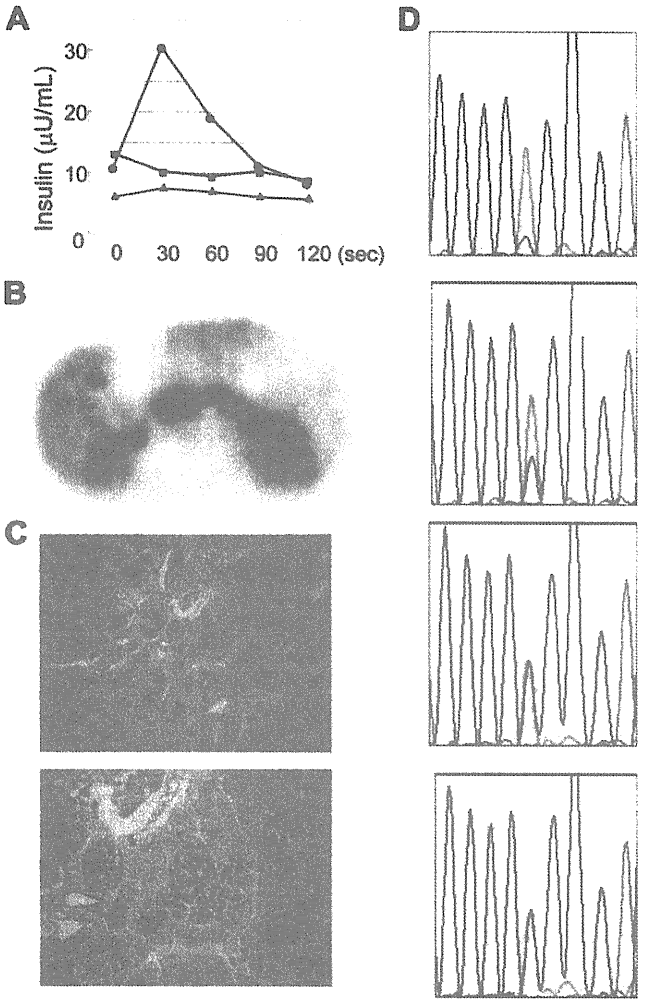

FIG. 1. Results of different diagnostic modalities in patient 10. A, Results of arterial stimulation venous sampling studies. The insulin concentration of the right hepatic vein was measured after the injection of calcium into the splenic (*filled circles*), gastroduodenal (*filled rectangles*), and superior mesenteric (*filled triangles*) arteries. An insulin response was observed only after stimulation of the splenic artery. B, A curved planar reconstruction of a [18F]-DOPA PET scan. The uptake in the head probably reflects an artifact. C, Chromogranin A staining of the resected pancreas showing the area in which abnormal islets were most densely distributed. Magnification, ×40 (*upper panel*), ×80 (*lower panel*). D, Mutational analysis of abnormal islet samples. The *upper two panels* show the results of two separate analyses of 30 (*upper panel*) and 40 (*lower panel*) islet samples. The *lower two panels* show the results of a similar analysis of an adjacent normal pancreatic area. The paternally inherited A allele (*green*) predominates in the abnormal islets, whereas the A and the wild-type G alleles (*black*) have similar intensities in the normal area of the pancreas.

single lesion composed of a solid β-cell cluster was identified by serial sections of the specimen (Fig. 1C). LCM was performed twice to collect samples from 30 and 40 of these islet clusters. Mutational analysis of the pooled DNA collected from these LCM samples revealed the predominance of the paternally inherited mutant allele within these scattered large islets compared with the surrounding normal pancreatic tissue (Fig. 1D).

Discussion

The most important finding of this study is the higher incidence of paternally inherited, monoallelic K_{ATP} channel mutations in Japanese patients with congenital hyperinsulinism ($P < 0.005$ by the sign test), which suggests that the majority of Japanese patients have the focal form. Although the number of patients is small, we believe our results represent the situation of the whole country for several reasons. First, a national survey in 2008–2009 conducted by the Ministry of Health, Labor, and Welfare of Japan estimated the incidence of persistent congenital hyperinsulinism as 1:35,400 births. Our study captured 23% of all cases during that period. Second, the patients were referred without geographical biases because ours is the only laboratory currently offering a comprehensive molecular diagnosis in Japan. Third, a previous report by Ohkubo *et al.* (13) also reported a high frequency (seven of 10) of monoallelic mutations in Japan. In contrast, patients with hyperinsulinism-hyperammonemia syndrome were collected somewhat arbitrarily over a longer period; therefore, the apparent higher incidence might not represent the actual incidence in Japan.

Conflicting results have been reported for the diabetogenesity of p.E1506K in *ABCC8* (12, 14, 15). The association might be a chance observation or might reflect a difference in the genetic background. If the association does exist, that might be due to the specific nature of the mutation, which confers the instability of the β-cells such as altered membrane potential of the cells.

Molecular diagnosis correctly predicted the histology in all patients who underwent pancreatectomy. On the contrary, the ability of [18F]-DOPA PET scans to identify focal lesions was inferior compared with the results of previous reports for other populations (16, 17). Histologically, at least two patients with ambiguous PET results had large focal lesions. The third patient (patient 10) appeared to have unusually scattered islets for a focal lesion. However, there remains the possibility that these islets are actually interconnected and represents a focal lesion with greater admixture of exocrine tissues. Although the number of patients was too small to draw a definite conclusion, larger lesions might be more common in the Japanese.

The reason that the incidence of the focal form of the disease is higher in Japanese is unclear. One possibility is that Japanese have a higher incidence of somatic isodisomy. If this occurred during the earlier stages of development, it would lead to the development of Beckwith-Wiedemann syndrome. However, the incidence of this syndrome caused by paternal isodisomy is not particularly higher in Japanese (18). Alternatively, cells with mutations common in Japanese might be more prone to develop into

a focal lesion, by either promoting a second hit of isodisomy or conferring a growth advantage after the disomic event. Further studies are necessary to address this question.

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Original Article

Neonatal lactic acidosis with methylmalonic aciduria due to novel mutations in the *SUCLG1* gene

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Abstract ***Background***: Succinyl-coenzyme A ligase (SUCL) is a mitochondrial enzyme that catalyses the reversible conversion of succinyl-coenzyme A to succinate. SUCL consists of an α subunit, encoded by *SUCLG1*, and a β subunit, encoded by either *SUCLA2* or *SUCLG2*. Recently, mutations in *SUCLG1* or *SUCLA2* have been identified in patients with infantile lactic acidosis showing elevated urinary excretion of methylmalonate, mitochondrial respiratory chain (MRC) deficiency, and mitochondrial DNA depletion.

Methods: Case description of a Japanese female patient who manifested a neonatal-onset lactic acidosis with urinary excretion of methylmalonic acid. Enzymatic analyses (MRC enzyme assay and Western blotting) and direct sequencing analysis of *SUCLA2* and *SUCLG1* were performed.

Results: MRC enzyme assay and Western blotting showed that MRC complex I was deficient. *SUCLG1* mutation analysis showed that the patient was a compound heterozygote for disease-causing mutations (p.M14T and p.S200F).

Conclusion: For patients showing neonatal lactic acidosis and prolonged mild methylmalonic aciduria, MRC activities and mutations of *SUCLG1* or *SUCLA2* should be screened for.

Key words lactic acidosis, methylmalonic acid, mitochondrial respiratory chain, *SUCLA2*, *SUCLG1*.

Urinary excretion of methylmalonic acid is caused by a defect in the isomerization of L-methylmalonyl-coenzyme A to succinyl-coenzyme A. The reaction is catalyzed by L-methylmalonyl-coenzyme A mutase (MCM), an enzyme that requires adenosylcobalamin as a cofactor.[1] Methylmalonic acidemia/aciduria is mainly classified into two types: one resulting from a defect in the MCM apoenzyme and another resulting from a defect in the steps leading to adenosylcobalamin synthesis. In some cases, other causes of methylmalonic acidemia/aciduria have been reported. Recently, deficiency of the succinyl-coenzyme A ligase (SUCL) has been reported in cases of infantile lactic acidosis with mild urinary excretion of methylmalonic acid.[2]

Succinyl-coenzyme A ligase is a mitochondrial enzyme associated with the Krebs cycle, catalyzing the reversible conversion of succinyl-coenzyme A to succinate. The enzyme consists of two subunits. The substrate specificity for guanosine diphosphate (GDP) or adenosine diphosphate (ADP) is determined by the β subunit. The α subunit is encoded by the *SUCLG1* gene, whereas the β subunit is encoded by *SUCLA2* for the ADP-specific subunit and by *SUCLG2* for the GDP-specific subunit. *SUCLG1* is ubiquitously expressed, but its expression is particularly high in the heart, brain, kidney, and liver. The SUCLA2 protein is primarily present in the brain, skeletal muscle, and heart, and the SUCLG2 protein is present in the liver and kidney. More than 20 cases of deficiency in the α subunit (mutation in *SUCLG1*) or ADP-forming β subunit (mutation in *SUCLA2*) have been reported.[3–5] These patients have mitochondrial respiratory chain (MRC) deficiency, mitochondrial DNA (mtDNA) depletion, encephalomyopathy, and mild methylmalonic aciduria.[6–9]

Here, we describe the case of a Japanese female patient who presented with neonatal-onset lactic acidosis with urinary excretion of methylmalonic acid. *SUCLG1* mutation analysis showed that the patient was a compound heterozygote for disease-causing mutations.

Case report

In 1993 a female infant was born at 38 weeks gestation (birthweight, 2640 g; birth length, 47.3 cm). Her Apgar scores were normal. On the day after birth, she developed problems. Her blood sugar was lower than 1.1 mmol/L, and hence, continuous glucose infusion was started. Mechanical ventilation and peritoneal dialysis were started when the infant was 2 days old because of cyanosis, severe metabolic acidosis (pH, 6.638, base excess,

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Table 1 Laboratory data

	2 days	4 days	4 months
WBC (/μL)	46 500	19 400	
RBC ($\times 10^6$/μL)	4.50	4.59	
Hb (g/dL)	18.0	18.0	
Ht (%)	59.0	51.5	
Plt ($\times 10^3$/μL)		105	
Total bilirubin (mg/dL)		8.8	
γ-GTP (IU/L)		136	
AST (IU/L)	607	217	
ALT (IU/L)	125	128	
LDH (IU/L)	5400	3860	
CK (IU/L)		6370	
CK-MB (IU/L)		216	
Na (mBq/L)		140	
K (mBq/L)		3.0	
TP (mg/dL)		4.7	
BUN (mg/dL)	23	24	
Cr (mg/dL)		1.2	
pH	6.638	7.477	
HCO_3^- (mEq/L)		14.5	
Base excess	−26.8	−4.9	
NH_3 (mmol/L)	191	45	
Lactate (mmol/L)	11	8.1	
Pyruvate (mmol/L)		0.41	
BS (mg/dL)		101	
Urine (organic acids excretion)		High, lactate, pyruvate; Moderate, methylmalonate, methylcitrate; Slight, glutarate, fumarate, succinate, 3-methylglutaconate	
Acylcarnitine (dried blood spots)			increase in C3 and C4DC
Methylmalonic acid (serum)			13 μmol/L (control, not detected MCM-deficient patients, 220–2900)
Methylmalonic acid (urine)			321 mmol/molCr (control, mean [SD], 2.0 [1.2])
^{14}C-propionate fixation (cultured fibroblasts)			8% of control

ALT, alanine aminotransferase; AST. aspartate aminotransferase; BS, blood sugar; BUN, blood urea nitrogen; CK, creatine kinase; γ-GTP, γ-glutamyltransferase; Hb, hemoglobin; Ht, hematocrit; LDH, lactate dehydrogenase; MCM, L-methylmalonyl-coenzyme A mutase; Plt, platelets; RBC, red blood cells; TP, total protein; WBC, white blood cells.

−26.8), lactic acidemia (11 mmol/L), and hyperammonemia (191 μmol/L; Table 1). She was transferred to Tohoku University Hospital at 4 days old.

Upon admission there was a swelling in the liver 4 cm below the costal margin. The lactate and pyruvate levels were 8.1 mmol/L and 0.41 mmol/L, respectively (L/P ratio, 20). Gas chromatography and mass spectrometry of urinary organic acid showed high levels of lactate and pyruvate excretion; moderate methylmalonate and methylcitrate excretion; and slight glutarate, fumarate, succinate, and 3-methylglutaconate excretion.

Acidosis improved on the following day, and mechanical ventilation and peritoneal dialysis were stopped. She developed prolonged hypotonia. At 1 month of age, auditory brainstem response was absent, and severe hearing impairment was noted. Head computed tomography showed diffuse atrophy. At 4 months of age, mild cardiac hypertrophy was seen on echocardiogram. The patient could not balance her head.

Lactic acidemia (4–9 mmol/L) with an elevated L/P ratio (20–25) and mild urinary excretion of methylmalonic acid persisted. An acylcarnitine profile of dried blood spots showed an increase in C3 (propionylcarnitine) and C4DC (isomers of methylmalonyl carnitine and succinylcarnitine). The serum level of methylmalonic acid was 13 μmol/L (control, not detected; MCM-deficient patients, 220–2900 μmol/L). The urinary levels of methylmalonic acid and methylcitrate were 321 mmol/molCr and 81.7 mmol/molCr, respectively (control, mean ± SD, 2.0 ± 1.2 mmol/molCr and 2.0 ± 0.9 mmol/molCr, respectively). A ^{14}C-propionate fixation assay using cultured fibroblasts showed that propionate fixation in the patient was 8% of that in the control. Enzymatic analyses of the pyruvate dehydrogenase complex and pyruvate carboxylase were normal.

Histology of a liver biopsy specimen indicated moderate macrovesicular and microvesicular steatosis in the hepatic parenchyma. There was no active inflammation or fibrosis. On electron microscopy hepatocytes containing lipid droplets were seen. Mitochondrial abnormalities and other specific findings were not apparent morphologically. Muscle biopsy samples were stained with hematoxylin and eosin, reduced nicotinamide adenine

dinucleotide tetrazolium reductase, modified Gomori-Trichrome, succinate dehydrogenase, periodic acid–Schiff, and cytochrome oxidase. No particular abnormalities were noted in the muscle biopsy specimens.

At 6 months of age, the patient was discharged from hospital. She was able to follow objects with her eyes. Because of feeding difficulty, a naso-gastric tube was used. She developed a social smile at 13 months of age but did not have head control. At 20 months of age, she suddenly died at home. Autopsy was not performed.

Because her clinical course was similar to that of previously reported SUCL-deficient patients,[3,4] we restarted diagnostic analysis using fibroblasts and biopsied muscle samples that had been stored for 16 years in liquid nitrogen.

Methods

Blue native polyacrylamide gel electrophoresis and Western blotting

Expression levels of the MRC complex (Co) I, II, III, and IV proteins in cultured fibroblasts were assessed on Western blotting using blue native polyacrylamide gel electrophoresis (BN-PAGE) according to previously described methods.[10] Immunostaining was performed using monoclonal antibodies specific for the 39 kD subunit of Co I, 70 kD subunit of Co II, core 1 subunit of Co III, and subunit 1 of Co IV (Invitrogen, Camarillo, CA, USA).

Determination of enzyme activities

Activities of MRC Co I, II, III, and IV were assayed.[10] The activity of each complex was presented as a percentage of the mean value obtained from 20 controls. The percentages of Co I, II, III, and IV activities relative to that of citrate synthase (CS) as a mitochondrial enzyme marker or Co II activity were calculated. Deficiency of each complex is confirmed when either the CS ratio and/or the Co II ratio is <45% (fibroblasts) or 35% (muscle).

Quantitative polymerase chain reaction

The mtDNA was quantitatively estimated on real-time amplification of ND1 fragments in the mtDNA genome, as described previously.[10] To determine the overall abundance of mtDNA, the real-time amplification result of ND1 was compared with that of exon 24 of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, as nuclear DNA (nDNA).

Direct sequencing of the SUCLG1 and SUCLA2 genes

Genomic DNA was extracted from cultured fibroblasts using a Sepa Gene Kit (Sanko Junyaku, Tokyo, Japan). All coding exons, including flanking introns, in *SUCLG1* and the *SUCLA2* genes were amplified using polymerase chain reaction (PCR). To facilitate cycle sequencing analysis, M13 universal and reverse primer sequences were attached to the 5′ ends of sense primers and antisense primers, respectively. PCR products were directly sequenced using a Big Dye Primer Cycle Sequencing kit and an ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

The Ethics Committee of the Tohoku University School of Medicine approved the present study.

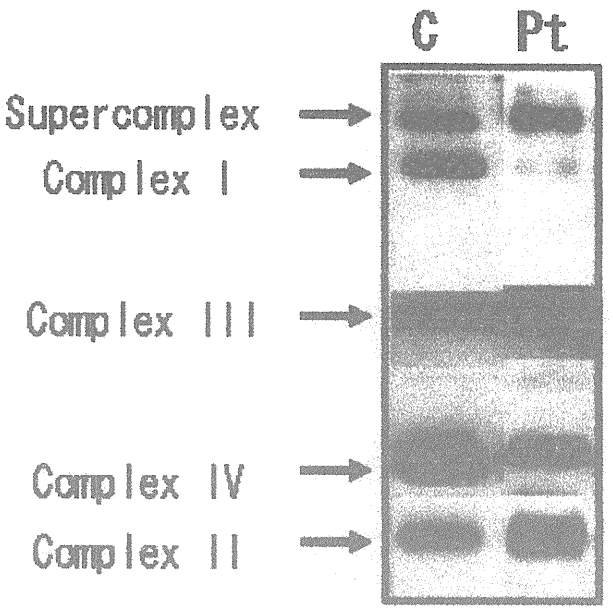

Fig. 1 Blue native polyacrylamide gel electrophoresis and subsequent Western blot analysis of mitochondrial respiratory chain complexes. The amount of assembled complex I was decreased. Complex I, anti-39 kDa subunit; complex II, anti-70 kDa subunit; complex III, anti-core 1 subunit; complex IV, anti-subunit 1.

Results

The amount of respiratory-chain complex in fibroblasts was determined on BN-PAGE Western blot. The intensity of the band corresponding to the assembled Co I of fibroblasts was decreased (Fig. 1). The intensity of the bands corresponding to Co II, III, and IV remained unchanged.

In fibroblasts, the enzyme activities of Co I and Co IV relative to that of Co II were decreased (<45%; Table 2). Even in the muscle biopsy samples, the ratios of (Co II + Co III)/CS, Co IV/CS, Co I/Co II, (Co II + Co III)/Co II, Co III/Co II, and CoIV/Co II were decreased.

Quantitative PCR showed that the ratio of mtDNA/nDNA of the fibroblasts did not decrease (72.9%; control, 76.4%). The ratio in the muscle biopsy specimen was also not decreased (270.1%).

Mutation analysis showed a heterozygous T-to-C substitution at position 41 in exon 1 of *SUCLG1* (c.41T > C; Fig. 2). This c.41T > C mutation changes the Met at position 14 to a Thr (p.M14T). Additionally, in exon 6, a heterozygous C-to-T substitution at position 599 in exon 1 of *SUCLG1* was found (c.599C > T). This mutation changes the Ser at position 200 to Phe (p.S200F). The p.M14T mutation was transmitted to the child from her mother; the other mutation (p.S200F) was transmitted to the child from her father (data not shown). Both substitutions were absent in the 100 alleles screened from healthy volunteers. No substitution was found in *SUCLA2*.

Table 2 Respiratory chain enzyme assay of the present patient

%	Co I	Co II	Co II + III	Co III	Co IV	CS
Fibroblasts						
% of normal	73	236	378	140	60	71
CS ratio	100	326	515	190	85	–
Co II ratio	**30**	–	158	58	**26**	–
Muscle						
% of normal	89	291	40	76	17	197
CS ratio	44	147	**20**	39	**8**	–
Co II ratio	**30**	–	**13**	**26**	**6**	–

Enzyme activities are expressed as % of mean normal control activity relative to protein, relative to CS, and relative to Co II. **Bold**, deficiency of the respective complex: <45% (fibroblasts) or 35% (muscle) of either CS ratio and/or Co II ratio. Reference range, fibroblasts 45–170; muscle 35–160.

Co I, complex I; Co II, complex II; Co III, complex III; Co IV, complex IV; CS, citrate synthase.

Discussion

The patient was identified to have a compound heterozygote mutation in *SUCLG1* (p.M14T and p.S200F). Clinical manifestations such as infantile lactic acidosis, mild methylmalonic aciduria, hypotonia, and hearing loss were compatible with symptoms previously reported in patients with *SUCLG1* or *SUCLA2* mutations.[3,6] The p.M14T and p.S200F mutations have not been reported previously. These substitutions were not found in the 100 alleles from healthy volunteers. p.M14 is located within the mitochondrial targeting sequence. Van Hove *et al.* reported a patient with a mutation at the same methionine (p.M14L) and speculated that the substitution of p.M14 would prevent proper translation initiation.[11] p.S200 is conserved across several species (Fig. 2). These data suggest that p.M14T and p.S200F are not polymorphisms but disease-causing mutations.

The amount of MRC complex I was decreased on BN-PAGE and Western blotting using fibroblasts, and multiple MRC defects were detected on enzyme assay. The ratios of mtDNA/nDNA of fibroblasts and muscle, however, did not decrease. Valayannopoulos *et al.* also reported that mtDNA depletion was not observed in two patients.[6] It is suggested that not all SUCL-deficient

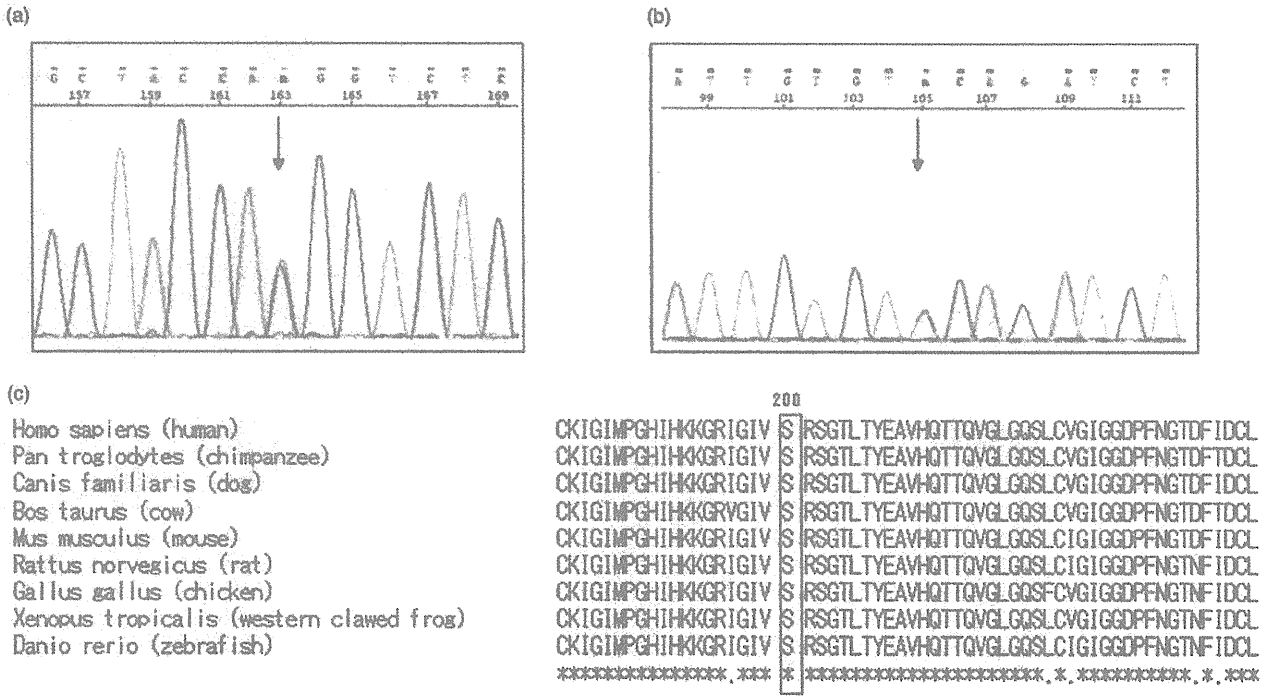

Fig. 2 (a) Heterozygous T-to-C substitution detected at c.41 in exon 1 of *SUCLG1*. This c.41T > C mutation changes the Met at position 14 to Thr (p.M14T). (b) Heterozygous C-to-T substitution detected at c.599 in exon 6 of *SUCLG1*. The c.599C > T substitution changes the Ser at position 200 to Phe (p.S200F). (c) Comparison of succinyl-coenzyme A ligase (SUCL) α subunits from several species. Serine at p.200 was conserved across all the species tested.

patients have mtDNA depletion, and that some mechanisms other than mtDNA depletion might participate in the multiple MRC deficiency observed in these patients.

In the present case, serum methylmalonic acid accumulation and low ^{14}C-propionate fixation capacity suggested disturbance of methylmalonic acid metabolism. Elevated methylmalonic acid may result from the accumulation of succinyl-coenzyme A under the assumption that accumulated succinyl-CoA inhibits the reaction catalyzed by MCM or causes an equilibrium shift, leading to the accumulation of methylmalonyl-coenzyme A, which is converted to methylmalonic acid. As usual, increased levels of C4DC are detected in patients with severe MCM deficiency during acute crises. It is suggested that the C4DC of the present patient was associated with an increased level of succinylcanritine due to accumulated succinyl-coenzyme A.

In conclusion, we identified two novel *SUCLG1* mutations in a Japanese female patient with neonatal lactic acidosis and prolonged mild methylmalonic aciduria. For patients showing these combined manifestations, MRC activities and mutations of *SUCLG1* or *SUCLA2* should be screened for.

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ARTICLE

Newborn Screening for Lysosomal Storage Disorders

KIMITOSHI NAKAMURA,* KIYOKO HATTORI, AND FUMIO ENDO

Lysosomes are intracellular organelles containing acid hydrolases that degrade biological macromolecules. Lysosomal storage disorders (LSDs) are caused by absent activity of one or more of these enzymes due to mutations of genes encoding lysosomal hydrolases or enzymes that process, target, and transport these enzymes. The specific signs and symptoms of each LSD derive from the type of material accumulated within the lysosome, the site (organ) of accumulation and the response of the body (sometimes in the form of an inflammatory or immune response) to the accumulated material. Interest for inclusion of these disorders in newborn screening programs derives from the availability of effective therapy in the form of enzyme replacement or substrate reduction therapy and bone marrow transplant that may improve long-term outcome especially if started prior to irreversible organ damage. Based on the availability of therapy and suitable screening methods, Gaucher disease, Fabry disease, Pompe disease, mucopolysaccharidosis I and II, Niemann–Pick disease, and Krabbe disease are candidates for newborn screening. Pilot newborn screening projects have been performed for some of these conditions that indicate the feasibility of this approach. This review will provide insight into these screening strategies and discuss their advantages and limitations.

KEY WORDS: tandem mass spectrometry; multiplex assays; mucopolysaccharidosis; Fabry disease; Pompe disease,

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INTRODUCTION

A lysosome is an intracellular organelle containing acid hydrolases that degrade proteins, glycoproteins, proteoglycans, lipids, and other complex macromolecules from phagocytosis, endocytosis, and autophagy [Futerman and van Meer, 2004; Fletcher, 2006; Eckhardt, 2010]. These macromolecules are degraded to smaller molecules through the action of various acid hydrolases. The resulting small molecules are then catabolized or recycled by the cell after export to the cytoplasm by passive diffusion or through the use of transporters. For some pathways, these recycled metabolites play a major role in the synthesis pathway. For example, almost 90% of sphingolipids are synthesized in this recycled pathway in many cells [Fredman, 1998; Gillard et al., 1998]. Lysosomal hydrolases are transported from the endoplasmic reticulum to the lysosome by a vesicular transporter. This vectorial transport is dependent on the presence of mannose 6-phosphate residues on their oligosaccharide chains attached to the lysosomal enzyme by a Golgi-localized phosphotransferase complex [Kollmann et al., 2010]. Mannose-6-phosphate receptors capture these processed enzymes into transport vesicles of the *trans*-Golgi network and deliver them to the lysosome. These enzymes can be endocytosed again by neighboring cells and delivered to the lysosome. This latter pathway plays a key role in allowing enzyme replacement therapy (ERT) to reach the lysosome of target cells.

More than 40 LSD are known and have a total estimated incidence of 1:7,000–1:9,000 [Meikle et al., 1999; Fletcher, 2006]. Symptom severity and disease onset of most LSD vary. This heterogeneity can be explained to some extent by the difference in organs affected and, in part, by the type of mutation. In general, mutations leaving very low residual enzyme activity cause the most severe early onset forms of the diseases. In contrast, higher residual enzyme activity delays disease onset [Kolter and Sandhoff, 1999]. Disease severity and onset are remarkably different in the late-onset forms of LSD and can vary even between siblings with identical mutations [Clarke et al., 1989; Wenger et al., 2000; Zhao and Grabowski, 2002]. The major lysosomal storage disorders (LSDs) for which a therapy is

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available and newborn screening is at different stages of development will be briefly described.

SELECTED LYSOSOMAL STORAGE DISORDERS

Fabry Disease

Fabry disease is an X-linked LSD that was initially described in 1898 [Anderson, 1898; Fabry, 1898]. Women can also have symptoms, but onset is generally later than for men and life expectancy is reported better. Fabry disease is caused by α-galactosidase A (Gal A) deficiency [Desnick et al., 2001]. The enzymatic defect leads to progressive accumulation of glycosphingolipids such as globotriaosylceramide (GL-3), especially in the brain, heart, kidney, eye, and skin. The classic disease phenotype consists of angiokeratomas, acroparesthesias, hypohidrosis, and corneal opacities during childhood. Accumulation of GL-3 in the vascular endothelium leads to renal and cardiac failure and cerebrovascular disease. Late-onset cardiac and renal variants with residual Gal A activity have been identified in individuals lacking some or all of the early classic manifestations mentioned above. Patients with the cardiac variant present with left ventricular hypertrophy (LVH), arrhythmia, and/or cardiomyopathy [Nakao et al., 1995], whereas patients with the renal variant develop proteinuria and end-stage renal disease (ESRD) [Kotanko et al., 2004] after 50 years of age. In addition, some patients with acute strokes after adolescence were found to have previously undiagnosed Fabry disease, 30% of whom had, retrospectively, classic manifestations. Fabry disease is diagnosed by measuring enzyme activity in white cells or plasma in males. Females can have normal enzyme activity and DNA testing is necessary to confirm or exclude the diagnosis in them.

ERT for Fabry disease was approved in Eng et al. [2001] and clinical trials are ongoing for pharmacologic enzyme enhancement therapy [Desnick and Schuchman, 2002]. The estimated incidence of classic Fabry disease is 1 in 50,000 males. Screening of males in hemodialysis, cardiac, and stroke clinics by determination of plasma Gal A activities detected previously undiagnosed Fabry disease in 0.25–1% of males undergoing hemodialysis, in 3–4% of males with LVH or hypertrophic cardiomyopathy, and in 5% of males with acute cryptogenic strokes [Brouns et al., 2010].

Newborn screening using a fluorometric enzyme assay in 37,104 males in Italy with follow-up mutation analysis identified 1 in 3,100 patients with Fabry disease. The mutations identified in this cohort predicted later-onset rather than classic Fabry disease with an 11:1 ratio [Spada et al., 2006]. In Japan, a newborn screening pilot program for Fabry disease has been carried out by Nakamura et al. (submitted for publication) using the fluorometric enzyme assay and subsequent mutation analysis. The incidence of the disease was approximately 1 in 4,700 males, with 88% of mutations being associated with a later-onset phenotype. In Taiwan [Sands and Davidson, 2006], a newborn screening pilot program for Fabry disease using the fluorometric enzyme assay found an incidence of approximately 1 in 1,250 males [Hwu et al., 2009; Lin et al., 2009]. All these studies suggest that Fabry disease may be underdiagnosed, especially the late-onset variants.

Mucopolysaccharidoses

Mucopolysaccharidoses (MPS) are LSDs that are characterized by the accumulation of glycosaminoglycans (GAGs) in urine, plasma, and various tissues. Primary treatment options for MPS include hematopoietic stem cell transplantation (HSCT) and ERT. ERT is now available for MPS I, MPS II, and MPS VI [Kollmann et al., 2010]. ERT reduces GAG accumulation, improves the clinical status and quality of life. Clinical trials of ERT for other types of MPS are underway.

Newborn screening for these conditions can be accomplished by measuring urinary GAG or directly by measuring enzyme activity in blood spots. Methods have been proposed for the quantification and qualitative evaluation of GAGs in urine by LC-MS/MS. This method can screen for MPS I, II, and VI by quantifying dermatan sulfate (DS) and heparan sulfate (HS) in urine.

In blood spots, eight lysosomal enzymes (α-L-iduronidase, iduronate sulfatase, arylsulfatase B, β-D-glucuronidase, β-D-galactosidase, α-D-mannosidase, α-L-fucosidase, and β-hexosaminidase), including those involved in selected MPSs, can be assayed. This can screen for MPS I, MPS II, MPS VI, MPS VII, GM1 gangliosidosis, galactosialidosis, MPS IV B, α-mannosidosis, fucosidosis, Sandhoff disease, and mucolipidosis II and III [Chamoles et al., 2001a,b, 2004]. Unfortunately, there are still no methods described for multiplexing these assays.

More recently, specific substrates have been developed to allow the use of MS/MS [Duffey et al., 2010a,b]. The advantage of this approach is that it allows multiplexing with simultaneous assays for MPS I, MPS II, MPS IIIA, and MPS VI.

Pompe Disease

Pompe disease, also known as glycogen storage disease type II, is an autosomal recessive disorder caused by deficiency

of the enzyme α-glucosidase (GAA), resulting in the accumulation of lysosomal glycogen in the skeletal muscles and heart [Kishnani et al., 2006]. This disorder causes a steady accumulation of glycogen substrate that leads to progressive muscle damage and organ failure. The rates of substrate accumulation and tissue damage are variable and reflect the residual enzyme activity and immune response to the accumulated material. In 2006, alglucosidase alfa was approved as the ERT for Pompe disease. A pilot program for Pompe disease newborn screening was started in Taiwan in 2005 that measures GAA activity using a fluorometric assay [Chien et al., 2009]. A thorough examination was performed to screen positive newborns. A diagnosis of Pompe disease was made clinically after the onset of symptoms. Screening revealed five severely affected infants with an incidence of approximately 1 in 41,000 screened newborns. ERT for Pompe disease was started in the five severely affected infants. In unscreened infants, the clinical diagnosis of Pompe disease was made later, at an average of 4 months of age. Initiation of earlier treatment of infants after newborn screening resulted in normal cardiac function and growth and acquisition of age appropriate milestones.

Krabbe Disease

Krabbe disease (globoid cell leukodystrophy) is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme galactosylceramide β-galactosidase (GALC). This results in the accumulation of galactosylceramide and psychosine that in most cases cause abnormalities of the brain white matter. Most patients present early in life with an early infantile or "classic" phenotype. Symptoms usually appear before 6 months of age and death occurs before 2 years of age. Other patients can present later in life with an attenuated phenotype. HSCT is the only available treatment for infants with early infantile Krabbe disease and must be performed prior to neurodegeneration. Newborn screening has been performed for Krabbe disease [Duffner et al., 2009]. Newborns treated with HSCT can have progressive central myelination and continued gains in developmental skills and cognitive function, whereas children who undergo transplantation after symptom onset experience minimal neurologic improvement. Transplantation is not effective in all cases of Krabbe disease and some transplanted patients have experienced developmental delays. Screening involves GALC activity detection by a fluorescent assay and subsequent DNA mutation analysis. Molecular analysis of the GALC gene is used for diagnostic confirmation.

THERAPEUTIC ADVANCES FOR LYSOSOMAL STORAGE DISORDERS

Hematopoietic Stem Cell Transplantation

Allogenic HSCT was one of the first therapies attempted in LSDs to introduce metabolic cross-correction. Therapy may also be useful for neurodegenerative LSDs because microglia cells are derived from hematopoietic stem cells [Asheuer et al., 2004; Boelens, 2006]. Clinical trials of HSCT have suggested that cells migrate across the blood–brain barrier. In animal models, it has been shown that donor cells produce the defective enzyme and that donor macrophages replace microglial cells in the brain [Kennedy and Abkowitz, 1997; Malatack et al., 2003]. Repopulation of transplanted cells in the brain is relatively slow because of the long lifespan of microglia [Kennedy and Abkowitz, 1997].

HSCT has shown efficacy in presymptomatic or mildly affected patients with some LSDs. It has been used in patients with MPS I, II, and VI; Gaucher disease; Wolman disease; metachromatic leukodystrophy; and Krabbe disease. Each LSD responds differently to HSCT, and transplantation timing relative to symptom onset seems critical for some disorders. HSCT is not effective for the patients with Fabry disease because secreted α-galactosidase lacks mannose-6-phosphate residues and the enzyme is seldom taken up by cells with the enzyme defect. Complications after HSCT are common and limit the usefulness of this treatment. These include graft versus host disease, toxicity of the conditioning regimen, and graft failure.

In addition to HSCT, transplantation of neural stem cells to the brain has been performed in an animal model for LSDs. This was first demonstrated in an MPS VII mouse model by injection of neural stem cells overexpressing β-glucuronidase into the ventricles of newborn mice [Snyder et al., 1995]. Clinical improvement has been observed after neural stem cell transplantation in animal models [Lee et al., 2007; Strazza et al., 2009]. There are no human data for this type of therapy.

Enzyme Replacement Therapy

Marked progress has been made in the treatment of LSDs over the past few decades [Brady et al., 1974; Achord et al., 1978; Brady, 2006]. Recombinant DNA techniques have allowed production of lysosomal enzymes in vitro. The recombinant enzymes are transported via the mannose-6 receptor pathway in Fabry disease, MPS I, II, and VI; and Pompe disease. In contrast,

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they are transported by macrophage mannose receptors in Gaucher disease. The exogenous enzymes are internalized by somatic cells and transferred to the lysosome where they degrade accumulated substrate and diminish the burden of the disease. ERT has been approved by regulatory agencies for Gaucher, Fabry, and Pompe disease in addition to MPS I, II, and VI. Before the introduction of ERT, no specific therapy was available for LSD patients, and supportive care and treatment were used only to manage complications. ERT consists in the regular administration of recombinant enzyme intravenously and since its success in patients with Gaucher disease, was extended to other LSDs. Clinical trials have demonstrated the clinical benefit of ERT in Fabry disease [Eng et al., 2001]; MPS I [Kakkis et al., 2001], II [Muenzer et al., 2006], and VI [Harmatz et al., 2005]; and in Pompe disease [Amalfitano et al., 2001].

The usefulness of ERT is limited because the enzyme is not always effective for all clinical symptoms. Clinical studies have shown that many symptoms of LSDs are irreversible in advanced cases despite the use of long-term ERT. Therefore, early diagnosis and treatment is important. In addition, recombinant proteins cannot cross the blood–brain barrier, and ERT has little or no effect on central nervous system (CNS) manifestations. Current clinical trials are assessing the effect of intrathecal enzyme replacement in MPS I and II.

Substrate Reduction Therapy

Substrate reduction therapy partially inhibits the biosynthesis of the accumulated product to reduce substrate influx into the catabolically compromised lysosome. A small-molecule oral substrate reduction therapy, miglustat, is available for Gaucher disease. The efficacy of substrate reduction therapy was evaluated in patients with Gaucher disease [Cox et al., 2000]. Adult Gaucher patients not treated with ERT were treated with *N*-butyldeoxynojirimycin for 12 months. Mean liver and spleen volumes were significantly decreased, and hematological parameters showed slight improvement. The most frequent adverse effect was diarrhea. In the extension study, statistically significant improvement was achieved in all major efficacy end points, indicating that treatment with *N*-butyldeoxynojirimycin was increasingly effective with time [Elstein et al., 2004]. The use of *N*-butyldeoxynojirimycin, known as miglustat (Zavesca), has been approved for Gaucher disease and is considered safe for adult patients, with mild or moderate symptoms, who are unwilling or unable to receive or to continue ERT or for patients with persistent signs of disabling disease activity despite maximal enzyme dosing. The drug may be applied in combination with ERT in these patients.

N-Butyldeoxynojirimycin is also considered an option for patients with Sandhoff disease, Tay–Sachs disease, or Niemann–Pick disease type C (NPC) because the drug is a small enough to cross the blood–brain barrier [Lachmann et al., 2004]. The drug is usually given at higher doses than in Gaucher disease to allow increased entry into the brain. A randomized clinical trial in patients with NPC demonstrated that miglustat improves or stabilizes horizontal saccadic eye movement velocity, a clinically relevant markers of NPC, with improvement in swallowing capacity, stable auditory acuity, and a slower deterioration in ambulatory index [Patterson et al., 2007; Wraith et al., 2010]. An open-label extension confirmed the persistence of clinical benefit that is more marked in patients with milder forms of the disease A lower dose of this drug was not effective in late-onset Tay Sachs [Shapiro et al., 2009]. Nevertheless, further developments in this area have the potential of developing effective an treatment for this condition.

Chemical Chaperons

Chemical chaperones can enhance the residual activity of the defective lysosomal enzyme. Imino sugars, such as deoxynojirimycin can act as both enzyme inhibitors and chaperones, which control the quality of newly synthesized proteins [Sawkar et al., 2002; Fan, 2008]. Under physiological conditions, chaperones help restore the native conformation of misfolded proteins. Chaperone therapy by using small molecules to stabilize and target a misfolded enzyme to the lysosome is in clinical trial for Gaucher, Fabry, and Pompe diseases caused by mutated but catalytically active enzymes. In animal models, these small molecules cross the blood–brain barrier and may be effective for CNS manifestations of LSDs. *N*-(*n*-nonyl)deoxynojirimycin for Gaucher disease and 1-deoxygalactonojirimycin for Fabry disease are good examples of chemical chaperones that show satisfactory response in vitro [Sawkar et al., 2002; Yam et al., 2005]. A similar effect was observed in fibroblasts from adult patients with Tay–Sachs disease and Sandhoff disease [Tropak et al., 2004]. Chemical chaperones may be therapeutically useful for treatment of various LSDs, although they are currently experimental and none is approved for the treatment of any LSD.

Gene Therapy

Many LSDs respond to HSCT and are excellent candidates for gene transfer therapy [Sands and Davidson, 2006], since they are generally well-characterized single gene disorders, the enzymes defective are usually not subject to complex regulation mechanisms, and enzyme activity even only a little higher than normal should be clinically sufficient. In vivo and ex vivo gene therapy techniques have been developed to administer the gene to defective organs in LSD animal models via the bloodstream or directly to the brain. Gene therapy using adenoassociated viral (AAV) or lentiviral vectors has been tested in small animal models of LSDs and resulted in normalized enzyme activity [Cachon-Gonzalez et al., 2006; Broekman et al., 2007]. However, gene therapy was initiated before the appearance of clinical symptoms in these studies. Testing in large animal models of LSDs is under current study [Haskins, 2009]. After intracerebral injection of

AAV-encoding human arylsulfatase A (ASA) into nonhuman primates, ASA expression could be detected [Colle et al., 2010]. The wide distribution of enzyme expression appears to be mediated by axonal transport and secretion by transduced neurons. At present, gene therapy in humans with their much larger brains has yet to be initiated.

SCREENING FOR DISEASES

Newborn Screening

Newborn screening for metabolic disorders started with Robert Guthrie's study of phenylketonuria (PKU) in the early 1960s. After demonstration that early diagnosis and therapy could prevent mental retardation in PKU, neonatal screening has become routine practice in developed countries as part of a public health program [Guthrie and Susi, 1963; Scriver and Kaufman, 2001]. Newborn screening identifies a high-risk group of patients from normal infants and then thoroughly investigate this group. Initial tests screened for one disorder at a time. The introduction of screening by tandem mass spectrometry permits the measurement of multiple analytes at the same time, allowing the detection of multiple classes of metabolic disorders.

The potential use of MS/MS for newborn screening was first suggested in 1990 [Millington et al., 1990], and early studies soon demonstrated its practicality [Chace et al., 1993; Rashed et al., 1995; Ziadeh et al., 1995]. MS/MS could simultaneously detect a number of disorders, making it possible to screen for some disorders that might otherwise have seemed too rare. Many compounds are initially separated by mass to charge ratio in MS/MS. Each compound is then fragmented for identification. The process requires roughly 2 min per sample and can detect 30 or more inborn errors of metabolism just screening for amino acids and acylcarnitines. At the present time, expanded screening is used to detect disorders of amino acid, organic acid, and fatty acid metabolism. However, the technology can be applied to a much wider range of compounds, and the field appears ready to expand. Table I summarizes the enzymes defective in several LSDs and those for which newborn screening assays have been developed.

Advances in Newborn Screening Technologies for LSD

Enzymatic assays. The initial system to diagnose LSD was the measurement of enzyme activity using a fluorescent artificial substrate [Meikle et al., 2006]. Diagnosis of MPS I is performed on leukocyte or cultured fibroblast homogenates to assay α-L-iduronidase activity by using 4-methyhylumbeliferyl-L-iduronide. For newborn screening, the standard method was adapted to measure α-L-iduronidase activity in dried blood spotted on filter paper [Chamoles et al., 2001a]. A 3-mm-diameter punchout of a blood spot on filter paper is added to elution buffer containing 4-methyhylumbeliferyl-α-L-iduronide as the substrate. Fluorescence of the enzyme product 4-methylumbelliferone is then measured. Methods for detection of other LSD, including MPS II, Pompe, Fabry, Sandhoff, Gaucher, Niemann–Pick (type A/B, not C), and Tay–Sachs diseases have been reported using the revised enzymatic assay of dried blood spot samples [Chamoles et al., 2001b, 2004]. The limitation of these approaches is that each assay uses 4-methylumbelliferone as an indicator of enzyme activity. In these assays, multiplexing is not possible because all assays (for MPS I, MPS II, Pompe, Fabry, Sandhoff, Gaucher, Niemann–Pick, and Tay–Sachs diseases) yield the same product (4-methylumbelliferone) as the fluorescent product of the enzyme reaction.

A variation of this approach includes the use of antibodies to enrich for the enzyme to be tested. In the case of Pompe disease, antibodies against GAA are used to coat microtiter plates. The endogenous GAA from the dried blood spots is eluted, attaches to the antibodies and is assayed for enzyme activity using fluorescent substrate [Umapathysivam et al., 2000]. Hypothetically, microtiter plates could be coated with several different primary antibodies to capture different endogenous enzymes. However, if all of the substrates produce the same fluorescent enzyme product (4-methylumbelliferone), then multiplexing is not possible. These limitations would work against practical newborn screening using this method.

Functional Detection of Enzymatic Products by Using MS/MS

The second advancement in LSD screening technology involves analyzing the activity of endogenous lysosomal enzymes with electrospray ionization-MS/MS [Gerber et al., 2001; Li et al., 2004]. This method, modified from the one for cell lysates for use with dried blood spots, was used in Krabbe disease to detect galactocerebroside β-galactosidase (GALC) activity. The substrate β-Gal-C8-Ccr is broken down by GALC to C8-Cer by the enzyme eluted from the dried blood spots. Both C8-Cer and C10-Cer, which is used as an internal standard, are quantified using MS/MS to detect GALC activity. The GALC enzyme on the dried blood spots is stable, allowing for sample transportation. A pilot program for Krabbe disease screening using MS/MS was started in 2006 [Orsini et al., 2009]. Out of 555,000 newborns, 10 were identified at risk for Krabbe disease. MS/MS has the advantage of being able to detect products of different mass to change ratio enabling the analysis of the results of different enzyme reactions. In theory, multiplexed assays can be developed for multiple diseases, including Pompe, Fabry, Gaucher, Niemann–Pick types A/B (NP A/B), Krabbe disease, and MPS-I [Zhang et al., 2008] and for five of them a multiplex assay has been proposed [Gelb et al., 2006]. In reality, the amount of activity measurable in a single blood spot is still limited. The assay for Pompe, Fabry and MPS-I can already be performed on the same blood spot [Duffey et al., 2010a]. MS/MS assays for blood spots have also been reported for MPS-VI [Duffey et al.,

TABLE I. Lysosomal Storage Disorders Amenable to Newborn Screening

Disease	Protein defect	Availability of screening strategies	Chromosomal localization	OMIM
Defects in glycosaminoglycan degradation (mucopolysaccharidoses)				
MPS I (Hurler, Scheie)	α-Iduronidase	Fluorometric, immune-quantification, multiplex	4p16.3	607015
MPS II (Hunter)	Iduronate sulfatase	Fluorometric, immune-quantification, multiplex	Xq28	309900
MPS IIIA (Sanfflippo A)	Heparan *N*-sulfatase	Immune-quantification, multiplex	17q25.3	252900
MPS IIIB (Sanfflippo B)	*N*-Acetylglucosaminidase	None	17q21	252910
MPS IIIC (Sanfflippo C)	Acetyl-CoA transferase	None	8p11.1	252930
MPS IIID (Sanfflippo D)	*N*-Acetylglucosamine-6-sulfatase	None	12q14	252940
MPS IVA (Morquio A)	*N*-Acetylgalactosamine-6-sulfatase	None	16q24.3	253000
MPS IVB (Morquio B9)	β-Galactosidase	None	3p21.33	230500
MPS VI (Maroteaux–Lamy)	*N*-Acetylgalactosamine-4-sulfatase	Fluorometric, MS/MS, immune-quantification, multiplex	5q11–13	253200
MPS IX	Hyaluronidase	None	3p21.3	601492
Defects in glycoprotein degradation (oligosaccharidoses)				
α-Mannosidosis	α-Mannosidase	None	19q12	248500
β-Mannosidosis	β-Mannosidase	None	4q22	248510
α-Fucosidosis	α-Fucosidase	None	1q34	230000
Sialidosis	α-Sialidase	None	6p21.3	608272
Galactosialidosis	Cathepsin A	None	20q13.1	256540
Aspartylglucosaminuria	Aspartylglucosaminidase	None	4q32	208400
Schindler disease, Kanzaki disease	α-Acetylglucosaminidase	None	22q13.1	104170
Others				
GM1-gangliosidosis	β-Galactosidase	None	3p21.33	230500
GM2-gangliosidosis (Tay–Sachs)	α-Subunit of β-hexosaminidase	Fluorometric	15q23	606869
GM2-gangliosidosis (Sandhoff)	β-Subunit of β-hexosaminidase	Fluorometric	5q13	606873
GM2-gangliosidosis (variant AB)	GM2 activator protein	None	5q31	272750
Gaucher disease	β-Glucocerebrosidase	Fluorometric, MS/MS, immune-quantification, multiplex	1q21	606463
Fabry disease	α-Galactosidase	Fluorometric, MS/MS, immune-quantification, multiplex	Xq22.1	301500
Pompe disease	Acid α-glucosidase	Fluorometric, MS/MS, immune-quantification, multiplex	17q25.2–q25.3	232300
Niemann–Pick type A and B	Sphingomyelinase	Fluorometric, MS/MS, immune-quantification, multiplex	11p15.2	607808
Krabbe disease	Galactosylceramidase	Fluorometric, MS/MS, immune-quantification, multiplex	14q31	245200

2010b] and Gaucher disease [Legini et al., 2011]. One issue with newborn screening is the identification of patients whose phenotype is not clear. For example, most patients identified by screening for Fabry disease have late-onset variants [Spada et al., 2006] and it is unclear whether they would have had clinical symptoms without treatment.

SUMMARY

Newborn screening is a major public health achievement that has improved the morbidity and mortality of inborn

errors of metabolism. The introduction of newborn screening for LSDs presents new challenges. The first is to be able to design a multiplex assay for multiple enzymes applicable to the limited amount of enzyme present in blood spots. These new assays must be validated in large numbers of newborns to confirm sensitivity and specificity. The second challenge is to have a better understanding of which forms of these diseases need treatment. This will allow us to determine if and when to start therapeutic interventions. In the absence of a family history, presymptomatic detection of an LSD can be achieved only through a newborn screening program. The efficacy and cost of the currently available therapies and the detection in newborns of diseases with later onset, often in adulthood, may raise ethical issues. The advancement of therapeutic options for treatment of LSD, especially in the field of small molecules, capable of entering he brain offers new hopes to affected patients in whom a timely diagnosis will become even more essential.

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幼児期に繰り返す嘔吐発作で発症したメチルマロン酸血症の同胞例

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要　旨

乳児期に全く症状を認めず，幼児期に繰り返す嘔吐発作，意識障害で発症したメチルマロン酸血症（以下MMA）の同胞例を経験した．妹は1歳時より嘔吐発作を繰り返しており，3回目の嘔吐発作時に尿有機酸分析を行いMMAと診断された．姉は3歳時より嘔吐発作を繰り返し，周期性嘔吐症として経過観察されていたが，妹がMMAと診断されたことを契機に精査を行い化学的にMMAと診断された．さらに，メチルマロニルCoAムターゼ遺伝子解析にて，p.G380E/p.G648Dの複合ヘテロ接合変異を同定した．感染等のストレス時に嘔吐発作を繰り返す症例の中には，診断に至っていないMMA例が潜在している可能性がある．

キーワード：メチルマロン酸血症，嘔吐発作，メチルマロニルCoAムターゼ遺伝子

はじめに

メチルマロン酸血症（methylmalonic acidemia：以下MMA）はメチルマロニルCoAムターゼ活性の低下により体内にメチルマロン酸を中心とする有機酸が蓄積する遺伝性疾患である．MMAは新生児期より嘔吐，体重増加不良，筋緊張低下，昏睡などの症状で発症することが多いとされている．しかし，近年タンデム質量分析などの検査が普及してきたことにより，感染や飢餓などを契機に間欠的に代謝性アシドーシスなどの発作を呈する軽症例の存在が明らかにされつつある．我々は乳児期には異常を認めず，幼児期より嘔吐発作を繰り返したMMAの同胞例を経験したので報告する．

症　例

症例1：1歳8か月，女児，第3子

主訴：意識障害，嘔吐

周産期歴：39週3日，3,316gにて仮死なく出生．

既往歴：1歳0か月時，1歳7か月時に意識障害を伴う嘔吐発作のため入院加療を要した．いずれも2～3日の輸液で軽快した．

家族歴：長姉（6歳）が周期性嘔吐症

現病歴：200X年12月31日より咳嗽，不機嫌を認め，翌年1月1日より食欲低下，1月2日に嘔吐を繰り返すようになったため近医受診した．内服処方を受け一旦帰宅したが，その後もぐったりとしていたため1月3日にA病院受診．受診時，意識障害（JCSIII-300）があり，血液検査にて低血糖，代謝性アシドーシスを認めたため入院となった．

入院時現症：身長79.0cm（－0.8SD），体重10.6kg（＋0.1SD）と身体発育に異常を認めなかった．心音，整，心雑音なし．肺にラ音を聴取した．腹部は平坦，軟であり肝脾腫は認めなかった．

入院時検査所見（表1）：WBC（23,380/μL），CRP（3.7mg/dl）の上昇を認めた．血液ガス検査（静脈血）にてpH7.183，BE－19.7mmol/L，HCO_3^- 6.7mmol/Lと代謝性アシドーシスを認めた．また高アンモニア血症（162μg/dl），低血糖（30mg/dl台），BUN異常高値（39mg/dl）も認めた．尿検査ではケトン（3＋）であった．髄液検査に異常を認めなかった．

入院後の経過：入院後，低血糖，代謝性アシドーシスに対し，20%ブドウ糖投与，重炭酸ナトリウムによる補正を行ったところ低血糖，代謝性アシドーシスの改善を認めた．輸液による加療を継続したところ，意識障害は徐々に改善し，同日中にJCSII-20，翌1月4日には意識は清明となった．意識障害の原因検索のため頭部MRI，脳波検査施行したが，器質的疾患や脳炎，脳症等を疑わせる所見を認めなかった．後遺症は認めず，1月7日に軽快退院となった．

既往歴，急性期の低血糖，代謝性アシドーシス，高アンモニア血症から代謝異常症を疑い，発作時検体を

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表1　症例1 入院時（発作時）検査所見

［静脈血ガス］		［血液生化学］	
pH	7.183	TP	6.3 g/dL
PCO_2	17.9 mmHg	Alb	4.1 g/dL
B.E.	43.2 mmol/L	AST	43 IU/L
HCO_3^-	6.7 mmol/L	ALT	16 IU/L
		LDH	338 IU/L
［血液一般］		T.Bil	0.3 mg/dL
WBC	23,380 /μL	BUN	39 mg/dL
RBC	355 万 /μL	Cre	0.45 mg/dL
Hb	9.8 g/dL	Glu	30 台 mg/dL
Ht	30.8 %	Na	132 mEq/L
Plt	32.6 万 /μl	K	5.4 mEq/L
		Cl	100 mEq/L
［血清］		Ca	8.1 mg/dL
CRP	3.7 mg/dL	アンモニア	162 μg/dL
［尿一般］（輸液開始後）		［髄液検査］	
比重	1.019	細胞数	1/3
潜血	（－）	タンパク	15 mg/dL
タンパク	（－）	糖	185 mg/dL
糖	（3＋）		
ケトン	（3＋）		

用いて尿有機酸分析を行った．その結果，メチルマロン酸，メチルクエン酸，3-ヒドロキシプロピオン酸の著明な排泄増加，乳酸・ピルビン酸の排泄増加，およびケトーシスの存在を示す3-ヒドロキシ酪酸やアセト酢酸の著明な排泄増加を認め（図1（a）），MMAと化学診断した．

退院後，非発作時に行った尿有機酸分析では，メチルマロン酸，メチルクエン酸，3-ヒドロキシプロピオン酸の排泄増加を認めたが，アシドーシスやケトーシスを示す代謝産物は認めなかった（図1（b））．また，濾紙血を用いたタンデム質量分析ではプロピオニルカルニチン（C3）/アシルカルニチン（C2）比0.35（カットオフ値0.22），プロピオニルカルニチン7.74μM（カットオフ値5.25）と上昇あり，MMAに特徴的な所見を認めた（表2）．

状態が回復した後，ビタミンB12に対する反応性の有無を検討するために，ビタミンB12（一般名：コバマミド，商品名：ハイコバール）投与前後の尿有機酸分析を行った．その結果，メチルマロン酸，メチルクエン酸の排泄の減少を認めた（表3）．しかし，ビタミンB12投与前のメチルマロン酸排泄量が13,814.4μmol/mol・Creatinineと著明高値であり，ビタミンB12投与後も4,628.3μmol/mol・Creatinineと1,000を下回っていないことから，ビタミンB12に対してある程度の反応はあるものの，その効果は乏しく，ビタミンB12非依存型MMAと考えられた．

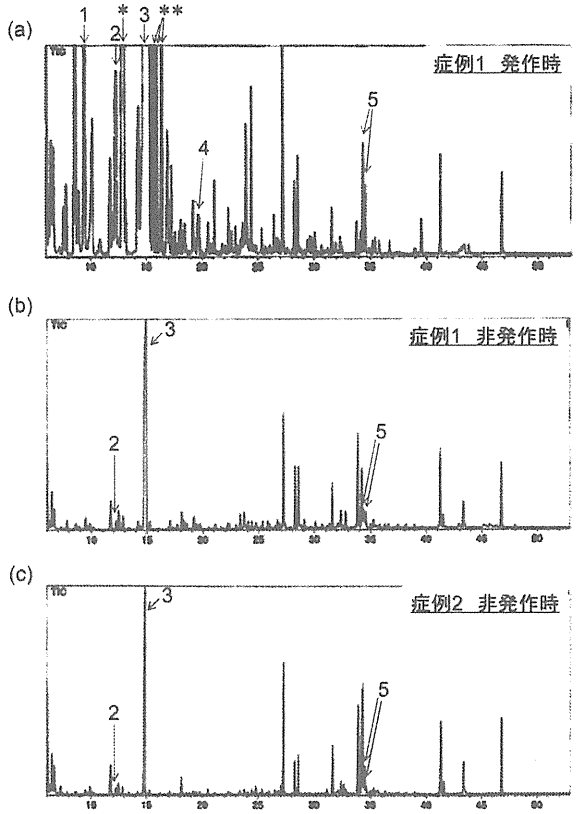

図1　尿有機酸分析

（a）症例1，発作時
1）乳酸，2）3-ヒドロキシプロピオン酸，3）メチルマロン酸，4）プロピオニルグリシン，5）メチルクエン酸などの著明な排泄増加を認める．また，ケトーシスを示す，3-ヒドロキシ酪酸（＊），アセト酢酸（＊＊）の著明な排泄増加を認める．
（b）症例1，非発作時
2）3-ヒドロキシプロピオン酸，3）メチルマロン酸，5）メチルクエン酸の排泄増加を認める．
（c）症例2，非発作時
2）3-ヒドロキシプロピオン酸，3）メチルマロン酸，5）メチルクエン酸の排泄増加を認める．

表2　タンデム質量分析

	症例1（非発作時）	症例2（非発作時）	カットオフ値
C3（μM）	7.74	7.98	＜5.25
C3/C2比	0.35	0.46	＜0.22
C0（μM）	23.45	18.23	10～60

C3：プロピオニルカルニチン
C2：アセチルカルニチン
C0：遊離カルニチン

MMAと診断後，食事療法によるタンパク摂取制限は行わず，カルニチン内服を開始したところ，嘔吐発作なく経過している．

表3　ビタミンB12投与前後の尿有機酸分析

	投与前	投与後
メチルマロン酸	13,814.4	4,628.3
メチルクエン酸-1	73.1	46.2
メチルクエン酸-2	54.1	31.7

（単位：μmol/mol・Creatinine）

症例2：6歳1か月，女児，第1子

主訴：嘔吐発作の精査

周産期歴：36週5日，2,216g，常位胎盤早期剝離のため緊急帝王切開にて出生．仮死は認めず，その後の発達も順調であった．

既往歴：3歳0か月時より嘔吐発作を6回繰り返し，入院や外来での治療を要した．毎回，輸液のみで軽快し周期性嘔吐症と診断されていた．

家族歴：妹（症例1）がMMA

現病歴：6歳1か月時，妹がMMAと診断された．3歳時より入院の上，持続点滴を必要とする嘔吐発作を繰り返し認めていたため，同疾患を疑い精査を行った．

身体所見：身長117.7cm（+0.9SD），体重19.3kg（−0.1SD）．心音・肺音に異常認めず，腹部所見も異常を認めなかった．

検査所見：尿有機酸分析ではメチルマロン酸，メチルクエン酸，3-ヒドロキシプロピオン酸の排泄増加を認め（図1（c）），MMAと化学診断した．濾紙血を用いたタンデム質量分析においても，プロピオニルカルニチン/アシルカルニチン比0.46，プロピオニルカルニチン7.98μMと上昇しており，MMAに特徴的な所見を認めた（表2）．

遺伝子解析結果：症例1において，ビタミンB12非依存型MMAと診断したことから，メチルマロニルCoAムターゼ異常症を疑いメチルマロニルCoAムターゼ遺伝子解析を行った．その結果，症例1，2のいずれにもc.1139G>A（p.G380E）とc.1943G>A（p.G648D）の複合ヘテロ接合変異を同定し，メチルマロニルCoAムターゼ異常症によるMMAと診断した．また両親の解析も行ったところ，父にはc.1139G>A（p.G380E）のヘテロ接合変異を，母にはc.1943G>A（p.G648D）のヘテロ接合変異をそれぞれ同定し，両親は保因者であった．

考　察

MMAはメチルマロニルCoAムターゼ活性の異常により発症する遺伝性疾患であり，アポ酵素であるメチルマロニルCoAムターゼ異常症と，メチルマロニルCoAムターゼの補酵素であるビタミンB12の代謝異常症に分類される．さらに，メチルマロニルCoAムターゼ異常症は，ムターゼ活性を認めない完全欠損症（mut^0）と，ムターゼ活性がある程度残存している部分欠損症（mut^-）とに細分される．臨床病型としては，新生児・乳児期早期から急激な経過で発症し，死亡率が高く神経学的予後が不良である重症型と，乳幼児期以降に発症する軽症型に分類され，mut^0の多くは重症型であり，mut^-は軽症型である[1)2)]．近年，「普段は正常だが，感染や飢餓などの際に間欠的に代謝不全に陥り，急性脳症様症状をきたした例」や，「繰り返す嘔吐発作を契機に診断された例」が報告されており，軽症型MMAは多彩な症状を呈することが明らかにされつつある[3)〜6)]．

本邦におけるMMAの頻度は，これまで約1/20万人と考えられていたが，濾紙血を用いたタンデム質量分析によるマススクリーニングの結果から，約1/9.6万人であるとの報告がなされている[7)]．これは無症状で経過している例，あるいは急性脳症や周期性嘔吐症として加療され，MMAの診断に至っていない例が潜在する可能性を示唆している．症例1では，意識障害を伴う嘔吐発作を3回繰り返していたが，初めの2回の発作では診断に至らなかった．また症例2も，3歳から嘔吐発作を繰り返していたが，周期性嘔吐症として加療されており，妹がMMAと診断されたことを契機としてMMAの診断に至った．繰り返す嘔吐発作を認めた場合は，MMAなどの代謝異常症を鑑別診断に入れて，血糖，アンモニア，血液ガスを測定し，さらにタンデム質量分析や尿有機酸分析を行うことが診断するうえで重要である．また，自験例では，診断確定後，カルニチン内服や長期間の飢餓を避けるなどの生活指導を行うことによって，嘔吐発作は認めていない．診断を確定することによって，カルニチン内服や感染などのストレス時の適切な処置を行い，代謝不全の発症あるいは増悪の予防が可能であった．

MMA症例の中には，ビタミンB12代謝異常症のみならず，メチルマロニルCoAムターゼ異常症であっても，その補酵素であるビタミンB12に対して反応性を示す場合がある[8)9)]．自験例（症例1）においてビタミンB12への反応性を評価したところ，ビタミンB12投与によって尿中メチルマロン酸濃度は13,814.4μmol/mmol・Creatinineから4,628.3μmol/mmol・Creatinineへと低下した．

これまで，ビタミンB12投与によってメチルマロン酸濃度が1,000μmol/mmol・Creatinine以下に低下した場合や，50%以上低下した場合などに，ビタミンB12に対する反応性があるとされてきた．しかしながら，これらの基準では真の反応を反映しているかどうか判断できないという議論もあり，現時点では，MMAにおけるビタミンB12への反応性に関する定義はま

だ確立されていない．また，メチルマロン酸濃度が10,000μmol/mmol・Creatinine以上を呈するMMA患者では，ビタミンB12への反応性を認める例は非常にまれではあるという報告もある[10]．これらの報告を基に症例1におけるビタミンB12への反応性について検討した結果，投与前の尿中メチルマロン酸濃度が10,000μmol/mmol・Creatinine以上の著明高値であり，投与後も1,000μmol/mmol・Creatinineを下回らないことから，ビタミンB12に対してある程度の反応性はあるものの，臨床的にはその効果は乏しいと考えた．

メチルマロニルCoAムターゼはメチルマロニルCoAムターゼ遺伝子によりコードされている．この遺伝子は6p21に存在し，13のエクソンからなる．MMAの原因となる変異は，これまで100以上も同定されており，本症例で認めたc.1943G>A（p.G648D）変異は本邦における複数の家系で同定されている．このc.1943G>A（p.G648D）変異をヘテロ接合変異でもつ患者は，mut⁻と報告されており，本症例の臨床症状と一致する[11]．一方，c.1139G>A（p.G380E）は現在まで報告のない新規の変異である．メチルマロニルCoAムターゼは2つのサブユニットからなり，各サブユニットは基質の結合部位であるβ/α barrelと，ビタミンB12結合部位の2つのドメインをもつ．p.G380EはN末端のβ/α barrelドメインに位置するミスセンス変異であるが，この近傍にはβ/αドメインの構造を変化させると考えられているp.A377Eというミスセンス変異が存在する[12]．またp.A377Eを保有するMMA患者の表現型はmut⁰となることが報告されている[13]．これらのことよりp.G380E変異はβ/αドメインの構造の変化に関与し，疾患の原因となる酵素活性低下をきたす変異であると結論した．

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