Genetic variation in the methylenetetrahydrofolate reductase gene, *MTHFR*, does not alter the risk of visual failure in Leber’s hereditary optic neuropathy

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Purpose: Focal neurodegeneration of the optic nerve in Leber hereditary optic neuropathy (LHON) is primarily due to a maternally inherited mitochondrial DNA mutation. However, the markedly reduced penetrance of LHON and segregation pattern of visual failure within families implicates an interacting nuclear genetic locus modulating the phenotype. Folate deficiency is known to cause bilateral optic neuropathy, and defects of folate metabolism have been associated with nonarteritic ischemic optic neuropathy.

Methods: Methylenetetrahydrofolate reductase (*MTHFR*) catalyzes a critical step in folate metabolism, and genetic variation in *MTHFR* has been associated with several late-onset neurodegenerative diseases.

Results: We therefore determined whether functional genetic variants in *MTHFR* could account for the reduced penetrance in LHON by studying 414 LHON mtDNA mutation carriers. We found no evidence of association between visual failure in LHON and *MTHFR* polymorphisms or the *MTHFR* haplotype.

Conclusions: Genetic variation in *MTHFR* does not provide an explanation for the variable phenotype in LHON.

Leber hereditary optic neuropathy (LHON; OMIM #535000) is a common cause of inherited blindness that typically presents with bilateral, painless, subacute vision failure in young adult males. Affected individuals develop focal degeneration of the optic nerve and present clinically with impaired color vision (dyschromatopsia), a dense visual field defect (central or cecocentral scotoma), and abnormal visual electrophysiology due to primary retinal ganglion cell loss [1]. The diagnosis is usually confirmed by molecular genetic analysis for one of three common mitochondrial DNA (mtDNA) mutations which all affect genes coding for complex I subunits of the respiratory chain: m.3460G>A, m.11778G>A, and m.14484T>C. However, only a few patients harboring a pathogenic LHON mtDNA mutation develop visual failure [2,3]. Segregation analysis of LHON pedigrees indicated a two-locus model: a mtDNA mutation as one locus and a modulating X-chromosomal locus [4]. Although an interacting X-chromosomal locus could explain the gender bias in LHON, not all pedigrees with LHON show linkage to the X-chromosome [5-7], and the segregation pattern in some pedigrees implicates one or more autosomal loci [8]. However, attempts to identify a nuclear modifying gene by both genetic mapping and functional genomics have so far failed to identify the interacting nuclear genes.

Folate is a necessary component for cellular maintenance and growth, especially important during early embryonic development, where it is involved in DNA synthesis. Methylenetetrahydrofolate reductase (*MTHFR*) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a critical step in the remethylation of homocysteine (Hcy) to methionine. Genetic variants in *MTHFR* are associated with hyperhomocysteinemia and cardiovascular disease [9] and are also associated with neural tube defects in the fetus [10]. c.677C>T, present at approximately 33%-37% heterozygously and roughly 10% homozygously in Europeans, leads to a substitution of alanine to valine (at position 222) in the catalytic domain of *MTHFR*, and subsequent reduction in enzyme activity [11]. This effect is magnified when c.677C>T is found as a compound heterozygote with homozygous c.1298A>C [12,13].

Previous studies have shown a link between oxidative stress and increased Hcy in neurodegenerative disorders [14, 15], with a pronounced increase in Hcy in homozygote c.677C>T Alzheimer disease [16] and Parkinson disease [17]. Elevated levels of Hcy have been shown to cause endothelial dysfunction by increasing oxidative stress or impairing nitric oxide metabolism [18,19]. Increased Hcy was shown to induce apoptotic death in retinal ganglion cells, hypothesized as a cause of LHON [20], by overstimulation of the N-methyl-D-aspartate receptors and caspase-3 activation [21].

Increased Hcy, but not the c.677C>T variation, was identified as a risk factor in nonarteritic ischemic optic neuropathy and central retinal vein occlusion [22,23]. Folate...
## Table 1. Non-synonymous MTHFR variants in LHON.

| Genotype frequency | Patients | WT | Het | MT | P   |
|--------------------|----------|----|-----|----|-----|
| rs45438591         | A        | 173| 8   | 0  | 0.323 |
|                    | C        | 222| 6   | 0  |      |
| rs1801133          | A        | 113| 56  | 13 | 0.479 |
|                    | C        | 151| 60  | 21 |      |
| rs45571736         | A        | 127| 50  | 3  | 0.585 |
|                    | C        | 172| 54  | 3  |      |
| rs1801131          | A        | 81 | 78  | 23 | 0.143 |
|                    | C        | 112| 102 | 16 |      |
| rs2274976          | A        | 151| 25  | 5  | 0.104 |
|                    | C        | 207| 19  | 3  |      |
| rs35737219         | A        | 177| 2   | 3  | 0.171 |
|                    | C        | 221| 9   | 2  |      |

| Allele frequency   | Patients | WT | MT | P   |
|--------------------|----------|----|----|-----|
| rs45438591         | A        | 354| 8  | 0.2854 |
|                    | C        | 450| 6  |      |
| rs1801133          | A        | 282| 82 | 0.867 |
|                    | C        | 362| 102|      |
| rs45571736         | A        | 304| 56 | 0.364 |
|                    | C        | 398| 60 |      |
| rs1801131          | A        | 240| 124| 0.131 |
|                    | C        | 326| 134|      |
| rs2274976          | A        | 327| 35 | 0.03 |
|                    | C        | 433| 25 |      |
| rs35737219         | A        | 356| 8  | 0.66 |
|                    | C        | 451| 13 |      |

Comparison of MTHFR variant genotype and allele frequencies between LHON patients (A) and controls (C; where WT and MT are homozygous wild-type and mutant, respectively and Het is heterozygous. P is an uncorrected Pearson's chi-square probability).
deficiency is known to cause bilateral optic neuropathy [24, 25]. Evidence is accumulating that implicates folate metabolism in optic neuropathies, particularly those affecting the retinal ganglion cell, making MTHFR a strong autosomal candidate genetic modifier in LHON, despite not localizing to the X chromosome and therefore less likely to contribute directly to the gender bias in LHON.

METHODS

We studied 12 common nonsynonymous MTHFR (NM_005957.3) single nucleotide polymorphisms (SNPs): (rs2066472, rs45550133, rs45438591, rs45571736, rs45496998, rs45449298, rs2274974, rs45590836, rs2274976, rs35737219, rs1801133, and rs1801131) in a European cohort of 414 LHON mtDNA mutation carriers (182 affected, 232 unaffected). All subjects were recruited from two European centers with local ethical review board approval in accordance with the declaration of Helsinki. 70% of the attached individuals were male, and 41% of the unaffected individuals were male, in keeping with the gender bias that characterizes LHON. All were homoplasmic for m.3460G>A, m.11778G>A, or m14484T>C. rs1801133 corresponds to c.677C>T, and rs1801131 corresponds to c.1298A>C. The additional ten SNPs were selected using the following criteria: 1) nonsynonymous substitutions predicted to affect MTHFR function; and 2) present in control subjects at >0.1% (dbSNP) [26].

The clinical phenotype was determined by a local ophthalmologist [1] and the genetic diagnosis was confirmed in affected individuals by mtDNA direct sequencing of the MTND genes or PCR-RFLP analysis. Control participants (unaffected mutation carriers) had no visual symptoms and were older (>30 years) than the median age of onset for LHON (24 years). The frequency of sequence variants was determined in European controls by primer extension of multiplex polymerase chain reaction products with the detection of the allele-specific extension products by matrix-associated laser desorption/ionization time of flight (MALDITOF; Sequenom, San Diego, CA) mass spectrometry. Genotype and allelic associations were compared using SPSS v15.0 using Fishers exact test. The p values given are two-tailed. To correct for multiple testing bias, we performed permutation testing using Haploview 4.0. Statistical power calculation is available at DSS research.

RESULTS

We analyzed SNP frequencies in 12 non-synonymous SNPs in a large LHON cohort. Six of the SNPs (rs2066472, rs45550133, rs45496998, rs45449298, rs2274974, rs45590836, rs2274976, rs35737219, rs1801133, and rs1801131) in a European cohort of 414 LHON mtDNA mutation carriers (182 affected, 232 unaffected). All subjects were recruited from two European centers with local ethical review board approval in accordance with the declaration of Helsinki. 70% of the attached individuals were male, and 41% of the unaffected individuals were male, in keeping with the gender bias that characterizes LHON. All were homoplasmic for m.3460G>A, m.11778G>A, or m14484T>C. rs1801133 corresponds to c.677C>T, and rs1801131 corresponds to c.1298A>C. The additional ten SNPs were selected using the following criteria: 1) nonsynonymous substitutions predicted to affect MTHFR function; and 2) present in control subjects at >0.1% (dbSNP) [26].

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DISCUSSION

We found no statistically robust association between any of the 12 functional MTHFR SNPs, individually or as complex
Comparison of LHON mutation specific MTHFR variant genotype and allele frequencies between LHON patients (A) and controls (C) where WT and MT are homozygous wild-type and mutant respectively and Het is heterozygous. P is an uncorrected Pearson’s chi-square probability.

| Allele | 3460 | 11778 | 14484 | Other |
|--------|------|-------|-------|-------|
|        | A    | C     | P     | A     | C     | P     | A     | C     | P     |
| rs45438591 |       |       |       |       |       |       |       |       |       |
| WT     | 32   | 47    | 0.231 | 118   | 149   | 0.333 | 8     | 8     | 1     |
| HET    | 1    | 0     |       | 6     | 4     | 1     | 1     | 1     | 0     |
| MT     | 0    | 0     |       | 0     | 0     | 0     | 0     | 0     | 0     |
| rs1801133 |       |       |       |       |       |       |       |       |       |
| WT     | 24   | 33    | 0.224 | 74    | 104   | 0.199 | 7     | 7     | 1     |
| HET    | 10   | 12    | 0.38  | 43    | 38    | 2     | 2     | 1     |
| MT     | 0    | 4     | 15    | 0     | 0     | 0     | 0     | 0     |
| rs45571736 |       |       |       |       |       |       |       |       |       |
| WT     | 31   | 34    | 0.018 | 83    | 118   | 0.166 | 1     | 6     | 0.028 |
| HET    | 3    | 15    | 41    | 35    | 6     | 6.1   | 0     |
| MT     | 0    | 0     | 1     | 1     | 2     | 2     | 0     |
| rs1801131 |       |       |       |       |       |       |       |       |       |
| WT     | 15   | 26    | 0.395 | 58    | 71    | 0.365 | 5     | 5     | 0.05 |
| HET    | 14   | 20    | 52    | 71    | 4     | 4     | 8     |
| MT     | 2    | 3     | 17    | 13    | 0     | 0     | 1     |
| rs2274976 |       |       |       |       |       |       |       |       |       |
| WT     | 27   | 41    | 0.624 | 111   | 143   | 0.13  | 4     | 7     | 0.148 |
| HET    | 5    | 6     | 15    | 10    | 2     | 2     | 3     |
| MT     | 2    | 1     | 0     | 2     | 0     | 0     |
| rs35737219 |       |       |       |       |       |       |       |       |       |
| WT     | 32   | 48    | 0.356 | 124   | 149   | 0.383 | 9     | 8     | 0.303 |
| HET    | 0    | 0     | 2     | 7     | 0     | 0     | 0     |
| MT     | 2    | 1     | 1     | 1     | 0     |

Comparison of LHON mutation specific MTHFR variant genotype and allele frequencies between LHON patients (A) and controls (C) where WT and MT are homozygous wild-type and mutant respectively and Het is heterozygous. P is an uncorrected Pearson’s chi-square probability.
genotypes, and vision failure in LHON families, or when affected individuals were compared to controls. It is intriguing that specific SNPs appeared to be associated with vision failure when considered in subgroup analyses separating the different LHON mtDNA mutations and different genders, but these associations did not stand up to the rigors of a correction for multiple significance testing. We therefore interpreted our findings conservatively, but larger studies may show that these associations are pathophysiologically relevant.

Although we cannot exclude the possibility that MTHFR contributes to the pathophysiology of LHON, our findings indicate that the gene is unlikely to be the major nuclear genetic modifier interacting with the primary mtDNA mutations. Genes encoding other enzymes involved in folate metabolism may be relevant, as could dietary intake of folate. Biochemical and epidemiological studies would address these issues. Further genetic studies on a genome-wide level are required to define the nuclear-mitochondrial interaction in LHON.

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### Table 4. c.677C>T and c.1298A>C complex genotypes in LHON

| rs1801131 :rs1801133 | Total | A   | U   | P   |
|---------------------|-------|-----|-----|-----|
| AA:CC               | 130   | 50  | 79  | 0.137 |
| AA:CT               | 54    | 24  | 30  | 1    |
| AA:TT               | 12    | 7   | 5   | 0.381 |
| CA:CC               | 109   | 48  | 61  | 1    |
| CA:CT               | 52    | 26  | 26  | 0.372 |
| CA:TT               | 19    | 4   | 15  | 0.056 |
| CC:CC               | 26    | 15  | 11  | 0.157 |
| CC:CT               | 10    | 6   | 4   | 0.345 |
| CC:TT               | 3     | 2   | 1   | 0.584 |
| Total               | 415   | 182 | 232 |

Comparison of c.677C>T and c.1298A>C (rs1801133:rs1801131) compound genotypes between LHON patients (A) and controls (C). P is an uncorrected Pearson’s chi-square probability.
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