ONLINE MUTATION REPORT

Prevalence of optineurin sequence variants in adult primary open angle glaucoma: implications for diagnostic testing

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Glaucoma, the leading cause of irreversible blindness world wide, affecting about 70 million people,[1 2] is characterised by progressive loss of optic nerve axons and visual field damage. As the condition is insidious, the diagnosis is often missed and the disease detected only later when patients have severe and irreversible visual impairment. Adult primary open angle glaucoma (POAG) is a major form of glaucoma world wide. Most POAG in white and Afro-Caribbean populations is of the high tension glaucoma (HTG) type, with raised intraocular pressure (IOP) being a major contributory factor for visual loss.[3–6] Normal tension glaucoma (NTG) is another important subtype of POAG in which typical glaucomatous cupping of the optic nerve head and visual field loss are present, but IOPs are consistently within the statistically normal population range. This accounts for about a third of all patients with POAG.[4–7]

Although the proportion of cases of glaucoma with a genetic basis has not been precisely defined, an increasing body of evidence derived from a range of populations indicates that glaucoma has a substantial heritable basis. It has been estimated that 20%–60% of patients with the disease have a family history, and under-reporting of a family history has been well documented in glaucoma.[8–11] In 1997, myocilin (*MYOC*, MIM 601652), located on chromosome 1q25,[12] was the first POAG gene to be characterised and found to be mutated in patients with juvenile and adult onset POAG.[13] Subsequent studies found that *MYOC* mutations account for fewer than 5% of cases of adult POAG,[13–17] with lower frequencies of *MYOC* mutations in Chinese and Japanese populations compared to white populations.[16 18] Rezaie *et al*[19] recently identified a second POAG gene, optineurin (*OPTN*, MIM 602432) in the GLC1E interval on chromosome 10p,[20] and showed that variations in this gene predominantly resulted in NTG. The most common *OPTN* mutation, Glu50 →Lys (E50K) was identified in 13.5% of families, 18% of whom had high IOP.[20] A second *OPTN* variant, Met98 →Lys (M98K) was identified in 13.6% of familial and sporadic cases of POAG compared to 2.1% of controls, making it a considerable risk associated genetic factor for glaucoma.[20] Such prevalence rates could potentially make the E50K/M98K variants more frequent than the Gln368Stop *MYOC* mutation, the most common mutation found in POAG, identified in 1.6% of unrelated glaucoma probands.[16] The purpose of this study was to determine the prevalence of these two *OPTN* sequence variants in a large cohort of unrelated British patients with adult onset POAG to assess the feasibility of developing diagnostic testing for these variants in patients with glaucoma.

Key points

- Recently, optineurin *(OPTN)* mutations were identified in patients with adult onset primary open angle glaucoma (POAG), a leading cause of irreversible blindness world wide. These included an E50K mutation in 13.5% of pedigrees, predominantly with normal tension glaucoma (NTG), and a M98K sequence variant in 13.6% of unrelated subjects.
- The purpose of this study was to investigate the prevalence of these two *OPTN* sequence variants in a large cohort of 315 unrelated British patients with glaucoma (132 NTG, 183 high tension glaucoma (HTG), and 95 control subjects).
- Using bidirectional sequencing and restriction enzyme analysis respectively, the E50K mutation was identified in two of 132 (1.5%) patients with NTG, none of 183 patients with HTG, and none of 95 control subjects, whereas the prevalence of the M98K variant was significantly higher in NTG (14/132, 10.6%) compared to HTG (8/183, 4.4%) (χ^2=4.6, p=0.03) or control subjects (3/95, 3.2%) (χ^2=4.4, p=0.04). Haplotype analysis of four single nucleotide polymorphisms within a 12 kb region of the *OPTN* gene showed that the changes arose independently.
- Our data indicate that E50K is a relatively infrequent cause of sporadic NTG in the white population in the United Kingdom, and the association of M98K with NTG but not HTG suggests genetic heterogeneity between these two phenotypes.

METHODS

Ascertainment of patients

Written informed consent was obtained from all subjects and the study was approved by the Moorfields Eye Hospital ethics committee. It was performed in accordance with the Helsinki Declaration. The patients comprised an unselected cohort of 315 unrelated white patients with adult onset POAG that included 186 women, with ages ranging from 52 to 81 years. All were from the greater London area and were attending tertiary referral glaucoma clinics at Moorfields Eye Hospital. Glaucoma was defined by the following strict criteria: the presence of typical glaucomatous optic neuropathy with compatible visual field loss, open drainage angles on gonioscopy, and absence of a secondary cause for glaucomatous optic neuropathy such as a previously raised IOP after trauma, a period of steroid administration, or uveitis. Patients also did not have

Abbreviations: HTG, high tension glaucoma; IOP, intraocular pressure; *MYOC*, myocilin; NTG, normal tension glaucoma; *OPTN*, optineurin; POAG, primary open angle glaucoma; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism

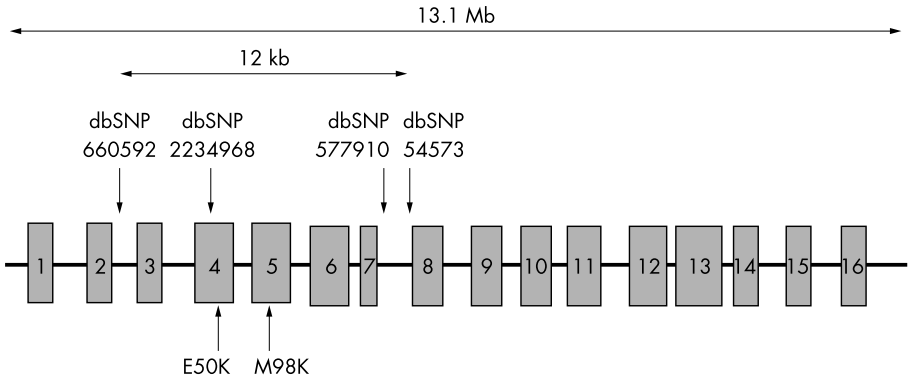

Figure 1 Optineurin gene: position of SNPs genotyped, in relation to E50K and M98K variants (not to scale). Exons are numbered in boxes.

evidence of high myopia or congenital abnormality, and had no other cause for their visual loss. Patients with NTG had a mean IOP without treatment that was consistently 21 mm Hg or less on diurnal testing, and patients with HTG had a mean IOP consistently above 21 mm Hg. Control DNA samples were obtained from 95 white spouses of probands participating in genetic research at the Hospital.

Molecular studies

Genomic DNA, extracted from venous blood using the Nucleon II extraction kit (Scotlab, Shelton, CT, USA), was subjected to 35 cycles of polymerase chain reaction (PCR) amplification using oligonucleotide primers in 50 µl reaction volumes (20 ng genomic DNA, 10 pmol of each primer, 200 mmol/l dNTPs, 1.5 mmol/l MgCl, and 2 units of *Taq* DNA polymerase (Promega, Madison, USA)). To detect the E50K mutation, exon IV of the *OPTN* gene was amplified using the primers (5'CAGGTGACTTTTCCACAGGA3') and (5'GATTTAGCATTTGGCAAGGC3'), and amplified exons purified with QuickStep columns (Edge Biosystems, Gaithersburg, MD, USA), then sequenced bidirectionally with fluorescent dideoxynucleotides (PE Biosystems, Foster City, USA) on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA) using standard conditions. To detect the M98K sequence variant, exon V of the *OPTN* gene was amplified using the primers (5'TCCACTTTCCTGGTGTGTGA3') and (5'CAGACCGATCCATTGTGATG3'). The 273 base pair polymerase chain amplification product was then digested with 1.0 U *Stu*I restriction enzyme (Promega, Corporation, Madison, WI, USA). The M98K nucleotide change resulted in the gain of the *Stu*I restriction site with the production of two fragments of 98 bp and 175 bp in size. For patients heterozygous for this change, three bands were produced (the third band being the 273 bp fragment).

Haplotype analysis: single nucleotide polymorphism (SNP) characterisation

To establish whether those with the E50K and M98K variants share a common ancestral haplotype or represent independent mutation events, four single nucleotide polymorphisms (SNPs) located within the *OPTN* gene were typed. The SNPs flanked E50K/M98K and were located within a 12 kb interval (fig 1). There were three non-coding SNPs with the respective reference dbSNP (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp) numbers (660592, 577910, and 545734) and one coding SNP (2234968).

The SNPs were characterised by minisequencing reactions. Briefly, this relies on extension of a primer that ends one base short of a polymorphic site, with fluorescent labelled dideoxy nucleotides, which are complementarily incorporated according to the sequence of the amplified target. The minisequencing reaction (5 µl SNaPshot Multiplex Ready Reaction reagent (Applied Biosystems, Foster City, CA, USA), 5 pmol of each primer, and 3 µl purified PCR product) was performed as follows: 25 cycles at 96°C for 10 seconds, 50°C for five seconds, and 60°C for 30 seconds. After extension, the samples were treated with shrimp alkaline phosphatase according to the manufacturer's protocol. The samples were electrophoresed on an automated ABI PRISM 3100 genetic analyser and analysed with ABI GeneScan 3.1 analysis software (Applied Biosystems). Size determinations were performed using the GeneScan-120 LIZ size calibrator with Genotyper Version 2 data collection software.

Table 1 Prevalence of E50K and M98K by subgroup

	E50K	χ2 test Odds ratio (95% CI)	M98K	χ2 test Odds ratio (95% CI)
HTG (n=183)	0 (0%) (0–2.0)	χ2=2.8, p=0.18 RR=1.02 (0.99 to 1.04)	8 (4.4%) (1.9–8.4)	χ2=4.6, p=0.03 OR=2.6 (1.1 to 6.4)
NTG (n=132)	2 (1.5%) (0.2–5.4)	χ2=1.5, p= 0.51 RR=1.02 (0.99 to 1.04)	14 (10.6%) (5.9–17.2)	χ2=4.4, p=0.04 OR=3.6 (1.02 to 13.0)
Controls (n=95)	0 (0%) (0–3.8)		3 (3.2%) (0.7–9.0)	

Table 2 Haplotypes of subjects with E50K and M98K variants

SNP	Alleles segregating with E50K change		Alleles segregating with M98K change		
660592	G/G	G/G	G/G	T/T	C/G
2234968	A/G	G/G	G/G	G/G	G/G
577910	G/G	A/A	G/G	A/A	A/A
545734	G/G	G/G	G/G	G/G	G/G

Statistical analysis

Genotypic frequencies among cases and controls were tested for significant differences using standard χ^2 analysis. Differences between odds ratios were tested for significance with a Breslow-Day test (version 8.02, SAS software package, SAS Institute, Cary, NC, USA).

RESULTS

A total of 315 patients with POAG (132 patients with NTG and 183 patients with HTG) and 95 control subjects were examined. Overall the E50K change was found in two out of 315 patients with POAG (0.6%; 95% confidence interval (95% CI) 0.08 to 2.3) and none out of 95 control subjects (χ^2=0.61, p=1.0). The M98K variant was found in 22 out of 315 patients with POAG (7.0%; 95% CI 4.4 to 10.4) and three out of 95 (3.2%; 95% CI 0.7 to 9.0) control subjects (χ^2=1.87, p=0.17).

The prevalence of the E50K change was two of 132 (1.5%; 95% CI 0.2 to 5.4) patients with NTG and none of 183 (0%; 95% CI 0 to 2) patients with HTG (table 1). The M98K variant was present in 14 of 132 (10.6%, 95% CI 5.9 to 17.2) patients with NTG, compared to eight of 183 (4.4%, 95% CI 1.9 to 8.4) patients with HTG (χ^2=4.6, p=0.03, odds ratio (OR)=2.6 (95% CI 1.1 to 6.4)) and three of 95 (3.2%, 95% CI 0.7 to 9.0) control subjects (χ^2=4.4, p=0.04, OR=3.6 (95% CI 1.02 to 13.0)). The difference in frequency of M98K between patients with HTG and control subjects was not significant (χ^2=0.17, p=1.0, OR =1.3 (95% CI 0.34 to 5.1)).

Haplotype analysis of a 12 kb region within the *OPTN* gene disclosed no common haplotype between patients with either the E50K or M98K variants (table 2).

DISCUSSION

Our data indicate that the E50K mutation is an infrequent cause of sporadic NTG, accounting for 1.5% of cases, and it is not associated with HTG in the United Kingdom population studied. This concurs with recent data from smaller studies of patients with POAG in the United States.[21 22] Based on these results, as only a small proportion of patients with NTG (95% CI 0.2 to 5.4%) would be expected to have the E50K change, diagnostic testing of sporadic cases of NTG for E50K is unlikely to be useful in the United Kingdom (and probably the United States) population. Similarly presymptomatic screening in the general population would be expected to yield an exceedingly low detection rate, as an abnormal test result would be rare and a normal test result would be meaningless. Commercially available kits such as the OcuGene test (InSite Vision), which screen for *MYOC* variations, have also been found to have low sensitivity for detecting mutations causing glaucoma,[17] and illustrate the current limitations of genetic testing in glaucoma.

Although the E50K mutation was found to be a rare cause of NTG, Rezaie *et al*[19] identified E50K in seven out of 52 families with POAG (13.5%), most of whom had NTG. The high prevalence of E50K in that study may be related to their investigation of only autosomal dominant NTG pedigrees. The 10-fold higher prevalence of E50K mutations in familial compared to sporadic cases (as found in this study) could also highlight the enrichment in inherited cases that is associated with a family history. Such a marked difference in prevalence between familial and sporadic cases supports the introduction of targeted diagnostic testing in those with a family history of NTG, although not at present in sporadic cases. As the cost of screening for known mutations declines with the continuing rapid advances in mutation detection techniques, it seems likely that the cost/benefit considerations of screening individual patients with NTG for common mutations such as E50K may become more favourable in the future.

The second sequence change studied, M98K, has previously been reported to be an attributable risk factor for POAG, present in 13.6% of both familial and sporadic POAG cases compared to 2.1% of controls.[19] However, our study did not identify an overall difference in prevalence between patients with POAG and controls, but did show that the M98K variant was associated specifically with NTG but not HTG. About 10% of patients with NTG were found to have this variant, compared to 4% of patients with HTG and 3% of controls. The difference in results between the two studies may be attributable to the panel of patients in the earlier study consisting of predominantly patients with NTG (only 13% of the patients with POAG used in that comparison actually had IOP values above normal).[19]

There is some controversy as to whether NTG and HTG are separate disease entities, or if they represent different ends of the phenotypic range of POAG. Whereas some studies have found that the conditions cannot be distinguished in terms of clinical behaviour or pathophysiology,[23–26] other reports have noted optic disc and visual field differences between patients with HTG and those with NTG.[27–31] The association of M98K with NTG but not HTG suggests genetic or allelic heterogeneity between these two phenotypes. Such genetic differences may imply different mechanisms of optic nerve damage, possibly by affecting susceptibility to factor(s) that mediate glaucoma.

In view of the high prevalence of M98K and that reported for E50K,[19] we investigated whether this was caused by a founder effect. Using intragenic SNPs spanning a 12 kb interval of the *OPTN* gene (fig 1), we identified three different M98K and two E50K haplotypes, indicating that these sequence changes arose independently.

A possible limitation of this study was that whereas the POAG population was well characterised using strict diagnostic criteria and is considered typical of patients with POAG, control subjects were not subjected to identical clinical examination. We cannot exclude the possibility that the patients with POAG may harbour other mutations or sequence changes in *OPTN*. Complete sequencing of the *OPTN* gene may show further mutations, and other differences between these groups. It is hoped that further research efforts are directed towards investigating the role of *OPTN* as an adult onset glaucoma gene.

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