Published in final edited form as:

Mult Scler. 2012 February ; 18(2): 240–243. doi:10.1177/1352458511416838.

Genetic variations within the *OPA1* gene are not associated with neuromyelitis optica

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Abstract

Neuromyelitis optica (NMO) is an idiopathic demyelinating disease which predominantly affects the optic nerve and spinal cord. Multiplex NMO pedigrees have been reported but the genetic risk factors conferring this increased familial susceptibility have not yet been determined. *OPA1* mutations have recently been identified in families with progressive visual failure and spastic paraparesis, raising the possibility that *OPA1* genetic variants could contribute to the aetiology of NMO. We therefore screened for *OPA1* in 32 patients with NMO. No pathogenic mutations were found, and none of the 13 single nucleotide polymorphisms identified were associated with an increased risk of developing NMO.

Keywords

Demyelination; Devic's disease; dominant optic atrophy; mitochondria; neuromyelitis optica; *OPA1*

Introduction

Neuromyelitis optica (NMO), also known as Devic's disease, is an idiopathic inflammatory demyelinating disease of the central nervous system (CNS) that preferentially targets the optic nerve and spinal cord.¹ The discovery of a specific NMO-IgG autoantibody marker against the aquaporin-4 (AQP4) water channel and the consistent presence of longitudinally-extensive myelitis on magnetic resonance imaging have further refined the diagnostic criteria for this disorder.² Recent clinicopathological studies have also revealed striking differences between NMO and prototypic multiple sclerosis (MS), establishing them as two distinct disease entities.³ Compared with MS, NMO is a relatively rare cause of CNS demyelination and the prevalence has been estimated at 0.5 to 1.0 per 100,000 among white caucasians.⁴

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Conflict of interest: None

The localised pathology observed within the optic nerve and spinal cord in NMO is intriguing and it is reminiscent of the more complicated phenotypes we have recently described in a subgroup of patients harbouring pathogenic *OPA1* mutations – the major causative gene for autosomal dominant optic atrophy (DOA, OMIM 165500).⁵ The cardinal feature of DOA is progressive visual failure secondary to the focal loss of retinal ganglion cell (RGC) axons and optic nerve demyelination. However, up to 20% of *OPA1* mutational carriers will develop multi-system organ involvement in later life, with a predilection for the neuraxis.⁶ In two of these families with DOA+, the *OPA1* mutation segregated with both optic atrophy and a severe progressive form of spastic paraplegia.⁶ Transcranial magnetic stimulation has also revealed subclinical demyelination of the corticospinal tract in patients with pure DOA, further highlighting the increased susceptibility of long neuronal pathways, including RGC axons, in *OPA1* disease.⁷ In one British and one French *OPA1*-positive family, the probands developed an MS-like illness, strikingly similar to the association between the primary mitochondrial DNA mutations causing Leber hereditary optic neuropathy and demyelination (Harding's disease).⁶ *OPA1* is an inner mitochondrial membrane protein, reinforcing the emerging links between mitochondrial dysfunction, oligodendrocyte survival, and neurodegeneration.⁵ Interestingly, multi-generational NMO pedigrees have been reported with clinical features indistinguishable from the more common sporadic variant.⁸ Candidate gene analysis has excluded pathogenic *AQP4* mutations and the major genetic risk determinants in these familial cases remain to be determined.^{8, 9} Our clinical observations relating to DOA+ therefore imply that some patients diagnosed with NMO could be harbouring pathogenic *OPA1* mutations, or that the expression of NMO could be influenced by specific *OPA1* single nucleotide polymorphisms (SNPs). To investigate these hypotheses, we screened a large case series of patients with NMO for possible disease-modifying *OPA1* genetic variants.

Patients and methods

Study groups

Genomic DNA samples, extracted from whole blood, were available from the following groups of white British subjects: (i) patients with NMO (N=32), identified as part of a British Neurological Surveillance Unit (BNSU) national case ascertainment study. All these patients fulfilled the revised diagnostic criteria,² with clinically- and radiologically-confirmed episodes of optic neuritis and transverse myelitis.^{4, 9} NMO anti-AQP4 antibodies were detected in 12 of these patients; (ii) patients with primary open angle glaucoma (POAG, N=137), diagnosed with either the high tension (HTG, N=67) or normal tension (NTG, N=70) form of the disease;¹⁰ and (iii) healthy controls (N = 75) with no evidence of ophthalmological or neuromuscular disorders. This study had the relevant institutional approval and written informed consent was obtained from all the subjects involved.

OPA1 genotyping

The entire coding region of the *OPA1* gene, including flanking exon-intron boundaries, was amplified by the polymerase chain reaction (PCR), using a set of 27 M13-tagged primer pairs, which is available on request. PCR products were sequenced using BigDye™ terminator cycle chemistries on an ABI3100 Genetic Analyser (Applied Biosystems, UK).

The sequence electropherograms obtained were compared with the Genbank *OPA1* reference sequence (Accession number NG_011605.1, mRNA transcript variant 1, NM_015560.2), using SeqScape™ software v2.6 (Applied Biosystems, UK).

Statistical analysis

The Hardy-Weinberg equilibrium for *OPA1* genotypes was assessed for patient and control groups (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). Allele and genotype SNP frequencies were compared with χ^2 analysis using Gra phPad™ v.4 statistical software (San Diego, CA).

Results

OPA1 variants identified

No pathogenic *OPA1* mutations were identified in the NMO cohort. A total of 13 *OPA1* variants were identified, and of these, nine were previously reported SNPs (Table 1). The remaining four variants were novel heterozygous changes found in four different patients with NMO. The exon 11 (c.1071A>G, p.A357A) and exon 22 (c.2256G>T, p.L752L) variants were synonymous, and the two novel intronic variants were not predicted to affect the neighbouring splice donor sites.

SNP associations

The SNP frequency in the NMO cohort was compared with healthy controls and the POAG group. No significant allele or genotype associations were detected with the following three *OPA1* SNPs: c.473A>G, IVS8+4c>t, and IVS8+32t>c (e-Tables 1, 2, and 3). Analysis of both the IVS8+4c>t and IVS8+32t>c SNPs showed no significantly increased risk of developing NMO with specific compound genotypes (e-Table 4). There was no significant allele or genotype associations for the remaining SNPs identified in patients with NMO compared with healthy controls (Data not shown). Further subgroup analysis did not reveal any significant *OPA1* SNP association between: (i) anti-AQP4 antibody-seropositive NMO patients and controls; (ii) anti-AQP4 antibody-seronegative NMO patients and controls; and (iii) anti-AQP4 antibody-seropositive and -seronegative NMO patients (Data not shown).

Discussion

No pathogenic *OPA1* mutations were identified in this well-characterised group of patients with NMO. Although we cannot exclude the possibility of large-scale *OPA1* genomic rearrangements, the latter is rare in DOA, accounting for less than 1% of singleton cases with suspected inherited optic atrophy.⁵ *OPA1* mutations have only been reported within the first ten intronic nucleotide positions, making it also unlikely that our PCR-based sequencing strategy has missed functional intronic variants influencing mRNA splicing or transcriptional activity.⁵ Only one non-synonymous *OPA1* SNP (c.473A>G, p.N158S) was present in patients with NMO, but it is an evolutionarily poorly-conserved amino acid residue. No significant allele and genotype associations were found for c.473A>G and the remaining SNPs identified in this NMO cohort when compared with both healthy controls and patients with POAG. Our findings contrast with recent observations in POAG, where specific *OPA1* variants at IVS8+4c>t and IVS8+32t>c have been linked with an increased

risk of developing NTG, suggesting a role for *OPA1* in glaucomatous optic nerve degeneration.¹⁰ Theoretically, these two intronic SNPs could still exert a minor influence on the expression of the NMO disease phenotype. However, NMO is a relatively rare form of CNS demyelination, and the sample size required to demonstrate these more subtle genetic modulatory effects will prove challenging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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

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Table 1
***OPA1* variants identified in the neuromyelitis optica (NMO) cohort**

cDNA	Location	AA Change	N =	Homozygous	Heterozygous
c.473A>G	Exon 4	p.N158S	25/32	15/25 (60%)	10/25 (40%)
c.557-19t>c	Intron 4	-	17/32	7/17 (41%)	10/17 (59%)
IVS8+4c>t	Intron 8	-	9/32	2/9 (22%)	7/9 (78%)
IVS8+32t>c	Intron 8	-	17/32	7/17 (41%)	10/17 (59%)
c.1071A>G*	Exon 11	p.A357A	1/32	0/1 (0%)	1/1 (100%)
c.1312+32a>g	Intron 13	-	1/32	0/1 (0%)	1/1 (100%)
c.1589+22t>g*	Intron 16	-	1/32	0/1 (0%)	1/1 (100%)
c.1770+16t>g	Intron 18	-	6/32	0/6 (0%)	6/6 (100%)
c.1770+47t>a*	Intron 18	-	1/32	0/1 (0%)	1/1 (100%)
c.1770+51t>g	Intron 18	-	17/32	7/17 (41%)	10/17 (59%)
c.2109C>T	Exon 21	p.A703A	25/32	15/25 (60%)	10/25 (40%)
c.2256G>T*	Exon 22	p.L752L	1/32	0/1 (0%)	1/1 (100%)
c.2707+25t>a	Intron 26	-	17/32	7/17 (41%)	10/17 (59%)

Identified *OPA1* variants were confirmed by reverse sequencing and checked against available databases to determine whether they had previously been reported: (i) the eOPA1 Database; (ii) the NCBI dbSNP Database; and (iii) the Human Genome Mutation Database.

* = novel *OPA1* variant, AA = amino acid; cDNA = complementary DNA; N = number of NMO patients harbouring the *OPA1* variant.