was born to parents who are first cousins and he is the first child to the parents. The development at 11 months was appropriate. Ten days prior to the admission he had diarrhea and dehydration and was treated with intravenous fluids. Following 2 days of febrile episodes, he developed tachypnea, poor perfusion and tachycardia, and unconsciousness. Initial laboratory tests indicated metabolic acidosis with an arterial pH of 6.9, Pco_2 of 10 mmHg, and bicarbonate level of 4.4 mM. The blood glucose was low at 1.8 mmol/L. Urine ketones were strongly positive (180 mg/dL). Serum lactate was normal. On the second hospital day, he had generalized tonic–clonic seizures and was treated with levetriacetam 20 m/kg and then started on maintenance dose of 10 mg/kg/dose. Brain CT showed hypodensities in the bilateral lentiform nucleus and caudate head, suggestive of metabolic encephalopathy (Fig. 1). The child was intubated and kept on a ventilator on the second hospital day. There was no improvement with sodium bicarbonate correction and the child was put on dialysis for 2 days. Fluid and electrolyte balance was maintained and the child received a glucose infusion stepwise in 2 mg/kg/min increments up to 12–15 mg/kg/min with monitoring of blood glucose levels. Following dialysis, the biochemical parameters improved. The child was extubated on the fourth hospital day. Acylcarnitine analysis showed a C5OH concentration of 3.08 μM (cut-off value 1.0) and a C5:1 concentration of 1.69 μM (cut-off value 0.3). Urinary organic acid analysis showed elevated levels of 2-methyl-3-hydroxybutyrate, 2-methylacetoacetate, and tiglylglycine. A tentative diagnosis of T2 deficiency was made. The child regressed, with loss of social smile, recognition, and the ability to sit or crawl. Management following the acute stage included a low-protein (1.5 g/kg), high-carbohydrate diet supplemented with 50 mg/kg carnitine. The child was discharged on the 15th hospital day on the same diet with the antiepileptic drug and baclofen for dystonia.

One week after discharge, dystonia of all four limbs, predominant in lower limbs and mild irritability were noted. A month later, irritability subsided and the child could follow objects and started recognizing the parents. Physiotherapy was started. At 15 months of age, social smile with partial head control was attained but central hypotonia persisted. Dystonia of the trunk with intermittent arching was also noted by 15–16 months of age. Trihexyphenydyl was used at dose of 4 mg twice a day. At 18 months, good head control was achieved and the child could sit and stand with support. At 24 months, he could walk with support; social interac-

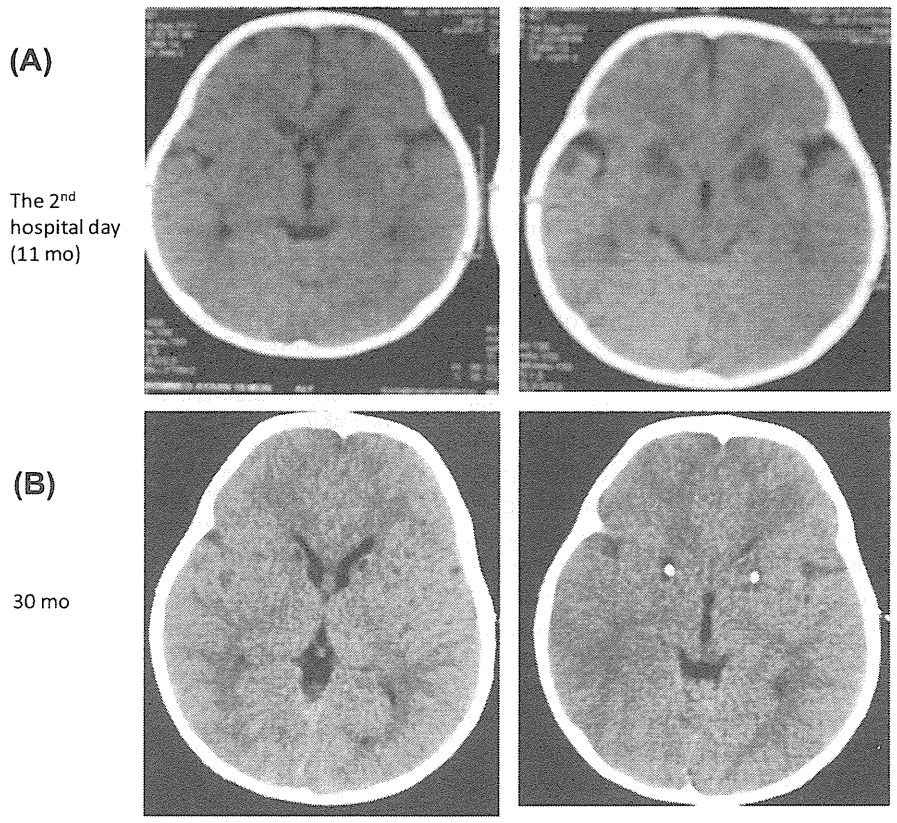

Fig. 1. Brain CT findings in patient GK95. (A) Plain axial images show symmetrical hypodensities involving bilateral basal ganglia, suggestive of metabolic encephalopathy. (B) Plain axial images show bilateral symmetrical calcification involving the anterior part of the lentiform nucleus with surrounding low-density areas.

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tion was good and he was able to say 4–6 two-syllable words. On a recent follow-up, at 30 months of age, speech had improved to 24–30 two-syllable words and the child was toilet trained. Although the child had truncal hypotonia with mild bilateral lower limb dystonia, he was able to walk with support. Social quotient was 96 and developmental quotient was 74 with predominant motor delay. Height was 84 cm (85th centile) and body weight was 13 kg (85th centile). Head circumference was 48 cm (50th centile). Follow-up brain CT showed bilateral calcification in the basal ganglia (Fig. 1).

An acetoacetyl-CoA thiolase assay was done in the absence and presence of potassium ion using cultured fibroblasts. Potassium ion-activated acetoacetyl-CoA thiolase activity was absent in GK95's fibroblasts ($-K^+$ 6.3, $+K^+$ 5.5 nmol/min/mg protein; control fibroblasts $-K^+$ 5.6, $+K^+$ 8.9 nmol/min/mg protein), confirming the diagnosis of T2 deficiency. Mutation analysis was then performed at the genomic level. We identified a homozygous c.578T>G (M193R) mutation. We confirmed that both parents were heterozygous carriers of the mutation. Transient expression of mutant T2 cDNA showed that the M193R mutant retained no residual T2 activity.

3. Discussion

Beta-ketothiolase deficiency, or T2 deficiency, was first described in 1971 [5], and more than 100 patients have been identified worldwide (including unpublished patients). Although most reports have come from Western countries, the Middle East, and Japan [1], T2 deficiency has recently been reported in other countries including China and Vietnam [6]. Furthermore, a possible founder mutation, R208X, was identified in the Vietnamese population [6]. The incidence of T2 deficiency is not yet defined in most populations. Newborn screening by tandem mass spectrometry is now ongoing in several countries and regions. The incidence of T2 deficiency was reported to be 1 in 232 000 over the period January 2001 to November 2010 in one study from Minnesota, USA [4]. A Japanese pilot study found no T2-deficient patients among roughly 2 million newborns screened by tandem mass spectrometry (Yamaguchi et al., unpublished data). However, newborn screening can yield false negative results, especially in individuals with mutations that allow some residual T2 activity [2–4]. Six of seven T2-deficient probands had such "mild" mutations in the Japanese population [2,3]. This is characteristic for the Japanese population. In India, a pilot screening of 5000 newborns from the state of Andhra Pradesh by tandem mass spectrometry detected several disorders but not T2 deficiency [7]. Because most T2-deficient patients can be identified by urinary organic acid analysis or acylcarnitine analysis during acute metabolic decompensation, popularization of these analyses in India may increase the detection of T2 deficiency.

Unconsciousness during severe ketoacidosis is one of the common clinical symptoms of T2 deficiency [1]. "Metabolic stroke-like episodes" comprises acute focal neurological deficits in connection with acute metabolic decompensation and associated focal lesions on brain imaging. Metabolic encephalopathy involving basal ganglia has been reported in other types of organic acidemia such as propionic acidemia and methylmalonic acidemia [8]. The basal ganglia have high energy requirements in childhood and this may make them particularly vulnerable to damage by impaired energy metabolism. Bilateral basal ganglia lesions in T2 deficiency have been reported only in patients GK06 [1,9] and GK70 [6] among our records of about 100 T2-deficient patients; both of these patients presented a severe ketoacidotic crisis. One T2-deficient patient (GK85) showed basal ganglia lesions without apparent severe ketoacidosis, perhaps because of chronic metabolic insufficiency, and presented with non-progressive choreiform movements [10]. The present patient, GK95, is the fourth patient to have basal ganglia lesions among our records. Because this patient showed no neurological problems before the severe ketoacidotic episode, it is likely that the metabolic encephalopathy was a sequela of the severe ketoacidotic episode.

In conclusion, we report a case of T2 deficiency with a novel mutation. This case with T2 deficiency had the uncommon presentation of metabolic encephalopathy with neurological sequela.

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Effects of idursulfase enzyme replacement therapy for Mucopolysaccharidosis type II when started in early infancy: Comparison in two siblings

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ABSTRACT

Mucopolysaccharidosis type II (MPS II) is a lysosomal storage disorder that is progressive and involves multiple organs and tissues. While enzyme replacement therapy (ERT) with idursulfase has been shown to improve many somatic features of the disease, some such as dysostosis multiplex and cardiac valve disease appear irreversible once established, and little is known about the preventative effects of ERT in pre-symptomatic patients. We report on two siblings with severe MPS II caused by an inversion mutation with recombination breakpoints located within the *IDS* gene and its adjacent pseudogene, *IDS-2*. The siblings initiated treatment with idursulfase at 3.0 years (older brother) and 4 months (younger brother) of age, and we compared their outcomes following 2 years of treatment. At the start of treatment, the older brother showed typical features of MPS II, including intellectual disability. After 34 months of ERT, his somatic disease was stable or improved, but he continued to decline cognitively. By comparison, after 32 months of ERT his younger brother remained free from most of the somatic features that had already appeared in his brother at the same age, manifesting only exudative otitis media. Skeletal X-rays revealed characteristic signs of dysostosis multiplex in the older brother at the initiation of treatment that were unchanged two years later, whereas the younger brother showed only slight findings of dysostosis multiplex throughout the treatment period. The younger brother's developmental quotient trended downward over time to just below the normal range. These findings suggest that pre-symptomatic initiation of ERT may prevent or attenuate progression of the somatic features of MPS II. Follow-up in a larger number of patients is required to confirm the additive long-term benefits of ERT in pre-symptomatic patients.

1. Introduction

Mucopolysaccharidosis (MPS) type II (Hunter syndrome, OMIM #309900), is an inborn error of glycosaminoglycan (GAG) metabolism caused by deficient activity of lysosomal iduronate 2-sulfatase (IDS, EC 3.1.6.13). The responsible gene, IDS, is located on chromosome Xq28, and the disease shows classic X-linked recessive inheritance. Rarely, females may be affected as a result of biallelic mutations, skewed X-inactivation, uniparental isodisomy, or X-autosome translocations [1,2]. Dermatan sulfate and heparan sulfate, the substrates for IDS, accumulate in the lysosomes of various tissues and organs of affected patients, leading to the development of characteristic signs and symptoms of MPS II after the first year of life. (HOS reference). Somatic features include coarse facies, straw-like hair, rough and thickened skin, macrocephaly, disproportionate short stature due to dysostosis multiplex, decreased joint mobility, cardiac valve disease and left ventricular hypertrophy, hepatosplenomegaly, obstructive sleep apnea, and restrictive lung disease. Frequent otitis media and hernias (inguinal and umbilical) may be the earliest presenting signs, but are non-specific. Patients with little to no IDS activity (severe form) exhibit progressive somatic disease, cognitive decline, and death during adolescence (HOS). Patients with some residual IDS activity (mild form) have largely somatic disease with normal intellectual development [3].

In recent years, enzyme replacement therapy (ERT) with recombinant human iduronate-2sulfatase (idursulfase, Elaprase®, Genzyme, a Sanofi Company and Shire Human Genetic Therapies, Cambridge, MA) has been available for the treatment of MPS II. Weekly infusions of idursulfase have been shown to improve walking capacity, hepatosplenomegaly, and urinary GAG levels [4]. However, ERT appears to be less effective in correcting disease manifestations once developed in the skeletal system and heart valves [5,6]. Intravenously administered ERT has not been shown to slow or prevent the deterioration of the central nervous system in patients with the severe phenotype, most likely because it does not cross blood–brain barrier at the labeled dose [7]. Although idursulfase is approved for use only in patients who are at least 5 years of age, a recent report from the Hunter Outcome Survey (HOS) suggests that it can safely reduce urinary GAG levels and hepatomegaly in young children, some

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of whom were below 1 year of age [8]. Recognizing that MPS II is a progressive disease that has some irreversible features, a panel of MPS II experts has recommended starting ERT as early as possible to achieve the best outcomes [9].

In this report, we describe our treatment experience in two Japanese brothers with the severe form of MPS II who started ERT at 4 months of age (pre-symptomatic) and 3 years of age (symptomatic). Our findings suggest that early, pre-symptomatic treatment is associated with a better clinical outcome as evidenced by the amelioration or prevention of certain somatic manifestations, e.g. dysostosis multiplex and cardiac valve disease, which once established, appear to be irreversible.

1.1. Case report

1.1.1. Patient 1 (older brother)

A 2 year 7 month old boy presented to our metabolism clinic with dysmorphic features, cardiac and skeletal disease, and severe developmental delay. He was the first child born to non-consanguineous Japanese parents. Following an uneventful pregnancy and neonatal period, he was noted to have a small ventricular septal defect during a febrile illness at 3 months of age. At 9 months of age, the ventricular septal defect had closed but mild mitral valve regurgitation was present. His parents noticed a gibbus deformity at approximately 1 year of age, and by age 2 he had developed stiffness in his elbow and fingers. His psychomotor development was moderately delayed: he walked at 1.5 years and was still non-verbal. Other past medical history was notable for a febrile seizure, umbilical hernia, enlarged adenoids, and bilateral otitis media. On physical examination, the boy had a coarse facies and disproportionately short limbs. His was above average in height (92.2 cm, +0.6 SD), overweight (17.0 kg, +3.2 SD), and had macrocephaly (50 cm, +0.5 SD). He had marked hepatomegaly and a nonpalpable spleen. Urinary GAG analysis revealed an elevated uronic acid level of 254 mg/g creatinine (normal mean ± SD, 30.0 ± 12.8) with increased amounts of dermatan sulfate (63%) and heparan sulfate (12%) relative to chondroitin sulfate (25%), consistent with MPS I or II. The diagnosis of MPS II was confirmed by the absence of detectable I2S activity in leukocytes.

No potential disease-causing mutation was found by sequencing all 9 exons of the IDS gene and their intron-exon junctions by conventional PCR-based methods [10]. To detect a recombination mutation between IDS and its adjacent putative pseudogene, IDS-2, that leads to an inversion and non-functional *IDS* gene, we performed a simple and rapid assay involving two PCR reactions. The first reaction selectively amplifies a 2.8 kb DNA fragment from the recombinant gene but not the wild type IDS gene, while the second reaction selectively amplifies a 3.5 kb DNA fragment from the wild type IDS gene but not the recombinant gene (Fig 1a) [11]. Genetic testing of the patient revealed an abnormal banding pattern indicative of recombination between the IDS gene and the IDS-2 pseudogene (Fig 1b).

1.1.2. Patient 2 (younger brother)

The younger brother was born just after his older brother was diagnosed with MPS II. Birth weight (2.966 kg) and length (47 cm) were normal for his gestational age of 39 weeks. There were no abnormal findings on initial physical examination, but the urinary uronic acid level was elevated at 423 mg/g creatinine (normal mean ± SD, 43.4 ± 12.9), and urinary GAG analysis showed increased amounts of dermatan sulfate (55%) and heparan sulfate (11%) relative to chondroitin sulfate (34%). IDS activity in leukocytes was below the detectable limit. As expected, Patient 2 had the same recombination mutation as his older brother.

1.1.2.1. Enzyme replacement therapy. Treatment with intravenous recombinant idursulfase was started at 3.0 years of age for Patient 1 and 4 months of age for Patient 2. Although the recommended dose of idursulfase is 0.5 mg/kg/week, Patient 1 received only 0.3–0.4 mg/kg/week for the first 1.5 years until his weight reached 20 kg (4.5 years of age) because of a restriction by the health insurance system; subsequently, he received 0.5 mg/kg/week of idursulfase. The dose for Patient 2 was 0.5 mg/kg/week from the start of treatment. As of December 2012, Patients 1 and 2 had received ERT for 34 and 32 months, respectively. Both patients have tolerated ERT well with only mild and intermittant urticaria.

2. Results

2.1. Urinary GAG

The uronic acid in urine was measured at several time points after initiation of ERT using the carbazole reaction method (SRL Medisearch, Tokyo, Japan). Fig. 2 shows the changes observed in both patients over time. In Patient 1, the uronic acid level decreased to approximately half of the baseline level after 3 months and then plateaued at 130–180 mg/g creatinine (29–49% reduction from baseline) (Fig. 2a). The uronic acid level in Patient 2 showed a continuous decrease to below 100 mg/g creatinine (76% reduction from baseline), but remains above the normal range (Fig. 2b).

2.2. Liver and spleen size

The liver edge of Patient 1 extended 4 cm below the right costal margin at baseline, and it rapidly became non-palpable after the initiation of ERT. The spleen was not palpable at any time, and by ultrasound, it was at the upper limit of normal size for age and remained stable during the first 28 months of ERT. Patient 2's liver and spleen were normal in size before and during ERT.

2.3. Cardiac function

At baseline, Patient 1's echocardiogram revealed moderate mitral valve regurgitation and a mildly distorted left ventricular wall, although the ejection fraction was normal at 69 %. These findings showed little change after 22 months of ERT. In Patient 2, no abnormalities were detected by echocardiography before and after 11 months of ERT.

2.4. Respiratory and Hearing

Patient 1 had bilateral exudative otitis media and adenoid hypertrophy at baseline that did not respond well to ERT. Although an adenoidectomy was performed at 3.5 years of age, exudative otitis media and hearing impairment persisted. Patient 2 also had exudative otitis media during the ERT period. Neither patient developed sleep apnea.

2.5. Skeletal X-rays

At baseline, dysostosis multiplex was already apparent in Patient 1. The most prominent findings were hypoplastic changes of the vertebral bodies giving rise to a characteristic protrusion of the antero-inferior surface, the so-called inferior tongue. Other mild signs of dysostosis multiplex included oar-like ribs, bullet-shaped phalanges, and iliac flaring. After 27 months of ERT, these findings showed little change. Similar, but milder findings of oar-like ribs and bullet-shaped phalanges were present in Patient 2 at 3 months of age. After 25 months of ERT, "inferior tongue" had become notable and oar-like ribs had progressed (data not shown).

2.6. Joints

Patient 1 had stiffness in multiple joints of his extremities at baseline. There was no obvious change with ERT, although accurate

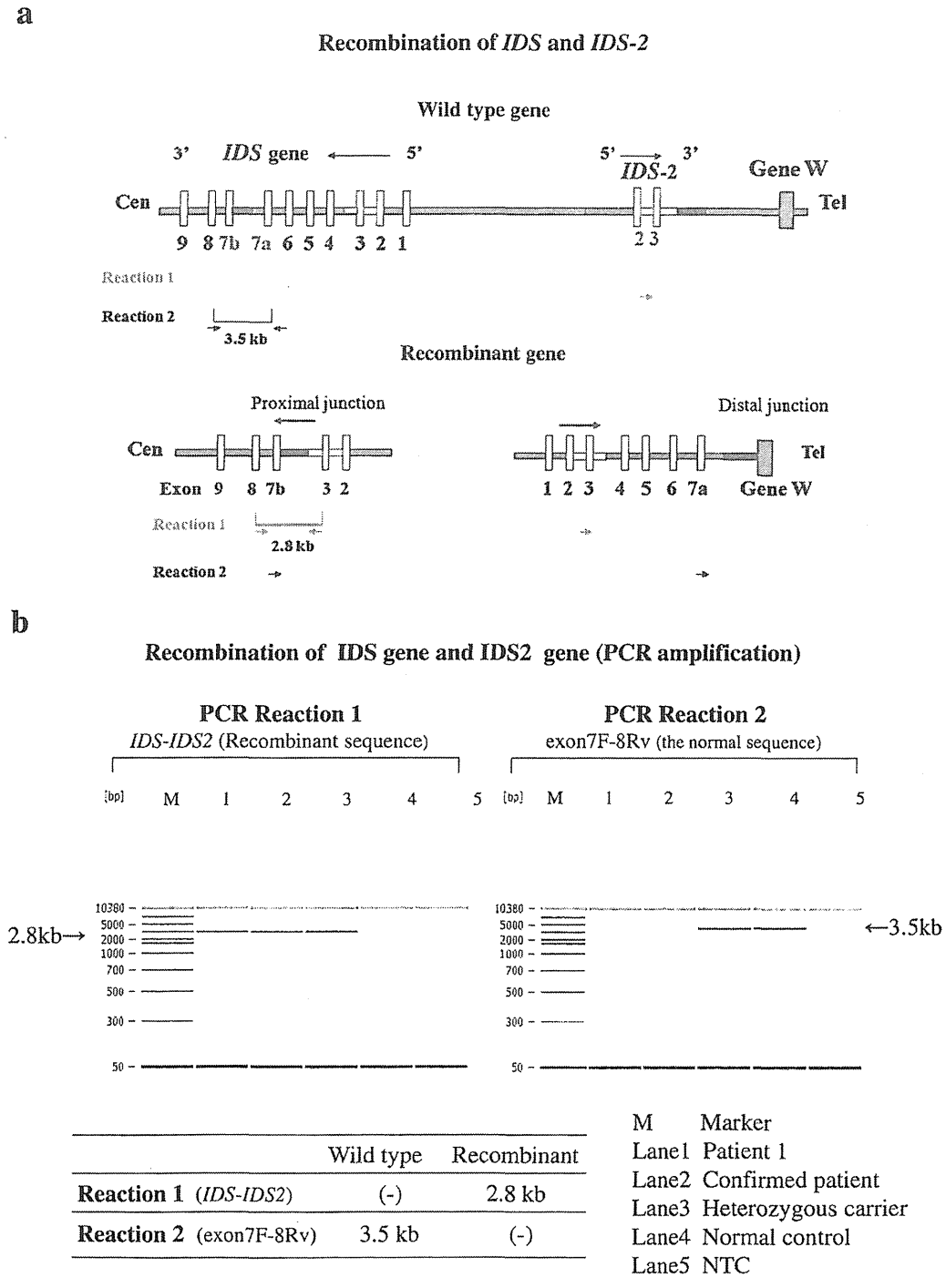
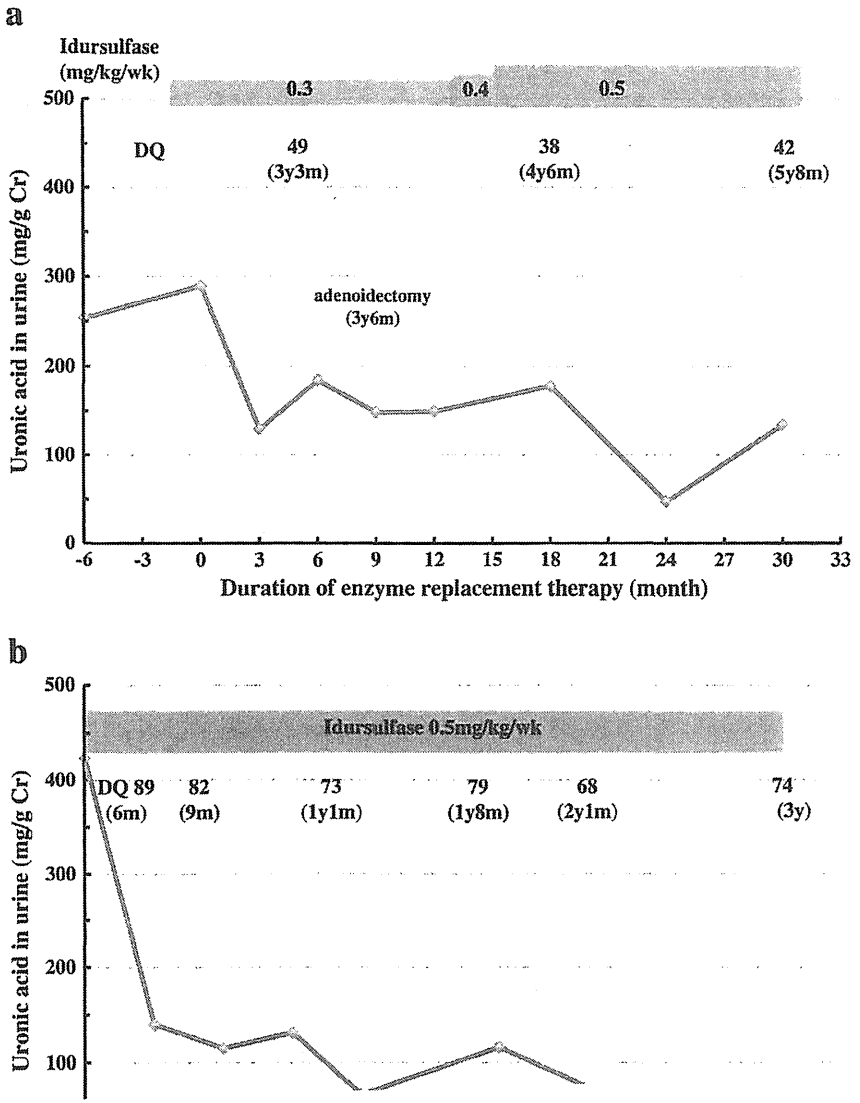


Fig. 1. Genetic diagnosis of MPS II by detecting recombination of the IDS and IDS-2 genes. The pseudogene IDS-2, which consists of sequences that are homologous to exons 2 and 3 and intron 7 of the IDS gene, is located ~20 kb telomeric to *IDS* in Xq27.3–q28. In the recombinant gene, exons 1, 4, 5, 6, 7a are translocated to the IDS-2 locus, thereby grossly altering the structure of the IDS gene. PCR reaction 1 amplified a 2.8 kb fragment of the recombinant gene in Patients 1 and 2 and the heterozygous carrier, but not in the normal control. PCR reaction 2 amplified a 3.5 kb fragment of the wild-type *IDS* gene in the heterozygous carrier and normal control, but not in the two patients.

measurement was difficult. Patient 2 had normal joint mobility that was maintained during ERT.

3. Magnetic resonance imaging of the central nervous system

By MRI, Patient 1 had dilated perivascular spaces in the cerebral white matter both at baseline and after 22 months of ERT, and at the latter timepoint, mild dilatation of the lateral ventricles also was apparent. At baseline, Patient 2's MRI showed only subtle changes in the corpus callosum that were suggestive of dilated perivascular spaces. After 14 months ERT, the dilated perivascular spaces became more typical and resembled those of his brother. Patient 2 did not show any evidence of hydrocephalus or cerebral atrophy (Data not shown).


a
Idursulfase
(mg/kg/wk)
0.3
0.4
0.5
DQ
49
(3y3m)
38
(4y6m)
42
(5y8m)
adenoidectomy
(3y6m)
Uronic acid in urine (mg/g Cr)
500
400
300
200
100
0
-6
-3
0
3
6
9
12
15
18
21
24
27
30
33
Duration of enzyme replacement therapy (month)
b
Idursulfase 0.5mg/kg/wk
DQ 89
(6m)
82
(9m)
73
(1y1m)
79
(1y8m)
68
(2y1m)
74
(3y)
Uronic acid in urine (mg/g Cr)
500
400
300
200
100

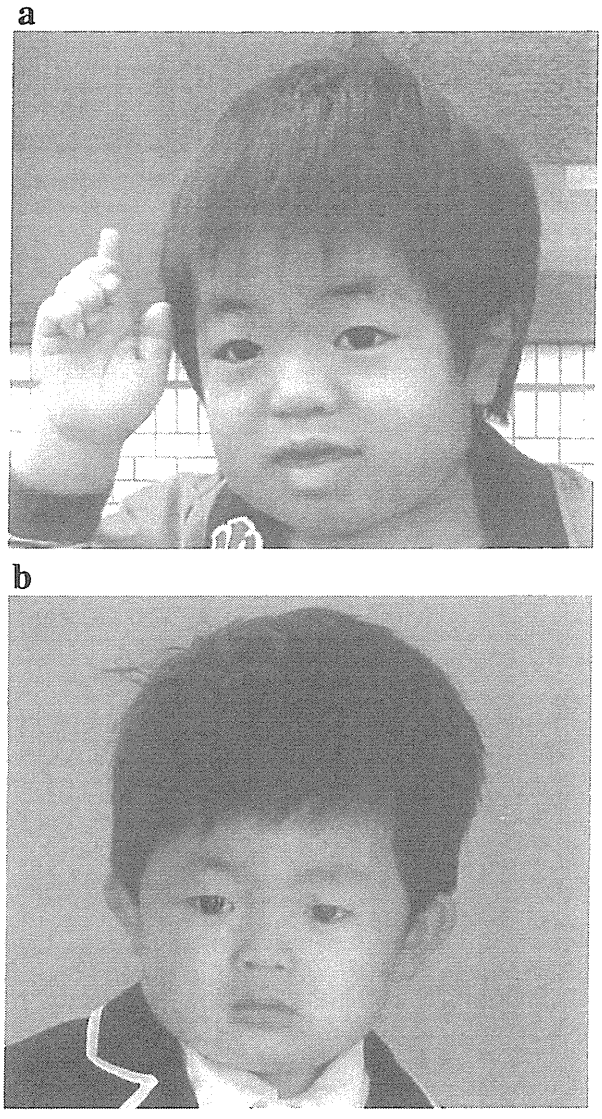


Fig. 3. Facial appearances of the brothers. (a) Patient 1 at the age of 2 years and 9 months, before initiation of ERT. (b) Patient 2 at the age of 2 years and 10 months, after 31 months of ERT.

Patient 1 (Fig. 3a; 2 years and 9 months old). Patient 1 had coarse features affecting his nose, lips, and tongue, whereas Patient 2 did not show any MPS II-related facial features.

4. Discussion

Since idursulfase ERT for MPS II became commercially available (2006 in the US; 2007 in the European Union and Japan), there has been an increasing number of reports on its clinical effects. Idursulfase has been shown to improve walking capacity while reducing hepatosplenomegaly and urinary GAG levels. The most common adverse events have been infusion-related reactions, including some reports of anaphylactic reactions [5]. However, most of the treatment effects described to date have been in patients above age 5 who manifested typical symptoms of MPS II before initiation of ERT [12,13]. The results suggest that once established, pathological changes in certain organs and tissues, e.g. the bones, joints, heart valves, and central nervous system are difficult to correct [9]. A recent analysis of the effects of ERT in patients younger than 6 years old enrolled in the Hunter Outcome Survey (HOS) has shown a similar safety profile and reduction in hepatomegaly as in older patients [8]. Of these 124 children treated ERT, 11 initiated treatment during the first year of life, and the youngest treated was 1 month of age. However, no individual outcome data have been reported.

There is limited information on the ability of idursulfase to prevent the occurrence of disease manifestations in pre-symptomatic MPS II patients. MPS II is difficult to diagnose in early infancy before the development of typical signs and symptoms due to the insidious progression of disease [14,15]. The few patients that have been diagnosed early usually had a previously affected relative that prompted pre-symptomatic testing, as was the case for our siblings. Only one recent case report has described the effects of idursulfase ERT initiated in an asymptomatic infant with MPS II [16]. This boy was diagnosed at 14 days of life on the basis of an older affected sister, who interestingly, was found to have low IDS activity and be heterozygous for a missense mutation, p.Tyr523Cys/c.1568A>G in exon 9, with almost totally skewed X-inactivation of the normal *IDS* gene. Idursulfase (0.5 mg/kg/wk) was initiated at 3 months of life and 3-year follow-up was provided. The affected boy did not develop coarse facial features, joint disease, or organomegaly, and his cardiac function remained normal; the only abnormal finding was a mild deformity of one vertebrae. In contrast, the older sister showed typical clinical features of MPS II when she was diagnosed at age 3, including severe intellectual disability (IQ=50) that worsened over time (IQ=24 at age 10) despite 5 years of ERT. Considering her severe phenotype, it is surprising that her affected brother has maintained a normal IQ of 98 at 3 years of age. An earlier report had described this mutation as mild [17]. It is possible that the sister had other unknown central nervous system complications or effects of skewed X-inactivation that affected her cognitive status, or that the original assignment as a mild mutation was incorrect. Another possibility is that ERT started in early infancy had a protective effect on the central nervous system, but animal data suggest that intravenously administered idursulfase is unable to cross the blood–brain barrier at this dose.

Our experience has been similar to this recent case report, with a better outcome observed when treatment was initiated at 4 months of age instead of at 3 years of age, a difference of 2.7 years. The reduced dose that the older brother received for the initial 15 months of treatment may have contributed to some of the differences in outcomes. Nevertheless, somatic symptoms were present in Patient 1 before 2 years of age, but none were seen in Patient 2 at the same age except for possibly exudative otitis media. The only other somatic finding has been slight signs of dysotosis multiplex by X-ray. The prognosis for his mental development seems less promising, given the gradual decline in DQ from normal to slightly below normal. Although hearing problems due to chronic exudative otitis media may have contributed to the apparent decline in DQ, it has been reported that speech development is less affected in patients with mild compared to severe MPS II despite similar otological findings [18]. The inversion mutation is predicted to be a severe mutation that leads to a non-functional *IDS* gene, and in one series it was present in 13% of boys with MPS II [19]. Treatment options to prevent further deterioration of his intellectual abilities appear limited at this time. Previous reports on the therapeutic effects of hematopoietic stem cell transplantation (HSCT) in MPS II patients have generally been negative, but most patients had pre-existing CNS disease and little clinical data exists on the use of this procedure as a preventative measure in patients with normal cognitive function [20]. According to a recent report, donor-derived cells were detected in the brain of a transplanted MPS II patient [21]. To determine whether HSCT may be beneficial to MPS II patients at risk for CNS involvement, additional data must be collected on cases in which HSCT is performed as early as possible. Intrathecal delivery of ERT to treat MPS II-related CNS disease is currently being investigated in an ongoing Phase 1 clinical trial, and the results have not yet been published.

In summary, this is the second detailed case report of idursulfase ERT started in early infancy in a patient with MPS II. In contrast to

the older brother who had typical features of MPS II at the initiation of ERT that did not completely resolve after 2 years of treatment, we believe that the near absence of somatic findings in the younger brother after 2 years of treatment is attributable to early ERT administered in the pre-symptomatic state. The effect of early ERT on the younger brother's intellectual development is less clear. Long-term observation of these and other similar cases should help to clarify the extent of the preventative effects of ERT on the somatic and CNS aspects of MPS II as well as to define the optimal timing of treatment to achieve the best possible outcomes.

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O-3

ポンペ病の新生児マス・スクリーニング検査の運用

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【はじめに】

ポンペ病は，ライソゾーム酵素の一つである酸性α-グルコシダーゼ（acid α-glucosidase: GAA）の欠損あるいは活性低下により，グリコーゲンが筋肉内に蓄積する疾患である．乳児型ポンペ病は，早期の酵素補充療法が効果的であるため，早期診断が重要である．一方，日本人の約3%に酵素活性値が正常の10～20%を示すが臨床症状を呈さない，Pseudodeficiencyと呼ばれる集団が存在し，ポンペ病との鑑別が問題となる．

当センターでは4MU法とGAA遺伝子のG576S多型解析を併用したポンペ病のスクリーニング法を確立し，新生児マス・スクリーニング検査を運用しているので報告する．

【対　象】

パイロットスタディは2011年1月から4月までに当センターで出生した新生児361人を対象とした．また，2011年5月より有料スクリーニング検査を開始し，希望者1,089名（全出生の76.4%）に行った．

【方　法】

乾燥ろ紙血から血液を抽出後，抽出液中のGAAに4-methylumbellifery-α-D-glucopyranoside（4MUG）を基質として反応させ，加水分解され遊離した4-methylumbelliferon（4MU）の蛍光強度を測定することで，GAA活性を算出した．

一次スクリーニング陽性基準として①GAA活性値が20％未満②阻害率60%以上③pH活性比30倍以上と設定した．また，乾燥ろ紙血を用いてPCRダイレクトシーケンス法によりGAA遺伝子のG576S多型解析を行った．

【結　果】

新生児361人を対象として行われたパイロットスタディによりGAA活性の基準値を40.5 pmol/punch/hr., SDは±16.9と設定した．その後，2011年5月1日から2012年4月30日までに1,089名の有料スクリーニング検査を実施した．その内GAA活性が基準値の20%未満を示しG576S多型解析の対象となったのは21名（1.93%）であった．19名はPseudodeficiencyと確認され，1名はリンパ球での酵素活性測定とGAA遺伝子検査を実施した．

【考　察】

4MU法とGAA遺伝子のG576S多型解析を併用したポンペ病のスクリーニング法を確立し運用している．本法は簡便迅速で，本疾患のスクリーニング検査法として有効と考えた．

【結　語】

4MU法とGAA遺伝子G576S多型解析を併用した新生児マス・スクリーニングを開始した．本法はPsudodeficiencyの鑑別も可能でありポンペ病の新生児マス・スクリーニング検査法として有用である．

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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 ⩾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 ⩾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 ⩾18 years had heights below the third percentile. The mean height of female GSD Ia patients aged ⩾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.

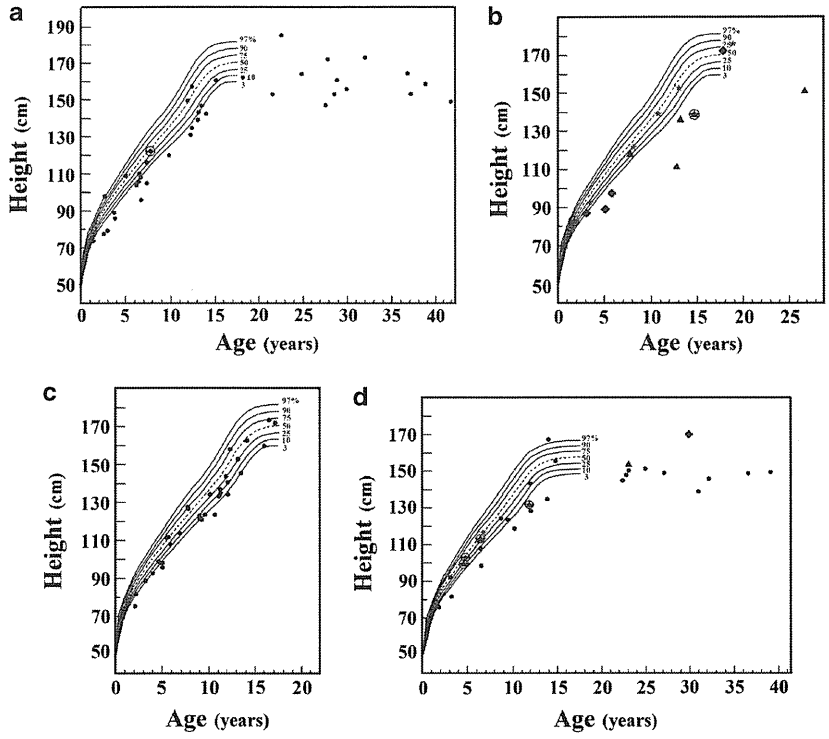

Figure 1 Stature of hepatic glycogen storage disease (GSD) patients. This figure was constructed with the age of GSD patients on the abscissa and the stature of patients with GSD on the ordinate. Percentiles are based on data from Japanese 2000 growth reports provided by the Ministry of Health, Labor 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.

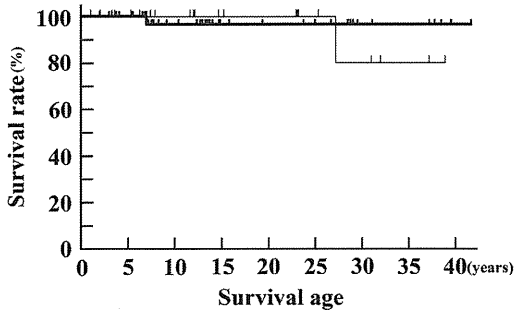

Figure 2 Long-term survival rates in patients with glycogen storage disease (GSD) Ia. The survival rates of 63 patients with different ages are shown by Kaplan–Meier survival curves. Two GSD Ia patients aged 6 years 10 months (male) and 27 years (female) died of liver failure after liver transplant. 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.

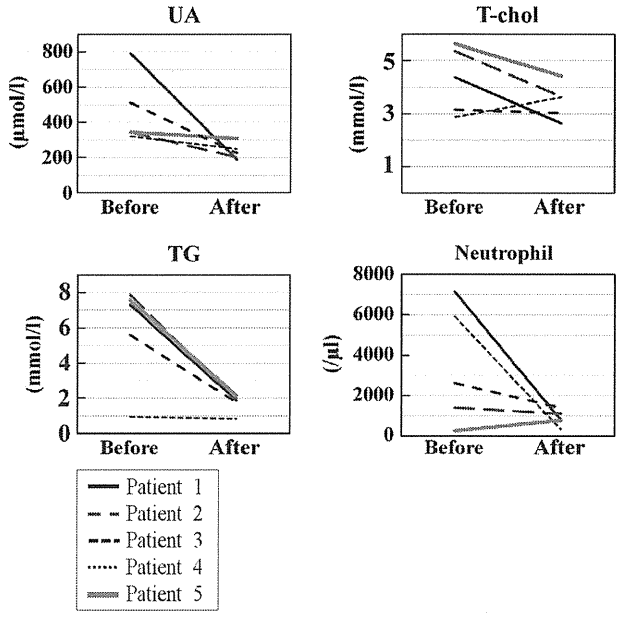

Figure 3 Comparison of data immediately before and 1 year after liver transplantation in glycogen storage disease (GSD) Ib patients. The age at liver transplantation was 1 year 1 month in patient 1 (male), 3 years 6 months in patient 2 (female), 3 years 6 months in patient 3 (male), 3 years 11 months 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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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).

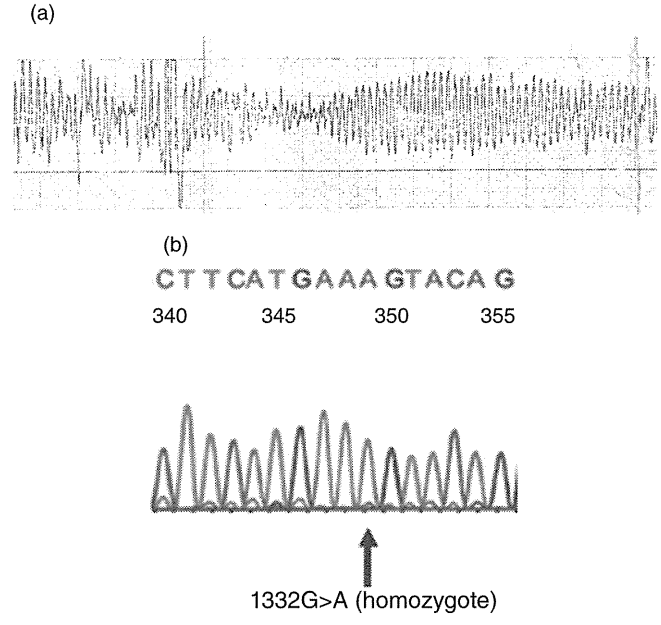

Fig. 1 (a) Electrocardiograph monitoring at the onset of ventricular fibrillation. (b) Gene analysis of the acyl-CoA dehydrogenase, very-long-chain (ACADVL) gene, indicating a homozygote c.1332G>A mutation in the exon–intron junction.

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.