Leber hereditary optic neuropathy (LHON; OMIM #535000) is a mitochondrial genetic disease that causes blindness in young adults, with an estimated minimum prevalence of 3.2 per 100 000 in the north east of England. It classically presents as bilateral subacute loss of central vision due to the focal neurodegeneration of the retinal ganglion cell layer. Over 95% of cases are principally due to one of three “primary” mtDNA point mutations: 3460G→A, 11778G→A, and 14484T→C, all of which involve genes that encode complex I subunits of the mitochondrial respiratory chain. However, less than ~50% of male and ~10% of female LHON carriers will develop the optic neuropathy.

This marked incomplete penetrance and gender bias clearly indicates that additional genetic and/or environmental factors influence the penetrance of the primary mtDNA mutations that cause focal degeneration of the optic nerve in LHON. There is evidence that the mtDNA background could be relevant for the phenotypic expression of LHON.

We carried out a meta-analysis of 297 published LHON pedigrees and confirmed the strength of the association between one specific mtDNA lineage, haplogroup J, and two of the primary LHON mutations: 11778G→A (odds ratio (OR) = 3.48, 95% confidence interval (CI) 2.36 to 5.15) and 14484T→C (OR = 27.53, 95% CI 14.53 to 52.13), and confirmed the absence of an association with the 3460G→A mutation (OR = 1.29, 95% CI 0.57 to 2.90).

The most compelling explanation is that the risk of visual loss in LHON carriers with the 11778G→A and 14484T→C mutations is increased by haplogroup J, and by extension, one or more of the mtDNA polymorphisms that define this haplogroup.
controls from the north east of England had also been determined previously using the same protocol.\textsuperscript{21}

**Systematic review**

Published studies that had analysed haplogroup distribution in LHON pedigrees were identified by searching the main electronic databases (MedLine and Web of Science) from 1988, when the first LHON mutation was reported.\textsuperscript{22} The keywords used in the search strategy were “optic atrophy”, “mitochondrial DNA”, “haplogroup”, and “phylogeny”. The reference lists of relevant papers were also assessed for the presence of additional studies not listed in these databases. Finally, the main investigators in the field of mitochondrial genetics were also contacted personally to obtain any unpublished data that might clarify some of their published results, especially if the raw data were not provided in the original paper.

**Study selection**

For the purpose of this review, studies (published or unpublished) were included only if sufficient data had been collected by the investigators to allow the haplogroup to be clearly deduced for their LHON pedigrees. In some of these studies, haplogroup status was not reported directly. However, pedigrees could still be grouped as being either J or non-J, as long as the polymorphic status at nucleotide positions 4216 and 13708 had been determined.\textsuperscript{26} As far as possible, we tried to ascertain that all included pedigrees were unrelated and of European extraction.

**Statistical analysis**

A meta-analysis was carried out using the Mantel-Haenszel method as implemented in the Cochrane Review ManagerTM software (version 4.1). A fixed effect model was adopted given the lack of significant heterogeneity between the included studies (http://www.cochrane.de/cochrane/hbook.htm). A meta-analysis was carried out using the Mantel-Haenszel method as implemented in the Cochrane Review ManagerTM software (version 4.1). A fixed effect model was adopted given the lack of significant heterogeneity between the included studies (http://www.cochrane.de/cochrane/hbook.htm). Moreover, none of these mtDNA mutations has been found to confer a competitive advantage, leading to persistence of haplogroup J and the 11778G\textsuperscript{R}A and 14484T\textsuperscript{R}C mutations? It is conceivable that this could be due to an early founder effect, whereby the 11778G\textsuperscript{R}A and 14484T\textsuperscript{R}C mutations arose early in the evolution of haplogroup J, leading to its over-representation on that mitochondrial lineage. There is some evidence in support of this hypothesis in the Dutch population,\textsuperscript{13} but this cannot provide a complete explanation because all three primary LHON mutations have arisen multiple times on different mitochondrial backgrounds\textsuperscript{14} (see also the discussion in Howell et al\textsuperscript{15} and Brown et al\textsuperscript{16}).

**RESULTS**

In our population based LHON cohort from the north east of England, there was a trend towards haplogroup J being over-represented in 11778G\textsuperscript{R}A and 14484T\textsuperscript{R}C pedigrees compared with our control population, but this was not statistically significant because of the small number of pedigrees involved (table 2). A total of 10 other haplogroup J association studies were identified through our search strategy (table 1). The main findings of our statistical meta-analysis are summarised graphically by a forest plot (fig 1).

**DISCUSSION**

This meta-analysis confirms the reported association between two of the primary LHON mutations, 11778G\textsuperscript{R}A and 14484T\textsuperscript{R}C, and haplogroup J. This was particularly marked for 14484T\textsuperscript{R}C, with pedigrees harbouring this mutation being \(\sim\)30 times more likely to belong to this particular haplogroup than controls. While it is important to consider the possibility that these results are influenced by the method of ascertainment, particularly through a publication bias following the identification of the so-called “secondary” LHON mtDNA mutations in 1991, the magnitude of the association is so great that it seems unlikely that this can be the sole explanation. Moreover, our epidemiological study of LHON in a defined geographical region revealed the same trend towards a haplogroup J association, adding weight to our conclusion.

How can we explain the association between mtDNA haplogroup J and the 11778G\textsuperscript{R}A and 14484T\textsuperscript{R}C mutations? It is conceivable that this could be due to an early founder effect, whereby the 11778G\textsuperscript{R}A and 14484T\textsuperscript{R}C mutations arose early in the evolution of haplogroup J, leading to its over-representation on that mitochondrial lineage. There is some evidence in support of this hypothesis in the Dutch population,\textsuperscript{13} but this cannot provide a complete explanation because all three primary LHON mutations have arisen multiple times on different mitochondrial backgrounds\textsuperscript{14} (see also the discussion in Howell et al\textsuperscript{15} and Brown et al\textsuperscript{16}).}

**Table 1** Summary of haplogroup association studies in LHON

<table>
<thead>
<tr>
<th>Study</th>
<th>Reference</th>
<th>Year</th>
<th>n J</th>
<th>n Non-J</th>
<th>n J</th>
<th>n Non-J</th>
<th>n J</th>
<th>n Non-J</th>
<th>n J</th>
<th>n Non-J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1991</td>
<td>175</td>
<td>16</td>
<td>159</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>1991</td>
<td>175</td>
<td>16</td>
<td>159</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>1992</td>
<td>179</td>
<td>25</td>
<td>174</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>25</td>
<td>1993</td>
<td>175</td>
<td>16</td>
<td>159</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>26</td>
<td>1994</td>
<td>160</td>
<td>16</td>
<td>144</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>27</td>
<td>1995</td>
<td>175</td>
<td>16</td>
<td>159</td>
<td>10</td>
<td>1</td>
<td>17</td>
<td>4</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>28</td>
<td>1997</td>
<td>67</td>
<td>5</td>
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<td>4</td>
<td>1</td>
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<td>10</td>
<td>13</td>
<td>21</td>
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<tr>
<td>29</td>
<td>1999</td>
<td>28</td>
<td>8</td>
<td>20</td>
<td>2</td>
<td>3</td>
<td>13</td>
<td>4</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Current study</td>
<td>1999</td>
<td>175</td>
<td>25</td>
<td>174</td>
<td>5</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In most of these studies, haplogroup distribution for a representative sample of normal controls from the local population was also reported. If the latter was not provided, this was sought either directly from the original investigators or from other published sources: Finland\textsuperscript{20} and the Netherlands (Dr G Barbujani, personal communication).
explanation is that the risk of visual loss is increased by haplogroup J.

If this mtDNA background does have a deleterious effect, it would be expected that haplogroup J should result in a more pronounced respiratory chain defect, and thus influence the phenotype of LHON. Cybrid cell lines carrying the 11778G→A mutation and haplogroup J were shown to have a lower oxygen consumption and a longer doubling time compared with cell lines with the 11778G→A mutation alone. However, a recently published study showed no difference in respiratory chain function between cybrid cell lines harbouring mtDNA from different haplogroups on the same

<table>
<thead>
<tr>
<th>Study</th>
<th>3460G→A</th>
<th>Controls</th>
<th>OR (95% CI fixed)</th>
<th>OR (95% CI fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>2/9</td>
<td>16/175</td>
<td>0.84 (0.54, 1.24)</td>
<td>0.84 (0.54, 1.24)</td>
</tr>
<tr>
<td>26</td>
<td>0/3</td>
<td>25/179</td>
<td>0.87 (0.42, 1.82)</td>
<td>0.87 (0.42, 1.82)</td>
</tr>
<tr>
<td>22</td>
<td>0/8</td>
<td>16/160</td>
<td>0.52 (0.03, 9.34)</td>
<td>0.52 (0.03, 9.34)</td>
</tr>
<tr>
<td>8</td>
<td>1/10</td>
<td>16/175</td>
<td>1.12 (0.67, 1.87)</td>
<td>1.12 (0.67, 1.87)</td>
</tr>
<tr>
<td>28</td>
<td>1/4</td>
<td>5/67</td>
<td>4.10 (0.37, 47.41)</td>
<td>4.10 (0.37, 47.41)</td>
</tr>
<tr>
<td>10</td>
<td>0/7</td>
<td>7/99</td>
<td>0.42 (0.04, 15.83)</td>
<td>0.42 (0.04, 15.83)</td>
</tr>
<tr>
<td>29</td>
<td>0/3</td>
<td>33/529</td>
<td>2.12 (0.11, 41.84)</td>
<td>2.12 (0.11, 41.84)</td>
</tr>
<tr>
<td>30</td>
<td>0/2</td>
<td>9/78</td>
<td>1.46 (0.07, 32.84)</td>
<td>1.46 (0.07, 32.84)</td>
</tr>
<tr>
<td>Current study</td>
<td>0/5</td>
<td>25/179</td>
<td>0.55 (0.03, 10.27)</td>
<td>0.55 (0.03, 10.27)</td>
</tr>
</tbody>
</table>
nuclear genetic background. In vivo magnetic resonance spectroscopy in patients harbouring the 11778G→A mutation also failed to detect any deleterious effect in brain and skeletal muscle from haplogroup J. The influence of haplogroup J on the biochemical features of the 14484T→C mutation has not yet been determined. This result would be interesting in order to clarify the much stronger association of haplogroup J with 14484T→C compared with 11778G→A. However, these studies will require cautious interpretations, given that both in vitro and in vivo biochemical studies have produced conflicting results regarding the extent of respiratory chain dysfunction in LHON. There is currently no evidence that haplogroup J influences age of onset or final visual outcome in LHON, although this trend requires further confirmation in a larger LHON cohort. Haplogroup J is one of nine European specific haplogroups, and therefore it would also be expected that LHON should be more common in populations of European extraction. This hypothesis will be difficult to test, given the paucity of data regarding the prevalence of LHON in different ethnic groups, and potential confounding factors such as a population bottleneck.

The analysis presented here also provides strong statistically based evidence that there is no association between haplogroup J and the 3460G→A mtDNA mutation. This is a most intriguing finding, given the strong haplogroup affiliation of the other primary LHON mutations. However, the 3460G→A mutation seems to behave differently in a number of ways. Firstly, 3460G→A is the LHON mutation most consistently associated with a significant biochemical complex I defect; secondly, compared with the 14484T→C mutation, it is associated with a poorer visual outcome; and thirdly, it is associated with a less prominent gender bias. This evidence suggests that 3460G→A is perhaps a “stronger” mutation—that is, less susceptible to the epistatic and epigenetic factors influencing the expression of the 14484T→C mutation and possibly the 11778G→A mutation.

Based on our meta-analysis of all published and unpublished datasets, there seems to be little doubt that the 14484T→C mutation, and to a lesser extent the 11778G→A mutation, are over-represented in haplogroup J, but several additional questions remain unanswerable. What is the combination of polymorphisms within haplogroup J that increases the risk of disease expression? Unfortunately, there are insufficient published data available to carry out haplogroup J sub-cluster analysis and explore further the differences reported in sub-clusters J1 and J2. Why should the 3460G→A mutation prove refractory to the mitochondrial genetic background? We have as yet no answer. Addressing these important issues will not only advance our understanding of LHON, but will also have broader relevance for other pathogenic mtDNA mutations.

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Authors’ affiliations

P Y W Man, D M Turnbull, P F Chinnery, Department of Neurology, The Medical School, University of Newcastle Upon Tyne, UK
N Howell, Mitokor, San Diego, CA, USA

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P Y W Man, N Howell, D A Mackey, S Nørby, T Rosenberg, D M Turnbull and P F Chinnery

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Correction


The first author’s name of all three papers has been corrected to Patrick Yu-Wai-Man.

doi:10.1136/jmg-2003-011247corr1, doi:10.1136/jmg-40.4.e41corr1, and doi:10.1135.jmg.39.3.162corr1

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