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SHORT REPORT

Refining the diagnosis of mitochondrial HMG-CoA synthase deficiency

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Summary Mitochondrial HMG-CoA synthase deficiency is an inherited metabolic disorder caused by a defect in the enzyme that regulates the formation of ketone bodies. Patients present with hypoketotic hypoglycaemia, encephalopathy and hepatomegaly, usually precipitated by an intercurrent infection or prolonged fasting. The diagnosis may easily be missed as previously reported results of routine metabolic investigations, urinary organic acids and plasma acylcarnitines may be nonspecific or normal, and a high index of suspicion is required to proceed to further confirmatory tests. We describe a further acute case in which the combination of urinary organic acids, low free carnitine and changes in the plasma acylcarnitine profile on carnitine supplementation were very suggestive of a defect in ketone synthesis. The diagnosis of mitochondrial HMG-CoA synthase deficiency was confirmed on genotyping, revealing two novel mutations: c.614G>A (R188H) and c.971T > C (M307T). A further sibling, in whom the diagnosis had not been made acutely, was also found to be affected. The possible effects of these mutations on enzyme activity are discussed.

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Introduction

Deficiency of mitochondrial HMG-CoA synthase (HMGCS) (McKusick 600234) is a metabolic disease inherited as an autosomal recessive trait. Mitochondrial HMG-CoA synthase catalyses the first and rate-limiting step of ketogenesis, condensing acetyl-CoA and acetoacetyl-CoA to 3-hydroxy-3-methylglutaryl-CoA (EC 2.3.3.10) (Hegardt 1999). Ketone body production is the normal physiological response to fasting, providing the body, and particularly the brain, with fuel in a usable form for energy. The brain cannot utilize fatty acids directly. In contrast, absent or defective mHMGCS activity results in hypoketotic hypoglycaemia during fasting that can lead to coma, which responds to glucose administration (Thomson et al 1997). Therefore, patients are asymptomatic until decompensation is precipitated by intercurrent infection or prolonged fasting.

The diagnosis of HMGCS deficiency is hampered by nonspecific clinical signs and the lack of a specific diagnostic test. Urinary organic acids reveal increased dicarboxylic and 3-hydroxydicarboxylic acids in the absence of significant ketonuria or diagnostic acylglycines. The combination of normal acylcarnitines in dried blood spots and the absence of urinary ketone bodies has been stated to indicate the diagnosis (Zschocke et al 2002). Use of molecular studies to confirm the diagnosis avoids the need for an invasive liver biopsy and the limitations of the enzyme assay (Mitchell

and Fukao 2001). We describe a further case, and the subsequent diagnosis in an elder sibling, in which acute changes in the acylcarnitine profile in conjunction with urinary organic acids were very suggestive of a defect in ketone synthesis.

Case reports

Case 1

The third child of unrelated parents presented at 7 months of age with encephalopathy following a 4-day choryzal illness with oral intake limited to clear fluids only. He had previously been well with normal growth and development. On examination he was comatose, poorly perfused with deep sighing respirations, and a 4 cm hepatomegaly. Initial investigations revealed hypoketotic hypoglycaemia with a true blood glucose of <1.0 mmol/L in the absence of ketones on urinary dipstick, and an associated metabolic acidosis. He was managed with intravenous 10% dextrose and fluid resuscitation, and referred for further investigation and management with a presumed diagnosis of MCAD deficiency. Subsequent investigations; however, revealed a normal lactate 0.65 mmol/L and ALT 30 μ/L. The ratio of nonesterified fatty acid to 3-hydroxybutyrate (NEFA:BOHB) ratio was elevated (7.3 mEq/mmol, 95th centile 6.6 mEq/mmol). Urinary organic acids revealed a massive dicarboxylic aciduria with only a trace of 3-hydroxybutyrate and acetoacetate. The plasma free carnitine was low (3.8 μmol/L, normal range 25–60) with a significantly elevated acetylcarnitine (C_2). After carnitine supplementation, acetylcarnitine increased dramatically (see Table 1).The combination of low ketones, dicarboxylic aciduria and especially the high pool of acetylcarnitine suggested a block in fat metabolism subsequent to fat oxidation. HMG CoA synthase deficiency was considered the most likely candidate. As expected, fat oxidation studies on fibroblasts were normal, emphasizing a fat oxidation disorder post acetyl-CoA.

Mutation analysis was performed by amplifying and sequencing all coding sequences and exon–intron boundaries of the HMGCS2 gene, as described (Hegardt 1999), revealing two novel mutations: c.614G>A (R188H) and c.971T>C (M307T) verified by restriction analysis after PCR amplification of the mutated exons (data not shown).

Clinically the patient responded to a dextrose bolus (0.65 g/kg) and infusion (7 mg/kg per min), with the encephalopathy resolving over several hours. Oral feeds recommenced 23 h after admission, and intravenous dextrose stopped 19 h later. The elevated acetylcarnitine and dicarboxylic aciduria were unusually persistent over the next few days, further suggesting a problem in the clearance of the acetyl pool. Liver function showed no deterioration. A 2 cm hepatomegaly remained at the time of discharge 5 days later, but had resolved completely when next reviewed in the clinic. He has remained well (4.5 years follow up) with no specific dietary measures apart from avoidance of prolonged fasting, and the occasional use of glucose polymer (10% Maxijul solution, 0.5 g/kg per h at diagnosis, adjusted for age) during intercurrent infections.

Case 2

At the time of presentation of the index case, questioning highlighted that one of the older siblings had been admitted previously at the age of 1 year with extreme lethargy following a vomiting illness that improved with intravenous fluids. The notes were not available from the local hospital for review. Non-acute organic acid and acylcarnitine analyses were normal. Genotyping confirmed the clinical suspicion that he too was affected, being a compound heterozygote c.614G>A (R188H) and c.971T>C (M307T) like his brother. He is managed in the same way as his brother and has had no further problems (4.5 years follow-up). The parents and remaining brother were confirmed as carriers.

Discussion

HMG-CoA synthase deficiency is a life-threatening, treatable cause of hypoketotic hypoglycaemia. Table 2 summarizes the phenotype and genotype of all reported patients. Initial clinical presentation is indistinguishable from the much commoner fat oxidation defect medium chain acyl-CoA dehydrogenase (MCAD) deficiency, with encephalopathy and hepatomegaly in a child who has previously been well with normal growth and development. However, lactate and liver transaminases are not elevated during severe decompensations. Urinary organic acids demonstrate increased

Table 1 Changes in free and acetylcarnitine in response to carnitine supplementation. Carnitine (100 mg/kg/day) commenced following the day 1 result

	Day 1	Day 2	Day 3	Day 5	Day 6
Free carnitine (μmol/L)	3.8	33.9	188.6	95.6	85.0
Acetylcarnitine (μmol/L)	39.5	108.8	139.0	35.0	29.6
Non esterified free fatty acids (mEq/L)	2.3			1.9	
3 Hydroxybutyrate (mmol/L)	0.316			0.015	

Table 2 Clinical, biochemical and genetic profile of reported cases of HMG-CoA synthase deficiency

Patient	Sex	Age at first presentation	Genotype	Symptoms	Organic acids	Acylcarnitines
Case 1 Bouchard et al (2001), (Thompson et al 1997)	M	6 y	F174L/F174L	Gastroenteritis, hypoketotic hypoglycaemic seizure	Normal (sample 2 days after presentation)	Carnitine normal
Case 2 Bouchard et al (2001) (Morris et al 1998)	M	16 mo	R424X/unknown	Diarrhoea, vomiting, hepatomegaly hypoketotic hypoglycaemic coma	Dicarboxylic aciduria (sample 2 days after presentation)	Normal
Case 3 Aledo et al (2001)	M	11 mo	G212R/R500H	Gastroenteritis, vomiting, hepatomegaly, hypoketotic hypoglycaemic coma	Dicarboxylic aciduria	Normal (blood spot)
Case 4 Zschocke et al (2002)	F	9 mo	G212R/IVS5+1g>a	Gastroenteritis, diarrhoea, vomiting, hepatomegaly, hypoketotic hypoglycaemic coma	Dicarboxylic aciduria	Normal
Case 5 Wolf (2003)	F	4.5 y	V54M/Y167C	Gastroentereritis, diarrhoea, vomiting, hepatomegaly, hypoglycaemic coma	Dicarboxylic aciduria	Normal
Case 6 (sister of no. 5)	F	19 mo	V54M/Y167C	Hepatomegaly, abnormal liver function	Dicarboxylic aciduria (controlled fast)	Normal (controlled fast)
Case 7 Present study	M	7 mo	M307T/R188H	Vomiting, hepatomegaly, hypoketotic hypoglycaemic encephalopathy	Dicarboxylic aciduria	Low free carnitine. Raised C_2 carnitine on carnitine supplementation
Case 8 (brother of no. 7)	N	1 y	M307T/R188H	Vomiting, lethargy	Normal (non-acute)	Normal (non-acute)

[a]M, male; F, female
[b]mo, month; y, year

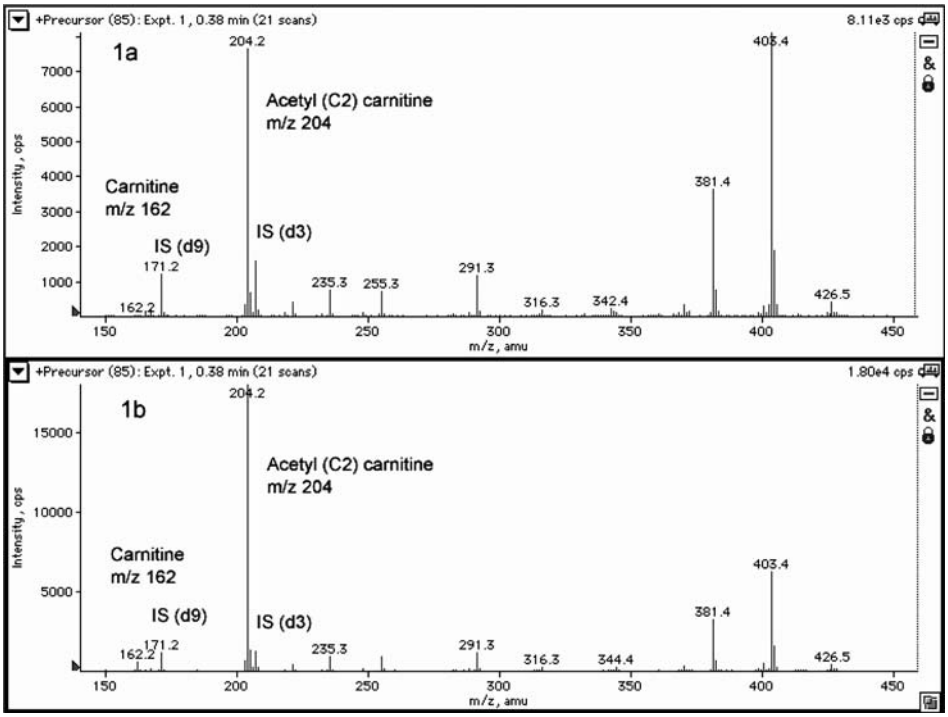

Fig. 1 (a) Acylcarnitine profile on presentation, showing low free carnitine. (b) Acylcarnitine profile following carnitine supplementation (100 mg/kg per day), revealing elevated acetylcarnitine (C_2)

dicarboxylic and 3-hydroxydicarboxylic acids in the absence of significant ketonuria and diagnostic acylglycines. Acylcarnitines have always been reported as 'normal', although no spectra have been published in the literature. Our index case is unusual in that the free carnitine was low on presentation. The C_2, although significantly elevated at 39.5μmol/L (normal range < 10) was not remarkable. We have observed C_2 concentrations up to 65 μmol/L (99th centile 53 μmol/L) in children during intercurrent illnesses, diagnostic fasts, and carnitine supplementation. In view of the carnitine depletion, carnitine was supplemented at 100mg/kg per day between four divided doses and the acylcarnitine profile was repeated the next day (see Fig. 1). The repeat profile showed that restoration of the free carnitine to normal levels was accompanied by a large and unexpected increase in C_2 (108.8 and 139.0 μmol/L after 24 and 48 h supplementation, respectively), indicating that there had been a build-up of acetyl-CoA, the final product of fat oxidation. This suggested that the metabolic block was perhaps subsequent to fat oxidation, raising the possibility of a ketone synthesis defect. HMG-CoA synthase deficiency was the most likely candidate in the absence of acidosis, raised lactate, deranged liver function and the organic acid profile of HMG-CoA lyase deficiency.

Genotyping has been suggested as the confirmatory test of choice, obviating the need for invasive liver biopsy and a technically difficult enzyme assay complicated by interference by several other enzymes within the liver homogenate (Bouchard et al 2001). Sequence analysis of the whole coding region of the *HMGCS2* gene revealed two novel mutations. A heterozygous c.614G > A (R188H) was identified in both probands and the mother, while c.971T > C (M307T) was observed in both probands and the father. Restriction enzyme analysis confirmed the mutations found by sequencing. Neither of the two mutations was found in 100 Spanish control chromosomes. The mutations affect conserved residues in mitochondrial HMG-CoA synthases. Methionine 307 is conserved in all mitochondrial HMG-CoA synthases except for mouse. Change to threonine (M307T), a polar residue, could affect the structure and ionic environment of the catalytic site. R188H affects an arginine, which is conserved in all mitochondrial and cytosolic HMG-CoA synthases and probably plays a role as an anion ligand in the active centre.

The low plasma free carnitine seen on the initial acylcarnitine sample taken at presentation has not been reported previously. Although the plasma acetylcarnitine was elevated, the true extent of the accumulated acetyl-CoA pool was not appreciated until revealed by carnitine supplementation. Previous reports have stated that acylcarnitine profiles are normal; however, the rise in acetylcarnitine is subtle, not uncommon and easily overlooked. On its own, a raised acetylcarnitine is a nonspecific finding, but in combination with hypoketotic hypoglycaemia, hepatomegaly and dicarboxylic aciduria a ketone synthesis defect must be considered.

HMG-CoA synthase deficiency is a rare cause of hypoketotic hypoglycaemia but may be commoner than reported as investigations previously described are nonspecific and therefore the diagnosis may be missed. The key to diagnosis

in this case was the grossly elevated acetylcarnitine that only became apparent after carnitine supplementation. Genotyping is the preferred method for confirmation of the diagnosis, being an easier and safer alternative to hepatic enzymology.

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