



## **Analysis of mitochondrial DNA mutations as a possible cause of Multiple Sclerosis**

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### **Summary**

As with chromosomal DNA, the DNA located in mitochondria (mitochondrial DNA or mtDNA) can contain mutations that are highly pathogenic. In fact, many diseases of the central nervous system are known to be caused by mutations in mtDNA. The possibility that mtDNA mutations contribute to susceptibility to MS has been approached from several different angles, the most well-studied of which has been the apparent connection between MS and the mitochondrial disease Leber hereditary optic neuropathy.

At this point, no clearly defined connection has been established between mtDNA mutations and the development of MS. However, the potential for such a connection still exists, perhaps in a subset of MS patients. There is also a possibility that mtDNA sequences influence the MS phenotype toward optic nerve involvement and/or that somatic mtDNA mutations induced by inflammation play a role in subsequent neurodegeneration. Each of these possibilities must be further clarified before the role played by mtDNA mutations in MS can be fully understood.

Note: Details of most of the studies mentioned in this document can be found summarized in the Cure Map genetic studies database, which can be downloaded from the Accelerated Cure Project web site:  
<http://www.acceleratedcure.org/downloads/phase2-genetic-studies.xls>

### **Hypotheses**

(1) Mutations (either inherited or somatic) or common alleles or haplogroups in mitochondrial DNA either directly cause MS or influence susceptibility to MS. (2) Mitochondrial DNA mutations, alleles or combinations of alleles influence the phenotype or progression of MS.

### **Experimental tests of the hypotheses**

Association between MS and the LHON mutations: Many of the experiments investigating associations between mtDNA genotypes and MS have explored a possible connection between MS and the mitochondrial disease Leber hereditary optic

neuropathy (LHON)\*. The possibility that MS and LHON might have a common genetic basis has been suggested by the several observations of LHON patients or carriers of LHON mutations who also have an “MS-like illness” or other features characteristic of MS<sup>1-10</sup>. These patients exhibit clinical manifestations of MS and/or demyelination as confirmed by the presence of lesions in MRI, CSF abnormalities or evoked potential results. One report noted an MS-like illness in 45% of the female LHON patients studied who had a mutation at position 11778, a much higher percentage than normally would be expected<sup>11</sup>. A recent analysis of LHON pedigrees in Belgium also revealed a higher number with MS than the authors predicted would have occurred by chance<sup>12</sup>.

To further explore the connection between MS and LHON, many scientists have performed genetic screens on MS patients for the mutations involved in or associated with LHON<sup>12-33</sup>. Despite the numerous reports of LHON patients with MS-like illness, none of the studies investigating the “primary” pathogenic mutations at 3460, 11778 and 14484 showed a significant association with MS; in fact, most did not even detect the presence of these mutations in any of the subjects. Seventeen of the LHON screens examined one or more of the “secondary” (non-pathogenic) LHON mutations at positions 4216, 4917, 13708 and 15257. Of the twelve case-control studies conducted for these mutations, seven found no significant differences between patients and controls, while five found an increased frequency in MS patients of at least one of the secondary mutations. One of these studies used a very large sample size (5,209 individuals from five European countries) to study seven mtDNA variants, including two LHON mutations. Of these, 13708 was found to be significantly associated with MS, but only in cohorts that were well-matched for age and geography<sup>32</sup>.

Finally, one study turned the MS/LHON question around and investigated whether HLA-DR (a region associated with MS) was also associated with LHON, but no evidence for such an association was found<sup>34</sup>.

Associations between MS and other mitochondrial loci: In addition to the LHON mutation studies, scientists have searched for associations with MS elsewhere in the mitochondrial DNA. For example, researchers have looked at the loci coding for a homologue for a murine transplantation antigen<sup>35</sup>, and at the loci coding for COII, ATPase 6 and 8, ND3, and ND4L subunits of oxidative phosphorylation as well as rRNA and tRNA<sup>36</sup>. However, no evidence for any association with MS was found in either study. Another group looked at the mtDNA that encodes subunits for Complex I; they found no evidence for association in their first study<sup>29</sup> but did find associations with nt4917 (a secondary LHON mutation) and nt9055 in a follow-up study<sup>33</sup>. Other studies have reported an association with MS for a polymorphism in the mt tRNA threonine gene<sup>18</sup>, and a possible connection in a small subgroup of MS subjects of first hypervariable D-loop (displacement loop) sequences in mtDNA<sup>37</sup>.

Sequencing studies have also detected novel mutations in people with MS. For instance, sequencing of a single MS subject detected a novel mutation at position

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\* LHON is an early adult-onset disease often resulting in legal blindness. 95% of cases are associated with 3 primary mutations in mtDNA. Penetrance of homoplasmic mutations ranges from 8%-43% depending on specific mutation and gender (women have lower penetrance), indicating that other genetic and/or environmental factors are required. LHON is a relatively rare disease; prevalence in Europe has been estimated at 1-2/100,000.

4298<sup>38</sup>; sequencing of 26 optic neuritis cases revealed potentially pathogenic novel mutations at ten locations<sup>30</sup>; and sequencing of six mitochondrial genes in 20 MS subjects detected four new point mutations. One particularly large study sequenced the entire mitochondrial genome of 159 MS subjects and identified 62 novel variants, none of which were seen in more than one subject<sup>39</sup>. On the other hand, a mutation at position 13966 found during a full mtDNA sequencing of an MS subject was detected once again in a separate screening of 49 MS subjects<sup>40</sup>. It is unclear whether any of these novel mutations may have played a role in the development of disease in these subjects, although in the optic neuritis study mentioned above, those subjects with potentially pathogenic mutations had worse residual visual function after recovery than the other subjects.

Associations between MS and mitochondrial haplogroups: Other genetic studies have investigated related groups of mtDNA loci, or haplogroups, with respect to MS. As with the individual-locus studies mentioned above, these investigations have produced mixed results. Six studies have found an association between MS or MS with optic neuritis and haplogroups J or K (or J\* or K\*)<sup>32, 33, 41-44</sup>. Because many of the mutations associated with LHON occur in haplogroup J, it is conceivable that the LHON-MS association is due to susceptibility alleles for the two diseases sharing the same haplogroup. However, other studies have found no association between these haplogroups and MS<sup>29, 31, 39, 45</sup>. Furthermore, speculating that the link between MS and certain mtDNA genotypes is due to differences in reactive oxygen species production, one team of researchers searched for a correlation between mtDNA haplogroup J\* or K\* and reactive oxygen species production, but no such correlation was found<sup>46</sup>.

Other haplogroups that have been associated with MS include haplogroups A<sup>44</sup>, BM<sup>43</sup>, and U<sup>39</sup>. Many other haplogroups, such as H, I, M, and V, have been analyzed in various studies but not associated with MS. One possible reason for the discrepancies among these studies is that subjects from a variety of regions and ethnic groups were analyzed (e.g., North American, Persian, Basque, and Spanish Caucasian).

Contributions of mutations in mtDNA to phenotype or progression: A few of the studies referred to above involving the LHON mutations found higher incidences of mutations in patients with optic neuritis compared to those without optic neuritis. However, others found no meaningful differences between these two groups of patients. Similarly, of those LHON screens that included only MS patients with visual involvement, some showed evidence for association with LHON mutations while others did not.

mtDNA mutations may also influence disease progression in MS. For instance, it has been suggested that oxidative species released as part of the inflammatory process may induce somatic mutations in mtDNA, which themselves may then go on to influence the progression of the disease in certain ways. Three studies demonstrated that nitric oxide (NO) induces damage to mitochondrial DNA in oligodendrocytes; one of these studies showed that oligodendrocytes are more sensitive to the effects of NO than are astrocytes or microglia<sup>47-49</sup>. Another two studies documented the presence of oxidative species or oxidative damage to mitochondrial DNA in plaque tissue<sup>50, 51</sup>. However, in another study, accumulation of mtDNA deletions, which has been associated with neurodegeneration and aging, was found to be equally prevalent in MS normal-appearing brain tissue compared with plaque tissue, and in MS normal-appearing brain tissue compared with tissue from age-matched controls<sup>52</sup>.

Gene expression studies: A small number of studies have assessed mitochondrial gene expression and activity in tissues from people with MS. For instance, a study of skeletal muscle detected reductions of complex I activities in MS subjects compared with controls<sup>53</sup>, and another showed that complex I and III activities were decreased in the motor cortex of people with MS<sup>54</sup>. However, these alterations may result from something other than mtDNA mutations (and in fact, one of these two studies did not find any mtDNA deletions in skeletal muscle from the MS subjects<sup>53</sup>).

Copy number variations: One study analyzed the copy number of a particular mtDNA gene compared with that of an invariable nuclear gene, and found this ratio to be increased in normal-appearing gray matter in MS subjects (compared with normal-appearing white matter and plaques from MS subjects or gray matter from non-neurological disease controls). The authors suggested that this increase in mtDNA copy numbers may reflect an attempt to compensate for axonal loss in MS gray matter<sup>55</sup>. A similar study of 26 optic neuritis subjects (six of whom had clinically definite MS) reported that the ON group had a higher lymphocyte mtDNA content, but lower mitochondrial respiratory activity, than controls<sup>30</sup>. Sixteen of the ON subjects were also found to harbor potentially pathologic mtDNA variants.

## Conclusions

Because transmission of mtDNA is maternally-based, diseases that are caused solely by defects in mtDNA are marked by the absence of father-child affected pairs. Father-child affected pairs do exist in MS, and indeed some (although not all) studies have found them to be relatively overrepresented in MS compared with mother-child affected pairs<sup>56</sup>. Therefore, MS is not caused *solely* by inherited mtDNA mutations. (It is hypothetically possible, if we assume that MS is a group of diseases, that inherited mtDNA mutations could be the sole and sufficient cause in a subset of MS families where the transmission is maternal. In such a situation where an mtDNA mutation alone can trigger a form of MS, heteroplasmy might explain why some of the offspring in these families develop MS and some do not. At present there is no evidence for this particular model of MS.)

A more realistic possibility is that susceptibility to MS is *influenced* by inherited mtDNA mutations or alleles. Circumstantial evidence in the form of patients with LHON reporting MS-like symptoms has led to multiple studies searching for associations with MS in the mitochondrial genome. At this time no locus has been definitively linked with MS although the “secondary” LHON mutations, which showed mixed results, may merit further research, as may other polymorphisms that have been identified in single studies. The primary LHON mutations have largely produced negative results in screens of MS patients. However, the increased prevalence of MS in LHON families suggests that primary LHON mutations may nevertheless increase susceptibility to MS, and that the failure to find an association in the MS patient studies is due to the rarity of these mutations. Alternatively, the LHON mutations may belong to a haplogroup which also contains a mutation predisposing to MS.

Because many of the studies of mtDNA in MS patients were limited in population size or number of loci examined, a comprehensive screen including all candidate loci and a sufficient number of patients and controls would be helpful in resolving whether certain inherited mtDNA genotypes increase MS risk. Careful matching of cases to controls is important, as highlighted in one analysis<sup>32</sup> which found that variants differed in frequency

between older vs. younger people and among people from different geographic locations to a degree that can affect the outcome of genetic studies. In addition, results from some of the LHON screening studies raise the possibility that mtDNA mutations influence MS phenotype, specifically the presence and extent of visual involvement. However, the nature of this influence is not yet well-defined and would also benefit from further study.

The possibility that *somatic* mtDNA mutations accumulating over time may be an initial cause of MS has not been extensively explored. It is possible that tissue-specific clonal expansion of mtDNA point mutations or deletions may take place in CNS or other tissue leading to CNS degeneration. This phenomenon appears to become more pronounced with age and has been suggested to play a role in aging or degenerative disease<sup>57</sup>. As mentioned above, however, one study found no evidence that the type of deletions associated with aging were more common in the brain tissue of people with MS than in age-matched controls. It is also possible that some factor (e.g., a mutagen or defect in mtDNA repair) acting on mtDNA in a tissue-specific manner may trigger MS.

With regard to MS progression, the few studies examining the inducibility and presence of mtDNA somatic mutations in MS suggest that mtDNA damage incurred during the course of MS may contribute further to the pathogenesis of the disease. It has been demonstrated that oxidative species are capable of damaging mtDNA *in vitro*, that oxidative markers are present in MS plaques and proximal areas, and that oxidative damage to mtDNA can be found in chronic active plaques. Again, more research is needed to define the exact contribution of this damage to the neurodegeneration seen in MS.

## References:

- 1 A. E. Harding, M. G. Sweeney, D. H. Miller, C. J. Mumford, H. Kellar-Wood, D. Menard, W. I. McDonald and D. A. Compston, *Occurrence of a multiple sclerosis-like illness in women who have a Leber's hereditary optic neuropathy mitochondrial DNA mutation*. Brain, 1992. **115**(Pt 4): p. 979-89. PubMed ID: 1393514.

The authors describe eight women with white matter lesions as shown by MRI, optic neuropathy with or without an MS-like illness, and the most common mtDNA mutation observed in LHON (11778).

- 2 F. M. Meire, R. Van Coster, P. Cochaux, B. Obermaier-Kusser, C. Candaele and J. J. Martin, *Neurological disorders in members of families with Leber's hereditary optic neuropathy (LHON) caused by different mitochondrial mutations*. Ophthalmic Genet, 1995. **16**(3): p. 119-26. PubMed ID: 8556281.

An individual from an LHON family with the 3460 mutation is described as having periventricular MS-like white matter lesions.

- 3 E. K. Nikoskelainen, R. J. Marttila, K. Huoponen, V. Juvonen, T. Lamminen, P. Sonninen and M. L. Savontaus, *Leber's "plus": neurological abnormalities in*

*patients with Leber's hereditary optic neuropathy.* J Neurol Neurosurg Psychiatry, 1995. **59**(2): p. 160-4. PubMed ID: 7629530.

Two of 38 LHON patients studied had an MS-like syndrome, exhibiting apparent periventricular white matter changes in MRI.

- 4 N. K. Olsen, A. W. Hansen, S. Norby, A. L. Edal, J. R. Jorgensen and T. Rosenberg, *Leber's hereditary optic neuropathy associated with a disorder indistinguishable from multiple sclerosis in a male harbouring the mitochondrial DNA 11778 mutation.* Acta Neurol Scand, 1995. **91**(5): p. 326-9. PubMed ID: 7639060.

The authors describe a male patient with LHON and the 11778 mutation who also had an MS-like illness. They recommend that the MS diagnostic process be expanded to include mitochondrial genotyping.

- 5 P. H. Jansen, M. S. van der Knaap and I. F. de Coo, *Leber's hereditary optic neuropathy with the 11 778 mtDNA mutation and white matter disease resembling multiple sclerosis: clinical, MRI and MRS findings.* J Neurol Sci, 1996. **135**(2): p. 176-80. PubMed ID: 8867076.

This report describes a mother and her son, both affected with LHON through the 11778 mutation, who also exhibited clinical and paraclinical evidence compatible with MS.

- 6 M. T. Bhatti and N. J. Newman, *A multiple sclerosis-like illness in a man harboring the mtDNA 14484 mutation.* J Neuroophthalmol, 1999. **19**(1): p. 28-33. PubMed ID: 10098545.

The authors describe a young male patient with clinical and paraclinical signs of demyelination and bilateral visual loss who harbored the 14484 LHON mutation.

- 7 R. Horvath, A. Abicht, E. A. Shoubbridge, V. Karcagi, C. Rozsa, S. Komoly and H. Lochmuller, *Leber's hereditary optic neuropathy presenting as multiple sclerosis-like disease of the CNS.* J Neurol, 2000. **247**(1): p. 65-7. PubMed ID: 10701903.

An MS-like disease was found in two of 39 Hungarian LHON patients, one homoplasmic for the 11778 mutation and the other for the 14484 mutation.

- 8 M. Tran, R. Bhargava and I. M. MacDonald, *Leber hereditary optic neuropathy, progressive visual loss, and multiple-sclerosis-like symptoms.* Am J Ophthalmol, 2001. **132**(4): p. 591-3. PubMed ID: 11589893.

This paper reported the case of a 34-year-old man with LHON, MS-like symptoms, and multiple brain and spinal cord lesions on MRI harboring a mutation at 11778.

- 9 D. Lev, M. Yanoov-Sharav, N. Waternberg, E. Leshinsky-Silver and T. Lerman-Sagie, *White matter abnormalities in Leber's hereditary optic neuropathy due to the 3460 mitochondrial DNA mutation.* Europ J Paediatr Neurol, 2002. **6**(2): p. 121-3. PubMed ID: 11995959.

A case is described of a female LHON patient with the 3460 mutation and with white matter lesions.

- 10 C. Buhmann, J. Gbadamosi and C. Heesen, *Visual recovery in a man with the rare combination of mtDNA 11778 LHON mutation and a MS-like disease after mitoxantrone therapy*. Acta Neurol Scand, 2002. **106**(4): p. 236-9. PubMed ID: 12225323.

A case is reported of a man with the LHON mutation 11778 and confirmed MS. The patient experienced unusual visual recovery during treatment with mitoxantrone. The authors suggest evaluating all MS patients with predominant visual impairment for presence of the LHON mutation.

- 11 A. E. Harding, P. Riordan-Eva and G. G. Govan, *Mitochondrial DNA diseases: genotype and phenotype in Leber's hereditary optic neuropathy*. Muscle Nerve, 1995. **3**(4): p. S82-4. PubMed ID: 7603533.

A study of 107 LHON patients from 79 families found that an MS-like illness occurred in 45% of the female patients with LHON mutation 11778.

- 12 L. Vanopdenbosch, B. Dubois, M. B. D'Hooghe, F. Meire and H. Carton, *Mitochondrial mutations of Leber's hereditary optic neuropathy: a risk factor for multiple sclerosis*. J Neurol, 2000. **247**(7): p. 535-43. PubMed ID: 10993496.

A literature review concerning the connection between LHON and MS, combined with clinical analysis of 103 LHON patients and 40 MS patients, leads the authors to conclude that having a primary LHON mutation is a risk factor for developing MS and that all three primary mutations have been associated with an MS-like illness. The data suggests that severe bilateral visual symptoms justifies screening MS patients for LHON, but screening LHON patients for MS is likely to be more rewarding.

- 13 H. Kellar-Wood, N. Robertson, G. G. Govan, D. A. Compston and A. E. Harding, *Leber's hereditary optic neuropathy mitochondrial DNA mutations in multiple sclerosis*. Ann Neurol, 1994. **36**(1): p. 109-12. PubMed ID: 8024249.

The LHON pathogenic mutations 11778 and 3460 were not found in a group of 307 randomly selected MS patients. However, three of 20 patients selected for prominent and early optic nerve involvement carried either the 11778 or 3460 mutation. The secondary mutation 13708 was found at similar frequencies in the 307 patients and a pool of 129 healthy controls.

- 14 B. Kalman, F. D. Lublin and H. Alder, *Mitochondrial DNA mutations in multiple sclerosis*. Mult Scler, 1995. **1**(1): p. 32-6. PubMed ID: 9345467.

A higher frequency of class II LHON mutations was observed in MS patients over controls (21% of the 53 patients compared with 10% of the 74 controls were positive for at least two (4216 and 4917 or 13,708) or three (4216, 13,708, 15,257) simultaneous class II LHON mutations). The presence of these mutations did not correlate with visual involvement. No class I mutations with primary pathogenic significance were detected in these MS patients.

- 15 M. Nishimura, H. Obayashi, M. Ohta, T. Uchiyama, Q. Hao and T. Saida, *No association of the 11778 mitochondrial DNA mutation and multiple sclerosis in Japan*. *Neurology*, 1995. **45**(7): p. 1333-4. PubMed ID: 7617193.  
None of the 80 Japanese MS patients screened, including 18 women with MS with bilateral optic neuropathy, carried the 11778 LHON mutation.
- 16 M. Sartore, M. Grasso, G. Piccolo, R. Fasani, R. Bergamaschi, A. Malaspina, M. Ceroni, M. Kobayashi, A. Semeraro, E. Arbustini and et al., *Leber's hereditary optic neuropathy (LHON)-related mitochondrial DNA sequence changes in italian patients presenting with sporadic bilateral optic neuritis*. *Biochem Mol Med*, 1995. **56**(1): p. 45-51. PubMed ID: 8593537.
- 18 Italian patients presenting with optic neuritis (11 of which went on to develop MS) were examined for 14 mtDNA polymorphisms. None carried the primary LHON mutations; single and multiple secondary LHON mutations at 4216, 4917, and 13708 were seen, but at the same frequencies as MS controls without optic nerve symptoms and healthy controls.
- 17 R. M. Chalmers, N. Robertson, D. A. S. Compston and A. E. Harding, *Sequence of mitochondrial DNA in patients with multiple sclerosis*. *Ann Neurol*, 1996. **40**(2): p. 239-43. PubMed ID: 8773606.  
Based on evidence that MS transmits preferentially through maternal line, the mtDNA of 9 MS patients with family history consistent with maternal transmission was sequenced. Four base-pair changes were found: 4216 and 4917, which may play a role in LHON, and 11447 and 14766, which were found in all 9 patients. A larger follow-on study involving 175 MS patients and 233 healthy controls showed similar frequencies for each of these polymorphisms in patients and controls.
- 18 U. Mayr-Wohlfart, C. Paulus, A. Henneberg and G. Rodel, *Mitochondrial DNA mutations in multiple sclerosis patients with severe optic involvement*. *Acta Neurol Scand*, 1996. **94**(3): p. 167-71. PubMed ID: 8899049.  
Primary LHON mutations were not detected in restriction enzyme analysis of 100 MS patients with altered VEP and 100 controls. However, MS patients show a higher percentage of secondary mutations, particularly in combination. Two neighboring base pair substitutions in a mt tRNA haplotype were significantly more frequent ( $p = 0.00018$ ).
- 19 H. Cock, R. Mandler, W. Ahmed and A. H. Schapira, *Neuromyelitis optica (Devic's syndrome): no association with the primary mitochondrial DNA mutations found in Leber hereditary optic neuropathy*. *J Neurol Neurosurg Psychiatry*, 1997. **62**(1): p. 85-7. PubMed ID: 9010406.  
A screen of 4 women with Devic's syndrome did not detect either the primary LHON mutations 3460, 11778 and 14484 or the secondary LHON mutation 4160.
- 20 B. Kalman, J. L. Rodriguez-Valdez, U. Bosch and F. D. Lublin, *Screening for Leber's hereditary optic neuropathy associated mitochondrial DNA mutations in*



*patients with prominent optic neuritis.* Mult Scler, 1997. **2**(6): p. 279-82.  
PubMed ID: 9065918.

LHON associated mutations were excluded in 22 patients with prominent optic neuritis whose optical atrophy developed in association with benign or severely disabling forms of MS.

- 21 V. Leuzzi, C. Carducci, M. Lenza, M. Salvetti, G. Ristori, S. Di Giovanni and A. Torroni, *LHON mutations in Italian patients affected by multiple sclerosis.* Acta Neurol Scand, 1997. **96**(3): p. 145-8. PubMed ID: 9300066.

Primary LHON mutations were excluded in a study of 74 Italian MS patients and 99 controls except for one patient with a virtually homoplasmic mutation at 11778. The secondary LHON mutation 15257 was found in a homoplasmic state in 5.4% of patients and 5.1% of controls.

- 22 A. Ohlenbusch, E. Wilichowski and F. Hanefeld, *Characterization of the mitochondrial genome in childhood multiple sclerosis. I. Optic neuritis and LHON mutations.* Neuropediatrics, 1998. **29**(4): p. 175-9. PubMed ID: 9762692.

Eight novel missense mutations were found in a complete sequencing of the mtDNA in six children with MS and visual involvement. Five of the six children carried a total of nine secondary LHON mutations at 4216, 4917, and 13708. The authors concluded that secondary LHON mutations are unlikely to contribute to genetic predisposition for MS, but may predispose for prominent optic nerve involvement.

- 23 E. Wilichowski, A. Ohlenbusch and F. Hanefeld, *Characterization of the mitochondrial genome in childhood multiple sclerosis. II. Multiple sclerosis without optic neuritis and LHON-associated genes.* Neuropediatrics, 1998. **29**(6): p. 307-12. PubMed ID: 10029350.

Sequencing of mtDNA in 13 children with MS and 20 controls for genes known to be the loci for LHON-associated mutations did not find evidence for involvement of nucleotide substitutions within the ND1, ND2, ND4, ND5, ND6, COI, COIII or cytochrome b genes in the etiology of MS. No primary LHON mutations were found, and secondary LHON mutations were found at a higher frequency in controls than in patients.

- 24 D. S. Mojon, K. Fujihara, M. Hirano, C. Miller, N. S. Lincoff, L. D. Jacobs and S. J. Greenberg, *Leber's hereditary optic neuropathy mitochondrial DNA mutations in familial multiple sclerosis.* Graefes Arch Clin Exp Ophthalmol, 1999. **237**(4): p. 348-50. PubMed ID: 10208269.

42 patients with familial MS were tested for presence of primary LHON mutations 3460, 11778 and 14484. None were found although two patients with unilateral optic neuritis harbored the secondary 15257 mutation.

- 25 D. S. Mojon, J. Herbert, S. A. Sadiq, J. R. Miller, M. Madonna and M. Hirano, *Leber's hereditary optic neuropathy mitochondrial DNA mutations at nucleotides 11778 and 3460 in multiple sclerosis.* Ophthalmologica, 1999. **213**(3): p. 171-5. PubMed ID: 10202290.

103 patients with clinically definite MS were screened for 11778 and 3460 mutations; neither mutation was found in any of the patients.

- 26 X. Feng, W. Pu, D. Gao, Y. Isashiki and N. Ohba, *Diagnostic potential of mitochondrial DNA assessment in patients with optic neuropathy*. Chin Med J (Engl), 2000. **113**(8): p. 743-6. PubMed ID: 11776061.

Four patients with MS in a group of 79 patients with some type of neuropathy were not found to be carriers of LHON mutations 3460, 11778 and 14484.

- 27 J. M. Hwang, B. L. Chang and S. S. Park, *Leber's hereditary optic neuropathy mutations in Korean patients with multiple sclerosis*. Ophthalmologica, 2001. **215**(6): p. 398-400. PubMed ID: 11741103.

20 Korean patients were screened for some or all of the LHON mutations 11778, 3460, 14484 and 15257; none were found.

- 28 M. Houshmand, M. H. Sanati, I. Rashedi, F. Sharifpanah, E. Asghari and J. Lotfi, *Lack of association between Leber's hereditary optic neuropathy primary point mutations and multiple sclerosis in Iran*. Eur Neurol, 2004. **51**(2): p. 68-71. PubMed ID: 14671420.

A study of 31 Iranian MS subjects with optic nerve involvement and 25 control subjects without optic nerve involvement showed no association of mtDNA variants 11778, 3460 or 14484 with MS.

- 29 T. Vyshkina, I. Banisor, Y. Y. Shugart, T. P. Leist and B. Kalman, *Genetic variants of Complex I in multiple sclerosis*. J Neurol Sci., 2005. **228**(1): p. 55-64. PubMed ID: 15607211.

None of the mtDNA variants tested were associated with MS, but certain haplotypes of nuclear-encoded Complex I genes were more common in mothers of MS subjects than in fathers.

- 30 T. M. Bosley, C. S. Constantinescu, C. R. Tench and K. K. Abu-Amero, *Mitochondrial changes in leukocytes of patients with optic neuritis*. Mol Vis., 2007. **13**(p. 1516-28. PubMed ID: 17893651.

Sixteen of 26 optic neuritis subjects were found to have potentially pathologic mtDNA changes. mtDNA content in lymphocytes was increased in ON subjects compared with controls, while mitochondrial respiratory activity was decreased.

- 31 S. M. Mihailova, M. I. Ivanova, L. M. Quin and E. J. Naumova, *Mitochondrial DNA variants in Bulgarian patients affected by multiple sclerosis*. Eur J Neurol., 2007. **14**(1): p. 44-7. PubMed ID: 17222112.

mtDNA variants T4216C was significantly more frequent in MS subjects than controls. None of the other secondary LHON mutations were associated with MS; nor was any mtDNA haplogroup.

- 32 X. Yu, D. Koczan, A. M. Sulonen, D. A. Akkad, A. Kroner, M. Comabella, G. Costa, D. Corongiu, R. Goertsches, M. Camina-Tato, H. J. Thiesen, H. I. Nyland, S. J. Mork, X. Montalban, P. Rieckmann, M. G. Marrosu, K. M. Myhr, J. T. Epplen, J. Saarela and S. M. Ibrahim, *mtDNA nt13708A variant increases the risk of multiple sclerosis*. PLoS ONE., 2008. **3**(2): p. e1530. PubMed ID: 18270557.

In a large study (over 5,000 subjects) of seven mtDNA variants, only 13708 was associated with MS. Further study supported the involvement of this SNP in MS rather than a linked variant.

- 33 T. Vyshkina, A. Sylvester, S. Sadiq, E. Bonilla, J. A. Canter, A. Perl and B. Kalman, *Association of common mitochondrial DNA variants with multiple sclerosis and systemic lupus erythematosus*. Clin Immunol., 2008. **129**(1): p. 31-5. Epub 2008 Aug 16. PubMed ID: 18708297.

K\* haplotype was significantly associated with MS; J\* was only associated with MS at the trend level.

- 34 G. G. Govan, P. R. Smith, H. Kellar-Wood, A. H. Schapira and A. E. Harding, *HLA class II genotypes in Leber's hereditary optic neuropathy*. J Neurol Sci, 1994. **126**(2): p. 193-6. PubMed ID: 7853025.

No evidence for an association between LHON and any HLA-DR genotype was found in a genetic study involving members of 79 LHON families.

- 35 R. M. Chalmers, N. Robertson, H. Kellar-Wood, D. A. Compston and A. E. Harding, *Sequence of the human homologue of a mitochondrially encoded murine transplantation antigen in patients with multiple sclerosis*. J Neurol, 1995. **242**(5): p. 332-4. PubMed ID: 7643143.

This study searched for evidence that polymorphisms for a homologue for the murine N-terminal portion of NADH-dehydrogenase subunit 1 encoded in mtDNA play a role in MS. No such evidence was found. The experiment included 87 MS patients, 10 LHON patients with MS-like illness, and 31 controls.

- 36 A. Ohlenbusch, E. Wilichowski and F. Hanefeld, *Characterization of the mitochondrial genome in childhood multiple sclerosis. III. Multiple sclerosis without optic neuritis and the non- LHON-associated genes*. Neuropediatrics, 1998. **29**(6): p. 313-9. PubMed ID: 10029351.

Sequencing of mtDNA in 13 children with MS and 20 controls found no evidence that increased susceptibility to MS is conferred by COII, ATPase 6 and 8, ND3, or ND4L subunits of oxidative phosphorylation as well as rRNA and tRNA.

- 37 B. Kalman, H. Alder, U. F. Bosch, F. D. Lublin and D. Chatterjee, *The evolutionary relationship among Caucasian MS patients and controls*. Mult Scler, 1996. **1**(5): p. 288-95. PubMed ID: 9345431.

An analysis was performed of the first hypervariable D-loop sequences of the mtDNA in patients with MS selected at random, MS patients with prominent optic neuritis, and

controls. Patients in both groups were generally representative of the Caucasian phylogeny, although a small cluster of unrelated MS patients was identified.

- 38 R. W. Taylor, P. F. Chinnery, M. J. Bates, M. J. Jackson, M. A. Johnson, R. M. Andrews and D. M. Turnbull, *A novel mitochondrial DNA point mutation in the tRNA(Ile) gene: studies in a patient presenting with chronic progressive external ophthalmoplegia and multiple sclerosis*. *Biochem Biophys Res Commun*, 1998. **243**(1): p. 47-51. PubMed ID: 9473477.

A new mutation was observed at position 4298 in the mitochondrial tRNA gene in a patient with MS and chronic progressive external ophthalmoplegia. This mutation was heteroplasmic in the skeletal muscle but not found in the patient's blood. This gene appears to be a mutational hotspot.

- 39 M. Ban, J. Elson, A. Walton, D. Turnbull, A. Compston, P. Chinnery and S. Sawcer, *Investigation of the role of mitochondrial DNA in multiple sclerosis susceptibility*. *PLoS ONE*, 2008. **3**(8): p. e2891. PubMed ID: 18682780.

Sequencing of 159 MS subjects and haplogroup analysis of 835 MS cases and 1506 controls revealed only a trend toward association with MS for super-haplogroup U.

- 40 B. Kalman, F. D. Lublin and H. Alder, *Characterization of the mitochondrial DNA in patients with multiple sclerosis*. *J Neurol Sci*, 1996. **140**(1-2): p. 75-84. PubMed ID: 8866430.

Three MS patients underwent full mtDNA sequencing; 8 unusual variants were found and investigated in a larger sample (49 patients and 63 controls). Two of these mutations warranted further investigation. The mutation at 13966 was found in two MS patients but in none of the controls nor in the large control population in the literature, and the mutation at 14798 demonstrated a higher frequency in patients with optic nerve involvement. The authors concluded that mtDNA mutations are not necessary for developing MS but may participate in the etiology or influence the phenotype in some subgroups.

- 41 P. Reynier, I. Penisson-Besnier, C. Moreau, F. Savagner, B. Vielle, J. Emile, F. Dubas and Y. Malthiery, *mtDNA haplogroup J: a contributing factor of optic neuritis*. *Eur J Hum Genet*, 1999. **7**(3): p. 404-6. PubMed ID: 10234520.

mtDNA haplogroup J was found only in European MS patients with optic neuritis but not in patients without visual symptoms.

- 42 B. Kalman, S. Li, D. Chatterjee, J. O'Connor, M. R. Voehl, M. D. Brown and H. Alder, *Large scale screening of the mitochondrial DNA reveals no pathogenic mutations but a haplotype associated with multiple sclerosis in Caucasians*. *Acta Neurol Scand*, 1999. **99**(1): p. 16-25. PubMed ID: 9925234.

Large-scale mtDNA screening performed in 77 Caucasian MS patients and 84 controls by restriction site polymorphism and haplotype analysis revealed an association between MS and the combination of haplogroups K\* and J\*, defined by simultaneous presence of Ddel restriction sites at nucleotides 10,394 and 14,798 in haplotype K and J (p-value 0.001). These haplogroups when analyzed singly were also increased in MS

patients over controls (p-values both < 0.05). No correlation existed between these haplogroups and phenotypic characteristics. The authors postulate that the association between LHON and MS may be due to an overlap in the genetic background for the diseases.

- 43 M. Houshmand, M. H. Sanati, F. Babrzadeh, A. Ardalan, M. Teimori, M. Vakilian, M. Akuchekian, D. Farhud and J. Lotfi, *Population screening for association of mitochondrial haplogroups BM, J, K and M with multiple sclerosis: interrelation between haplogroup J and MS in Persian patients*. *Mult Scler.*, 2005. **11**(6): p. 728-30. PubMed ID: 16320736.

Haplogroups BM and J were associated with MS in a study of 70 MS subjects and 70 controls; no association was found for M and K.

- 44 H. H. Kumleh, M. Houshmand, M. S. Panahi, G. H. Riazi, M. H. Sanati, K. Gharagozli and M. Ghabaee, *Mitochondrial D-Loop Variation in Persian Multiple Sclerosis Patients: K and A Haplogroups as a Risk Factor!!* *Cell Mol Neurobiol*, 2006. **6**(p. 6. PubMed ID: 16680528.

Haplogroups A and K were significantly more common in 54 MS subjects compared with 100 controls.

- 45 O. Fernandez, V. Fernandez, V. Martinez-Cabrera, C. Mayorga, A. Alonso, A. Leon, C. Arnal, M. Hens, G. Luque, E. de Ramon, A. Caballero and L. Leyva, *Multiple sclerosis in Gypsies from southern Spain: prevalence, mitochondrial DNA haplogroups and HLA class II association*. *Tissue Antigens.*, 2008. **71**(5): p. 426-33. Epub 2008 Feb 28. PubMed ID: 18312478.

No significant differences in mtDNA sequences were found in Gypsy MS cases compared with Gypsy controls.

- 46 O. Vladimirova, F. M. Lu, L. Shawver and B. Kalman, *The activation of protein kinase C induces higher production of reactive oxygen species by mononuclear cells in patients with multiple sclerosis than in controls*. *Inflamm Res*, 1999. **48**(7): p. 412-6. PubMed ID: 10450792.

The release of reactive oxygen species (ROS) from peripheral blood mononuclear cells in MS patients in relapse and remission did not show a correlation with mtDNA haplogroup. ROS production characteristics did show certain differences between relapse and remission and also differed in healthy controls and controls with other neurological diseases.

- 47 A. J. Mackenzie-Graham, B. Mitrovic, A. Smoll and J. E. Merrill, *Differential sensitivity to nitric oxide in immortalized, cloned murine oligodendrocyte cell lines*. *Dev Neurosci*, 1994. **16**(3-4): p. 162-71. PubMed ID: 7535681.

Mitochondrial DNA damage was observed in differing degrees in five different lines of murine oligodendrocytes exposed to the nitric oxide donor SNAP. DNA damage was found in viable cells and thus was independent of cell death.

- 48 B. Mitrovic, J. Parkinson and J. E. Merrill, *An in Vitro Model of Oligodendrocyte Destruction by Nitric Oxide and Its Relevance to Multiple Sclerosis*. *Methods*, 1996. **10**(3): p. 501-13. PubMed ID: 8954860.

An in vitro experiment found that nitric oxide-induced damage to oligodendrocytes was more severe than that induced in astrocytes and microglia as measured by DNA strand breaks, mitochondrial dysfunction, morphological changes and cell death.

- 49 N. M. Druzhyna, S. I. Musiyenko, G. L. Wilson and S. P. LeDoux, *Cytokines induce nitric oxide-mediated mtDNA damage and apoptosis in oligodendrocytes. Protective role of targeting 8-oxoguanine glycosylase to mitochondria*. *J Biol Chem.*, 2005. **280**(22): p. 21673-9. Epub 2005 Apr 4. PubMed ID: 15811855.

Introduction of inflammatory cytokines to cultured rat oligodendrocytes resulted in elevated nitric oxide production by the cells as well mtDNA damage. mtDNA damage could be reduced via application of the DNA repair enzyme hOGG1.

- 50 O. Vladimirova, J. O'Connor, A. Cahill, H. Alder, C. Butunoi and B. Kalman, *Oxidative damage to DNA in plaques of MS brains*. *Mult Scler*, 1998. **4**(5): p. 413-8. PubMed ID: 9839301.

The oxidative marker 8-OH-dG was found to be elevated in MS plaques compared with normal appearing white matter. It was also found to be elevated in normal appearing cortical tissues in the vicinity of plaques compared to control cortical tissue. Irreversible degeneration may result from DNA damage due to reactive oxygen species and nitric oxide produced during inflammatory episodes.

- 51 F. Lu, M. Selak, J. O'Connor, S. Croul, C. Lorenzana, C. Butunoi and B. Kalman, *Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis*. *J Neurol Sci*, 2000. **177**(2): p. 95-103. PubMed ID: 10980305.

The presence of damage to cytoplasmic DNA, in conjunction with impaired NADH activity and a possible increase in complex IV activity, was confirmed in cells within chronic active plaques. The authors suggest that oxidative damage to mtDNA may result in decreased ATP synthesis and ultimately cell death or degeneration.

- 52 A. Blokhin, T. Vyshkina, S. Komoly and B. Kalman, *Lack of mitochondrial DNA deletions in lesions of multiple sclerosis*. *Neuromolecular Med*, 2008. **10**(3): p. 187-94. PubMed ID: 18286391.

Examination of brain tissue samples from MS subjects and controls revealed no increased accumulation of mtDNA deletions that are associated with aging.

- 53 H. H. Kumleh, G. H. Riazi, M. Houshmand, M. H. Sanati, K. Gharagozli and M. Shafa, *Complex I deficiency in Persian multiple sclerosis patients*. *J Neurol Sci.*, 2006. **243**(1-2): p. 65-9. Epub 2006 Jan 18. PubMed ID: 16413582.

Testing of skeletal muscle from MS and control subjects found a reduction in complex I activities in the MS tissue but no excess mtDNA deletion.

- 54 R. Dutta, J. McDonough, X. Yin, J. Peterson, A. Chang, T. Torres, T. Gudz, W. B. Macklin, D. A. Lewis, R. J. Fox, R. Rudick, K. Mirnics and B. D. Trapp, *Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients*. *Ann Neurol.*, 2006. **59**(3): p. 478-89. PubMed ID: 16392116.

Gene transcription analysis in six MS and six control brains showed a reduction in Complex I and III activity in MS motor cortex, specific for neurons.

- 55 A. Blokhin, T. Vyshkina, S. Komoly and B. Kalman, *Variations in mitochondrial DNA copy numbers in MS brains*. *J Mol Neurosci.*, 2008. **35**(3): p. 283-7. Epub 2008 Jun 20. PubMed ID: 18566918.

mtDNA copy number values were higher in neurons of normal-appearing gray matter of MS tissue compared with neurons from other regions. Values were also higher in MS NAGM compared with control GM.

- 56 O. H. Kantarci, L. F. Barcellos, E. J. Atkinson, P. P. Ramsay, R. Lincoln, S. J. Achenbach, M. De Andrade, S. L. Hauser and B. G. Weinshenker, *Men transmit MS more often to their children vs women: the Carter effect*. *Neurology.*, 2006. **67**(2): p. 305-10. PubMed ID: 16864824.

A study of familial MS found that transmission of MS from fathers to children was more common than transmission from mothers to children.

- 57 E. Nekhaeva, N. D. Bodyak, Y. Kraytsberg, S. B. McGrath, N. J. Van Orsouw, A. Pluzhnikov, J. Y. Wei, J. Vijg and K. Khrapko, *Clonally expanded mtDNA point mutations are abundant in individual cells of human tissues*. *Proc Natl Acad Sci U S A*, 2002. **99**(8): p. 5521-6. PubMed ID: 11943860.

This paper demonstrates the significant presence of clonally expanded somatic mtDNA point mutations in diverse human tissues such as buccal epithelium and heart muscle. The ability of these mutations to expand, combined with their high incidence, raises the possibility of their involvement in degenerative disease.

**Terms searched in conjunction with “multiple sclerosis”:**  
mitochondria\* DNA