Mitochondrial Disease and Stroke

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Background and Purpose—It is well known that some mitochondrial disorders are responsible for ischemic cerebral infarction in young patients. Our purpose was to determine, in this prospective ongoing study, whether ischemic stroke is the only manifestation of a mitochondrial disorder in young patients.

Methods—Patients aged ≤50 years, admitted to the Stroke Unit from January 1999 to May 2000 with a diagnosis of ischemic stroke of unknown origin, were included in the study. All of them had full biochemical and hematologic tests, neuroimaging studies, transesophageal echocardiography, and extracranial and transcranial Doppler sonography. Patent foramen ovale was ruled out. Lactic acid concentrations were measured after anaerobic exercise of the forearm, and a morphological, biochemical, and molecular study after biceps muscle biopsy was performed.

Results—Of the 18 patients so far included, 3 (17%) presented lactic acid hyperproduction after physical exercise, and 6 (33%) showed deficit of the mitochondrial respiratory chain complexes. The molecular analyses have confirmed mitochondrial mutations at base pairs 3243 (characteristic of mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes [MELAS]), 4216, and 15 928.

Conclusions—These results suggest that ischemic stroke may be the only manifestation or the initial manifestation of a mitochondrial disorder. (Stroke. 2001;32:2507-2510.)

Key Words: cerebral infarction ■ mitochondrial myopathies ■ stroke ■ young adults

It is well known that some mitochondrial disorders are responsible for ischemic cerebral infarction in young patients. In the present prospective ongoing study, we sought to determine whether ischemic stroke is the only manifestation of a mitochondrial disorder in young patients.

Subjects and Methods

Patients aged ≤50 years, admitted to the Stroke Unit from January 1999 to May 2000, with a final diagnosis of ischemic stroke of unknown origin were included after signing an informed consent approved by the ethics committee. Mild hypertension, glucose intolerance, and/or hyperlipidemia remaining stable without pharmacological treatment were not considered criteria of exclusion. All patients underwent exhaustive biochemical, serological, hematological, and coagulation studies, as well as immunologic analysis, CT scan and/or MRI, transesophageal echocardiography (TEE), and extracranial and transcranial Doppler sonography were also performed.

Pyruvate, lactate, and ammonia blood levels, both at rest and during a modified Munsat’s forearm ischemic exercise test (FIET), were measured. Samples were obtained basally and at 1, 3, 5, 10, and 20 minutes after the test. Normal basal lactate values (BLVs) for our laboratory were 0.94 to 2.72 mmol/L (L.M. Jimenez, unpublished data, 1997). BLVs above the upper limit of the normal range were considered abnormal. Test results were assessed by comparing progressive increments of lactate values with the BLVs. The value of lactate after FIET was considered abnormal when it was at least 3.25 times greater than the BLV according to our own controls (mean control value of increment 2.77±0.48 mmol/L).2

Brachial biceps muscle samples were obtained and immediately frozen in liquid nitrogen. Morphological and histochemical analyses included hematoxylin-eosin, NADH–tetrazolium reductase, succinate dehydrogenase, oil red O, cytochrome c oxidase, and Gomori trichrome stain. The biochemical analyses determined enzymatic activity of the mitochondrial respiratory chain (MRC) complexes according to the technical procedures described by other authors.3 The mean control values of our own controls (30 males and 20 females, aged 16 to 55 years) were as follows: 20±4.5% (complex I), 10.3±2.1% (complex II), 63±18% (complex III), 41.5±11% (complex IV), and 140±22 nmol/min per milligram (complex V) (J. Arenas, Y. Campos, unpublished data, 1998).

A molecular analysis of mitochondrial DNA was also performed.4 High-frequency mutations found in mitochondrial encephalomyopathies—A3243G, A8344G, and those associated with Leber hereditary optic neuropathy—were studied by means of a polymerase chain reaction and subsequent digestion with restriction enzymes. The tRNAAsp and tRNAVal genes and adjacent regions were sequenced and studied. The Southern blotting technique was used to find deletions of mitochondrial DNA.

To explore the validity of lactic acid values, both at baseline and after FIET, we compared those results against those of the muscular biochemical analysis as the diagnostic gold standard of mitochondrial diseases in a 2×2 table.

Results

Patient Data

Eighteen patients (16 males and 2 females), aged between 16 and 50 years (mean 40±10 years), were studied. Other than

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ischemic stroke in the patients, neither the patients nor their relatives had symptoms suggesting a mitochondrial disorder. Transient ischemic attack occurred in 5 patients (28%), minor stroke in 5 (28%), and established stroke in 8 (44%). The stroke was considered lacunar in 8 patients (45%), cortical-subcortical in 6 (33%), and vertebrobasilar in 4 (22%). Nine patients had repetitive ischemic symptoms. Three patients had mild arterial hypertension, 3 had dyslipidemia, and 1 had glucose intolerance corrected by diet and physical exercise. Five patients (28%) had migraine, and 1 suffered from myalgia. Moderately high creatine kinase values were found in 3 patients (17%), and basal hyperlactacidemia was found in 12 (67%). In the first 5 minutes of exercise, a relative hyperproduction of lactate was observed in 3 patients (17%). A very high absolute value (11 mmol/L), although $>3.25$ times the basal value, was observed in case 6 (Table 1). All other biochemical and hematologic analyses were normal. TEE was always normal. Doppler studies showed normal results in all but 1 patient, who had total occlusion of the left internal carotid artery (ICA) (case 4).

In the muscle biopsy, 8 patients (44%) showed morphological signs of metabolic disorder: lipid droplets (6 of 8 patients) and increased subsarcolemmal oxidative activity (5 of 8 patients). Four of them had findings that strongly suggested a mitochondrial disorder: ragged red fibers (RRFs) (3 of 8 patients) and cytochrome c oxidase–negative fibers (3 of 8 patients). A deficit of $\geq1$ MRC complexes was found in 6 patients (33%). Mitochondrial mutations were established in 2 patients. Seven patients had biochemical or morphological abnormalities that justified the diagnosis of a mitochondrial disorder (Table 1).

The BLV was more sensitive in predicting an unsuspected mitochondrial disease than was the lactate value after FIET, but its specificity was low. However, the relative increment of lactate after FIET showed 100% specificity and positive predictive value (Table 2).

### Case Reports

**Case 1**
A 32-year-old female with a previous history of migraine presented sensitive aphasia and mood disorder. A left parietotemporal ischemic lesion was found in the CT. She recovered, and 2 weeks later she deteriorated again. A CT showed a new lesion involving the left occipital lobe. Six months later, she was readmitted because of sudden loss of vision in the left side of the visual field. The examination revealed a left homonymous hemianopia, lack of attention

### TABLE 1. Data of Patients With Biochemical or Morphological Abnormalities Suggesting Mitochondrial Disease

<table>
<thead>
<tr>
<th>Risk factors, symptoms, clinical diagnosis, follow-up, and biochemical findings</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRFs</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>HT</td>
<td>HL</td>
<td>–</td>
</tr>
<tr>
<td>Migraine</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Myalgia</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Diagnostic stroke</td>
<td>Stroke</td>
<td>TIA</td>
<td>Stroke</td>
<td>TIA</td>
<td>Stroke</td>
<td>TIA</td>
<td>TIA</td>
</tr>
<tr>
<td>Repetitive stroke</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Location</td>
<td>PCA</td>
<td>MCA</td>
<td>MCA</td>
<td>Lacunar</td>
<td>MCA</td>
<td>MCA</td>
<td>MCA</td>
</tr>
<tr>
<td>Follow-up</td>
<td>Tremor, epilepsy</td>
<td>–</td>
<td>–</td>
<td>Polyneuropathy</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CK</td>
<td>Normal</td>
<td>↑</td>
<td>Normal</td>
<td>Normal</td>
<td>↑</td>
<td>Normal</td>
<td>↑</td>
</tr>
<tr>
<td>BLV</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Normal</td>
<td>↑</td>
<td>Normal</td>
</tr>
<tr>
<td>FIET</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Normal</td>
<td>↑</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Signs of mitochondrial disease in muscle biopsy**

| Lipids | – | + | – | + | + | – | + |
| SDH | – | + | – | – | + | – | + |
| RRFs | + | – | – | + | + | – | – |
| Cytochrome c oxidase negative | + | – | + | + | – | – | – |
| MRC activity (%) | I (11), IV (16) | I (11), I (10) | – | III (23), IV (12) | III (24), IV (13) | – | IV (13) |
| Mutations | 3243 | ND | ND | – | – | 4216, 15,928 | – |

VRF indicates vascular risk factor; CK, creatine kinase; SDH, succinate dehydrogenase; PCA, posterior cerebral artery; I to IV, complexes I to IV; TIA, transient ischemic attack; MCA, middle cerebral artery; ND, still not determined; HT, arterial hypertension; and HL, hyperlipemia.

*High absolute value.

### TABLE 2. Validity of BLV and Its Relative Increment After FIET as a Marker of Mitochondrial Disorder

<table>
<thead>
<tr>
<th>BLV</th>
<th>FIET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>67%</td>
</tr>
<tr>
<td>Specificity</td>
<td>33%</td>
</tr>
<tr>
<td>PPV</td>
<td>33%</td>
</tr>
<tr>
<td>NPV</td>
<td>67%</td>
</tr>
</tbody>
</table>

PPV indicates positive predictive value; NPV, negative predictive value.
and concentration, and recent memory deterioration. CT showed an additional recent right temporo-occipital ischemic infarction. Abnormal cortical hyperintensity, as described in MELAS, with relative preservation of the underlying white matter was evident by MRI. Focal epileptic seizures and an action tremor appeared in the follow-up. Biochemical evaluation showed high BLV (3.5 mmol/L) that increased >3.25 times after FIET. Muscle biopsy showed RRFs and cytochrome c oxidase–negative fibers. Molecular analyses confirmed the tRNA^Leu^ mutation (A → G) at base pair 3243, which is characteristic of mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS). The patient had no relatives with symptoms suggesting a mitochondrial disease, but the molecular analysis of her 9-year-old daughter revealed 78% mutated mitochondria in peripheral blood (33% in the index patient). There was no detectable mitochondrial mutation in her mother’s peripheral blood.

Case 5
A 44-year-old male experienced language difficulties, and 20 minutes later, a mixed aphasia with slight right hemiparesis was patent. CT showed a hypodense ischemic lesion in the upper branch of the left middle cerebral artery. Total occlusion of the left ICA was detected by Doppler sonography. Creatine kinase values were high, with normal MB isoenzyme. Despite the fact that the physical exercise was not intense enough, the lactate value was elevated to 4.26 mmol/L, with a BLV of 1.88 mmol/L. Increased subsarcolemmal oxidative activity, lipid droplets, and RRFs appeared in the muscle sample. A deficit of complexes III and IV of the MRC was also found.

Case 6
A 49-year-old male experienced, during 1 hour, 3 short episodes of weakness and tingling in the left limbs; the last episode occurred with speech difficulties. CT was normal. Mild hypertriglyceridemia was detected. BLV was 4.36 mmol/L rising to 10.94 mmol/L at 1 minute after FIET. Muscle biopsy showed type 2 fiber predominance. A deficit of complexes III and IV of the MRC was found. Molecular analysis was positive for mutations at base pair 4216 (T → C) and 15 928 (G → A) of tRNA^Leu^.

**Discussion**

The prevalence of mitochondrial disease in patients with stroke is not well known.5,6 Whereas some authors found a prevalence of 7.2%7 in a series including patients aged <19 years, other authors found a prevalence of only 0.8% in a study including females aged between 15 and 45 years8 (Table 3). In patients with occipital infarction, it was as high as 10%, probably because mitochondrial disease affects mainly that territory.9 Although MELAS is typically related to ischemic stroke,10 it is very likely that other mitochondrial disorders are also responsible for ischemic infarction in young patients. In the present study, 22% and 33% of the patients had morphological and biochemical data, respectively, that could justify the diagnosis of a mitochondrial disease. A possible explanation for such a high prevalence is that we have included only young patients with an ischemic stroke for which all currently known causes were meticulously excluded. On the other hand, the diagnosis of mitochondrial disease was based on not only the morphological study, which may be normal in these patients, but also the biochemical study, which is a more accurate marker of the disease.

Only 1 patient with mitochondrial pathology showed the typical mutation of MELAS (case 1). Case 6 had 2 mutations of mitochondrial DNA unrelated to MELAS. The mutation at location 15 928 is a polymorphism of the encoding region of mitochondrial DNA.11 Mutation 4216 is a secondary mutation of Leber hereditary optic neuropathy, which increases the risk of disease expression.12 This mutation is also frequently found in patients with stroke and migraine with aura.13 Young patients with mild vascular risk factors for ischemic stroke should not necessarily be excluded from studies of mitochondrial disease. In fact, diabetes mellitus is a usual component of the mitochondrial diseases,14 and patients having hypertriglyceridemia have also been described.15 Case 5 showed mild arterial hypertension, and case 6 suffered from dyslipidemia. However, the high prevalence of MRC defects in our series compared with the results obtained in our control series of healthy people highly suggests a causal relationship between stroke and mitochondrial disorder.

One of our patients had a total occlusion of the left ICA (case 7). Although this association has been described only once in patients with MELAS,10 mitochondrial disease may predispose a patient to arterial obstruction of nonatheromatous origin.

Some data from the present study may help to find patients suffering from mitochondrial disease. Among the clinical characteristics, migraine and repetitive ischemic infarctions16 were frequently associated with a mitochondrial disorder: 60% and 55% of the patients having migraine and repetitive strokes, respectively, had biochemical mitochondrial abnormalities. Biochemically, the relative increment of lactate after FIET seems to be a good marker of the disease.

Although the present study may show some limitations that were due to the number of patients, we can conclude that mitochondrial diseases may be responsible for ischemic infarctions currently considered as cryptogenic. Stroke may be the expression of either an oligosymptomatic type of a well-known mitochondrial disease or the initial expression of another disease, so far unknown. Our diagnostic procedures

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**TABLE 3. Prevalence of MDs in Young Patients With Strokelike Episodes**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients, N</th>
<th>Age, y</th>
<th>MD, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bogouslavsky and Regli,5 1987</td>
<td>41</td>
<td>&lt;30</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Riikonen and Santavuori,4 1994</td>
<td>44</td>
<td>&lt;16</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td>Henderson et al,4 1997</td>
<td>128</td>
<td>15–44</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Majamma et al,9 1997†</td>
<td>38</td>
<td>18–45</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Lanthier et al,2000</td>
<td>55</td>
<td>&lt;18</td>
<td>4 (7.2)</td>
</tr>
<tr>
<td>Present study, 2001</td>
<td>18</td>
<td>&lt;51</td>
<td>4 (22)‡</td>
</tr>
</tbody>
</table>

MD indicates mitochondrial disorder.

*Females with ischemic stroke. †Patients with occipital stroke. §Morphological study. ¶Biochemical analysis.
should be followed in at least those patients aged <50 years suffering from ischemic cerebral infarction of unknown etiology.

References
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