used non-invasive positive pressure ventilation (NIPPV). One patient who used NIPPV died after 42 years of disease duration from respiratory failure.

DISCUSSION

Three mutations (c.2997G>T (p.Trp999Cys), c.1566C>G (p.Tyr522X) and c.4497delT) were relatively more prevalent in the present Japanese patients with LGMD2B. Also, in Japanese patients with MM, these three mutations were relatively more prevalent in our previous study.[13] We identified the c.3373delG mutation that occurred with high frequency in Japanese patients with MM, as well as these three mutations,[13] in only one allele in Japanese patients with LGMD2B.

In MM, patients with the c.2997G>T (p.Trp999Cys) mutation had a significantly late onset.[13] Tagawa *et al*[6] reported that age of onset in patients homozygous for the c.2997G>T (p.Trp999Cys) mutation was later than the third decade of life. In this study, especially in patients homozygous for the c.2997G>T (p.Trp999Cys) mutation, onset was significantly late. Only 10% of patients homozygous for the c.2997G>T (p.Trp999Cys) mutation developed muscle symptoms by 30 years. In contrast, 90% of patients without this mutation developed muscle symptoms by the same age. However, it is difficult to explain why the c.2997G>T (p.Trp999Cys) mutation is related to late onset forms. In an immunohistochemical analysis by Tagawa *et al*,[6] although one patient with the homozygous c.2997G>T mutation (p.Trp999Cys) showed an 'abnormal' pattern, cytoplasmic accumulation of immunopositive material with deficiency of membrane staining or positive/negative mosaic membrane staining, three patients with the same mutation showed a negative pattern. Guglieri *et al*[21] reported that patients carrying two truncating mutations showed the first muscular symptoms earlier in life than subjects harbouring double missense substitutions. Indeed, the c.2997G>T mutation is theoretically deduced to be a missense mutation (p.Trp999Cys). Meanwhile, in this study, there were only two patients carrying missense mutations other than the c.2997G>T (p.Trp999Cys) mutation. Thus it is difficult to discuss statistically whether an association between the c.2997G>T (p.Trp999Cys) mutation and late onset forms is due to a missense mutation or other inherent characteristics. Nonetheless, it is important that the late onset phenotype is found with prevalent mutations.

Although patients homozygous for the c.2997G>T (p.Trp999Cys) mutation had late onset, no difference in progression was observed in those harbouring the c.2997G>T (p.Trp999Cys) mutation, except for difficulty in standing on tiptoe. This suggests that the c.2997G>T (p.Trp999Cys) mutation is related to late onset but not to the slow progression of the disease. Furthermore, the c.2997G>T (p.Trp999Cys) mutation may be relevant to the proximal dominant impairment in dysferlinopathy, diagnosed as the limb girdle type. Interestingly, there was only one patient homozygous for the c.2997G>T (p.Trp999Cys) mutation in 27 families with MM.[13] Moreover, patients homozygous for the c.2997G>T (p.Trp999Cys) mutation had a high probability that the muscle weakness started in the upper limbs. In addition, serum CK levels in patients homozygous for the c.2997G>T (p.Trp999Cys) mutation were lower than those in the other two groups.

We investigated the clinical features of 40 patients in 36 families with LGMD2B in whom dysferlin mutations were confirmed. Disease duration was long (26.6±9.9 years) in this study. The clinical features of LGMD2B in this study were as follows: (1) onset in the late teens or early adulthood except in patients homozygous for the c.2997G>T (p.Trp999Cys) mutation; (2) lower limb weakness at onset in most patients; (3) distal change of lower limbs on muscle CT in the early stage; (4) impairment of lumbar erector spinal muscles on muscle CT in the early stage; (5) predominant involvement of the proximal upper limbs; (6) preservation of function of the hands in late stage; (7) preservation of strength in the neck muscles in late stage; (8) lack of facial weakness or dysphagia; (9) avoidance of scoliosis; (10) hyper-Ckaemia; (11) preservation of cardiac function; and (12) tendency for respiratory function to decline with disease duration and occasional necessity for ventilatory assistance.

Age at onset was similar to that reported for various types of mutations.[18 19 21] However, patients with disease onset at 73 years[24 29] or congenital onset[30] have been reported. Clinicians may not have taken into account analyses of the dysferlin gene for such patients.

Nishida *et al* proposed the name 'distal limb girdle type muscular dystrophy' for patients with MM that develop proximal muscle involvement relatively early.[31 32] Nguyen *et al*[17] classified patients for whom it was not possible to distinguish between a distal phenotype of MM and a limb girdle phenotype, even when examined at onset, as a distinct 'proximodistal' phenotype group. Although patients with the typical features of MM[13 33] or asymptomatic hyper-CKaemia were excluded from the present study, variable patterns of weakness in the lower limbs were observed, as in previous studies.[17 19 20 22 24] However, the predominant muscle weakness was in proximal sites of the upper limbs in the present study.

The results of muscle CT scan in this study were similar to those of detailed imaging studies in LGMD2B at a relatively early stage.[23 34 35] In this study, we had only one case in whom a low density change in the dorsal muscle in the neck region occurred in the early stage. In contrast, two patients showed fatty muscle degeneration of the cervical elector spinae muscles after 9 or 10 years of disease duration, in a study performed using 3.0 T MRI.[35] Another exceptional finding was a case in which the quadriceps femoris muscles were more severely damaged than the hamstrings, which was reported in two of five patients.[34]

In the present study, only two patients showed levels of EF that were <50% and the patient who showed the lowest EF (32.7%) had no symptoms or signs of cardiomyopathy.[36] Guglieri *et al*[21] reported cardiac rhythm changes in three patients and left ventricular hypertrophy in four patients from a total of 22 LGMD2B patients. Wenzel *et al*[37] reported ECG abnormalities (repolarisation abnormalities or left ventricular hypertrophy) in four patients and pathological echocardiographic parameters in five of seven LGMD2B patients. Furthermore, two patients had symptoms and signs of dilated cardiomyopathy. Choi *et al*[38] reported left ventricular hypertrophy on ECG in two patients, no cardiac signs in one patient and mildly decreased EF (45%) in one patient among five MM patients. Therefore, we think that in most patients with LGMD2B, in spite of the existence of laboratorial abnormalities, cardiac function was clinically preserved.

Mahjneh *et al*[16] reported that patients with LGMD2B with disease durations of up to 7 years might show slight restrictive lung disease. Cagliani *et al*[39] reported that respiratory tests showed mild obstructive signs at the small airways in a 20-year-old man with LGMD2B. Illa *et al*[12] reported that pulmonary function tests revealed a mild reduction in VC in two

patients with DACM. In this study, many patients showed pathological levels of respiratory parameters and levels of %VC decreased with disease duration. Furthermore, some patients needed NIPPV and one patient died of respiratory failure. The change in muscles related to respiratory function worsens with disease duration. Therefore, it is important to pay attention to respiratory function in patients with dysferlinopathy.

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of 12 autopsy subjects with infantile polyarteritis nodosa,[13,15] now considered indistinguishable from KD. Although scrotal redness and tenderness are important signs of testicular torsion, careful observation should be made to avoid unnecessary surgical exploration. Color Doppler imaging and radionuclide testicular scanning may be helpful to differentiate epididymo-orchitis or hydrocele from testicular torsion. A similar suspected pathogenesis was discussed in a report on acute scrotum in Henoch–Shönlein purpura; acute scrotum is a relatively well-known complication of this disease.[12]

Five of the 10 reviewed patients and the two present patients had edema of the extremities. Although data on serum albumin level were available only for the present two patients, both patients had low serum albumin. This suggested an association between increased vascular permeability in acute phase of the disease and acute scrotum. The present two patients had acute scrotum after diagnosis of KD. In contrast, eight of 10 reviewed patients had acute scrotum on admission or before diagnosis, suggesting the diagnostic value of this condition.

Although the incidence of acute scrotum in patients with KD is unknown, careful observation may identify additional patients with the complication. Most of the reported patients were free of tenderness and the condition resolved spontaneously over time, suggesting the potential presence of overlooked patients with this complication of KD.

In summary, based on the 10 reported cases and the two present cases, acute scrotal symptoms in KD may be extracardiac findings of the acute phase of the disease, and must be reported in the list of possible KD complications.

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VLCAD deficiency in a patient who recovered from ventricular fibrillation, but died suddenly of a respiratory syncytial virus infection

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Abstract VLCAD deficiency is an autosomal recessive disorder caused by a defect of fatty acid oxidation. The phenotype is classified into three clinical forms on the basis of the onset of symptoms: a severe form with neonatal onset; a milder form with childhood onset; and a late-onset form. The neonatal form is the most common, and has a higher mortality rate

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than the others. We report the case of a newborn infant with VLCAD deficiency who developed ventricular fibrillation, which was successfully treated by intensive care, but who suddenly died after a respiratory syncytial virus infection. Early institution of i.v. glucose treatment and active immunization with vaccine, such as palivizumab (anti-RSV mAb), may be important to reduce the frequency and severity of life-threatening episodes.

Key words arrythmia, fatty acid oxidation disorder, neonatal sudden death, respiratory syncytial infection, VLCAD deficiency.

Patients with fatty acid oxidation disorders may present with early onset of a severe form usually associated with cardiomyopathy and leading to sudden death in some cases. In infants, the disease course can be rapid and is difficult to diagnose in the emergency department. Very-long-chain acyl-coenzyme A (CoA) dehydrogenase (VLCAD) deficiency is an autosomal recessive disorder caused by a defect of ACADVL gene affecting fatty acid oxidation. The prevalence of the disease has been estimated to be 1 in 150 000. Symptoms of VLCAD deficiency appeared during infancy or childhood are hypoglycemia, lethargy, muscle weakness, liver failure and heart failure. Here, we report the case of a newborn infant with VLCAD deficiency who developed ventricular fibrillation, which was successfully treated by intensive care, but who suddenly died after a respiratory syncytial virus infection.

Case report

The present patient was a boy weighing 3566 g at birth who was born at 39 weeks 4 days of gestation following an unremarkable pregnancy. There was no significant family history or consanguinity. On the first day of life, tachypnea and grunting were noted. These findings suggested pneumonia, but the patient's clinical condition did not improve with i.v. antibiotics. The patient did not respond well and was therefore transferred to the pediatric emergency center for further examination. He had slightly delayed capillary refilling time, oxygen saturation of 99%, heart rate 118 beats/min, and respiratory rate 80 breaths/min. Laboratory analysis indicated blood glucose and potassium levels of 42 mg/dL (2.33 mmol/L) and 7.05 mmol/L, respectively, and blood gas measurement showed metabolic acidosis with pH 7.294, pCO_2 29.4 mmHg, pO_2 35.6 mmHg, HCO_3 13.8 mmol/L, base excess −11.1 mmol/L, and anion gap 25.2 mEq/L. Electrocardiograph (ECG) monitoring indicated a sudden onset of ventricular fibrillation (VF) (Fig. 1a). Cardiac pulmonary resuscitation was attempted, with calcium gluconate and epinephrine, and after 30 min the patient showed recovery to sinus rhythm. Sodium bicarbonate followed by glucose-insulin therapy was initiated. The patient was then transferred to the neonatal intensive care unit (NICU) at Kumamoto University Hospital. After arrival, hypoglycemia, hyperkalemia, and metabolic acidosis recovered quickly. Cardiac function required more time for complete recovery, but the patient did not experience arrhythmia. Acylcarnitine analysis on tandem mass spectrometry (MS/MS), using a dried blood spot taken on admission, indicated elevated long-chain acylcarnitines, with a C14-1-acylcarnitine level of 4.08 μmol/L (control, <0.40; Table 1).

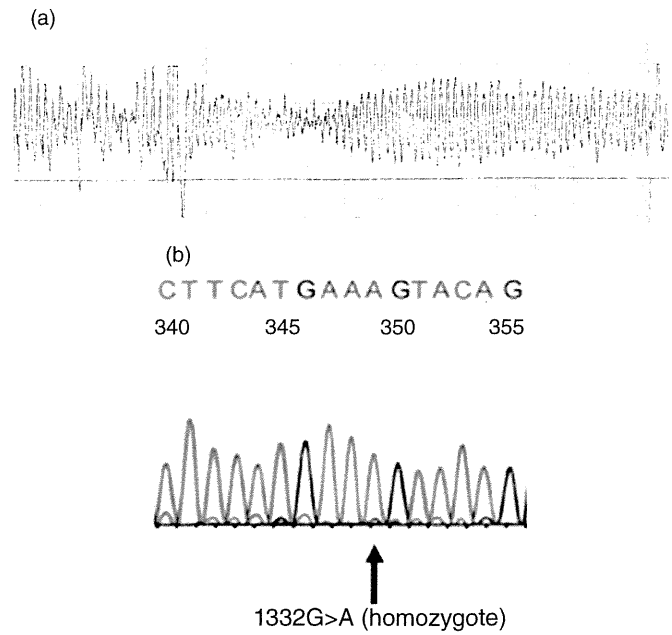

Fig. 1 (a) Electrocardiograph monitoring at the onset of ventricular fibrillation. (b) Gene analysis of the acyl-CoA dehydrogenase, very-long-chain (ACADVL) gene, indicating a homozygote c.1332G>A mutation in the exon–intron junction.

Table 1 Acylcarnitine concentration on MS/MS

Acylcarnitine	Concentration (nmol/mL)	Control (nmol/mL)
C0	27.86	>10
C2	9.59	21.16 ± 5.26 (mean ± SD)
C4	0.15	<1.0
C5	0.13	<1.0
C6	0.06	<0.30
C8	0.17	<0.30
C10	0.78	<0.35
C12	1.09	<0.35
14	6.15	<0.40
C14:1	4.08	<0.40
C16	13.38	<6.0
C16-OH	0.12	<0.05
C18	2.64	<3.0
C18:1	3.3	<3.0
C18:1-OH	0.05	<0.05

MS/MS, tandem mass spectrometry.

Table 2 A VLCAD activity assay†

Subject	Palmitoyl-CoA dehydrogenase activity (pmol/min/10^6 lymphocytes)
Patient	0.42
Control	25.1
Normal (n = 31)	54.5 ± 17.5

†Using lymphocytes and palmitoyl-CoA as a substrate. VLCAD, very-long-chain acyl-coenzyme A (CoA) dehydrogenase.

These data suggested that the newborn patient may have very-long-chain acyl-coenzyme A (CoA) dehydrogenase (VLCAD) deficiency; enzyme assay[1] was then performed. Palmitoyl-qCoA dehydrogenase activity in lymphocytes was reduced to approximately 1% that of the mean in normal control subjects (Table 2). The diagnosis of VLCAD deficiency was confirmed on these findings. Gene analysis identified a homozygote c.1332G>A mutation in the exon–intron junction of the acyl-CoA dehydrogenase, very-long-chain (ACADVL) gene (Fig. 1b), indicating a splicing abnormality. After confirming the diagnosis, the patient had normal development with long-term dietary therapy and supplementation of L-carnitine and medium-chain triglyceride (MCT) oil. After the neonatal period, echocardiography and ECG were normal. Vomiting and diarrhea were sometimes associated with metabolic acidosis, but the patient recovered quickly after rapid transfusion of glucose and electrolytes. The day before his death at 2 years old, he had cough and low-grade fever. He presented to the emergency department, which he often visited for regular treatment. He was diagnosed as having respiratory syncytial virus (RSV) infection according to the RSV detection kit. Given that his respiratory condition was satisfactory and blood gas analysis was normal, it was decided that hospitalization was not necessary. He was therefore returned his home with some medicine for cough and fever, but he had only half the usual quantity of MCT milk that night. He coughed and woke up early in the morning, and he was conscious until just before the attack. His breathing sounded normal, then he suddenly stood up and fell down and became unconscious. He seemed to be in cardiopulmonary arrest when he arrived at the emergency department. After efforts at resuscitation we confirmed his death at the hospital. A cardiogenic cause, particularly arrhythmia, was most likely the cause of the sudden death. The death was too sudden to be due to breathing problems or brain lesion. Because there was no sign of vomiting, no sign of abuse, no congestion due to suffocation, we speculated that his death was due to arrhythmia induced by VLCAD deficiency.

Discussion

VLCAD deficiency is an autosomal recessive disorder and the prevalence of VLCAD deficiency has been estimated to be 1 in 150 000. The phenotype of VLCAD deficiency is heterogeneous. It is classified into three clinical forms on the basis of the onset of symptoms: a severe form with neonatal onset; a milder form with childhood onset; and a late-onset form. The neonatal form is the most common, and patients present with cardiomyopathy, hepatopathy, and skeletal myopathy. This form has a higher mortality rate than the others.[2] VF and respiratory arrest have been reported in patients who develop VLCAD deficiency within 1 year of birth.[3] In the present case, the patient developed VF and was rescued by cardiopulmonary resuscitation, because the pediatrician was at his bedside during the development of VF. When the patient was transferred to NICU, metabolic acidosis was improved by glucose transfusion. First, we suspected mitochondrial disease and secondary cardiac disorder. MS/MS was very useful for the final diagnosis of VLCAD deficiency.

In Kumamoto, MS/MS analysis was initiated as a pilot study 5 years ago, and MS/MS was introduced for mass screening of newborns with approximately 100% agreement. Because clinical manifestations in the present case were observed 2 days after birth, the patient was not covered by standard screening. The abnormality was detected only when post-symptom high-risk screening was performed. Elevations in C14:1, C16, and C16+18/C2 were identified on MS/MS, and VLCAD deficiency was suspected. At this point, the patient was given MCT milk and carnitine. Next, we performed a fatty acid β-oxidation assay and found that the metabolism of C14 to C12 was abnormal. We also performed a VLCAD enzyme assay and ACADVL gene analysis.[1] Palmitoyl-CoA dehydrogenase activity in the present patient was found to be severely decreased. Molecular analysis of the ACADVL gene encoding VLCAD showed that the patient had a single base mutation, c,1332G>A, at the exon–intron junction. To the best of our knowledge, this case presents a novel mutation. We examined the sequences by calculating splicing score (http://www.fruitfly.org/seq_tools/splice.html). In the normal sequence (CTTCATGAAGGTACAGGACGGT), splice site was recognized with donor score 0.9. The false-positive (FP) rate and the correlation coefficient (CC) were 1.1% and 0.73. In the present patient's sequence (CTTCATGAAAGTACAGGACGGT), the splicing was not recognized. As a result of the mutation, abnormal splicing of the mRNA would occur. We assumed that it was a mutation causing exon-skipping or connection to a new junction.[4]

An inborn error in metabolism is one of the differential diagnoses of unknown cardiomyopathy or arrhythmia. In this case, MS/MS was insufficient for preclinical diagnosis because of the delayed time of sampling to detect early-onset VLCAD, but it was very useful for accurate diagnosis.[5] It is possible to prevent secondary complications of VLCAD with intake of MCT milk and carnitine supplementation and with diet therapy. In past reports, patients surviving the initial episode have nearly normal cardiac function by avoidance of fasting, and using a low-fat diet with frequent meals and vigilance during intercurrent illness.[6] We can expect normal development with careful follow up for most patients.[7] It is important to start a glucose infusion, not only in cases of gastroenteritis and starvation, but also in cases of general infection with the potential for exacerbation.

The prognosis for control of VLCAD deficiency is very challenging, even after successful resolution of several crises. Early institution of i.v. glucose treatment may be important to

reduce the frequency and severity of life-threatening episodes. In addition, active immunization with vaccine, such as palivizumab (anti-RSV mAb), might be necessary.

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Transverse myelitis and acute motor sensory axonal neuropathy due to *Legionella pneumophila*: A case report

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Abstract Guillain–Barré syndrome is a rapidly progressive symmetrical muscle weakness associated with acute inflammatory disease. Transverse myelitis (TM) is the inflammation of the spinal cord characterized by rapidly evolving muscle weakness in the lower extremities, defects in sensory level and sphincter dysfunction. Guillain–Barré syndrome, and TM association occurs very rarely in childhood. A 7-year-old girl presented with complaints of neck pain, spout-style vomiting, cough, shortness of breath, and acute paraparesis with sensory and sphincter disturbance. The patient was intubated because of increased respiratory distress. A positive direct fluorescein antigen test in bronchoalveolar lavage confirmed *Legionella pneumophila* infection. Imaging and neurophysiologic studies were diagnostic for TM with acute motor and sensory axonal neuropathy. She was treated with a combination of high-dose methylprednisolone and intravenous immunoglobulins, and we observed incomplete recovery. The presented case is the first child with concomitant TM and acute motor and sensory axonal neuropathy related to *L. pneumophila* infection.

Key words acute motor and sensory axonal neuropathy, child, immune modulation, *Legionella pneumophila*, transverse myelitis.

Demyelinating disorders can affect any part of the nervous system. Transverse myelitis (TM), which is characterized by focal spinal cord inflammation, may be idiopathic, parainfectious or disease-associated. Diseases associated with TM include demyelinating conditions and connective tissue disorders. Apart from TM attributable to direct spinal cord infection, TM is autoimmune. Demyelination limited to the spine is known as TM, whereas radicular and peripheral nerve demyelination is recognized as the acute inflammatory demyelinating form of Guillain–Barré Syndrome (GBS).[1,2] Acute motor and sensory axonal neuropathy (AMSAN), a subtype of GBS, is an autoimmune and usually post-infectious disease characterized by endoneurial inflammation with both primary demyelination and axonal degeneration.[2]

To our knowledge, this is the first presentation of a child with simultaneous TM and AMSAN related to *Legionella pneumophila* infection in the English-language medical literature.

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ORIGINAL ARTICLE

Current status of hepatic glycogen storage disease in Japan: clinical manifestations, treatments and long-term outcomes

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Many reports have been published on the long-term outcome and treatment of hepatic glycogen storage diseases (GSDs) overseas; however, none have been published from Japan. We investigated the clinical manifestations, treatment, and prognosis of 127 hepatic GSD patients who were evaluated and treated between January 1999 and December 2009. A characteristic genetic pattern was noted in the Japanese GSD patients: most GSD Ia patients had the g727t mutation, and many GSD Ib patients had the W118R mutation. Forty-one percent (14/34) of GSD Ia patients and 18% (2/11) of GSD Ib patients of ages $\geqslant$13 years 4 months had liver adenoma. Among subjects aged 10 years, 19% (7/36) of the GSD Ia patients and none of the GSD Ib patients had renal dysfunction. The mean height of male GSD Ia patients aged $\geqslant$18 years was 160.8 ± 10.6 cm (n = 14), and that of their female counterparts was 147.8 ± 3.80 cm (n = 9). Patients with hepatic GSDs develop a variety of symptoms but can survive in the long term by diet therapy, corn starch treatment and supportive care. Liver transplantation for hepatic GSDs is an important treatment strategy and can help improve the patients'quality of life.
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Keywords: adenoma; glycogen storage disease; g727t; height; hepatocellular carcinoma; liver transplantation; renal dysfunction; W118R

INTRODUCTION

Glycogen storage diseases (GSDs) are inherited metabolic diseases caused by the deficiency of enzymes regulating glycogenolysis or gluconeogenesis. As glycogen primarily accumulates in the liver and muscle, the disorders of glycogen degradation affect the liver, muscles or both. Hypoglycemia is the main symptom of hepatic GSDs, whereas muscle weakness or elevated muscle enzyme is the main symptom of myopathic GSDs. Hepatic GSDs, except for GSD IXa, are autosomal recessive, and GSD IXa is an X-linked recessive disorder. GSD Ia, GSD III and GSD IXa account for 80% of hepatic GSDs.

GSD Ia (Mendelian Inheritance in Man (MIM) no. 232200) is caused by a deficiency of glucose-6-phosphatase (EC 3.1.3.9) in the endoplasmic reticulum. GSD Ib (MIM no. 232220) is caused by a deficiency of glucose-6-phosphate transporter, which leads to the dysfunction of glucose-6-phosphatase in the endoplasmic reticulum. GSD Ia is the most common GSD, and its frequency is 1/100 000 to 1/400 000 births in the general Caucasian population; GSD Ib is much less frequent than GSD Ia. The manifestations of GSD Ia are short stature, hypoglycemia, hepatomegaly, hyperlipidemia, hyperuricemia, hyperlactacidemia, hepatoadenoma, renal disorder[1,2] and hepatocellular carcinoma.[3,4] Most GSD Ib patients have neutropenia and neutrophil dysfunction in addition to these symptoms. GSD III (MIM no. 232400) is caused by a deficiency of the debranching enzyme, which consists of amylo-1,6-glucosidase (EC 3.2.1.33) and oligo-1,4-1,4-glucantransferase (EC 2.4.1.25). The incidence of GSD III has been reported to be 1 per 83 000 live births in Europe and 1 per 100 000 live births in North America.[5] There are two major GSD III subtypes: GSD IIIa, which affects both the liver and muscle and accounts for 80% of all GSD III cases, and GSD IIIb, which affects only the liver and comprises approximately 15% of them.[6] The manifestations of GSD III are similar to those of GSD Ia, and many patients with GSD IIIa have hypertrophic cardiomyopathy.[7]

GSD IV (MIM no. 232500) is caused by a deficiency of amylo-1,4 to 1,6-transglucosidase (EC 2.4.1.18), which leads to the absence of branched glycogen. GSD IV, which is the most severe type of GSD, represents 0.3% of all GSDs.[8] This disease rapidly progresses to cirrhosis early in life and causes death between 3 and 5 years of age because of liver failure.[9] If signs of GSD IV, such as cervical cystic hygroma, are detected,[8] the patients are likely to die in the neonatal period. The effective treatment for progressive GSD IV is liver

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transplantation.[10] GSD VI (MIM no. 232700), which is rarer and milder than the other hepatic GSDs, is caused by a deficiency of glycogen phosphorylase (EC 2.4.1.1) in the liver. GSD IXa (MIM no. 306000) is caused by a deficiency of phosphorylase kinase α2 (PHKA2)—a subunit of phosphorylase kinase (EC 2.7.11.19), which consists of four subunits, namely, α, β, γ and δ. The clinical course of GSD IXa is benign, and most adult patients are asymptomatic.[11] With aging, clinical and biochemical abnormalities gradually disappear. The other subtypes of GSD IX include subtypes caused by a deficiency of phosphorylase kinase β, phosphorylase kinase γ or δ, or muscle phosphorylase kinase. The Fanconi–Bickel syndrome, GSD XI (MIM no. 227810), is caused by a deficiency of glucose transport 2 and is characterized by hepatorenal glycogen accumulation and proximal renal tubular dysfunction.[12]

The treatment for these hepatic GSDs comprises the prevention of hypoglycemia. The basic treatment is the consumption of frequent meals and uncooked cornstarch.[13–15] Moreover, restriction of the intake of sugars, such as fructose, galactose, sucrose and lactose, is important mainly for GSD I.

Complete blood glucose control by these measures is unlikely to ameliorate complications, such as hyperuricemia and hyperlipidemia.[16] GSD patients are administered allopurinol for hyperuricemia and statin, fibrates or niacin formulations for hyperlipidemia.[17,18] Administration of angiotensin-converting enzyme inhibitor or/and angiotensin receptor blocker, which have a renoprotective effect, is recommended for GSDs with possible renal complications.[19] Gene therapy can be an effective as a radical treatment measure for GSDs.[20,21] However, the definitive treatment of GSDs is only liver transplantation.[22–25]

Many reports have been published overseas on the long-term outcome and treatment of GSD patients.[11,17,26–28] However, no report has yet been published on the long-term outcome of GSDs in Japan, wherein GSD Ia with a mutation causing mild symptoms has been detected in many cases. We studied the current status of clinical manifestations, treatment, and long-term outcome of hepatic GSDs in Japan.

MATERIALS AND METHODS

Study patients

In 2009, we sent a questionnaire to 928 Japanese institutions, including the departments of pediatrics, endocrinology and metabolism, neonatology, genetics, and transplant surgery, asking doctors if they had diagnosed or provided medical care to hepatic GSDs patients. Each institution was the medical center for a locality and had 300 or more beds. Of the 928 institutions, 668 (72%) responded. Of these 668 institutions, 97 had treated patients with GSDs. A second questionnaire was then sent to these 97 institutions in 2009, and responses were received from 53 (55%) of them. On the basis of the received reports, 127 cases of GSDs diagnosed and treated between January 1999 and December 2009 were studied. We excluded patients who were not definitely diagnosed and considered patients visiting multiple institutions as single patients. The 127 cases of GSDs (types Ia, Ib, III, IV, VI, IXa and others) were diagnosed on the basis of clinical manifestations, family history, enzyme activity, metabolite analysis (75 g oral glucose tolerance test (OGTT) or/and glucagon test) and/or DNA analysis. This study was approved by the ethical committee of the Faculty of Life Science, Kumamoto University.

The definition of clinical manifestations of GSD applied in this study was the same as that proposed by Smit *et al.*[27] In addition, we used the following definitions. Hyperlactacidemia was defined as a blood lactate level $>2.2\,mmol\,l^{-1}$. Hyperuricemia was defined by a history of receiving drugs for hyperuricemia and/or blood uric acid level $>420\,\mu mol\,l^{-1}$. Hyperlipidemia was defined by a history of medical treatment for hyperlipidemia, blood total cholesterol level $>5.9\,mmol\,l^{-1}$, or blood total triglyceride level $>1.7\,mmol\,l^{-1}$. Mental retardation was diagnosed if the patient's intelligence quotient was <70, as per standardized tests, such as the Wechsler Intelligence Scale for Children and the Wechsler Adult Intelligence Scale. Proteinuria was defined by protein levels $>30\,mg\,dl^{-1}$ in 1 spot urea test or $>500\,mg\,day^{-1}$. Renal dysfunction was defined by blood creatinine levels $>90\,\mu mol\,l^{-1}$. Increased susceptibility to infection was defined as a neutrophil count of $<1500/\mu l$ and/or hospitalization more than three times a year because of infection.

Statistical analysis

The age at onset of hepatic GSD patients was expressed in terms of the median and interquartile range, and the age of onset was analyzed by the Mann–Whitney *U*-test of IBM SPSS Statistics Version 19.[29] A *P*-value of <0.05 was considered statistically significant. The height of hepatic GSD patients was expressed in terms of mean ± s.d. values. Kaplan–Meier curves of estimated survival rate were generated by SPSS.

RESULTS

Age at onset and methods for definitive diagnosis of hepatic GSDs

Table 1 indicates the age, onset age and methods used for definitive diagnosis in each of the 127 cases of hepatic GSD. GSD Ib and GSD IV patients manifested symptoms earlier than those with other types of GSD (GSD Ia vs GSD Ib, $P=0.001$; GSD Ia vs GSD IV, $P=0.022$; GSD Ia vs GSD XIa, $P=0.002$). Enzyme activity was measured in 50% (64/127) of the patients with GSDs, and genotype analysis was performed in 50% (63/127); genotypes could be identified in 40% (51/127) of the patients with GSDs. DNA analysis was performed in the case of 52 patients with GSD Ia, 7 patients with GSD Ib, 1 patient with GSD III, 1 patient with GSD VI, 5 patients with GSD IXa and 2 patients with GSD XI. Thereafter, identifiable mutations were detected at a rate of 79% (41/52) in GSD Ia patients, 86% (6/7) in GSD Ib patients, 40% (2/5) in GSD IXa patients and 100% (2/2) in GSD XI patients. Of the GSD Ia patients with recorded identifiable mutations, 81% (29/36) had g727t homozygote mutations and 17% (6/36) had compound heterozygotes with g727t mutations. Of the GSD Ib patients with recorded identifiable mutation, 83% (5/6) had homozygote or compound heterozygote mutations of W118R. Eight patients with GSD Ia, one patient with GSD IXa and one patient with GSD XI were diagnosed by DNA-based and enzymatic analyses.

Clinical manifestations of hepatic GSD

Table 2 indicates the frequency of clinical manifestations in hepatic GSD patients. In GSD Ia patients, growth retardation (78%; 51/65), hypoglycemia (69%; 45/65), hyperuricemia (88%; 57/65) and hyperlipidemia (94%; 61/65) were observed at the frequency of >50% (Table 2a). Convulsions (9%; 6/65), mental retardation (9%; 6/65), liver tumors (22%; 14/65), proteinuria (26%; 17/65), renal dysfunction (11%; 7/65) and increased susceptibility to infection (5%; 3/65) were not frequently observed (Table 2b). Of the 14 GSD Ia patients with liver tumors, 4 had a single adenoma, 9 had 3 or more multifocal adenomas and 1 patient had hepatocellular carcinoma with multiple adenomas. Only one patient with GSD Ia developed acute pancreatitis.

Height of hepatic GSD patients

Figures 1a–d show the height of male and female hepatic GSD patients. The height of 56% (14/25) of the male GSD Ia patients aged <18 years and 43% (6/14) of the male GSD Ia patients aged ⩾18 years was below the third percentile. The mean height of male GSD Ia patients aged ⩾18 years was 160.8 ± 10.6 cm ($n=14$; Figure 1a). Fifty-seven percent (4/7) of the male GSD Ib patients, 50% (2/4) of the GSD III patients aged <18 years and 19% (6/32) of the male GSD IXa patients had heights below the third percentile (Figures 1b and c).

Table 1 Age of onset, diagnosis and definitive diagnosis of hepatic GSD patients

	Patient's age: median (minimum–maximum)	*Age at onset: median (minimum–maximum)*	*Age of diagnosis: median (minimum–maximum)*	*Enzyme activity (%)*	*Identifiable mutation (%)*	*Dead patients*	*Surviving patients*	*No. of patients*
GSD Ia	13 y 8 mo (0 d–11 y 1 mo)	9 mo (0 d–11 y 1 mo)	1 y 2 mo (0 d–11 y 2 mo)	19/65 (29%)	41/65 (63%)	2 (3%)	63 (97%)	65 Patients (male: 41, female: 24)
GSD Ib	12 y 1 mo (1 y–27 y)	3 mo (0 d–4 mo)**	5.5 mo (2 mo–6 y 6 mo)**	3/11 (27%)	6/11 (55%)	1 (9%)	10 (91%)	11 Patients (male: 7, female: 4)
GSD III	12 y (3 y 7 mo–29 y 10 mo)	10.5 mo (7 mo–2 y 3 mo)	1 y (7 mo–2 y 3 mo)	4/6 (67%)	0/6 (0%)	1 (17%)	5 (83%)	6 Patients (male: 4, female: 2)[a]
GSD IV	1 y 1 mo (2 d–14 y 2 mo)	2 mo (0 d–5 mo)*	4 mo (0 d–9 mo)*	4/4 (100%)	0/4 (0%)	3 (75%)	1 (25%)	4 Patients (male: 3, female: 1)
GSD VI	9 y 10 mo (3 y 10 mo–19 y 6 mo)	1 y 3 mo (1 mo–3 y 4 mo)	1 y 4 mo (1 mo–6 y 6 mo)	4/6 (67%)	0/6 (0%)	0 (0%)	6 (100%)	6 Patients (male: 5, female: 1)
GSD IXa	9 y 9 mo (2 y 6 mo–17 y 11 mo)	1 y 7 mo (1 mo–5 y)**	2 y (1 mo–11 y)**	29/32 (91%)	2/32 (6%)	0 (0%)	32 (100%)	32 Patients (male: 32)
Others	11 y 9 mo (11 y 9 mo–29 y 9 mo)	1 y (5 d–1y 6 mo)	1 y 8 mo (1 y 8 mo–1 y 10 mo)	1/3 (33%)	2/3 (67%)	0 (0%)	3 (100%)	3 Patients (male: 2, female: 1)
Total				64/127 (50%)	51/127 (40%)	7 (6%)	120 (94%)	127 Patients (male: 94, female: 33)

Abbreviations: d, days; GSD, glycogen storage disease; mo, months; y, years.
The category 'Others' includes the GSD IX (one patient), other than those with GSD IXa and Fanconi–Bickel syndrome (GSD XI; two patients).
*$P<0.05$.
**$P<0.01$.
[a]Includes four patients each with GSD IIIa (male, 2; female, 2) and two male patients with an unknown subtype.

One hundred percent (5/5) of the male GSD VI patients had height greater than the tenth percentile (Figure 1b). Thirty-three percent (5/15) of the female GSD Ia patients aged <18 years and 44% (4/9) of the female GSD Ia patients aged $\geqslant 18$ years had heights below the third percentile. The mean height of female GSD Ia patients aged $\geqslant 18$ years was 147.8 ± 3.80 cm ($n=9$; Figure 1d).

Long-term survival of patients with hepatic GSD

Table 1 presents the number of hepatic GSD patients who survived and died. Two patients with GSD Ia (age of death: 6 years 10 months, male; 27 years, female), a male GSD Ib patient (13 years 5 months), a female GSD IIIa patient with cardiomyopathy (24 years 8 months) and a male GSD IV patient (1 year 11 months) died because of liver failure after liver transplantation. The other two patients with GSD IV died of liver failure 2 months after birth.

The long-term survival rate of GSD Ia patients at 20 years after birth was 97% for male patients and 100% for female patients (Figure 2). The survival rate of GSD Ib patients at 20 years after birth was 80% (Supplementary Figure 1).

Treatment for hepatic GSD

Table 3 indicates the treatment received by the hepatic GSD patients. Among the patients with GSD Ia, uncooked corn starch was administered to 98% (64/65) of the patients; allopurinol, to 74% (48/65); lipid-lowering drugs, to 42% (27/65); and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, to 15% (10/65). Dietary management with restriction of the intake of galactose, fructose and saccharose was used for 63% (41/62) of the patients. Most patients were not taking the GSD formula when they were taking corn starch. Lipid-lowering drugs were administered to 66% (18/27) of the GSD Ia patients with hyperlipidemia and aged 14 years or more. The youngest patient who received lipid-lowering drugs was 5 years old.

Liver transplantation for hepatic GSD

Table 4 shows the ages at which liver transplants were performed for hepatic GSD patients. As many metabolic disorders, such as hypoglycemia, were improved in the two patients with GSD Ia who underwent successful liver transplantation, symptoms such as nasal bleeding and growth disorder were ameliorated. These two patients needed allopurinol, but not diet and corn starch treatment.

Figure 3 and Supplementary Figure 2 present the comparison between the data obtained immediately before liver transplant and 1 year after liver transplant in five patients with GSD Ib. Blood levels of uric acid, total cholesterol and triglyceride in GSD Ib patients improved after liver transplantation, but the abnormalities in the neutrophil count were not ameliorated. All the five patients received granulocyte colony-stimulating factor after liver transplants; however, the frequency of granulocyte colony-stimulating factor administration after liver transplantation was lower than that before transplantation, as was the susceptibility to infection. No patients in this study received bone-marrow transplantation.

DISCUSSION

Most patients with hepatic GSD, except for GSD VI and IXa, which were mild types, manifested symptoms before 2 years of age. Further, the age of onset for GSD Ib and IV was lower than that for the other hepatic GSDs. However, two male GSD Ia patients presented with symptoms at 11 years and 9 years, thereby indicating that GSD may be detected at any age. Enzyme activity in the erythrocytes or leukocytes was measured in patients with GSD III, VI, IXa and XI, without performing invasive liver biopsy. Genome sequencing for GSD III, VI and IXa was difficult and not likely to be performed. Among the GSD I patients, the g727t mutation of the glucose-6-phosphatase gene has been detected in almost 90% alleles of GSD Ia,[30] and the W118R mutation of glucose-6-phosphate transporter gene is highly frequent in GSD Ib patients.[31] Therefore, we performed DNA analysis rather than enzyme assay, which requires invasive liver biopsy in GSD I patients. As this study focused on GSD patients younger than 18 years, we did not include many GSDs patients older than 18 years. Thus, the exclusion of GSD patients older than 18 years and GSD III patients may have introduced a bias in the results.

We investigated the statures of patients with hepatic GSD. Among the hepatic GSDs, GSD I commonly presents with short stature. Height <3 percentile were noted in 56% of the male GSD Ia patients and 33% of female GSD Ia patients aged <18 years. Mean stature in patients with GSD Ia aged >18 years was 160.8 ± 10.6 cm ($n=14$) and 147.8 ± 3.80 cm ($n=9$) for male and female patients, respectively.

Table 2 (a) Frequent manifestations of hepatic GSD; (b) Infrequent manifestations of hepatic GSD

(a)

	Growth disorder	*Hypo- glycemia*	*Hyper- lactacidemia*	*Hyper- uricemia*	*Hyper- lipidemia*	*Hepato- megaly*	*Fatty liver*	*Liver disorder*
GSD Ia	78% (51/65)	69% (45/65)	92% (60/65)	88% (57/65)	94% (61/65)	92% (60/65)	65% (42/65)	97% (63/65)
GSD Ib	55% (6/11)	91% (10/11)	91% (10/11)	64% (7/11)	55% (6/11)	100% (11/11)	64% (7/11)	64% (7/11)
GSD III	50% (3/6)	83% (5/6)	83% (5/6)	67% (4/6)	50% (3/6)	100% (6/6)	50% (3/6)	83% (5/6)
GSD IV	25% (1/4)	50% (2/4)	25% (1/4)	0% (0/4)	0% (0/4)	50% (2/4)	0% (0/4)	50% (2/4)
GSD VI	17% (1/6)	67% (4/6)	50% (3/6)	17% (1/6)	17% (1/6)	100% (6/6)	17% (1/6)	83% (5/6)
GSD IXa	44% (14/32)	34% (11/32)	34% (11/32)	6% (2/32)	41% (13/32)	97% (31/32)	47% (15/32)	84% (27/32)
Others	67% (2/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	67% (2/3)	0% (0/3)	33% (1/3)
Total	61% (78/127)	61% (77/127)	71% (90/127)	56% (71/127)	66% (84/127)	93% (118/127)	54% (68/127)	87% (110/127)

(b)

	Bleeding tendency	*Convulsion*	*Mental retardation*	*Gout*	*Liver tumor*	*Protein- uria*	*Renal dysfunction*	*Hyper- tension*	*Cardio- myopathy*	*Myopathy*	*Osteoprosis*	*Increased susceptibility to infection*
GSD Ia	31% (20/65)	9% (6/65)	9% (6/65)	11% (7/65)	22% (14/65)	26% (17/65)	11% (7/65)	3% (2/65)	6%(4/65)	1.5% (1/65)	3% (2/65)	5% (3/65)
GSD Ib	18% (2/11)	36% (4/11)	27% (3/11)	9% (1/11)	18% (2/11)	9% (1/11)	0% (0/11)	9% (1/11)	9% (1/11)	0% (0/11)	0% (0/11)	100% (11/11)
GSD III	0% (0/6)	67% (4/6)	33% (2/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	17% (1/6)	17% (1/6)	50% (3/6)	0% (0/6)	0% (0/6)
GSD IV	75% (3/4)	0% (0/4)	25% (1/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	50% (2/4)	50% (2/4)	0% (0/4)	25% (1/4)
GSD IV	17% (1/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	17% (1/6)	0% (0/6)	0% (0/6)
GSD IXa	0% (0/32)	0% (0/32)	0% (0/32)	0% (0/32)	0% (0/32)	6% (2/32)	3% (1/32)	0% (0/32)	0% (0/32)	3% (1/32)	0% (0/32)	0% (0/32)
Others	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	33% (1/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
Total	20% (26/127)	11% (14/127)	9% (12/127)	6% (8/127)	13% (16/127)	16% (20/127)	7% (9/127)	3% (4/127)	6% (8/127)	6% (8/127)	1.6% (2/127)	12% (15/127)

Abbreviation: GSD, glycogen storage disease.

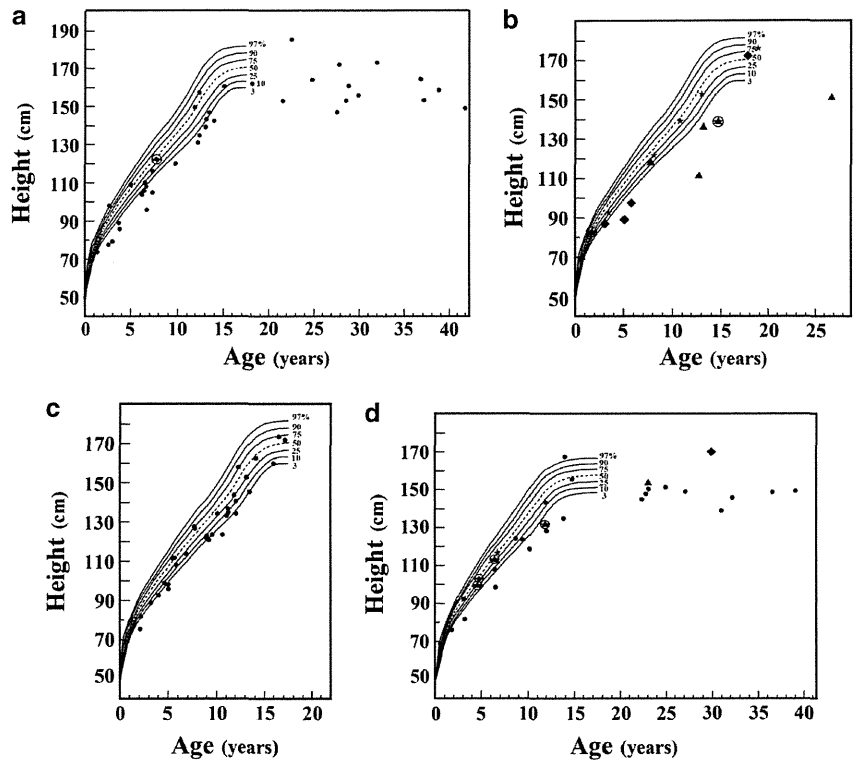

Figure 1 Stature of hepatic glycogen storage disease (GSD) patients. This figure was constructed with the age of GSD patients on the abscissa and the stature of patients with GSD on the ordinate. Percentiles are based on data from Japanese 2000 growth reports provided by the Ministry of Health, Labor and Welfare in Japan. (**a**) Stature of male patients with GSD Ia. The height of the male GSD Ia patient aged 7 years 11 months was measured after liver transplantation. ●: GSD Ia patients ($n=39$); ⊖: patients after liver transplant. (**b**) Stature of male patients with GSD Ib, GSD III and GSD VI. The heights of male GSD Ib patients aged 1 year 10 months and 14 years 10 months were measured after liver transplantation.▲: GSD Ib patients ($n=7$); ◆: GSD III patients ($n=4$); *: GSD VI patients ($n=5$); ⊖: patients after liver transplant. (**c**) Stature of male patients with GSD IXa. ●: GSD IXa patients ($n=32$). (**d**) Stature of female patients with GSD Ia, GSD Ib, GSD III and GSD VI. The heights of female GSD Ia patient aged 4 years 10 months and GSD Ib patients aged 4 years 7 months, 6 years 6 months and 11 years 11 months were measured after liver transplantation.●: GSD Ia patients ($n=24$), ▲: GSD Ib patients ($n=4$), ◆: GSD III patients ($n=1$), *: GSD VI ($n=1$), ⊖: patients after liver transplant.

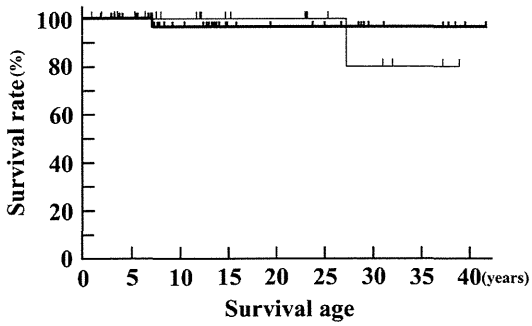

Figure 2 Long-term survival rates in patients with glycogen storage disease (GSD) Ia. The survival rates of 63 patients with different ages are shown by Kaplan–Meier survival curves. Two GSD Ia patients aged 6 years 10 months (male) and 27 years (female) died of liver failure after liver transplant. Male GSD Ia patients (black bold line), $n=41$; female GSD Ia patients (black fine line), $n=22$.

Therefore, we can expect that the final stature of patients with GSD Ia ranges from 3 to 10 percentile of the Japanese height.

Liver tumor and renal dysfunction, which are not frequently observed, are important determinants of the prognosis in patients with GSD.[1] It has been reported that liver adenomas are detected in 22 to 75% of patients with GSD Ia,[28,32] and some of these adenomas developed to hepatocellular carcinoma.[33,34] In this study, liver tumors, which have been reported to be less frequent overseas, were detected in 22% (14/65) of the patients with GSD Ia and in 18% (2/11) of patients with GSD Ib. The youngest GSD Ia patient with liver adenoma was a male patient aged 13 years 4 months, and 41% (14/34) of GSD Ia patients older than this patient had liver adenoma. Nakamura *et al.*[35] reported that 57.9% (11/19) of adult GSD Ia patients with the g727t homozygote mutation had liver adenomas, and 16% (3/19) of them had hepatocellular carcinoma. In this study, only one patient developed hepatocellular carcinoma, which was treated by percutaneous ethanol injection therapy and radiofrequency ablation, and did not recur.

Proteinuria, which is detected in many patients with GSD I, may progress to renal dysfunction or renal failure. In this study, two of the seven GSD Ia patients with renal dysfunction underwent hemodiafiltration. Chen *et al.* reported that 70% of GSD Ia patients aged >10 years presented with renal dysfunction and that 40% of GSD Ia patients with renal dysfunction developed progressive renal failure. The incidence of renal dysfunction, which was 11% (7/65) in GSD Ia patients of this study and 19% (7/36), in GSD Ia patients >10 years old, was very low.

As GSD Ia with g727t mutation is considered to be a mild type of GSD Ia, patients with the g727t mutation may develop only proteinuria but are not likely to develop renal dysfunction. It has been reported that transforming growth factor-β expression increases in the tubular epithelial cells and is involved in the pathophysiology of

Table 3 Treatment for hepatic GSD

Treatment	Dietary management	Uncooked corn starch	Sodium and potassium citrate	Allopurinol	Lipid- lowering drugs	ARB or ACE-I	Hypoglycemic medication	L- carnitine	G-CSF
GSD Ia	63% (41/65)	98% (64/65)	37% (24/65)	74% (48/65)	42% (27/65)	15% (10/65)	6% (4/65)	0% (0/65)	0% (0/65)
GSD Ib	64% (7/11)	82% (9/11)	0% (0/11)	9% (1/11)	9% (1/11)	0% (0/11)	9% (1/11)	27% (3/11)	55% (6/11)
GSD III	33% (2/6)	67% (4/6)	17% (1/6)	17% (1/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
GSD IV	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)
GSD VI	17% (1/6)	67% (4/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
GSD IXa	16% (5/32)	50% (16/32)	0% (0/32)	0% (0/32)	31% (10/32)	0% (0/32)	0% (0/32)	0% (0/32)	0% (0/32)
Others	33% (1/3)	67% (2/3)	33% (1/3)	0% (0/3)	33% (1/3)	0% (0/3)	0% (0/3)	67% (2/3)	0% (0/3)

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; GSD, glycogen storage disease; G-CSF, granulocyte colony-stimulating factor.

Table 4 Age at liver transplant for hepatic GSD

Age	< 1 y	1 y to < 6 y	6 y to < 12 y	12 y to < 18 y	⩾ 18 y	Total
GSD Ia	0	3	0	0	1	4
GSD Ib	0	4	1	2	0	7
GSD III	0	0	0	0	1	1
GSD IV	2	0	0	0	0	2
Total	2	7	1	2	2	14

Abbreviations: GSD, glycogen storage disease; y, years.

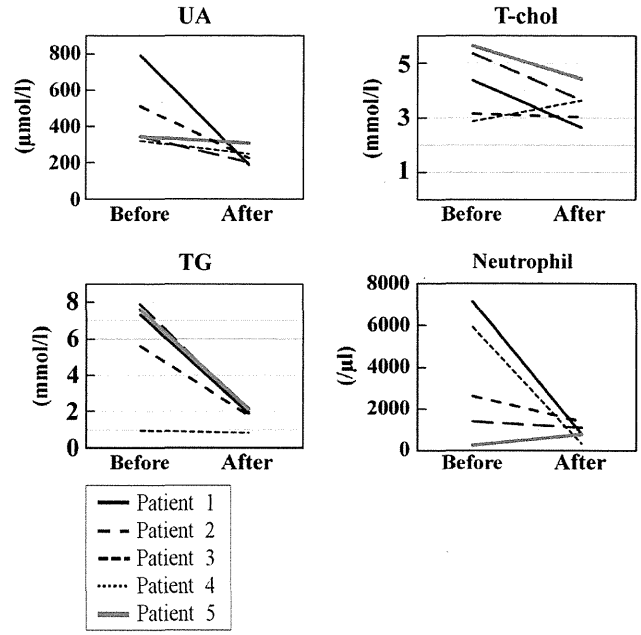

Figure 3 Comparison of data immediately before and 1 year after liver transplantation in glycogen storage disease (GSD) Ib patients. The age at liver transplantation was 1 year 1 month in patient 1 (male), 3 years 6 months in patient 2 (female), 3 years 6 months in patient 3 (male), 3 years 11 months in patient 4 (female) and 8 years 6 months in patient 5 (female). Only patient 3 received allopurinol after liver transplant. T-chol, total cholesterol; TG, triglyceride; UA, uric acid.

renal interstitial fibrosis, which results from the increase in the expression of extracellular matrix proteins in GSD I patients.[36] Angiotensin receptor blocker, angiotensin-converting enzyme inhibitor and alloprinol have been considered drugs with the highest potential of interfering with transforming growth factor-β expression because the renin angiotensin–aldosterone system and uric acid have been known to involved in the expression of transforming growth factor-β.[37,38] Moreover, it has been recognized that the small, dense low-density lipoprotein and modified low-density lipoprotein induce the development of glomerular sclerosis and renal dysfunction.[39]

Liver tumor is related to constant stimulation by hormones, such as insulin and glucagon, by persistent peripheral hypoglycemia. Therefore, the expression of renal dysfunction and liver tumor negatively correlates with metabolic control.[40] Important treatment strategies are restriction of the intake of galactose, fructose, and saccharose and blood glucose control by consumption of frequent meals and uncooked cornstarch.[40] Moreover, allopurinol, lipid-lowering drugs, and angiotensin receptor blocker or angiotensin-converting enzyme inhibitor have been reported to be significantly important in delaying the progression of kidney disease in GSD I patients.[19,39,41]

Recent reports have indicated that GSD patients may present with diabetes. Two GSD Ia patients who were brothers and had the g727t homozygote mutation developed type II diabetes and received therapy involving an α-glucosidase inhibitor and an insulin secretagogue. They monitored themselves for hypoglycemia attacks and corrected the same by consuming food or glucose. As shown in Table 1 and Figure 2, patients with hepatic GSD, except for those with GSD IV, can survive in the long term. Further, reports have also shown that GSD Ib and GSD III patients developed type II diabetes.[42,43] Therefore, physicians must pay attention to the development of obesity- and lifestyle-related diseases in GSD patients.

Table 3 indicates the treatments received by patients with hepatic GSD. As treatment after liver transplantation was recorded in Table 3, none of patients with GSD IV take dietary treatment and corn starch treatment. Use of lipid-lowering drugs has been recommended for adult GSD patients overseas.[18] Although definitive criteria for the use of lipid-lowering drugs in Japan have not yet been established, the youngest patient who received hypoglycemic medication was 5 years old.

Fourteen patients with hepatic GSD received liver transplants. According to overseas reports, the indications for liver transplantation in GSD patients are the progression of adenomatous lesions or multiple adenomas, suspicion or detection of malignant transformation of an adenoma, unresponsiveness to medical therapy, insufficient control of hypoglycemia, and growth or sexual retardation.[17,24,44] In Japan, the definitive criteria for liver transplants are controversial; many pediatricians and transplant surgeons follow the same indications reported overseas for liver transplantation. GSD I patients with uncontrolled hypoglycemia, which leads to convulsions and mental retardation, should receive liver transplants. Ninety-one

percent (10/11) of patients with GSD I received liver transplants because of insufficient control of hypoglycemia and metabolic disorders, despite medical therapy. GSD III and GSD IV patients received liver transplants because of liver failure, which was considered an indication of liver transplant, as per the pediatric end-stage liver disease scores. In this study, all GSD I patients with multiple liver adenomas underwent hepatectomy, and only one patient with GSD I received a liver transplant because of adenoma recurrence after adenoma resection. Five of 14 GSD patients died because of liver failure <2 months after liver transplantation. The other nine patients survived and improved such that they did not develop hypoglycemia without medication and showed better increase in height. The frequency of infection decreased in GSD Ib patients after transplantation, as described previously.[45] Liver transplants contributed to an improved quality of life (QOL) in GSD patients. We believe that liver transplants should be proactively performed in patients with GSD Ib. Although the success rate of liver transplantation for hepatic GSD in this study was lower than that reported abroad,[24,46–49] the low success rate of liver transplants may be attributed to the severe liver failure in the fatal GSD cases before transplantation.

In conclusion, we discussed the diagnosis, treatment and long-term outcome of hepatic GSDs and the present status of hepatic GSD patients in Japan. We found a characteristic genetic pattern with many GSD Ia patients presenting with the g727t mutation and GSD Ib patients showing the W118R mutation. Although patients with hepatic GSD, except for those with GSD IV, develop a variety of symptoms, they can survive in the long-term by diet therapy, corn starch treatment and supportive care. Liver transplantation is an important therapeutic strategy for hepatic GSD and can help improve the patients' QOL.

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ORIGINAL ARTICLE

Clinical features and management of organic acidemias in Japan

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Organic acidemias (OAs) are rare inborn errors of metabolism. The clinical presentations of methylmalonic acidemia (MMA) and propionic acidemia (PA) in Japan have not yet been examined in detail. We aimed to investigate the clinical presentations of OAs in Japan and evaluate current therapies for improving long-term outcomes, especially in MMA and PA cases. Questionnaires were sent to 928 institutions in 2009 inquiring about OAs, and secondary questionnaires were sent to those who confirmed that they had diagnosed and/or treated such cases; 119 cases were eventually included for analysis. In Japan, the majority of OAs was MMA, which was associated with a high mortality rate. The survival rates at 20 years of age in vitamin B12-unresponsive MMA, vitamin B12-responsive MMA and PA patients were 69.8%, 94.4% and 95.8%, respectively. Factors associated with mortality in MMA were failure to thrive, hypoglycemia and pancreatitis. Factors associated with mental retardation in vitamin B12-unresponsive MMA, vitamin B12-responsive MMA, and PA were seizure and liver dysfunction, seizure and failure to thrive, and failure to thrive, respectively. We advocated that avoiding failure to thrive due to too restricted protein diet, hypoglycemia and pancreatitis associated with mortality lead to improve outcome, especially in vitamin B12-unresponsive MMA patients.
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INTRODUCTION

Organic acidemias (OAs) are rare inborn errors of metabolism that can be life-threatening and have severe complications.[1] In Japan, studies have focused on the long-term outcomes of inherited metabolic diseases, such as urea cycle disorders and glycogen storage disease.[2,3] As OAs usually present with common symptoms due to metabolic acidosis, early diagnosis and appropriate treatment are essential for obtaining optimal outcomes. In the diagnosis of OAs, gas chromatography mass spectrometry (GC/MS) is typically used to analyze urinary organic acids. Furthermore, in recent years, tandem mass spectrometry (MS/MS) has also become available for newborn screening (NBS) and advanced metabolic tests, which are performed as indicated based on family history and/or routine laboratory test results.[4–8]

Methylmalonic acidemia (MMA) and propionic acidemia (PA) are the most frequent types of organic acid metabolism disorders and are inherited in an autosomal recessive pattern. These conditions are caused by severe inborn errors of catabolism of the amino acids isoleucine, valine, methionine and threonine, as well as metabolism of odd-chain fatty acids and cholesterol side chains. MMA is characterized by an abnormal accumulation of methylmalonyl-CoA and methylmalonic acid. It is caused by a defect of the methylmalonyl-CoA mutase (MCM) or a defect in the synthesis of 5′-deoxyadenosylcobalamin, which is the cofactor of MCM (cblA, cblB, cblD).[1] Moreover, some disorders involving methylcobalamin metabolism result in MMA and homocysteinuria (cblC, cblD, cblF). The deficiencies of MCM are subdivided into mut^0, with complete loss of MCM activity, and mut$^-$, with partial residual activity. Clinically, vitamin B12-responsive MMA is considered when a patient with MMA responds effectively to vitamin B12 supplementation and exhibits a defective synthesis of cobalamin and the presence of a part of mut$^-$. In contrast, vitamin B12-unresponsive MMA is considered when a patient with MMA responds poorly to vitamin B12 supplementation and exhibits the presence of mut^0 or a residual part of mut$^-$. PA is characterized by an abnormal accumulation of propionic acid and metabolites such as methylcitrate, 3-hydroxypropionic acid and propionyl glycine. It is caused by a defect of the enzyme propionyl-CoA carboxylase (PCC), which converts propionyl-CoA to methylmalonyl-CoA.

The clinical presentations of MMA and PA can be similar in many ways. Most patients with the early-onset type present in the neonatal

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period, within 1 month after birth, with nonspecific signs such as appetite loss, recurrent vomiting, lethargy, hypotonia and tachypnea. Some patients have more severe complications such as hyperammonemia, seizure, deep coma and death. Patients with the late-onset type have similar clinical pictures as the early-onset type and can be diagnosed with failure to thrive and/or developmental delay. Regardless of the time of onset, decompensation, manifesting as impaired consciousness, can be life-threatening and indicates a severe clinical condition.[5]

Treatment options include a restricted protein diet to reduce the levels of harmful amino acids in affected patients. Moreover, administration of antibiotics can result in a reduction in the propionate produced from gut bacteria. Arginine and sodium benzoate are also prescribed to treat hyperammonemia. Hemodialysis must be implemented without delay. Carnitine supplementation is effective to release trapped CoA and prevent secondary carnitine deficiency.[9] However, as there is no specific therapy for MMA and PA, patients who recover from decompensation often experience neurologic sequelae. In the present study, we aimed to investigate the clinical presentations of organic acidemias in Japan and evaluate current therapies for improving long-term outcome, especially in cases with MMA and PA.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Faculty of Life Science, Kumamoto University. In 2009, a questionnaire was sent to 928 institutions, including the Departments of Pediatrics, Neonatology, Genetics, and Transplant surgery, asking doctors if they diagnosed or provided medical care to OAs patients. In response, 201 institutions confirmed that they had diagnosed and/or treated inborn errors of metabolism. A secondary detailed questionnaire concerning OAs was then sent to these 201 institutions, of which 41 responded. In 2012, we conducted follow-up research to reveal outcome. In total, 119 cases of OAs were collected. The definition of clinical manifestations of OAs used in this study was the same as that previously described.[3] In addition, mental retardation was diagnosed if the patient's intelligence quotient was <70, in standardized tests, such as the Wechsler Intelligence Scale for Children and the Wechsler Adult Intelligence Scale.

Statistical analysis

Data were analyzed using IBM SPSS statistics 21.0. Descriptive and inferential statistics were used in this study. Significance of differences was assessed using the Mann–Whitney *U*-test or Kruskal–Wallis test. For data with small values, the Fisher exact test was used for a more precise *P*-value. Independence was tested using the chi-square test. Mortality rates were compared using Kaplan–Meier analysis with the logrank test.

RESULTS

General characteristics

Characteristics of affected individuals were shown in Table 1. There were 17 deaths, including 10 patients with vitamin B12-unresponsive MMA (58.8%), 2 with vitamin B12-responsive MMA (11.8%) and 1 with MMA whose response to vitamin B12 was unknown (Table 1). The deceased patients comprised 13 of 65 MMA patients and 2 of 30 PA patients. There was statistical difference in survival rates. In 2012, at a subsequent follow-up, the median age of all patients with OAs was 128 months (interquartile range (IQR), 68.3–191.8). There was no significant difference in age distribution between MMA and PA patients. The median age of the 17 deceased patients was 11 months (IQR, 11–84 months; range, 5 days to 23 years). There was also no significant difference in sex between MMA and PA patients. The number of all male OA patients was 55 (46%). In Table 2, characteristics of onset and diagnosis of OAs were shown.

Table 1 Characteristic of affected individuals

	N *(%)*	*Death case (%)*
Methylmalonic acidemia		
Vitamin B12-unresponsive	42 (35.2)	10 (58.8)
Vitamin B12-responsive	20 (16.8)	2 (11.8)
Unidentified on response to vitamin B12	3 (2.51)	1 (5.9)
Propionic acidemia	30 (25.1)	2 (11.8)
Holocarboxylase synthetase (HCS) deficiency	6 (5.0)	—
Glutaric aciduria type II	5 (4.0)	1 (5.9)
Isovalericacidemia	3 (2.5)	—
3-Methylcrotnyl-CoA carboxylase (3MCC) deficiency	3 (2.5)	1 (5.9)
Glutaric aciduria type I	2 (1.7)	—
Multiple carboxylase deficiency	2 (1.7)	—
Ethylmalonic aciduria	1 (0.8)	—
β-Ketotiolase deficiency	1 (0.8)	—
3-Hydroxy-3-methylglutaric aciduria	1 (0.8)	—
Total	119	17

Table 2 Characteristics of onset and diagnosis of OAs

	N *(%)*
(a) Onset	
First month of life	48 (44)
1 month–1 year	34 (31)
Older than 1 year	13 (12)
Total	95[a]
(b) Diagnosis	
Within a month	72 (74)
1 month–1 year	19 (20)
Over 1 year	6 (6)
Total	97

[a]Fifteen in 110 cases did not have an onset and were diagnosed by newborn screening or advanced metabolic tests.

NBS by MS/MS and advanced metabolic tests were used to diagnose 11 and 4 cases, respectively (vitamin B12-responsive MMA, 1 and 3 cases; vitamin B12-unresponsive MMA, 1 and 0 case; PA, 7 and 1 cases; HCS deficiency, 1 and 0 case; and 3MCC deficiency, 1 and 0 case, respectively). In those cases, 12 cases were already treated with some kinds of drugs before onset (vitamin B12-responsive MMA, 4; vitamin B12-unresponsive MMA, 1; PA, 6; HCS deficiency, 1; and 3MCC deficiency, 0). There was no significant difference in the age of onset between MMA and PA patients; however, patients with vitamin B12-responsive MMA had a lower rate (33%) of onset within a month of life (data not shown).

We received 116 valid responses concerning the method(s) of diagnosis. There were similar diagnostic tools available in each institution. Nearly all patients ($n=113$, 97.4%) underwent analysis of urinary organic acids using gas chromatography mass spectrometry (GC/MS). Other available methods used were tandem mass spectrometry (MS/MS) ($n=38$, 32.8%), enzyme activity measurement ($n=47$, 40.5%) and genetic analysis ($n=16$, 13.8%). Three cases of PA were diagnosed using enzyme activity measurement, genetic analysis and MS/MS without GC/MS. In this study, the detailed results of enzyme activity measurement and genetic analysis could not be collected; however, in previous reports, some kinds of OAs had

relationship between genotype and phenotype. Table 2 shows the interval time required for diagnosis. Of 97 valid responses, 72 cases (74.2%) were diagnosed within a month from onset, 19 cases (19.6%) from 1 month to 1 year and 6 cases (6.2%) took over a year. These last six cases included two of vitamin B12-unresponsive MMA (one patient died at 12 years of age) and one each of vitamin B12-responsive MMA, PA, glutaric aciduria type I and ethylmalonic aciduria.

Clinical characteristics and manifestations

In MMA and PA, clinical and laboratory findings noted through the patients' clinical course are shown in Table 3a. Most patients had a history of vomiting and appetite loss. In contrast, hypoglycemia and seizure were observed in fewer than 30% of all cases. Liver dysfunction were found in many cases of vitamin B12-unresponsive MMA (23/42, 54.8%, $P<0.05$), but fatty liver and hepatomegaly were not statistically significant in this group (17/42, 40.5% for each). Furthermore, renal dysfunction was more likely to occur in MMA, regardless of the response to vitamin B12, than in PA ($P<0.05$).

Mental retardation is a characteristic clinical feature in OAs. Patients with vitamin B12-unresponsive MMA were significantly more likely to experience mental retardation compared with those with vitamin B12-responsive MMA and PA ($P<0.05$). Factors associated with mental retardation are shown in Table 3a. In patients with vitamin B12-unresponsive MMA, factors associated with mental retardation were seizure ($P<0.05$), liver dysfunction ($P<0.01$), fatty liver ($P<0.01$) and hepatomegaly ($P<0.01$). In patients with vitamin B12-responsive MMA, associated factors were failure to thrive ($P<0.05$) and seizure ($P<0.05$). In patients with PA, associated factors were failure to thrive ($P<0.05$). In addition, liver dysfunction, fatty liver, hepatomegaly and failure to thrive as associated factors were significant indicators regardless of suffering from seizure. Factors associated with mortality are shown in Table 3a. Failure to thrive ($P<0.05$), pancreatitis ($P<0.05$) and hypoglycemia ($P<0.01$) were significantly associated with deceased patients of MMA. The median age of the 10 deceased patients of MMA with failure to thrive was 55 months (IQR, 17–83; range, 2 months to 276 months). Failure to thrive, pancreatitis and hypoglycemia as associated factor with mortality were not significantly related to the maximum value of blood ammonium and blood pH value at onset and seizure, respectively ($P>0.05$). The maximum values of blood ammonium at onset are shown in Figure 1a. The median blood ammonium values of vitamin B12-unresponsive MMA ($n=18$), vitamin B12-responsive MMA ($n=8$) and PA ($n=10$) patients were 115 μmol l^{-1} (IQR, 76–343), 103 μmol l^{-1} (IQR, 74–169) and 120 μmol l^{-1} (IQR, 72 −354), respectively. There was no significant difference in the blood ammonium values between the groups. In cases of mortality and mental retardation, there was no significant difference in the maximum values of blood ammonium at onset among those with MMA. The blood pH values at onset are shown in Figure 1b. Within the limit of valid data, the median blood pH values of vitamin B12-unresponsive MMA ($n=18$), vitamin B12-responsive MMA ($n=7$) and PA ($n=9$) patients were 7.15 (IQR, 7.03–7.20), 7.31 (IQR, 7.22–7.36) and 7.23 (IQR, 7.11–7.37), respectively. Patients with vitamin B12-unresponsive MMA were more likely to have a lower blood pH, without a statistically significant difference, compared with vitamin B12-responsive MMA or PA, but with a statistically significant difference, compared with both vitamin B12-responsive MMA and PA ($P<0.05$). In cases of mortality and mental retardation, there was no significant change in blood pH values at the onset of MMA. In addition, there was no significance in outcomes between mental retardation and blood pH values at onset.

Table 3 The features of clinical and laboratory findings, managements in chronic phase

(a)

Clinical findings (%)	*Vitamin B12-unresponsive MMA* N = *42*	*Vitamin B12-responsive MMA* N = *20*	*PA* N = *30*
Vomiting**	95.2 (40/42)	70.0 (14/20)	63.3 (19/30)[a]
Appetite loss**	95.2 (40/42)	65.0 (13/20)	66.7 (20/30)[b]
Seizure	21.4 (9/42)[b]	20.0 (4/20)[b]	16.7 (5/30)
Mental retardation*	69.0 (29/42)	40.0 (8/20)	43.3 (13/30)
Visual impairment	2.4 (1/42)	5.0 (1/20)	6.7 (2/30)
Liver dysfunction**	54.8 (23/42)[a]	25.0 (5/20)	20.0 (6/30)
Fatty liver	40.5 (17/42)[a]	10.0 (2/20)	13.3 (4/30)
Hepatomegaly	40.5 (17/42)[a]	10.0 (2/20)	16.7 (5/30)
Renal dysfunction	35.7 (15/42)	10.0 (2/20)	6.7 (2/30)
Heart failure	7.1 (3/42)	5.0 (1/20)	10.0 (3/30)
Compromised	7.1 (3/42)	5.0 (1/20)	0
Failure to thrive**[c]	69.0 (29/42)	30.0 (6/20)[b]	30.0 (9/30)[b]
Pancreatitis[c]	4.8 (2/42)	0	3.3 (1/30)
Laboratory findings			
Hypoglycemia[d]	28.6 (12/42)	10.0 (2/20)	13.3 (4/30)
Hyperuricemia	31.0 (13/42)	5.0 (1/20)	13.3 (4/30)
Neutropenia/ thrombocytopenia	26.2 (11/42)	10.0 (2/20)	13.3 (4/30)

(b)

Drugs (%)	*Vitamin B12-unresponsive MMA* N = *25*	*Vitamin B12-responsive MMA* N = *15*	*PA* N = *19*
L-carnitine	100 (25/25)	86.7 (13/15)	94.8 (18/19)
Arginine	0	0	5.3 (1/19)
Sodium benzoate	0	6.7 (1/15)	5.3 (1/19)
Vitamin B12	28 (7/25)	93.3 (14/15)	5.3 (1/19)
Metronidazole	40 (10/25)	6.7 (1/15)	5.3 (1/19)
Urinary alkalizer [e]	56 (14/25)	6.7 (1/15)	15.8 (3/19)
Low-protein diet (%)			
At 2 weeks after onset	88 (22/25)	38.5 (5/13)	46.7 (7/15)
Chronic phase	93 (14/15)	25 (3/12)	50 (8/16)

Independence was tested by chi-square test. On small numbers, data were analyzed by Fisher exact test for a more precise *P*-value.
[a]Related factor to mental retardation on MMA, PA ($P<0.01$).
[b]Related factor to mental retardation on MMA, PA ($P<0.05$).
[c]Related factor to mortality on overall MMA ($P<0.05$).
[d]Related factor to mortality on overall MMA ($P<0.01$).
[e]Urinary alkalizer: ularyt, sodium bicarbonate.
*$P<0.05$, **$P<0.01$.

Treatment and outcome

We investigated the required intensive care treatments, such as hemodialysis, exchange transfusion, peritoneal dialysis, respirator use and catecholamine administration, during the acute phase. In total, 40.0% (12/30), 13.3% (2/15) and 23.8% (5/21) of patients with vitamin B12-unresponsive MMA, vitamin B12-responsive MMA and PA, respectively, required intensive care treatments. Vitamin B12-

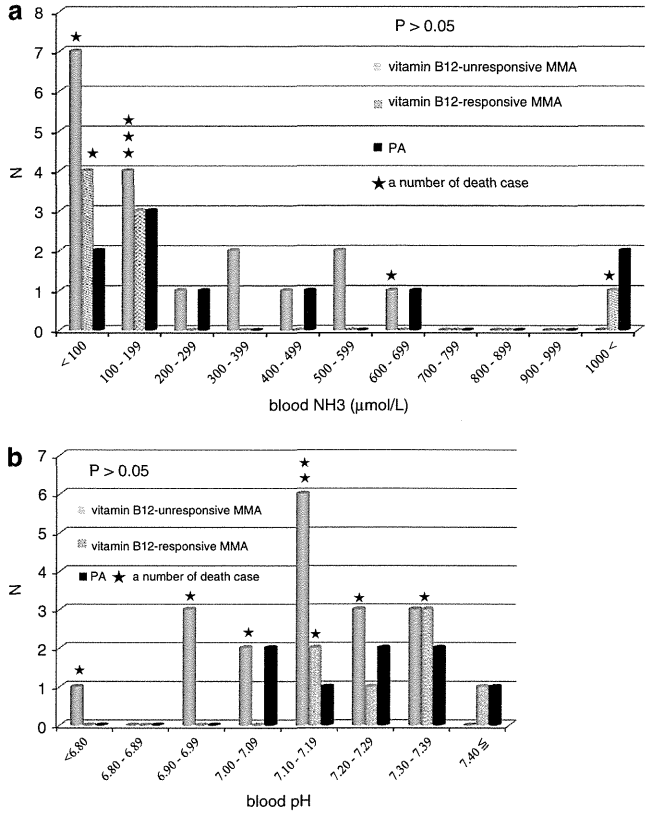

Figure 1 Histogram of methylmalonic acidemia (MMA) and propionic acidemia (PA) patients with different blood ammonium values ($\mu mol\,l^{-1}$) (**a**) and blood pH values (**b**) at onset. (**a**) Histogram of MMA and PA patients with different blood ammonium values ($\mu mol\,l^{-1}$) at onset. A total of 36 patients had available data. Over 50% had blood ammonium levels $<200\,\mu mol\,l^{-1}$. Most of the deceased patients did not have significantly high blood ammonium levels ($P>0.05$). There was no significance between the groups, after analysis by the Kruskal–Wallis test ($P>0.05$). (**b**) Histogram of MMA and PA patients with different blood pH values at onset. A total of 33 patients had available data. Vitamin B12-unresponsive MMA patients had lower blood pH compared with vitamin B12-responsive MMA and PA patients. Blood pH values did not have relationship between the deceased MMA patients and survived MMA patients ($P>0.05$). There was no significance between groups on analysis with the Kruskal–Wallis test ($P>0.05$).

unresponsive MMA patients were significantly more likely to undergo intensive care treatment ($P<0.05$). In these patients, hemodialysis was performed in 16.7% (5/30), exchange transfusion in 16.7% (5/30), peritoneal dialysis in 20.0% (6/30), respirator use in 20.0% (6/30) and catecholamine administration in 17.2% (5/30) of cases. Among all the OAs, deceased patients required hemodialysis, exchange transfusion or peritoneal dialysis significantly ($P<0.05$).

Table 3b shows the management for the chronic phase, including the administration of medications and a low-protein diet. L-Carnitine was prescribed for almost all patients (94.9%) of MMA and PA. Arginine and sodium benzoate was seldom prescribed for MMA and PA patients (0%, 2.5% for MMA and 5.3%, 5.3% for PA, respectively). Vitamin B12-responsive MMA patients were treated with vitamin B12, except for one patient who underwent liver transplantation. Metronidazole and a combination of sodium bicarbonate and citrate were more often prescribed to vitamin B12-unresponsive MMA patients at rates of 40% and 56%, respectively, compared with vitamin B12-responsive MMA and PA. With regard to

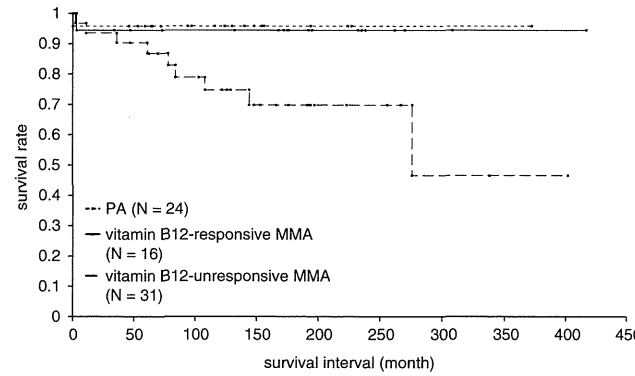

Figure 2 Survival rates in patients with methylmalonic acidemia (MMA) and propionic acidemia (PA). The survival rates of MMA and PA patients without liver transplantation are illustrated as Kaplan–Maier curves. The survival rates of PA, vitamin B12-responsive MMA and vitamin B12-unresponsive MMA patients at 20 years of age were 95.8, 94.4 and 69.8%, respectively. Significant values were revealed by logrank testing between groups ($P<0.05$).

nutrition management, we assessed the administration of a restricted protein diet at 2 weeks after onset and during the chronic phase (Table 3b). Vitamin B12-unresponsive MMA patients were given a strictly restricted protein diet, in comparison with vitamin B12-responsive MMA and PA patients. During the chronic phase, feeding support, such as a naso-gastric tube and gastrostomy tube, was required in 40.0% of vitamin B12-unresponsive MMA, 20.0% of vitamin B12-responsive MMA and 23.5% of PA patients. The survival rate was compared between MMA and PA using the Kaplan-Meier curves (Figure 2). There was valid data for the outcomes of 31 vitamin B12-unresponsive MMA, 16 vitamin B12-responsive MMA and 24 PA patients. Ten vitamin B12-unresponsive MMA patients died due to severe acidosis ($n=3$), cardiomyopathy ($n=1$) and unknown causes ($n=6$). Of the 65 MMA patients with valid data regarding liver transplantation, 13 received liver transplantation (11 vitamin B12-unresponsive MMA patients and 2 vitamin B12-responsive MMA patients). In addition, six PA patients underwent liver transplantation. As the number of liver transplantation cases was limited in this study, the survival rate was estimated in non-transplanted cases. The survival rate of vitamin B12-unresponsive MMA was significantly lower than those of vitamin B12-responsive MMA and PA. In vitamin B12-unresponsive MMA patients, the survival rate gradually decreased to 69.8% by the age of 144 months, following which the survival rate was steady until 276 months. Moreover, the survival rate at 10 years of age in the early-onset type and the late-onset type of vitamin B12-unresponsive MMA were 61.0% and 81.0%, respectively, which was no significant difference between the types. In contrast, the survival rates at 20 years of age in vitamin B12-responsive MMA and PA patients were 94.4% and 95.8%, respectively.

DISCUSSION

OAs are often life-threatening immediately after disease onset and can result in severe sequelae.[1] These rare diseases require further investigation to improve the current management and outcomes of OAs. This study revealed the clinical features of OAs in Japan, especially in cases with MMA and PA. We found that the majority of patients with OAs had MMA, followed by PA, together accounting for ~80% of all OAs in Japan.

There are several limitations to this study. First, most patient information available was restricted to neonates, infants and children. Second, tandem mass spectrometry (MS/MS) was not used as a general screening tool in all OAs. Since the early 1990s, MS/MS has been considered an effective tool for diagnosing inborn errors of metabolism.[4,10,11] In Japan, a pilot study examining the use of MS/MS was started in 1997; however, MS/MS is just becoming a part of government screening in recent years. In the present study, 15 cases of OAs were diagnosed by NBS using MS/MS or advanced metabolic tests before onset. Seven possible mild PA patients could be involved in this study. Third, there may have been some bias as most patients with OAs were managed by specialists in inherited metabolic diseases, and therefore might have good outcomes. It is possible that there were some patients who received limited treatment and/or management and others who were not diagnosed; however, in Japan, patients with OAs are typically referred to specialists in inherited metabolic diseases.

Blood glucose as an important indicator of a poor prognosis in MMA and PA

Previous studies report that the majority of MMA and PA patients were diagnosed in the neonatal period.[12–15] In the present study, the majority of MMA and PA patients had an early onset, and almost all of the remaining patients manifested clinical signs within the first year of life. OAs are characterized by common symptoms, possibly leading to decompensation.[1,15] The present study indicated a significant relationship between hypoglycemia and mortality in vitamin B12-unresponsive MMA patients. Hypoglycemia was not a common feature of MMA and PA, but it was a significant prognostic factor of mortality in vitamin B12-unresponsive MMA patients. Hypoglycemia might be induced by graduated severity of decompensation because it was the results of decompensation regarding glucose metabolism. Furthermore, hyperglycemia in MMA patients was also indicative of a poor prognosis.[16,17] Thus, for careful monitoring of MMA and PA, blood glucose is an important marker.[17]

Difference in the management of patients with MMA and PA

Aggressive, broad therapy is often necessary to treat OAs at onset because of the possibility of severe sequelae and death. Affected individuals should be referred to a metabolic center where intensive care is available.[18–20] In the present study, the frequency of intensive care treatment was greater in MMA patients than in PA patients. Hemodialysis, exchange transfusion or peritoneal dialysis were used significantly in all mortality cases. Furthermore, in the present study, L-carnitine was prescribed for almost all affected individuals, which is similar to the recommended treatment.[14,19,21] The importance of preventing secondary carnitine deficiency is relatively well understood in the management of OAs. Moreover, it is important to intake adequate amount of protein while managing nutrition.[22] When affected patients are in a state of decompensation, a strict restrictive protein diet is necessary for recovery. However, after recovery from the decompensation, infants and children require sufficient protein for growth. In the present study, many vitamin B12-unresponsive MMA patients were given a restricted protein diet, and 70% of them were found to have failure to thrive, which is likely a result of the limited diet.

Clinical features associated with mental retardation and mortality in MMA and PA

Failure to thrive was associated with mortality in patients with MMA. Because the 10 deceased patients of MMA with failure to thrive involved three infants under the 12 months of age, failure to thrive was important factor to management for not only after infant period but also during infant period. Furthermore, failure to thrive as associated factor with mortality was not related to blood ammonium value and pH value at onset, and seizure. Vitamin B12-responsive MMA and PA patients received a restricted protein diet less frequently than vitamin B12-unresponsive MMA. However, failure to thrive was significantly related to mental retardation in vitamin B12-responsive MMA and PA patients. In MMA patients, seizures were associated with mental retardation regardless of the response to vitamin B12, similarly to a previous report.[23] Moreover, liver dysfunction, hepatomegaly and fatty liver were related to mental retardation in vitamin B12-unresponsive MMA patients, but not to mortality. In addition, these associated factors to mental retardation were indicators regardless of suffering from seizure. In this study, because of limited valid data, influence of the blood ammonium value and pH value at onset on the clinical feature as associated factors to mental retardation was unclear. In PA patients, vomiting and appetite loss were related to mental retardation significantly. However, those were common and nonspecific symptoms in metabolic disease.

The majority of deaths occurred in vitamin B12-unresponsive MMA patients. Therefore, there was significant difference in mortality rates between vitamin B12-unresponsive MMA, vitamin B12-responsive MMA and PA patients. Concerning treatment of OAs, more MMA patients required intensive care treatment compared with PA patients, especially hemodialysis, exchange transfusion or peritoneal dialysis were used significantly in all mortality cases. There was no significance found in blood ammonium values at the onset in MMA and PA patients. In comparison, progressive acidosis was observed in vitamin B12-unresponsive MMA patients, who had significantly lower blood pH compared with patients with vitamin B12-responsive MMA and PA ($P<0.05$). This was likely due to the effects of toxic metabolites such as methylmalonate on secondary mitochondrial dysfunction, as previously reported.[15,24]

Outcome

We analyzed the outcomes of MMA and PA patients using Kaplan-Maier curves (Figure 2). Because of improving therapies including nutrition management, drugs and hemodialysis, the survival rates of MMA and PA patients have increased in comparison with previous reports. In the present study, there was a significant difference in mortality among vitamin B12-unresponsive MMA, vitamin B12-responsive MMA and PA patients. The survival rate in vitamin B12-unresponsive MMA patients gradually decreased to 69.8% by the age of 144 months. In contrast, that in PA patients at 20 years of the age was 95.8%. MMA was much severe than PA, but clinically classical PA was more severe than or at least as severe as vitamin B12-unresponsive MMA. In this study, though the survival rate in PA patients was better than that of vitamin B12-unresponsive MMA, PA patients could involve seven possible mild PA patients. Those possible mild form PA patients might have the mild form specific mutation (Y435C) in the *PCC* gene and might not develop acute decompensation.[25] Because of including mild PA patients in analysis, the survival rate of PA might be higher than that excluding mild PA patients. In the future, as the use of NBS with MS/MS increases, it is our hope that the mortality rates of MMA and PA patients will continue to decrease as previously described.[5] Furthermore, factors associated with mental retardation and death may affect the indications for liver transplantation.