

# Primary open angle glaucoma due to T377M MYOC: Population mapping of a Greek founder mutation in Northwestern Greece

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**Background:** Mutations in the *MYOC* gene have been shown to explain 5% of unrelated primary open angle glaucoma (POAG) in different populations. In particular, the T377M *MYOC* mutation has arisen at least three separate times in history, in Great Britain, India, and Greece. The purpose of this study is to investigate the distribution of the mutation among different population groups in the northwestern region of Greece.

**Materials and methods:** We explored the distribution of the "Greek" T377M founder mutation in the Epirus region in Northwestern Greece, which could be its origin. Genotyping was performed in POAG cases and controls by PCR amplification of the *MYOC* gene, followed by digestion with restriction enzyme. Statistical analyses were performed by an exact test, the Kaplan–Meier method and the t-test.

**Results:** In the isolated Chrysovitza village in the Pindus Mountains, a large POAG family demonstrated the T377M mutation in 20 of 66 family members while no controls from the Epirus region ( $n = 124$ ) carried this mutation ( $P < 0.001$ ). Among other POAG cases from Epirus, 2 out of 14 familial cases and 1 out of 80 sporadic cases showed the mutation ( $P = 0.057$ ). The probability of POAG diagnosis with advancing age among mutation carriers was 23% at age 40, and reached 100% at age 75. POAG patients with the T377M mutation were diagnosed at a mean age of 51 years ( $SD \pm 13.9$ ), which is younger than the sporadic or familial POAG cases: 63.1 ( $SD \pm 11$ ) and 66.8 ( $SD \pm 9.8$ ) years, respectively.

**Conclusions:** The T377M mutation was found in high proportion in members of the Chrysovitza family (30.3%), in lower proportion in familial POAG cases (14.2%) and seems rare in sporadic POAG cases (1.2%), while no controls (0%) from the Epirus region carried the mutation. Historical and geographical data may explain the distribution of this mutation within Greece and worldwide.

**Keywords:** primary open angle glaucoma, *GLC1C*, myocilin, *MYOC*, founder mutation

## Introduction

Glaucoma is a heterogeneous group of disorders resulting from optic nerve damage and is the second leading cause of irreversible blindness worldwide. Primary open angle glaucoma (POAG) is the most common form, with reported prevalence rates ranging from 1.1%<sup>1</sup> to 3.8%.<sup>2</sup> The definition of glaucoma has changed many times as the etiology and pathogenetic pathways are still largely unknown. A high intraocular pressure (IOP) is no longer considered part of the definition of POAG, but represents a risk factor for the development of POAG.<sup>3</sup> Other risk factors are old age, race, and positive family history.<sup>4</sup> At the genetic level, a distinction between juvenile onset (age at diagnosis 10–35 years) and adult onset (age at diagnosis above 35 years) has been made.

Many juvenile onset cases have autosomal dominant inheritance whilst adult onset cases are typically multifactorial.

The first locus related to juvenile onset glaucoma was mapped to chromosome 1q21-q31 (*GLCIA*).<sup>5</sup> A common POAG locus for both juvenile and adult onset POAG was suggested from the study of a large Canadian family with linkage to the *GLCIA* locus and different ages of onset.<sup>6</sup> Other pedigrees with adult onset POAG excluded linkage to the *GLCIA* locus.<sup>7,8</sup> The gene encoding *myocilin* (*MYOC*), located in 1q23-q24, consists of 3 exons, and shows high expression in the trabecular meshwork and ciliary body.<sup>9</sup> Mutations in the *MYOC* gene were finally identified as responsible for glaucoma at the *GLCIA* locus.<sup>9</sup> Large studies have demonstrated that point mutations in *MYOC* account for 4.6% of unrelated POAG patients.<sup>10</sup> Patients with POAG due to *MYOC* mutations show marked phenotypic variation with age at diagnosis ranging from 8 to 77 years and highest recorded IOP ranging from 12 to 77 mm Hg.<sup>10</sup> To date approximately 60 disease-causing *MYOC* mutations have been reported, and a genotype-phenotype correlation exists.<sup>11</sup>

Evidence for founder effects of several *MYOC* mutations has been reported in different populations.<sup>12–16</sup> The T377M mutation was first described in 1998 and has since been identified in populations from five different continents.<sup>17–25</sup> An investigation of founder effects for this mutation has demonstrated that it has arisen at least three separate times, namely in Great Britain, India, and Greece.<sup>16</sup> The purpose of the present study is to determine the prevalence of the Greek T377M founder mutation in familial and sporadic cases of POAG and unaffected controls in Epirus, the Northwestern region of Greece, and to discuss geographical and historical data to assist explaining the propagation of this mutation in Greece and worldwide. We also describe the clinical characteristics of the T377M mutation carriers in our material.

## Materials and methods

Sporadic and familial POAG patients from Epirus, Northwest Greece, as well as unaffected controls from the same region were recruited. POAG patients were recruited from the Glaucoma Unit of the Department of Ophthalmology, University of Ioannina School of Medicine, or from the Department of Ophthalmology, General Hospital of Ioannina “G. Chatzikosta”. All 200 POAG patients seen in follow-up between April 2005 and January 2006 at the two hospitals were asked to participate in the study and 94 of them gave their consent and had blood drawn for genetic testing. The 106 non responders for blood testing, had POAG of the same severity (max IOP, c/d ratio, visual fields) and their ethnicity,

their age at diagnosis, age at examination and sex matched the group that gave their consent. These two hospitals cover the entire Epirus region. Of the 94 cases, 80 were sporadic and 14 had a positive family history of POAG, which was confirmed by clinical examination of family members.

Members of a large family with POAG segregating as a monogenic trait (*GLC1C*) were recruited from a previous study.<sup>26</sup> The T377M *MYOC* mutation was found to segregate in a large proportion of the members of this family.<sup>24</sup> The family originated from the Chrysovitsa village of the Pindus Mountains, Epirus. A total of 66 out of 73 family members were available for the present study. Furthermore, we included 4 sporadic POAG cases and 4 individuals without POAG (controls) from the Chrysovitsa village, who were not related to the pedigree described above, and which are included in the sporadic POAG and control groups, respectively.

Unaffected Epirus residents were selected as controls from patients admitted to cataract surgery in the Department of Ophthalmology, General Hospital of Ioannina “G Chatzikosta”, between April 2005 and January 2006. A total number of 124 controls were recruited, of which 24 individuals had pseudoexfoliative material in the anterior chamber of one or both eyes (without glaucoma).

The protocol was approved by the Ethics Committees of the University of Ioannina School of Medicine, the General Hospital of Ioannina “G Chatzikosta”, and the Institute of Child Health, Athens. The clinical examination protocol included an interview to determine the presence of family history of POAG, full medical history, and history of ophthalmologic disorders. Applanation tonometry was performed with a recently calibrated Goldmann applanation tonometer (Haag Streit AG, Bern, Switzerland). The anterior segment was examined by slit lamp biomicroscopy including gonioscopy. Optic disc appearance was evaluated and the cup/disc ratio was measured. Qualitative signs of POAG, such as bared circumpapillary vessels, disc hemorrhages, focal neuroretinal rim thinning or a notch extending to the margin, as well as retinal fiber layer defects, were assessed with a stereoscopic fundus lens (Volk Optical Inc., Mentor, Ohio). Automated perimetry by Humphrey 24-2 (Humphrey Inc., San Leandro, CA, USA) was performed to determine visual field defects in the POAG patients. Staging of the perimetry results (Early Glaucomatous Loss, Moderate Glaucomatous Loss and Advanced Glaucomatous Loss) was done according to Hodapp classification, as suggested by the European Glaucoma Society.<sup>27,28</sup> Ocular hypertension (OHT) was defined as IOP  $\geq$  21 mm Hg, with all other ocular findings within normal limits. The criteria for the diagnosis of POAG were according to the guidelines of the European

Glaucoma Society.<sup>27</sup> Patients with pseudoexfoliation glaucoma were excluded from the study. The clinical examination protocol, except for the automated perimetry, was also performed in the controls.

Venous blood was drawn from patients and controls following informed consent, and DNA was obtained by a salt extraction method.<sup>29</sup> Genotyping of the T377M MYOC mutation was performed by PCR amplification of genomic DNA using a modified protocol of Melki et al.<sup>22</sup> The primers used were MYOC-F (5'-GATTATGGATTAAGTGGTGCTTCG-3') and MYOC-R (5'-TGAGCATCTCCTTCTGCCATTGCC-3'). A fragment of 874 bp was amplified according to the following protocol: 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute, and final extension of 72°C for 7 minutes. The unpurified PCR product was subsequently digested with Hsp92 II enzyme (Promega Corporation, Madison, Wisconsin) at 37°C for 24 hours and digests were run on a 9% polyacrylamide

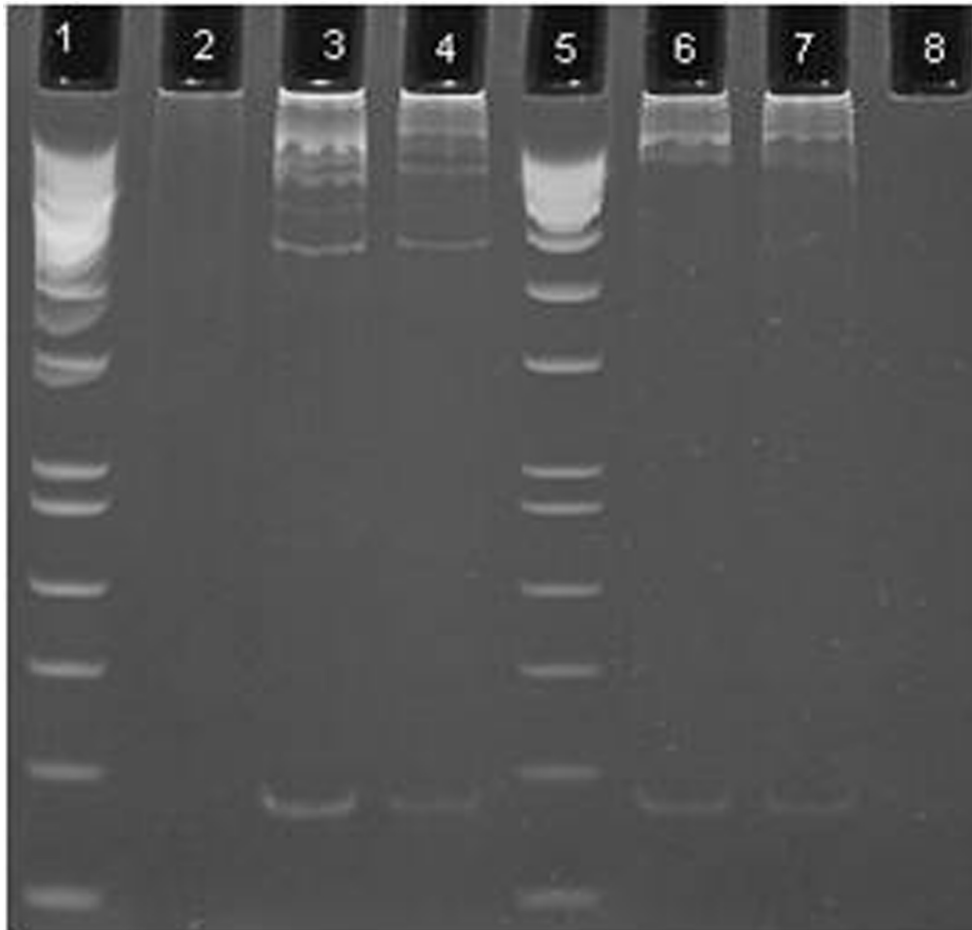
gel in TBE buffer at 90 V for 3 hours. HSP92II recognizes a CATG restriction site, and therefore, the wild-type DNA yielded 3 fragments of 740, 80, and 54 bp, while the T377M mutation creates a restriction site with fragments of 443, 297, 80, and 54 bp (Figure 1).

Mutation carrier rates between groups were compared with an exact test. Time-to-POAG diagnosis analysis used the Kaplan–Meier method. Comparison of continuous measures used the t-test. *P* values were two-tailed.

## Results

### Prevalence of T377M in various groups

The results of T377M MYOC genotyping in POAG patients and unaffected controls are summarized in Table 1. In the Chrysovitsa family segregating POAG as an autosomal dominant trait, 20 out of 66 family members (30.3%) carried the T377M mutation. Two out of 14 Epirus POAG cases (14.2%) with positive family history had the T377M



**Figure 1** Examples of the results obtained from the molecular analysis. Lanes 1 and 5: PhiX174 DNA/HinI marker; Lanes 2 and 8: blank; Lanes 3 and 4: heterozygotes for the T377M mutation (740 bp, 443 bp, 297 bp, and 80 bp bands are visible); Lanes 6 and 7: normal DNA samples not harboring the T377M mutation (740 bp, and 80 bp bands are visible). The 54 bp band was not visible in the gel.

**Table 1** Frequency of the T377M *MYOC* mutation in different categories of examined individuals from Epirus, Greece

Groups	Number of individuals	Number of T377M carriers	Proportion (95% CI)
Sporadic POAG (Epirus)	80 <sup>a</sup>	1	1.2 (0.0–6.8)
Familial POAG (Epirus)	14 <sup>b</sup>	2	14.2 (1.8–42.8)
Chrysovitsa family	66 <sup>c</sup>	20 <sup>d</sup>	30.3 (19.6–42.9)
Epirus controls	124 <sup>e</sup>	0	0.0 (0.0–2.9)
Sporadic POAG (Chrysovitsa)	4	0	0.0 (0.0–60.2)
Chrysovitsa controls	4	0	0.0 (0.0–60.2)

<sup>a</sup>Number includes four cases from the Chrysovitsa village; <sup>b</sup>From unrelated families; <sup>c</sup>Fifteen family members had been diagnosed with POAG; <sup>d</sup>Twelve T377M carriers had POAG; <sup>e</sup>Number includes 4 individuals from the Chrysovitsa village.

**Abbreviation:** POAG, primary open-angle glaucoma.

mutation, whereas only one out of 80 sporadic Epirus POAG cases (1.2%) carried this mutation (exact  $P = 0.057$  for the comparison of familial versus sporadic POAG cases). Furthermore, the T377M mutation was not detected among 124 Epirus inhabitants without POAG (controls) (exact  $P < 0.001$  for the comparison against POAG cases). Moreover, none of the four sporadic POAG cases from the Chrysovitsa village (not belonging to the large family described above) had the T377M mutation.

### Clinical features for T377M carriers with POAG

The clinical findings in carriers of the T377M mutation from Epirus are presented in Table 2. Of these, 15 had a diagnosis of POAG (12 from the Chrysovitsa family, 2 other familial cases, 1 sporadic case). Eight cases of POAG had severe visual defects and 7 had modest visual defects. In Kaplan-Meier analysis, the probability of POAG diagnosis with advancing age among mutation carriers was 23% at age 40, and reached 100% at age 75. Three individuals had undergone surgery in both eyes (trabeculectomy) and one (F3-38) definitely needed surgical therapy but was unwilling to comply. Also, in T377M mutation carriers, the probability of developing glaucoma with advancing age in *GLC1C* and non-*GLC1C* individuals is shown in a Kaplan-Meier plot in Figure 2.

### Clinical features in POAG: T377M carriers versus non-carriers

The mean age at diagnosis  $\pm$  SD, mean age at examination  $\pm$  SD, mean maximum IOP  $\pm$  SD, and mean maximum cup/disc ratio  $\pm$  SD in the various groups of Epirus individuals are presented in Table 3. POAG patients with the T377M mutation were diagnosed at  $51 \pm 13.9$  years of age, which is younger than the sporadic or familial POAG cases without T377M:  $63.1 \pm 11$  and  $66.8 \pm 9.8$  years of age, respectively ( $P < 0.001$  for POAG cases with versus without T377M).

The mean maximum IOP ( $31.9 \pm 8.5$  mm Hg) was not much higher in the T377M POAG group than in the sporadic or familial POAG groups without T377M ( $28.1 \pm 7.1$  mm Hg and  $29.1 \pm 6.3$  mm Hg, respectively). Finally, there were no significant differences in the cup/disc ratio for T377M carriers versus non-carriers with POAG (Table 3).

### Discussion

We have examined the prevalence of the T377M *MYOC* mutation in diverse groups of patients with POAG and unaffected controls in the region of Epirus in Northwestern Greece. Our findings suggest that T377M is largely concentrated in the originally described family from Chrysovitsa, where almost one in four members (15/66) are affected by POAG.<sup>26</sup> However, some other familial glaucoma cases from the region also carry this mutation, while the mutation is very rare in sporadic POAG cases and virtually absent in unaffected controls from the general Epirus population. POAG associated with T377M in this region is associated with early onset in a substantial proportion of cases and practically complete penetrance of the disease by age 75, but otherwise the severity of the illness is not much worse compared with cases without T377M from the same region.

Hereditary diseases are occasionally frequent in small populations that have been living in relative isolation for a long time. When a mutation was introduced by a founder in an isolated population, it could have a chance to become frequent even in spite of selection against it. One such example of an isolated population is the Northwest region of Greece, Epirus. The Chrysovitsa village in the Pindus Mountains of the Epirus region of Greece has been isolated for centuries. Eleven pedigrees from Epirus segregating adult onset POAG in an autosomal dominant mode were described by Kitsos et al.<sup>30</sup> Linkage analysis in the largest of these pedigrees, habitating the small village of Chrysovitsa (population of 993 in 2001) on Mount Pindus, showed linkage to the *GLC1C* locus

**Table 2** Clinical findings in carriers of the T377M MYOC mutation from Epirus, Greece

ID <sup>a</sup>	Year of birth	POAG	GLCIC	Age at examination	Age at diagnosis	Max intraocular pressure	Max cup/disc ratio	Visual fields	Surgery (trabeculectomy)
F2-3	1909	Yes	Yes	85	75	28	0.9	SD	No
F2-5	1917	Yes	Yes	84	67	56	0.95	SD	No
F2-6	1924	Yes	Yes	77	60	23	0.8	SD	No
F2-7	1927	Yes	Yes	74	57	26	0.4	MD	No
F2-8	1930	Yes	Yes	71	54	43	0.9	SD	No
F3-6	1949	Yes		55	36	30	0.6	MD	No
F3-11	1940	No		69		18	0.3		No
F3-14	1949	No		60		19	0.2		No
F3-19	1951	No		58		20	0.2		No
F3-22	1948	No	Yes	61		19	0.3		No
F3-25	1957	Yes		44	34	36	0.5	MD	Yes OD/OS
F3-38	1960	Yes	Yes	41	36	30	0.7	SD	No
F3-27	1947	No		62		14	0.3		No
F3-29	1955	Yes	Yes	54	39	27	0.5	MD	No
F3-37	1958	Yes		51	46	32	0.6	MD	No
F3-31	1958	Yes		43	43	26	0.5	MD	No
F3-32	1959	OHT		50		22	0.2		No
F3-34	1964	No	Yes	45		20	0.4		No
F3-35	1970	Yes	Yes	35	32	36	0.9	SD	No
F4-27	1980	No		29		16	0.4		No
20DC	1928	Yes		80	70	27	0.8	SD	Yes OD/OS
K151	1946	Yes		59	58	24	0.7	MD	No
K153	1925	Yes		80	67	35	0.9	SD	Yes OD/OS

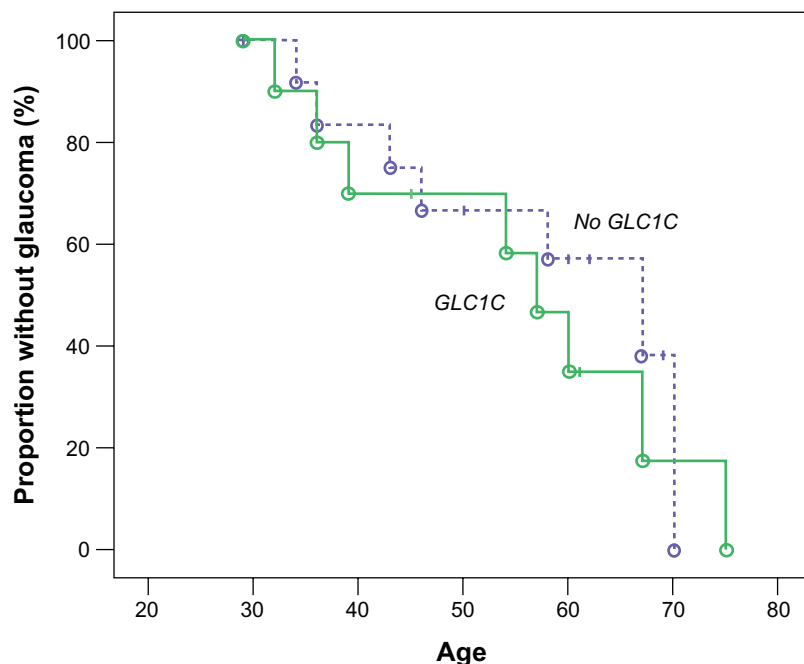
<sup>a</sup>The F individuals indicate members of the Chrysovitsa family, the 20DC individual is a sporadic POAG case, whereas the K individuals are familial POAG cases.

**Abbreviations:** SD, severe defect – advanced glaucomatous loss; MD, moderate defect – moderate glaucomatous loss; OHT, ocular hypertension; POAG, primary open-angle glaucoma; OD, right eye; OS, left eye.

at 3q21-q24,<sup>26</sup> which had originally been described in a large family from Oregon, USA.<sup>31</sup> Due to the usually multifactorial mode of inheritance of adult onset POAG, both *GLC1C* families were screened for mutations in the *MYOC* gene.<sup>24</sup> Interestingly, the T377M mutation was found in 67% (10/15) of the POAG patients of the Chrysovitsa family in an earlier evaluation of the same family.<sup>24</sup> Taxiarchis, another small Greek village (~1100 inhabitants) on Mount Holomondas in the Chalkidiki region, known to be enriched for familial glaucoma,<sup>32</sup> was recently studied by Wirtz et al.<sup>33</sup> The incidence of glaucoma was 17% (22/126) and the T377M *MYOC* mutation was detected in 59% (13/22) of the POAG patients. The haplotype of the T377M mutation in Taxiarchis villagers was the same as in the Chrysovitsa villagers.<sup>33</sup>

As evident from Table 1, acknowledging that some of the data are sparse, the mutation seems to occur in a considerable proportion of familial POAG cases from Epirus, as compared

to the low proportion found in the sporadic POAG cases from the same region. One of the T377M mutation carriers (K151 – Table 2), a 63-year-old lady of Vlach ancestry (both parents) with familial glaucoma, was born in Kefalovryso (father and her 2 sisters: POAG patients). The other familial case (K153 – Table 2), an 84-year-old man, was born in Perdika (4/5 brothers and his son: POAG patients). The T377M sporadic case (20DC – Table 2) originates from Kastritsa (both parents and all 5 brothers/sisters: healthy – no POAG). The above 3 small villages are located at a 60-70 km distance from Chrysovitsa (Figure 3). None of these individuals has any known relation to Chrysovitsa going back 2 generations (origin of grandparents). The Taxiarchis village is located about 300 km from Chrysovitsa and there is oral information from Taxiarchis villagers that a route existed between the two villages, which was traveled frequently, at times to bring young women from Chrysovitsa to Taxiarchis for marriage



**Figure 2** T377M carriers: Probability of developing glaucoma with advancing age for *GLC1C* and non-*GLC1C* individuals (Kaplan–Meier plot).

to local men in order to bring “new blood” into the village. In the study by Hewitt et al,<sup>16</sup> six pedigrees shared the same “Greek” haplotype: the Chrysovitsa family, 2 Australian families, 2 USA families of Greek origin, and 1 family from FYROM, which is in close geographic proximity to Epirus. Furthermore, we had oral information from the examined patients that the two Australian families as well as one from the two US families originated from the Epirus region. In another recent paper from Croatia,<sup>25</sup> a small isolated village called Veli Brgud in the mountains of the Istrian peninsula

(population 550), with a previously reported high prevalence of early onset POAG, was investigated. Nine out of 16 POAG patients that were examined, carried the T377M *MYOC* mutation. There has been no haplotype analysis so far to link the above cases to the known “Greek” haplotype, but the distance from this village to the Epirus Region is about 800 km in straight line. It is conceivable that all these cases in southeastern Europe may point to a common founder, with family members migrating to different places of the region over the last several centuries.

**Table 3** Comparison of clinical features in T377M *MYOC* carriers with POAG versus other groups of individuals from Epirus, Greece

Groups	Mean (±SD) age at diagnosis (years)	Mean (±SD) age at examination (years) <sup>a</sup>	Mean (±SD) max IOP (mmHg) <sup>a</sup>	Mean (±SD) max cup/disc ratio <sup>a</sup>
T377M carriers with POAG (n = 15)	51.0 (13.9)	61.5 (17.5)	31.9 (8.5)	0.67 (0.2)
Sporadic POAG without T377M (n = 79)	63.1 (11.0)	70.0 (10.0)	28.1 (7.1)	0.60 (0.21)
Chrysovitsa POAG and OHT without T377M (n = 12) <sup>a</sup>	48.0 (16.9)	51.7 (17.3)	24.9 (4.2)	0.49 (0.28)
Familial POAG without T377M (n = 12)	66.8 (9.8)	76.4 (12.9)	29.1 (6.3)	0.69 (0.18)
Controls (n = 124)	–	74.2 (8.4)	14.6 (2.2)	0.25 (0.1)

<sup>a</sup>Four individuals were sporadic POAG cases and 8 were from the Chrysovitsa *GLC1C* family (3 with POAG and 5 with OHT).

**Abbreviations:** OHT, ocular hypertension; POAG, primary open-angle glaucoma.



**Figure 3** Map of Greece and bordering countries showing the location of the T377M MYOC carriers.

In summary, 2 isolated small Greek villages, known to be enriched for glaucoma, Chrysovitsa from our study and 1 at 300 km distant from it, were shown to harbor the T377M MYOC mutation on the same haplotype, a Greek founder mutation probably originating from the Epirus region. Furthermore, in our study, the mutation was found in relatively high proportion in familial POAG cases but seems rare among sporadic cases from the same region, while there was no mutation carrier found in the control group.

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## Disclosures

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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