SCIENTIFIC CORRESPONDENCE

The phenotype of normal tension glaucoma patients with and without *OPA1* polymorphisms

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Aim: Polymorphisms in *OPA1*, the gene responsible for autosomal dominant optic atrophy, were recently found to be strongly associated with normal tension glaucoma (NTG). The aim of this study was to determine whether *OPA1* polymorphisms affect the phenotype of NTG patients.

Methods: A retrospective analysis was performed of 108 well characterised NTG patients who had been genotyped for *OPA1* variations, and who had previously undergone automated perimetry and Heidelberg retina tomography (HRT). 25 NTG patients had the at-risk *OPA1* genotype (IVS 8 +4 C/T; +32 T/C) and 83 NTG patients did not. Differences between groups were sought in a wide range of structural, psychophysical, and demographic factors. These included sex, age at diagnosis, family history of glaucoma, history of ischaemic risk factors and vasospasm, laterality of glaucoma, presenting and highest diurnal intraocular pressure (IOP), initial cup-disc (CD) ratio, baseline visual field global indices, and optic disc parameters as measured by HRT. For a subgroup of patients with at least 5 years of follow up and 10 visual field tests, point-wise linear regression analysis (PROGRESSOR for Windows software) was applied to the visual field series.

Results: There was no significant difference in the two groups with respect to sex, age at diagnosis, family history of glaucoma, history of ischaemic risk factors and vasospasm, or laterality of glaucoma. The comparison of IOP, CD ratio and visual field global indices, MD and CPSD in the two groups showed no significant difference. There were no differences in the mean values for any of the HRT parameters analysed. For the subgroup of patients with at least 5 years of follow up, there was also no significant difference in the number of patients with progressing locations, the mean number of progressing locations per subject, the mean slope of the progressing locations or the mean slope for whole visual field.

Conclusions: The absence of phenotypic differences in normal tension glaucoma patients with and without the *OPA1* polymorphisms IVS 8 +4 C/T; +32 T/C suggest that these *OPA1* polymorphisms do not underlie any major phenotypic diversity in these patients.

Glaucoma, the leading cause of irreversible blindness worldwide,^{1,2} is typified by progressive loss of optic nerve axons and visual field damage. Normal tension glaucoma (NTG) is an important subtype of glaucoma in which intraocular pressures (IOP) are within the statistically normal population range, and accounts for approximately a third of all primary open angle glaucoma cases.^{3–6} Although there is evidence for the role of genetic factors in NTG,^{7–10} the molecular mechanisms underlying NTG are unknown. The discovery of optineurin as a causative gene suggests that one

mechanism producing NTG may involve apoptosis of retinal and optic nerve ganglion cells.¹⁰

Autosomal dominant optic atrophy (ADOA) is another condition with slowly progressive optic nerve damage.^{11–14} In ADOA patients, excavation of the optic nerve may develop, and such patients are occasionally misdiagnosed as having NTG.¹⁵ Mutations in *OPA1* (MIM No 605290), the gene responsible for ADOA, were recently found to result in primary degeneration of retinal ganglion cells.¹⁶ The similarities between glaucoma and ADOA (in terms of the cell type affected and the phenotype) make *OPA1* a good candidate gene for glaucoma, particularly NTG.

A recent study found an association between polymorphisms in the *OPA1* gene and NTG.¹⁷ Approximately 20% of NTG subjects were found to carry two single nucleotide polymorphisms (SNPs) on intervening sequence (IVS) eight of the *OPA1* gene (IVS 8 +4 C/T; +32 T/C), compared to only 3.7% of control subjects ($\chi^2 = 22.04$, $p = 0.00001$), indicating that this genotype is strongly associated with NTG, and may represent a diagnostic marker for the disease.¹⁷ This observation raises the possibility that different subgroups of NTG exist, one of which is distinguished by genetic variations in *OPA1*. The aim of this study was to compare clinical features of NTG patients with and without such polymorphisms in order to determine whether *OPA1* polymorphisms impart a characteristic phenotype in NTG patients.

METHODS

A retrospective analysis was performed of 108 white NTG patients who had been previously genotyped for *OPA1* variations. There were 25 NTG patients (group 1) with, and 83 NTG patients (group 2) without the at-risk *OPA1* genotype IVS 8 +4 C/T; +32 T/C. These patients attended a tertiary referral glaucoma clinic and had undergone various investigations including automated perimetry and Heidelberg retina tomography (HRT, Heidelberg Engineering, Heidelberg, Germany). NTG cases were defined by the following strict criteria¹⁸: the presence of typical glaucomatous optic neuropathy with compatible visual field loss; mean IOP without treatment that was consistently equal to or less than 21 mm Hg on diurnal testing, open drainage angles on gonioscopy, and absence of a secondary cause for glaucomatous optic neuropathy, such as a previously raised IOP following trauma, a period of steroid administration or uveitis. Only one eye from each patient was included. For bilateral cases, the right eye was analysed. The study had the approval of the Moorfields Eye Hospital ethics committee and was performed in accordance with the Helsinki Declaration.

Patients' hospital records were reviewed and the following data collected: demographic characteristics including sex and age at diagnosis; family history of glaucoma; history of ischaemic risk factors such as hypertension, diabetes mellitus, ischaemic heart disease and smoking; history of vasospasm such as migraine and cold hands and feet; the presenting and

Table 1 Demographic features and systemic history

	Group 1 (n=25)	Group 2 (n=83)	p Value
Sex			
Male	8	21	0.51
Female	17	62	
Age of onset			
<60 years	11	31	0.67
>60 years	14	52	
Family history of glaucoma			
Positive	7	28	0.59
Negative	18	55	
Ischaemic risk factors			
Positive	12	33	0.46
Negative	13	50	
Vasospasm			
Positive	7	17	0.41
Negative	18	67	
Laterality			
Bilateral	19	65	0.81
Unilateral	6	18	

Table 2 Presenting clinical features

	Group 1 (n=25)	Group 2 (n=83)	p Value
Mean presenting IOP (mm Hg)	16.2 (1.8)	17.0 (2.8)	0.11
Mean highest diurnal IOP (mm Hg)	17.9 (2.3)	18.9 (2.6)	0.06
Mean presenting cup disc ratio	0.75 (0.1)	0.76 (0.1)	0.93
Mean presenting MD (dB)	-8.7 (8.4)	-8.0 (6.5)	0.96
Mean presenting CPSD (dB)	8.5 (4.8)	8.0 (4.3)	0.60

IOP = intraocular pressure, MD = mean deviation, CPSD = corrected pattern standard deviation.

highest recorded diurnal intraocular pressure (IOP); cup-disc (CD) ratio at presentation; and interocular symmetry of glaucoma.

Visual field analysis

Subjects underwent static automated white on white threshold perimetry (program 24-2, model 640, Humphrey Instruments, Dublin, CA, USA). The first visual field test for all subjects was discarded from the analyses to allow for learning effects, and the subsequent first reliable visual field was used as the baseline. The global indices such as mean deviation (MD) and corrected pattern standard deviation (CPSD) of the baseline visual fields were analysed for all cases.

The visual fields of a subgroup of 88 subjects (18 from group 1 and 70 from group 2) who had at least 5 years of follow up with at least 10 visual field tests performed during this time were also analysed for progression. Pointwise linear regression analysis was applied to the field series of each of these subjects using PROGRESSOR for Windows software.¹⁹ Progression was defined as the presence of a significant regression slope

($p < 0.01$) showing 1 dB per year or more of sensitivity loss at the same test location with the addition of two out of three successive field tests to the series starting with the first three. The mean number of progressing locations per subject, the mean slope for the progressing locations as well as the mean slope of the whole visual field per year was evaluated.

Optic disc analysis

The Heidelberg retina tomograph (HRT, Heidelberg Engineering, Heidelberg, Germany) was used to image the optic disc and the baseline optic disc parameters were analysed. The mean topography of three images was generated in the 10×10 degree frame and the disc edge delineated on the mean image by a single trained observer (DP), using a drawn contour line. Images with significant movement artefact were rejected. Global and segmental disc and cup areas were analysed directly by means of HRT software (version 2.01b) using the standard reference plane. Rim area was calculated by subtracting the cup area from the disc area. Six predefined segments were used (0 degrees always temporal, 90 degrees always superior): temporal quadrant (-45 to $+45$ degrees), temporal superior octant ($+45$ to $+90$ degrees), temporal inferior octant (-90 to -45 degrees), nasal quadrant ($+135$ to $+225$ degrees), nasal superior octant ($+45$ to $+90$ degrees), and nasal inferior octant (-135 to -90 degrees).

Statistics

Statistical analysis was carried out using Statistical Package for Social Sciences version 9.0 (SPSS Inc, Chicago, IL, USA). Parametric and non-parametric tests of significance were carried out where appropriate. Comparisons between groups were done with Mann-Whitney U tests for continuous variables that were not normally distributed. χ^2 analysis was used for comparison of proportions. Statistical significance was assumed at the $p < 5\%$ level.

RESULTS

There was no significant difference in the two groups with respect to demographic factors such as sex and age at diagnosis. There was also no significant difference with respect to family history of glaucoma, history of ischaemic risk factors, history of vasospasm, or laterality of glaucoma (Table 1).

The comparison of IOP, CD ratio and visual field global indices, MD, and CPSD in the two groups is summarised in Table 2. There was no significant difference found although the difference in mean highest diurnal IOP between the two groups approached significance ($p = 0.06$).

The mean HRT parameters are summarised in Table 3. There were no differences in the mean values for any parameter analysed.

Comparing the visual fields of the subgroup of 88 subjects (18 from group 1 and 70 from group 2) who had at least 5 years of follow up (with at least 10 visual field tests performed during this time) revealed no difference in the number of patients with progressing locations, the mean number of progressing locations per subject, the mean slope of the progressing locations, or the mean slope for whole visual field (Table 4).

Table 3 Presenting optic disc parameters, as measured by HRT

	Group 1 (n=25)	Group 2 (n=83)	p Value
Disc area (mm ²)	2.04 (0.41)	2.11 (0.45)	0.77
Global rim area (mm ²)	0.90 (0.31)	0.89 (0.31)	0.88
Temporal rim area (mm ²)	0.13 (0.07)	0.14 (0.07)	0.57
Temporal superior rim area (mm ²)	0.10 (0.04)	0.10 (0.05)	0.80
Temporal inferior rim area (mm ²)	0.10 (0.08)	0.08 (0.06)	0.36
Nasal rim area (mm ²)	0.29 (0.12)	0.30 (0.11)	0.49
Nasal superior rim area (mm ²)	0.13 (0.06)	0.14 (0.05)	0.63
Nasal inferior rim area (mm ²)	0.16 (0.06)	0.13 (0.06)	0.14

Table 4 Visual field progression of subjects with at least 5 years of follow up and at least 10 visual fields performed during this time

	Subgroup 1 (n=18)	Subgroup 2 (n=70)	p Value
Number of patients with progressing locations	16 (88.9%)	54 (77.1%)	0.98
Mean number of progressing locations per subject	7.89 (14.67)	7.28 (7.77)	0.46
Mean slope of progressing locations per year (dB/year)	-0.51 (0.91)	-0.37 (0.56)	0.91
Mean slope for whole visual field per year (dB/year)	-1.99 (1.62)	-1.92 (1.13)	0.39

DISCUSSION

This study did not detect a significant difference in a range of phenotypic features in normal tension glaucoma patients with and without the *OPA1* polymorphisms IVS 8 +4 C/T; +32 T/C. This suggests that these specific genetic variations in *OPA1* do not underlie any major phenotypic diversity in NTG, although the possibility of more subtle phenotypic differences, such as variable rates of response to treatment cannot be excluded. The mean presenting IOP and the highest diurnal IOP appeared to be lower in NTG patients with the *OPA1* polymorphisms (almost reaching significance for the latter), which may indicate that the glaucoma in such patients is less IOP dependent. If this were to be the case, it would suggest that NTG patients have different IOP thresholds for glaucomatous damage to occur, and that those with *OPA1* polymorphisms may be at risk of glaucoma at lower IOP levels.

Polymorphisms are associated with a variety of other diseases including Alzheimer's dementia,²⁰ age related macular degeneration,²¹ diabetes mellitus,²² and schizophrenia.²³ Although intronic polymorphisms, of which *OPA1* (IVS 8 +4 C/T and +32 T/C) is an example, are associated with conditions like intracerebral haemorrhages and cerebral aneurysms,²⁴ little is known about how intronic polymorphisms influence disease phenotype. Possible mechanisms include regulation of transcription,^{25–26} effects on protein function, or indirectly by conferring susceptibility in patients to other factor(s) that mediate disease.¹⁷

The biochemical mechanisms by which *OPA1* may influence NTG remain obscure. The pathogenic characteristics of *OPA1* resemble those of Leber hereditary optic neuropathy, which results from a defect of the mitochondrion. Alexander *et al* hypothesised that mutations in the *OPA1* gene affect mitochondrial integrity, resulting in an impairment of energy supply.¹⁶ Occurring in the highly energy demanding neurons of the optic nerve, notably the papillomacular bundle, this would presumably lead to damage of retinal ganglion cells and visual loss.

A variety of factors may contribute to the development of optic neuropathy in glaucoma. In the apparent absence of elevated IOP, which is the main risk factor identified for glaucoma, non-IOP related factors are advocated to predominate in eyes with NTG including abnormal blood flow,^{27–29} systemic hypotension,^{30–32} and an abnormal coagulability profile.^{33–36} A strong genetic component is likely to be significant in NTG.^{7–10} It is hypothesised that several interacting genes contribute to the development of disease, with the putative role of each polymorphic sequence variation influenced by an individual's genetic and environmental background. However the number and identity of genes contributing to NTG has yet to be fully determined. Much remains to be learned about the phenotypic effects of specific genes and alleles in this condition. It is hoped that further research efforts will be directed towards investigating the association of *OPA1* polymorphisms found in a substantial proportion of NTG patients, as it may lead to greater understanding of the factors underlying this important cause of blindness.

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REFERENCES

- Thylefors B, Negrel AD, Pararajasegaram R, *et al*. Global data on blindness. *Bull World Health Organ* 1995;**73**:115–21.
- Quigley HA. Number of people with glaucoma worldwide. *Br J Ophthalmol* 1996;**80**:389–93.
- Shiose Y, Kitazawa Y, Tsukahara S, *et al*. Epidemiology of glaucoma in Japan: a nationwide glaucoma survey. *Jpn J Ophthalmol* 1991;**35**:133–5.
- Sommer A, Tielsch JM, Katz J, *et al*. Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans: the Baltimore Eye Survey. *Arch Ophthalmol* 1991;**109**:1090–5.
- Klein BE, Klein R, Sponsel WE, *et al*. Prevalence of glaucoma. The Beaver Dam Eye Study. *Ophthalmology* 1992;**99**:1499–504.
- Bonomi L, Marchini G, Marraffa M, *et al*. Prevalence of glaucoma and intraocular pressure distribution in a defined population. The Egna-Neumarkt Study. *Ophthalmology* 1998;**105**:209–53.
- Bennett SR, Alward WL, Folberg R. An autosomal dominant form of low tension glaucoma. *Am J Ophthalmol* 1989;**108**:238–44.
- Stoilova D, Child A, Trifan OC, *et al*. Localization of a locus (GLC1B) for adult onset primary open angle glaucoma to the 2cen-q13 region. *Genomics* 1996;**36**:142–50.
- Sarfazli M, Child A, Stoilova D, *et al*. Localization of the fourth locus (GLC1E) for adult onset primary open angle glaucoma to the 10p15-p14 region. *Am J Hum Genet* 1998;**62**:641–52.
- Rezaie T, Child A, Hitchings R, *et al*. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science* 2002;**295**:1077–9.
- Votruba M, Fitzke FW, Holder GE, *et al*. Clinical features in affected individuals from 21 pedigrees with dominant optic atrophy. *Arch Ophthalmol* 1988;**106**:351–8.
- Brown J, Fingert JH, Taylor CM, *et al*. Clinical and genetic analysis of a family affected with dominant optic atrophy (OPA1). *Arch Ophthalmol* 1997;**115**:95–9.
- Johnston RL, Seller MJ, Behnam JT, *et al*. Dominant optic atrophy. Refining the diagnostic criteria in light of genetic linkage studies. *Ophthalmology* 1999;**106**:123–8.
- Johnston PB, Gaster RN, Smith VC, *et al*. A clinicopathologic study of autosomal dominant optic atrophy. *Am J Ophthalmol* 1979;**88**:868–75.
- Fournier AV, Damji KF, Epstein DL, Pollock SC. Disc excavation in dominant optic atrophy. Differentiation from normal tension glaucoma. *Ophthalmology* 2001;**108**:1595–602.
- Alexander C, Votruba M, Pesch UE, *et al*. A gene encoding a dynamin-related GTPase is mutated in autosomal dominant optic atrophy (OPA1) linked to chromosome 3q28. *Nat Genet* 2000;**26**:211–5.

- 17 Aung T, Ocaka L, Ebenezer N, et al. A major marker for normal tension glaucoma: association with polymorphisms in the OPA1 gene. *Hum Genetics* 2002;**110**:52–6.
- 18 Kamal D, Hitchings R. Normal tension glaucoma- a practical approach. *Br J Ophthalmol* 1998;**82**:835–40.
- 19 Fitzke FW, Hitchings RA, Poinoosawmy D, et al. Analysis of visual-field progression in glaucoma. *Br J Ophthalmol* 1996;**80**:40–8.
- 20 Bullido MJ, Artiga MJ, Recuero M, et al. A polymorphism in the regulatory region of APOE associated with risk for Alzheimer's dementia. *Nat Genet* 1998;**18**:69–71.
- 21 Allikmets R. Further evidence for an association of ABCR alleles with age-related macular degeneration. The International ABCR Screening Consortium. *Am J Hum Genet* 2000;**67**:487–91.
- 22 Horikawa Y, Oda N, Cox NJ, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 2000;**26**:163–75.
- 23 Wei J, Hemmings GP. The *NOTCH4* locus is associated with susceptibility to schizophrenia. *Nat Genet* 2000;**25**:376–7.
- 24 Takenaka K, Sakai H, Yamakawa H, et al. Polymorphism of the endoglin gene in patients with intracranial saccular aneurysms. *J Neurosurg* 1999;**90**:935–8.
- 25 Cruts M, Backhovens H, Martin JJ, van Broeckhoven C. Genetic analysis of the cellular oncogene fos in patients with chromosome 14 encoded Alzheimer's disease. *Neurosci Lett* 1994;**174**:97–100.
- 26 Bailly S, Israel N, Fay M, Gougerot-Pocidal MA, Duff GW. An intronic polymorphic repeat sequence modulates interleukin-1 alpha gene regulation. *Mol Immunol* 1996;**33**:999–1006.
- 27 Drance SM, Sweeney VP, Morgan RW, Feldman F. Studies of factors involved in the production of low tension glaucoma. *Arch Ophthalmol* 1973;**89**:457–65.
- 28 Phelps CD, Corbett JJ. Migraine and low-tension glaucoma. A case-control study. *Invest Ophthalmol Vis Sci* 1985;**26**:1105–8.
- 29 Drance SM, Douglas GR, Wijsman K, et al. Response of blood flow to warm and cold in normal and low-tension glaucoma patients. *Am J Ophthalmol* 1988;**105**:35–9.
- 30 Hayreh SS, Zimmerman MB, Podhajsky P, et al. Nocturnal arterial hypotension and its role in optic nerve head and ocular ischaemic disorders. *Am J Ophthalmol* 1994;**117**:603–24.
- 31 Meyer JH, Brandt-Dohrn J, Funk J. Twenty four hour blood pressure monitoring in normal tension glaucoma. *Br J Ophthalmol* 1996;**80**:864–7.
- 32 Graham SL, Drance SM, Wijsman K, et al. Ambulatory blood pressure monitoring in glaucoma: the nocturnal dip. *Ophthalmology* 1995;**102**:61–9.
- 33 Drance SM. some factors in the production of low tension glaucoma. *Br J Ophthalmol* 1972;**56**:229–42.
- 34 Carter CJ, Brooks DE, Doyle DL, Drance SM. Investigations into a vascular etiology for low-tension glaucoma. *Ophthalmology* 1990;**97**:49–55.
- 35 Hamard P, Hamard H, Dufaux J, et al. Optic nerve head blood flow using a laser Doppler velocimeter and haemorheology in primary open angle glaucoma and normal pressure glaucoma. *Br J Ophthalmol* 1994;**78**:449–53.
- 36 O'Brien C, Butt Z, Ludlam C, et al. Activation of the coagulation cascade in untreated primary open-angle glaucoma. *Ophthalmology* 1997;**104**:725–30.

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