EXTENDED REPORT

The role of the Met98Lys optineurin variant in inherited optic nerve diseases

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Aims: To investigate the role of the common *OPTN* Met98Lys variant as a risk allele in open-angle glaucoma (OAG), autosomal dominant optic atrophy (ADOA) and Leber's hereditary optic neuropathy (LHON).

Methods: The presence of the Met98Lys variant was determined in a total of 498 (128 with normal-tension glaucoma (NTG)) patients with OAG, 29 patients who had myocilin-related OAG, 101 patients from ADOA pedigrees, 157 patients from LHON pedigrees and 218 examined OAG age-matched normal controls.

Results: 17 of 218 (7.8%) controls had the Met98Lys variant. 28 (5.6%) patients with OAG were Met98Lys positive. More Met98Lys carriers were found in the NTG group than in the high-tension glaucoma (HTG) group ($p=0.033$). However, no significant difference was observed between the NTG and control cohorts ($p=0.609$). Two *MYOC* mutation carriers were found to have the variant. The variant was found in 1 of 10 pedigrees with ADOA and in 8 of 35 pedigrees with LHON.

Conclusion: Data from this study do not support a strong role for the *OPTN* Met98Lys variant in glaucoma, ADOA or LHON. However, a weak association was observed of the variant with NTG compared with that with HTG. Meta-analysis of all published data on the variant and glaucoma confirmed that the association, although weak, is highly statistically significant in the cohort with glaucoma versus controls.

Primary open-angle glaucoma (POAG) is the most common optic neuropathy.¹ Although POAG has a strong genetic influence, to date only two genes have been unequivocally identified as causing POAG.²⁻⁴ POAG can be subclassified into high-tension glaucoma (HTG), with intraocular pressure (IOP) increased to >21 mm Hg or normal-tension glaucoma (NTG). The *Myocilin* (*MYOC*) gene on chromosome 1q23 was the first gene identified as causing POAG,³ and typically manifests an HTG phenotype.⁵ *Optineurin* (*OPTN*), located at chromosome 10p13, was the second gene identified as causing POAG. *OPTN* has been shown to be mutated in some families with NTG,⁴ whereas investigations into other polymorphisms (in particular *OPTN* Met98Lys) have yielded conflicting results.⁴⁻⁹ In the original paper describing *OPTN*, it was proposed that the Met98Lys variant was very strongly associated with glaucoma.⁴ The Met98Lys allele alters the binding affinity¹⁰ of *OPTN* to RAB8, a protein which interacts with huntingtin protein. Furthermore, it has recently been reported that *OPTN* is associated with neural degeneration by metabotropic glutamate receptor signalling via interaction with huntingtin protein.¹¹

The hereditary optic atrophies are a heterogeneous group of conditions that cause loss of vision. Optic atrophy is characterised by optic nerve pallor owing to retinal ganglion cell axon degeneration and a loss of the surrounding microvasculature. POAG shares many phenotypic features with the hereditary optic atrophies. Recently, there has been renewed interest in the possible association between autosomal dominant optic atrophy (ADOA; OMIM: 165500) and NTG.¹² Although people with ADOA typically have poor visual acuity and reduced colour vision (not usually found in POAG), patients with both ADOA and NTG can have similar visual field defects, similar optic disc morphology, parapillary atrophy and an IOP <21 mm Hg.¹³

ADOA (Kjer's optic atrophy), the most common form of hereditary optic atrophy, has a prevalence varying between

1:10 000 and 1:50 000 in different populations.¹⁴⁻¹⁵ Dominant optic atrophy is a genetically heterogeneous disease, although most pedigrees have mutations of the *OPA1* gene on chromosome 3q28.¹⁶⁻¹⁷ A genetic association between NTG and the *OPA1* gene was suggested initially through work carried out by Aung *et al.*¹⁸⁻¹⁹ In a similar Caucasian cohort, Powell *et al.*²⁰ refined the association to an intronic *OPA1* polymorphism. However, this association was not replicated in a separate Korean cohort.²¹

Leber's hereditary optic neuropathy (LHON; OMIM: 535000) is the second leading cause of hereditary optic atrophy, with most affected patients being legally blind. Although the primary predisposing factor is a mutation in the mitochondrial DNA (mtDNA),²² thereby explaining the matrilineal inheritance for risk of visual loss, other genetic and environmental factors contribute to the expression of LHON. These include background mitochondrial haplotype, nuclear genes and environmental factors, such as drugs toxic to mitochondria.¹³

The underlying genetic predispositions for POAG, ADOA and LHON are probably proapoptotic and may overlap. Because *OPTN* is associated with both the apoptotic FAS ligand and the tumour necrosis factor α pathway,²³ dysfunction of *OPTN* could be deleterious to any mitochondrial disruption, whether due to primary mtDNA mutations or ADOA secondary *OPA1* changes. Given the overlap in the clinical features of ADOA, LHON and POAG, as well as the differing results relating to the relationship between *OPTN* polymorphisms in POAG, we investigated the role of the *OPTN* Met98Lys polymorphism as a risk factor in three large cohorts of hereditary optic neuropathies. Although other

Abbreviations: ADOA, autosomal dominant optic atrophy; GIST, Glaucoma Inheritance Study in Tasmania; HTG, high-tension glaucoma; IOP, intraocular pressure; LHON, Leber's hereditary optic neuropathy; mtDNA, mitochondrial DNA; NTG, normal-tension glaucoma; OAG, open-angle glaucoma; POAG, primary open-angle glaucoma

Table 1 Mitochondrial mutation, haplogroup and phenotype of *OPTN* Met98Lys carriers from pedigrees with Leber's hereditary optic neuropathy

Pedigree	mtDNA mutation	mtDNA haplogroup	Age at most recent examination (years)	Sex	Manifesting LOV	Age at LOV (years)
Vic2	14484T→C (heteroplasmy)	J	19	F	Yes	6
Vic2	14484T→C (heteroplasmy)	J	50	F	Yes	14
Vic2	14484T→C (Heteroplasmy)	J	26	F	Yes	17
Vic19	11778G→A	B	22	M	Yes	24
Qld1	11778G→A	T	41	M	Yes	28
Tas1	11778G→A	H	45	M	No	
Tas1	11778G→A	H	47	F	No	
Tas1	11778G→A	H	21	M	No	
Vic5	11778G→A	K	35	F	No	
Vic5	11778G→A	K	62	F	No	
Vic5	11778G→A	K	35	F	No	
Vic6	11778G→A	H	48	F	No	
Vic7	11778G→A	I	24	M	No	
WA3	11778G→A	I	30	M	No	

F, female; LOV, loss of vision; M, male; mtDNA, mitochondrial DNA.

OPTN sequence variations have been reported in glaucoma, they have not been evaluated in this study.

MATERIAL AND METHODS

Patient selection

Written informed consent was obtained from each patient and this study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions. Approval was obtained from the relevant Institutional Review Boards of the Royal Victorian Eye and Ear Hospital, Melbourne, Australia, and the Royal Hobart Hospital, Hobart, Australia.

Pedigrees with ADOA were recruited from southeastern Australia. A diagnosis of ADOA was made on the basis of clinical features including reduced visual acuity; characteristic colour vision and visual field abnormalities measured using a Humphrey Visual Field Analyser (Humphrey Instruments, San Leandro, California, USA); characteristic temporal or generalised optic disc pallor; and evidence of autosomal dominant inheritance of the disease on pedigree analysis. *OPA1* mutations had previously been identified in all pedigrees included in this study to ensure homogeneity and diagnostic accuracy in the cohort.²⁴

Pedigrees with LHON were recruited from the same geographical region as the patients with ADOA. A cohort of patients with LHON, from pedigrees that have been described previously and from whom DNA was available, were recruited.^{25–27} The age at which vision was lost was recorded for each patients affected with LHON. An mtDNA mutation had been previously identified in each patient or the mutation carrier status was inferred through genealogy.

Patients with POAG were recruited through the Glaucoma Inheritance Study in Tasmania (GIST). GIST subjects were derived from a predominantly Caucasian population. POAG was defined by the presence of the following in at least one eye: optic disc cupping (cup:disc ratio ≥ 0.7); or a 0.2 inter-eye disparity in cup:disc ratio; or focal rim notching with corresponding visual field loss. Visual field assessments were conducted using the Humphrey Visual Field Analyzer threshold 24–2 algorithm and graded as abnormal if the mean deviation or pattern standard deviation had a probability of normality of $<5\%$, or if the Glaucoma Hemifield Test was abnormal. Patients were required to have an open irideocorneal angle on gonioscopic examination. HTG was diagnosed in patients who had glaucoma and an untreated IOP >21 mm Hg. Participants diagnosed with NTG had never been found to have an applanation IOP measurement >21 mm Hg. A diagnosis of NTG or HTG was made by the treating ophthalmologist and this subclassification was then confirmed

in the GIST follow-up. The severity of the disease phenotype was determined in the POAG group by the age at diagnosis, severity of optic disc cupping and GIST severity score. In brief, the GIST score is a combined assessment of visual field severity, optic disc cupping and degree of IOP elevation.²⁸ GIST scores of 0.7, 0.8, 0.9 and 1.0 imply mild, moderate, severe or very severe disease, respectively. For a more detailed description of the derivation of the POAG diagnostic criteria or the GIST score, the reader is referred to Coote *et al.*²⁸

A subset of the POAG group had previously been identified as having a disease-causing mutation in the *MYOC* gene.^{29–30} Given the possibility for phenotypic modification effects between *MYOC* and *OPTN* and the fact that a proportion of the patients with *MYOC* were recruited from the same pedigree, the *MYOC* cohort was analysed separately from the NTG and HTG POAG groups.

Controls were recruited from the same geographical region and were matched by age to the POAG cohort. The control cohort comprised 152 people who resided in local retirement homes and 66 unrelated people who were recruited through an adjuvant genetic study. Controls were included if they were found on examination to be free of ocular hypertension, POAG and optic atrophy.

Laboratory techniques

For the *OPTN* gene screening, a 621 bp fragment spanning the Met98Lys mutation site was amplified using a Hybaid PCR Express Thermal Cycler (Hybaid, Ashford, Middlesex, UK). Primer sequences were as follows: F: 5'-GACCAGGCAAAACACCAATCC-3' and R: 5'-CCTTAGCTCCTAGTAACCATAG-3' (Geneworks, Adelaide, South Australia, Australia). After PCR restriction, digest was carried out on the products using the *StuI* enzyme. The 603T→A nucleotide change present in the Met98Lys polymorphism carriers creates a *StuI* restriction site, thus resulting in digested fragments of 416 bp and 205 bp, respectively.

Statistical analysis

Allele frequencies were analysed using the χ^2 test through Intercooled Stata V.7.0. The non-parametric Kruskal–Wallis test was used to examine the disease severity in patients with POAG. Unless otherwise indicated, data are presented as mean (standard deviation (SD)).

RESULTS

OPTN Met98Lys in controls

In all, 218 (151 female) controls, who had no clinical sign of optic atrophy on examination, were recruited. The mean (SD)

Table 2 Participants who did not carry the *OPTN* Met98Lys variant but who were from pedigrees with Leber's hereditary optic neuropathy in which other family members did carry the variant

Pedigree	mtDNA mutation	mtDNA Haplogroup	n	Mean (SD) age at examination (years)	Male, n (%)	Manifesting LOV, n	Mean (SD) age at LOV (years)
Vic2	14484T→C (heteroplasmy)	J	1	49	1 (100)	1	17
Vic19	11778G→A	B	0	—	0 (0)	0	—
Qld1	11778G→A	T	4	26.5 (15.70)	3 (75.0)	4	22.5 (14.9)
Tas1	11778G→A	H	43	43.7 (23.3)	14 (32.6)	7	25.9 (10.8)
Vic5	11778G→A	K	3	29.7 (3.1)	2 (66.7)	1	14
Vic6	11778G→A	H	1	28	1 (100)	0	—
Vic7	11778G→A	I	1	60	0 (0)	0	—
WA3	11778G→A	I	2	36.0 (24.0)	1 (50.0)	0	—

LOV, loss of vision; mtDNA, mitochondrial DNA.

age at recruitment was 70.9 (16.6) years. Of them, 17 (7.8%) controls were found to have the *OPTN* Met98Lys variant in the heterozygotic state.

OPTN Met98Lys in pedigrees with ADOA

A total of 101 patients from 10 genealogically separate ADOA pedigrees were recruited, of whom 61 (60.4%) were female. Sixty three (62.4%) were known to carry an *OPA1* disease-causing mutation and were 45.5 (18.2) years old at examination. The mean (SD) age across all pedigrees for onset of ADOA symptoms was 15.2 (15) years (range 4–55).

The *OPTN* Met98Lys change was found in one family (ADOA Vic12), which harboured a splice site mutation in intron 9 (c985–1 g→a) of the *OPA1* gene and had severe ADOA disease expression. Four of the five *OPA1* mutation carriers in this pedigree were found to have the *OPTN* Met98Lys change. However, all the five *OPA1* mutation carriers had clinical manifestations of ADOA beginning in the first decade of life. The age range for the cohort at most recent examination was 34–67 years (mean 45.4), and at the time of examination, the mean visual acuity was 6/60 (range 6/24 to count fingers). No other pedigrees with ADOA were found to carry the *OPTN* Met98Lys variant.

OPTN Met98Lys in patients with LHON

A total of 157 patients from 35 genealogically separate LHON pedigrees were recruited. The mean (SD) age at review was 38.5 (19.8) years. Fifty nine (37.5%) patients were diagnosed with visual loss from LHON, of whom 15 (25.4%) were female. The age across all pedigrees for loss of vision was 24.1 (2.6) years.

Of the 59 patients diagnosed with LHON, 5 (8.5%) were found to be positive for Met98Lys. The proportion of affected Met98Lys carriers did not differ from those who did not manifest disease (9/98; $p = 0.89$) or from the frequency in the normal population ($p = 0.92$).

To further investigate if this variant influenced the severity of the LHON phenotype, all patients who were found to have the *OPTN* Met98Lys variant were reviewed with respect to the individual mtDNA variation (table 1). The phenotype did not differ markedly from those in the same pedigrees who did not have the *OPTN* Met98Lys variant (table 2). It is noteworthy that three women in one pedigree (LHON Vic2) were heteroplasmic for the mtDNA 14484T→C mutation, were heterozygotic for *OPTN* Met98Lys and manifested severe visual loss at young ages. Heteroplasmy for the mtDNA 14 484T→C mutation would normally confer a low risk of visual loss, particularly in women.³¹

OPTN Met98Lys in POAG

In all, 498 patients with POAG were studied, of whom 128 (25.7%) were classified as having NTG. There were 222 (60.2%) and 82 (64.1%) women in the HTG and NTG groups, respectively. The mean (SD) age at diagnosis for the overall

POAG cohort was 62.9 (12) years (table 3). Compared with the HTG group, patients with NTG were diagnosed at an older age ($p = 0.005$) and had larger cup:disc ratios in their worst eye ($p = 0.001$).

Twenty eight patients with POAG were identified as having the *OPTN* Met98Lys variant. Analysing the POAG group by the presence of the *OPTN* Met98Lys variant showed that there was a trend towards a lower maximum mean (SD) IOP (24.0 (8.3) v 20.9 (4.4); $p = 0.072$). The presence of the Met98Lys variant in the POAG group did not influence age at diagnosis, GIST severity score or maximum cup:disc ratio in the worst eye (table 3).

We found significantly more *OPTN* Met98Lys carriers in the NTG group (12/128; 9.4%) than in the HTG group (16/370; 4.3%; $p = 0.033$). This association was, however, not significant after Bonferroni correction. In addition, there was no significant difference between the NTG and control cohorts ($p = 0.609$). The difference in frequency between the HTG group and control group approached significance ($p = 0.077$). The presence of the *OPTN* Met98Lys variant did not seem to modify the POAG phenotype in either the HTG or NTG subgroups (table 3).

OPTN Met98Lys in MYOC POAG

Twenty nine patients (15 female) who had the *MYOC* Gln368Stop mutation and POAG were recruited from 14 pedigrees, and 16 patients (10 female) who had POAG with the *MYOC* Thr377Met mutation were recruited from three pedigrees.

Two of the *MYOC* Gln368Stop mutation carriers were found to also carry the *OPTN* Met98Lys variant and had maximum recorded IOPs of 22 and 24 mm Hg, respectively, with corresponding maximal cup:disc ratios of 0.7 and 0.8, respectively. The maximum recorded mean (SD) IOP and cup:disc ratio in the *MYOC* Gln368Stop mutation carriers who did not have the *OPTN* Met98Lys variant were 33.8 (10.5) and 0.81 (0.17) mm Hg, respectively. One patient who had both the Thr377Met *MYOC* mutation and the *OPTN* Met98Lys variant was found to have a maximum recorded IOP of 24 mm Hg and a maximum cup:disc ratio of 0.8 in the worst eye. The maximum recorded mean (SD) IOP and cup:disc ratio in the *MYOC* Thr377Met mutation carriers who did not have the *OPTN* Met98Lys variant were 28.5 (7.6) and 0.82 (0.17) mm Hg, respectively. However, analysis of pooled *MYOC* mutation carriers showed no significant difference in maximal recorded IOP ($p = 0.066$) or cup:disc ratios ($p = 0.283$) in the Met98Lys carriers.

DISCUSSION

Investigation of the *OPTN* Met98Lys polymorphism in POAG has yielded conflicting findings. Although in our study more *OPTN* Met98Lys carriers were found in the NTG group than in the HTG group ($p = 0.033$), this finding did not remain significant after correction for multiple testing. Importantly,

Table 3 Phenotypic breakdown of patients with primary open-angle glaucoma with and without the *OPTN* Met98Lys variant

	n	Mean (SD) age at examination	p Value	Mean (SD) age at diagnosis	p Value	Mean (SD) GIST severity score	p Value	Mean (SD) maximum IOP	p Value	Mean (SD) maximum CDR	p Value
POAG without Met98Lys	470	72.5 (10.9)	0.49	62.8 (12.1)	0.24	0.85 (0.11)	0.27	24.0 (8.3)	0.07	0.78 (0.16)	0.76
POAG with Met98Lys	28	73.9 (8.9)		65.7 ± 10.1		0.83 (0.12)		20.9 (4.4)		0.77 (0.14)	
HTG without Met98Lys	354	72.0 (11.06)	0.55	61.9 ± 12.2	0.74	0.86 (0.11)	0.28	26.1 (8.3)	0.71	0.77 (0.17)	0.66
HTG with Met98Lys	16	71.6 (7.1)		63.7 ± 7.7		0.83 (0.13)		24.4 (3.4)		0.76 (0.16)	
NTG without Met98Lys	116	74.2 (10.5)	0.22	65.4 ± 11.3	0.30	0.83 (0.10)	0.91	16.9 (2.7)	0.78	0.83 (0.12)	0.14
NTG with Met98Lys	12	77.1 (10.5)		68.1 (12.3)		0.83 (0.10)		17.3 (1.4)		0.79 (0.10)	
Overall (POAG)*	498	72.6 (10.8)		62.9 (12.0)		0.85 (0.11)		23.8 (8.2)		0.78 (0.16)	

CDR, worst eye cup:disc ratio; GIST, Glaucoma Inheritance Study in Tasmania; HTG, high-tension glaucoma; IOP, intraocular pressure; NTG, normal-tension glaucoma; POAG, primary open-angle glaucoma;.

*Excluding myocilin mutation carriers.

we observed no significant difference in carrier frequency between the NTG and control cohorts ($p = 0.609$). We carried out a meta-analysis of all published work to date on the *OPTN* Met98Lys variant in glaucoma. A weak but highly significant association with POAG was found (odds ratio (OR) 1.35; 95% confidence interval (CI) 1.16 to 1.58; $p = 0.001$; fig 1).^{4 6-9 32-42} This finding remains significant after exclusion of the original study⁴ describing the positive association (OR 1.29; 95% CI 1.10 to 1.51; $p = 0.001$).

With the numbers available in this study, a significant difference was not reached between maximal recorded mean (SD) IOP and presence of the *OPTN* Met98Lys variant (24.0 (8.3) for non-carriers v 20.9 (4.4) for carriers; $p = 0.07$). The response of *OPTN* expression to increased IOP remains controversial.^{43 44} Melki *et al*³³ found that patients positive for Met98Lys have a significantly lower IOP than *MYOC* mutation carriers who do not have the Met98Lys variant. It should, however, be noted that *MYOC* mutations are almost invariably associated with substantially raised IOP.⁵ We found that our double mutant patients tended to have lower IOP than their single *MYOC* mutation-carrying counterparts. Such a finding may explain the previous case report

describing an NTG *MYOC* mutation patient, and adds to the emerging evidence for gene–gene interaction in POAG.^{45 46}

Throughout our whole series, no patient was found to be homozygotic for *OPTN* Met98Lys. The *OPTN* Met98Lys variant was found in one ADOA family who harboured a splice site mutation in intron 9 (c985–1 g→a) of the *OPA1* gene and had severe disease expression. This *OPA1* mutation abolishes the acceptor splice site and results in either skipping exon 10 entirely, with no frameshift subsequently in exon 11, or a frameshift with a premature stop at the first codon of exon 10. It has been described in other studies, which have not commented on the disease severity.⁴⁷ Given that only four of the five *OPA1* mutation carriers in this two-generation pedigree were found to have the *OPTN* Met98Lys change, no firm conclusions can be made regarding its effect on disease severity. Incomplete disease-gene segregation can occur in complex heterogeneous diseases due to the multiple additive effects of minor risk alleles.²

From our large LHON cohort of 35 genealogically separate pedigrees with LHON, the Met98Lys variant was identified in 14 (8.9%) patients, five of whom manifested the disease. The variant was not more common in those who manifested

Figure 1 Meta-analysis of all published case-control studies of the *OPTN* Met98Lys variant in open-angle glaucoma. Diamonds represent odds ratio (OR) point estimates, with the dashed and solid bars indicating the respective 95% CI. Dashed bars indicate patients with normal-tension glaucoma, solid bars indicate pooled case patients with normal or high-tension glaucoma. Note that the overall analysis has an OR > 1.

visual loss. However, it was unexpected to find females heteroplasmic for mtDNA14484T→C manifesting visual loss.

Conclusion

Our large study of the *OPTN* Met98Lys variant in optic neuropathies found no major gene effect. However, a trend towards an association with NTG was again found, prompting a meta-analysis of all published work examining this variant in POAG. This disclosed a weak but highly significant association with POAG. It is expected that multiple genetic modifiers may act collectively to influence optic nerve diseases. Very large well-characterised cohorts of cases and matched controls are required to dissect these potentially additive complex interactions, which are unlikely to segregate in typical mendelian patterns.

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