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 533 000) 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; Flanagan 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)
in the NMO patients fell within the control range of synonymous and non-synonymous substitutions in the 32 NMO cases 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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**Table 2** **MTCYB** substitutions in 32 cases of neuromyelitis optica

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(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...
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References
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