·Basic Research·

Anticipation, anti-glaucoma drug treatment response and phenotype of a Chinese family with glaucoma caused by the Pro370Leu myocilin mutation

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Abstract

• AIM: To describe the anticipation and anti-glaucoma drugs response of a Chinese family with juvenile-onset open angle glaucoma (JOAG) caused by the Pro370Leu myocilin (MYOC) mutation.

• METHODS: Fifteen members of a three-generation Chinese family with JOAG were recruited to this study. They all underwent ophthalmic common examinations. Patients suspected to have JOAG got an assessment of visual field and optical coherence tomography. Intraocular pressures (IOPs) of four patients were measured at 8, 10, 12, 14, 17 o´clock respectively after using anti-glaucoma drugs. Mutation screening of all MYOC gene coding exons of the participants was performed by using direct sequencing of PCR products.

• RESULTS: Clinical examinations and pedigree analysis revealed eight family members were suffered from JOAG. Apparent genetics anticipation phenomenon was observed in this family. Their clinical features included elevated IOP of 35-55mmHg, loss of visual field, thinning of retinal nerve fiber layer, and glaucomatous optic disc damage. Noticeably, their intraocular pressure levels could be controlled within normal range at 8 and 10 o´clock by anti-glaucoma drugs, but their IOPs would elevate >21mmHg after 12 o´clock. Seven patients received trabeculectomy produced thin-walled, pale, and saccate filtering blebs maintaining lower intraocular pressure efficiently. Mutation screening indentified a heterozygous C→T missense mutation in the MYOC gene at position 1 109 in exon 3, corresponding to a substitution of a highly conserved proline to leucine at codon 370 in the olfactomedin domain of MYOC.

• CONCLUSION: The clinical characteristics of JOAG in this family were 1) genetics anticipation; 2) high IOP; 3) temporay response to anti-glaucoma drugs; 4) filtering surgery produced thin-walled and saccate filtering blebs, helping maintain lower IOP.

• **KEYWORDS:** phenotype; anticipation; anti-glaucoma drugs; myocilin

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INTRODUCTION

Glaucoma affects approximately 65 million people worldwide and is considered the second leading cause of blindness[1]. This disease comprises of a group of disorders characterized by a progressive defect of the visual field. Although unnecessary for diagnosis, elevated intraocular pressure (IOP) is often associated with glaucoma[2]. Primary open angle glaucoma (POAG) is the most common type and classified as adult-onset (AOAG) and juvenile-onset (JOAG) types, mainly distinguished by the age of onset of the disease. The initiation of the diseases between 3 and 35 years old is commonly considered as JOAG and it is categorized as POAG (adult onset) if it manifests after 35 years old[3]. Moreover, juvenile-onset POAG usually has a more severe disease phenotype compared to the adult onset type.

Genetic factors play an important role in the pathogenesis of glaucoma. Typically JOAG displayed an autosomal dominant pattern of inheritance with variable expressivity and incomplete penetrance[4]. About 30% to 56% of patients with glaucoma have a positive family history. First-degree

relatives of POAG patients are seven to ten times more likely to have POAG compared to the general population [5]. Up to now, 17 chromosomal loci have been linked to JOAG or POAG by linkage analysis, and were defined as GLC1A to GLC1Q[3], and association studies have identified many gene mutations that might contribute to JOAG. Three genes responsible for POAG have been identified: MYOC (coding for myocilin, GLC1A; OMIM 601652), OPTN (coding for optineurin, GLC1E; OMIM 602432), and WDR36 (coding for WD-repeat-containing protein 36, GLC1G; OMIM 609669) [6-8]. Though sequence variants in NTF4 (GLC1O) have been reported as a cause of POAG, it has not been confirmed in other studies[9-12].

Recently, genome-wide association studies (GWAS) for POAG have identified common variants in CDKN2B-AS and SIX/SIX6 regions in individuals of European ancestry, in TMCO1 and CDKN2BAS1 in an Australian cohort[13,14]. And then, TMCO1 was identified significance associations with IOP by GWAS and meta-analysis in 6,000 subjects of Eruopean ancestry collected in three datasets[15]. These results also confirm the common variants in multiple genomic regions in regulating IOP and/or glaucoma risk including: CDKN2BAS1, GAS7, CAV1/CAV2 and SIX1/SIX6[15].

Of these three loci identified, MYOC was the first gene linked with JOAG. In 1997, Stone *et al* [6] first reported a gene, named TIGR, associated with JOAG. TIGR lies in the GLC1A interval and is expressed in the ciliary body, trabecular meshwork, retina, sclera, choroids, and some extraocular tissues such as the mammary gland, thymus, testis, heart, and skeletal muscles[16]. TIGR was subsequently called MYOC or myocilin. To date, at least 226 different mutations in MYOC have been previously described, and in selected advanced POAG subgroups, the prevalence of MYOC mutations rose from 16% to 40%[17,18]. The P370L MYOC mutation was found in many JOAG patients, but their clinical characteristics and treatment options were not describe in detail.

In this manuscript, we describe the clinical features and the genetic causes in a three-generation Chinese family with JOAG carrying the P370L mutation in the MYOC gene. Genetic anticipation and short-term efficient response to anti-glaucoma treatment are noticeable clinical characteristics of this JOAG family.

SUBJECTS AND METHODS

Subjects Fifteen members of a three-generation Chinese family participated this study. They were recruited by the Zhongshan Ophthalmic Center (Sun Yat-sen University, Guangzhou, China). The Zhongshan Ophthalmic Center Joint Committee on Clinical Investigation approved this study. The authorized family members all consented to participate after being informed of the nature of the research. All participants underwent ophthalmic examinations that included measurements of best-corrected visual acuity (BCVA), intraocular pressure (IOP); evaluation of the iridocorneal angle; and ophthalmoscopy. They received an assessment of IOP using a Goldman tonometer. Patients suspected to have JOAG then got an assessment of visual field using automated threshold perimetry (Humphrey visual field analyzer) and an assessment of retinal thickness by optical coherence tomography (OCT).

Four of JOAG patients treated with anti-glaucoma drugs twice a day, including 0.2% brimonidine tartrate (Allergan) and brinzolamide (Alcon) eye drops. In addition, the proband (IV: 5) received 0.2% timolol eye drop additional twice daily. These anti-glaucoma drugs were used around 7:00 and 19:00 daily and 15min time interval for different eye drop. Their IOPs were measured at 8, 10, 12, 14, 17 o'clock respectively at least 15d post treatment. Seven patients (14 eyes) received MMC (mitomycin C, 0.286mg/mL,) trabeculectomy. The surgical sponges with MMC solution were placed the scleral bed under the scleral flap for 4min. The whole treated area was irrigated by 200mL BSS after removing all sponges.

Diagnosis of POAG will meet open anterior chamber angle and two of the following criteria: elevation of intraocular pressure (IOP>21mmHg) in at least one eye; characteristic glaucomatous optic nerve head changes and/or retinal nerve fiber layer (RNFL) changes; characteristic visual field defects. Elevated IOPs were not attributable to other causes. JOAG was purely defined by age (ages 10 to 35 years old). Two hundred Chinese individuals older than 40 years old, without a family history of ocular diseases, were also recruited as normal controls.

Genetic Analysis Genomic DNA was extracted from blood samples. Exon 1, 2, and 3 of the MYOC gene and their flanking intronic regions, were amplified by PCR using the primer pairs previously described in a published study [9]. PCR was performed by using a 20μL reaction containing 40ng of genomic DNA mixed with 10X buffer, 30pmol/L primers, 2.5mmol/L dNTP, and 0.1U Taq polymerase (TaKaRa Taq™ DR001A). PCR condition was as follows: 94℃ for 5min, followed by 35 cycles at 94℃ for 30s, at 55℃ for 30s, at 72℃ for 45s, and a final extension at 72℃ for 7min. The PCR products were then sequenced by the Applied Biosystems 370 DNA Analyzer (Applied Biosystems) according to the BigDye Terminator version 3.1 protocol.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Table 1 Clinical findings of eight members of a family with juvenile POAG

Pedigree (*n*)	Age (a)	Sex	Age of diag (a)	BCVAR/L	Max IOP (mmHg)R/L	C/D ratio R/L	Medical therapy	IOP after medicine	Trabeculotomy
II-1	76	F	NA	NLP/NLP	NA	NA	NA	NA	NO
III-1	56	M	25	0.5/0.4	NA	0.7/0.8	NO	NA	Yes
III-5	41	F	27	LP/1.0	55/45	1.0/0.9	S	NA	Yes
IV-1	21	M	18	0.9/1.0	51/55	0.5/0.5	S	NA	Yes
IV-3	19	F	19	0.9/1.0	52/18	0.5/0.3	S	Pos	Yes
IV-5	16	M	15	1.0/1.0	53/55	0.6/0.7	S	Pos	Yes
IV-6	15	F	14	1.0/1.0	35/42	0.4/0.5	S	Pos	Yes
IV-7	12	M	11	1.0/1.0	32/35	0.6/0.5	S	Pos	Yes

BCVA: Best-corrected visual acuity; Diag: Diagnosis; IOP: Intra-ocular pressure; C/D ratio: Cup/disc ratio; LP: Light perception; NLP: No light perception; R: Right eye; L: Left eye; NA: Unavailable; S: Sensitive; Pos: IOPs above 21mmHg.

Table 2 Average Intraocular Pressure values in different time phase by $\bar{x} \pm s$

Time (o'clock)	8	10	12	14	17
Average IOPs (mmHg)	15.63±1.31	16.63±0.85	27.5±3.19	33.75±2.18	35.13±3.57

Table 3 Intraocular pressure values in different time phase by *q*-test

Comparison among groups	$\bar{x}_A - \bar{x}_B$	$S_{\bar{x}_A - \bar{x}_B}$	a	*q*	*P*
8 and 17 o'clock	19.5	0.9744	5	20.01	$P<0.01$
8 and 14 o'clock	18.12	0.9744	4	18.60	$P<0.01$
8 and 12 o'clock	11.87	0.9744	3	12.18	$P<0.01$
8 and 10 o'clock	1.0	0.9744	2	1.03	$P>0.05$

RESULTS

Genetics Anticipation Of the fifteen subjects examined, eight were diagnosed with JOAG, including four females and four males (Figure 1). The pedigree revealed autosomal-dominant transmission of the disease. The clinical characteristics of the eight JOAG patients were summarized in Table 1. Ages of family members ranged from 12 to 76 years. The parents were diagnosed with JOAG at an average of 26 years old. The filial generations were diagnosed with JOAG at an average age of 16.6 years old, ten years earlier than their parents. Apparent genetics anticipation phenomenon was observed in this family. The severity of glaucoma phenotype in filial generation is increased. The parent generations (III) did not feel any uncomfortable until they lost peripheral vision, while filial generations (IV) complained about blurred vision and asthenopia at the beginning of glaucoma.

Short –term Control of IOP with Anti –glaucoma Medicines Four of JOAG patients received anti-glaucoma drug treatment for at least 15d have shown normal range of IOP at 8 and 10 o'clock. But their IOP would elevate >21mmHg after 12 o'clock, and even reach 40mmHg at 14 or 17 o'clock (Table 2, Figure 2). By *q* -test, IOPs increased significantly after 12 o'clock ($P<0.01$, Table 3). All the four patients got trabeculectomy at last.

Other Clinical Characterization Of the fifteen subjects examined, visual acuity ranged from no light perception (NLP) to 20/20. All structures of the anterior chamber were visible in all subjects examined. An elevated IOP of 46.36± 8.98mmHg (35-55mmHg) was present in all JOAG patients. Except for IV-6 (Figure 1), she was diagnosed as glaucoma in the early stage, the patients all had visual-field loss characteristic of glaucoma including central scotoma, tubular vision, and temporal-sideisland. Thinning of the retinal nerve fiber layer and glaucomatous optic disc damage were observed in all patients through OCT.

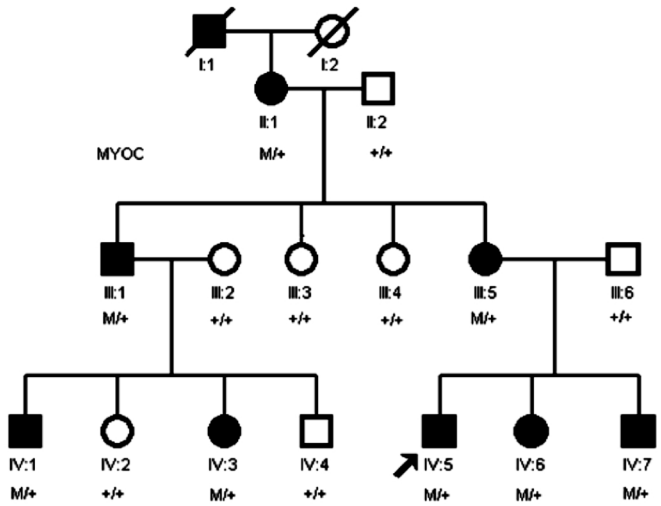

Figure 1 Pedigree of the study family shows an autosomal dominant pattern of juvenile primary open angle glaucoma. Individuals are identified by generation and individual numbers +: normal MYOC gene; M: mutant MYOC gene (Pre370Leu).

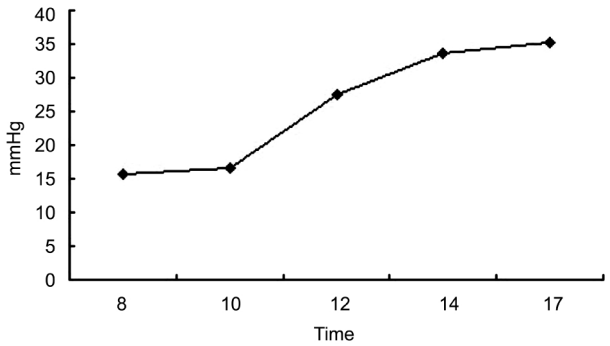

Figure 2 Average IOPs of four JOAG patients at 8, 10, 12, 14 and 17 o´clock respectively. IOP level could be controlled within normal range at 8 and 10 o´clock, but would elevate > 21mmHg after 12 o´clock.

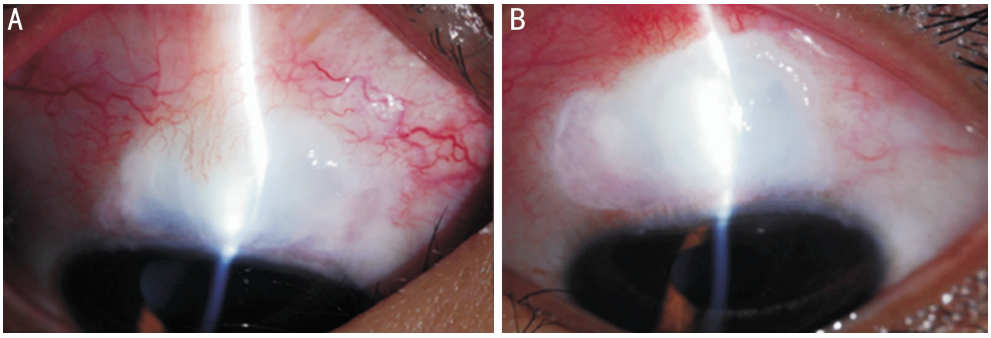

Figure 3 Eye images of a proband showing thin-walled, pale, and saccate filtering bleb after combined trabeculectomy A: Right eye; B: Left eye.

Seven patients (14 eyes) received surgeries trabeculectomy. After the operation, the mean IOPs of the patients were 13.4±3.67mmHg within 3 months. IV: 4 (Figure 1) had an IOP of 5mmHg even at the last examination. None of the patients required any anti-glaucoma medications after the operation. The filtering blebs were thin-walled, pale, and saccate (Figure 3).

Mutation Detection Direct sequencing revealed a heterozygous C→T missense mutation at position 1 109 in exon 3 of the MYOC gene (Figure 4), which causes the substitution of a highly conserved proline to leucine at codon 370 in the olfactomedin domain of MYOC. The Pro370Leu change cosegregated with the phenotype of POAG in the study family and was not found in the 200 control participants. Therefore, the mutation was inherited in an autosomal dominant manner. A heterozygous C→T missense mutation at position 1 058 in exon 3 of the MYOC gene was found by sequecing in VI:1 patient (Figure 5). This mutation causes a change of threonine to isoleucine in condon 353 in the same domain of Pro370Leu, but Thr353Ile mutation did not cosegregated with the phenotype of POAG. Moreover, this change has been identified in 2 normal control participants, which suggests it is non-disease causing mutation.

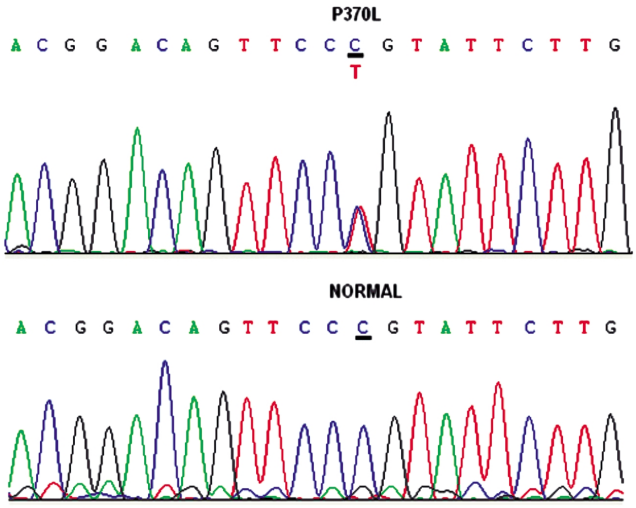

Figure 4 DNA tracing from a segment of exon 3 in MYOC in an affected individual (at top), and in a control subject (at bottom). Sequencing revealed a heterozygous C (T missense mutation at position 1 109 of the MYOC gene.

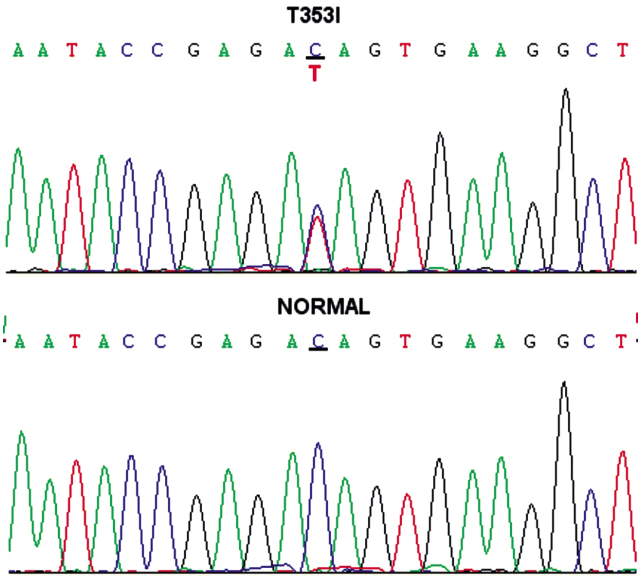

Figure 5 DNA tracing from a segment of exon 3 in MYOC in IV: 1 patient (at top), and in other patients (at bottom). Sequencing revealed a heterozygous C (T missense mutation at position 1 058 of the MYOC gene.

DISCUSSION

We have sequenced all exons of MYOC gene in a JOAG family and 200 unaffected controls in the same region of Guangdong, China. Our findings suggest that Pro370Leu MYOC mutation is responsible for JOAG in this family. Clinical phynotype of glaucoma associated with Pro370Leu is characteristic, including earlier onset, different effects for anti-glaucoma drugs and lower IOP after filtering surgery.

The pathogenesis of MYOC Pro370Leu mutation is still unclear. Myocilin is a secreted glycoprotein with an unidentified function. This glycoprotein is organized into three modular domains each of which is encoded by one of its three exons. These exons consist of an N-terminal leucine zipper-like domain, a central linker region, and a C-terminal olfactomedin-like (OLF) domain [19]. The P370L mutation occurs in the highly conserved olfactomedin-like domain.

The disease-causing myocilin forms large extracellular aggregates linked by disulfide bonds [20,21]. Three reported OLF disease variants, A246T, G246R, and A445V, exhibit properties indistinguishable from those of the WT OLF. However, an increased aggregation propensity *in vitro* in the OLF disease variants relative to that of the WT OLF suggests that biophysical factors other than thermal stability such as kinetics and unfolding pathways may be involved in myocilin glaucoma pathogenesis [22]. The P370L mutation distributed in populations worldwide including Japanese, French, English, North American, Indian, Chinese, Canadian, and Spanish patients [2]. The wide distribution of this mutation could be the result of independent de novo events in different ethnic backgrounds rather than the result of a founder effect since this transition affects a CpG dinucleotide that could be a mutation hotspot[23].

Thr353Ile mutation occurs in a residue located in the conserved olfactomedin homology region of the myocilin protein, but it was found in 1 patient and 2 normal individuals. Though VI:1 patient had two missense mutations, his POAG phynotype is not different from other patients. These evidences suggested Thr353Ile is not a disease-causing mutation, though it had been found in some POAG and primary angle-closure glaucoma (PACG) patients[24,25]. More studies need to verify it.

With our study, genetic anticipation is very remarkable in this JOAG family. Genetic anticipation is a phenomenon in which the age of onset of a disorder is reduced and/or the severity of the phenotype is increased in successive generation. The molecular mechanisms underlying anticipation are unknown, but it has been typically associated to trinucleotide repeat expansions in several genetic diseases[26]. Anticipation in glaucoma first was described between the forth and fifth generations of two glaucoma families in 1979[27]. Since then, there were few reports about anticipation of glaucoma. In our research, the onset of POAG in the filial generation (IV) was at an average age of 16.6 years old, and about ten years younger than that in the parental generations (III), at an average of 26 years old. Genetic anticipation should be the major reason for this earlier onset in the younger generation.

An unstable dynamic mutation expands with each generation has been regarded as a possible mechanism of anticipation in 40 diseases [28]. In addition, telomere length attrition has also been proposed as another mechanism of anticipation in different inherited diseases, such as dyskeratosis congenital[29]. Although, the mechanism of anticipation in JOAG was not discovered yet, we believe that further studies of these JOAG families will provide valuable insights into the genetic anticipation.

In this study, we identified the P370L mutation in a family with a higher IOP average of 44.29±5.38mmHg (35-55mmHg). It is higher than another Chinese JOAG family. In that family, mild IOPs (average value of 34.18±2.93mmHg) were measured in eight JOAG patients with the P370L mutation, but glaucoma optic disc cupping and visual field defects observed in all patients[2].

There are several anti-glaucoma drugs current available, including brimonidine tartrate (an alpha-adrenergic agonist) brinzolamide drop (a carbonic anhydrase inhibitor) and timolol drop (a beta-blocker). They all work by decreasing aqueous humour production and reducing pressure in side the eye. Several studies indicated that the IOPs of POAG patients with the P370L mutation could not be controlled with these anti-glaucoma medications [30]. There were not reports about the mutiple IOPs measurements of JOAG patients caused by P370L mutation during a day. We found lowing IOPs in 8 and 10 o'clock, which demonstrated these eye drops worked and aqueous fluid production decreasing. IOPs increased about 12 o'clock, which indicated the effects of eye drops are reducing or aqueous produce increasing. The patients promised that they used these eye drops with exact schedule, so increasing aqueous produce may be responsible for high IOP in afternoon. However, most researches revealed that insoluble aggregates of misfolded mutant myocilin caused the death of trabecular meshwork, and therefore impairing the aqueous humor ourflow [31]. We did not know the glaucoma pathogenesis caused by P370L, but we can speculate that changed myocilin protein effects not only aqueous outflow but also produce. The medicines are not enough to inhibit the continuous aqueous humour production. From the IOPs curves of patients (Figure 2), we can look at P370L glaucoma from a new point. Excessive production of aqueous humor may be the main reason for MYOC mutation glaucoma. More research needs to be done.

Anti-glaucoma surgeries served as the last-line of treatment [30,32,33]. MMC trabeculectomy was selected as the operation method. In high pressure glaucoma of young patients, success of this operation was achieved in 91% at 5 years; however, the complication rate was high [34]. After the operation, the mean IOPs of the patients were maintained within the lower range. IV: 4 (Figure 1) had an IOP of 5mmHg even at the last examination. Though we do not know the exact reason, MMC maybe play an important role for lower IOPs. As we observed, the filtering blebs of all patients in this family were thin-walled, pale, and saccate after operation.

Thin-walled and avascular blebs are more common with MMC trabeculectomy when the conjunctival flap is

limbus-based (90%) than fornix-based (21%), particularly in pediatric cases and in young adults [35]. Thin-walled blebs are prone to late leakage and infection. Bleb-related endophthalmitis is relatively common after MMC trabeculectomy [36]. Both prolonged hypotony and hypotony maculopathy are common after MMC trabeculectomy. The reported frequency of prolonged hypotony was 21.3% [37]. Fortunately IV: 4 patient (Figure 1) did not suffer from macular edema, even if the IOP<5mmHg for several months.

Based on the clinical characteristics of the JOAG family, there appears to be both intra- and inter-family variations in the phenotype associated with this mutation. The findings in this study enrich what is currently known about the clinical phenotypes of the MYOC P370L mutation and further supports MYOC being involved in the development of POAG. By the five time daily IOP curve, we may suppose abnormal myocilin protein increase aqueous that could not been completely inhibited by anti-glaucoma medicines.

In conclusion, the clinical characteristics of JOAG patients in the family were 1) genetic anticipation: the offspring generation were diagnosed with JOAG ten years earlier than their parents and tended to have a more severe pathogenetic condition; 2) anti-glaucoma medical treatments were effective, but could not maintain normal IOPs for all day long; 3) all patients suffered from high IOPs (35-55mmHg); 4) after filtering surgery, the filtering blebs were thin-walled, saccate, and lower IOP was able to maintain.

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