

Malonyl coenzyme A decarboxylase deficiency

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Abstract

Two new cases of malonyl coenzyme A (CoA) decarboxylase deficiency are described. Hitherto, the worldwide experience of the disorder has been confined to reports on two affected Australian children. The new cases are Scots born and are the offspring of consanguineous parents of Scots/Irish origin. They were investigated during episodes of vomiting and febrile convulsions associated with concomitant developmental delay. Malonic aciduria and grossly reduced malonyl CoA decarboxylase activity were demonstrated and the total ion current chromatograms of urinary organic acid profiles obtained by gas chromatography-mass spectrometry are presented. The clinical and biochemical features of the Scots and Australian patients are compared.

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Malonyl coenzyme A (CoA) decarboxylase deficiency is an extremely rare inborn error of fatty acid metabolism characterised by the excretion of excess malonate in the urine of affected subjects during episodes of vomiting or febrile convulsions. The primary defect is reduced activity of mitochondrial malonyl CoA decarboxylase (EC 4.1.1.9.), the enzyme responsible for conversion of intramitochondrial malonyl CoA to acetyl CoA.

Two Australian cases have been reported previously,¹⁻³ and in this paper we describe the

clinical and biochemical findings of two new cases of Scots/Irish descent. Similarities with the Australian patients are discussed.

Case reports

CASE 1

A boy, the eldest son of three children of a second cousin marriage of Irish parents was born in 1986 by caesarean section at 37 weeks' gestation. Although his birth weight was 3500 g he required nasogastric feeding for four days.

At the age of 3.9 years after a febrile convulsion he was admitted to hospital. He had signs of an upper respiratory tract infection together with profuse diarrhoea and vomiting. The child exhibited developmental delay with rather unusual facies consisting of prominent epicanthic folds and a long face. He also had a mild systolic murmur. The possibility of Williams' syndrome or fragile X syndrome was excluded on further investigation.

Four days after admission during his inpatient recovery from gastroenteritis, urinary organic acid screening revealed an increased excretion of malonate (775 mg/g creatinine) together with the presence of adipate (270 mg/g creatinine) and suberate (130 mg/g creatinine), which was confirmed by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis also identified a smaller amount of methylmalonate (fig 1).

Only 24 hours later, urinary malonate excretion had fallen to a trace concentration (20 mg/g creatinine) with no evidence of the dicarboxylic acids, adipate and suberate on this occasion. Analysis of his urinary amino acids showed increased excretions of the dibasic amino acids, lysine and cystine, together with methionine and the methyl-histidines.

Six months later the patient was given an oral L-carnitine load (100 mg/kg body weight). Before the carnitine load the noteworthy features of the urinary organic acid chromatogram were the presence of suberate (60 mg/g creatinine) and a trace of malonate (30 mg/g creatinine). After carnitine the chromatogram revealed hippurate (1105 mg/g creatinine) as the major component with only a modest concentration of malonate (45 mg/g creatinine). Thin layer chromatography for acylcarnitines showed a faint ultraviolet absorbing spot corresponding to acetylcarnitine, which is a normal finding.

A skin biopsy specimen was obtained for culture of fibroblasts for assay of malonyl CoA decarboxylase. Grossly reduced enzyme activity (table 1) confirmed the diagnosis of malonyl CoA decarboxylase deficiency.

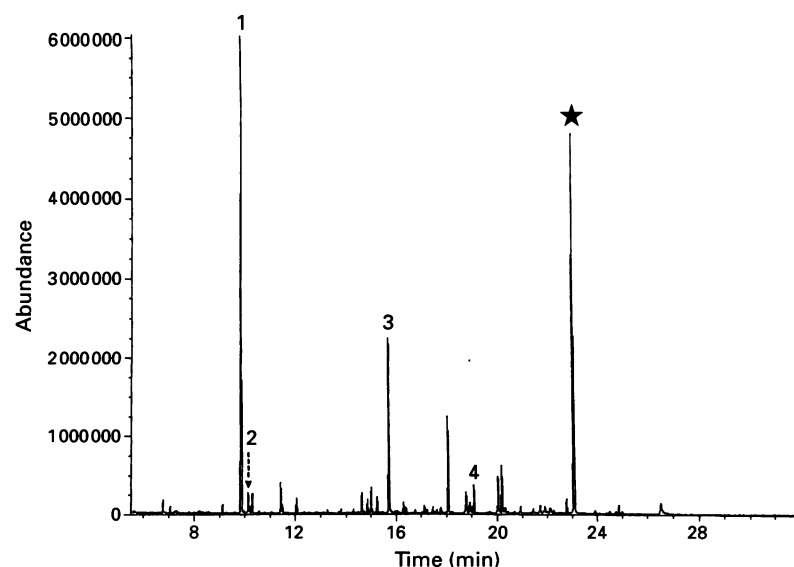


Figure 1 Total ion current chromatogram of urinary organic acid metabolites from case 1. Peaks identified are (1) malonic acid, (2) methylmalonic acid, (3) adipic acid, and (4) suberic acid; *pentadecanoic acid (internal standard).

Table 1 Malonyl CoA decarboxylase activity (cultured fibroblasts)

Cell line	Enzyme activity (nmol/hour/mg protein)	% Control
Normal control 1	11.6	100
Normal control 2	15.2	
Mildly deficient control	3.3	25
Severely deficient control	0.55	4
Case 1	0.72	5
Case 2	0.59	4

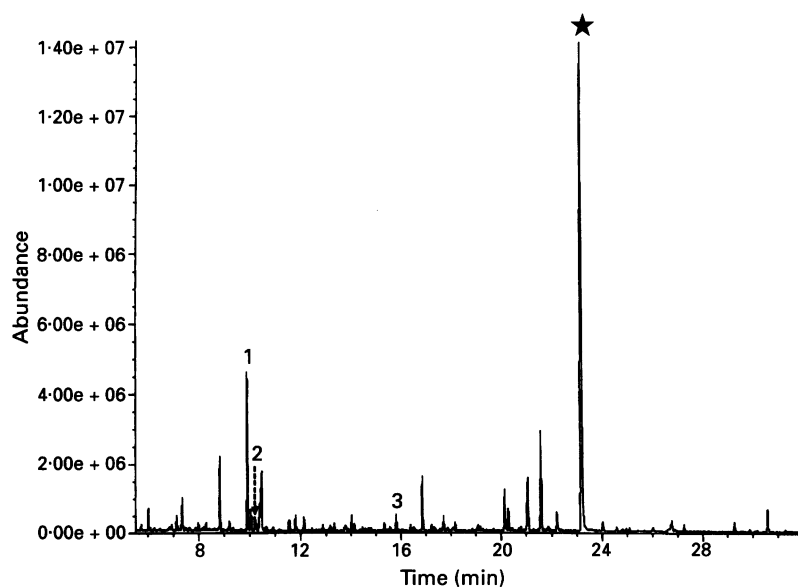


Figure 2 Total ion current chromatogram of urinary organic acid metabolites from case 2. Peaks identified are (1) malonic acid, (2) methylmalonic acid, and (3) adipic acid; *pentadecanoic acid (internal standard).

CASE 2

A girl, the elder daughter of two children of a first cousin marriage of Scots parents, was born in 1976 at term by normal delivery with a birth weight of 4000 g. She was well until 1 year of age when she was admitted to hospital with a 24 hour history of vomiting, fever, and increasing drowsiness. She was found to be hypoglycaemic and ketotic with a palpable liver. Her blood gases at this time revealed a partially compensated metabolic acidosis and the plasma urea was raised. The response to intravenous glucose was initially good but there was a later relapse. She showed a marked degree of hypotonia and floppiness and convulsions had also occurred.

After correction of the hypoglycaemia and mild dehydration, the plasma urea, electrolyte, and glucose concentrations returned to normal. Urine chromatography for amino acids did not show any evidence of a specific defect. Various microbiological investigations all gave negative results and her cerebrospinal fluid protein was normal, as were her serum proteins.

Radiography of the skull and chest was normal but her electroencephalogram was abnormal, displaying a pattern that could be due to many different forms of encephalopathy. Four days after admission the transaminase activities were slightly raised and at one stage she had a raised blood pyruvate but normal lactate concentration. Her condition had stabilised three weeks after admission but she

remained extremely floppy with choreoathetoid movements.

Her present condition includes the presence of spastic choreoathetosis and ataxia, which result in severe dysarthria with very little intelligible speech. Review of the parental consanguinity prompted investigation of the patient for urinary organic acid excretion. An oral L-carnitine loading test (100 mg/kg body weight) was performed and malonate (125 mg/g creatinine) and adipate (25 mg/g creatinine) were the noteworthy features of the urinary organic acid chromatogram after carnitine, which was verified by GC-MS (fig 2). A trace amount of methylmalonate was also identified by GC-MS. However, thin layer chromatography for acylcarnitines revealed only acetylcarnitine. In the next day's urine specimen there was no evidence of malonate excretion but a little adipate (15 mg/g creatinine) remained present.

Malonyl CoA decarboxylase activity was measured using cultured fibroblasts. Grossly reduced malonyl CoA decarboxylase activity (table 1) confirmed the tentative diagnosis of the enzyme deficiency.

Methods

URINARY ORGANIC ACIDS

Urine organic acids were assayed by gas chromatography as described by Tanaka *et al*^{4,5} with column modifications. The organic acids were extracted from aliquots of urine containing 250 µg creatinine with ethyl acetate and diethyl ether, taken to dryness, and trimethylsilyl derivatives prepared. The separation of the organic acids by gas chromatography was achieved using a wide bore capillary column with a flame ionisation detector relating the peak areas to a standard solution of pentadecanoic acid.

Identification of the peaks obtained by this screening method was confirmed by combined GC-MS using a Hewlett Packard 5890 Series II gas chromatograph with an HP-1 25 m cross linked methyl silicone gum capillary column attached to an HP 5971A mass selector detector and microcomputer.

URINARY ACYLCARNITINES

Urinary acylcarnitines were prepared and identified essentially as described by Bhuiyan *et al*.⁶

CULTURED FIBROBLASTS

Skin biopsy tissue was set up in culture in Ham's F-10 medium with 20 mM Hepes buffer and L-glutamine supplemented with 20% fetal calf serum and containing 1% penicillin/streptomycin. Fibroblast cells were grown in 25 ml flasks to about 70% confluency before air transport to Australia. Later this was adjusted to 80% confluency before transport.

MALONYL COA DECARBOXYLASE ASSAY

This was performed by using cultured fibroblasts as the enzyme source, [1, 3-¹⁴C] malonyl

CoA as substrate and the measurement of labelled carbon dioxide ($^{14}\text{CO}_2$) released.¹ To give the best comparison of the residual activities in the two new cases and the two previously described Australian cases cell lines from all four patients were assayed together in a single batch of assays along with two control cell lines (table 1).

Discussion

The Scots born patients described in this article were hospitalised in infancy during episodes of vomiting and febrile convulsions. They are children of consanguinous parents and are mentally retarded with developmental delay. On metabolic screening excess malonic aciduria was demonstrated and reduced activity of fibroblast malonyl CoA decarboxylase confirmed.

Similarities in certain aspects of the clinical and biochemical features of the malonyl CoA decarboxylase deficiency patients become apparent when compared with the two Australian cases (table 2). The first Australian child did not, however, show any abnormalities on formal testing of development,¹ although his height was below the 3rd centile, whereas the other cases all had signs of developmental delay apparent. Normal development was also reported in case 2 by her parents until her severe encephalitic illness at 1 year of age.

In reports on the second Australian patient it was demonstrated that a low fat, high carbohydrate diet (18% and 67% of energy intake respectively) given for a period of five months led to near normalisation of the urinary organic acid excretion.^{2,3} It was further proposed

that the long term development in affected individuals might be improved by implementing this diet as a permanent regimen. The outcome has been poor in this child with regard to intellectual development, but there have been no further hypoglycaemic episodes while on the diet or in the four years since the diet was discontinued (D M Danks, personal communication). Hospital admission is recommended during periods of infection as it is during febrile illness that the harmful effects of this disorder become apparent.

The urinary excretion of excess malonate by case 1 was much greater during his acute illness than the amount detected in case 2 who was clinically well when diagnosed (table 3). Indeed, the chance discovery of malonic aciduria in case 2 after the carnitine load was fortuitous as the next day the urinary malonate was undetectable. So, too, the malonic acid excretion in case 1 had fallen from 775 mg/g creatinine to a concentration of 20 mg/g creatinine 24 hours later.

These findings again illustrate the importance of obtaining urines for organic acid analysis during acute episodes of illness for reliable detection of abnormal metabolites as their excretion in this disorder can drop markedly within a short time and may be missed. It has been found that a high fat, low carbohydrate diet promoted malonic aciduria in affected individuals.^{2,3} When patients are well, therefore, a ketogenic (high fat) diet may be considered to promote the urinary excretion of excess malonate for diagnostic purposes. The mother of one of our patients was put on a ketogenic diet but developed diarrhoea within 24 hours and the diet was discontinued. Urine collected during the diet showed excess malonate excretion, although neither parent of the other case displayed abnormal organic acid excretion on the ketogenic challenge and it is considered that the ketogenic challenge, although of proved diagnostic value in homozygous subjects, is not a useful measure in the investigation of obligate heterozygotes.

The oral L-carnitine loading test was used for possible detection of a medium chain or long chain fatty acyl CoA dehydrogenase defect and the determination of urinary acylcarnitines was employed to assist in this differential diagnosis. Malonyl CoA is, however, the only physiological compound known to be capable of inhibiting carnitine acyltransferase I,⁷ the enzyme responsible for the formation of acylcarnitines.

It has been suggested that although the mitochondrion is impermeable to cytoplasmic malonyl CoA, mitochondrial malonyl CoA might be able to contribute to the cytoplasmic pool³ and presumably inhibit the action of carnitine acyltransferase I. The appearance of excess malonic aciduria by case 2 cannot be explained by carnitine loading but she may have had a meal of high fat content before urine collection. Dicarboxylic acids (adipate and suberate) usually appear during episodes of illness or fat challenge confirming that malonyl CoA interferes with fatty acid β -oxidation and stimulates alternative ω -oxidation. There

Table 2 Malonyl CoA decarboxylase deficiency: comparison of cases

Features	Scottish cases		Australian cases	
	1	2	1	2
Sex	M	F	M	M
Age at presentation of symptoms (years)	3.9	1.0	1-2	0.9
Parental consanguinity	+	+	+	-
Vomiting	+	+	+	+
Gastrointestinal symptoms	+	+	+	+
Infection at presentation	+	+	-	+
Convulsions	+	+	-	+
Developmental delay and/or mental retardation	+	+	*	+
Abnormal facial features	+	-	-	+
Physical problems	-	+	-	+
Metabolic acidosis	NT	+	+	+
Hypoglycaemia	NT	+	-	+
Malonicaciduria	+	+	+	+
Presence of methylmalonic acid	+	†	+	+

+ = Present; - = absent.

*Short stature but comparable with parents' heights.

† = Trace amount of methylmalonic acid present.

NT = not tested.

Table 3 Urinary organic acids (mg/g creatinine) by gas chromatography with flame ionisation detection (GC-FID)

Case No	Clinical status	Date (day/month)	Malonate	Adipate	Suberate	Methylmalonate	Hippurate
1	Unwell	25/04	775	270	130	Present*	NE
		26/04	20	ND	ND	ND	NE
	Well	18/10	30	ND	ND	ND	NE
	Well	18/10	45	ND	ND	ND	1105
2	Well	03/06	125	25	ND	Present*	NE
	Well	04/06	ND	15	ND	ND	NE

ND = None detected on GC-FID.

NE = Minor peak on GC-FID therefore not estimated.

*Detected by GC-MS.

is, however, not total inhibition of fatty acid β -oxidation, as ketosis was evident with case 2 during her encephalitic illness at 1 year of age.

Mammalian malonyl CoA decarboxylase is usually located in the mitochondrial matrix and protects key mitochondrial enzymes such as methylmalonyl CoA mutase and pyruvate carboxylase from inhibition by malonyl CoA.⁸ This may explain the raised blood pyruvate concentration encountered in one of the patients and the hypoglycaemia observed predominantly during acute episodes of illness when methylmalonic acid was also demonstrated in the urine of all the affected cases (table 2). Secondary dibasicaminoaciduria, which was found in the urine of case 1, has been observed previously in the more familiar primary organic acidopathies, propionicacidaemia and 3-methylcrotonylglycinuria.⁹

Malonyl CoA decarboxylase deficiency is inherited as an autosomal recessive disorder¹ and presumably as a nuclear coded protein. Jang *et al* observed that in goose uropygial gland there must be two populations of mRNA, one encoding the mitochondrial enzyme and the other encoding the cytoplasmic form.⁸ Southern blot analysis indicated a single copy gene for the decarboxylase suggesting that alternative promoter utilisation and/or splicing may produce the two forms of the enzyme. Isolation of a cDNA clone for the mitochondrial enzyme will clarify the issue.

The two new cases have doubled the worldwide experience of malonyl CoA decarboxylase deficiency. Although this disorder appears to be very rare, we consider that it cannot be confined to the populations of Melbourne and Glasgow as there is no apparent racial connection between the Australian and Scottish patients. A ketogenic challenge to asymptomatic patients suspected of harbouring a fatty acid disorder and especially this deficiency would aid detection by stimulating accumulation of the abnormal organic acids which could

be identified by GC-MS. Confirmation by specific enzyme assay could then follow.

Addendum

It has been drawn to our attention (DRT) since preparing the manuscript that Professor Reuben Matalon of the Children's Hospital in Miami has recently had a patient confirmed as suffering from malonyl CoA decarboxylase deficiency.

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