SCIENTIFIC REPORT

The role of mitochondrial haplogroups in primary open angle glaucoma

R Andrews, T Ressiniotis, D M Turnbull, M Birch, S Keers, P F Chinnery, P G Griffiths

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Aim: To investigate a possible association between mitochondrial haplogroups and primary open angle glaucoma (POAG).

Methods: Genomic DNA was extracted from 140 POAG patients and 75 healthy individuals. Restriction enzyme digest analysis of polymerase chain reaction (PCR) amplified fragments was used to determine the mitochondrial haplogroup of each patient and control.

Results: The median age was 73 years for the POAG patients (range 51–87, SD 8.01) and 78 years for the controls (range 68–90, SD 4.4). Mean IOP was 20.8 mm Hg for the patients (SD 2.6) and 16.2 mm Hg for the controls (SD 3.4). Median cup/disc ratio was 0.8 and 0.3 for patients and controls respectively. No statistically significant difference was found in the haplogroup distribution between the POAG patients and the healthy individuals (Fisher's exact test).

Conclusion: In this cohort, mitochondrial haplogroups do not appear to contribute to the pathogenesis of POAG.

Epidemiological studies demonstrate that a significant proportion of typical late onset glaucoma is genetically determined. Although the prevalence of the disease in first degree relatives is greater than in the general population, it is not high enough to indicate a simple Mendelian pattern of inheritance, suggesting rather a polygenic or multifactorial mode of transmission.[1]

Some studies have shown the prevalence of a maternal family history is six to eight times greater than a paternal history.[2–4] This occurs despite the prevalence of the disease being equal in both sexes[5] and in the absence of any maternal influence upon IOP.[3] It is difficult to explain this difference in strict Mendelian terms. Matrilineal inheritance is, however, a characteristic of mitochondrial genetics.[6]

Mitochondria are unique among organelles in that they contain their own DNA.[7] Mitochondrial DNA (mtDNA) has a number of unusual features which impart to mitochondrial disorders a novel set of genetic characteristics, one of which is that mtDNA is transmitted exclusively through the maternal line.[8]

During evolution, a number of mutations have accumulated on the mtDNA, representing specific single nucleotide polymorphisms (SNPs), according to which human populations can be categorised into various mtDNA ''haplogroups.''[7 9] These haplogroups were recently found to influence energy dependent processes such as sperm motility and affecting the risk of developing late onset neurodegenerative diseases.[8 10]

Studies on the inheritance of glaucoma have concentrated on the nuclear genome. The higher prevalence of maternal compared to paternal transmission of primary open angle glaucoma (POAG) observed in some family studies suggested to us that mtDNA haplogroups or polymorphisms could play a part in the pathogenesis of POAG. Other studies have also demonstrated influence of mtDNA on the pathophysiology of optic neuropathies such as Leber's disease[11] and neurodegenerative diseases, such as Parkinson's disease (PD).[10]

More than 100 pathological defects in human mtDNA have now been described.[12–14] Ophthalmic involvement is common.[15] Optic atrophy is a prominent ocular manifestation and, in Leber's hereditary optic neuropathy (LHON), is the predominant and frequently sole presentation of the disease. Optic atrophy is also associated with a number of other defects in mtDNA.[15 16] Chloramphenicol, a specific inhibitor of mitochondrial protein synthesis, may also produce a clinical picture virtually indistinguishable from LHON.[17] This is also the case with tobacco-alcohol amblyopia.[18] Taken together, these observations suggest that the retinal ganglion cells are exquisitely sensitive to a variety of disruptions of mitochondrial metabolism.[19] This propensity for optic nerve damage suggests that mitochondrial dysfunction could also contribute to the pathogenesis of other optic neuropathies in which the aetiology remains to be defined.

METHODS

Case selection

To be included in the study all 140 affected white people from the north east of England had to meet the following criteria: presentation over the age of 60 years, untreated intraocular pressures (IOPs) all below 30 mm Hg, disc cupping, progressive nerve fibre bundle visual field loss, and open angles on four mirror gonioscopy. Individuals with high myopia,[20] a history of severe blood loss and vascular hypoperfusion,[21] or a past history of uveitis or topical steroid use were excluded from the study. In each case a full medical history and ophthalmic examination were undertaken. Ophthalmic assessment included refraction, Goldmann applanation tonometry, gonioscopy, ophthalmoscopy, and Humphrey (24:2) visual field analysis.

The control group consisted of 75 individuals having no family history of POAG and, on examination by an experienced glaucoma specialist, had IOPs below 21 mm Hg with normal optic discs and visual fields, and no other ocular pathology. In each case a full medical history and glaucoma assessment was undertaken.

Mitochondrial haplogroup analysis

Genomic DNA was extracted from 10 ml of venous blood drawn with informed consent from each patient and control subject following approval from our regional ethics committee.

Abbreviations: IOP, intraocular pressure; LHON, Leber's hereditary optic neuropathy; mtDNA, mitochondrial DNA; PCR, polymerase chain reaction; PD, Parkinson's disease; POAG, primary open angle glaucoma; SNPs, single nucleotide polymorphisms

Table 1 mtDNA haplogroup analysis

Haplogroup	Primer	Position		PCR (bp)	Anneal (°C)	Digest	RFLP +	RFLP −
H	F ATTTAGCTGACTCGCCACAC R CATCCATATAGTCACTCCAGG	6863–6882 7396–7376	14F 14R	533	58	− 7025 *Alu*1	5, 188, 340	5, 31, 157, 340
T	F GCCCTTCTAAACGCTAATCC R GGAGGTTGAAGTGAGAGG	12940–12959 13453–13435	27F 27R	513	58	+ 13366 *Bam*H1	87, 426	513
J	F AGTCTTGTAAACCGGAGATG R TGCTGTACTTGCTTGTAAGC	15914–15933 16215–16196	J (F) J (R)	301	58	− 16065 *Hin*f1	86, 215	65, 86, 150
UK	F GCCACATAGCCCTCGTAGT R TATTTGGAGTTGCACCAAGATT	11633–11651 12330–12309	UK (F) UK (R)	697	58	+ 12308 *Hin*f1	22, 138, 221, 316	160, 221, 316
K	F ACCACCCAACAATGACTAATC R GTTGTCGTGCAGGTAGAGG	8656–8676 9201–9183	18F 18R	545	58	− 9052 *Hae*II	545	145, 400
WI	F TAACATCTCAGACGCTCAGG R GTTAGCTTTACAGTGGGCTC	7744–7763 8309–8290	16F 16R	565	58	+ 8249 *Ava*II	41, 524	565
I	F CTCAACTATCACACATCAACTG R AGATACTGCGACATAGGGTG	16223–16244 129–110	D2F D2R	474	58	+ 16389 *Bam*H1	308, 166	474
V	F GCAGGCACACTCATCACAG R GATTTTGCGTAGCTGGGTTTG	4512–4530 5003–4983	9F 9R	491	58	− 4580 *Nla*III	355, 136	68, 136, 287
X	F ACTTAACTTGACCGCTCTGAG R ATTGGTGGCTGCTTTTAGGC	1651–1671 2193–2174	3F 3R	542	57	− 1715 *Dde*I	17, 28, 113, 156, 228	17, 28, 48, 113, 156, 180
M	F CAACACCCTCCTAGCCTTAC R TGGCGGCAAAGACTAGTATG	1008–5–10104 10674–10655	21F 21R	589	57	+ 10397 *Alu*I	75, 148, 164, 202	75, 148, 366

Restriction enzyme digest analysis of polymerase chain reaction (PCR) amplified fragments spanning specific informative sites was used to determine the mitochondrial haplogroup of each patient and control. The haplogroup analysis was based on the phylogenetic network for European mtDNA, as described by Finnila *et al*.[22] The details of the oligonucleotide primers, the polymorphic sites, the PCR conditions, and the restriction enzymes that were employed, are illustrated in table 1.

RESULTS

Our cohort consisted of 140 POAG patients and 75 controls. The median age was 73 years for the POAG patients (range 51–87, SD 8.01) and 78 years for the controls (range 68–90, SD 4.4). Mean IOP was 20.8 mm Hg for the patients (SD 2.6) and 16.2 mm Hg for the controls (SD 3.4). Median cup/disc ratio was 0.8 and 0.3 for patients and controls, respectively. There was no statistically significant difference in the haplogroup distribution between the POAG patients and the healthy individuals, as illustrated in table 2 (Fisher's exact test).

DISCUSSION

Sequence variants defining mtDNA haplogroups have been regarded as benign polymorphisms. Haplogroup and phylogenetic analysis of LHON patients have, however, shown that two of the three primary LHON mutations, at np 11778 and 14484, tend to be associated with European mtDNA haplogroup J.[11 23 24] Additional evidence supporting a role for mtDNA haplogroups as a risk factor in disease expression has also recently been reported with the observation that migraine associated stroke is more common in individuals belonging to haplogroup U than would be expected on a random basis.[25] The site, or sites, within these haplogroups that influence penetrance or expression have not been identified.

Mitochondria play a crucial part in neurodegenerative diseases, such as PD. Complex I is the first site of the respiratory chain, produced by the assembly of 35–37 nDNA and 7 mtDNA encoded subunits. Decreased complex I activity has been found to cause parkinsonism and nigrostriatal dopaminergic degeneration in humans. Van der Walt and colleagues have reported that mitochondrial haplogroups J and K have a protective role in PD, where in another study, Pyle and colleagues attribute this reduced risk to the haplogroup cluster UKJT.[26] In two smaller studies, however, haplogroup J was associated with increased risk for PD.[27 28]

POAG is a complex neurodegenerative disease, where cell death occurs by apoptosis, in a similar manner as in PD and Alzheimer's disease. Patients with Alzheimer's disease and Parkinson's disease may have an increased occurrence rate of glaucoma. In our study, a possible association between mtDNA haplogroups and POAG was investigated, but no such evidence was detected.

Our study does not preclude the possibility that mitochondrial DNA haplotypes could have a role in some matrilineal

Table 2 Haplogroup distribution in POAG patients and healthy individuals

POAG patients		Controls	p Value*
H	58 (41.4%)	37 (49.3%)	0.5226
T	20 (14.3%)	13 (17.3%)	0.6966
J	17 (12.1%)	5 (6.7%)	0.3449
U	28 (20.0%)	10 (13.3%)	0.3548
K	1 (0.7%)	4 (5.3%)	0.0571
W	4 (2.9%)	1 (1.3%)	0.6614
I	3 (2.1%)	1 (1.3%)	1
V	0 (0.0%)	0 (0.0%)	1
X	3 (2.1%)	2 (2.7%)	1
M	0 (0.0%)	0 (0.0%)	1
Others	6 (4.3%)	2 (2.7%)	0.7175
Number	140	75	

*Fisher's exact test.

pedigrees though it seems unlikely. Though our study contained some members of larger pedigrees the numbers were too small to justify separate analysis.

The cause of the reported differential rates of maternal and paternal inheritance observed in glaucoma still remain to be defined. The greater life expectancy of females is one factor which could contribute to an apparently increased rate of maternal transmission in any late onset disease. Closer offspring contact with mothers than fathers is a further possible source of reporting bias. This influence would be expected to confer an apparent predominance of maternal eye history in other ophthalmic patient groups. While both Shin *et al*[3] and Morgan and Drance[2] found a predominant maternal eye history in patients with glaucoma, this parental bias was not observed in those with ocular hypertension drawn from the same population background. This suggests that closer maternal offspring contacts are also unlikely to contribute to the increased prevalence of a maternal family history.

Genetic influences other than haplotype variants of the mitochondrial genome that could account for this observation must be considered. Genomic imprinting is a plausible explanation[29] and unstable expansions of trinucleotide repeats, involved in the pathogenesis of a number of neurological disorders, are now also known to display parental bias.[30]

Authors' affiliations

R Andrews, T Ressiniotis, M Birch, P G Griffiths, Department of Ophthalmology, Royal Victoria Infirmary, Newcastle upon Tyne, UK
R Andrews, T Ressiniotis, D M Turnbull, S Keers, P F Chinnery, Department of Neurology, The Medical School, The University of Newcastle upon Tyne, UK

Correspondence to: Dr P F Chinnery, Neurology, The Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK; p.f.chinnery@ncl.ac.uk

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