Phosphorus MR spectroscopy shows a tissue specific in vivo distribution of biochemical expression of the G3460A mutation in Leber’s hereditary optic neuropathy

R Lodi, V Carelli, P Cortelli, S Iotti, M L Valentino, P Barboni, F Pallotti, P Montagna, B Barbiroli

Occipital lobe and calf muscle energy metabolism were studied in vivo by magnetic resonance spectroscopy (1H-P-MRS) in four members of a family harbouring the mitochondrial DNA G3460A mutation causing Leber’s hereditary optic neuropathy (LHON). Three siblings carried 100% mutated mitochondrial DNA (heteroplasmy), while their mother had coexistence of mutated and wild-type mitochondrial DNA (heteroplasmy). Indices of brain energy metabolism on 31P-MRS were abnormal in all subjects examined, but the muscle oxidative phosphorylation rate was normal. These findings indicate a tissue specific distribution of the biochemical expression of the G3460A LHON mutation and suggest that extramitochondrial factors, such as nuclear genes, may influence expression of this mutation in vivo.

Leber’s hereditary optic neuropathy (LHON), the commonest cause of isolated blindness in young men, is a maternally inherited form of bilateral acute or subacute loss of central vision associated with retinal ganglion cells and optic nerve degeneration. A mitochondrial DNA point mutation at one of three nucleotide positions (11778, 14484, or 3460) is found in the majority of LHON pedigrees. All three mutations affect genes coding for different subunits of complex I of the mitochondrial respiratory chain. The G3460A mutation affects the ND1 subunit and is found in 4–19% of all complex I of the mitochondrial respiratory chain. The G3460A mutations affect genes coding for different subunits of carrying the G3460A mutation.

The normal rate of mitochondrial ATP production in fibroblasts showed that reduced complex I activity was associated with a mitochondrial ATP synthesis is still far from clear. Cock et al showed that normal mitochondrial ATP production rate in a single LHON patient homoplasmic for the G3460A mutation. Cybrid cell studies suggested that the nuclear background may modulate the expression of the biochemical deficit associated with the G3460A mutation.

To gain insight into the biochemical expression of the G3460A mutation in vivo we used phosphorus magnetic resonance spectroscopy (1H-P-MRS), a sensitive method of evaluating mitochondrial function in intact tissues. We studied energy metabolism in neural and extraneural tissues in four members of a LHON pedigree harbouring the G3460A mutation.

METHODS

Patients

We studied four individuals (I-2, II-1, II-2, and II-3) from a family harbouring the G3460A mutation previously shown to have a reduced complex I activity in platelets (family 4 in Carelli et al). The proband (II-1) suffered typical LHON at 18 years of age. His visual acuity is now reduced to counting fingers in both eyes, with severe optic atrophy. His twin sisters (II-2, II-3) never complained of any visual disturbance. The proband’s mother never complained of visual loss, nor shows optic atrophy on fundoscopic examination. A proband’s maternal aunt, now deceased, also suffered LHON in her twenties.

Informed consent was obtained from each patient and from normal volunteers (see below), and the studies were carried out with the approval of the local hospital ethics committee.

Molecular genetic studies

Total DNA was extracted from multiple tissues (whole blood, leucocyte or platelet enriched pellets, urinary epithelium, and liver biopsy, as shown in table 1) by a standard phenol-chloroform method. Heteroplasmy of mitochondrial DNA was evaluated by polymerase chain reaction (PCR) amplification of a convenient mitochondrial DNA fragment encompassing the G3460A mutation, followed by restriction fragment

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mutation load and brain phosphorus magnetic resonance (MR) spectroscopy data</th>
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<tbody>
<tr>
<td>Case</td>
<td>Mutated mtDNA (%)</td>
</tr>
<tr>
<td></td>
<td>Whole blood</td>
</tr>
<tr>
<td>I2</td>
<td>56/F</td>
</tr>
<tr>
<td>II-1</td>
<td>33/M</td>
</tr>
<tr>
<td>II-2</td>
<td>25/F</td>
</tr>
<tr>
<td>II-3</td>
<td>25/F</td>
</tr>
<tr>
<td>Controls</td>
<td>(n=36)</td>
</tr>
</tbody>
</table>

na, not available; PCr, phosphocreatine; Pi, inorganic phosphate; PP, phosphorylation potential ([ATP]/[ADP]+[Pi]).
**Phosphorus MR spectroscopy**

In vivo P-MRS was performed on the right calf muscle by the pulse and acquire technique (repetition time of five seconds) at rest, during in-magnet aerobic incremental exercise of plantar flexion as previously described. Muscle P-MRS was performed on the right calf muscle by the pulse and acquire technique (repetition time of five seconds) at rest, during in-magnet aerobic incremental exercise of plantar flexion as previously described. Postexercise spectra were collected for five minutes, with a time resolution of 10 seconds during the first 60 seconds and 30 seconds thereafter. The limits of all the peaks were marked manually on each spectrum after phasing, and areas calculated between the limits. Muscle mitochondrial ATP production was assessed by measuring the rate of entirely oxidative PCr resynthesis during recovery. The rate of PCr resynthesis was calculated from the monoexponential equation best fitting the experimental points, and reported as time constants. Intracellular pH was calculated from the chemical shift of Pi relative to PCr.

Control subjects were healthy volunteers: n 36 (mean (SD) age 37 (17) years) for brain studies, and n 35 (38 (16) years) for skeletal muscle studies. Data are presented as mean (SD). Individual results were taken as abnormal when they fell outside the normal range.

**RESULTS**

All tissues examined were homoplasmic for the G3460A mutation in the index case (II-1) and in his twin sisters (II-2 and II-3), but their mother (I-2) showed a variable degree of heteroplasy in the examined tissues (table 1). Brain indices of mitochondrial function such as [PCr], free [ADP], and PP were abnormal in all individuals carrying the G3460A mutation (table 1, fig 1B). Occipital lobe [Pi] was within the normal range only in case I-2. We do not know the load of mutated mitochondrial DNA in the brain of case I-2. However, the milder degree of brain bioenergetic impairment found in case I-2 compared with her offspring suggests the presence of some degree of heteroplasy in her brain, which in turn would modulate the extent of the energy metabolism deficit (table 1). The rate of PCr postexercise resynthesis was within normal range in all four subjects carrying the G3460A mutation (fig 2).

**DISCUSSION**

Our observations indicate that the biochemical expression of the G3460A mitochondrial DNA mutation in vivo is tissue specific. We found a severe defect of brain bioenergetics in both heteroplasmic and homoplasmic individuals carrying the

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**Figure 1** [A] Sagittal spin-echo T1 weighted magnetic resonance (MR) image showing the location of the 7.5 cm diameter surface coil. The region of interest was selected by the DRESS localisation technique for P-MRS acquisitions. Vertical lines define in the occipital region a 2.5 cm thick coronal slice. (B) 31P MR spectra (400 free induction decays) from occipital lobes of a healthy volunteer, and case II-1 carrying the 3460/ND1 mtDNA mutation. DRESS, depth resolved surface coil spectroscopy; PCr, phosphocreatine; Pi, inorganic phosphate. The phosphomonoesters peak is located to the left of the Pi peak; the phosphodiesters peak is located between Pi and PCr. The abscissa reports the chemical shift in parts per million (ppm) and the ordinate the relative intensity.

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**Figure 2** Rate of phosphocreatine (PCr) resynthesis after exercise in the four subjects carrying the A3460G mtDNA mutation. In view of the influence of the cytosolic pH on the rate of PCr resynthesis after exercise, the time constant (TC) of PCr recovery is plotted against the minimum pH reached in the postexercise period. The two lines represent the 95% confidence interval defined in healthy subjects.
G3460A mitochondrial DNA mutation, but we could not demonstrate an impairment of skeletal muscle mitochondrial oxidative metabolism in any subject. All subjects apart from case 1-2 were heteroplasmic for the G3460A mitochondrial DNA mutation. Muscle biopsy was not performed in these LHON patients and we do not know the level of mutated mitochondrial DNA in their skeletal muscle. However, at least for the three siblings in the second generation there is consistent evidence that they are homoplasmic mutant in all tissues examined, suggesting that the same probably also applies to skeletal muscle or any other tissue. Thus it seems very unlikely that normal muscle ATP production in these cases could be related to heteroplasmic mitochondrial DNA mutation. The proband's mother showed heteroplasmic mitochondrial DNA in blood cells and—considering the rarity of skewed segregation among tissues in LHON—it is conceivable that a fairly similar mutant load would be present in her skeletal muscle.

There is a complex relation between mitochondrial DNA mutations, respiratory chain enzyme specific activity, and the rate of mitochondrial ATP synthesis. The role played by nuclear background has recently been underscored by cybrid cell studies. The expression of defective complex I specific activity associated with the G3460A mutation was modulated by changing the nuclear background of the cybrid cell system used in one study. Similarly, the comparision of respiratory function between LHON lymphoblast cell lines and lymphoblast derived cybrids harbouring each of the three LHON pathogenic mutations showed differences related to the change in nuclear background. These observations also seem to apply to mitochondrial DNA mutations in RNA genes. Previous in vivo 31P-MRS studies have shown that the rate of mitochondrial ATP production is severely impaired in the skeletal muscle of subjects carrying the G11778A LHON mitochondrial DNA mutation, and to a lesser extent in those carrying the T14484C mutation. However, complex I enzymatic assays revealed normal or mildly reduced activity with the G11778A mutation, and unaltered complex I activity with the T14484C mutation. In a previous 31P-MRS study of three subjects carrying the 3460 mutant DNA we showed a negative correlation between percentage of mutated mitochondrial DNA and the rate of skeletal muscle mitochondrial ATP production, measured—as in this study—from the rate of PC recovery after exercise. However, the rate of mitochondrial ATP production was within the normal range in all subjects, indicating a very small effect of the 3460 mitochondrial DNA mutation on the modulation of the rate of muscle mitochondrial ATP production in vivo.

The relation between primary LHON mutations and complex I dysfunction is still debated, and various factors, of both mitochondrial and nuclear origin, may play a role in their biochemical expression in vivo. We provided in vivo evidence of a tissue specific distribution of the biochemical expression of the G3460A LHON mutation which is associated with mitochondrial dysfunction in the occipital lobes, but normal oxidative metabolism in an extraneural tissue (skeletal muscle). This finding—and those of another recent in vivo study investigating the A3243G mitochondrial DNA mutation—supports the role of the nuclear genetic environment in the biochemical expression of mitochondrial DNA mutations in vivo. Our observations may be important in understanding the molecular mechanisms responsible for the variable penetrance of LHON, and for the fact that its clinical expression is highly restricted to retinal ganglion cells and the optic nerve; it appears that these tissues may express the oxidative phosphorylation deficit produced by specific mitochondrial DNA mutations more severely than other tissues.

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REFERENCES

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