Association of POAG Risk Factors and the Thr377Met *MYOC* Mutation in an Isolated Greek Population

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Purpose. To characterize the *MYOC* genotype correlation with phenotypes in an isolated Greek population with a high incidence of glaucoma.

Methods. Five hundred thirty-one villagers were enrolled in the study. Participants underwent a comprehensive ophthalmic examination. All three exons of myocilin were bidirectionally sequenced. Power calculations and measured genotype analysis was conducted using the genetic variance analysis program, SOLAR version 4.2, to account for the relatedness between individuals.

Results. The participants, 376 of whom were linked in a single 11-generation pedigree, ranged in age from 10 to 95 years with a mean age of 49. Sixty-five individuals had POAG, and 27 of those carried the Thr377Met *MYOC* mutation. Both peak intraocular pressure and vertical cup-to disratio were significantly associated with the *MYOC* Thr377Met variant ($P = 9 \times 10^{-14}$ and $P = 9 \times 10^{-8}$, respectively), whereas central corneal thickness showed no significant association ($P < 0.7$).

Conclusions. This village had a high frequency of glaucoma, with 12% of the participants aged 10 to 95 years having the disease. In this cohort, the Thr377Met *MYOC* mutation was significantly associated with both high intraocular pressures and high vertical cup-to-disc ratios. No association was found with central corneal thickness. (*Invest Ophthalmol Vis Sci.* 2010;51:3055–3060) DOI:10.1167/iovs.09-4652

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Primary open-angle glaucoma (POAG) is a chronic optic neuropathy that is a significant cause of blindness throughout the world.[1,2] By 2020, it is predicted that close to 60 million people will have POAG.[2] The clinical diagnosis of POAG is based on a combination of findings, including specific changes in the appearance of the optic nerve head, characteristic visual field loss, and a slow and generally asymptomatic progression.[3] Risk factors for glaucoma include elevated intraocular pressure (IOP), central cornea thickness (CCT), advanced age, race, and family history.[4] Although a uniform definition is often applied to glaucoma, the disease is extremely heterogeneous and probably results from a variety of causal mechanisms.

More than 20 genetic loci for POAG have been identified by genetic linkage and association studies.[5–11] Of these, two in particular have been reported in the Greek POAG population, *MYOC* (*GLC1A*)—that is, the Thr377Met variant and the *GLC1C* locus, which was found specifically in a family in Epirus in the northwestern part of Greece.[12,13] In a previous study we showed that the Thr377Met *MYOC* mutation was particularly prevalent in Taxiarchis, a Greek village in northeastern Greece.[14] This small village is unique in that it is isolated, and most of the current population has not been examined for glaucoma. The objective of this study was to investigate the role of the *MYOC* mutation in the occurrence of POAG in this village. We expanded the study population from 122 to 531 participants and characterized the Thr377Met *MYOC* mutation in relation to POAG disease status and the glaucomatous risk factors IOP, CCT, and optic nerve findings.

Methods

Five hundred thirty-one villagers from a small Greek village were enrolled in the study according to the tenets of the Declaration of Helsinki. Informed consent was obtained from the participants after explanation of the nature and possible consequences of the study. The protocol was approved by the Bioethics Committee of Aristotle University (Thessaloniki, Greece). An initial examination at the community center in Taxiarchis included ophthalmic and general history, IOP, CCT, and evaluation of the optic nerve status, as previously described.[14] Systolic and diastolic blood pressures were evaluated with a digital automatic blood pressure monitor (3ACI-PCC05; Microlife, Kent, WA). Participants were invited to participate in a more comprehensive examination at the Glaucoma Unit of the First University, Department of Ophthalmology, American Hellenic Educational Progressive Association (AHEPA) Hospital (Thessaloniki). To date, 239 of the original 531 participants have been examined at the glaucoma unit.

MYOC Screening

The promoter region and exons 1 to 3 of myocilin were sequenced bidirectionally at the Portland Veterans Administration Medical Center (Portland, OR), as previously described.[15]

Statistical Analyses

All statistical analyses including power calculations were conducted using the genetic variance analysis program SOLAR version 4.2.[16] Quantitative trait association analysis between the Thr377Met *MYOC* mutation and maximum IOP, vertical cup-to-disc ratio (CDR), and CCT was performed in SOLAR by using the measured genotype approach,[17] testing for genotype-specific differences in the means of traits while allowing for nonindependence among family members. Covariates age, sex, age–sex interaction, systolic blood pressure, and diastolic blood pressure were included in all analyses.

Results

The participants ranged in age from 10 to 95 years with a mean age of 49 ± 20 years. Of the 531 villagers, 307 were female and 224 were male. Two hundred thirty-nine subjects participated in a more comprehensive examination at the University of Thessaloniki, including all but two of the individuals with a diagnosis of POAG. Sixty-five participants were confirmed to have POAG as shown in Table 1. Five of the POAG patients had received the diagnosis before this study; three of those had undergone surgery (trabeculectomy). Eighteen individuals had exfoliation syndrome (XFS) or exfoliative glaucoma (XFG); none had received prior IOP-lowering treatment. Congenital glaucoma was not found in this population. The distribution of the sexes was very similar among those with glaucoma, ocular hypertension (OHT), or suspected glaucoma. However, almost twice as many females as males were present in the normal population. The overall prevalence of POAG and XFS/XFG in the village was 12% and 3.4%, respectively.

Inheritance of POAG

The pedigree structure of the village is very complex with 376 participants in 11 generations linked together. Because of the complexity of relationships within the village, there are many connections across Figures 1 to 4 that are not shown, in an attempt to simplify the village pedigree. In the figures the relationship between 243 family members in nine generations is depicted. The 133 individuals not shown either had normal ocular findings or had suspected glaucoma and were omitted to simplify the figure. The pedigrees in Figures 1 to 3 are all connected, indicated by the colored backgrounds surrounding the symbols of individuals duplicated in the panels. The pedigrees include 60 of the 65 POAG patients; 5 appeared to be unrelated, although further study is necessary to confirm this finding. Almost all villagers with the Thr377Met *MYOC* mutation (73/74) are included in the four figures.

POAG occurred in nine parent–child sets, in one grandparent–grandchild set, and in three consecutive generations in one nuclear family. In the nine parent-child sets, two were concordant for the wild-type *MYOC*, two were concordant for the Thr377Met *MYOC* mutation, and five were discordant, with the parents having the Thr377Met *MYOC* mutation and the children inheriting the wild-type form. The grandparent (A54) and grandchild (A448) both had the Thr377Met *MYOC* variant and POAG. In the three-generation nuclear family, the grandparent had the wild-type, whereas the grandchild and parent had the Thr377Met *MYOC* mutation.

Fourteen sets of siblings had two or more individuals with POAG. Nine of the POAG sibships were concordant for *MYOC* status: six for the Thr377Met *MYOC* mutation, and three for wild-type *MYOC*. Five sibships were discordant for the Thr377Met *MYOC* mutation and POAG: one in Figure 1 (A125/A213), three in Figure 2 (A33/A17, A23/A75, and A530/A558), and one in Figure 3 (A547/A34).

Table 1. Clinical Profile of Participants

Sex	POAG	XFS	Glaucoma*	OHT	Suspect	Normal	Total
M	33	9	1	11	24	146	224
F	32	9	1	16	23	226	307
Total	65	18	2	27	47	372	531

* Has not participated in more comprehensive examination at AHEPA.

Age at Onset of POAG

Age at onset of POAG ranged from 16 to 82 years. Three patients under the age of 40—two with the wild-type *MYOC* and one with the Thr377Met *MYOC* mutation— had an aggressive course of disease consistent juvenile open-angle glaucoma (JOAG). Glaucoma prevalence increased from 2% in the 10- to 19-year-old group to 23% in the 60- to 69-year-old group as shown in Table 2. This represented a nearly 12-fold increase in POAG diagnosis with age and was very similar between individuals with the Thr377Met variant and those with wild-type *MYOC* (data not shown). The youngest affected individual was 16 and was homozygous for the Thr377Met *MYOC* mutation, as reported previously.[14]

Thr377Met *MYOC* Variant

Seventy-four (13%) villagers had the Thr377Met *MYOC* variant, as shown in Table 3. These 74 villagers comprised 27 individuals with POAG (16–95 years), 6 with XFS/XFG, 6 OHT, 6 with suspected glaucoma, 28 with normal ocular findings (11–79 years), and 1 with glaucoma who had not been evaluated for exfoliation. The average age of POAG diagnosis for villagers with the Thr377Met *MYOC* mutation was 59 ± 18 years compared with 57 ± 14 for individuals with the wild-type *MYOC*.

Of the 65 individuals with POAG, 27 had the Thr377Met mutation. A DNA sample was not available for one of the POAG patients, and so the *MYOC* status of this person could not be evaluated. Two of the POAG patients were homozygous for the Thr377Met variant, as previously reported.[14] Two individuals with glaucoma diagnosed in the village had not had an extensive examination to differentiate between XFG and POAG. One had the Thr377Met *MYOC* mutation, and one had wild-type *MYOC*. Of the 18 individuals with XFS/XFG, 6 had the Thr377Met *MYOC* variant, 11 had wild-type *MYOC*, and 1 had no DNA sample available.

POAG Risk Factors

The POAG endophenotypes CDR, IOP, and CCT were characterized in the villagers. The maximum IOP in the POAG patients with the Thr377Met mutation ranged from 13 to 68 mm Hg, with an average of 26.8 ± 11 mm Hg, as shown in Table 4. The glaucoma patients with wild-type *MYOC* had a maximum IOP of 23.2 ± 5 mm Hg, ranging from 15 to 36 mm Hg. The mean maximum IOP in all the individuals with the Thr377Met *MYOC* variant was 21.8 ± 8.6 mm Hg compared with 18.0 ± 3.7 in the individuals with the wild-type *MYOC* gene. Systolic blood pressure was a significant covariate with maximum IOP. A significant association ($P = 9 \times 10^{-14}$) between maximum IOP and the Thr377Met *MYOC* variant was found with the mutation effectively increasing IOP by 4.4 mm Hg. Systolic blood pressure was used as a covariate.

The average maximum vertical CDR in POAG patients with the Thr377Met mutation was 0.74 ± 0.17 compared with

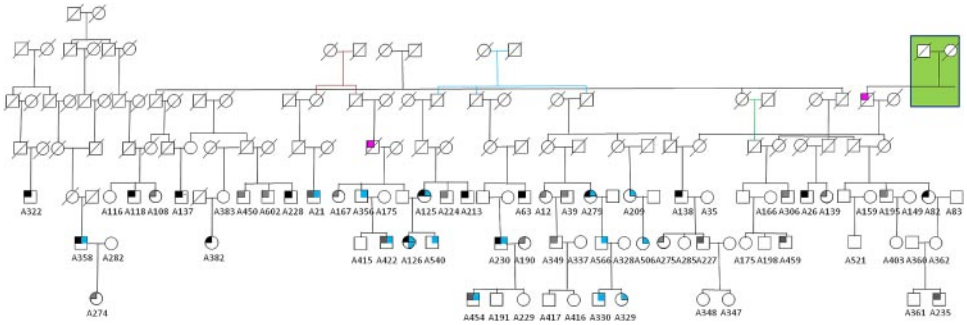

FIGURE 1

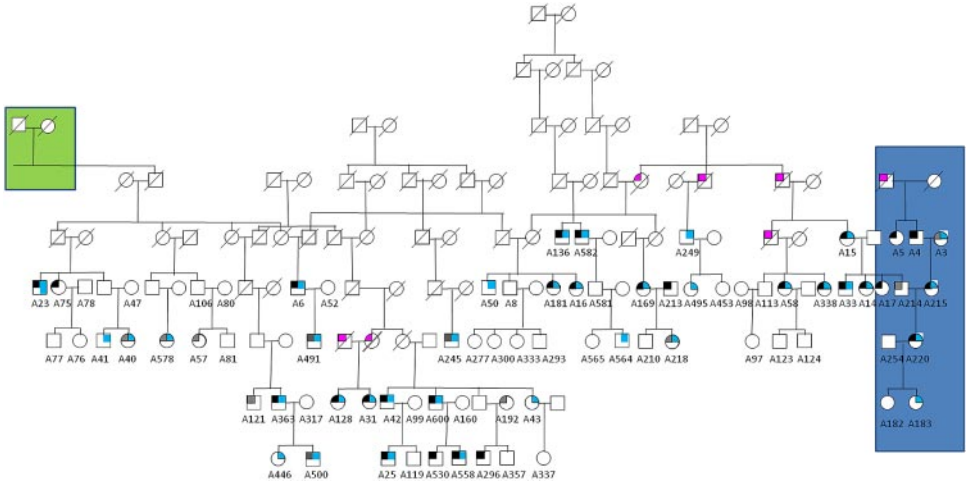

FIGURE 2

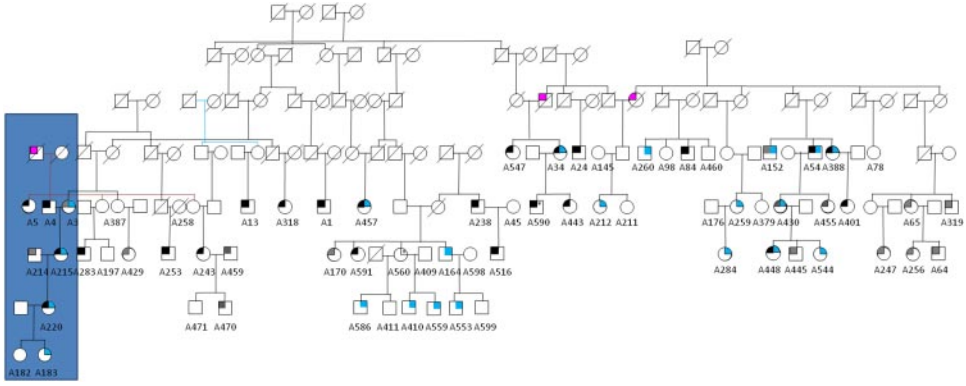

FIGURE 3

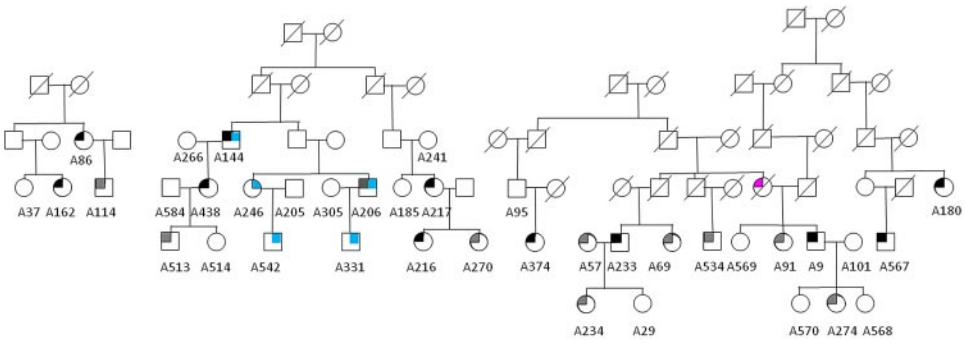

FIGURE 4

FIGURES 1–4. Pedigree structure of participants from the village. *Circle*: female; *square*: male. *Black upper left quadrant in symbol*: POAG; *gray upper left quadrant*: suspected glaucoma; *violet upper left quadrant*: reported history of glaucoma; *blue upper right quadrant*: presence of Thr377Met *MYOC* mutation. *Asterisk in upper left quadrant*: no DNA sample available and therefore no test conducted for the presence of Thr377Met MYOC mutation. *Right half of the symbol filled with blue*: two individuals homozygous for the Thr377Met *MYOC* mutation. *Numbers starting with A below symbol*: individual participated in the study. *Symbol with slash*: individual is deceased. *Green or blue background*: individuals repeated in the panels, showing relationship between pedigrees in the individual panels.

Table 2. Number of POAG Cases in Relation to Age

Age Range	Total Number POAG	*n*	Total % POAG
70 and older	15	101	0.15
60–69	19	82	0.23
50–59	12	87	0.14
40–49	11	74	0.15
30–39	5	79	0.06
20–29	2	61	0.03
10–19	1	47	0.02
Total	65	531	0.12

0.66 ± 0.14 in POAG patients with wild-type *MYOC*. In all the individuals with the Thr377Met *MYOC* variant, the average maximum vertical CDR was 0.51 ± 0.26, compared with 0.36 ± 0.17 in all the individuals with wild-type *MYOC*. Age was a significant covariate with CDR. A significant association ($P = 9 \times 10^{-8}$) was also found between maximum CDR and the Thr377Met *MYOC* mutation, including age as a covariate.

The average CCT in POAG patients with the Thr377Met variant was 541 ± 27 versus 560 ± 38 in the patients with wild-type *MYOC*. Overall, the average CCT in the Thr377Met mutation group was 555 ± 36 compared with 561 ± 38 in everyone with the wild-type gene ($P < 0.7$). Age, sex, and diastolic blood pressure (but not systolic blood pressure) were significant covariates with CCT; no significant association was found between CCT and the Thr377Met *MYOC* mutation ($P < 0.7$).

Penetrance of Thr377Met MYOC Mutation

Analysis of the Thr377Met *MYOC* mutation carriers showed that the number of affected individuals including POAG, OHT, XFS, and suspected glaucoma increased with age (Table 5). The youngest age group (10–19 years) had a penetrance of 14% for POAG, which more than doubled to 33% in the 20 to 29 age group. By ages 50 to 59 years, 85% of the mutation carriers had clinical signs of glaucoma, and by age 80 the mutation increased to 100% penetrance.

Discussion

This report extends our previous account of glaucoma and the Thr377Met *MYOC* mutation in this remote village in Greece.[14] We expanded the number of participants from 126 to 531 and identified an additional 44 affected subjects for a total of 65 POAG patients. In addition, the number of individuals with the Thr377Met *MYOC* mutation increased from 21 to 74, although we did not find any additional Thr377Met homozygotes (two had been identified originally). In this article, we look at the pedigree structure of this village in relation to POAG and the Thr377Met *MYOC* variant. In addition, we focus on the POAG clinical characteristics in this population and the interaction between the Thr377Met mutation and these characteristics.

Interviewing the new participants allowed us to connect the original five families into one large pedigree, including 376 of the participants.[14] This POAG pedigree is one of the most complex reported in the literature. All the individuals in this article with the Thr377Met *MYOC* mutation share the same haplotype, which has been identified in all Greek POAG patients with this variant.[12] A large family in Epirus in northwestern Greece, as well as several Australian families who emigrated from Epirus to Australia, share this Thr377Met mutation and haplotype.[12] Thus, it is likely that a common founder introduced this mutation to northern Greece.

Another common finding among the families with the Thr377Met *MYOC* mutation reported in the literature is that many have several members with POAG, but not all affected individuals carry the *MYOC* variant.[13,18,19] Moreover, some individuals with the mutation have normal ocular findings at the time of examination, suggesting that other genetic or environmental factors may affect the presentation of the disease. Because of the large number of participants, this village offers a fascinating possibility for identifying protective genes in those individuals with normal ocular findings and the Thr377Met *MYOC* mutation and additional POAG susceptibility genes.

To the best of our knowledge, there is no other epidemiologic cohort in which a specific mutation has been investigated in relation to POAG. This isolated population has a surprisingly high number of POAG subjects, with a rate of 12% across all ages and reaching 23% in the 60- to 69-year age group. Prevalence rates for POAG in other Caucasian populations across all ages are much lower, ranging from 0.8% to 2.49%, as reported in the Beaver Dam Eye Study,[20] Baltimore Eye Survey,[21] Blue Mountain Eye Study,[22] Vision Impairment Project,[23] and the Rotterdam Study.[24] The highest rates occur in older age groups—for example, 6.94% of those 80 years of age and older.[25] The incidence of POAG in Thessaloniki, 1 hour by car from Taxiarchis, is similar to other Caucasian populations, with 3% in the 60- to 64-year group and 8.3% in those older than 80.[26] Historically, the village was a population isolate created by geography and the economy. Thus, the difference in frequency of glaucoma between the village and Thessaloniki is understandable, but still striking.

The genetic architecture of POAG is complex with more than one gene variant likely to influence disease risk, and each variant likely to influence different components of the disease. As such, we investigated the association between the *MYOC* Thr377Met variant and three separate clinical components of the disease phenotype; IOP, vertical CDR, and CCT. We included the covariates age, sex, and systolic and diastolic blood pressure in our modeling to account for their effects on the variance of each of these clinical measures. In our sample, age was the only covariate that influenced more than one trait measure—significant for both vertical CDR and CCT.

Table 3. *MYOC* Status and Clinical Characteristics

	Sex	POAG	XFS	Glaucoma	OHT	Suspect	Normal	Total
Thr 377 Met	Male	13	2		2	6	12	35
MYOC	Female	14	4	1	4	0	16	39
Wild-type	Male	19	6	1	9	18	134	187
	Female	18	5		12	22	210	267
Total		64	17	2	27	46	372	528

No DNA was available on three individuals, one with POAG, one with XFS and one suspect. Therefore, these individuals were excluded from the data in this table.

TABLE 4. POAG Risk Factors and MYOC Status

Risk Factor	POAG (n = 27)	OHT (n = 6)	Suspect (n = 6)	XFS (n = 6)	Normal (n = 29)	All (n = 74)
Thr377Met MYOC						
Max IOP	27.0 ± 11	24.2 ± 2.6	18.3 ± 1.8	21.2 ± 7.1	17.2 ± 3.6	21.8 ± 8.6
Max CDR	0.74 ± 0.17	0.38 ± 0.14	0.58 ± 0.09	0.63 ± 0.21	0.26 ± 0.08	0.51 ± 0.26
CCT	542 ± 26	565 ± 19	577 ± 51	545 ± 30	564 ± 40	555 ± 36
Risk Factor	**POAG (n = 37)**	**OHT (n = 30)**	**Suspect (n = 40)**	**XFS (n = 11)**	**Normal (n = 334)**	**All (n = 454)**
Wild-type MYOC						
Max IOP	23.2 ± 5.4	23 ± 1.7	18.6 ± 1.8	20.5 ± 4.9	16.0 ± 2.5	18.0 ± 3.7
Max CDR	0.66 ± 0.14	0.41 ± 0.12	0.54 ± 0.13	0.54 ± 0.19	0.29 ± 0.11	0.36 ± 0.17
CCT	560 ± 38	571 ± 38	553 ± 30	536 ± 36	562 ± 39	561 ± 38

Three people with clinical data (one with POAG, one with XFS and one suspect) but no DNA are omitted from this table.

Myocilin mutations in general have been associated with high IOPs[27]; however, this is the first report of a significant association of a specific *MYOC* mutation, Thr377Met, with IOP. The finding that systolic blood pressure is a significant covariate for maximum IOP is consistent with previous findings in other populations.[28–30] Sex and age have been reported not to be significantly related to IOP, consistent with our findings.[30]

Consideration of CDR as a risk factor for POAG may be somewhat controversial, since some would say that it is an indicator of early glaucomatous damage. However, we agree with findings in The Ocular Hypertension Treatment Study that when a clinician examines a patient for the first time, there is no way to determine whether the observed CDR has been stable over the patient's life or has enlarged as part of the disease process.[31] Thus, an individual with a large CDR may not yet have glaucoma but may be at higher risk for development of the disease. Using vertical CDR as a risk factor, we found a significant association with the Thr377Met variant, with age as a covariate. The relationship of vertical CDR with age is controversial; some studies support this association, whereas other studies find no correlation.[32,33] No correlation of CDR was found with sex or systolic or diastolic pressure, which is consistent with some reports,[32,34] but inconsistent with the correlation between vertical CDR and diastolic blood pressure found in an Asian population.[35] This result may simply reflect ethnic differences.

CCT has only recently been considered a POAG risk factor[31,36] and has not been examined in relation to *MYOC* mutations. Our finding of no association of CCT with the Thr377Met *MYOC* variant suggests that the mutation has little impact on CCT. Of interest, sex, age, and systolic blood pressure were found to be significant covariates with CCT. Our finding that CCT is associated with age is consistent with that in The Ocular Hypertension Treatment Study, which reported that CCT decreases over time.[37] We are unaware of any reports in which systolic blood pressure covaries with CCT. If CCT correlates with systolic blood pressure in other populations it could be an indicator of clinical damage from hypertension and would be worthy of further investigation.

TABLE 5. Penetrance of Thr377Met MYOC Mutation

Age Group	Affected (n)*	Normal (n)	Total (n)	%
80 and older	2	0	2	100
70–79	11	2	13	85
60–69	7	4	11	64
50–59	11	2	13	85
40–49	6	5	11	55
30–39	2	1	3	67
20–29	4	8	12	33
10–19	1	6	7	14

* Number of affected includes those with POAG, OHT and suspects.

The population of this isolated village presents a unique opportunity to unravel the complexities of glaucoma due to the presence of the Thr377Met *MYOC* mutation and POAG in a significant proportion of the villagers. The discordance between the Thr377Met *MYOC* mutation and POAG within some of the families suggests that other mitigating factors for POAG may be present within the village. Analysis of the genetic profile of the participants has the potential for uncovering additional susceptibility genes as well as protective ones. Thus, this village represents a rich resource for a better understanding of the pathophysiology of POAG.

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