Published in final edited form as:

J Med Genet. 2010 February ; 47(2): 120–125. doi:10.1136/jmg.2009.067512.

***OPA1* increases the risk of normal but not high tension glaucoma**

P Yu-Wai-Man^{1,2}, JD Stewart¹, G Hudson¹, RM Andrews³, PG Griffiths², MK Birch², and PF Chinnery^{1,4}

¹Mitochondrial Research Group, The Medical School, Newcastle University, NE2 4HH, UK

²Department of Ophthalmology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, UK

³Moorfields Eye Hospital, London, EC1V 2PD, UK

⁴Institute of Human Genetics, Newcastle University, NE1 3BZ, UK

Abstract

Background—Primary open angle glaucoma (POAG) is a progressive optic neuropathy characterised by the selective loss of retinal ganglion cells (RGCs), pathological optic disc cupping and visual field defects. The *OPA1* gene encodes for an inner mitochondrial membrane protein crucial for normal mitochondrial function and pathogenic mutations cause autosomal dominant optic atrophy (DOA) by specifically targeting RGCs. This raises the distinct possibility that more subtle genetic variations in *OPA1* could alter the risk of developing glaucoma.

Methods—We studied 137 POAG patients, 67 patients with high tension glaucoma (HTG), 70 patients with normal tension glaucoma (NTG), and 75 controls from the North East of England. Three single nucleotide polymorphisms (SNPs) in intron 8 (IVS8+4c>t and IVS8+32t>c) and exon 4 (c.473A>G) of the *OPA1* gene were genotyped in our study group. In addition, the entire *OPA1* coding region was sequenced in 24 individuals with the CT/TT compound genotype using standard BigDye™ chemistries.

Results—There was no difference in either allele or genotype frequency for the IVS8+32t>c SNP between patients and controls, but there was a significant association between the T allele at IVS8+4c>t and the risk of developing NTG (Odds ratio (OR) = 2.04, 95% confidence interval (CI) = 1.10–3.81, P = 0.004), but not HTG. Logistic regression analysis also confirmed a strong association between the CT/TT compound genotype at IVS8+4 and IVS8+32 with NTG (OR = 29.75, 95% CI = 3.83–231.21, P < 0.001).

Conclusions—The CT/TT compound genotype at IVS8+4 and IVS8+32 is a strong genetic risk determinant for NTG but not HTG.

Keywords

Dominant optic atrophy; glaucoma; haplogroup; mitochondria; optic nerve; *OPA1*

Corresponding author: Professor Patrick Francis Chinnery, Mitochondrial Research Group, The Medical School, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK, Tel: +44-191-282-4375, Fax: +44-191-282-4373, P.F.Chinnery@ncl.ac.uk.

Competing Interests: None

Introduction

Primary open angle glaucoma (POAG) is the second most common cause of blindness in developed countries and accounts for about 10% of all blind registration in the United Kingdom.¹ It affects over 60 million people worldwide and with an ageing population, the prevalence of POAG is expected to increase by 30% in the next 20 years.² POAG is characterised by the selective, progressive loss of retinal ganglion cells (RGCs), leading to structural changes at the optic nerve head in the form of pathological cupping and visual field loss. The most significant risk factors for the development of glaucoma are raised intraocular pressure (IOP), increasing age, a positive family history in a first degree relative and ethnicity (Black > Hispanic > Caucasian > Asian).³⁴ In the non-glaucomatous population, IOP follows a normal distribution with a mean of 16.0 mmHg and a standard deviation (SD) of 2.5 mmHg, giving a statistical upper limit of 21.0 mmHg. About two thirds of POAG patients have IOPs > 21.0 mmHg at initial presentation i.e. high tension glaucoma (HTG), whereas the remainder with IOPs ≤ 21.0 mmHg are classified as normal tension glaucoma (NTG).³⁴

POAG is a complex disease with a strong genetic component and multiple susceptibility loci have been identified in populations from different ethnic backgrounds.³⁵ Four causative genes have been identified so far: *optineurin* (*OPTN*, OMIM 602432) on chromosome 10p14-15, *myocilin* (*MYOC*, OMIM 610652) on chromosome 1q24-25, *CYP11B* (OMIM 601771) on chromosome 2p21-22, and *WDR36* (OMIM 609669) on chromosome 5q21-22, but these account for less than 5-10% of patients with sporadic, adult-onset POAG.

Interestingly, a maternal family history of POAG is 6-8 times more likely than a paternal family history, which suggests a possible mitochondrial genetic influence.⁶⁻⁸ Furthermore, mitochondrial abnormalities have been identified in patients with POAG, with an increase in mitochondrial DNA (mtDNA) content and a reduction in mitochondrial respiratory chain activities.⁹ The preferential loss of RGCs in glaucoma is also a key pathological feature seen in Leber hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy (DOA), the two most common inherited optic neuropathies. Both these conditions are the result of mitochondrial dysfunction, LHON from primary mtDNA mutations affecting the respiratory chain complexes and the majority of DOA families have mutations in the *OPA1* gene (3q28-q29), which codes for an inner mitochondrial membrane protein critical for mitochondrial maintenance, oxidative phosphorylation (OXPHOS) and regulation of apoptosis.¹⁰

Two single nucleotide polymorphisms (SNPs) within intron 8 of the *OPA1* gene (IVS8+4c>t and IVS8+32t>c) have recently been linked with an increased risk of developing POAG in some, but not all, populations studied.¹¹⁻¹⁵ To further clarify the influence of *OPA1* in modulating susceptibility to glaucoma, we have performed an analysis of *OPA1* SNPs in a well-characterised cohort of POAG patients, and in addition reviewed the literature to compare the strength of the association identified in other study groups.

Methods

Patient and control samples

We investigated a well-characterised white Caucasian cohort consisting of 137 POAG patients (Mean age = 71.6 yrs, SD = 8.0 yrs) and 75 controls (Mean age = 79.3 yrs, SD = 4.4 yrs) from the North East of England, a region which has been relatively stable in terms of migratory flux.¹⁶¹⁷ The POAG group consisted of 67 HTG patients with a mean pre-treatment IOP of 23.1 mmHg (SD = 2.0 mmHg, range 22-28 mmHg) and 70 NTG patients with a mean pre-treatment IOP of 17.7 mmHg (SD = 2.2 mmHg, range 13-21 mmHg). Both cases and controls underwent a full ophthalmological examination including (i) IOP measurement by Goldmann applanation tonometry, (ii) gonioscopy to confirm open drainage angles, (iii) dilated fundal examination, and (iv) Humphrey full-threshold 24:2 visual field perimetry. All POAG patients had unequivocal evidence of glaucoma with both pathological optic disc cupping and characteristic visual field defects. HTG patients with intraocular pressures greater than 30mmHg were not included in this study. Control subjects were unaffected spouses of POAG patients and other ocular pathologies were carefully excluded. Genomic DNA was extracted from whole blood using established methods¹⁶¹⁷ and stored at -80°C for future molecular genetic investigations, with prior approval having been obtained from our local research ethics committee.

OPA1 genotyping

The two SNPs in intron 8 (IVS8+4c>t and IVS8+32t>c) were genotyped using the following primers to generate a 383 base pair polymerase chain reaction (PCR) product: (i) forward 5'-TGA AGT TCT TGA TGT TCT CTC TG-3' and (ii) reverse 5'-ATG GCT AAT TTA ATC CAC TGT TC-3'. In the second phase of this study, the entire coding region of the *OPA1* gene was amplified in 24 individuals with the CT/TT compound genotype (HTG N=10, NTG N=12, controls N=2), using a set of 27 M13-tagged primer pairs (available on request). Genotyping for the c.473A>G SNP located in exon 4 was also determined for the entire study cohort, using the following primers to generate a 341 base pair PCR product: (i) forward 5'-GGG TTG TCA TGA GGA TTA AAC AA-3' and (ii) reverse 5'-AAA AAT GTC CTG TTT TTC ATT GG-3'. For all three SNPs (IVS8+4c>t, IVS8+32t>c and c.473A>G) and the entire *OPA1* coding region, PCR products were purified and sequenced using BigDye™ terminator cycle chemistries on an ABI3100 Genetic Analyser (Applied Biosystems). Sequence results were then compared with the Genbank *OPA1* reference sequence (Accession number AB011139) using SeqScape™ software v2.1 (Applied Biosystems).

MtDNA haplogroups

The mtDNA haplogroup status for both POAG patients and controls had previously been determined by restriction fragment analysis of amplified PCR fragments spanning specific informative sites within the mitochondrial genome.¹⁸ Haplogroups K, W, I, V, X and M were present in less than 5% of our control population and these were analysed as one single group.

Statistical analysis

The Hardy-Weinberg equilibrium for *OPA1* genotypes was assessed for patients and controls (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>) and statistical analyses were performed using SPSS™ v.15 statistical software (Chicago, Illinois). Allele and genotype SNP frequencies were compared with Fisher's Exact Test and χ^2 analysis, with Bonferonni correction for multiple comparisons where applicable. Binary logistic regression was also used for multivariate analysis of other possible confounding factors that could influence the risk of developing glaucoma. This form of analysis assumes that the logarithm of the odds ratio is a linear function of the predictor variables included in the model:

$$\text{Log}(P/1 - P) = B_0 + B_1X_1 + B_2X_2 + \dots B_nX_n$$

where P is the probability of developing glaucoma; $X_1, X_2 \dots X_n$ represent the chosen predictor variables; and $B_0, B_1 \dots B_n$ are coefficients reflecting the nature of each predictor.¹⁹ The independent variables used in our model were: (i) age, (ii) gender, (iii) maximum pre-treatment IOP, (iv) mtDNA haplogroup, and (v) IVS8+4 and IVS8+32 compound genotype.

Results

Intron 8 SNPs

Both POAG and control groups were in Hardy-Weinberg equilibrium at IVS8+4c>t and IVS8+32t>c. The T allele at IVS8+4c>t was over-represented among NTG patients compared to controls (Odds ratio (OR) = 2.04, 95% confidence interval (CI) = 1.10-3.81, $P = 0.03$), but there was no significant difference in allele frequency for the HTG and whole POAG groups. Similarly, there was a significant difference in the distribution of the three IVS8+4c>t genotypes for the NTG group ($P = 0.025$) but not for the HTG or whole POAG groups (Table 1A). There was no significant difference in either allele or genotype frequency for the IVS8+32t>c SNP when comparing all POAG cases, HTG and NTG subgroups to controls (Table 1B).

Compound genotype

Analysis of both IVS8+4c>t and IVS8+32t>c SNPs showed a statistically significant increased risk of glaucoma in subjects with the CT/TT compound genotype for the whole POAG group (OR = 6.98, 95% CI = 1.59-30.59, $P = 0.003$) and NTG group (OR = 7.55, 95% CI = 1.63-35.10, $P = 0.004$). Although there was a trend towards a higher risk of HTG with the CT/TT compound genotype ($P = 0.013$), this was not significant following Bonferroni correction (Table 2). The CT/TT compound genotype was also not associated with higher pre-treatment IOPs or worse CDRs, in both the HTG and NTG groups ($P > 0.05$, data not shown).

Logistic regression

Binary logistic regression confirmed a statistically significant increased risk of developing glaucoma with the CT/TT compound genotype for the whole patient group (OR = 56.52, 95% CI = 5.98-533.78, $P < 0.001$) and those with NTG (OR = 29.75, 95% CI = 3.83-231.21,

$P < 0.001$), but not for the HTG group (Table 3). None of the mtDNA haplogroups were significant risk factors for glaucoma. Although haplogroup J was over-represented among NTG patients compared to controls ($P=0.045$), this fell below the level for statistical significance with Bonferroni correction.

***OPA1* haplotype**

We sequenced the entire *OPA1* coding region of all 24 individuals with the CT/TT compound genotype at IVS8+4 and IVS8+32 (HTG N = 10, NTG N = 12, controls N = 2) and no previously described pathogenic mutations or polymorphisms were identified. However, all of them shared the same haplotype, with homozygosity at both the c.473A>G (p.N158S) and c.2109C>T (p.A703A) SNPs, except for one NTG patient (Figure 1A). The latter was heterozygous for the c.473A>G and c.2109C>T SNPs, and also had two additional heterozygous SNPs at c.575C>T (p.A192V) and c.1608A>C (p.A536A). Further analysis of the non-synonymous c.473A>G (p.N158S) SNP in our entire study cohort, did not show any statistically significant difference between our patient and control groups (Table 4).

Discussion

This study has identified a strong association between specific *OPA1* polymorphisms at IVS8+4c>t and IVS8+32t>c and the risk of glaucoma. Subgroup analysis based upon pre-treatment IOPs further indicated that the susceptibility conferred by these two SNPs was largely restricted to the NTG group. This is compatible with the notion that non-IOP related factors are probably more important in the pathophysiology of NTG compared to HTG.³⁵ The T allele at IVS8+4 led to a two-fold increased risk of NTG and although the IVS8+32 SNP on its own was not a risk factor, compound genotype analysis suggested a synergistic influence with the IVS8+4 SNP and identified CT/TT as the high-risk compound genotype.

To confirm our initial findings, we performed a more rigorous logistic regression analysis to control for the possible influence of other variables that might affect the risk of developing glaucoma, thereby minimising the chance of identifying a spurious association. The odds ratio for the CT/TT compound genotype remained highly significant, and was associated with a 30-fold increased risk of NTG, independent of age, gender, maximum pre-treatment IOP and mtDNA haplogroup. However, the CT/TT compound genotype was not associated with markers of disease severity i.e. higher pre-treatment IOPs or worse optic disc cupping, supporting the conclusions of a previous report.²⁰ MtDNA haplogroup J was also over-represented among the NTG group compared to controls, although this became non-significant with Bonferonni correction. Nevertheless, this is an interesting observation given that the mtDNA background is thought to exert a direct effect on the assembly of the mitochondrial respiratory chain complexes²¹ and haplogroup J specifically has been linked with an increased risk of visual loss among Caucasian LHON mutation carriers²², the classic example of an inherited mitochondrial optic neuropathy.

We performed a literature review to identify all previous *OPA1* glaucoma association studies, including unpublished meeting abstracts, and there is a consistent lack of association between the IVS8+4 and IVS+32 SNPs and HTG (Table 5). A high-risk compound

genotype for developing NTG was found in two studies involving British Caucasian subjects¹¹¹³ and one Japanese study²³, with the latter reporting a relatively weaker association. However, no association was identified in other populations of Asian¹⁴¹⁵²⁴ and African extraction²⁵²⁶, possibly indicating that the influence of *OPA1* polymorphisms on NTG is limited to certain ethnic groups or it has a smaller effect (OR < 1.5), not detectable by the sample size (< 100) used in most of these studies. In this respect, it is worth noting that the control groups in these studies indicate a marked variation in the frequency of the minor T allele at IVS8+4c>t in different populations: Caucasian (7-18%), African (2-5%) and Asian (1-2%). Of note, the three other studies which have found a significant association between *OPA1* and NTG have all identified different high-risk compound genotypes to the one identified in our study. As case-control studies are open to several sources of bias which can be difficult to control, this could therefore indicate a spurious population stratification effect.

What are the possible explanations for the increased risk of NTG in individuals harbouring the CT/TT compound genotype? The majority of pathogenic *OPA1* mutations are truncative (> 70%) and the reduction in protein level implicates haploinsufficiency as the underlying process leading to optic nerve degeneration in DOA.²⁷ Opa1 is a multi-functional protein and its crucial pro-fusion properties contribute to the maintenance of a highly interconnected mitochondrial network within cells.¹⁰ It is therefore not surprising that mitochondrial fragmentation is a prominent feature in fibroblasts cultured from DOA patients, with the release of cytochrome *c* from the mitochondrial compartment precipitating the onset of apoptotic cell death.²⁸²⁹ A recent study has also found murine RGC cells to be more sensitive to the downstream events of mitochondrial fragmentation and pro-apoptotic stimuli when compared to other neuronal populations. Since the IVS8+4 SNP is located within the donor splice site region, the CT/TT compound genotype could exert an effect, albeit unproven, on mRNA transcript levels, which would then potentiate RGC loss by disrupting the delicate balance between mitochondrial fusion and fission. However, the more pronounced effect seen in NTG compared to HTG clearly implicates other mechanisms and additional investigations are required.

Unlike other association studies, we also sequenced the whole *OPA1* coding region, firstly to exclude the possibility of pathogenic mutations since DOA is not infrequently misdiagnosed as NTG in clinical practice³⁰, and secondly to explore the possibility that IVS8+4 and IVS8+32 are actually tagging SNPs in linkage disequilibrium with another functional *OPA1* variant. Except for one NTG patient, all individuals carrying the CT/TT compound genotype shared the same *OPA1* haplotype, with homozygosity at both the c.473A>G (p.N158S) and c.2109C>T (p.A703A) SNPs, suggesting a common ancestral event. Subsequent analysis of our entire cohort did not identify any significant difference in allele or genotype frequency at the poorly conserved, non-synonymous c.473A>G SNP in exon 4 (figure 1B). However, our study has not excluded the possibility that the IVS8+4 and IVS8+32 SNPs are in linkage disequilibrium with another causative gene in the vicinity of *OPA1* or the presence of *OPA1* intronic variants regulating mRNA splicing or transcriptional activity.

The finding of a significant association between specific combination of *OPA1* polymorphisms and NTG does not imply causation and although biologically plausible, our results require additional functional confirmation, for example by comparing the bioenergetics and mitochondrial network morphology of fibroblasts obtained from patients with and without the high-risk CT/TT compound genotype. With a rapidly ageing population, glaucoma will remain an important cause of visual morbidity and a greater understanding of the complex genetic influences that lead to RGC loss will have important implications both for the identification and screening of high-risk groups, and in identifying pathophysiological pathways that could be amenable to therapeutic intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

PYWM is an MRC Clinical Research Fellow and PFC is a Wellcome Trust Senior Fellow in Clinical Science. PFC also receives funding from the Parkinson's Disease Society (UK), the Medical Research Council Translational Muscle Centre, and the UK NIHR Biomedical Research Centre in Ageing and Age related disease.

References

1. Bunce C, Wormald R. Leading causes of certification for blindness and partial sight in England & Wales. *BMC Public Health*. 2006; 6:58. [PubMed: 16524463]
2. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006; 90(3):262–7. [PubMed: 16488940]
3. Hewitt AW, Craig JE, Mackey DA. Complex genetics of complex traits: the case of primary open-angle glaucoma. *Clinical and Experimental Ophthalmology*. 2006; 34(5):472–84. [PubMed: 16872346]
4. Caprioli J, Garway-Heath DF. A critical reevaluation of current glaucoma management. *Ophthalmology*. 2007; 114(11):S1–S41. [PubMed: 17980762]
5. Wiggs JL. Genetic etiologies of glaucoma. *Arch Ophthalmol*. 2007; 125(1):30–7. [PubMed: 17210849]
6. Morgan RW, Drance SM. Chronic Open-Angle Glaucoma and Ocular Hypertension - Epidemiological Study. *Br J Ophthalmol*. 1975; 59(4):211–5. [PubMed: 1138846]
7. Shin DH, Becker B, Kolker AE. Family History in Primary Open-Angle Glaucoma. *Arch Ophthalmol*. 1977; 95(4):598–600. [PubMed: 849183]
8. Charliat G, Jolly D, Blanchard F. Genetic risk factor in primary open-angle glaucoma: a case-control study. *Ophthalmic Epidemiol*. 1994; 1(3):131–8. [PubMed: 8790619]
9. Abu-Amero KK, Morales J, Bosley TM. Mitochondrial abnormalities in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2006; 47(6):2533–41. [PubMed: 16723467]
10. Yu-Wai-Man P, Griffiths PG, Hudson G, Chinnery PF. Inherited Mitochondrial Optic Neuropathies. *J Med Genet*. 2009; 46(3):145–58. [PubMed: 19001017]
11. Aung T, Ocaka L, Ebenezer ND, Morris AG, Krawczak M, Thiselton DL, Alexander C, Votruba M, Brice G, Child AH, Francis PJ, Hitchings RA, Lehmann OJ, Bhattacharya SS. A major marker for normal tension glaucoma: association with polymorphisms in the *OPA1* gene. *Hum Genet*. 2002; 110(1):52–6. [PubMed: 11810296]
12. Aung T, Ocaka L, Ebenezer ND, Morris AG, Brice G, Child AH, Hitchings RA, Lehmann OJ, Bhattacharya SS. Investigating the association between *OPA1* polymorphisms and glaucoma: comparison between normal tension and high tension primary open angle glaucoma. *Hum Genet*. 2002; 110(5):513–4. [PubMed: 12073024]

13. Powell B, Toomes C, Scott S, Yeung A, Marchbank N, Spry P, Lumb R, Inglehearn C, Churchill A. Polymorphisms in OPA1 are associated with normal tension glaucoma. *Mol Vis.* 2003; 9(58): 460–4. [PubMed: 14551537]
14. Sato M, Kawase K, Yamamoto T, Dubo S, Shink EE, Si E, Raymond V. Lack of association between normal-tension glaucoma and intron 8 polymorphisms in the gene causing autosomal dominant optic atrophy, OPA1, in Japan. *Invest Ophthalmol Vis Sci.* 2003; 44:1124.
15. Woo SJ, Kim DM, Kim JY, Park SS, Ko HS, Yoo T. Investigation of the association between OPA1 polymorphisms and normal-tension glaucoma in Korea. *J Glaucoma.* 2004; 13(6):492–5. [PubMed: 15534475]
16. Ressiniotis T, Griffiths PG, Birch M, Keers S, Chinnery PF. The role of apolipoprotein E gene polymorphisms in primary open-angle glaucoma. *Arch Ophthalmol.* 2004; 122(2):258–61. [PubMed: 14769603]
17. Ressiniotis T, Griffiths PG, Birch M, Keers S, Chinnery PF. Primary open angle glaucoma is associated with a specific p53 gene haplotype. *J Med Genet.* 2004; 41(4):296–8. [PubMed: 15060108]
18. Andrews R, Ressiniotis T, Turnbull DM, Birch M, Keers S, Chinnery PF, Griffiths PG. The role of mitochondrial haplogroups in primary open angle glaucoma. *Br J Ophthalmol.* 2006; 90(4):488–90. [PubMed: 16547333]
19. Bland, M. *An Introduction to Medical Statistics.* 3rd ed.. Oxford University Press; Oxford: 2000.
20. Aung T, Okada K, Poinosawmy D, Membrey L, Brice G, Child AH, Bhattacharya SS, Lehmann OJ, Garway-Heath DF, Hitchings RA. The phenotype of normal tension, glaucoma patients with and without OPA1 polymorphisms. *Br J Ophthalmol.* 2003; 87(2):149–52. [PubMed: 12543739]
21. Pello R, Martin MA, Carelli V, Nijtmans LG, Achilli A, Pala M, Torroni A, Gomez-Duran A, Ruiz-Pesini E, Martinuzzi A, Smeitink JA, Arenas J, Ugalde C. Mitochondrial DNA background modulates the assembly kinetics of OXPHOS complexes in a cellular model of mitochondrial disease. *Hum Mol Genet.* 2008; 17(24):4001–11. [PubMed: 18806273]
22. Hudson G, Carelli V, Spruijt L, Gerards M, Mowbray C, Achilli A, Pyle A, Elson J, Howell N, La Morgia C, Valentino ML, Huoponen K, Savontaus ML, Nikoskelainen E, Sadun AA, Salomao SR, Belfort R, Griffiths P, Man PYW, de Coe RFM, Horvath R, Zeviani M, Smeets HJT, Torroni A, Chinnery PF. Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. *Am J Hum Genet.* 2007; 81(2):228–33. [PubMed: 17668373]
23. Mabuchi F, Tang S, Kashiwagi K, Yamagata Z, Iijima H, Tsukahara S. The OPA1 gene polymorphism is associated with normal tension and high tension glaucoma. *American Journal of Ophthalmology.* 2007; 143(1):125–30. [PubMed: 17188046]
24. Kumaramanickavel G, Aung T, Sripriya S, Waseem N, Okada K, Seah SKL, Baskaran M, Mishima HK, Vithana EN, Bhattacharya S. Lack of association of IVS8+4 C/T and IVS8+32 T/C polymorphisms in the OPA1 gene with normal tension glaucoma in patients from Singapore, India and Japan. *Invest Ophthalmol Vis Sci.* 2005; 46:3811.
25. Yao WL, Jiao XD, Hejtmancik J, Leske M, Hennis A, Nemesure B. Evaluation of the association between OPA1 polymorphisms and primary open-angle glaucoma in Barbados families. *Mol Vis.* 2006; 12(73-75):649–54. [PubMed: 16785854]
26. Liu Y, Schmidt S, Qin XJ, Gibson J, Munro D, Wiggs JL, Hauser MA, Allingham RR. No association between OPA1 polymorphisms and primary open-angle glaucoma in three different populations. *Mol Vis.* 2007; 13(242-45):2137–41. [PubMed: 18079692]
27. Ferre M, Bonneau D, Milea D, Chevrollier A, Verny C, Dollfus H, Ayuso C, Defoort S, Vignal C, Zanlonghi X, Charlin JF, Kaplan J, Odent S, Hamel CP, Procaccio V, Reynier P, Amati-Bonneau P. Molecular screening of 980 cases of suspected hereditary optic neuropathy with a report on 77 Novel OPA1 mutations. *Hum Mutat.* 2009; 30:E692–705. [PubMed: 19319978]
28. Olichon A, Landes T, Arnaune-Pelloquin L, Emorine LJ, Mils V, Guichet A, Delettre C, Hamel C, Amati-Bonneau P, Bonneau D, Reynier P, Lenaers G, Belenguer P. Effects of OPA1 mutations on mitochondrial morphology and apoptosis: relevance to ADOA pathogenesis. *J Cell Physiol.* 2007; 211(2):423–30. [PubMed: 17167772]

29. Zanna C, Ghelli A, Porcelli AM, Karbowski M, Youle RJ, Schimpf S, Wissinger B, Pinti M, Cossarizza A, Vidoni S, Valentino ML, Rugolo M, Carelli V. OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain*. 2008; 131:352–67. [PubMed: 18222991]
30. Buono LM, Foroozan R, Sergott RC, Savino PJ. Is normal tension glaucoma actually an unrecognized hereditary optic neuropathy? New evidence from genetic analysis. *Curr Opin Ophthalmol*. 2002; 13(6):362–70. [PubMed: 12441838]

Key Points

- Primary open angle glaucoma (POAG) is the second leading cause of blindness in developed countries, and based upon their pre-treatment intraocular pressures (IOPs), patients are classified as having either high tension glaucoma (HTG, IOP > 21.0 mmHg) or normal tension glaucoma (NTG, IOP ≤ 21.0 mmHg).
- Although POAG is a late-onset acquired optic neuropathy, it has a major genetic basis and it shares striking clinical and pathological overlap with autosomal dominant optic atrophy (DOA), which is the result of mutations in the *OPA1* gene.
- In this study, we demonstrate a strong association between two specific *OPA1* SNPs (IVS8+4c>t and IVS8+32t>c) and the risk of developing NTG but not HTG. The high-risk CT/TT compound genotype conferred a 30-fold increased risk of disease, supporting a possible role of the Opa1 protein in the pathophysiology of NTG.

<i>H. sapiens</i>	EKIRKALPNS ED LVKLA---PDFDKIVESLSLLKDFFTS
<i>P. troglodytes</i>	EKIRKALPNS ED LVKLA---PDFDKIVESLNLLKDFFTS
<i>M. musculus</i>	EKIRKALP SS EDLASLA---PDLKITESLSLLKDFFTA
<i>G. gallus</i>	EKLIKALPDADDLAKLL---PDFEKIGESFTSLKGIFSP
<i>D. rerio</i>	DKLASALPELEEIAKLL---PDMEKIGENFTFLKS-LLS
<i>D. melanogaster</i>	IEVGSLVKNAIEVDPKLK-QLGEDKLSEWRNWFD SRL DD
<i>A. gambiae</i>	LAVKDAVKDSIEIDPRLK-QLSEHKLN EW RQWFDQRLDN
<i>C. elegans</i>	SQKMKGIKDGFGADGQNKWAEWMAKFEQFKQ Q KEDQNGN

Figure 1.

A: Shared *OPA1* haplotype in individuals with the IVS8+4 and IVS8+32 CT/TT compound genotype.

B: Alignment of the 36 amino acids encoded by exon 4, indicating the poor evolutionary conservation of the codon altered by the c.473A>G SNP (p.N158S) in both lower vertebrates and invertebrates.

Table 1
Allele and genotype frequencies for the IVS8+4c>t and IVS8+32t>c *OPAI* SNPs

(A) IVS8+4c>t

	Controls (N=75)	Whole Group (N=137)		HTG (N=67)		NTG (N=70)	
Allele	P value			P value		P value	
C	131 (87.3%)	222 (81.0%)		114 (85.1%)		108 (77.1%)	
T	19 (12.7%)	52 (19.0%)	0.096	20 (14.9%)	0.581	32 (22.9%)	0.030*
Genotype	P value ^a			P value ^a		P value ^a	
CC	59 (78.7%)	90 (65.7%)		49 (73.1%)		41 (58.6%)	
CT	13 (17.3%)	42 (30.7%)		16 (23.9%)		26 (37.1%)	
TT	3 (4.0%)	5 (3.6%)	0.106	2 (3.0%)	0.061	3 (4.3%)	0.025*

(B) IVS8+32t>c

	Controls (N=75)	Whole Group (N=137)		HTG (N=67)		NTG (N=70)	
Allele	P value			P value		P value	
T	79 (52.7%)	155 (56.6%)		60 (44.8%)		59 (42.1%)	
C	71 (47.3%)	119 (43.4%)	0.440	74 (55.2%)	0.666	81 (57.9%)	0.375
Genotype	P value ^b			P value ^b		P value ^b	
TT	21 (28.0%)	41 (29.9%)		12 (17.9%)		11 (15.7%)	
TC	37 (49.3%)	73 (53.3%)		36 (53.7%)		37 (52.9%)	
CC	17 (22.7%)	23 (16.8%)	0.578	19 (28.4%)	0.769	22 (31.4%)	0.566

χ^2 analysis of all three possible genotypes at ^a IVS8+4C>T and ^b IVS8+32T>C;

* Significant P value.

Table 2
Compound genotype frequencies for the IVS8+4c>t and IVS8+32t>c *OPAI* SNPs

(A) Whole group

IVS8+4	IVS8+32	Patients	Controls	P value [*]	Odds ratio	95% CI
TT	TT	5 (3.6%)	3 (4.0%)	1.000 [*]	0.91	0.21-3.92
CT	TT	22 (16.1%)	2 (2.7%)	0.003 [*]	6.98	1.59-30.59
CT	TC	20 (14.6%)	11 (14.7%)	0.989 [*]	0.99	0.45-2.21
CC	TT	14 (10.2%)	16 (21.3%)	0.026 [*]	0.42	0.19-0.92
CC	TC	53 (38.7%)	26 (34.7%)	0.563 [*]	1.19	0.66-2.14
CC	CC	23 (16.8%)	17 (22.7%)	0.296 [*]	0.69	0.34-1.39

(B) HTG

IVS8+4	IVS8+32	Patients	Controls	P value	Odds ratio	95% CI
TT	TT	2 (3.0%)	3 (4.0%)	1.000	0.74	0.12-4.56
CT	TT	10 (14.9%)	2 (2.7%)	0.013	6.40	1.35-30.40
CT	TC	6 (9.0%)	11 (14.7%)	0.316	0.57	0.20-1.64
CC	TT	7 (10.4%)	16 (21.3%)	0.110	0.43	0.17-1.12
CC	TC	30 (44.8%)	26 (34.7%)	0.216	1.53	0.78-3.01
CC	CC	12 (17.9%)	17 (22.7%)	0.483	0.74	0.33-1.70

(C) NTG

IVS8+4	IVS8+32	Patients	Controls	P value [*]	Odds ratio	95% CI
TT	TT	3 (4.3%)	3 (4.0%)	1.000 [*]	1.08	0.21-5.51
CT	TT	12 (17.1%)	2 (2.7%)	0.004 [*]	7.55	1.63-35.10
CT	TC	14 (20.0%)	11 (14.7%)	0.396 [*]	1.46	0.61-3.46
CC	TT	7 (10.0%)	16 (21.3%)	0.062 [*]	0.41	0.16-1.07
CC	TC	23 (32.9%)	26 (34.7%)	0.818 [*]	0.92	0.46-1.84
CC	CC	11 (15.7%)	17 (22.7%)	0.289 [*]	0.64	0.27-1.48

* Significant with Bonferroni correction: $P < 0.008$.

Table 3
Logistic regression analysis comparing POAG patients with controls

Predictor variables	Whole group		HTG		NTG	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
MtDNA haplogroup^a						
H	0.62 (0.26-1.50)	0.292	0.97 (0.43-2.18)	0.934	0.51 (0.20-1.28)	0.151
T	0.42 (0.12-1.48)	0.176	0.65 (0.21-2.00)	0.457	0.37 (0.10-1.45)	0.154
J	3.79 (0.86-16.63)	0.077	1.64 (0.34-8.05)	0.540	4.42 (1.04-18.92)	0.045
U	1.68 (0.50-5.62)	0.401	2.02 (0.62-6.62)	0.246	1.85 (0.53-6.38)	0.332
Others	0.82 (0.24-2.82)	0.757	0.65 (0.19-2.21)	0.490	0.72 (0.21-2.52)	0.609
Compound genotype^b						
CT/TT	56.52 (5.98-533.78)	< 0.001*	9.91 (1.74-56.57)	0.010	29.75 (3.83-231.21)	< 0.001*

Our logistic regression models also incorporated the following predictor variables with the controls acting as the reference group: (i) age, (ii) gender, and (iii) maximum IOP, except in the HTG subgroup where maximum IOP was not included;

* Significant with Bonferroni correction:

^a $P < 0.01$

^b $P < 0.008$.

Table 4
Allele and genotype frequencies for the c.473A>G *OPAI* SNP

	N =	Allele		P value	Genotype			P value ^a
		A	G		AA	AG	GG	
Whole group	137	128 (46.7%)	146 (53.3%)		26 (19.0%)	76 (55.5%)	35 (25.5%)	
Controls	75	75 (50.0%)	75 (50.0%)	0.543	19 (25.3%)	37 (49.3%)	19 (25.3%)	0.531
HTG	67	65 (48.5%)	69 (51.5%)		13 (19.4%)	39 (58.2%)	15 (22.4%)	
Controls	75	75 (50.0%)	75 (50.0%)	0.813	19 (25.3%)	37 (49.3%)	19 (25.3%)	0.548
NTG	70	63 (45.0%)	77 (55.0%)		13 (18.6%)	37(52.9%)	20 (28.6%)	
Controls	75	75 (50.0%)	75 (50.0%)	0.818	19 (25.3%)	37 (49.3%)	19 (25.3%)	0.613

^a χ^2 analysis of all three possible genotypes at c.473A>G.

Table 5
Summary of previous studies investigating the role of *OPAI* polymorphisms in POAG

Study ^a	Population	Significant association				High-risk compound genotype (NTG)		
		HTG	N =	NTG	N =	IVS8+4/+32	Odds ratio ^b	95% CI
[11,12]	Caucasian	No	90	Yes	163	CT/TC	6.25	2.67-14.59
[13]	Caucasian	N/A	-	Yes	61	CC/CC	2.71	1.31-5.57
[14]	Japanese	N/A	-	No	337	-	-	-
[15]	Korean	N/A	-	No	65	-	-	-
[24]	Indian	N/A	-	No	50	-	-	-
[24]	Chinese	N/A	-	No	53	-	-	-
[24]	Japanese	N/A	-	No	42	-	-	-
[25]	African-Caribbean	No	48	No	61	-	-	-
[23]	Japanese	No	191	Yes	194	CC/TC	2.14	1.32-3.45
[26]	Caucasian	No	279	N/A	-	-	-	-
[26]	African-American	No	193	N/A	-	-	-	-
[26]	African	No	170	N/A	-	-	-	-

N/A: Not assessed; N: Number of patients analysed;

^aStudies in chronological order

^bOdds ratio were derived by χ^2 analysis using published figures and additional unpublished data provided by Dr Vincent Raymond (Personal communication) for study [14].