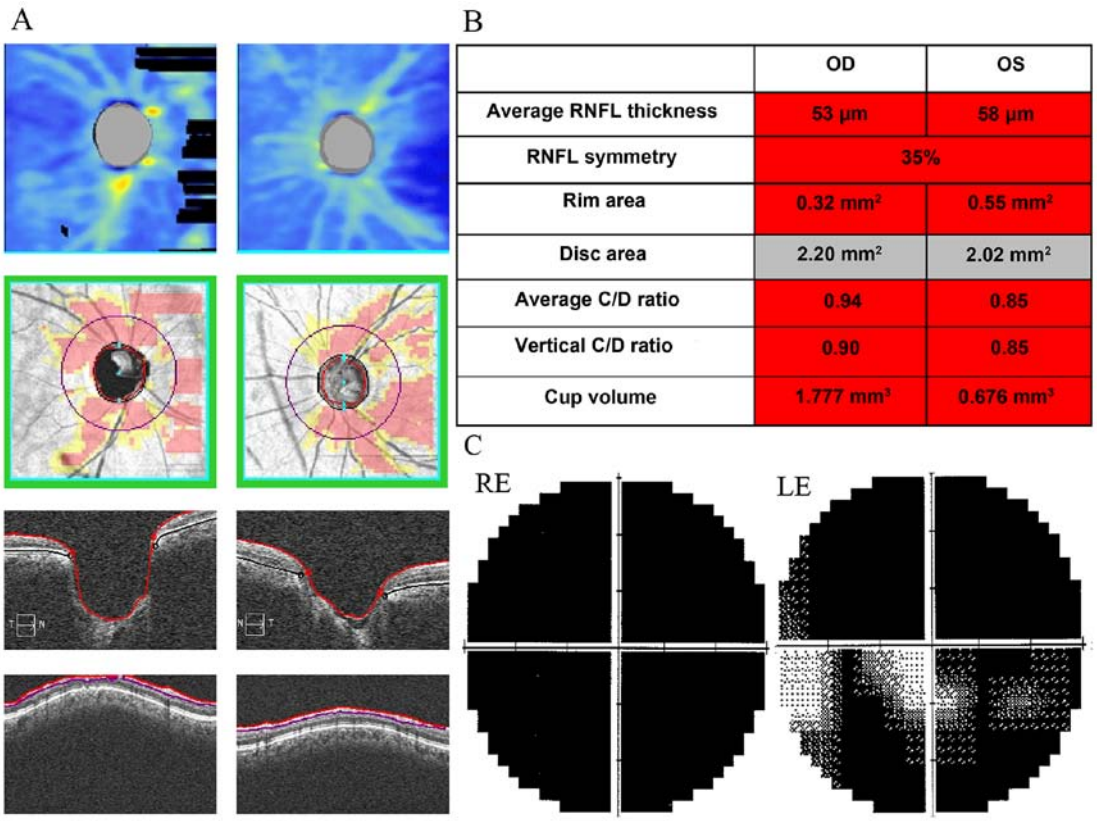


Figure 1. Pedigree of the family with primary open-angle glaucoma.



	OD	OS
Average RNFL thickness	53 μm	58 μm
RNFL symmetry	35%	
Rim area	0.32 mm²	0.55 mm²
Disc area	2.20 mm²	2.02 mm²
Average C/D ratio	0.94	0.85
Vertical C/D ratio	0.90	0.85
Cup volume	1.777 mm³	0.676 mm³

Figure 2. Examination of proband II3. Optical coherence tomography examination (A) images and (B) results of proband II3. (C) Visual field examination images of proband II3. RE, right eye; LE, left eye; RNFL, retinal nerve fiber layer; OD, oculus dexter; OS, oculus sinister; C/D, cup-to-disc ratio.

and 0.8 (OD and OS, respectively). Optical coherence tomography revealed a significant thinning of the patient's RNFL thickness (Fig. 2A and B). The patient exhibited severe visual field damage (Fig. 2C) and low vision (OD, light perception; OS: 1.0). Of the remaining 19 family members examined, four were diagnosed with POAG, one exhibited OHT, whereas the remaining 14 were unaffected and exhibited normal clinical features, without obvious signs of glaucoma; the ophthalmic

Table I. Demographic data and clinical characteristics of patients with the D208Y mutation.

A, Patients with POAG

Pedigree number (n=5)	Sex	Age at study, years	IOP at study, nCT OD/OS, mm hg	Age at diagnosis, years	BCVA, OD/OS	C/D ratio, OD/OS	Visual field damage, OD/OS	Highest IOP, nCT OD/OS, mm hg	Operation eye/age, years
I2	Female	75	18/17	33	NLP/NLP	1.0/1.0	NA	55/60	OU/33
II1	Male	54	10/18	22	FC/50 cm; HM/30 cm	0.9/0.9	NA	53/55	OU/22
II3	Female	50	17/15	28	LP/1.0	0.9/0.8	S/S	50/20	OU/28
II5	Female	43	18/15	22	1.0/1.0	0.7/0.6	E/E	35/30	OU/27
III5	Male	26	8/31	26	1.0/0.08	0.5/0.9	E/S	8/31	OS/26
Mean	-	49.6±15.91	-	26.2±4.12	-	-	-	40.2±17.57/ 39.2±15.51	-

B, Unaffected family members

Pedigree number (n=3)	Sex	Age at study, years	IOP at study, nCT OD/OS, mm hg	Age at diagnosis, years	BCVA, OD/OS	C/D ratio, OD/OS	Visual field damage, OD/OS	Highest IOP, nCT OD/OS, mm hg	Operation eye/age, years
III2	Male	29	14/17	-	1.0/1.0	0.3/0.4	Normal	14/17	-
III6	Male	11	13/16	-	1.0/1.0	0.3/0.3	Normal	13/16	-
III8	Male	10	18/19	-	1.0/1.0	0.3/0.3	Normal	18/19	-

C, Patients with ocular hypertension (n=1)

Pedigree number	Sex	Age at study, years	IOP at study, nCT OD/OS, mm hg	Age at diagnosis, years	BCVA, OD/OS	C/D ratio, OD/OS	Visual field damage, OD/OS	Highest IOP, nCT OD/OS, mm hg	Operation eye/age, years
III9	Male	17	28/29	-	0.2/0.12	0.5/0.4	Normal	28/29	-

POAG, primary open-angle glaucoma; IOP, intraocular pressure; C/D, cup-to-disc ratio; NLP, no light perception; FC, fingers counting; LP, light perception; BVCA, best-corrected visual acuity; NA, not available; S/S, severe visual field defect/severe visual field defect; E/E, early visual field defect/early visual field defect; E/S, early visual field defect/severe visual field defect; nCT, non-contact tonometer; OU, ocular uterque; OD oculus dexter; OS, oculus sinister.

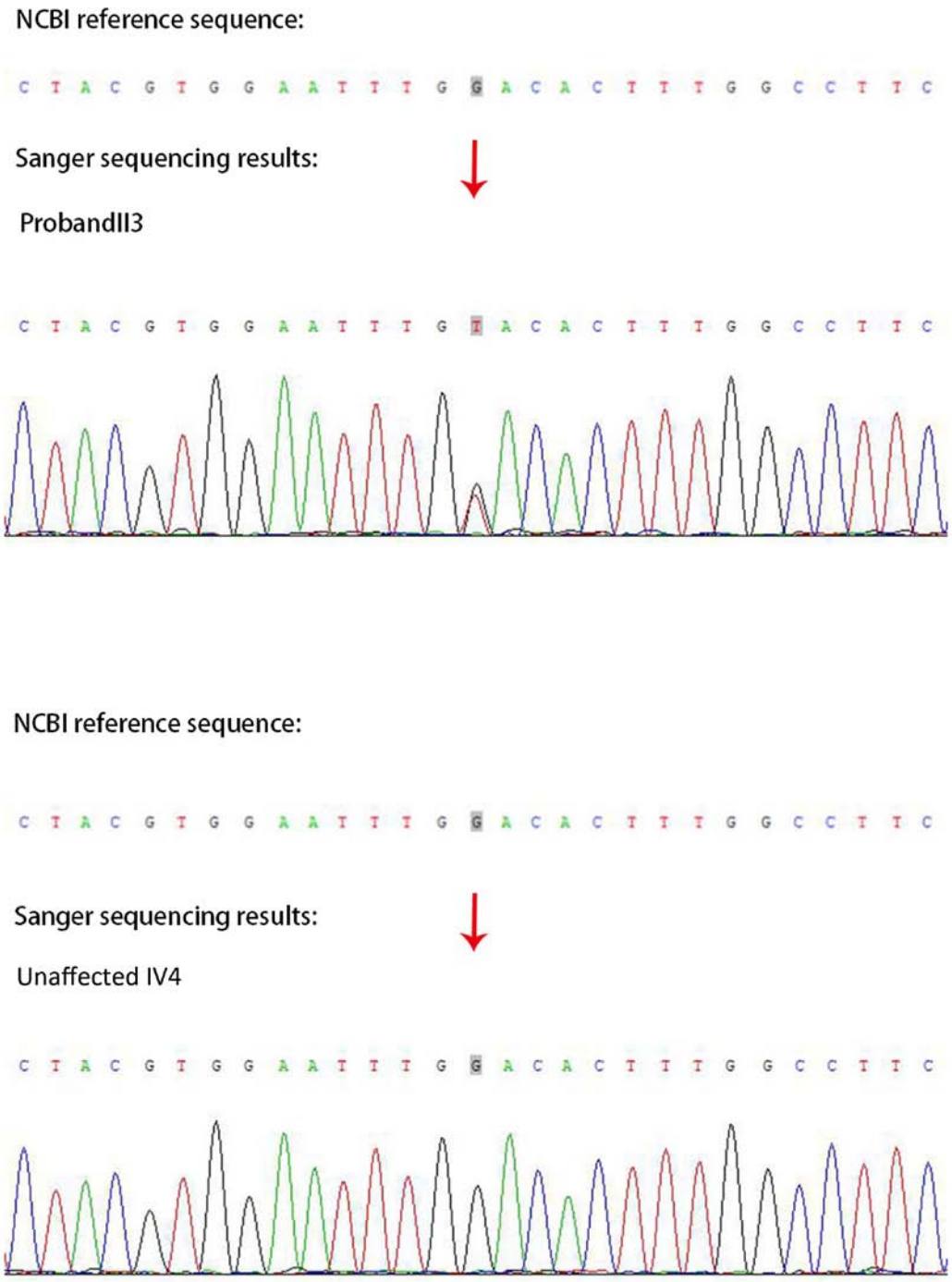


Figure 3. Sequencing results of *MYOC*. The sequences of the proband (II3) and an unaffected member (IV4) are shown. A heterozygous mutation (c.G622T: p.D208Y) is detected in exon 2 of MYOC in the proband. MYOC, myocilin.

examination results of five patients with POAG and one individual with OHT are listed in Table I. All five patients with POAG had a wide anterior chamber angle and normal iris. Among these five patients, three were female. The mean age of all POAG patients at diagnosis was 26.2±4.12 years (range, 22-33 years). All patients showed symptoms of eye distention, headache and vision loss before diagnosis. The mean maximum IOP values were 40.2±6.53 mmHg (range, 8-55 mmHg) and 39.2±15.51 mmHg (range, 20-60 mmHg) (OD and OS, respectively); two patients were blind. Severe visual field defects were observed in three eyes of two patients (II3, III5). A total of nine eyes in five patients (I2, II1, II3, II5, III5) underwent surgery (80%), at an average age of 27.2±3.54 years (range, 22-33 years). No optic disc or visual field defects were observed for the OHT individuals (III9).

Mutation screening of MYOC in POAG. WES was performed for the human genome, covering >20,000 genes and 85% of the human heritage diseases. The detection range included mutation types such as single nucleotide variants and indels. MYOC is the most common POAG-related pathogenic gene (14); the reference sequence of the MYOC gene can be found in the NCBI gene databank (ID: 4653). After comparing with the reference sequence, a heterozygous MYOC missense mutation

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CLUSTAL O(1.2.4) multiple sequence alignment

Xenopus     ---------------MGSL-ALHIVVALWVAQGISGQFRRTSDGSGQCTYSFTVPSATEGG   45
Homo        MRFFCARCCSFGPEMPAVQLLLLACLVWDVGARTAQLRKANDQSGRCQYTFSVASPNESS   60
Macaca      ---------------MPAVQLLLLACLVWDVGARTAQLRKANDRSGRCQYTFSVASPNESS   46
Rattus      -MPSCAYCCSCGPKMPALQLLFLACLVWGMGARTAQFRKANDRSGRCQYTFTVASPSESS   59
Mus         ---------------MPALHLLFLACLVWGMGARTAQFRKANDRSGRCQYTFTVASPNESS   46
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Xenopus     CTEPAQAKAAIQDLQREVSVRHKEMEGLQIRLGLLEKLVNRLLGGEGLVKPSLGPQAGAD   105
Homo        CPEQSQAMSVIHNLQRDSSTQRLDLEATKARLSSLESLL-HQLTLDQAARPQE---TQEG   116
Macaca      CPEQSQAMSVIHNLQKDSSTQRLDLEATKARLSSLESLLHHQLTLDRAAGPQE---TPEG   103
Rattus      CPREDQAMSAIQDLQRDSSIQHADLESTKARVRSLESLL-HQMTSGGVTGTQE---VQEG   115
Mus         CPREDQAMSAIQDLQRDSSIQHADLESTKARVRSLESLL-HQMTLGRVTGTQE---AQEG   102
            * .  ** :.*::**:: * :: ::*. : *:  **.*: : :     .  .    .  .

Xenopus     LQLEVQKLRMEKDEWEGQRGSLEMAYADLLKEKESLEEEKQQLSQRLE-------------   153
Homo        LQRELGTLRRERDQLETQTRELETAYSNLLRDKSVLEEEKKRLRQENENLARRLESSSQE   176
Macaca      LQRELGTLRRERDQLETQTRELETAYSNLLRDKSVLEEEKKRLRQENENLARRLESSSQE   163
Rattus      LQGQLGALRRERDQLETQTRDLEVAYNNLLRDKSALEEEKRQLEQENKDLARRLEGSSQE   175
Mus         LQGQLGALRRERDQLETQTRDLEAAYNNLLRDKSALEEEKRQLEQENEDLARRLESSSEE   162
            ** ::  ** *:*: * *  .** ** :**::*. *****::* *. :

Xenopus     --RDVQGQCPQVPGSSRAQAADSSKTRVPDTSRVKDQQAPSRQVSRWGADPVGYQELKSE   211
Homo        VARLRRGQCPQTRDT--------------------ARAVPPGSREVSTWNLDTLAFQELKSE   218
Macaca      VARLRRGQCPQTRDT--------------------ARDVPPGSREVSTWNLDTLAFQELKSE   205
Rattus      VARLRRGQCPSTHHP-------------------SQDMLPGSREVSQWNLDTLAFQELKSE   217
Mus         VTRLRRGQCPSTQYP-------------------SQDMLPGSREVSQWNLDTLAFQELKSE   204
              *  :****..                       :     **:** *. *:.:******

Xenopus     LTALPASRMIPETQSTNHSSETIRADGACGELTWIGEPTTYRKADNIAGKYGVWMKDPKP   271
Homo        LTEVPASRILKESPSGYLR--SGEGDTGCGELVWVGEPLTLRTAETITGKYGVWMRDPKP   276
Macaca      LTEVPASRILKESPSGHLQ--SREGDNGCGELVWVGEPLTLRTAETITGKYGVWMRDPKP   263
Rattus      LTEVPASQILK-NQSGHPR--SKEGDKGCGVLMWVGEPVTLRTAETITGKYGVWMRDPKP   274
Mus         LTEVPASQILKENPSGRPR--SKEGDKGCGALVWVGEPVTLRTAETIAGKYGVWMRDPKP   262
            ** :***:::  . *      : ..* .** * *:*** * *.*:.*:********:****

Xenopus     LAPYTLDTVWRVNTVGADIRQVFEYENIDQLIKGYPGKVYVLPRSMESNGAVVYKGSLYY   331
Homo        TYPYTQETTWRIDTVGTDVRQVFEYDLISQFMQGYPSKVHILPRPLESTGAVVYSGSLYF   336
Macaca      TYPYTRETTWRIDTVGTDVRQVFEYDLISQFMQGYPSKVHILPRPLESTGAVVYSGSLYF   323
Rattus      THPYTQETTWRIDTVGTGIRQVFEYSQISQFEQGYPSKVHVLPQALESTGAVVYSGSLYF   334
Mus         THPYTQESTWRIDTVGTEIRQVFEYSQISQFEQGYPSKVHVLPRALESTGAVVYAGSLYF   322
              *** ::.**::***: :******. *.*: :***.**::**: :**.***** ****:

Xenopus     PRRKSRILVKYDFKTESVAVQREIPNAGYQGQYPYSWGGYTDIDLAVDEVGLWVIYSTEK   391
Homo        QGAESRTVIRYELNTETVKAEKEIPGAGYHGQFPYSWGGYTDIDLAVDEAGLWVIYSTDE   396
Macaca      QGAESRTVIRYELNTETVKAEKEIPGAGYHGQFPYSWGGYTDIDLAVDESGLWVIYSTDE   383
Rattus      QGAESRTVLRYELNTETVKAEKEIPGAGYHGQFPYAWGGYTDIDLAVDESGLWVIYSTEE   394
Mus         QGAESRTVVRYELDTETVKAEKEIPGAGYHGHFPYAWGGYTDIDLAVDESGLWVIYSTEE   382
               :** :::*::.**:* .::***.***:*::**:************** *********::

Xenopus     AKGSIVLSLLDSESLEVKQSWETQIRKQSVANAFMICGTLYTVGSYSSSSTTVNFAFDTS   451
Homo        AKGAIVLSKLNPENLELEQTWETNIRKQSVANAFIICGTLYTVSSYTSADATVNFAYDTG   456
Macaca      AKGAIVLSKLNPENLELEQTWETNIRKQSVANAFIICGTLYTVSSYSSADATVNFAYDTG   443
Rattus      TRGAIVLSKLNPENLELESTWETNIRKQSVANAFVICGILYTVSSYSSVHATINFAYDTN   454
Mus         AKGAIVLSKLNPANLELERTWETNIRKQSVANAFVICGILYTVSSYSSAHATVNFAYDTK   442
            ::*:**** *:  .**:: :***:**********:*** ****.**:*  :*:***:**

Xenopus     TGVQRPVGIPFKNQYGYASMIDYNPTEKKIYGWDNFNMVAYDVRLSKM      499
Homo        TGISKTLTIPFKNRYKYSSMIDYNPLEKKLFAWDNLNMVTYDIKLSKM      504
Macaca      TGISKTLTIPFKNRYKYSSMIDYNPLEKKLFAWDNLNMVTYDIKLSKM      491
Rattus      TGISKTLTIPFKNRYKYSSMVDYNPLERKLFAWDNFNMVTYDIKLSEM      502
Mus         TGTSKTLTIPFTNRYKYSSMIDYNPLERKLFAWDNFNMVTYDIKLLEM      490
            ** .: : ***.*:* *:**:**** *:*::.***:***:**::* :*
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: Striking similarity

. Weak similarity

* Identical

Figure 4. Multiple sequence alignment analysis showed that the myocilin protein has a high degree of conservation amongst different species. The red rectangle indicates the position of p.D208Y in humans.

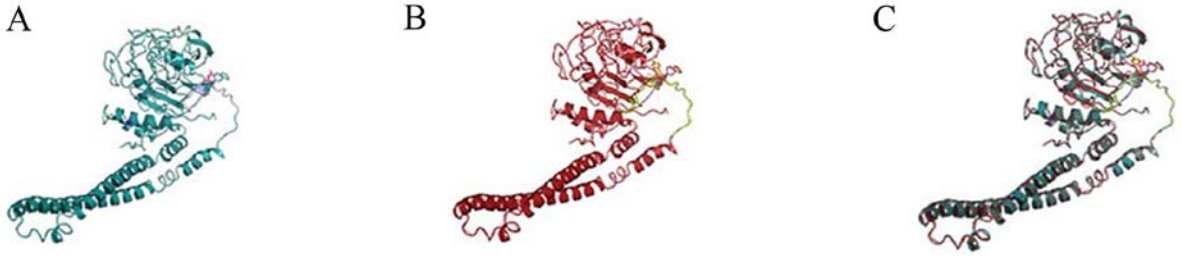

Figure 5. Three-dimensional structure of myocilin. (A) Mutant type. (B) Wild-type. (C) Overlay of mutant and wild-type.

(c.G622T: p.D208Y) was detected in the proband with POAG (II3; Fig. 3). The disease associated with the MYOC gene variation in the OMIM database is the primary open-angle glaucoma 1A type and is an autosomal dominant genetic (https://www.omim.org/entry/137750). The mutation (G to T) was located in the coding region of exon 2 at base 622, leading to the mutation of amino acid 208 of the encoded protein from aspartic acid to tyrosine. Multiple sequence alignment analyses indicated that this gene shows a high degree of conservation among different species (Fig. 4). This detected mutation locus was not found in several databases (ClinVar, 1000 genomes, ExAC and gnomAD). Protein function prediction software SIFT and MutationAssessor revealed that the mutation was deleterious. Based on protein structural and functional analysis with Phyre2, the aspartic acid was replaced by tyrosine due to the base mutation, but the secondary structure of the mutant protein did not change significantly compared with that of the wild type (Fig. 5). sA publicly available version of the database query HGMD, included in the reported MYOC gene variants, missense mutation rare benign variation (hgmd.cf.ac.uk/ac/index.php). Of the 20 family members, five individuals with POAG (I2, II1, II3, II5 and III5), one individual with OHT (III9) and three unaffected family members (III2, III6 and III8) were found to carry the mutation, whereas the remaining unaffected members did not harbor the mutation. Therefore, the mutation prevalence in this family was 45%. Three mutation carriers (III2, III6 and III8) did not exhibit elevated IOP values or glaucomatous defects (Table I).

Discussion

In the present study, a novel c.G622T: p.D208Y heterozygous mutation of the *MYOC* gene was identified in a Chinese family with a high incidence of POAG. In one previous study, a heterozygous variation of p.D208E in *MYOC* was found in one patient with OHT and two patients with POAG, although its relationship with POAG could not be clarified as it was also found in a 50-year-old individual without POAG (33). In the current pedigree, five patients with POAG and four unaffected individuals were found to harbor the p.D208Y mutation in *MYOC*. The incomplete penetrance suggests that, depending on the mode of inheritance, a DCM may cause related diseases in some individuals, but not in all DCM carriers. Age-related penetrance of POAG was defined as the ratio of the total number of POAG patients carrying the mutated gene to the total number of mutation carriers in a specific age group. The penetrance of the D208Y mutation was calculated as 0% in individuals <20 years old, 55.6% in patients 20-35 years old, 11.1% in patients 31-35 years old and 0% >45 years old. The penetrance of the majority of *MYOC* mutations is incomplete, which may be associated with age-related gene expression, environmental exposure time and gene-gene or gene-environment interactions (34). Compared with other evaluated *MYOC* mutations, the penetrance of the p.D208Y mutation in this family was low. In previous studies, the penetrance of *MYOC* p.Q368X was low, with 56.4% (35) of such patients developing glaucoma at 40 years of age and 78% developing glaucoma at 70 years of age (36). By contrast, *MYOC* p.P370L reached full penetrance by 27 years of age (37). It is possible therefore that the three non-affected carriers and one carrier with OHT of the mutated gene in the present study were too young to show signs of glaucoma. As aspartic acid is negatively charged and hydrophilic, whereas tyrosine is not charged and hydrophilic, the mutation of p.D208Y may change the local charge density of this protein. However, the exact function of MYOC and the physiological and pathological effects of MYOC in POAG remain unclear. For the cases in the present study, complete ophthalmological surveillance with optic disc photography, tonometry and automated perimetry every 6 months is recommended. This would facilitate further determination of whether the *MYOC* mutated gene is a pathogenic gene of POAG.

POAG is asymptomatic in its early stages and is often detected in the advanced stage with severe visual field damage and high IOP. Owing to the genetic characteristics of POAG, the association between its genotype and phenotype is of great significance for predicting the phenotypic variation range of specific mutations and for better diagnosis and treatment. Further studies on a larger number of families from different ethnic backgrounds are required to establish the genotypic-phenotypic associations for this blindness-causing disease. Although no pathogenic characteristics of p.D208Y were identified in the present study, the results expand the mutational spectrum of *MYOC*-induced POAG, which may be of clinical significance for disease prediction.

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Availability of data and materials

The data that support the findings of the present study are available from GenBank (accession no. MN335319), but restrictions apply to the availability of these data, which were used under license for the current study, and thus are not publicly available. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request and with permission from GenBank.

Authors' contributions

WF and FC conceived and designed the study. WF, FC and WZ conducted clinical examinations. WF, WL, CD and YG analyzed and interpreted the data. WF wrote the manuscript. WF, FC, CD and YG reviewed and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Union

Hospital (Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China). Written informed consent was obtained from all participants or their legal guardians, where required.

Patient consent for publication

Written informed consent for publication was obtained from all participants or their legal guardians, where required.

Competing interests

The authors declare that they have no competing interests.

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