Altered Cerebral Glucose Metabolism in a Family With Clinical Features Resembling Mitochondrial Neurogastrointestinal Encephalomyopathy Syndrome in Association With Multiple Mitochondrial DNA Deletions

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Objective: To determine the involvement of cerebral metabolism in 2 siblings with mitochondrial neurogastrointestinal encephalomyopathy syndrome (MNGIE)–like disease with multiple mitochondrial DNA (mtDNA) deletions.

Design: Case report.

Setting: Department of Neurology at a university medical center.

Patients: Two siblings with MNGIE-like disease with multiple mtDNA deletions.

Main Outcome Measures: Clinical, biochemical, genetic, and imaging findings, including cerebral magnetic resonance imaging, proton magnetic resonance spectroscopy, and positron emission tomography with fluorine 18–labeled deoxyglucose (FDG-PET).

Results: Genetic analysis of muscle DNA revealed multiple mtDNA deletions, while no mutations were detected in ECGF1, POLG1, ANT1, or Twinkle. Cerebral magnetic resonance imaging and proton magnetic resonance spectroscopy findings were unremarkable. Reduced regional glucose metabolism was found in a patchy and asymmetrical pattern predominantly in the frontotemporal region in both siblings by means of FDG-PET.

Conclusions: The discrepancy between absence of clinical signs of cerebral involvement and the substantial impairment of glucose metabolism reflects a chronic subclinical encephalopathy. To our knowledge, the predominantly frontotemporal distribution has not been described previously in mitochondrial disorders.

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Mitochondrial neurogastrointestinal encephalomyopathy syndrome (MNGIE) is known to be an autosomal recessive disease characterized by gastrointestinal symptoms, such as intestinal pseudo-obstruction, recurrent vomiting, diarrhea, or cachexia, and neurological signs, including chronic progressive external ophthalmoplegia (CPEO), generalized myopathy, and polyneuropathy.1 Multiple mitochondrial DNA (mtDNA) deletions found in MNGIE can also be seen in patients with familial CPEO, related to mutations in nuclear genes, such as the mitochondrial polymerase gamma (POLG1), adenine nucleotide translocase 1 (ANT1), and Twinkle (C10orf2).2 Patients presenting with multiple mtDNA deletions and/or depletion and clinical features undistinguishable from MNGIE but without leukoencephalopathy on brain magnetic resonance imaging (MRI) do not show mutations in the ECGF1 gene. This entity is defined as MNGIE-like disease.1,3 Since clinical signs of cerebral involvement are uncommon in MNGIE, and MNGIE-like disease presents without morphological abnormalities on MRI, this study aimed to clarify functional aspects of brain metabolism in MNGIE-like disease by means of proton magnetic resonance spectroscopy (1H-MRS) and positron emission tomography with fluorine 18–labeled deoxyglucose (FDG-PET).

Methods

Clinical Findings

Patient 1 presented at 35 years of age with chronic diarrhea, cachexia (body mass index [BMI] [calculated as weight in kilograms di-
vided by height in meters squared], 14), and asthenia. The neurological examination revealed CPEO and proximal muscle weakness. Plasma creatine kinase (335 U/L [to convert to micromoles per liter, multiply by 0.0167]), normal range, <170 U/L) and lactate levels at rest (22.5 mg/dL [to convert to micromoles per liter, multiply by 0.111], normal range, 4.5-19.8 mg/dL) were elevated. Urinary thymidine and deoxyuridine concentrations showed no elevation. Electrophysiological workup revealed mixed myopathy and axonal neuropathy. Electroencephalography showed bitemporal focal slowing. Results of the cerebral spinal fluid examination, including lactate and protein levels, were normal. No cardiomypathy, endocrine disorder, or visual or hearing loss was detected. Scores on the Mini-Mental State Examination (28 of 30), DemTect (12 of 18), and Beck Depression Inventory (13) were found within normal ranges.

Patient 2, the index patient’s 33-year-old brother, presented with CPEO, modest cachexia (BMI, 17), and a history of rhabdomyolysis at the ages of 23 and 27 years. Diagnostic workup showed comparable results, including elevated plasma creatine kinase (205 U/L) and lactate (37.8 mg/dL) levels at rest, mixed myopathy, and axonal neuropathy as well as unremarkable findings on cerebrospinal fluid and neuropsychological assessment (Mini-Mental State Examination score, 28 of 30; DemTect score, 13 of 18, and Beck Depression Inventory score, 7).

Two brothers and 1 sister (BMI range, 24-28) and the nonconsanguineous parents were healthy, implying an autosomal recessive or X-linked recessive inheritance.

**MORPHOLOGICAL AND BIOCHEMICAL EXAMINATION OF SKELETAL MUSCLE**

Six-micrometer, serial cross-sections of muscle biopsy specimens were obtained for histochemical stains according to standard procedures. A frozen part of the biopsy specimen was used for biochemical examinations. The biopsy specimen was kept at −80°C until analysis. Activities of respiratory chain enzyme complexes I through IV were determined in skeletal muscle as described.

**MRI, 1H-MRS, AND FDG-PET**

Cerebral MRI and 1H-MRS were performed on a 1.5-T scanner (Interna; Philips Medical Systems, Best, the Netherlands). Parameters of 1H-MRS included volume of interest (2 × 2 × 2 = 8 cm³), repetition time (1.5 milliseconds), and echo time (135 milliseconds). Volumes of interest with a sufficient time-to-noise ratio were sampled from the parietooccipital and basal ganglia regions. The PET measurements were carried out on an ECAT EXACT HR (Siemens Medical Solutions, Knoxville, Tennessee) after 12 hours fasting in a resting state with eyes closed. After 30 minutes’ rest, a rapid bolus of 370 million Bq (to convert to curies, multiply by 2.7 × 10⁻¹⁰) of FDG was injected intravenously (specific activity, 18.5 billion Bq/µmol). Scanning was immediately initiated, with a total scan time of 40 minutes. Image analysis was performed using IDL (Research Systems Inc, Boulder, Colorado) and MIPTool (U. Pietrzyk, PhD; K. Herholz, PhD; A. Schuster, MD; H. M. von Stockhausen, MD; H. Lucht, MD; W. D. Heiss, 1996). All data were spatially normalized by affine 12-parameter transformation, using the SPM99 (Wellcome Department of Cognitive Neurology, London, England) standard PET brain template. Defining a cutoff to distinguish between normal and pathological FDG-PET findings, thus providing an objective analysis procedure, Herholz et al14 introduced a diagnostic user-independent analysis of FDG-PET scan abnormalities. It is based on age-adjusted t sum statistics and an automated voxel-based procedure, which was validated in a large data set comprising of 110 normal controls.5 Local critical t values were calculated for a significance level of P = .05 (1-sided, uncorrected). Age regression was performed (controls, n=10) and abnormal voxels were defined in individual images as those voxels whose values were lower than 95% of the age-adjusted prediction limits. Corresponding t sum maps, with reference to the values expected by the regression, were calculated. The absolute regional cerebral metabolic ratio of glucose (CMRglu) (in micromoles per 100 g per minute) was determined in areas predicted as abnormal by the resulting single image and compared with the corresponding area in the age-adjusted control group. For further methodological parameters, see Herholz et al.3

**RESULTS**

**DNA ANALYSIS**

DNA extraction from muscle and blood of the 2 affected brothers and additionally from blood of their family members was performed according to standard purification protocols (Qiagen GmbH, Hilden, Germany). Restriction fragment length polymorphism analysis of the frequent transfer RNA mutations and long-range polymerase chain reaction to detect mtDNA deletions as well as real-time polymerase chain reaction for mtDNA depletion were performed by standard methods followed by sequencing of ECGF1, POLG1, ANT1, and Twinkle (C10orf2).

**MORPHOLOGICAL, BIOCHEMICAL, AND GENETIC FINDINGS**

Open muscle biopsy specimens of both patients revealed numerous ragged red fibers on Gomori trichrome stain with partial cytochrome-c oxidase deficiency. Biochemical measurements of the muscle mitochondrial respiratory chain enzymes showed a deficiency of complexes II and III relative to citrate synthase activity in patient 1 and a slight deficiency of complex I in the younger brother (Table 1). Both patients demonstrated elevated citrate synthase activities suggestive of mitochondrial proliferation. Multiple mtDNA deletions were observed on Southern blot analysis of muscle DNA in both brothers. No mtDNA depletion was detected. No pathogenic mutations were found in ECGF1, POLG1, ANT1, or Twinkle.

**IMAGING FINDINGS**

Magnetic resonance imaging revealed a slight frontal cortical atrophy in the index patient (Figure 1) and normal cerebral MRI results in his sibling. The single-volume 1H-MRS investigation found neither evidence of lactate (1.33 ppm) nor a decrease of N-acetylaspertate (2.02 ppm) signal intensity or total choline (3.20 ppm) over total creatine (the sum of phosphocreatine and creatine, 3.04 ppm) signal intensity ratio in the parietooccipital and basal ganglia regions in both patients (Figure 1). Positron emission tomography with fluorine 18-labeled deoxyglucose exhibited a substantial reduction of global CMRGlut, with 27 µmol/100 g/min in the index patient and 24 µmol/100 g/min in the younger brother as compared with the age-adjusted control group (mean [SD], 35.2 [3.5] µmol/100 g/min; range, 27.1-
38.8 µmol/100 g/min; n=10). Comparison of the spatially normalized FDG uptake and the corresponding deviations from the normal reference samples showed significant reductions of CMRGlu in a patchy and asymmetrical fashion predominantly in the frontotemporal regions (Figure 2). Absolute CMRGlu values were selected from regions exhibiting the most prominent decrease in CMRGlu (Table 2). The clinically more affected index patient presented more widespread regional CMRGlu reductions, while the reduction of the global CMRGlu was pronounced in his younger brother. The most diminished glucose metabolism was found in the left frontopolar area of patient 1 (20.4 µmol/100 g/min in comparison with mean [SD] 35.6 [3.9] µmol/100 g/min in normal controls; range, 30.0-42.1 µmol/100 g/min; n=10).

**Table 1. Respiratory Chain Activities in Patients 1 and 2**

<table>
<thead>
<tr>
<th>Complex</th>
<th>U per Unit of CS</th>
<th>CS, U per Gram of NCP</th>
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<tbody>
<tr>
<td>Normal</td>
<td>0.17-0.56</td>
<td>0.08-0.45</td>
</tr>
<tr>
<td>Patient 1</td>
<td>0.17</td>
<td>1.27</td>
</tr>
<tr>
<td>Patient 2</td>
<td>0.11↓</td>
<td>1.42</td>
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Abbreviations: CS, citrate synthase; NCP, noncollagen protein; arrow, value of interest is out of the normal range in the Table.

a Patient 1 showed a complex II and III deficiency relative to the increased CS activity. Patient 2 presented with a slight deficiency of complex I.

b Reduced nicotinamide adenine dinucleotide–coenzyme Q10–oxidoreductase.

c Succinate cytochrome-c oxidoreductase.

d Cytochrome-c oxidase.

Van Goethem et al² reported for the first time mutations of POLG1 in patients with MNGIE-like disease, in contrast to the apparently homogeneous clinical picture of MNGIE caused by mutation in ECGF1. Likewise, gastrointestinal symptoms, including paralytic ileus and degenerative intestinal wall abnormalities, were occasionally described in patients with MELAS (mitochondrial encephalopathy, lactic acidosis, and strokelike episodes) caused by the mtDNA point mutation A3243G.6,7 The patients presented in this study confirm the complexity in categorizing distinct clinical phenotypes into propagated acronyms like MELAS or MNGIE. The clinical phenotype comprising gastrointestinal symptoms and CPEO may be shared with a variety of other mitochondriopathies of both nuclear and mtDNA inheritance.

[Image of Table 1 and diagrams]

Figure 1. Cerebral magnetic resonance imaging (A and B) and proton magnetic resonance spectroscopy (C and D) of the right parietooccipital region and the left basal ganglia of the index patient. Fluid-attenuated inversion recovery sequences display a mild frontal cortical atrophy. The proton resonance intensities exhibit normal spectroscopic features. Cho indicates choline; Cr, creatine; and NAA, N-acetylaspartate.

Figure 2. Regional absolute regional cerebral metabolic ratio of glucose reductions of the index patient (A and C) and his sibling (B and D). Transaxial positron emission tomography with fluorine 18-labeled deoxyglucose (FDG-PET) images (A and B) display a normal brain uptake in a gray scale and regions exhibiting significant decline of FDG uptake marked by black overlay (C and D) (P<.05, uncorrected).
Cerebral MRI in mitochondriopathies may reveal leukoencephalopathy, basal ganglia calcification, cortical atrophy, and stroke-like lesions, which occur predominantly in the posterior-temporal and occipital regions in MELAS. Proton magnetic resonance spectroscopy of brain metabolism is capable of demonstrating signal intensity alterations in mitochondriopathies, like the presence of lactate, decreased N-acetylaspartate signal intensity, and decreased total creatine over total choline signal intensity ratio. Previous PET studies have shown selective vulnerability of distinct brain regions regarding oxygen and glucose metabolism. Presence of an impaired glucose metabolism was described predominantly in occipital, parietal, and temporal regions as well as in the basal ganglia.

The FDG-PET results presented in this study on 2 patients with a possible autosomal recessive mitochondrial disease and multiple mtDNA deletions demonstrated extensive regional impairment of the cortical glucose metabolism without cerebral symptoms. To the best of our knowledge, the predominantly frontomesial and frontotemporal glucose hypometabolism has not been described in mitochondrial disorders before. The extent of the CMRGlu reduction is in good agreement with a study by Berkovic et al, who found a mean (SD) cortical CMRGlu of 25.7 (5.4) µmol/100 g/min (range, 21.0–33.3 µmol/100 g/min) in a series of 5 patients with myoclonus epilepsy and ragged red fibers. Molnár et al found the global CMRGlu values (25–32 µmol/100 g/min, n = 5) in all investigated patients with MELAS, CPEO, and pure mitochondrial myopathy and neuropathy to be lower than the applied normal value (34 µmol/100 g/min). However, no firm correlation between the severity of cerebral involvement and the degree of CMRGlu reduction could be established so far.

The presence of a subclinical central nervous system (CNS) involvement in different mitochondrialpathies was demonstrated in several previous FDG-PET and \textsuperscript{1}H-MRS-studies on adult patients. Molnár et al found the impaired glucose uptake present in patients with and without cerebral symptoms and independent from the duration of the disease. Using serial FDG-PET studies, Damian et al reported the presence of chronic subclinical encephalopathies in patients with MELAS without stroke-like episodes and unremarkable cerebral MRI findings over a 7-year follow-up. Salvan et al demonstrated a wide variability of cerebral metabolic alterations using \textsuperscript{1}H-MRS on patients with CPEO without clinical signs of cerebral involvement. However, Dinopoulos et al observed deep gray matter signal changes on brain MRI and the presence of lactate elevation in proton spectra only in association with clinical CNS involvement in a series of 24 children (median age, 0.5 years) with definite mitochondriopathies. Using a combined FDG-PET and \textsuperscript{1}H-MRS approach on 2 infants (aged 2 weeks and 14 months) with congenital lactic acidosis due to defective mitochondrial respiration, a corresponding increase of lactate signal intensity and CMRGlu could be observed accompanied by clinical deterioration. It may be hypothesized that the prevalence of subclinical involvement of cerebral metabolism is higher in the chronic state of energy failure in adult patients, whereas early diagnosis in infants and children often implies a more deleterious course of disease, including \textsuperscript{1}H-MRS and PET abnormalities alongside clinical CNS involvement.

In conclusion, we suggest that FDG-PET is a useful and sensitive technique to unveil subclinical alterations of cerebral metabolism in mitochondriopathies. Further multimodal MRS and PET studies are needed to elucidate the prevalence, spatial distribution, and natural course of subclinical encephalopathies in mitochondriopathies.

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Table 2. Regional and General CMRGlu Compared With Age-Adjusted Control Group

<table>
<thead>
<tr>
<th>VOI</th>
<th>CMRGlu, µmol/100 g/min, Mean (SD) [Range]</th>
<th>Patient 1</th>
<th>28.6 (4.3) [34.5-46.4]</th>
<th>28.2 (3.6) [37.3-47.9]</th>
<th>20.4 (3.9) [30-42.1]</th>
<th>35.6 (3.9) [30-42.1]</th>
<th>ND</th>
<th>ND</th>
<th>ND</th>
<th>ND</th>
<th>ND</th>
<th>27 (3.9) [30-42.1]</th>
<th>35.2 (3.9) [30-42.1]</th>
</tr>
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<tbody>
<tr>
<td>Frontomesial</td>
<td>Left Frontopolar</td>
<td>Left Temporopolar</td>
<td>General</td>
<td>CMRGlu, µmol/100 g/min, Mean (SD) [Range]</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>VOI</td>
<td>Control</td>
<td>Frontomesial</td>
<td>Left Frontopolar</td>
<td>Left Temporopolar</td>
<td>General</td>
<td>CMRGlu, µmol/100 g/min, Mean (SD) [Range]</td>
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<tr>
<td>VOI</td>
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<td>Left Temporopolar</td>
<td>General</td>
<td>CMRGlu, µmol/100 g/min, Mean (SD) [Range]</td>
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Abbreviations: CMRGlu, cerebral metabolic rate of glucose; ND, not determined; VOI, volume of interest (volume size, 20 × 20 × 20 mm).

a Control group, n = 10. The VOIs were selected from regions on t scan masks indicating reduced CMRGlu.

b Measuring the CMRGlu of the total brain activity excludes the use of VOI.
REFERENCES