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CASE REPORT

Consanguineous 3-methylcrotonyl-CoA carboxylase deficiency: Early-onset necrotizing encephalopathy with lethal outcome

T. Baykal[1]*, *G. Huner Gokcay*[1], *Z. Ince*[2], *M. F. Dantas*[3], *B. Fowler*[3], *M. R. Baumgartner*[4], *F. Demir*[1], *G. Can*[2], *and M. Demirkol*[1]

[1]Department Nutrition and Metabolism, [2]Department Neonatology, Children's Hospital, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; [3]University Children's Hospital, Basel; [4]University Children's Hospital, Zurich, Switzerland
*Correspondence: Istanbul University, Istanbul Medical Faculty, Capa 34390 Istanbul, Turkey. E-mail: tbaykal@istanbul.edu.tr

Summary: A patient with a severe neonatal variant of 3-methylcrotonyl-CoA carboxylase (MCC) deficiency is reported. The first child of healthy consanguineous Turkish parents presented on the second day of life with dehydration, cyanosis, no sucking, generalized muscular hypotonia, encephalopathy, respiratory depression requiring mechanic ventilation, macrocephaly, severe acidosis and hypoglycaemia. Elevated C_5-OH-carnitine in dried blood spot by tandem MS and elevated urinary excretion of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine suggested MCC deficiency, confirmed by enzyme analysis in cultured fibroblasts. Cerebral ultrasonography and cranial CT findings revealed progressive changes such as disseminated encephalomalacia, cystic changes, ventricular dilatation and cerebral atrophy. Treatment with high-dose biotin and protein-restricted diet was ineffective and the patient died at the age of 33 days with progressive neurological deterioration. Mutation analysis revealed a homozygous mutation in the splice acceptor site of intron 15 in the MCC β-subunit. Early-onset severe necrotizing encephalopathy should be included in the differential diagnosis of isolated MCC deficiency.

3-Methylcrotonyl-CoA carboxylase (MCC, EC 6.4.1.4) deficiency is an inborn error of leucine catabolism with autosomal recessive inheritance. Isolated MCC deficiency is caused by mutations in either *MCCA* (McKusick 210200) or *MCCB* (McKusick 210210), which encode the α- and β- subunits of MCC, respectively. The clinical course ranges from severe to benign forms. Patients with MCC deficiency usually have normal growth and development until they present with an acute episode between 6 months and 3 years of age. The episodes frequently follow minor infections and involve feeding difficulty, vomiting, lethargy, apnoea, muscle hypotonia and seizures. Typical laboratory findings of an acute episode are severe hypoglycaemia, hyperammonaemia, elevated hepatic transaminases, mild metabolic acidosis and moderate ketonuria. Detection of elevated 3-hydroxyisovaleric acid (3-HIVA) and 3-methylcrotonylglycine (3-MCG) in urinary

organic acids by gas chromatography--mass spectrometry (GC-MS) is diagnostic and may occur without elevation of isovalerylglycine or the distal leucine metabolites (Sweetman and Williams 2001). The analysis of acylcarnitines in dried blood samples by tandem MS consistently shows elevated 3-hydroxyisovaleryl-carnitine.

CASE REPORT

The patient was a male infant born postmaturely at the gestational age of 44 weeks, with normal spontaneous delivery at home. He was the first child of healthy Turkish parents who were first cousins once removed. The family history is otherwise unremarkable. There was absence of sucking or crying during the first day. He was brought to our hospital at the age of 31 h. Anthropometric evaluation showed a body weight of 3.5 kg (75th centile), height 51 cm (50th centile) and head circumference 38 cm (97th centile). Physical examination revealed dehydration, cyanosis, encephalopathy, respiratory depression and poor capillary circulation. Neonatal reflexes were depressed and hypertonic episodes were observed in spite of prominent hypotonia. He was intubated immediately and put on mechanical ventilation in the neonatal intensive care unit. After cultures had been taken, antibiotics and intravenous fluids were started, with a presumptive diagnosis of sepsis. Within an hour of hospital admission, cardiac arrest occurred with re-establishment of circulation after 2–3 min of resuscitation. Biochemical evaluation showed metabolic acidosis (pH 7.3, BE -15.3 mEq/L, P_{CO_2} 17.5 mm/Hg), hypoglycaemia (glucose 36 mg/dl) and hyperlactataemia (5.7 mmol/L, normal < 2). Urinary organic acid determination by GC-MS revealed elevated lactic acid, 3-HIVA, 3-MCG and 3-hydroxybutyric acid. Acylcarnitines and amino acids by tandem MS disclosed elevated C_5-OH-carnitine, normal free carnitine and normal amino acids profile in dried blood spots. Plasma biotinidase activity was normal (4.59 nmol/min per ml, normal 4.2–8.4). Sepsis work-up, blood and CSF cultures were negative. Cerebral ultrasonography showed cerebral oedema on the second day of admission and mannitol therapy was given for a short duration. EEG demonstrated disseminated amplitude depression. Doppler ultrasonography (US) (on the 7th day of admission) showed decreased cerebral blood flow, progression of oedema and ventricular dilatation. At the age of 3 weeks, depression of the cranial bones was observed and repeated cerebral US showed cystic changes and leukodystrophy. Cranial CT was planned because of the progression in cerebral US findings. Disseminated encephalomalacia, multiple cysts, ventricular dilatation and cerebral atrophy were observed. The infant was treated with protein-restricted diet (1 g/kg per day), high-dose biotin (30--60 mg/day) and carnitine (100 mg/kg per day) supplementation. There was no clinical or bio-chemical improvement with this treatment; the baby could not come off the ventilator and he was lost at the age of 33 days.

MATERIALS AND METHODS

Urinary organic acid determination was performed by GC-MS. The activities of biotin-dependent carboxylases were determined in fibroblasts, cultured in media

containing different amounts of biotin, by measuring the incorporation of [^{14}C]bicarbonate into acid nonvolatile products as previously described (Suormala et al 1985). Mutational analysis was performed by amplification of cDNA from fibroblast RNA followed by direct sequencing and confirmation of identified mutations on genomic DNA as described (Baumgartner et al 2001).

RESULTS

Table 1 shows the urinary excretion of 3-HIVA and 3-MCG detected with different biotin dosages and dietary protein intake. There was no clinical or biochemical response to therapy. While MCC activity was undetectable, the other two biotin-dependent carboxylases had activities in the normal range in cultured fibroblasts (Table 2). RT-PCR amplification and direct sequencing of *MCCB* cDNA resulted in a smaller product than wild-type owing to exon 16 skipping. Amplification of *MCCB* exon 16 from genomic DNA revealed a homozygous mutation in the splice donor site of intron 16 (IVS16+1G>A).

DISCUSSION

Patients with MCC deficiency show variable clinical phenotypes ranging from asymptomatic adults to severe neonatal phenotypes, leading to coma and death in early infancy. This patient was born postmaturely with macrocephaly. He was apathic soon after birth, did not cry and rejected feeding. The severity of clinical and biochemical abnormalities including lactic acidosis raised the suspicion of holocarboxylase synthetase deficiency, but the absence of 3-hydroxypropionic

Table 1 The urinary excretion of 3-hydroxyisovaleric acid (3-HIVA) and 3-methylcrotonylglycine (3-MCG) under biotin and dietary therapy

	Urinary organic acid excretion			
Age (days)	*3-HIVA* (mol/mol creatinine)	*3-MCG* (mol/mol creatinine)	*Biotin supplementation* (mg/day)	*Dietary therapy*[a]
2	0.153	0.044	–	Breast milk
18	6.703	13.653	30	Breask milk
21	5.267	9.389	40	Protein restriction: 0 g protein/kg per/day Energy: 440 kJ/kg per day
29	1.084	1.073	60	Protein restriction: 1 g protein/kg per day Energy: 440 kJ/kg per day

[a] Given with a nasogastric tube

Table 2 Activities of biotin-dependent carboxylases in cultured fibroblasts

	Carboxylase activity (pmol/min per mg protein) *at given biotin concentration*			
	0.1 nmol/L NB[a]	*10 nmol/L FCS*[b]	*10 μmol/L NB*	*10 μmol/L FCS*
Propionyl-CoA carboxylase				
Patient	216	637	346	647
Controls ($n = 31$, range)	183–1451	287–2150	201–1513	302–1600
Methylcrotonyl-CoA carboxylase				
Patient	ND[c]	ND	0.6	ND
Controls ($n = 31$, range)	52–642	160–696	120–776	130–647
Pyruvate carboxylase				
Patient	183	489	360	476
Controls ($n = 31$, range)	207–2373	229–2660	240–1845	225–2753

[a] NB, newborn calf serum
[b] FCS, fetal calf serum
[c] ND, not detectable

aciduria points to isolated MCC deficiency even in such severe cases. Very few patients with early-onset MCC deficiency have been reported (Bannwart et al 1992; Lehnert et al 1996). They had seizures, hypotonia, failure to thrive, hypoglycaemia and metabolic acidosis in the neonatal period. Our patient had the earliest presentation of all MCC cases reported to date. We recognize, however, that our patient is the product of a consanguineous union and could have an additional genetic disorder that was not detected, despite comprehensive evaluation.

Cranial MRI changes in isolated MCC deficiency are reported as marked brain atrophy in a 15-year-old patient and multiple foci of leukodystrophy in a 14-month-old patient (Dodelson de Kremer et al 2002; Murayama et al 1997). The striking cranial imaging findings of our patient included severe encephalomalacia, leukodystrophy, cystic lesions, ventricular dilatation and cerebral atrophy, indicating a rapid progression within the newborn period. During the last week of his life, this necrotizing encephalomalacia, even manifested as depression of cranial bones, was evident on physical examination.

MCC is a heteromeric mitochondrial enzyme composed of biotin-containing α-subunits and smaller β-subunits (Baumgartner et al 2001). Mutation analysis revealed a homozygous mutation in the splice donor site of intron 16 in *MCCB*. The functional consequences of this splice site mutation can be assumed to be deleterious, as suggested by undetectable MCC activity in cultured fibroblasts. The patient with severe phenotype and early onset described by Bannwart and colleagues was homozygous for another mutation in *MCCB*, the missense mutation E99Q, and was reported as proband 002 (Baumgartner et al 2001). According to our current knowledge, most patients carry private mutations (Baumgartner et al 2001).

These observations add early-onset severe necrotizing encephalopathy to the spectrum of clinical abnormalities in isolated MCC deficiency.

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