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Two cases of benign methylmalonic aciduria detected during a pilot study of neonatal urine screening

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Abstract

Two cases of benign methylmalonic aciduria (MMAuria) were found among 9780 neonatal screenings using the previously described screening method consisting of urease digestion, ethanol deproteinization and gas chromatography–mass spectrometry. Combining this screening method with the stable isotope dilution technique showed very specific and sensitive measurements of methylmalonic acid in urine. The concentrations of urinary methylmalonic acid were measured at several ages. The levels of urinary methylmalonic acid in two patients varied from 0.27 to 3.04 mol/mol creatinine (control<0.01 mol/mol creatinine). Methylcitrate and homocystine were not increased in the patient's urine or blood. Blood propionylcarnitine was also at normal levels. The urinary methylmalonate excretions were decreased to the levels of about 50% of the start point after vitamin B12 treatment in one patient, but the other patient showed no change. No clinical abnormalities were observed during these periods.

Keywords: Benign methylmalonic aciduria

1. Introduction

Methylmalonic aciduria (MMAuria) is caused by a deficiency in methylmalonyl-CoA mutase apoenzyme (McKusick 251000) or defective synthesis of 5′-deoxyadenosylcobalamin, the coenzyme of the mutase (McKusick 251100). MMAuria has severe clinical features including metabolic acidosis, vomiting, developmental delay, mental retardation or in some instances even neonatal death [1,2]. It is one of the most frequent organic aciduria found in neonates, and one of the causes of sudden infant death. Therefore, early diagnosis and appropriate treatment are very important for the prevention of neurological damage or death. Biochemical diagnosis of MMAuria is carried out by the detection of methylmalonic acid (MMA) in the urine or serum of the patient.

Several methods for mass screening of organic aciduria and amino aciduria have been reported. Recently, Matsumoto and Kuhara developed a simple and rapid screening method [3,4] which consists

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of urease treatment, ethanol deproteinization and gas chromatography–mass spectrometry (GC–MS). Using this method, we conducted a pilot study for newborn mass screening [5,6]. Two cases of benign MMAuria were identified in more than 9780 samples. These patients did not show clinical abnormalities and only excreted large amounts of methylmalonic acid in their urine (greater than 20 SD). Neither methylcitrate nor homocystine were increased in their urine; these are also quantifiable using this method [7]. The absolute concentrations of MMA in these cases were measured using deuterium labeled methylmalonic acid as an internal standard. Follow-up studies of these two cases of benign MMAuria are described in this report.

2. Case reports

2.1. Case 1 (T.N., female)

This patient was born after 41 weeks gestation and her birth weight was 3228 g. At 3 days old, she underwent phototherapy because of hyperbilirubinemia. No other clinical or biochemical abnormalities were detected. GC–MS neonatal urine screening was carried out at 5 days old and shows that she excreted abnormal amounts of MMA (+30 SD). The vitamin B12 administration test was carried out at 22 days old, but the excretion of MMA was not improved. Enzymatic measurement using patient fibroblasts showed a normal activity in methylmalonyl-CoA mutase. Repeated organic acid analyses were carried out at several ages under no substantial treatment, and she showed normal development and no clinical abnormalities at 3 years old. She is presently suspected of benign MMAuria.

2.2. Case 2 (T.Y., male)

This patient was born after 38 weeks gestation and his birth weight was 2942 g. He showed normal development and no clinical abnormalities. A urine specimen obtained at 5 days old was analyzed at GC–MS neonatal screening, and he was found to have excreted an abnormally large amount of MMA (+20 SD). He was also suspected of having benign MMAuria. The concentration of methylmalonic acid in urine also decreased to about 50% of that after three doses of vitamin B12 at 135 days old (from 2.60 to 1.28 mol/mol creatinine), which was significant ($P=0.0736$) as determined by the Mann–Whitney U test. Repeated organic acid analyses were also carried out at several ages without substantial treatment.

3. Methods

Details of the analytical procedures have been published elsewhere [3–6]. Trimethylsilyl derivatives of urinary metabolites were analyzed using a GC–MS (Shimadzu QP-5000 and Hewlett-Packard HP 5973 MSD) equipped with a fused-silica capillary column (J&W DB-5, 30 m×0.25 mm×0.25 mm). Quantitations of urinary metabolites were performed by mass chromatography. For quantitation of urinary methylmalonic acid, D3-methylmalonic acid (methyl-d_3-malonic acid (99.5 molar% D) from MSD Isotopes, Montreal, Canada) was used as the internal standard [7]. Fragment ions at m/z 247 [M-15] and m/z 250 [M-15] were used to quantify methylmalonic acid 2TMS and D3-methylmalonic acid 2TMS, respectively.

Isolation of urinary acylcarnitines was carried out according to the procedure described by Poorthuis et al. [8]. Methyl esters of acylcarnitine were analyzed by fast atom bombardment mass spectrometry (FAB MS) using a Jeol JMS DX 303 mass spectrometer.

4. Results and discussion

A combination of the improved urease treatment GC–MS analysis with mass chromatography stable-isotope dilution analysis was used for the determination of urinary MMA. The calibration curve consisted of the data points for 0–50 nmol of MMA using 25 nmol of labeled D3-MMA as the internal standard. The correlation coefficients of standard curves were 0.998. The recovery of MMA was greater than 95% using this urease treatment method at 25 nmol ($n=6$) and this method showed a low relative standard variation: RSD<6.3%. We used 0.1 ml urine (creatinine; 0.1–0.5 μmol) for analyses, and the detection limit for MMA by this method was 0.5

nmol/vial. The concentrations of MMA in control urine samples were lower than 0.01 mol/mol creatinine (estimated 1 nmol/0.1 ml urine). Therefore, the sensitivity of the present method was adequate for MMA detection.

The concentration of MMA in normal specimens is typically lower than 0.01 mol/mol creatinine in newborn infants [9–11]. Usually, extremely large quantities of MMA are found in the urine of patients with MMAuria. Very similar clinical conditions and metabolic abnormalities are found in propionic acidemia and MMAuria. The increased urinary metabolites except MMA are closely related in both diseases. Therefore, the detection and quantitation of MMA is very important for the chemical diagnosis.

Most MMAuria patients have the first clinical episode within 1 week of birth. For prevention of clinically severe conditions, both early diagnosis and treatment are very important. Therefore, we started a pilot study for newborn IEM (inborn errors of metabolism) screening using our simple and rapid screening technique, and found two cases of MMAuria (cases 1 and 2). These patients excreted large quantities of MMA (Fig. 1), but both patients showed no clinical abnormalities and no other abnormal metabolites except MMA. The urine samples at different ages were analyzed (Table 1 and Fig. 2). They excreted greater than 0.27 mol/mol creatinine but lower than 3.04 mol/mol creatinine.

Several studies have reported benign MMAuria [9–13]. Shapira et al. reported the concentrations of MMA in benign MMAuria are 0.09–3.25 mol/mol creatinine and clinical abnormalities appeared at greater than 5.3 mol/mol creatinine [9], and more recently, Sniderman et al. reported 122 cases of low–moderate MMAuria (urinary MMA greater than 1.4 mol/mol creatinine) during 25 years of newborn screening in Quebec, Canada, conducted at 21 days of age by thin-layer chromatography of urine samples [11]. The follow-up studies of these individuals showed that 13 had symptoms, and a biochemical abnormality was identified in 10 of these 13 individuals with symptomatic MMAuria. Treacy et al. reported that one benign MMAuria patient, classified as such because of a low concentration of MMA (0.29 mol/mol creatinine) and no clinical abnormality during the neonatal period, developed severe metabolic acidosis at 5 years old [10]. The patient was diagnosed with partial deficiency of methylmalonyl-CoA mutase with enzyme assay and the remaining mutase activity was 10%. These findings show the importance of screening for low–moderate MMAuria in neonatal screening programs and intensive follow-up.

Propionylcarnitine is a marker compound used in screening for MMA acidemia by acylcarnitine analysis using tandem mass spectrometry [14,15]. The blood acylcarnitines were analyzed in the case 1 patient using ESI-MS–MS, but an accumulation of propionylcarnitine was not detected in her blood. Urinary acylcarnitines were also analyzed using FAB MS, but the urinary propionylcarnitine level was within normal levels.

Methylcitrate is the other sensitive marker compound for the accumulation of propionyl CoA in body tissue. The enhanced urinary excretion of methylcitrate is observed in propionic acidemia and MMAuria. We examined the urinary excretion of methylcitrate in the present patients, but there were no abnormal excretions of methylcitrate at any measurement points. These findings show that propionyl-CoA is not accumulated at such a concentration as would produce methylcitrate in the present patients.

It is important to distinguish the following different types of MMAuria, (A) MMA CoA mutase^{-} or MMA CoA mutase^{0}, (B) deficiency of adenosylcobalamine synthetic process and (C) others. In the case of (A) and (B), MMA is excreted into the urine at high concentrations. In some cases of methylmalonic aciduria–homocystinuria caused by (B), however, low excretions of MMA were also reported. The present patients showed no homocystinuria and low excretions of MMA, and therefore it seems that they belong to type (C).

The concentrations of MMA in the present benign forms were 0.27–3.04 mol/mol creatinine, and one case was examined over a period of 3 years. The levels of excretion of MMA in the present two cases are shown in Fig. 2. They can be clearly distinguished from the typical MMA excretion of normal individuals. The values of MMA in the benign form were lower than the acute form (greater than 5 mol/mol creatinine), but if an acutely ill patient is treated with conventional emergency measures, a lower excretion of MMA would be detected also in

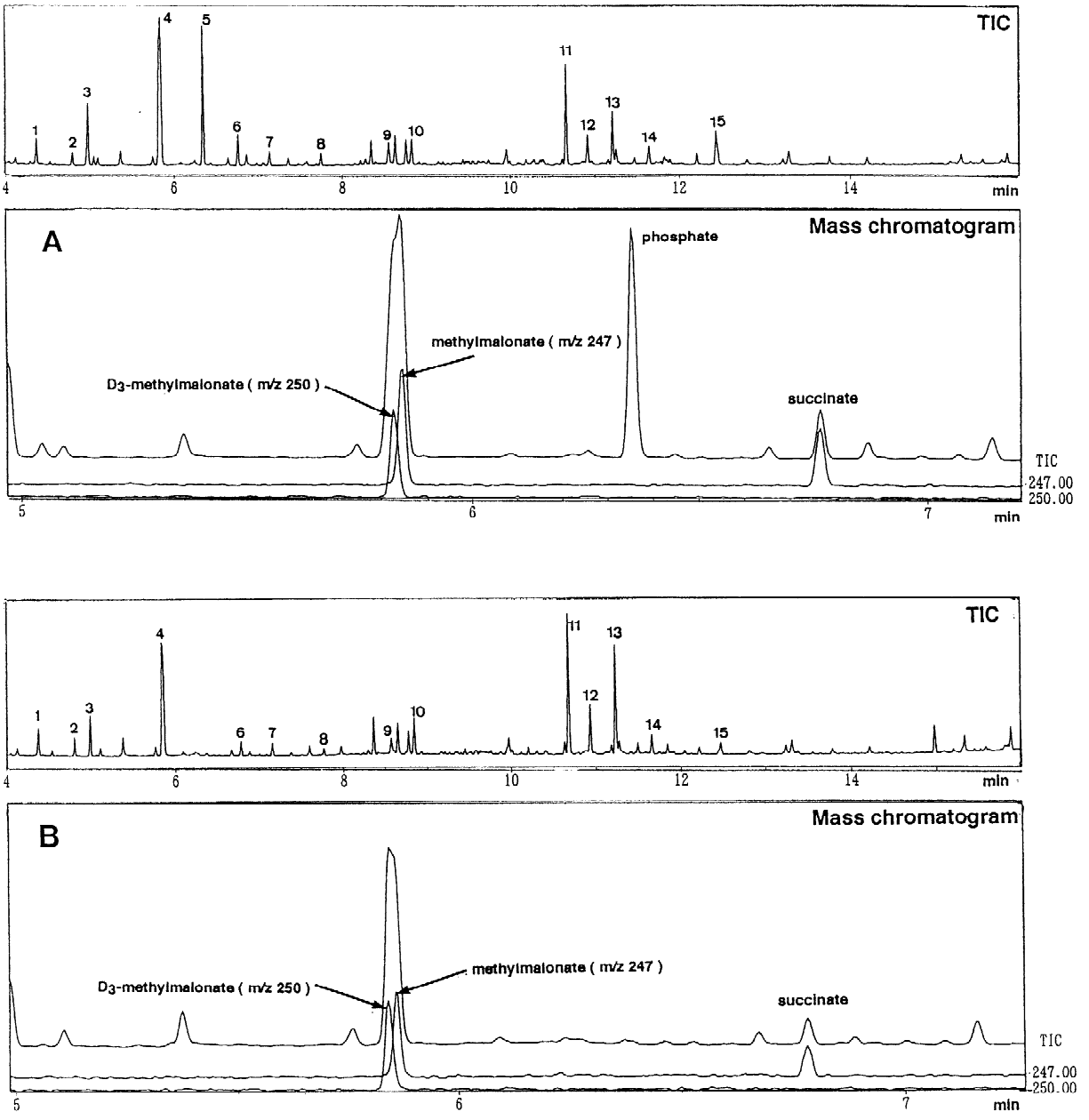

Fig. 1. Mass chromatography for benign methylmalonic aciduria (A) case 1 (B) case 2 (I.S., D3-methylmalonic acid, 25 nmol) 1=lactate; 2=alanine; 3=glycine; 4=methylmalonate; 5=phosphate; 6=succinate; 7=serine; 8=2-dehydrotetronate; 9=hydroxyproline; 10=creatinine; 11=citrate; 12,13=galactose; 14=glucose; 15=urate.

the acute form and the values may overlap with the benign form. It appeared that the excretion of MMA is dependent on the ingestion of protein and the clinical episodes are related to the accumulation of propionyl-CoA in body tissues. The monitoring of the urinary concentration of methylmalonate and methylcitrate is quite suitable for the inspection of clinical conditions, and the present screening tech-

Table 1
Comparison of urinary excretion of methylmalonic acid in benign methylmalonic aciduria

Patient	Age	Methylmalonate (mol/mol creatinine)	Other metabolites
Case 1 (T.N)	5 days–3 years	0.91±0.32 (n=24) 0.27–1.69	
Case 2 (T.Y)	4 days–5 months	1.51±0.69 (n=14) 0.61–3.04	
Control	5 days–10 months	<0.01	
Ledley et al. [12]	Eight patients (18 months–13 years)	0.96–3.26	
Shapira et al. [9]	4 years	0.17	3HBA, AAA
	6 years	4.14	3HBA, AAA
Treacy et al. [10]	21 days	0.29	
	5 years	1.56	MCA, 3HPA
Swell et al. [13]	10 years, girl	0.39–0.68	
	13 years, (sister)	0.60	
	18 years, (brother)	0.04	
	Mother	0.64	
Sniderman et al. [11]	22 patients	0.21–1.13	

3HBA, 3-hydroxybutyrate; AAA, acetoacetate; MCA, methylcitrate; 3HPA, 3-hydroxypropionate.

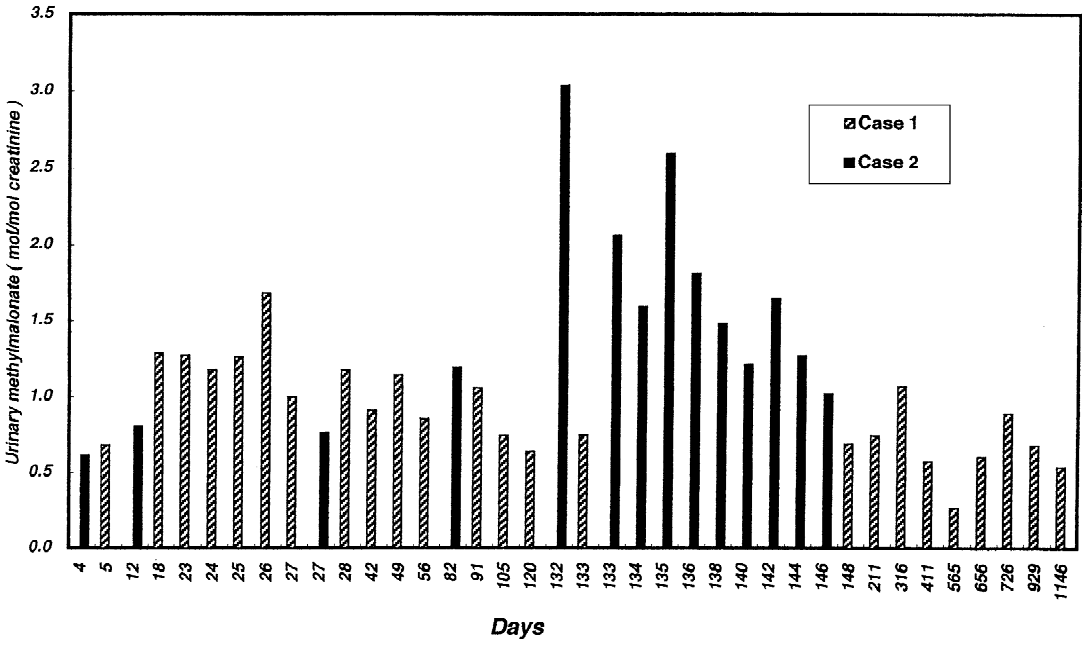

Fig. 2. Variation in urinary excretion of methylmalonic acid with age in two patients with benign methylmalonic aciduria.

nique is very useful for the detection of these compounds because this technique is able to examine not only MMA but also many other metabolites of amino acids and organic acids at the same time [4,6,16].

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