

## Diffuse leukodystrophy in an infant with cytochrome-*c* oxidase deficiency

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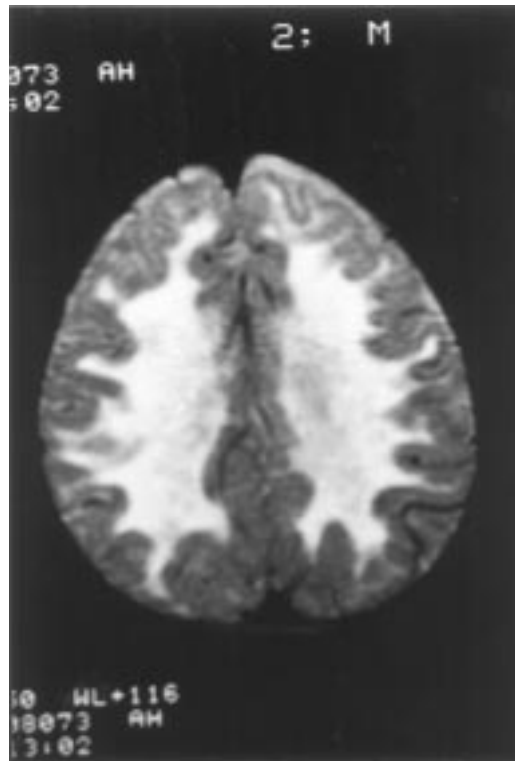
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**Summary:** A 25-month-old boy, born to consanguineous parents, had progressive spastic tetraplegia, and increased signal of the white matter on cerebral T2-weighted magnetic resonance imaging indicative of diffuse leukodystrophy. Elevated blood and cerebrospinal fluid lactate levels pointed to a respiratory chain defect. Cytochrome-*c* oxidase deficiency was demonstrated in cultured skin fibroblasts and skeletal muscle. This report extends the phenotype of COX deficiency in infancy. Systematic study of blood and CSF lactate should be carried out in every infant with leukodystrophy.

Leukodystrophies are severe degenerative diseases involving the brain white matter. They form a heterogeneous group of disorders, the common factor being the pathological process. The most common types of leukodystrophy in infancy are metachromatic and Krabbe leukodystrophies (Aicardi 1993). Leukodystrophy is a rare presentation of Leigh syndrome, which, when present, is usually associated with, at least, abnormalities of basal ganglia, (Bourgeois et al 1992; Zafeiriou et al 1995). Cytochrome-*c* oxidase (COX) (EC 1.9.3.1) deficiency (McKusick 220110) is the most commonly reported biochemical aetiology of Leigh syndrome (Shoffner and Wallace 1995). Here we report on a case, in an infant, of isolated diffuse leukodystrophy associated with COX deficiency, without the main clinical manifestations of Leigh syndrome and without the classical features of this syndrome on magnetic resonance imaging (MRI).



**Figure 1** Axial T2-weighted magnetic resonance imaging (MRI) scan of the brain showing diffuse increased signal intensity of the white matter

### CASE REPORT

A boy was born to healthy first-cousin parents, at term, after a normal pregnancy and delivery. He was the fourth boy of this family; the second one had died at age 9 months in a state of spastic tetraplegia with leukodystrophy of unknown aetiology. Our patient developed normally until age 3 months, when a progressive tetraplegia was noticed. At age 25 months he had severe spastic tetraplegia, with axial hyper-tonia, swallowing difficulties and severe growth failure, absent speech, and good visual contact. He did not have ptosis, oculomotor palsy, abnormal eye movements, optic atrophy or abnormal ventilation. He never had seizures. Echocardiography was normal. There was a sensorimotor peripheral demyelinating neuropathy. Auditory and visual evoked potentials were severely altered, electroretinogram was normal. Electroencephalogram showed a low-amplitude record. Axial T2-weighted MRI of the brain revealed an abnormal high signal throughout the white matter (Figure 1). Other T1- or T2-weighted MRI sections showed normal-appearing basal ganglia, brainstem, cerebellum and thalami, without intracerebral calcification, and cortical atrophy in the occipital and frontal lobes (not shown).

**Table 1** Enzyme investigation in skeletal muscle and cultured skin fibroblasts from patient and controls<sup>a</sup>

	Patient	Controls
<b>Skeletal muscle homogenate</b>		
		(n = 25)
Enzyme activities		
COX	84	65–138
SQDR	28	18–42
CS	152	38–112
LDH	2485	486–4166
Enzyme activity ratio		
COX/CS	<b>0.6</b>	3.0 ± 0.6
LDH/COX	<b>29.5</b>	13.8 ± 4.7
COX/SQDR	<b>3.0</b>	5.5 ± 0.9
<b>Isolated skeletal muscle mitochondria</b>		
		(n = 21)
Enzyme activities		
COX	714	515–1057
QCCR	1059	405–818
SQDR	200	57–161
NCCR	414	103–307
Enzyme activity ratio		
COX/NCCR	<b>1.7</b>	3.6 ± 0.4
COX/QCCR	<b>0.7</b>	1.5 ± 0.2
COX/SQDR	<b>3.6</b>	8.8 ± 1.5
<b>Cultured skin fibroblast homogenate</b>		
		(n = 19)
Enzyme activities		
COX	<b>25</b>	47–172
QCCR	119	58–133
SQDR	24	15–30
CS	115	58–147
LDH	7812	3695–9606
G3PDH	16	10–21
Enzyme activity ratio		
COX/QCCR	<b>0.2</b>	1.2 ± 0.2
COX/SQDR	<b>1.0</b>	4.9 ± 0.5
COX/CS	<b>0.2</b>	1.2 ± 0.2
LDH/COX	<b>314</b>	43.1 ± 6.6
SQDR/G3PDH	1.6	1.5 ± 0.2

<sup>a</sup> COX (complex IV), cytochrome-*c* oxidase; SQDR (complex II), succinate quinone dichlorophenolindophenol (DCPIP) reductase; CS, citrate synthase; LDH, lactate dehydrogenase; QCCR (complex III), quinol cytochrome-*c* reductase; NCCR (complexes I + III), NADH cytochrome-*c* reductase; G3PDH, glycerol 3-phosphate quinone DCPIP reductase. All enzyme measurements were carried out as previously described (Rustin et al 1994). Enzyme activities in nmol/min per mg protein. **Bold** type indicates abnormal values

## METABOLIC FINDINGS

Serum lactate was 6.6 mmol/L (normal <3), pyruvate was 166  $\mu$ mol/L (normal 41–67) and lactate/pyruvate ratio was 40 (normal <20). Cerebrospinal fluid (CSF) lactate was 7.4 mmol/L (normal <3); CSF protein was normal. There was no paradoxical ketonaemia after a carbohydrate-enriched meal. Blood ammonia, creatine phosphokinase and hepatic transaminases were normal.

Gas chromatography–mass spectrometry of urinary organic acids (Harpey et al 1983) showed (in  $\mu$ mol/mmol creatinine): lactate 280 (normal <40); 3-hydroxybutyrate 208 (normal <50); ethylmalonate 56 (normal <5); succinate 81 (normal <60); fumarate 67 (normal <10); adipate 54 (normal <7); suberate 8 (normal <2); 2-ketoglutarate 286 (normal <40); *cis*-aconitate 165 (normal <30); citrate 323 (normal <100).

Plasma and urinary amino acids were normal. There was no proteinuria or glucosuria. Arylsulphatase A activity in lymphocytes and  $\beta$ -galactocerebrosidase activity in cultured skin fibroblasts were normal.

Study of a biopsy specimen of vastus lateralis did not show ragged-red fibres but showed a clear lipid storage in type I fibres. COX immunocytochemical study in skeletal muscle was homogeneously negative.

Respiratory chain function was studied both in skeletal muscle and in cultured skin fibroblasts (Rustin et al 1994). Abnormal enzyme activity ratios of COX to other marker enzymes were found in muscle (tissue homogenate and isolated mitochondria), which pointed to a partial defect of COX in this tissue (Table 1). The defect was even more obvious in cultured skin fibroblast homogenate, since both absolute rate of COX activity and its activity relative to other marker enzymes were found defective (Table 1). Southern blot analysis of muscle mtDNA digested with restriction enzyme *Pvu*II and hybridized with a full-length mtDNA probe did not reveal any major rearrangement.

## DISCUSSION

In this patient, the normal activities of arylsulphatase A and  $\beta$ -galactocerebrosidase excluded metachromatic and Krabbe leukodystrophies, respectively. Lactic acidosis in blood and CSF pointed to a respiratory chain defect, as well as the urinary organic acid profile which showed evidence of secondary fatty acid  $\beta$ -oxidation defect (ethylmalonate, adipate, suberate), and evidence of altered tricarboxylic acid cycle function (succinate, fumarate, 2-ketoglutarate, *cis*-aconitate and citrate). A similar urinary organic acid profile has been reported in a case of Pearson marrow–pancreas syndrome associated with a mtDNA deletion of the COX II subunit gene (Ribes et al 1993).

Our patient had a COX deficiency more marked in cultured skin fibroblasts than in skeletal muscle. The expression of COX deficiency in his fibroblasts allows pre-natal diagnosis in his family on chorionic villi or amniocytes (Ruitenbeek et al 1996; Shoffner and Wallace 1995).

As suggested by van der Knaap et al (1996), such a case of diffuse leukodystrophy of infancy without detectable abnormalities of basal ganglia, thalami, brainstem and

cerebellum on MRI scan should not be considered as a case of Leigh syndrome but should be classified in a separate neuropathological entity: COX deficiency with leukodystrophy.

Finally, the parents' consanguinity, the history of the affected brother, the deficiency of a single complex of the respiratory chain in fibroblasts and muscle, the uniform COX deficiency by immunochemistry in muscle, and the absence of major mtDNA rearrangement point to a nuclear-encoded recessive disease (Shoffner and Wallace 1995).

This report of apparently pure leukodystrophy extends the phenotype of COX deficiency in infancy. Systematic screening for elevated blood and CSF lactate levels and urinary organic acid study should be carried out in every case of infantile leukodystrophy.

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