

# Mitochondrial Complex I Encephalomyopathy and Cerebral 5-Methyltetrahydrofolate Deficiency

**Author**V. T. Ramaekers<sup>1</sup>, J. Weis<sup>2</sup>, J. M. Sequeira<sup>3</sup>, E. V. Quadros<sup>3</sup>, N. Blau<sup>4</sup>**Affiliation**<sup>1</sup>Division of Pediatric Neurology, University Hospital, RWTH Aachen, Germany<sup>2</sup>Institute of Neuropathology, University Hospital, RWTH Aachen, Germany<sup>3</sup>Department of Medicine and Cell Biology, SUNY-Downstate Medical Center, Brooklyn, New York, USA<sup>4</sup>Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zürich, Switzerland**Key words**

- mitochondrial disorder
- folate
- methyltetrahydrofolate
- treatment

**Abstract**

Folate transport to the brain depends on ATP-driven folate receptor-mediated transport across choroid plexus epithelial cells. Failure of ATP production in Kearns-Sayre syndrome provides one explanation for the finding of low spinal fluid (CSF) 5-methyltetrahydrofolate (5MTHF) levels in this condition. Therefore, we suspect the presence of reduced folate transport across the blood-spinal fluid barrier in other mitochondrial encephalopathies. In the present patient with mitochondrial complex I encephalomyopathy a low 5-methyltetrahydrofolate level was found in the CSF. Serum folate receptor autoantibodies were negative and could not explain the low spinal fluid folate levels. The

epileptic seizures did not respond to primidone monotherapy, but addition of ubiquinone-10 and radical scavengers reduced seizure frequency. Add-on treatment with folic acid led to partial clinical improvement including full control of epilepsy, followed by marked recovery from demyelination of the brainstem, thalamus, basal ganglia and white matter. Cerebral folate deficiency is not only present in Kearns-Sayre syndrome but may also be secondary to the failure of mitochondrial ATP production in other mitochondrial encephalopathies. Treatment with folic acid in addition to supplementation with radical scavengers and cofactors of deficient respiratory enzymes can result in partial clinical improvement and reversal of abnormal myelination patterns on neuro-imaging.

**Introduction**

Reports of Kearns-Sayre syndrome documented normal plasma folate levels associated with isolated lowering of CSF 5-methyltetrahydrofolate (5MTHF), which was believed to result from failure of adequate ATP production necessary for the active ATP-dependent folate transport across the blood-brain barrier [2,5,7,12]. In two deceased patients with Kearns-Sayre syndrome autopsy studies documented oncocytic transformation of choroid plexus epithelial cells with an abundance of deletions in mitochondrial DNA and decreased expression of mitochondrial DNA encoded proteins [12]. However, one report demonstrated not only low CSF 5MTHF concentrations, but also low plasma folate concentrations [1].

Therefore, we investigated the folate metabolism in a patient with mitochondrial complex I encephalomyopathy, as well as the effect of add-on treatment with folic acid, radical scavengers and cofactors of complex I [11].

**Patient and Methods**

After diagnosis of mitochondrial encephalomyopathy in the patient, further investigations were performed to study folate transport mechanisms across the blood-brain barrier. After informed parental consent, CSF specimens were collected between 8:30 and 10:00 A.M. and analyzed for the end and intermediary metabolites of the biogenic monoamines dopamine and serotonin, pterins and 5-methyltetrahydrofolate (5MTHF). Simultaneously we also determined serum folate, vitamin B12 and homocysteine levels. The presence of blocking autoantibodies against the folate receptors was investigated using previously described methods [9].

Following add-on treatment with folic acid combined with the previously started treatment of the radical scavengers vitamin C and E and ubiquinone-10 as co-factor of the deficient respiratory enzyme complex I, the patient was followed up for a period of 3 years.

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**Correspondence**  
**Dr. V. T. Ramaekers**  
 Division of Pediatric Neurology  
 University Hospital Aachen  
 Pauwelsstrasse 30  
 52074 Aachen  
 Germany  
 Tel.: ■■■■■  
 Fax: ■■■■■  
 vramaekers@skynet.be

Cerebral MRI investigations were performed before and after treatment.

### Case Report

After a normal pregnancy the patient, now a 6-year-old boy, was born by Caesarian section after prolonged labour with an Apgar score of 7–7–9 after 1, 5 and 10 minutes, respectively, and an umbilical arterial pH of 7.25. His birth weight was 3950 g and his length 54 cm. Both parents were non-consanguineous and healthy, except for severe psoriasis of the mother. Because of feeding difficulties and generalized muscle weakness since birth, the patient was referred for a muscle biopsy after finding marked elevation of plasma lactate (8.2 mM; normal range 1–1.8). Morphological analysis of the muscle biopsy revealed myofibrillar anomalies and hypertrophic type I fibres in the presence of normal and atrophic type II fibres. Electron microscopy showed the presence of myofibrillary abnormalities as well as swelling and hypertrophy of mitochondria.

Biochemical investigations of a fresh muscle sample showed marked reduction of oxidation rates after preincubation with various <sup>14</sup>C-radiolabelled substrates, as well as a reduced production rate of ATP + creatine phosphate from pyruvate. Measurement of the activities of the pyruvate dehydrogenase complex and respiratory chain enzymes revealed an isolated moderate deficiency of complex I NADH-Q10 oxidoreductase at 50 mU/U CS (normal reference values for complex I activity: 70–250 mU/U CS [citrate synthetase]). Fibroblast cultures showed normal respiratory enzyme activities. Molecular genetic investigations could rule out deletions and mutations of mitochondrial DNA, tRNA for leucine and lysine, and the common mutations found in MELAS, MERRF and Leigh/NARP. Analysis of nuclear genes encoding for the subunits of the mitochondrial complex I have ruled out mutations of NDUFS 1, 2, 4 and 7. Further investigation of other nuclear encoded complex I units are pending.

From birth, neurodevelopment was characterized by generalized muscle hypotonia and weakness with marked delay in acquisition of motor milestones, ataxia, development of pyramidal deficits in the lower limbs and the onset of generalized and focal epileptic seizures from the age of 1 year. At the age of 1 year, anticonvulsant drug treatment with primidone failed to control seizures. MRI of the brain at the age of 14 months showed fronto-temporal atrophy with bilateral extensive signs of leukoencephalopathy affecting the supratentorial white matter, ventral part of the internal capsule, cerebral peduncles and brainstem. T<sub>2</sub>-weighted sequences showed bilateral hyperintensive signal changes of the thalamus, hypothalamus, quadrigeminal plate and dorsal pons. Minor bilateral signal changes were present at the medial lenticular nucleus. CSF analysis at the age of 1.5 years showed moderate lactate elevation at 3.6 mM (reference <1.5) and diminished CSF 5MTHF at 38.5 nM (normal reference range 64–182) in the presence of normal values for the intermediary and end metabolites of the monoamines dopamine and serotonin (5-hydroxytryptophane, 5-hydroxyindoleacetic acid, homovanillic acid, L-dopa, 3-O-methyldopa) and pterins. Serum folate, vitamin B 12 and homocysteine were normal. Serum blocking autoantibodies against the folate receptor were absent.

From the age of 6 months a multivitamin preparation without folate was started and had no effect. From the age of 1 year and

2 months this multivitamin preparation was replaced by the complex I cofactor ubiquinone-10 (2–3 mg/kg/day) in combination with high doses of the radical scavengers vitamin C (10 mg/kg) and E (25 U/kg). The frequency of akinetic and adverse motor seizures diminished but the other clinical signs and symptoms did not respond.

From the age of 1 year and 10 months, folinic acid (1.2 mg/kg BW) and riboflavin (0.5–1 mg/kg) were added. After the addition of folinic acid and riboflavin to the previously mentioned treatment the seizures became fully controlled after 1 year, with normalization of the EEG. Hypotonia and ataxia improved and the patient was able to sit alone after 4 years treatment with folinic acid. However, cognitive deficits and pyramidal signs of the lower limbs persisted.

MRI follow-up at the age of 4 years and 11 months, following treatment with folinic acid, cofactors of complex I and radical scavengers for more than three years, showed marked reversal of the earlier observed signs of de-/hypomyelination and the absence of an elevated lactate signal on MR spectroscopy (● Fig. 1).

### Results of treatment

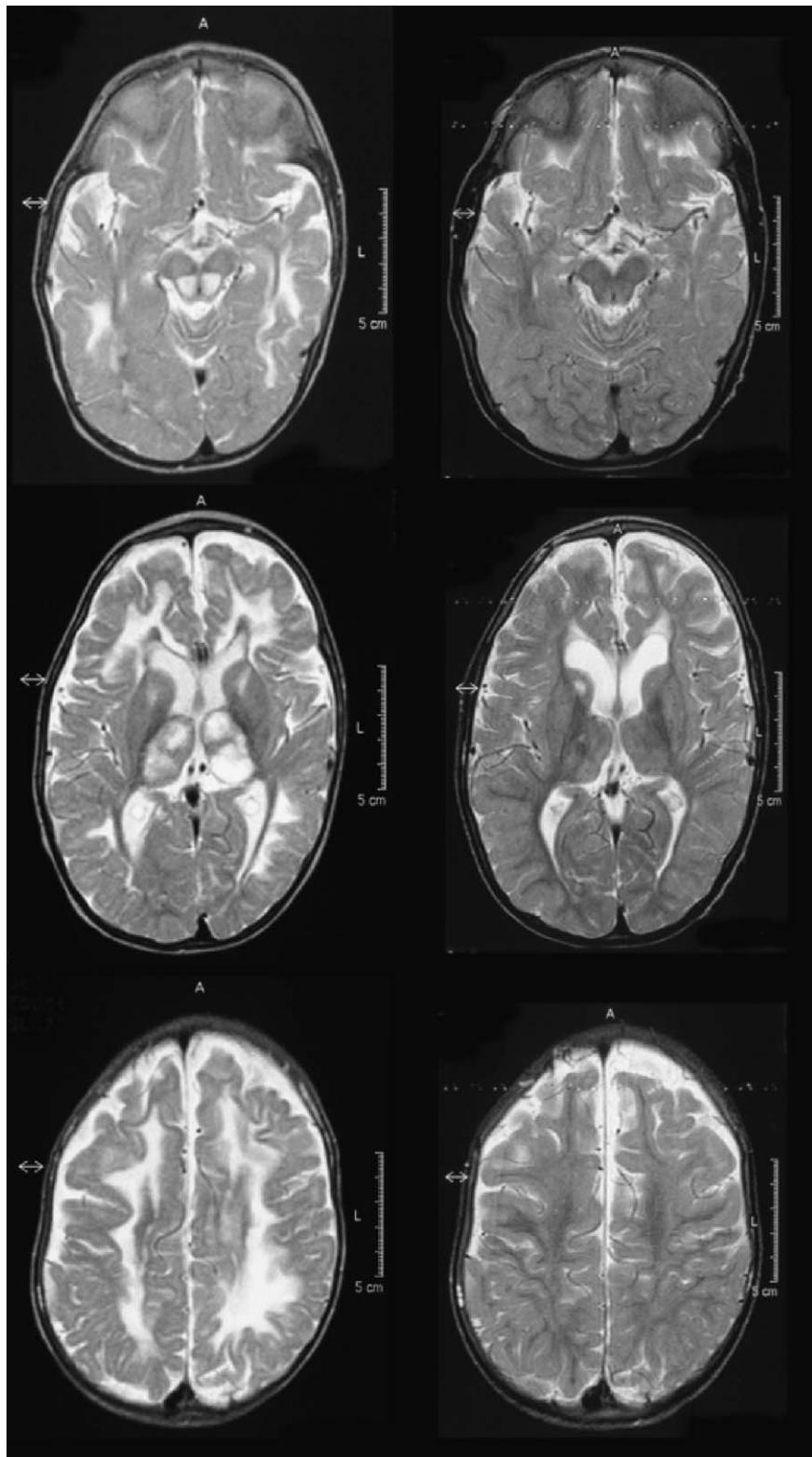
After add-on treatment with folinic acid there was partial clinical improvement with reduction of seizures, hypotonia and ataxia, whereas cognitive deficits and pyramidal signs in the lower limbs persisted. MRI follow-up showed reversal of abnormal myelination of brainstem, basal ganglia and white matter, while MR spectroscopy showed the absence of an elevated lactate peak.

### Discussion

This case report describes a severe mitochondrial complex I encephalomyopathy manifesting with psychomotor retardation, weakness, hypotonia and ataxia, spastic diplegia and refractory seizures and MRI changes of leukoencephalopathy with demyelination. The clinical picture of the patient with mitochondrial encephalopathy due to complex I deficiency resembles that of the children with the infantile-onset CFD syndrome ([3,4,6,8,9,13]; ● Table 1). The normal plasma homocysteine and normal hematological investigations with the absence of macrocytic anemia in the patient never suggested a disorder of folate metabolism. However, the 5MTHF measurement in CSF was low. The presence of low CSF folate levels prompted the analysis for blocking autoantibodies to the folate receptor in the serum which was found negative. The reduced transport of

**Table 1** Clinical findings in the patient compared to the major clinical features in the infantile-onset CFD syndrome

Clinical feature	Patient
deceleration of head growth	–
agitation, insomnia	+
psychomotor retardation	+
autistic features	–
epilepsy	+
pyramidal signs	+
extrapyramidal signs	–
ataxia and hypotonia	+



**Fig. 1** On the left the T<sub>2</sub>-weighted images of case 1 with complex I mitochondrial leukoencephalopathy are shown before treatment with radical scavengers, ubiquinone-10, riboflavin and folic acid. The images on the left side demonstrate marked signal changes at the brain stem, thalamus, anterior part of the internal capsule and white matter with marked reversal after treatment during more than three years, shown by the corresponding axial sections on the right (reprinted with editor's permission from New Encyclopedia of Neuroscience 2006. Chapter on "Folate Deficiency States" by V. T. Ramaekers [in press]).

5MTHF across the choroid plexus in this mitochondrial disorder can be tentatively attributed to a failure of ATP production.

The therapeutic effect of the combination of folic acid and radical scavengers could be demonstrated *in vivo* by the control of refractory epilepsy and by the reversal of leukoencephalopathic changes in the patient who received treatment for 3 years. In addition, the absence of an elevated lactate on follow-up by MR spectroscopy also confirms the beneficial effect of this treatment. Previous reports on treatment with cofactors of deficient

respiratory chain complexes combined with antioxidants showed conflicting results with some anecdotal reports of a curative effect [10]. A recent report also documented a dramatic reversal of white matter demyelination in a patient with Kearns-Sayre syndrome with low CSF folate who received folic acid over a period of 1 year [7]. Further studies are needed to confirm the effect of early intervention with folic acid combined with

antioxidants and respiratory chain cofactors among the heterogeneous group of mitochondrial disorders affecting the central nervous system.

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