Table 2. Number of prescriptions written for the 10 most commonly prescribed drug types

Medicines	Number of prescriptions
Probiotics	621
Anti-emetics	474
Antibiotics	206
Antipyretics	180
Expectorant agents	127
Antihistamine	109
Antitussive	106
Antidiarrhoeal agents	90
Antipruritic	55
Bronchodilator	52
Others	275

to increase between 2005 and 2007 (Fig. 1). In contrast, the percentage of paediatric gastroenteritis cases treated with anti-emetics and antipyretics remained fairly stable, and the percentage of cases treated with antibiotics underwent a modest decrease over the study period. Interestingly, the percentage of cases treated with antidiarrhoeal agents was small, and decreased over the study period. These results show that, for paediatric gastroenteritis, prescription trends changed over the period 1997–2007.

Prescription of anti-emetics and patient age

The percentage of cases that were treated with anti-emetics increased with patient age (Table 3). During the study period, 32·3% of cases for patients aged 0–2 years and 47·7% of cases for patients aged 3–5 years were treated with anti-emetics. There are no marked difference in the percentage of

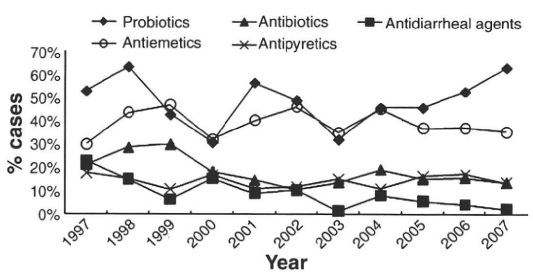

Fig. 1. Prescriptions for each of five common drug types given as a percentage of the total number of cases.

Table 3. Anti-emetic prescriptions for patients diagnosed with paediatric gastroenteritis during 1997–2007

		Prescriptions of anti-emetics	
		Number	%
Total patients	N = 1241	474	38·2
Age			
0	N = 238	67	28·2
1	N = 347	121	34·9
2	N = 183	62	33·9
3	N = 168	74	44·0
4	N = 163	78	47·9
5	N = 142	73	51·4
Sex			
Male	N = 704	256	36·4
Female	N = 537	218	40·2

prescription of anti-emetics between male and female. The anti-emetic prescribed was domperidone in almost all cases (n = 470, 99·6% of all anti-emetics prescriptions). For 83% of the cases for which a domperidone suppository was prescribed, the patient was administered this treatment only once (74%) or twice (9·0%) (Table 4). Although there was no difference between suppositories and dry syrup with respect to the total number of prescriptions, there was greater variation in the frequencies of the prescriptions for patients who were prescribed dry syrup.

DISCUSSION

Main results

The results of our study of prescription trends for paediatric gastroenteritis treatment at Hamamatsu University Hospital between 1997 and 2007 show that several types of drugs were used to treat symptoms, such as vomiting, diarrhoea and fever. More cases were treated with probiotics than with any other drug type, with the percentage of cases treated in this way having increased recently. Although several major guidelines for the treatment of paediatric gastroenteritis clearly recommend oral rehydration and advise against medications, we found that anti-emetics were commonly used in Japan. Furthermore, we found that the percentage of cases treated with anti-emetics remained fairly stable during the study

Table 4. Frequency of domperidone prescription for the treatment of paediatric gastroenteritis

	Once		Twice		Three times		Four times		Five times		More than six times		Total no. prescriptions
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Domperidone suppository	126	74	16	9	9	5	7	4	6	3	8	5	172
	1 day		2 days		3 days		4 days		5 days		>6 days		Total no. prescriptions
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Domperidone dry syrup	27	16	47	27	31	18	32	19	19	11	15	9	171

period. These results indicate that overseas guidelines have little effect on prescriptions for the treatment of paediatric gastroenteritis in Japan.

Trends in prescriptions for paediatric gastroenteritis

We found that the percentage of cases treated with probiotics has recently increased (particularly during the last 3 years of the study). During the 10-year study period, almost all of probiotics prescribed was *Enterococcus faecalis* (86·3%), followed by *Bifidobacterium* (11%). It is known that *E. faecalis* is a common component of the human intestinal flora (13).

Probiotics have been successfully used to prevent and reduce the severity or duration of paediatric rotaviral diarrhoea (14, 15), which indicates that probiotics may be an effective adjunct in the management of paediatric gastroenteritis. It is thought that probiotics act in part by competing with pathogens for receptor sites or intraluminal nutrients, and in part by enhancing the host's immune defenses (16, 17). Clinical trial data demonstrating the efficacy of probiotics for the treatment of paediatric gastroenteritis may have contributed to the recent increase in probiotic use observed in the present study. Over the past several years, some reviews of clinical trials of efficacy of probiotics indicate that the treatment of acute gastroenteritis with some probiotic strains was effective (18). However, as these trials tended to be of limited sample size and, given the large number of strains of probiotics available, the data is insufficient to confirm efficacy. Furthermore, well-controlled clinical trials are needed to verify the efficacy of probiotics for Japanese children with gastroenteritis.

In contrast to probiotic use, use of antibiotics declined during the study period. Even when a bacterial cause is suspected for gastroenteritis, the potential benefits of antibiotic therapy must be carefully weighed against the potentially harmful consequences, such as development of antimicrobial-resistant infections (19, 20). It is possible that Japanese paediatricians are widely aware of this risk, and are acting accordingly.

Over the study period, the percentage of cases treated with antidiarrhoeal agents remained very small, and decreased over time. The antidiarrhoeal agents prescribed included loperamide (75·6% of antidiarrhoeal prescriptions) and scopolia powder extracts containing hyoscyamine and scopolamine (24·4% of antidiarrhoeal prescriptions). Both agents reduce intestinal lumen motility, but loperamide is an opioid receptor agonist and hyoscyamine and scopolamine are muscarinic receptor agonists. There are some reports of loperamide causing adverse events including opiate-induced ileus, drowsiness and nausea caused by the effects of atropine (21, 22). Release of a document by the Japanese MHLW advising against the administration of these antidiarrhoeal agents (11) might have contributed to the limited use of these agents in the present study. Drugs not specific to gastroenteritis, including expectorant agents, antihistamine and antitussive, did not show any remarkable changes during the study period (data not shown). As it is well known that symptoms such as cough can be

involved frequently in patients with gastroenteritis, drugs not specific to gastroenteritis might have been prescribed when necessary (23, 24).

Prescription of anti-emetics

Currently in Japan, there are two anti-emetics that have been approved to reduce gastroenteritis-related vomiting: domperidone and metoclopramide (a dopamine antagonist). Other anti-emetics, including ondansetron and granisetron (a 5-HT_3 serotonin antagonist), have been approved for the treatment nausea and vomiting, induced by chemotherapy but not by gastroenteritis. Accordingly, the most commonly prescribed anti-emetic in this Japanese study was domperidone. In an Italian study, a similar trend was found, with the most common anti-emetic prescribed for paediatric gastroenteritis being domperidone, followed by metoclopramide (8). In the present study, we found that anti-emetics were more likely to be prescribed for older children. This trend might reflect prescribers' concerns about the adverse events associated with these dopamine antagonists, especially in young children, although these are not frequent (25, 26). We found that the majority of anti-emetic suppository prescriptions were for one or two doses. Similarly, in a previous US study, the majority of anti-emetics prescriptions (63·6%) were for after discharge and for 1 day or less (7).

Treatment of vomiting in children using anti-emetics is still a controversial issue. Ondansetron is reportedly effective in decreasing vomiting (27–29). In contrast, however, a systematic review of the effectiveness and safety of anti-emetics in children yielded only weak and unreliable evidence of the benefits of ondansetron and metoclopramide over placebo in reducing the number of episodes of vomiting due to gastroenteritis (30). Thus, anti-emetics might not be justified for the treatment of paediatric gastroenteritis, given the weak evidence of their effectiveness.

Limitations of the study

In this study, we obtained data from the database of a medical order entry system. Therefore, the patients' symptoms (e.g. dehydration, vomiting and diarrhoea) were not well characterized. Furthermore, the database does not include drugs for injection, but we assume that few of these would have been used for the treatment of gastroenteritis in children. The study cohort was defined based on assignment of the ICD-10 code at initial presentation; however, our methodology did not allow us to confirm the accuracy of the diagnosis. Finally, this study was conducted using prescription data obtained from a single hospital. However, despite Hamamatsu University Hospital serving as a secondary referral centre receiving patients with chronic disease, it also treats patients with other types of disease, just like a suburban clinic, therefore we think that our results are very likely to be representative of the general clinical situation in Japan.

CONCLUSIONS

There have been very few studies on prescription trends in Japan. In the present study, we established that prescription trends for the treatment of paediatric gastroenteritis changed little over a recent 10-year period. These results indicate that in the absence of any Japanese guidelines on the treatment of paediatric gastroenteritis, prescription patterns may reflect the individual preferences of physicians. Clearly, development of official, evidence-based Japanese guidelines would be a vital tool for improving the quality of medical practice. In Japan, we still lack strong evidence upon which to base recommendations relating to medical treatment (including probiotics and anti-emetics) of paediatric gastroenteritis. To redress this situation, we have initiated clinical research that aims to assess the effectiveness and safety of anti-emetics for gastroenteritis among children in Japan.

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Testicular thecoma in an 11-year-old boy with nevoid basal-cell carcinoma syndrome (Gorlin syndrome)

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Key words:
Thecoma;
Gorlin syndrome;
Testis

Abstract We report a case of testicular thecoma in an 11-year-old Japanese boy with nevoid basal-cell carcinoma syndrome (Gorlin syndrome). He presented with left testicular swelling and underwent a radical orchiectomy on suspicion of a malignant paratesticular tumor. The tumor arose from the testis exophytically and was diagnosed as a thecoma histopathologically. Ovarian thecoma-fibroma group tumors are closely associated with Gorlin syndrome or with abnormalities in *PTCH*, a candidate gene for the syndrome. The occurrence of an extremely rare testicular thecoma in this case (the second in the literature) suggests that such an etiological association may also exist in the pathogenesis of testicular tumors.

A thecoma is a benign stromal tumor typically seen in the ovary and characterized by spindle-shaped cells forming luteinization [1]. The pathogeneses of ovarian thecoma and fibroma are closely associated with a tumorigenic syndrome called *nevoid basal-cell carcinoma syndrome* (Gorlin syndrome) or abnormalities in its candidate gene, *PTCH* [2]. Herein, we report the first case of a testicular thecoma in a boy with Gorlin syndrome.

1. Case report

An 11-year-old boy was referred to us for painful scrotal swelling. His uncle had died of a cerebellar tumor. The boy had previously been diagnosed with a cerebellar medulloblastoma at 7 months of age and received a series of surgeries, adjuvant multidrug chemotherapies, and radiation therapy. Since the age of 7 years, he had been administered leuprolide as a treatment of premature elevation of testosterone. Concurrently, the boy was diagnosed with Gorlin syndrome based on the presence of skin lesions and odontogenic keratocysts, but without detectable *PTCH* gene mutations.

At presentation, his external genitalia were at the prepubertal stage. He had palpably normal bilateral testes but swelling in the left paratesticular area. He was diagnosed with epididymitis based on magnetic resonance imaging (Fig. 1A) and spontaneous resolution of the pain. Seven months later, however, the paratesticular lesion increased in size and formed a firm nontender mass. Magnetic resonance imaging revealed a heterogeneous 21-mm mass adjacent to

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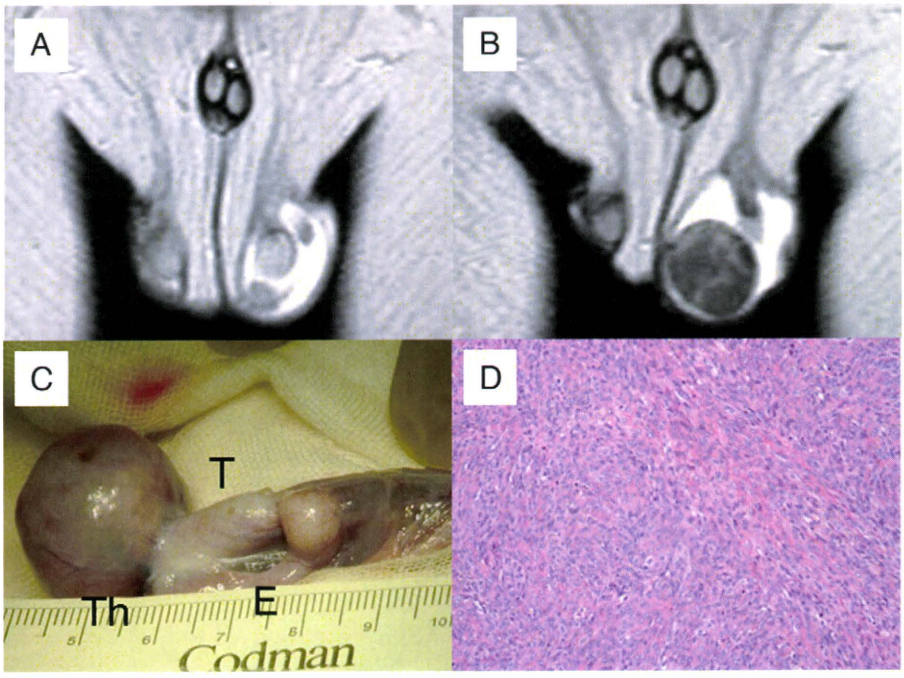

Fig. 1 Magnetic resonance imaging of the left intrascrotal mass (T2-weighted) at first (A) and second referral (B). C, Gross appearance of the tumor. D, Microscopically, the tumor consisted of spindle cells, with occasional luteinized cells that are immunoreactive for inhibin α (figure not shown). Th indicates thecoma; T, testis; E, epididymis.

the left testis (Fig. 1B). Germ cell tumor markers were negative. Testosterone was 29.9 ng/dL (reference range, 18-150 ng/dL), estradiol was 11.3 pg/mL (reference range, 5-16 pg/mL), luteinizing hormone was 0.9 mIU/mL (reference range, <1-5 mIU/mL), and follicular stimulating hormone was 1.4 mIU/mL (reference range, 2-7 mIU/mL). There were no signs of metastasis on radiography. An inguinal exploration revealed a well-circumscribed tumor located at the caudal end of the testis (Fig. 1C). Because the intraoperative pathologic examination could not exclude a malignant mesenchymal tumor, a left radical orchiectomy was performed. Histopathologically, the tumor arose from the tunica albuginea of the testis. Spindle cells were predominant, with occasional luteinization (Fig. 1D). The luteinized tumor cells were positive for inhibin α and calretinin and negative for MyoD and desmin. Consequently, the diagnosis of thecoma was established. The patient has remained free from recurrence for 2 years.

2. Discussion

Thecoma is an extremely rare tumor in the testis, with just one previously reported case [3]. Typically, thecomas are benign stromal tumors arising from ovarian theca cells and constitute 1% of all ovarian tumors [1]. Ovarian stromal tumors are classified into either thecoma or fibroma, and occasionally clumped together as a thecoma-fibroma group.

Gorlin syndrome is a disorder characterized by malformations of the skin, nerves, eyes, and bone [4], with frequent loss of heterozygosity at 9q22.3 or abnormalities in the *PTCH* gene, a homolog of the patched gene in *Drosophila* [5]. Gorlin syndrome patients without detectable *PTCH* mutations, as in the present case, are believed to have germinal mosaicism [6]. The syndrome is associated with basal cell carcinoma, ovarian tumor, and medulloblastoma (as found in the present case). Many women with this syndrome develop ovarian thecoma or fibroma at a mean age of 30 years [2,7]. In parallel with these findings, loss of heterozygosity at 9q22.3 is observed in 40% of sporadic ovarian thecoma-fibroma cases [8], suggesting a strong pathogenic association between *PTCH* abnormalities and thecoma-fibroma development.

A possible explanation for the occurrence of testicular thecoma is one analogous to the association between Gorlin syndrome and ovarian thecoma-fibroma, although the absence of a genetic linkage in the present case precludes a definitive conclusion. The cytotoxic chemotherapy against the prior cerebellar tumor may have enhanced a genetic predisposition to thecoma. Thus, thecoma-fibroma should be taken into the differential diagnosis for male patients with Gorlin syndrome presenting with an intrascrotal mass. Because bilateral lesions are seen in the ovary, testis-sparing surgery and contralateral surveillance should be recommended [7].

3. Conclusion

The present case may suggest the existence of an unreported tumorigenic mechanism in the male gonads.

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Pediatric surgical image

Preoperative diagnosis of congenital segmental giant megaureter presenting as a fetal abdominal mass

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Key words:
Segmental megaureter;
Imaging;
Prenatal diagnosis;
Abdominal mass

Abstract We describe a case of congenital segmental giant megaureter in a boy that presented as a fetal abdominal mass. He also had bilateral undescended testes, bilateral vesicoureteral reflux, and segmental aniridia. He presented with hypoglycemia in the neonatal period that resolved. Postnatal magnetic resonance imaging, voiding cystourethrography and radionuclide imaging established the diagnosis, and a ureteroureterostomy was performed at 12 months.

Congenital segmental giant megaureter (CSGM), which comprises an extremely rare subgroup of megaureter, has not been accurately diagnosed preoperatively [1-4]. Herein, we present the first report of successful preoperative diagnosis of this disease by a comprehensive imaging series.

1. Case report

The patient was born at 39 weeks gestation and had a birth weight of 4648 g. His mother was 32 years of age, and this first gestation was achieved by in vitro fertilization embryo transfer. Fetal echography at an early gestational stage revealed an abdominal cystic mass. Magnetic resonance imaging (MRI), which was performed prenatally and postnatally, revealed a cystic lesion between the right kidney and the urinary bladder. The intensity of the fluid in the cystic mass was identical to that of urine (Fig. 1).

At birth, the patient had several congenital abnormalities, including bilateral undescended testes, micropenis, and segmental aniridia. He also presented with hypoglycemia. No apparent chromosomal anomalies were detected.

A dimercaptosuccinic acid (DMSA) renal scan showed hypoplasia of the left kidney and hyperplasia of the right kidney, and indicated that the cystic mass was unlikely to be extrarenal pelvis (Fig. 2, left). Voiding cystourethrography (VCUG) showed bilateral vesicoureteral reflux (VUR). The contrast material that refluxed to the right ureter drained into a dilated space and subsequently into the right renal pelvis, establishing the diagnosis of CSGM (Fig. 2, right).

Diuretic renography was performed with the range of interest set to discriminate the overlap between the cystic lesion and the right renal pelvis. The radionuclide in the renal pelvis and cystic dilatation showed responses to furosemide, demonstrating the absence of a functional obstruction. The patient retained stable renal function (serum creatinine, 0.3 mg/dL) without any febrile urinary tract infection

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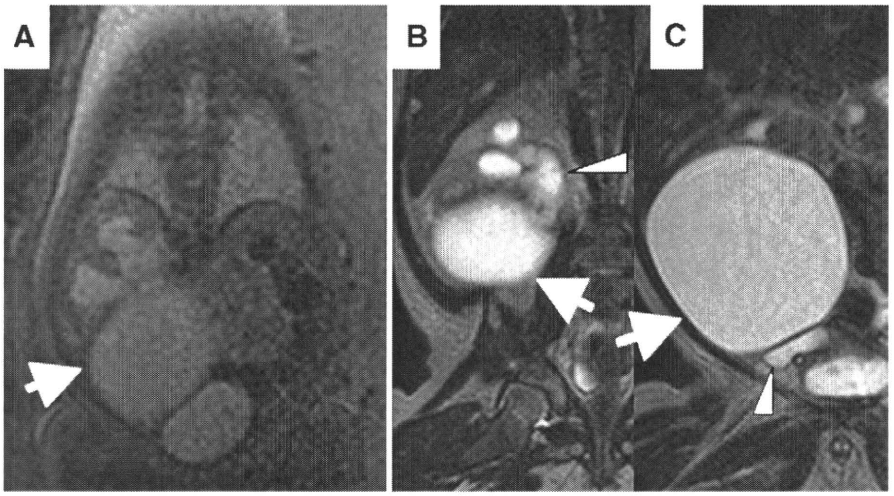

Fig. 1 A, Fetal MRI at 35 (right) weeks of gestation. Coronal single-shot fast spin-echo T2-weighted images showed a cystic mass (arrow) located between the right kidney and the urinary bladder. B. Postnatal MRI at day 2. Coronal T2-weighted images showed the proximal and distal ureter (triangles) located near the cystic mass (arrow).

episodes under prophylactic antibiotics. However, intractable pyuria persisted and the cystic lesion was considered to be the focus.

Based on these findings, we elected to perform cystoscopy, retrograde pyelography, ureteroureterostomy and bilateral orchidopexy simultaneously at age 12 months. During cystoscopy, the bilateral ureteral orifices opened ectopically into the bladder neck. Retrograde pyelography confirmed the diagnosis of CSGM, delineating the interphase of the noncystic part of the ureter with cystic dilatation (Fig. 3, left). Through a 5-cm flank incision, the dilatated portion was resected between the proximal duplicated renal collecting system and the distal ureter, and a stented end-to-end ureteroureterostomy was performed (Fig. 3, right). Postoperative diuretic renography showed no obstruction in the right upper urinary tract. However, he experienced two breakthrough febrile urinary tract infection episodes after the procedure, necessitating a VUR repair at 18 months of age. The ureters were dissected extravesically and anastomosed to the bladder cross-trigonally with tapering of the distal right ureter. Intraoperatively, the vas deferens was noted to enter into the ureters bilaterally.

2. Discussion

Urinary tract dilatation occurs in approximately 1.5% of all neonates, and is classified into hydronephrosis, hydroureteronephrosis and megacystis-megaureter, according to the obstruction point [5]. CSGM is an extremely rare condition, with less than 10 cases reported in the literature, and has not been accurately diagnosed preoperatively before this report [1-4]. Diagnosis of CSGM is challenging because it cannot be classified into any of the above categories and its etiology may not be explained by an obstruction.

This case presents several novel points for imaging of CSGM. First, this is the first reported case in which MRI

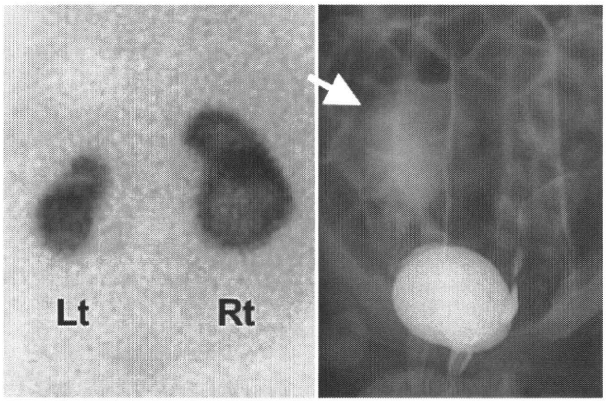

Fig. 2 Left, DMSA renal scan. The cystic mass was unlikely to be a dilated renal pelvis. Right, The VCUG revealed bilateral VUR. The refluxed contrast medium drained into the cyst (arrow).

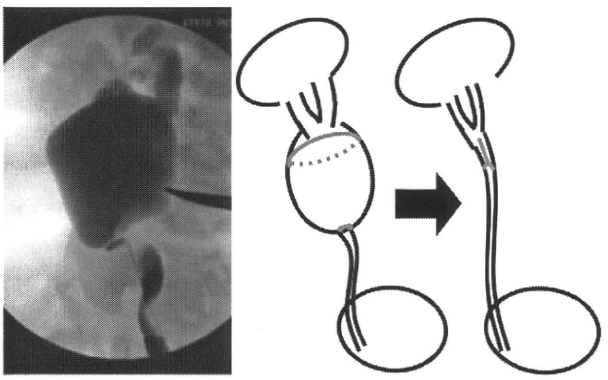

Fig. 3 Left, A retrograde pyelogram delineated the entire upper urinary tract. Right, Schema of the operation. The cystic dilatation was resected and the ureter was repaired by end-to-end anastomosis.

was fully used for prenatal and postnatal diagnosis of CSGM. The ureter proximal and distal to the cystic dilatation was visualized in postnatal images, thus proving the diagnostic utility of MRI (Fig. 1). Second, our prenatal diagnosis was hydronephrosis with a huge extrarenal pelvis, and DMSA was useful for negating this possibility. Third, VCUG was performed as part of the standard workup, and it was fortuitous that the presence of VUR assisted in the diagnosis (Fig. 2). Fourth, diuretic renography was useful to rule out a functional obstruction. Fifth, retrograde pyelography allowed us to develop a precise plan before making an incision (Fig. 3). Collectively, the establishment of a preoperative diagnosis enabled us to plan minimally required surgery under appropriate informed consent from the parents, instead of an emergency neonatal abdominal exploration, as has been frequently reported in previous cases.

The patient had multiple problems, including neonatal hypoglycemia, bilateral undescended testes, segmental aniridia and developmental retardation, all of which can be associated with chromosome 11 disorders (eg, WAGR and Beckwith-Wiedeman syndrome) [6]. Although there was no apparent genetic disorder at the genome level, genetic mosaicism could not be ruled out, and the patient is currently under surveillance for nephroblastoma.

This case illustrates that, although extremely rare, CSGM can be accurately diagnosed by a series of modern imaging techniques, if it is included in the differential diagnosis of prenatal and neonatal urinary tract dilatation.

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ORIGINAL

Phenotypical variety of insulin resistance in a family with a novel mutation of the insulin receptor gene

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Abstract. A novel mutation of insulin receptor gene (INSR gene) was identified in a three generation family with phenotypical variety. Proband was a 12-year-old Japanese girl with type A insulin resistance. She showed diabetes mellitus with severe acanthosis nigricans and hyperinsulinemia without obesity. Using direct sequencing, a heterozygous nonsense mutation causing premature termination at amino acid 331 in the α subunit of INSR gene (R331X) was identified. Her father, 40 years old, was not obese but showed impaired glucose tolerance. Her paternal grandmother, 66 years old, has been suffered from diabetes mellitus for 15 years. Interestingly, they had the same mutation. One case of leprechaunism bearing homozygous mutation at codon 331 was identified. These findings led to the hypothesis that R331X may contribute to the variation of DM in the general population in Japan. An extensive search was done in 272 participants in a group medical examination that included 92 healthy cases of normoglycemia and 180 cases already diagnosed type 2 DM or detected hyperglycemia. The search, however, failed to detect any R331X mutation in this local population. In addition, the proband showed low level C-peptide/insulin molar ratio, indicating that this ratio is considered to be a useful index for identifying patients with genetic insulin resistance. In conclusion, a nonsense mutation causing premature termination after amino acid 331 in the α subunit of the insulin receptor was identified in Japanese diabetes patients. Further investigations are called for to address the molecular mechanism.

Key words: Insulin receptor, Insulin resistance, Type 2 diabetes, Leprechaunism, C-peptide/insulin molar ratio

THE INTERACTION of insulin with its cell surface receptor is the first step in insulin action and the first identified target of insulin resistance. Mutations in the insulin receptor gene lead to the insulin resistance in several syndromic forms. The human insulin receptor is encoded by a single gene with 22 exons and is an assembly of a disulfide bond-linked tetramer composed of two α and two β subunits [1-5]. After binding of insulin to the extracellular α subunit, the tyrosine kinase of the membrane spanning β subunit is activated and the receptor is autophosphorylated [6]. Insulin receptor kinase regulates the action of insulin on metabolism and growth through signal transduction pathways and is therefore thought to be central to insulin action [7].

Some dozens of mutations in the human insulin receptor gene have already been identified to date [8-11]. Homozygous or compound-heterozygous mutations in the insulin receptor gene are found in patients with syndromes of severe insulin resistance [12]. More severe Donohue syndrome ("Leprechaunism" OMIM 246200) and the milder Rabson-Mendenhall syndrome (OMIM 262190) are characterized by intrauterine and postnatal growth retardation, facial dysmorphism, lack of subcutaneous fat and altered glucose homeostasis with hyperinsulinemia, acanthosis nigricans and reduced life expectancy [13-15]. Cells from most patients with Donohue syndrome show absent or

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severely reduced insulin binding, whereas those with Rabson-Mendenhall retain some insulin binding capacity. Therefore, it has been proposed that severity of the phenotype is determined by the degree of insulin resistance and that residual insulin binding capacity correlates with survival. Heterozygous mutations in the insulin receptor gene have been demonstrated in type A insulin resistance with the triad of insulin resistance, acanthosis nigricans, and hyperandrogenism (OMIM147670) [16].

In this study, we identified a heterozygous mutation causing premature termination at amino acid 331 substituting a termination codon for arginine in the L2 domain in α subunit of the insulin receptor gene in a Japanese patient with diabetes mellitus and hyperinsulinemia. Interestingly, her family members shared the same mutation but showed different clinical course.

Materials and Methods

Subjects

The proband, a girl of 12 years old, was referred to our hospital because of glucosuria detected by school urinary screening. She presented with mild symptoms of polydipsia and polyuria. She was born to unrelated Japanese parents at 37 weeks of gestation (birth weight 2495 g, birth length 48 cm). At birth, she did not have the dysmorphic features characteristic of leprechaunism or Rabson-Mendenhall syndrome, including intrauterine growth retardation, fasting hypoglycemia. Sensorineural hearing loss in right side was diagnosed when she was infant, but did not deteriorate.

At presentation, she was not obese, but showed severe acanthosis nigricans with scratching scar of her neck. It also mildly existed at the axilla and elbow. Hirsutism was not observed. Body mass index (BMI) was 21.6 (height 148.6 cm, weight 47.7 kg). Blood pressure was 110/70 mmHg. Pubertal stage was B2 and PH1. Laboratory tests revealed the following; HbA1c, 9.2 %; FPG, 124 mg/dL; IRI, 65.7 μU/mL; C-peptide, 3.18 ng/mL; AST, 20 IU/L; ALT, 18 IU/L; total cholesterol, 194 mg/dL; HDL cholesterol, 43.7 mg/dL; testosterone, 0.33 ng/mL. Islet associated autoantibodies were absent. Urine testing showed no ketonuria but proteinuria (microalbumin 64.4mg/g cr) and glucosuria. Ocular complication and retinopathy was not detected. Abdominal CT revealed no fatty liver and area of visceral fat on umbilical level was 41.8 cm^2 (normal: 60>). Although she showed diabetes mellitus with severe insulin resistance, her data of body composition was not suggested risk for obesity or metabolic syndrome. Self monitored blood glucose levels were 120-140 mg/dL at premeal time and 170-200 mg/dL at postprandial time. Her father, 40 years old, was healthy and no obesity (BMI 21.8) from a clinical point of view at the time of investigation. Her paternal grandmother, 66 years old, has been suffered from diabetes mellitus. She was also not obese (BMI 21.6) and has been treated with sulfonylureas for 15-years. She already developed retinopathy and presented vitreous hemorrhage 10 years ago. Her younger brother, seven years of age, had mild mental retardation and supported by special education. He showed mild obesity but normal response to oral glucose tolerance test without hyperinsulinemia (FPG, 86 mg/dL; IRI, 8.6 μU/mL; C-peptide, 1.53 ng/mL).

Measurements

The standard 75 g oral glucose tolerance test (OGTT) was performed, after overnight fast. Levels of glucose, insulin and C-peptide were measured at 0, 30, 60, 90 and 120 min. Insulin was measured using an enzyme immunoassay (E test TOSOH II; TOSOH Corporation, Tokyo, Japan). Cross-reactivity with proinsulin was 2 %. C-peptide was measured using a chemiluminescent enzyme immunoassay (LUMIPULSE Presto C-peptide; FUJIREBIO Inc., Tokyo, Japan). Proinsulin was measured using a RIA2 antibody method (HUMAN PROINSULIN RIA KIT; Linco Research Inc., St. Charles, MO).

We calculated C-peptide/insulin molar ratio from each molecular weight and international unit of insulin i.e. 26 IU/mg. We estimated molecular weight of insulin at 5800 and C-peptide at 3600. Consequently, 1 μU/mL of insulin is 6.09 pmol/L and 1 ng/mL of C-peptide is 0.278 nmol/L.

Sequence analysis

Informed consent was obtained from her family. Genomic DNA was extracted from peripheral blood lymphocytes using a DNA isolation kit for mammalian blood. Exon 1-2 of the insulin gene and Exons 1-22 of the insulin receptor gene were individually amplified using primer sets as described [17, 18]. PCR products were purified for direct sequence analysis on an ABI gene analyzer 310 or 3100 system according to the manufacturer's instructions (Applied Biosystems).

Analysis for prevalence of R331X mutant in population

We tested the frequency of R331X in type 2 DM or by chance hyperglycemia in adult people, living in the Akita prefecture located in northern Japan. We studied 272 participants of a group medical examination, comprised 92 healthy cases checked normoglycemia and 180 cases already diagnosed type 2 DM or detected hyperglycemia. These included 47 cases with family history of DM and 14 cases diagnosed before third decade. All participants gave informed consent, and the Ethics Committee of Kyoto University School of Medicine approved the study.

Genotyping of R331X was assayed with PCR restriction fragment length polymorphism. PCR reactions were conducted in a reaction volume of 7.5 μL with 20 ng genomic DNA, 2× GC buffer, 200 μM dNTPs, 10 pmol of each primer and 1 unit of LA Taq polymerase (Takara, Tokyo, Japan). The PCR primers used were 5′-AGATGTCTGAAGGACCTTGGA-3′ as a forward primer and 5′-ACAGCTCAGAGGGACATGGA-3′ as a reverse primer. PCR was performed with 39 cycles of the following 94°C for 45 s, 54°C for 45 s and 74°C for 1 min in a thermocycler. Obtained PCR products showed a single fragment at 285 bp. Six μL of 285-bp product were then digested with 2 units of BspCNI restriction enzyme at 25 °C for 2 h. Digestion products were visualized on a 3 % agarose gel. Wild-type allele produced double band at 269 and 16 bp and mutant allele produced three bands at 165, 104 and 16 bp.

Table 1 C-peptide/insulin molar ratio in family members

Patient

OGTT (1) on admission

Time (min)	0	30	60	90	120
PG (mg/dL)	124	224	263	280	262
IRI (μU/mL)	65.7	114.8	191.5	279.1	290.1
CPR (ng/mL)	3.18	4.62	7.01	9.80	10.00
CPR/IRI molar ratio	2.21	1.84	1.67	1.60	1.57

OGTT (2) 2 weeks after admission

Time (min)	0	30	60	90	120
PG (mg/dL)	85	190	224	220	202
IRI (μU/mL)	52.7	136.9	187.4	239.2	313.6
CPR (ng/mL)	2.68	5.80	8.00	8.99	10.50
CPR/IRI molar ratio	2.32	1.93	1.95	1.72	1.53

Father

OGTT

Time (min)	0	30	60	90	120
PG (mg/dL)	88	169	256	214	172
IRI (μU/mL)	10.1	37.0	100.5	108.1	110.5
CPR (ng/mL)	1.24	3.14	6.96	8.05	8.22
CPR/IRI molar ratio	5.60	3.87	3.16	3.40	3.40

Grandmother

Fasting time	
PG (mg/dL)	146
IRI (μU/mL)	30.1
CPR (ng/mL)	2.51
CPR/IRI molar ratio	3.84

Results

An OGTT revealed a diabetic pattern with hyperinsulinemia (Table 1). The homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance, was 20.1. The C-peptide/insulin molar ratio was extremely low. The fasting and 120 min levels were 2.21 and 1.57, respectively (normal level of fasting is 4.0<). An insulin tolerance test (0.1U/kg insulin i.v.) showed insulin resistance with only 37 % reduction in plasma glucose levels. Metformin was started from 250 mg/day and increased up to 500 mg/day. HbA1c levels improved to 5-6 % six months later. At that point in time, her fasting proinsulin level was 71.7 pmol/L when the IRI level was 49.1 μg/mL. Proinsulin/insulin molar ratio was 0.24 (normal 0.1-0.2). Her insulin levels were still high; however, the acanthosis nigricans had disappeared after she had regained diabetic control.

Her clinical course suggested two genetic diseases of glucose metabolism. One was the insulin gene mutation, as characterized by a low level C-peptide/insulin molar ratio, and sometimes presents as type 2 DM. The other was the insulin receptor gene mutation, which clinically demonstrated type A insulin resistance.

A sequencing analysis of the 22 exons as well as the intron-exon junctions identified a heterozygous mutation at nucleotide position 1072 substituting a termination codon for arginine 331, a conserved amino acid in the insulin-like growth factor I receptor and insulin receptor-related receptor, in the putative receptor L2 domain of the patient's insulin receptor (Fig. 1) [19]. No other mutations were found in any of the insulin receptor genes analyzed in this study.

Her father and grandmother also had the same

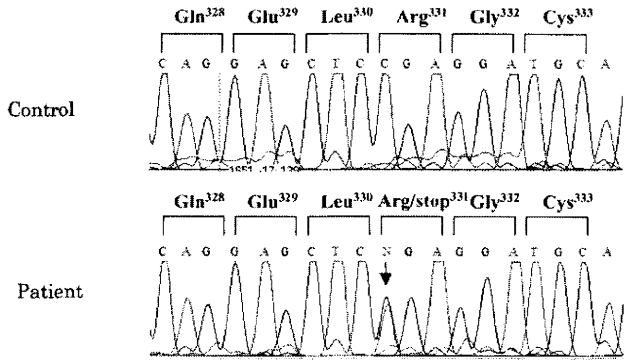

Fig. 1 Partial nucleotide sequence of the insulin receptor gene in the patient. Sequence from the patient is shown in comparison with that from the control. The patient is heterozygous for a mutation at the nucleotide position 1072, converting Arg 331(CGA) to a termination codon (TGA). An arrow indicates the position of mutation.

heterozygous mutation (data not shown). The fasting C-peptide/insulin molar ratio of her grandmother was relatively low under the treatment of sulfonylureas (3.84, IRI; 30.1 μU/mL, CPR 2.51 ng/mL). The HbA1c level of her father was 4.7 %, but OGTT showed impaired glucose tolerance (Table1). Although the fasting insulin level was 10.1 μU/mL, it increased up to 100 μU/mL in 60 -120 min. The C-peptide/insulin molar ratio was 5.60 in fasting and 3.40 in 120 min. They showed milder insulin resistance in comparison to the proband. The heterozygous mutation seemed to significantly affect the insulin resistance of the three subjects, even if no typical skin lesions were observed in either the father or grandmother.

One unrelated case of leprechaunism with R331X homozygous mutation was identified in Tokyo, Japan. The patient was born to unrelated parents at 39 weeks of gestation with a birth weight of 1743 g. She showed an extreme degree of insulin resistance (FPG, 200 mg/dL<; IRI 10,000 μU/mL<). She thereafter started to receive subcutaneous injections of recombinant human IGF-I. After treatment, her glucose metabolic abnormality was improved. Informed consent was obtained from her parents for sequence analysis. Her parents had R331X heterozygous mutation. They did not demonstrate any symptoms of diabetes mellitus. Information on the glucose tolerance including OGTT was unavailable.

These findings led to the hypothesis that insulin receptor genetic variants contribute to the variation of DM in the general population in Japan. An extensive search was done in 272 participants in a group medical examination that included 92 healthy cases of normoglycemia and 180 cases already diagnosed as type 2 DM or detected hyperglycemia. The search, however, failed to detect any R331X mutation in this local population.

Discussion

Type A insulin resistance was initially characterized in young female patients with acanthosis nigricans, ovarian hyperandrogenism and virilization [20]. Over 30 mutations have so far been described in these patients, which are mainly clustered in the tyrosine kinase domain of the insulin receptor [21, 22].

A nonsense mutation was identified in one allele of a patient substituting the termination codon (TGA) for the CGA codon normally encoding Arg^{331} located in a putative L2 domain, which is a single stranded right-hand beta-helix and is suggested to make up the bilobal ligand binding site [23]. The nonsense mutation at codon 331 truncated the C-terminal half of the receptor α subunit as well as the entire β subunit including the transmembrane anchor and the tyrosine kinase domain. Therefore, it is unlikely that this truncated receptor, translated from the mutant allele, would be either functional or located on the cell surface. In fact, extreme insulin resistance was observed in a female leprechaunism patient with homozygous R331X alleles.

Hyperinsulinemia is usually considered to be the result of resistance to the physiological effects of insulin and consequent compensatory increased insulin secretion. Recently, the C-peptide/insulin ratio is widely used as a surrogate of hepatic insulin clearance for the evaluation in type 2 DM or glucose intolerance [24, 25]. This index, should clarify whether impaired hepatic insulin clearance or increased insu-

lin secretion has a dominant effect on such patients. Insulin and C-peptide are secreted into the portal vein in a 1:1 molar ratio after β-cell stimulation by carbohydrate or other secretagogues. A large fraction of endogenous insulin is cleared by the liver, whereas C-peptide, which is cleared primarily by the kidney and has a lower metabolic clearance rate than insulin, and traverses the liver with essentially no extraction by hepatocytes [26, 27]. Diminished insulin clearance has been demonstrated to be an important underlying mechanism for the hyperinsulinemia found in various insulin-resistant conditions [28-30]. For example, to evaluate hyperinsulinemia in African Americans, at risk for type 2 DM, several studies used C-peptide/insulin molar ratio as an index of hepatic insulin clearance. African American children and adults showed lower C-peptide/insulin ratio than White Americans, thus suggesting that high insulin levels could be partly attributed to lower clearance [31, 32].

Therefore, the use of the C-peptide/insulin molar ratio reflects of hepatic insulin clearance [33]. A low C-peptide/insulin molar ratio of our patients suggests impaired hepatic insulin clearance because of, not only DM, but also abnormal insulin receptor expression in the liver. To this day, a low C-peptide/insulin molar ratio has not been substantially observed among individuals with type A insulin resistance. Two family cases with an insulin receptor gene mutation reported the presence of a low C-peptide/insulin molar ratio [34, 35]. They showed hyperinsulinemic hypoglycemia, severe insulin resistance and the C-peptide/insulin molar ratio ranged from 1.1 to 3.8.

As well as this reported cases, the molar ratio of the proband of our family was very low similar to that observed in subjects with insulin gene mutation. Previously, low C-peptide/insulin ratio was well reported to be a clinical feature of mutations in the human insulin gene causing either familial hyperinsulinemia or familial hyperproinsulinemia. The elevated circulating IRI consisted mainly of the unprocessed mutated proinsulin, which had accumulated because of proinsulin's relatively low clearance compared with insulin. In these subjects, proinsulin levels were tends to be extremely high, namely over three hundred pmol/L [36, 37]. Due to dramatic improvements in the assay techniques of IRI, cross-reactivity with proinsulin is normally seen at very low levels. Consequently, there have been no new reports regarding hyperproinsulinemia with insulin gene mutations for the last decade.

Recently, the fasting proinsulin/insulin ratio is used as a marker of β-cell dysfunction. In peripheral blood, fasting proinsulin accounts for 10-20% of insulin but it may reach values as high as 50 % in type 2 DM. Taura *et al.* evaluated the basal and dynamic proinsulin-insulin relationship to assess the β-cell function during OGTT in type 2 DM [38]. The proinsulin/insulin molar ratio was higher in type 2 DM (0.39 ± 0.05) subjects than normal (0.14 ± 0.01) and impaired glucose-tolerant (0.13 ± 0.02) subjects. In comparison to this study, the fasting proinsulin/insulin ratio of the proband, 0.20 was slightly higher than normal. It is difficult to consider that her low C-peptide/insulin molar ratio is derived from structural abnormalities in the proinsulin molecule.

We calculated the C-peptide/insulin molar ratio of several previous cases with insulin receptor gene mutation from data measured simultaneously. Severe cases, Rabson-Mendenhall syndrome or Donohue's syndrome, showed very low level (0.69 to 1.83) [14, 39-41]. Milder cases, type A insulin resistance or DM, also showed relatively low molar ratio (1.47 to 4.26) [35, 42]. However, most previous case reports only recorded the IRI data, more investigations are needed to discuss these clinical characteristics.

Interestingly, the patient's father did not show hyperinsulinemia while demonstrating a normal C-peptide/insulin molar ratio after fasting. However, after oral glucose ingestion, the insulin level increased 100.5 μU/mL/mL at 60 min and the molar ratio gradually decreased from 5.60 to 3.40. Meier *et al.* studied the C-peptide/insulin molar ratio as calculated at singular time points after oral glucose administration in non-diabetic subjects [37]. They reported that the molar ratio decreased to half level at 30 minutes and then it gradually increased up to the initial level through 120 min. In contrast to their data, the proband and her father showed a gradually decreasing pattern from 0 to 120 minutes. Receptor-mediated insulin endocytosis and degradation in hepatocyte underlie the basic mechanism of insulin clearance. Insulin is targeted for degradation after internalization, whereas the receptor recycles back to the cell surface [43]. CEACAM1, a transmembrane glycoprotein, plays a significant role in receptor-mediated insulin endocytosis [44]. *In vitro* studies suggest that upon its phosphorylation by the insulin receptor kinase, CEACAM1 binds indirectly to the receptor to undergo internalization in clathrin-coated vesicles as part of endocytosis complex [45].

CEACAM1 is considered to interact with two separate domains of the insulin receptor: a C-terminal for its phosphorylation, and cytoplasmic juxtamembrane domain required for internalization [46]. R331X mutant defects these important domains for endocytosis of insulin-insulin receptor complex. A reduction of endocytosis may also affect recycle of insulin receptor and may cause prolonged low hepatic extraction after glucose oral load observed in subjects having R331X mutation. Although her father showed normal data in fasting period, the oral glucose test may be a supplementary means for evaluating of insulin receptor mutant subjects.

As stated above, C-peptide is believed to be a better index of the pancreatic β-cell function than insulin because C-peptide levels are unaffected by hepatic clearance. When comparing the father's C-peptide levels of OGTT with proband, only a slight difference was observed. This result indicates that the insulin secreting function of β-cell is not substantially different and the cause of hyperinsulinemia in the proband is dominantly affected by impaired hepatic insulin clearance. The evaluation of the C-peptide/insulin molar ratio is thus considered to be a useful index for identifying genetic insulin resistance patients. On the other hand, a mild phenotype such as that observed in her father may not be effectively evaluated by the fasting data alone.

Unrelated Japanese patients with another mutation of the insulin receptor gene have been previously reported. They showed different phenotypes: one was detected as a heterozygous mutation in type A insulin resistance, while the other was detected as a compound heterozygous mutation in leprechaunism, thus indicating that the severity of such mutations will determine the phenotype [47]. The phenotype of heterozygous R331X differed substantially among the current family members. Although the proband and her grandmother showed diabetes mellitus with insulin resistance, the difference in the age of onset was around forty years. In addition, her father did not show insulin resistance after fasting. The reason for this difference may be conditioned by heredity and environment. The lifestyle for children has changed over the last few decades in Japan. The proband often consumed high caloric foods before detecting glucosuria. Numerous genetic factors related to diabetes mellitus have also been investigated. The insulin receptor pathway plays an important role in the glucose metabolism. The phenotype of a homozygous mutation, leprechaunism, revealed this important function in humans. However, a heterozygous mutation including Type A insulin resistance shows a mild phenotype. Variance in the current family case suggests that various genetic factors may therefore have played a role in their glucose metabolism. Contrary to expectations, the hypothesis that R331X determines the phenotype for glucose tolerance in Japanese people was ruled out. In addition, the influence of other reported mutations was unclear.

In conclusion, a nonsense mutation causing premature termination after amino acid 331 in the α subunit of the insulin receptor was identified in Japanese diabetes patients. The phenotype of R331X showed variety, and therefore further investigations, including determination of the mRNA level as well as ligand binding and receptor autophosphorylation, are thus called for to address the molecular mechanism by which this mutation leads to the occurrence of diabetes, as was observed in the current patient. In addition, the C-peptide/insulin molar ratio is considered to be a useful index for identifying genetic insulin resistance patients.

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Short Report

Partial paternal uniparental disomy of chromosome 6 in monozygotic twins with transient neonatal diabetes mellitus and macroglossia

Suzuki S, Fujisawa D, Hashimoto K, Asano T, Maimaiti M, Matsuo K, Tanahashi Y, Mukai T, Fujieda K. Partial paternal uniparental disomy of chromosome 6 in monozygotic twins with transient neonatal diabetes mellitus and macroglossia.
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Transient neonatal diabetes mellitus (TNDM) usually develops within the first few weeks of life and resolves at a median age of 3 months. In most of the cases, TNDM is caused by the over-expression of a paternally expressed imprinted *PLAGL1* locus on chromosome 6q24. The most frequent manifestation other than TNDM is intrauterine growth retardation (IUGR), and in some cases macroglossia. We investigated monozygotic twins who had macroglossia without IUGR. Both of the twins developed insulin-dependent hyperglycemia within the first week of life, which subsequently resolved. DNA profiling with polymerase chain reaction amplification was performed for polymorphic microsatellite markers of chromosome 6. The six informative markers, located between 6p24 and 6q15, showed normal biparental inheritance. However, the six distal informative markers, located between 6q23.2 and the 6q telomeric region, showed the absence of a maternal allele and the presence of a single paternal allele. The monosomy of the 6q telomeric region was not confirmed by chromosome banding showing 46, XX. These findings provide further evidence that partial paternal uniparental disomy of chromosome 6 (pUPD6) causes TNDM. The phenotypes other than diabetes observed in patients with partial pUPD6 may differ from those observed in patients with complete pUPD6.

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Transient neonatal diabetes mellitus (TNDM) is a rare disorder, in which insulin-dependent hyperglycemia usually develops within the first few weeks of life and resolves at a median age of 3 months with possible relapse as permanent diabetes mellitus in later childhood (1). Most patients with TNDM are born with intrauterine growth retardation (IUGR) and they sometimes exhibit macroglossia (2, 3). About 70% of the cases of TNDM are caused by over-expression of a paternally expressed imprinted *PLAGL1* locus on chromosome 6q24. This is because of three genetic causes: paternal uniparental disomy of chromosome 6 (pUPD6), paternal duplication of 6q24, and loss of maternal methylation of the differential methylated region at 6q24 (1). On the other hand, approximately 20% of the patients with TNDM have activating mutations in the *KCNJ11* and *ABCC8* genes that encode for the ATP sensitive potassium (K_{ATP}) channel (1).

Partial uniparental disomy (UPD) is a rare genetic abnormality. It is defined as UPD of a part of a chromosome (interstitial or telomeric) together

with biparental inheritance of the rest of this chromosomes' pair and a normal karyotype (4).

To date, there has been no report on TNDM due to partial pUPD6 although one case of neonatal diabetes mellitus due to partial pUPD6 has been reported; in that report, the type (i.e. TNDM or PNDM) was not confirmed because the patient died at 14 days of age (5).

Here, we describe the cases of a pair of female, monozygotic twins with TNDM due to partial pUPD6. IUGR was not observed in either of the twins, but they exhibited macroglossia. The identical phenotype between the monozygotic twins confirmed that partial pUPD6 is the genetic cause of TNDM.

Materials and methods

Case report

The patients were a pair of female monozygotic twins who were conceived after *in vitro* fertilization–embryo transfer. Monochorionic–diamniotic twin pregnancy resulted from the implantation of a single blastocyst. The twins were born at 29 weeks of gestation because of threatened premature delivery, and were the first children of their parents. The birth weight and length of twin 1 were 1472 g [+0.6 standard deviation (SD)] and 42 cm (+0.8 SD), and those of twin 2 were 1127 g (−0.8 SD) and 38 cm (−0.3 SD), respectively. The SD score was based on the data derived from a Japanese population (6). Both twins had macroglossia, and received mechanical ventilation for 5 days after birth because of respiratory distress syndrome. Intravenous feeding followed by tube feeding was initiated after birth. The blood glucose (BG) levels at birth were 2.8 and 1.3 mmol/l for twins 1 and 2, respectively. However, routine laboratory analysis revealed hyperglycemia without ketonuria at 2 days of age (twin 1: BG level, 12 mmol/l; twin 2: BG level, 15.5 mmol/l). Regular insulin was administered, and the BG levels decreased rapidly to within the normal range. However, persistent hyperglycemia developed at 12 days of age in twin 1 and 4 days of age in twin 2, and insulin was administered. Thereafter, the BG levels gradually decreased, and insulin administration was stopped at 29 days of age for twin 1 and 22 days of age for twin 2. At 1 year of age, euglycemia was maintained, and glycosylated hemoglobin levels were 4.9% for twin 1 and 4.7% for twin 2.

Microsatellite analysis of chromosome 6

The analysis was performed according to a previous report (2). Briefly, genomic DNA was extracted from the peripheral blood of the twins and both parents. DNA profiling with polymerase chain reaction (PCR) amplification was performed for polymorphic microsatellite markers by using the ABI Prism Linkage Mapping Set version 2 (Applied Biosystems, Foster City, CA). PCR products were electrophoresed by using the ABI Prism 310 Genetic Analyzer and analyzed by using GeneScan (Applied Biosystems). This study has been approved by the Ethical Review Board of Asahikawa Medical College. Written consent was obtained from the patients' parents.

Results

Microsatellite analysis confirmed that the twins were monozygotic. The six informative markers (D6S1574, D6S309, D6S470, D6S422, D6S1610, and D6S462), located between 6p24 and 6q15, showed normal biparental inheritance. However, the six distal informative markers (D6S292, D6S1569, D6S1577, D6S264, D6S1697, and D6S281), located between 6q23.2 and the 6q telomeric region, showed the absence of a maternal allele and the presence of a single paternal allele, indicating paternal uniparental isodisomy within this region (Table 1). The two microsatellite markers (D6S434 located at 6q21 and D6S287 located at 6q22.31, respectively) are heterozygous with different sizes indicating that these may be biparental. Mosaicism was not proven in all the regions. The monosomy of the 6q telomeric region was not confirmed by chromosome banding, which showed a normal karyotype (46, XX).

Discussion

To the best of our knowledge, only one case of neonatal diabetes due to partial pUPD6 has been reported to date; however, in that case, the type of neonatal diabetes (i.e. TNDM or PNDM) was not determined because the patient died at 14 days of age. Therefore, our study provides evidence that partial pUPD6 causes TNDM. In the present case, partial pUPD6 may be localized between markers D6S287 and D6S292, which map to 6q22.31 and 6q23.2, respectively. Our findings obtained in monozygotic twins with identical clinical courses provide valuable information to elucidate for clinical and genetic aspects of TNDM due to partial pUPD6. Furthermore, the same diabetic phenotype in the twins was a clinically important observation and confirmed the association between partial pUPD6 and TNDM. Moreover, differences in clinical manifestation exist between partial pUPD6 and either complete pUPD6 or paternal duplication