LETTER TO THE EDITOR

Does mitochondrial DNA predispose to neuromyelitis optica (Devic's disease)?

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Neuromyelitis optica (NMO), or Devic's disease, is a relapsing demyelinating disease of the central nervous system characterized by optic neuritis and myelitis with distinct clinical, imaging, CSF and serological features (Wingerchuk *et al.*, 2006). There is increasing evidence that NMO is an antibody-mediated organ-specific autoimmune disease associated with anti-aquaporin 4 antibodies detectable in serum (Lennon *et al.*, 2004), supported by four recent papers in the same edition of *Brain* (Matsuoka *et al.*, 2007; Misu *et al.*, 2007; Roemer *et al.*, 2007; Takahashi *et al.*, 2007) and the accompanying scientific commentary (Compston, 2007). However, it is still not known why the disorder specifically targets the optic nerves and spinal cord. Several siblings with NMO have been reported (McAlpine, 1938; Keegan and Weinshenker, 2000; Yamakawa *et al.*, 2000), raising the possibility of a genetic predisposition, but no pathogenic mutations have been identified in the *AQP4* gene on chromosome 18q11.2-q12.1 (Lu *et al.*, 1996).

NMO has similarities with Leber hereditary optic neuropathy (LHON, MIM 535 000) which is primarily due to mutations of mitochondrial DNA (mtDNA) that disrupt complex I of the respiratory chain (Carelli *et al.*, 2004). Although the genetic defect in LHON is present in all tissues, the pathology also is strikingly tissue-specific. Most affected individuals develop sub-acute painless visual failure due to focal involvement of both optic nerves (Newman *et al.*, 1991; Riordan-Eva *et al.*, 1995), but some also develop a progressive myelopathy, with high signal extending over multiple spinal levels on MR imaging, and the absence of oligoclonal bands in the CSF (Johns *et al.*, 1991; Jaros *et al.*, 2007). Tissue-specific susceptibility to mitochondrial dysfunction is thought to explain why the neurodegeneration in LHON only affects specific neuronal pathways, and recent evidence implicates a similar mechanism in the axonal loss that follows acute inflammatory lesions in multiple sclerosis (MS) (Dutta *et al.*, 2006).

A further link between LHON and central nervous system demyelination is the MS-like illness first described in women harbouring LHON mtDNA mutations (Harding *et al.*, 1992), characterized by severe and often irreversible bilateral visual failure. Patients with LHON-MS have typical brain imaging and unmatched oligoclonal bands in the cerebrospinal fluid (Riordan-Eva *et al.*, 1995). Although the majority of cases are female, males have been described with each of the common LHON mtDNA mutations (Lees *et al.*, 1964; Flanigan and Johns, 1993; Kellar-Wood *et al.*, 1994; Olsen *et al.*, 1995; Jansen *et al.*, 1996; Leuzzi *et al.*, 1997; Horvath *et al.*, 2000; Buhmann *et al.*, 2002).

Given the clinical similarities between NMO and LHON, previous investigators have looked for specific mtDNA mutations in a small number of patients with NMO (Johns *et al.*, 1991; Cock *et al.*, 1997; Kalman and Mandler, 2002; Ghezzi *et al.*, 2004), and others have studied polymorphic variation of mtDNA in NMO cases (Cock *et al.*, 1997; Kalman *et al.*, 1999; Kalman and Mandler, 2002; Celebisoy *et al.*, 2006). However, the largest case series only included four patients, so the role of mtDNA in the etiology of NMO has yet to be resolved. To address this issue we studied the mtDNA of 32 British patients with NMO fulfilling recent diagnostic criteria (Wingerchuk *et al.*, 2006). These patients are part of the United Kingdom NMO study cohort co-collected through the British Neurological Surveillance Unit (Jacob *et al.*, 2005). Two different hypotheses were tested: (i) that highly deleterious pathogenic LHON mtDNA mutations are a common cause of NMO; and, (ii) that mtDNA polymorphisms are associated with NMO. We consciously limited our study to patients

of European maternal ancestry to allow interpretation of the mtDNA genetic background in an appropriate population context.

Are pathogenic LHON mtDNA mutations a common cause of NMO?

We sequenced the *MTND1*, *MTND4* and *MTND6* genes which are known to harbour the vast majority (>99%) of LHON mutations, including the three most common: m.3260G>A, m.11778G>A, and m.14484T>C (Mackey *et al.*, 1996), and other primary pathogenic mutations found within the ND6 "hotspot" (Chinnery *et al.*, 2001). No LHON or other known pathogenic mutations were identified in the 32 NMO cases.

Are mtDNA polymorphisms associated with NMO?

There are a number of different ways that polymorphic variation of mtDNA could be associated with NMO. Human mtDNA is maternally inherited and has acquired extensive variation over time. Substitutions acquired >10 000 years before the present subdivide the phylogeny into a number of discrete clades called haplogroups. Specific haplogroups are preferentially associated with mtDNA mutations that cause LHON, and increase the clinical penetrance of the disorder (Brown *et al.*, 1997; Man *et al.*, 2004). Sub-haplogroup J1 is associated with an increased risk of visual failure in m.14484T>C pedigrees, and J2 is associated with an increased risk of visual failure in m.11778G>A pedigrees (Carelli *et al.*, 2006; Hudson *et al.*, 2007).

Epidemiological evidence has led to the suggestion that NMO is a prototypic form of MS which emerged out of Africa, and was shaped into its current form through a gene-environment interaction between Human leukocyte antigens and Epstein-Barr virus, either through genetic hitch-hiking or in parallel to the emergence of mtDNA haplogroup J from Western Asia (Compston, 2004; Cox *et al.*, 2005). In keeping with this, small studies have reported an association between optico-spinal MS and haplogroup J (Mayr-Wohlfart *et al.*, 1996; Reynier *et al.*, 1999; Kalman and Mandler, 2002), although this has not been a universal finding (Otaegui *et al.*, 2004).

To determine whether these deep-rooted haplogroup-defining polymorphisms are associated with NMO, the mtDNA haplogroup was defined in all 32 NMO cases by PCR-RFLP analysis (Torroni *et al.*, 1997), and compared to 1010 British controls (part of the 1958 UK-MRC birth cohort, Table 1). There was no significant difference in the overall haplogroup distribution (Exact $P = 0.117$), nor in the frequency of the individual haplogroups (Table 1). Direct sequencing of the mtDNA regions encompassing nucleotides 3010 (3010 in J1) and 15257 (15257A in J2)

Table 1 Haplogroup distribution for 32 patients with neuromyelitis optic and 1010 controls

Study Group	Haplogroup											TOTAL
	H	T	J	K	U (w/o K)	I	W	V	X	M	Other	
Neuromyelitis optica patients	16	3	1	1	5	1	2	0	1	1	1	32
Percentage (%)	50.0	9.4	3.1	3.1	15.6	3.1	6.3	0	3.1	3.1	3.1	
95% CI	33.63–66.37	2.46–25.00	0–17.11	0–17.11	6.39–32.23	0– 17.11	0.72 –21.16	0–12.73	0– 17.11	0–17.11	0– 17.11	
Controls Subjects	448	99	123	91	119	33	11	32	15	6	33	1010
Percentage (%)	44.0	9.8	12.2	9.0	11.8	3.3	1.1	3.2	1.5	0.6	3.3	
95% CI	41.26–47.48	8.04–11.80	10.22–14.36	7.32–10.95	9.86–13.93	2.32–4.56	0.54–1.94	2.18–4.44	0.83–2.44	0.22–1.29	2.26–4.56	
P-Value	0.577	1.00	0.166	0.355	0.588	1.00	0.051	0.607	0.424	0.18	1.00	

Ninty five percent confidence intervals (CI) calculated according to the Clopper-Pearson method. *P*-value = empirical *P*-value determined by Monte-Carlo simulation with 1000 iterations based on the method of Roff and Bentzen (1989) as described in Samuels *et al.* (2006). For historical reasons, haplogroups K and U are shown separately, but K is a sub-haplogroup of U. W/o = without, "Other" refers to European subjects who could not be classified into one of the ten major European haplogroups.

Table 2 *MTCYB* substitutions in 32 cases of neuromyelitis optica

Patient code	MtDNA Haplogroup	*MTCYB* substitutions					
1	H	m.15326A>G					
3	H	m.15326A>G					
4	H	m.15326A>G					
6	H	m.15326A>G					
7	H	m.15326A>G					
14	H	m.15326A>G					
16	H						
17	H	m.15326A>G					
18	H	m.15326A>G					
19	H	m.15001T>G	m.15326A>G				
24	H	m.15001T>G	m.15326A>G				
29	H	m.15326A>G					
72	H	m.15326A>G					
80	H	m.15064A>G	m.15326A>G	m.15833C>T			
86	H	m.15326A>G					
88	H	m.15326A>G					
36	I	m.14766C>T	m.14927A>G	m.15043G>A	m.15317G>A	m.15326A>G	m.15758A>G
85	J	m.14766C>T	m.14798T>C	m.15326A>G	m.15452C>A		
66	K	m.14766C>T	m.14798T>C	m.15326A>G			
64	M	m.14766C>T	m.14783T>C	m.14803C>T	m.15043G>A	m.15301G>A	m.15326A>G
33	T	m.14766C>T	m.14905G>A	m.15326A>G	m.15452C>A	m.15607A>G	
52	T	m.14766C>T	m.14905G>A	m.15326A>G	m.15452C>A	m.15607A>G	
56	T	m.14766C>T	m.14905G>A	m.15326A>G	m.15452C>A	m.15607A>G	
5	U	m.14766C>T	m.15326A>G	m.15631A>G	m.15721T>C		
34	U	m.14766C>T	m.14793A>G	m.15218A>G	m.15317G>A	m.15326A>G	m.15767C>T
59	U	m.14766C>T	m.15326A>G				
70	U	m.14766C>T	m.15326A>G				
75	U	m.14766C>T	m.14793A>G	m.15218A>G	m.15326A>G		
49	W	m.14766C>T	m.15326A>G				
77	W	m.14766C>T	m.15326A>G				
31	X	m.14766C>T	m.15326A>G				
58	Other	m.15326A>G					

(relative to the revised Cambridge reference sequence for mtDNA, Andrews *et al.*, 1999).

revealed that the one haplogroup J case of NMO belonged to sub-haplogroup J1c.

Recent phylogenetic analysis and association studies of extensive LHON pedigrees suggests that substitutions in the mtDNA gene coding for cytochrome *b, MTCYB*, are responsible for the increased risk of visual failure in haplogroup J m.11778G>A and m.14484T>C pedigrees (Carelli *et al.*, 2006; Hudson *et al.*, 2007). This may arise through the interaction between complex I and III (cyt b) subunits in super-complexes (Schagger and Pfeiffer, 2000), or the cumulative effect of deleterious mutations affecting serial components of the respiratory chain. We therefore sequenced the entire *MTCYB* gene in the 32 NMO patients and compared the result to 100 datasets of 32 randomly selected healthy control subjects identified from a subgroup of 527 healthy controls within MitoKor database (Herrnstadt *et al.*, 2002), calculating the number of synonymous, non-synonymous and the total number substitutions in *MTCYB* (Table 2, Fig. 1). The total number of *MTCYB* substitutions in the 32 NMO cases fell within the range of control values (Fig. 1a). Likewise, the number of synonymous and non-synonymous *MTCYB* changes in the NMO patients fell within the control range (Fig. 1b and 1c), as did the ratio of synonymous to non-synonymous substitutions (Fig. 1d), providing evidence against the hypothesis that the accumulation of deleterious substitutions predicted to alter cyt b function predisposes to NMO.

In conclusion, we found no evidence to support the hypothesis that ancient mtDNA polymorphisms are associated with or predispose to NMO. A study of this size cannot exclude a subtle increased susceptibility, especially if conferred by rare mtDNA variants in a region not directly sequenced here. However, this would be very difficult to demonstrate, given the rarity of NMO and the samples sizes required to show a convincing association between mtDNA variants and a complex disease (Samuels *et al.*, 2006).

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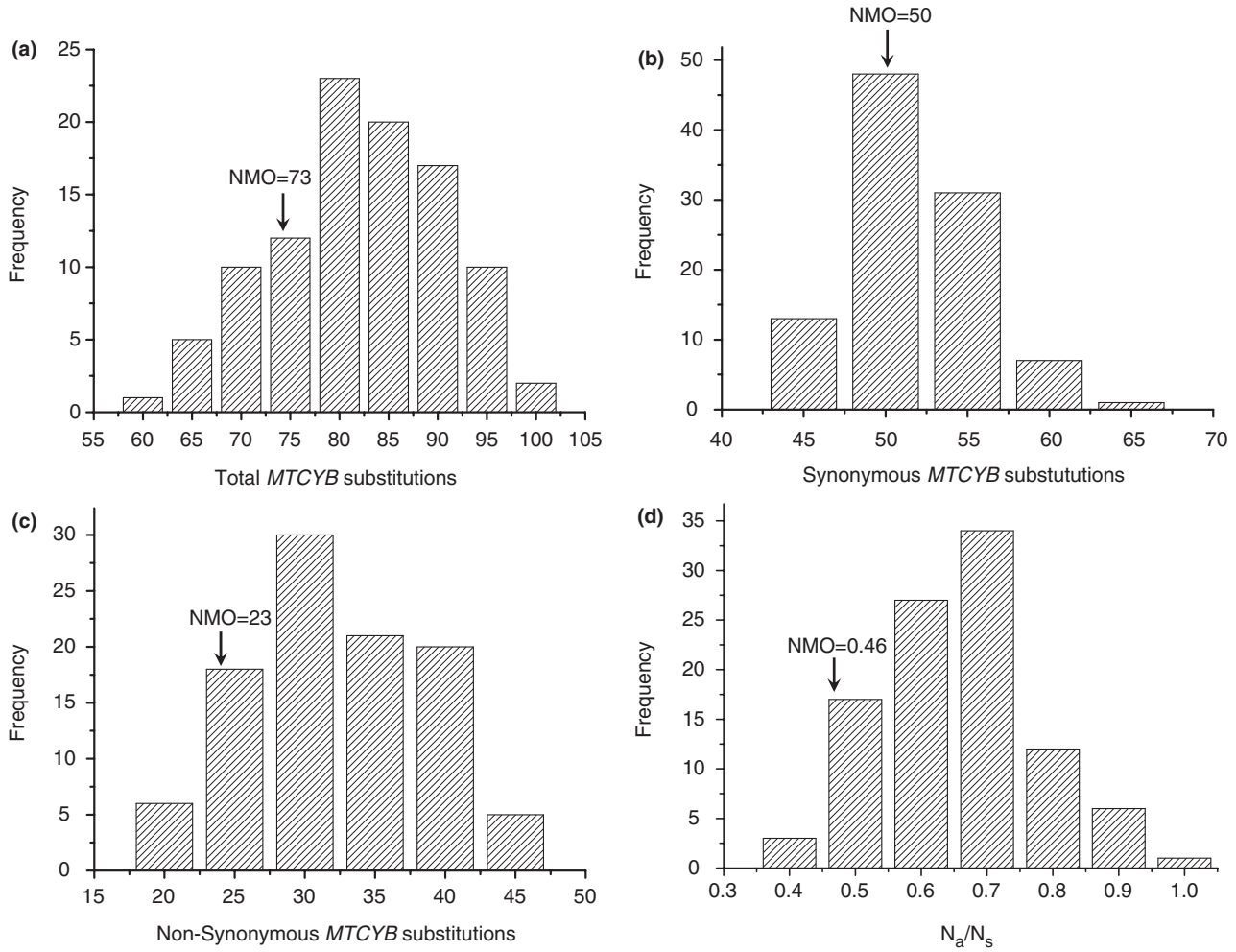

Fig. 1 Frequency distribution histograms showing the number *MTCYB* substitutions relative to the revised Cambridge reference sequence of mitochondrial DNA (Andrews *et al.*, 1999) in 100 randomly selected control subjects from a subgroup of 527 controls from the MitoKor database (Herrnstadt *et al.*, 2002): (**a**) total number of substitutions, (**b**) number of synonymous substitutions, (**c**) number of non synonymous substitutions, (**d**) the ratio of non-synonymous (N_a) to synonymous (N_s) substitutions in *MTCYB*. The corresponding value for the 32 patients with NMO is shown on each distribution with an arrow.

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