PRIMARY OPEN-ANGLE GLAUCOMA DUE TO MUTATIONS IN THE *MYOC* GENE

Vergaro A.[1,2], Rezková L.[1], Fichtl M.[1], Jedličková J.[2], Ďuďáková Ľ.[2], Růžičková E.[1], Lišková P.[1,2]

[1]Department of Ophthalmology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic
[2]Research Unit for Rare Diseases, Department of Pediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic

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MUDr. Andrea Vergaro
Oční klinika 1. LF UK a VFN
v Praze
U Nemocnice 2
12808 Praha 2
E-mail: andrea.vergaro@vfn.cz

SUMMARY

Aim: Mutations in the myocilin gene (*MYOC*) cause trabecular dysfunction and thus are involved in the pathogenesis of primary open-angle glaucoma (POAG). The aim of this study was to characterize and describe the clinical findings in two Czech families with POAG due to pathogenic variants in the *MYOC* gene.
Material and methods: Members of the two families affected by POAG underwent complete ophthalmological examination. In the proband from the first family, a direct sequencing of the three most frequent mutations in the *MYOC* gene was performed, and in the proband from the second family, an exome sequencing was performed. Other family members underwent targeted tests using direct sequencing.
Results: In total, 10 individuals diagnosed with POAG aged 20–70 years (mean 32.2 years, SD ±10.9 years) were examined. Eight of them showed advanced glaucomatous neuropathy with severe changes in the retinal nerve fiber layer. Clinical signs of POAG were present in six individuals in the third decade of life already; another four developed POAG during the fourth decade of life. Eight out of 10 patients had to undergo filtration surgery. Surgery was performed within 1 to 7 years of diagnosis, but mostly was performed within 2 years of glaucoma diagnosis. In the first family, *MYOC* variant c.1099G>A p.(Gly367Arg) was shown in the affected family members; in the second family *MYOC* variant c.1440C>A p.(Asn480Lys), both in heterozygous state. The changes were assessed as pathogenic.
Conclusion: Our study is the first to describe mutations in the *MYOC* gene causing POAG in Czech patients. Genetic testing may be recommended for this diagnosis, especially in individuals with early presentation and a positive family history. Carriers of pathogenic variants of the *MYOC* gene have a lifetime risk of developing POAG of more than 50% and the course of their disease is often more aggressive, requiring surgical intervention to permanently control the intraocular pressure.
Key words: Myocilin, mutation, primary open angle glaucoma, juvenile glaucoma

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INTRODUCTION

Glaucoma is a neurodegenerative disease that is one of the leading causes of severe irreversible vision loss worldwide [1]. Primary open-angle glaucoma (POAG) is the most common form. The etiopathogenesis of POAG has not yet been fully elucidated. However, a significant influence of genetic factors has been demonstrated [2]. In patients with early manifestation up to 40 years of age, Mendelian type of transmission should be considered, i.e. disease onset is caused by a single gene alteration, whereas in individuals with later manifestation, a polygenic type of inheritance is most often involved [3].

Mutations in the *MYOC* gene are the most common cause of the monogenic type of glaucoma, accounting for 10 % of all juvenile open-angle glaucomas and 2–4 % of adult-onset POAG, according to the literature data [4]. To date, more than 70 pathogenic variants have been identified in the *MYOC* gene [5]. *MYOC* encodes a myocilin protein, whose physiological function has not yet been fully clarified. Myocilin is intracellularly proteolytically degraded and its fragments are then released into the extracellular matrix. By interacting with intercellular proteins, they participate in the homeostasis of the trabecular meshwork. Although myocilin is found in many tissues, mutations in *MYOC* cause solely diseases of the

eye [4]. The main hypothesis of the pathophysiological mechanisms involves apoptotic cell loss in the trabecular meshwork, leading to its dysfunction and subsequent elevation of intraocular pressure (IOP) [5].

The aim of this study was to describe the clinical signs and symptoms of POAG associated with mutations in the *MYOC* gene in two families of Czech origin.

MATERIAL AND METHODS

The research described in this study was approved by the Ethics Committee of the General University Hospital in Prague and all participants or their legal representatives signed an informed consent.

Subjects from two mutually unrelated families with a history of POAG were referred to our Department for a specialist consultation. The primary reason for the referral was uncontrolled intraocular pressure with maximal pharmacological treatment. The complete ophthalmological workup included assessment of best corrected visual acuity on Snellen charts with values in the decimal system, intraocular pressure measurement using Goldmann applanation tonometry, visual field examination using static perimeter Medmont M700 (Medmont International, Nunawading, Australia) and spectral-domain optic coherence tomography (SD-OCT) - Spectralis (Heidelberg Engineering GmbH, Heidelberg, Germany). Apart from macula section, also peripapillary retinal nerve fiber layer (RNFL) thickness, using circular scans located 3.5 to 3.6 mm from the center of the optic nerve head, was assessed. The physiological range of total RNFL thickness was considered to be 97.41 ±11.73 µm in children and 97.52 ±9.83 µm in adults [6]. Other family members were examined in the same manner.

Furthermore, a detailed genealogical analysis was carried out, including plotting the pedigree of both families and molecular genetic testing. DNA was extracted from a venous blood sample, using the Gentra Puregene Blood Kit (Qiagen GmbH, Hilden, Germany), or from a saliva sample, using the Oragene (Oragene OG-300, DNA Genotek, Canada). Sanger sequencing of three PCR products of the *MYOC* gene (reference sequence NM_000261.2)

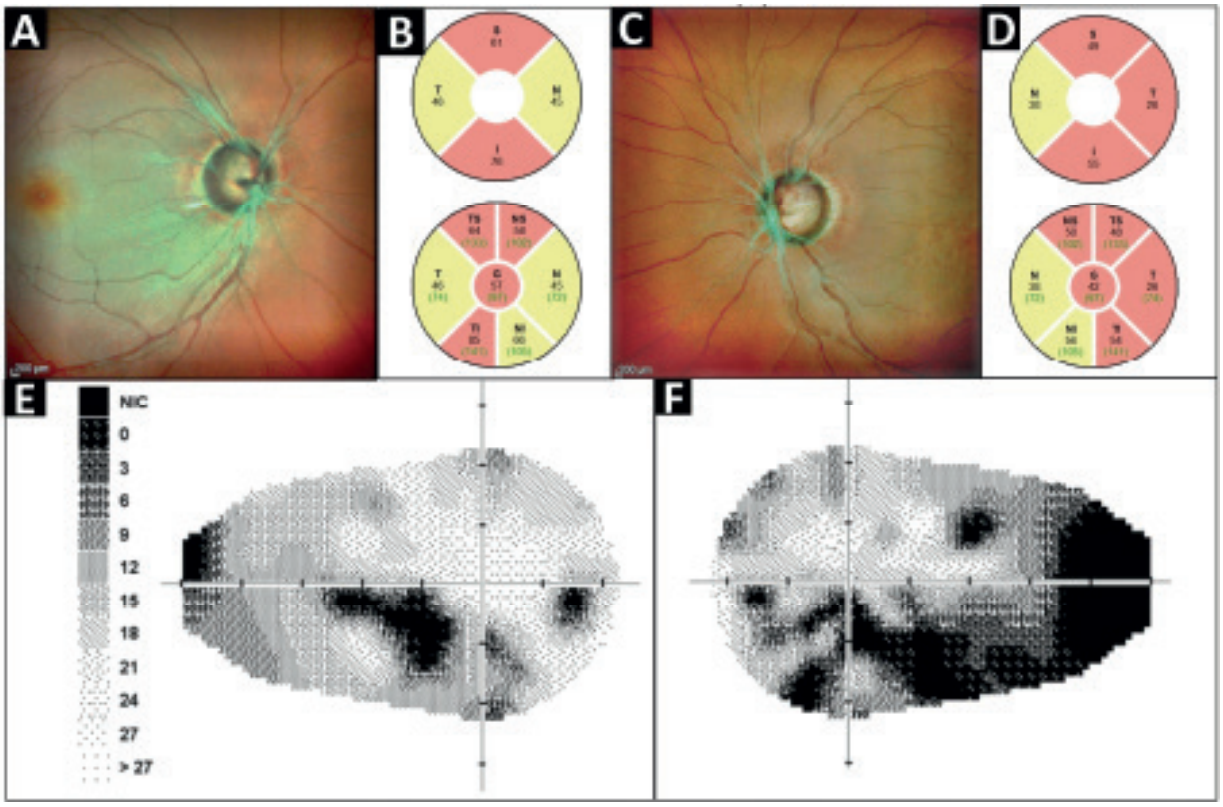

Figure 1. Clinical findings in proband from family 1 at time of the first examination at our department. SD-OCT MultiColor fundus photography of the right (**A**) and left eye (**C**) showing glaucomatous cupping in both eyes, measurements of RNFL thickness in right eye (**B**) and left eye (**D**) demonstrating significant RNFL thinning to values located respectively under the 5th (yellow sectors) and 1th (red sectors) percentile of the normative database, Automated visual field examination (Glaucoma program) of the right eye reporting absolute scotomas located within the inferior Bjerrum's area (**E**) and of the left eye (**F**) where visual field defects are located only partly within the Bjerrum's area although strictly correlate with the RNFL thinning

RNFL – peripapillary nerve fiber layer

covering its third exon, which, according to the literature, contains the majority of pathogenic mutations, was performed in a proband from Family 1 [4]. DNA was amplified, using primers designed by us (sequences available on request in the laboratory) and sequenced on an ABI PRISM 3100 Genetic Analyzer capillary sequencer (Applied Biosystems, Foster City, California, USA). Exome sequencing was performed in a proband from Family 2. Sequencing libraries were generated by the SureSelect Human All Exome V6 capture kit (Agilent, Santa Clara, California, USA). Libraries were sequenced with the Novaseq6000 device (Illumina, San Diego, California, USA). Sanger sequencing was also used for targeted testing of the presence of the detected mutations in other relatives. The effect of the detected variants was determined, based on the criteria of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [7].

RESULTS

The proband from Family 1 was diagnosed with POAG at the age of 42 years and was referred to our Department at the age of 49 years for elevation of intraocular pressure, unresponsive to a combination of three topical anti-glaucoma medications. His clinical examination showed glaucomatous neuropathy with asymmetric vertical excavation of the optic nerve head (Figure 1A, C). On SD-OCT examination, there was a significant decrease in RNFL thickness, with total value of 57 µm on the right and 42 µm on the left (Figure 1B, D). Using automated perimetry, absolute scotomas corresponding to segments with decreased RNFL thickness were identified bilaterally. Maximum changes of the visual field were in the inferior Bjerrum area (Figure 1E, F). Gonioscopy showed open anterior chamber angle.

Bilateral trabeculectomy with Ologen implant (Aeon Astron Europe BV, Leiden, The Netherlands) was subsequently performed in our Department. After surgery, intraocular pressure in the right eye remained in the range of 8 to 16 mmHg without any anti-glaucoma mediations in the six-month follow-up period after surgery. In the left eye, intraocular pressure increased to 24 mmHg one year after the filtration surgery, and the IOP decreased only after initiation of treatment using a fixed combination of brinzolamide and brimonidine.

Furthermore, three males from Family 1 were examined. They were diagnosed with open-angle glaucoma at a mean age of 39.25 years (range 22–47). Thus, three of the four family members were diagnosed with POAG during the fourth decade of life and one in the third decade. Consistent with this diagnosis, we detected significant bilateral RNFL atrophy in all these individuals (Table 1). At the time of the first examination in our Department, two of the patients (Figure 2A, I:1, II:1) had elevated intraocular pressure, despite treatment using three to four anti-glau-

Table 1. Clinical findings in the heterozygote carriers of pathogenic MYOC gene variants from both families examined in our study. Patients are coded according to the provided pedigrees (Figure 2)

Family/ subject	Age at diagnosis	Age at first examination	IOP (mmHg)		RNFL (µm)		Surgical procedures/age at time of surgery	
			RE	LE	RE	LE	RE	LE
1/I:1	47	70	17*	37*	ND	67 (67 y.)	TE/48	TE+Express/48
1/II:1	46	47	26*	24*	46	62	TE/47	TE+Ologen/47
1/II:3	42	49	24*	21*	57	42	TE/49	TE/48
1/III:1	22	24	11*	13*	35 (29 y.)	39 (29 y.)	TE/25	TE/25
2/IV:4	25	55	12	15	39	30	TE/25	TE/25
2/IV:10	42	46	21*	55*	97	54	TE+MMC/46	TE+MMC/46
2/V:3	29	29	34*	> 50	47	ND	Express+Ologen/29 Express+Ologen/30	CPC/29
2/V:5	28	30	18*	19*	104	106	None	None
2/V:6	21	23	36*	32*	118	116	DS+Healaflow/38	Express P200+Ologen/38
2/V:7	20	20	24	24	99	98	None	None

**IOP measured while on three or more IOP lowering medications*
Age expressed in years, RNFL values represent global peripapillary RNFL thickness, age at time of examination is provided (in brackets) for patients who were tested at a time different from the first examination
IOP – intraocular pressure, CPC – cyclophotocoagulation surgery, MMC – 0.04% mitomycin C, RE – Right eye, OL – Left eye, RNFL – peripapillary nerve fiber layer, TE – trabeculectomy, DS – Deep sclerectomy, ND – No data available

coma mediations. Finally, filtration surgery was necessary to control the IOP in all four affected family members. Basic clinical data and surgeries performed on the examined individuals from Family 1 are summarized in Table 1.

In the proband from Family 1 (II:3, Figure 2A), a mutation c.1099G>A p.(Gly367Arg) in heterozygous state was detected in the *MYOC* gene, which has been repeatedly described in association with POAG [8–12]. The variant was classified as pathogenic, which was consistent with the Clinvar database (https://www.ncbi.nlm.nih.gov/clinvar/), where it is also interpreted as causal under the identifier VCV000007952.2. This mutation was found in all family members diagnosed with POAG, i.e. in males: I:1, II:1, II:3, III:1. On the contrary, it was not found in female III:2, who had no signs and symptoms of glaucoma (Figure 2A).

The proband from Family 2 (IV:10, Figure 2B) was 46 years old at the time of the first examination in our Department. He was referred for bilateral asymmetric elevation of IOP that was refractory to maximal anti-glaucoma therapy: a combination of three topical anti-glaucoma medications and systemic acetazolamide 250 mg TID. He was diagnosed with POAG at the age of 42 years. In accordance with asymmetric IOP elevation, also other clinical examination methods showed significant asymmetry between the two eyes; concentric narrowing of the visual field to 10 degrees and a significant thinning of RNFL thickness in the worse left eye in contrast to normal visual field and the absence of significant glaucomatous cupping of the optic nerve head and physiological RNFL curve in the right eye. (Figure 3). The patient underwent immediate bilateral trabeculectomy with intraoperative use of mitomycin C 0.04 %. During the course of the follow-up period of one year, there was no elevation of IOP or progression of visual filed scotomas in either eye. OCT examination of the RNFL in the right eye showed no progression, while in the left eye a further significant thinning in the RNFL layer was identified in all quadrants, which may be explained by the advanced stage of the disease at the time of surgery and the very high preoperative IOP.

Overall, filtration surgery was necessary to achieve IOP control in four of the six family members examined in our Department (Figure 1). The reason for referral was the elevation of IOP unresponsive to three or more topical anti-glaucoma medications in four patients and the presence of a family history of the disease in two patients. Four of the six patients were diagnosed with POAG during the third decade of life and the youngest patient was 21 years old. In this patient, filtration surgery was

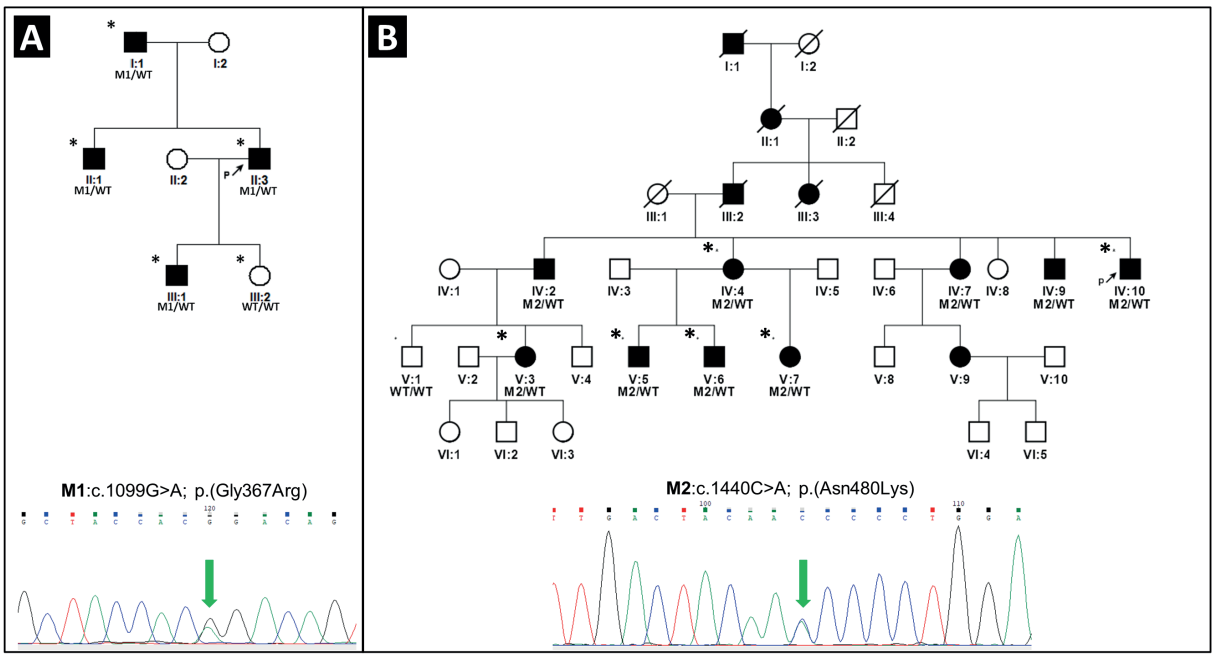

Figure 2. Pedigrees of the two families with POAG associated with pathogenic variants in the *MYOC* gene. Family 1 harboring variant M1 c.1099G>A in heterozygote state detected in four affected subjects (**A**). Family 2 harboring variant M2 c.1440C>A in heterozygous state identified in nine affected subjects (**B**). Probands are marked with a black arrow, subjects examined by at least one of the authors are marked with an asterisk, black circles represent female subjects with POAG and black squares represent male subjects affected with POAG. Results of genetic tests for variants of the *MYOC* gene are shown only for subjects who underwent molecular genetics testing. All carriers of pathogenic variants in the *MYOC* gene confirmed by genetic test showed clinical signs and symptoms of POAG (POAG diagnosis in the subjects, who were not examined at out department [i.e. IV:2, IV:7, IV:9] was established by a different healthcare provider and confirmed by reviewing available clinical documentation)
POAG – primary open angle glaucoma WT – wild type

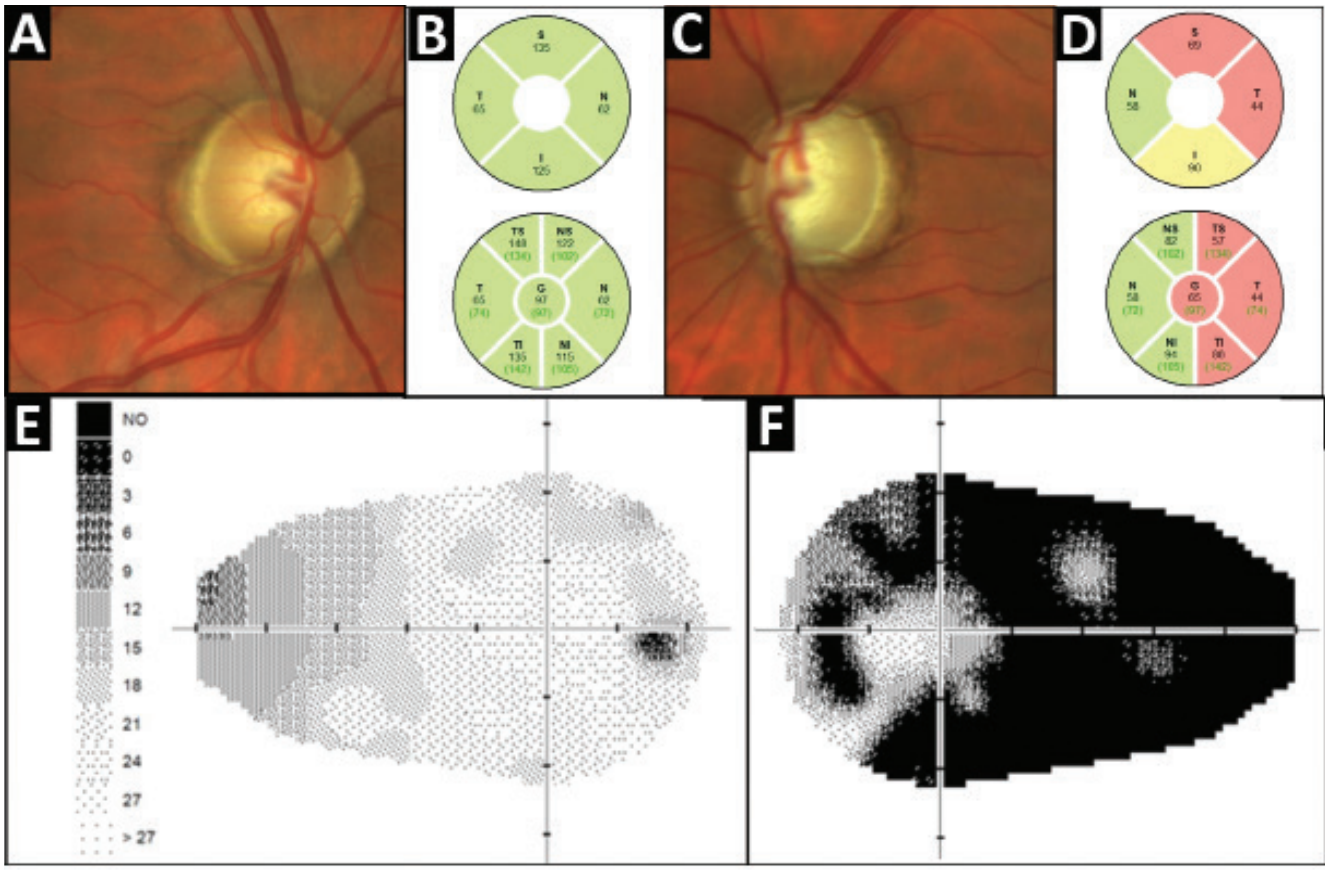

Figure 3. Clinical findings in proband from family 2 at time of the first examination at our department. Color fundus photography of the right (**A**) and left eye (**C**) showing striking asymmetry in optic disc cupping between the two eyes. Automated visual field examination (Glaucoma program) with corresponding asymmetrical visual field damage, strictly correlating with RNFL thickness measurements in the right eye (**B**) and left eye (**D**)

RNFL – peripapillary nerve fiber layer

performed bilaterally after two years of follow-up. Female patient V:7 (Figure 2B) was diagnosed with ocular hypertension at 20 years of age and repeated elevations of IOP above 30 mmHg and a positive family history for glaucoma, leading to the initiation of anti-glaucoma monotherapy using a prostaglandin analog.

In the proband from Family 2, a pathogenic variant c.1440C>A; p.(Asn480Lys) in heterozygous state was identified in the *MYOC* gene. This mutation was also detected in other family members IV:2, IV:4, IV:7, IV:9, V:3, V:5, V:6 a V:7 (Figure 2B). Variant c.1440C>A has also been repeatedly described in association with the occurrence of POAG [13–16]. We classified the variant as pathogenic, as did the Clinvar database, where it is interpreted as causal under the identifier VCV000007951.1.

DISCUSSION

In our study, we have described for the first time causal mutations in the *MYOC* gene and related clinical findings in patients of Czech descent.

Patients suffering from POAG caused by mutations in the *MYOC* gene usually have an elevation of IOP, which is refractory to pharmacological or laser treatment alone, and in most cases it is necessary to proceed to surgical treatment, most often by trabeculectomy.

POAG caused by *MYOC* gene mutations exhibits incomplete penetrance, which means that only some carriers of pathogenic mutations develop clinically detectable glaucoma disease during their lifetime. The likelihood of developing POAG varies according to the specific variant and increases with age, with more severe mutations leading to POAG onset more frequently and earlier, e.g. p.(Thr377Met), which causes disease in 88% of carriers by age 30 [17]. It should be emphasized that there is no known pathogenic variant in the *MYOC* gene that is completely penetrant at the age of 30 years.

The p.(Gly367Arg) mutation found in members of Family 1 has been repeatedly described in patients with POAG in different populations. It is estimated that its carriers have a more than 90 % risk of developing glaucoma by age 50 [8–11,18]. The diagnosis of POAG was usually

made during the fourth decade of life in carriers of this pathogenic variant. However, the lowest recorded age at presentation was 12 years [9]. More than 50 % of patients with POAG due to p.(Gly367Arg) must undergo trabeculectomy to achieve IOP control [8–11,18]. The data in the literature are consistent with our observations. The mean age of diagnosis in Family 1 was 39.25 years (SD ±10.13 years), with a mean IOP at first examination of 21.63 mmHg (SD ±7.71 mmHg), despite the use of a combination of three or more anti-glaucoma medications in all four patients examined. The highest recorded IOP was 37 mmHg. All affected family members required trabeculectomy to control IOP, and all had significant glaucomatous changes in the visual field and a significant decrease in RNFL at the time of the first examination.

The pathogenic variant p.(Asn480Lys) found in the second family is rarer; it has so far only been found in a few families in France, the Netherlands, Malaysia, India and Peru [13,15,16,19,20]. The average age of diagnosis in the published articles was less than 40 years and the lowest age of diagnosis was 10 years [19]. The percentage of patients requiring filtration surgery for IOP control ranged from 28 to 70 % [13,15,16]. Consistent with data from the literature, the mean age of diagnosis of POAG in Family 2 was 27.5 years (SD ±7.97 years), and 66 % of patients required filtration surgery for IOP control, all bilaterally within 2 years of POAG diagnosis. Moreover, it was necessary to perform a cyclodestructive procedure in one patient. Given the very high intraocular pressure unresponsive to three or more anti-glaucoma medications and the family history, filtration surgery was performed in two individuals younger than 30 years, even though they did not show signs of glaucomatous neuropathy.

CONCLUSION

This study demonstrates for the first time the role of *MYOC* gene mutations in glaucoma disease in the Czech population. The results of our study showed that *MYOC* mutations c.1099G>A and c.1440C>A in the heterozygous state are the cause of POAG in the studied families. POAG due to mutations in the *MYOC* gene is an autosomal dominant disease, with a 50 % risk of transmission to the next generation. Consistent with the published literature, we confirm the severity of the clinical course of POAG caused by *MYOC* mutations, including the high likelihood of the need for filtration surgery to achieve IOP control. Identification of mutation carriers in families allows for the individual adjustment of the schedule of prophylactic ophthalmological follow-up examinations aimed at early detection of the onset and progression of glaucoma disease, and for consideration of performing filtration surgery at early signs of POAG before the onset of neuropathy.

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