Homoplasmic and Exclusive ND4 Gene Mutation in Japanese Pedigrees With Leber's Disease

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Purpose. To preliminarily examine whether mitochondrial heteroplasmy or synergism of multiple mitochondrial (mt) DNA mutations are related to the symptoms manifested in Japanese pedigrees with Leber's hereditary optic neuropathy (LHON), 90 percent of which have an mtDNA mutation at position 11778 in the NADH dehydrogenase subunit 4 (ND4) gene. This would be a first step toward clarifying why not all individuals with the 11778 mutation are affected in this ethnically unique population.

Methods. Seven ND4 11778 mutant Japanese pedigrees, including 17 maternal line members, were analyzed by restriction fragment length polymorphisms and Southern blot hybridization using mutant and wild-type sequence-specific oligonucleotide probes of leukocyte DNA amplified by polymerase chain reaction.

Results. All of the members, regardless of symptoms, were revealed to possess only the homoplasmic ND4 11778 mutation with no other mtDNA mutation tested. On the other hand, all of the affected individuals were male, and conversely, all of the unaffected were female, except for an 18-year-old male with only peripapillary microangiopathy.

Conclusions. Neither heteroplasmy of the ND4 11778 mutation nor simultaneous mutations reported in the different complex I genes can account for the variation in the clinical phenotype in our series. Taken together with the sex bias in symptom manifestation, the results indirectly suggest that an extramitochondrial factor, such as an X-chromosome-linked gene, possibly contributes to the development of optic atrophy in the Japanese LHON pedigrees tested. Invest Ophthalmol Vis Sci. 1993;34:488-495.

Leber's hereditary optic neuropathy (LHON) is a maternally transmitted mitochondrial disease, characterized by acute or subacute bilateral, usually permanent loss of central vision, mostly developing during adolescence.1 Wallace et al2 first found a G-to-A mutation at nucleotide position (nt) 11778 of mitochondrial (mt) DNA in 9 of 11 LHON families, which causes a substitution of the 340th amino acid histidine for a highly conserved arginine in NADH dehydrogenase subunit 4 (ND4). Approximately one-half of white LHON pedigrees carry this mutation,2-6 and some of the remaining pedigrees have an alternative LHON-specific mutation at nt 3460 or 4160 in ND1 gene,7-9 whereas about 90% of Japanese LHON pedigrees possess the ND4 11778 mutation,10-15 indicating a highly significant ethnic difference in the genetic heterogeneity of LHON.15

However, not all individuals with one of the LHON-specific mtDNA mutations manifest symptoms. Thus, the presence of a single mtDNA mutation...
Three possibilities have been proposed as candidates for an additional factor or factors necessary for LHON expression. The first is heteroplasmy of the ND4 11778 mutation. Each cell contains thousands of mitochondria and mtDNAs, and the proportion of mutant to wild-type mtDNA molecules coexisting differs among cells, organs, and individuals. Some LHON patients are demonstrated to have a higher proportion of mutant mtDNA molecules compared with their asymptomatic maternal relatives. The second possibility is synergism or antagonism of simultaneous multiple mutations in one or more complex I genes. Third, genealogical data regarding the characteristic mode of inheritance in LHON, such as male preference and reduced penetrance with late onset in females, raise the possibility of involvement of an X-chromosome-linked genetic factor. The two-locus mitochondrial and X-linked gene model has been proposed. None of these three issues have been substantially studied in Japanese LHON pedigrees.

As a first step toward clarifying which of the above scenarios plays a key role in symptom manifestation in our ethnically unique population, we have investigated whether or not the mitochondrial genotype (heteroplasmy or homoplasmy of the ND4 11778 mutation, or the simultaneous presence or absence of multiple mtDNA mutations) correlates to clinical phenotype. Our result that all matrilineage members, irrespective of phenotypes, harbor only the homoplasmic ND4 11778 mutation without the other reported mtDNA mutations in complex I genes, indicates that there is no apparent correlation of mitochondrial genotype to clinical phenotype. This indirectly suggests that an extra-mitochondrial genetic factor possibly contributes to the sex bias in LHON expression in the Japanese pedigrees tested.

**MATERIALS AND METHODS**

**Subjects**

We collected seven Japanese LHON pedigrees, including six reported pedigrees (families A to F in Table 1) and a new pedigree (family G in Table 1), based on the criteria of Vilkki et al. Table 1 shows 18 individuals examined in the pedigrees: nine male LHON patients (seven probands, two maternal line males [D1, D3]), one microangiopathic male (A2), seven asymptomatic maternal line females, and one internal control of D6 (a son of the affected male D1). Ten unrelated apparently healthy donors also were examined.

**Table 1. Family Members Examined in Seven LHON Pedigrees**

<table>
<thead>
<tr>
<th>Family</th>
<th>Case No.</th>
<th>Sex</th>
<th>Age*</th>
<th>Family Relation</th>
<th>Phenotype</th>
<th>Age at Onset</th>
<th>Visual Acuity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>M</td>
<td>16</td>
<td>Proband</td>
<td>Optic atrophy</td>
<td>16</td>
<td>8/200 4/200</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>18</td>
<td>Brother</td>
<td>Microangiopathy</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>F</td>
<td>42</td>
<td>Mother</td>
<td>No symptom</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>M</td>
<td>52</td>
<td>Proband</td>
<td>Optic atrophy</td>
<td>18</td>
<td>2/200 HM</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>46</td>
<td>Sister</td>
<td>No symptom</td>
<td>4/200</td>
<td>4/200</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>F</td>
<td>56</td>
<td>Sister</td>
<td>Optic atrophy</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>M</td>
<td>60</td>
<td>Proband</td>
<td>No symptom</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>69</td>
<td>D2’s brother</td>
<td>Optic atrophy</td>
<td>16</td>
<td>HM 2/200</td>
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<tr>
<td>D‡</td>
<td>1</td>
<td>M</td>
<td>67</td>
<td>Grandmother</td>
<td>No symptom</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>69</td>
<td>D2’s brother</td>
<td>Optic atrophy</td>
<td>17</td>
<td>2/200 20/200</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>43</td>
<td>Mother</td>
<td>No symptom</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>M</td>
<td>42</td>
<td>D1’s son</td>
<td>Optic atrophy</td>
<td>18</td>
<td>2/200 2/200</td>
</tr>
<tr>
<td></td>
<td>6§</td>
<td>M</td>
<td>18</td>
<td>Proband</td>
<td>No symptom</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>M</td>
<td>19</td>
<td>Proband</td>
<td>Optic atrophy</td>
<td>17</td>
<td>6/200 4/200</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>21</td>
<td>Sister</td>
<td>No symptom</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>F</td>
<td>45</td>
<td>Mother</td>
<td>No symptom</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>M</td>
<td>52</td>
<td>Proband</td>
<td>Optic atrophy</td>
<td>17</td>
<td>10/200 10/200</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>52</td>
<td>Proband</td>
<td>Optic atrophy</td>
<td>15</td>
<td>8/200 4/200</td>
</tr>
</tbody>
</table>

* Age at the molecular and ophthalmologic test.
† Visual acuity at the molecular and ophthalmologic test. HM indicates hand motion. Corrected visual acuities of asymptomatic individuals are 20/20 in all cases.
‡ Family D is the representative pedigree reported.
§ Internal control of an unaffected male offspring of the affected male D1.
The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants. Human experimentation committee approval of Kobe University School of Medicine was granted.

All the patients displayed optic atrophy with a history of the typical clinical course, including similar age at onset (Table 1). Bilateral uncorrectable visual acuity ranged from hand motion to 20/200 in all cases (Table 1). Automated static perimetry examination revealed that the visual field impairment was bilateral cecocentral scotoma, uniformly confined to approximately within 15° of the central region in all cases. None of the patients had an episode of significant recovery or amelioration of the visual function. No maternal line members exhibited apparent systemic disturbances. However, we could not exclude the existence of subtle or occult disorders, such as mild cardiac arrhythmia, because a detailed systemic examination, including electrocardiogram and muscle biopsy, was not conducted in this study.

**DNA Isolation, Polymerase Chain Reaction (PCR), and Analysis of Restriction Fragment Length Polymorphism (RFLP)**

Peripheral leukocytes were obtained from the already mentioned donors, from which total DNA was extracted and purified as described.15

Table 2 shows the LHON-associated mtDNA mutations found so far in the complex I genes2-7,9,21 and examined in this study. All of the mutations other than the ND1 4160 T-to-C mutation have the specific restriction endonucleases for RFLPs (Table 2). The PCR primers for the complex I genes, the sequences of which were adapted from Anderson et al27 (Table 2), were synthesized by an Applied Biosystems (Tokyo, Japan) model 391 automated DNA synthesizer. The mtDNA fragments encompassing each of the above mutations were amplified by PCR using an automated thermal cycler (Iwaki Glass, Tokyo, Japan), according to the methods reported by Saiki et al28 and Vilki et al.13 The 100 μl reaction buffer contained 500 ng total DNA, 100 pmol each of forward and reverse PCR primers (Table 2), and 5 U of AmpliTaq DNA polymerase (Perkin Elmer Cetus, Emeryville, CA). Thirty cycles of amplification were completed (94°C denaturation for 1 min, except for 5 min in the first cycle, annealing for 1.5 min at the temperatures indicated in Table 2, and 72°C extension for 2 min, except for 5 min in the last cycle).

For RFLPs, a 10 μl aliquot of PCR-amplified segments was digested with 10 U of either Bsa HI (New England Biolab, Beverly, MA), Sph I (Takara Shuzo, Tokyo, Japan), Nsp7524 I (Takara Shuzo), Mae I (Boehringer Mannheim Yamanouchi, Tokyo, Japan), or Bst NI (New England Biolab; Table 2), and electrophoresed in 2% agarose gels with a size marker of 123 base pair Ladders (lane M in Fig. 1 and 2; Bethesda Research Labs., Gaithersburg, MD). The gels were stained with ethidium bromide and photographed under UV transillumination.

**Southern Blot Hybridization**

**Sequence-Specific Oligonucleotide (SSO) Probe Hybridization.** A 10 μl aliquot per lane of PCR-amplified fragments was electrophoresed in duplicate and capillary-transferred onto Nytran-N membranes (Schleicher & Schuell, Keene, NH) after denaturation in 0.5 N NaOH, 1.5 mol/l NaCl and subsequent neutralization in 0.5 mol/l Tris-Cl (pH 7.5), 1.5 mol/l

<table>
<thead>
<tr>
<th>Mutations Found in Complex I Gene</th>
<th>PCR Primers*</th>
<th>Annealing Temp. (°C)†</th>
<th>Product Length (base pairs)</th>
<th>Enzymes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND1 3460 (G → A)§</td>
<td>3357 ~ 3377</td>
<td>3663 ~ 3643</td>
<td>55</td>
<td>307</td>
</tr>
<tr>
<td>ND1 4136 (A → G)§</td>
<td>4021 ~ 4044</td>
<td>4274 ~ 4251</td>
<td>60</td>
<td>254</td>
</tr>
<tr>
<td>ND1 4160 (T → C)§</td>
<td>4021 ~ 4044</td>
<td>4274 ~ 4251</td>
<td>60</td>
<td>254</td>
</tr>
<tr>
<td>ND1 4216 (T → C)§</td>
<td>4021 ~ 4044</td>
<td>4274 ~ 4251</td>
<td>60</td>
<td>254</td>
</tr>
<tr>
<td>ND2 4917 (A → G)§</td>
<td>4875 ~ 4895</td>
<td>5117 ~ 5097</td>
<td>55</td>
<td>243</td>
</tr>
<tr>
<td>ND4 11778 (G → A)¶</td>
<td>11646 ~ 11666</td>
<td>11957 ~ 11937</td>
<td>55</td>
<td>312</td>
</tr>
<tr>
<td>ND5 13708 (G → A)¶</td>
<td>13492 ~ 13512</td>
<td>13844 ~ 13824</td>
<td>55</td>
<td>353</td>
</tr>
</tbody>
</table>

* Nucleotide sequences are adapted from Anderson et al.27 The PCR primers were common for the ND1 4136, 4160, and 4216 sites.
† The annealing temperatures (T) were calculated from a GC content-dependent equation, T = [4 × (G + C) + 2 × (A + T)] / 5. The selected temperatures indicated were lower when compared between the forward and reverse primers.
¶ Restriction endonucleases used. Bsa HI and Nsp7524 I were determined by GeneBank Gene Sequence Database (Hitachi Software Engineering), and the others were determined from the references indicated below.
§ From Huoponen et al27 and Howell et al.8
¶ From Johns and Berman21
# From Wallace et al.21

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FIGURE 1. Analysis of ND4 11778 mutation in seven Japanese LHON pedigrees. (A) PCR products of 312 bp length. Lanes correspond to cases in Table 1. Lane N, normal control. Lane M, size marker. (B and C) Southern blot hybridization of the above fragments to wild-type and mutant, respectively, SSO probe (Table 3). (D) Conventional Southern blot hybridization of Sphi NI-digested total DNA to the probe of the 312 bp fragment immunologically labeled.

NaCl. The 19-base wild-type and mutant SSO probes for ND1 4160 and ND4 11778 (Table 3) were synthesized as described above, 5'-end-labeled separately with [γ-32P]-adenosine triphosphate (222 TBq/mmol; New England Nuclear-Daiichi Kagaku, Tokyo, Japan) by T4 polynucleotide kinase (Toyobo, Osaka, Japan) at

FIGURE 2. Search for other mtDNA mutations in complex I genes (Table 1). (A) 254 bp PCR fragments spanning ND1 4136, 4160, and 4216 (Table 2). No bands were cut by Sphi I or Nsp7524 I. (B) Southern blot hybridization of the above products to wild-type ND1 4160 SSO probe (Table 3). (C) 307 bp ND1 3460 PCR fragments digested by Bsa HI to 104 and 203 bp fragments (Table 2).
TABLE 3. ND1 and ND4 Sequence-Specific Oligonucleotide (SSO) Probes for Hybridization

<table>
<thead>
<tr>
<th>SSO Probe</th>
<th>Nucleotide Sequence*</th>
<th>Temp. (°C)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND1 4160</td>
<td>5'-AGGTGTATGAGTTGGTCGT-3'†</td>
<td>51</td>
</tr>
<tr>
<td>Wild-type</td>
<td>5'-AGGTGTATGAGTTGGTCGT-3'†</td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>5'-ACGACCAACCCATACACCT-3'</td>
<td>53</td>
</tr>
<tr>
<td>ND4 11778</td>
<td>5'-ATTATGATCGAGCACTGTGAG-3'†</td>
<td>49</td>
</tr>
<tr>
<td>Wild-type</td>
<td>5'-ATTATGATCGAGCACTGTGAG-3'†</td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>5'-CTCAGAGTCACATCATAAT-3'</td>
<td>47</td>
</tr>
</tbody>
</table>

* Nucleotide sequences are adapted from Anderson et al.27 The normal and mutated bases at distinct sites were indicated by the underlined letters.
† Washing temperatures at high stringency after hybridizations, calculated as in Table 2.
‡ Opposite polarities were selected to achieve the least mismatch pairing.

37°C for 1 hr, and purified through Bio-Gel P-2 (Bio-Rad Labs Japan, Tokyo, Japan) spun columns. According to the method of Saiki et al., hybridization was carried out using each radiolabeled SSO probe with a total radioactivity of 107 counts per minute. Membranes were washed twice at room temperature and twice at high stringency (51°C and 53°C for the ND1 4160 wild-type and mutant SSO probes; 49°C and 47°C for the ND4 11778 counterparts, respectively) for 15 min each in 6X SSC-0.1% sodium dodecyl sulfate, and exposed overnight to Fuji (Tokyo, Japan) RX X-ray film with intensifying screens at -80°C.

Conventional Southern Blotting. It is likely that some limited templates happen to be exclusively amplified during early PCR cycles. Such a bias may be misinterpreted as authentic homoplasmy (Table 2). To rule out any possible amplification bias, the conventional Southern blotting29 was carried out as follows. Five micrograms of total DNA with mtDNA was digested with 10 U of Sfa NI and electrophoresed through 0.7% agarose gels, followed by blot hybridization to the wild-type ND4 probe of the PCR products labeled with Genius Nonradioactive DNA Labeling and Detection Kit (Boehringer Mannheim Yamanouchi). If the ND4 11778 mutation is homoplasmic, a 1595 bp fragment without 916 bp and 679 bp wild-type fragments should be detected because of an Sfa NI-site loss at nt 11778 between the cut sites at nt 10863 and 12457 of mtDNA.27

RESULTS

We previously demonstrated by Sfa NI and Mae III RFLPs that matrilineage members examined in families A to F possess the ND4 11778 mutation,15 as does a family G proband (our unpublished data). However, we have not studied in detail whether heteroplasmy of the ND4 11778 mutation or simultaneous presence of multiple mutations in the complex I genes correlate to the clinical phenotype of the maternal line members in these pedigrees.

First, for the analysis of heteroplasmy, we performed hybridization of PCR-amplified 312 bp-ND4 fragments (Fig. 1A and Table 2) of leukocyte mtDNAs from the 17 matrilineages (Table 1) to the 19 base wild-type or mutant SSO probes; 49°C and 47°C for the ND4 11778 counterparts, respectively) for 15 min each in 6X SSC-0.1% sodium dodecyl sulfate, and exposed overnight to Fuji (Tokyo, Japan) RX X-ray film with intensifying screens at -80°C.

Conventional Southern Blotting. It is likely that some limited templates happen to be exclusively amplified during early PCR cycles. Such a bias may be misinterpreted as authentic homoplasmy (Table 2). To rule out any possible amplification bias, the conventional Southern blotting29 was carried out as follows. Five micrograms of total DNA with mtDNA was digested with 10 U of Sfa NI and electrophoresed through 0.7% agarose gels, followed by blot hybridization to the wild-type ND4 probe of the PCR products labeled with Genius Nonradioactive DNA Labeling and Detection Kit (Boehringer Mannheim Yamanouchi). If the ND4 11778 mutation is homoplasmic, a 1595 bp fragment without 916 bp and 679 bp wild-type fragments should be detected because of an Sfa NI-site loss at nt 11778 between the cut sites at nt 10863 and 12457 of mtDNA.27

Second, we analyzed the six other LHON-associated mutations of the complex I genes (Table 2) in the above 17 members. RFLPs of the ND1 3460, 4136, and 4216; ND2 4917; or ND5 13708 site-encompassing PCR products after digestion with the respective enzymes (Table 2) were as follows.

1. In Figure 2A, the 254 bp PCR products bracketing ND1 4136 and 4216 between the common primers (Table 2) were not digested with Sph I and Nsp7524 I, respectively, which indicates that both sites make up the wild-type sequence in all
of the 17 maternal lines, as in D6 and control N. Likewise, the 243 bp PCR products encompassing ND2 4917 (Table 2) were not cut by Mse I (data not shown) and thus were wild-type in all the cases.

2. Figure 2C presents evidence that the 307 bp PCR products encompassing ND1 3460 also were wild-type in all the cases, because Bst HI cut them into the two 104 and 203 bp fragments. Likewise, the 353 bp PCR products bracketing ND5 13708 (Table 2) were not restricted with Bst NI (data not shown). Thus, the sequence was normal in any case. On the other hand, for the ND1 4160 T-to-C mutation, which does not affect the recognition site for any enzyme available, 19-base SSO probe hybridization to the 254 bp ND1 PCR products was employed (Table 2). Figure 2B shows that the ND1 PCR products from all of the cases hybridized selectively to the wild-type probe (Table 3), but not to the mutant probe (data not shown). Thus, there was no mutation at this specific site in any subject.

In conclusion, the above results clearly indicate that all of the 17 maternal line members in the 7 Japanese pedigrees have only the homoplasmic ND4 11778 mutation, but none of the 6 non-ND4 mutations in the complex I genes.

**DISCUSSION**

In some white LHON pedigrees, intra- or interfamilial variations in clinical expression are suggested to be caused by heteroplasmic of the ND4 11778 G-to-A mutation, which is produced by mitotic or meiotic segregation with unequal distribution of mutant and wild-type mtDNA in different cells, tissues, and individuals. Although heteroplasmic pedigrees are much less frequent than homoplasmic pedigrees,7,19 Newman et al6 have suggested that the incidence of heteroplasmy is likely to be underestimated. We may have failed to detect the possible existence of heteroplasmic matrilineage members in the previous screening study15 using PCR-RFLP technique. The present study, however, revealed that the ND4 gene mutation was homoplasmic in all of the 17 maternal relatives in the seven currently available pedigrees. Such mitochondrial homoplasm was demonstrated not only in the nine affected individuals but also in the microangiopathic case (A2) and the seven asymptomatic maternal lines (Fig. 1 and Table 1). Thus, the phenotypic variation in our cases cannot be explained by heteroplasmy of leukocyte mtDNA. However, the possibility of heteroplasmy in the target ocular tissue not available for examination cannot be excluded.

Johns and Berman21 have presented evidence that one-third of the 64 LHON probands have simultaneous multiple mutations in some complex I genes. If LHON is a multi-step mitochondrial genetic disorder, accumulation of mtDNA mutations could synergistically affect the clinical expression.5 In their study, about 20% of ND4 11778 mutant probands have one or two additional mutations at ND1 4216, ND2 4917, or ND5 13708.21 Regarding the ND5 13708 base change, a recent study by another group further supported its association with both ND4 11778 mutation-positive and negative LHON pedigrees. Taken together, 18.6% of the ND4 11778 mutant white patients were calculated to have the ND5 13708 mutation. In contrast, our results in Figure 2 by RFLP analysis and SSO probe hybridization show that none of the above three nonND4 mutations coexisted with each other or with the ND4 11778 mutation in the LHON patients as well as in the asymptomatic maternal relatives. On the other hand, during preparation of this report, four additional mutations have been found to be associated with ND4 11778 mutation negative patients,9,32 which are G-to-A transitions at nt 5244 in ND2 gene of complex I, at nt 15257 and 15812 in cytochrome b gene, and at nt 7444 in one of the cytochrome c oxidase genes. They are postulated to exert a synergistic effect on LHON expression, like the three aforementioned mutations. Except for the nt 7444 mutation, the other three are found only in patients who harbor the ND5 13708 mutation. None of the four mutations are detected in ND4 11778 mutant patients. Hence, the coexistence of these four mutations in our ND4 11778 mutant and ND5 13708 wild-type LHON pedigrees is unlikely, although the rigorous conclusion cannot be drawn until confirmative experiments are completed in our series.

Three LHON-specific mutations at ND1 3460, 4160, and 4160 have been disclosed as alternatives to the ND4 11778 mutation.23 In contrast with the seven aforementioned mutations that have been detected in some normal LHON-unrelated individuals, these three mutations are found to be only in LHON pedigrees, which indicates their primary role in the pathogenesis of LHON. Both the 3460 and 4160 mutation attenuate the specific activity of complex I in platelet mitochondria,8,33 whereas the 4136 mutation may ameliorate the biochemical defect of complex I and neurologic abnormalities as an "intragenic suppressor" mutation.8 We also examined whether any of these three LHON-specific mutations coexisted with the ND4 11778 mutation in the Japanese pedigrees from the viewpoint of the aforementioned synergistic effect of multiple mtDNA mutations, although such a coexistence has not been found in white patients. However, Figure 2 shows that none of these mutations were detected in the seven pedigrees tested.

Our findings strongly suggest that heteroplasmy of the ND4 11778 mutation and simultaneous muta-
tions in the different complex I genes cannot account for the variation in the clinical phenotype in our series. The homogeneity of the ND4 11778 mutation, without genetic locus heterogeneity and with homoplasy, suggests that the mutation might be derived from one or a few common ancient ancestors in Japan, which is conceivable in the close society of this country. On the other hand, regarding the clinical status of the affected individuals, such mitochondrial homogeneity is in accordance with the uniform symptom manifestation, such as similar age at onset, severe visual impairment confined to the central region of the visual field, and lack of recovery. Although we unfortunately did not conduct a detailed systemic examination that included electrocardiogram, none of the patients exhibited the apparent severe systemic disorders reported in some white patients.6,8

All the affected individuals were men, and conversely, all the asymptomatic individuals were women, exception for one microangiopathic 18-yr-old male, who is at risk of developing optic atrophy. We have emphasized that all of these matrilineage members have the same mitochondrial genotype. Such a sex bias in symptom manifestation is compatible with the previous genealogical data1,22 and directs our interest to the third possibility of involvement of an X-chromosome-linked genetic factor, as mentioned in the introduction. Although the previous results of X-linkage analyses are controversial,24,25 Bu and Rotter23 recently proposed the two-locus model in which both the mitochondrial and X-linked recessive genes are responsible for the development of optic atrophy. They have postulated that an affected female offspring is not only an mtDNA mutant but also a homozygote at the X-linked locus or a heterozygote affected because of the inactivation of a normal allele on an X-chromosome. A male offspring from an abnormal X homozygous mother is postulated to be affected with a 100% probability, whereas a male offspring from an X heterozygous mother is postulated to be affected with a 50% probability.25 Such a model is very attractive for explaining the characteristic inheritance pattern of this disease. The present study, which excludes mitochondrial heteroplasmy and its influence on the phenotype, serves as an important base applying such a model to Japanese LHON pedigrees.35

The above concept of the two locus regulation of LHON expression, however, still leaves confusing aspects regarding the etiology of LHON—how the two genes interact for LHON expression; how the model explains the presence of individuals who exhibit only microangiopathy without visual impairment; and the reason the apparent symptom is confined to eyes. Further studies are needed to explain these difficult problems and to determine the precise pathogenesis of LHON.

Key Words
Japanese pedigree, Leber’s hereditary optic neuropathy, mitochondrial heteroplasmy, simultaneous mutations, 11778 mutation

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References


