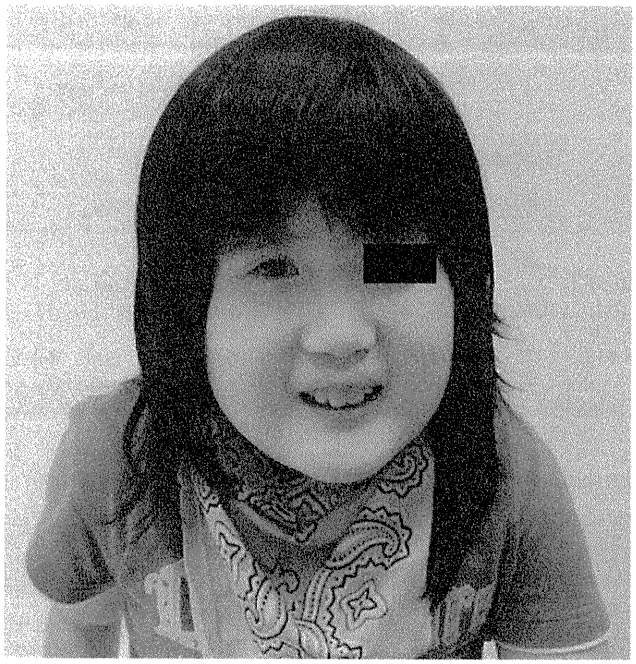

Fig. 1 Patient 1 at age 9. Note the deep-set eyes associated with almond-shaped palpebral fissures, straight eyebrows, prominent forehead, broad and flat nasal root, and pointed chin.

had small and narrow hands with a straight ulnar border and small feet with short toes. Her skin was generally hypopigmented. On initial evaluation the physical features and behavioral characteristics suggested PWS because she was given a score of 8.5 using the consensus diagnostic criteria for PWS (Table 1).[12,13]

Patient 2

Patient 2 was a boy aged 10 years, who was born at 41 weeks of gestation to non-consanguineous parents of Russian descent after an uneventful pregnancy. The mother was 21 years old and the father was 31 years old at the time of his birth. His birthweight was 2780 g (−1.19 SD) and length was 51.0 cm (+0.24 SD). He had hypotonia and difficulty in sucking during the neonatal period. His psychomotor development was apparently delayed: he walked at 19 months and could speak repeated words at 5 years. At the age of 6 years he developed generalized tonic–clonic seizures, easily controlled with valproate, but electroencephalogram and magnetic resonance imaging were normal. At school-going age he developed hyperphagia, which resulted in obesity. His mother conducted hard dietary restriction, which in turn caused malnutrition. Behavioral problems included temper outbursts and impulsivity. On physical examination at age 10 his height was 122.0 cm (−2.9 SD), weight was 24.5 kg (−1.4 SD), and OFC was 51.0 cm (−0.29 SD). He had deep-set eyes, straight eyebrows, hypopigmentation, strabismus, and a pointed chin (Fig. 2). He was given a score of 7.5 using the consensus diagnostic criteria for PWS (Table 1). Laboratory findings, including insulin-like growth factor-1 and growth hormone (GH) provocative tests indicated subnormal GH secretion. Low serum prealbumin, retinol binding protein indicated malnutrition.

Table 1 Scoring based on the diagnostic criteria for PWS[12]

	Patient 1	Patient 2
Major criteria		
Infantile central hypotonia	+	+
Infantile feeding problems/failure to thrive	+	−
Rapid weight gain between 1 and 6 years	+	+
Characteristic facial appearance	+	+
Hypogonadism: genital hypoplasia, pubertal deficiency	−	−
Developmental delay/mental retardation	+	+
Hyperphagia/food foraging/obsession with food	+	+
Cytogenetic or molecular diagnostic testing	−	−
Minor criteria		
Decreased fetal movement and infantile lethargy	−	−
Typical behavior problem	−	+
Sleep disturbance/sleep apnea	−	−
Short stature for the family by age 15 years	+	+
Hypopigmentation	+	+
Small hands and feet for height age	+	−
Narrow hands with straight ulnar border	+	−
Esotropia, myopia	−	+
Thick, viscous saliva	−	−
Speech articulation defects	+	+
Skin picking	−	−
Total scores	8.5	7.5

PWS, Prader–Willi syndrome.

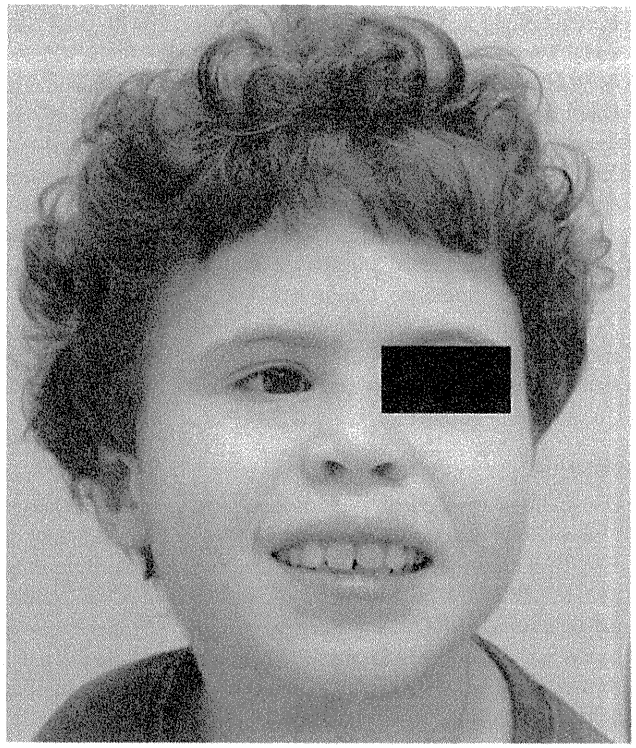

Fig. 2 Patient 2 at 10 years of age.

Molecular analysis

Genomic DNA was purified from whole blood and treated with sodium bisulfite according to the standard methods. Methylation-specific polymerase chain reaction (MS-PCR) of the *SNURF-SNRPN* exon 1 and promoter region was performed with primers described previously.[14]

Cytogenetics and fluorescence in situ *hybridization*

Cytogenetics of chromosomes from phytohemagglutinin-stimulated peripheral blood lymphocytes was performed according to the standard protocols.

Deletion screening for the PWS critical region was performed using the commercially available LSI *SNRPN* probe (Vysis; Abbot Molecular, Des Plaines, IL, USA). For screening of the terminal deletion of the short arm of chromosome 1, FISH was carried out using the probe D1Z2 mapped on 1p36.3. BAC clones were used as the probes for FISH to characterize the range of deletion; these clones were selected by the University of California, Santa Cruz (UCSC) Genome Browser from the Human March 2006 assembly (http://genome.ucsc.edu/). Bacterial stabs of the BAC clones were streaked onto Luria-Bertani plates with an appropriate antibiotic. For probes, DNA was isolated from overnight cultures with the appropriate antibiotic using the QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany). All DNA were labeled by nick translation according to the manufacturer's instructions (Nick Translation Mix; Roche Diagnostics, Basel, Switzerland). The probes were blocked with Cot-1 DNA (Roche Diagnostics) to suppress repetitive sequences. Slides were baked at 65°C for proper aging. Chromosomes and probes were denatured on a hotplate at 75°C for 3 min and then hybridized overnight at 37°C. The slides were washed with 0.4X SSC and 0.3% NP-40 at 70°C for 2 min, washed with 0.2X SSC and 0.1% NP-40 at room temperature for 30 s, and then stained with DAPI for 3 min. Hybridization, post-hybridization washing, and counterstaining were carried out according to the standard procedures. The slides were analyzed using a completely motorized epifluorescence microscope (Leica DMRXA2) equipped with a CCD camera. Both the camera and the microscope were controlled with Leica CW4000 M-FISH software (Leica Microsystems Imaging Solutions, Cambridge, UK).

Written informed consent was obtained from the parents of both patients participating in the study, in accordance with the Kanagawa Children's Medical Center Review Board and Ethics Committee.

Results

Conventional cytogenetic analysis demonstrated a normal karyotype in both patients. FISH using a probe corresponding to *SNRPN* within the PWS region of 15q11–q13 showed no deletion. MS-PCR of chromosome 15 showed biparental methylation patterns at the *SNRPN* exon 1 region, withdrawing the diagnosis of PWS. Subsequent FISH using D1Z2 corresponding to 1p36.3 showed deletion of the region, confirming the diagnosis of 1p36 deletion syndrome in both patients.

We further applied molecular cytogenetic techniques using the BAC clones to characterize the size of the deletions. The

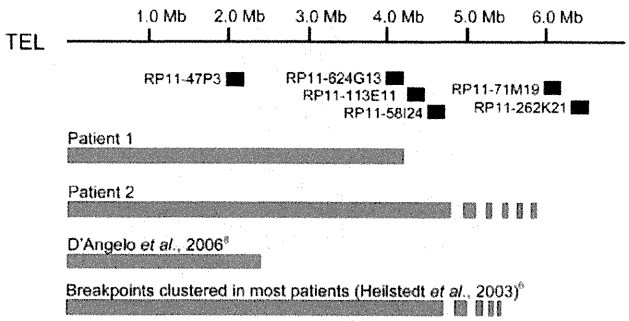

Fig. 3 Characterization of 1p36 deletion. Gray bars, deleted regions. The location of the BAC analyzed are shown according to the University of California, Santa Cruz (UCSC) Genome Browser from the Human March 2006 assembly.

results of these analyses on both patients are summarized in (Fig. 3). In both the patients, the deletion breakpoints were common within the chromosomal band 1p36.32 but at different regions between RP11-624G13 (4 000 095–4 178 764) and RP11-113E11 (4 366 091–4 546 558) in patient 1 and between RP11-58I24 (4 722 126–4 898 111) and RP11-71M19 (6 097 961–6 283 696) in patient 2. These analyses established the 1p36 deletions as being located between 4.17 and 4.36 Mb in patient 1 and between 4.89 and 6.09 Mb in patient 2. The parents of both patients had normal karyotype.

Discussion

Given the clinical history and neurological features of both the patients, we arrived at a preliminary diagnosis of PWS. The facial appearance, hypopigmentation, mental retardation, feeding difficulties in the neonatal period, and hypotonia together with the characteristic behavior, including hyperphagia, were suggestive of PWS (Table 1). In children older than 3 years of age with 8 points in the consensus diagnostic criteria (4 from the major criteria), PWS should be suspected.[12,13] We were unable, however, to demonstrate deletion of the critical region of PWS (proximal long arm of chromosome 15 [15q11–q13]) and the methylation pattern of *SNRPN* exon 1 for PWS. Considering the distinctive facial features, we decided to perform FISH using D1Z2 mapped on 1p36.3, and we found a de novo deletion of this region in both patients.

Patients with 1p36 deletion syndrome have clinical features overlapping those of PWS. Furthermore, the PWS-like phenotype has been described in patients with chromosome Xq duplication,[15,16] fragile X syndrome,[17] upd(14)mat,[9,10] and 6q deletion syndrome.[11] Slavotinek *et al.* reviewed 39 patients reported to have pure 1p36 deletion, and found 2 (5.1%) with the PWS-like phenotype.[2] Using FISH and/or microsatellite markers, D'Angelo *et al.* screened 41 patients with negative results for PWS, presenting with hypotonia, developmental delay, obesity and/or hyperphagia, and behavioral problems, and detected a patient with a subtelomeric deletion of 1p.[8] Mitter *et al.* analyzed a cohort of 33 patients with low birthweight, feeding difficulties, and consecutive obesity for whom PWS was excluded on methylation analysis of *SNRPN*, and detected upd(14)mat in four of the

patients.[10] PWS is known to be one of the most common microdeletion syndromes, one of the most frequent disorders seen in genetics clinics, and the most commonly recognized genetic form of obesity.[18] Therefore additional screening on FISH with the appropriate probes combined with MS-PCR at the maternally expressed gene 3 (*MEG3*; also referred to as *GTL2* for gene trap locus 2) promoter region in patients with PWS-like phenotype should be considered for alternative diagnoses.

We determined the deletion breakpoints on FISH using the BAC clones mapped on the critical regions in both of the present patients: the breakpoints were different in both patients. D'Angelo *et al.* demonstrated that their patient with the 1p36 deletion and PWS-like phenotype had a terminal deletion of 2.5 Mb (Fig. 3); the authors suggested that the chromosomal segment 1p36.33-p36.32 is the critical region for the manifestation of obesity and hyperphagia.[8] Genotype–phenotype correlations may be useful to locate the genes responsible for several clinical features of the syndrome;[6] the degree of mental retardation is dependent on the deletion size. Heilstedt *et al.* analyzed the breakpoints in 61 patients with the 1p36 deletion, and elucidated potential critical regions for the clinical findings of facial clefts, hypothyroidism, cardiomyopathy, hearing loss, large fontanel, and hypotonia.[6] In the Battaglia *et al.* study, behavioral disorders were commonly observed (47%) in the patients with the 1p36 deletion, including self-biting of hands and wrists (30%), temper tantrums (22%), and hyperphagia (13%), overlapping the typical phenotype of PWS.[4] Reduced social interaction and severe–profound mental retardation, however, are distinct features of 1p36 deletion from PWS. Together with the present results and the D'Angelo *et al.* study, we suggest that the critical region for the PWS-like phenotype is within 4 Mb from 1pter.

In summary, the clinical features of 1p36 deletion syndrome overlap those of PWS, recognized as the PWS-like phenotype. We mapped the aberrations in two patients with the 1p36 deletion associated with the PWS-like phenotype using molecular cytogenetics. We hypothesize the possible involvement of the terminal 4 Mb region of chromosome 1p36 in the PWS-like phenotype.

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CASE REPORT

Brachmann-de Lange syndrome with congenital diaphragmatic hernia and *NIPBL* gene mutation

Shinichi Hosokawa[1], Nobumasa Takahashi[1], Hiroyuki Kitajima[1], Masahiro Nakayama[2], Kenjirou Kosaki[3], and Nobuhiko Okamoto[4]

Departments of [1]Neonatology, [2]Pathology, and [4]Developmental Pediatrics, Osaka Medical Center and Research Institute for Maternal and Child Health, and [3]Department of Pediatrics, Keio University School of Medicine, Osaka, Japan

ABSTRACT **We report herein a case of Brachmann-de Lange syndrome complicated with congenital diaphragmatic hernia in which a *NIPBL* gene mutation was identified. A female infant born at 37 weeks of gestation died 134 min after delivery, even though endotracheal intubation and resuscitation were performed immediately after the scheduled caesarean operation. We diagnosed the infant with Brachmann-de Lange syndrome from her physical characteristics. An abnormal peak at the 29th exon in the translation area of the *NIPBL* gene was detected using denaturing high-performance liquid chromatography. In addition, a mutation of cytosine to thymine (nonsense mutation) at the 5524th base was identified using the direct sequence method. This variation was likely the cause of the syndrome.**

Key Words: Brachmann-de Lange syndrome, congenital diaphragmatic hernia, denaturing high-performance liquid chromatography, direct sequence method, gene mutation

INTRODUCTION

Brachmann-de Lange syndrome (BDLS) is a multiple congenital anomaly syndrome characterized by growth and mental retardation, variable anomalies of the upper limbs and a peculiar face with hypertrichosis. A pediatrician named de Lange (1933) reported two cases of this disease while working at Amsterdam University in the Netherlands, and termed the disease Cornelia de Lange syndrome. It was subsequently revealed that Brachmann (1916) had reported on a patient exhibiting the same symptoms. As a result of these two reports, the condition is currently known as Brachmann-de Lange syndrome (Opitz 1985).

Brachmann-de Lange syndrome was originally thought to be related to 3q partial trisomic syndrome, as the clinical manifestations of the two diseases are relatively similar. More recently, Krantz *et al.* (2004) and Tonkin *et al.* (2004) reported a variation in the *NIPBL* gene in a BDLS patient, allowing the two diseases to be more easily distinguished.

We report herein a case of BDLS with congenital diaphragmatic hernia caused by a mutation in the *NIPBL* gene that was identified using denaturing high-performance liquid chromatography.

Case report

A 21-year-old woman delivered a female infant at 37 weeks and 2 days of gestation by scheduled caesarean operation due to intrauterine growth retardation and congenital diaphragmatic hernia diagnosed by fetal echography at a gestational age of 30 weeks and 2 days. The infant's birthweight was 1766 g (–2.6 SD) and her Apgar score was 1 at 1 min and 3 at 5 min. When the infant was born, her entire body was pale and she did not demonstrate spontaneous breathing patterns. Endotracheal intubation was immediately performed and artificial ventilation with high frequency oscillation (HFO) and nitric oxide inhalation therapy was initiated. Unfortunately, there was no improvement in her condition, even following the administration of resuscitative medication, including adrenaline and surfactant, and she died 134 min after birth.

We considered that the patient had BDLS due to her characteristic facial features, including synophrys, brachyrrhinia, long philtrum, thin lip, small mandible and short cervix, and the presence of hirsutism and a congenital diaphragmatic hernia. Although her limbs were small and short, and a bilateral single transverse palmar crease was recognized on each hand, the BDLS characteristics of syndactyly and limb reduction defects were not observed (Fig. 1).

At laboratory examination at birth, we identified slight acidosis; however, significant abnormal findings, including anemia and electrolyte imbalance in the cord blood were not observed. The infant's blood gas (venous blood) at 47 min after birth was also recognized as mixed acidosis of pH 6.763, PCO_2 188.0 mmHg, PO_2 3.2 mmHg and BE –16.5 mmol/L. Her hemoglobin was 6.8 g/dL, her C-reactive protein (CRP) was negative and there was no elevation in liver enzyme levels. Hypernatremia was observed in her electrolytes (Table 1). Amniotic fluid chromosomes were of a normal karyotype of 46, XX. X-ray of the entire body revealed a hanging bell-shaped thoracic cage, low pneumatization in the bilateral lungs and a stomach bubble in the middle thorax (Fig. 2).

Pathological autopsy of the infant was undertaken after we obtained informed consent from her parents. The placental weight was 190 g, which was small for the number of gestational weeks (our center average is 514 g), villi were immature and the umbilical cord contained a single umbilical artery. The left diaphragm was almost entirely defective and the liver, stomach, spleen, pancreas, small intestine and large intestine protruded into the intrathoracic area. Marked hypoplasia of the lungs was also recognized with a pulmonary weight ratio of 0.003 (normal is 0.012). In addition, the lungs were histologically immature. Bilateral hydroureter, annular pancreas and atrial septal defect were also observed. We did not examine the brain, as the parents did not consent to craniotomy.

After we obtained written informed consent from the parents for gene diagnosis, we extracted genomic DNA from the patient's blood and amplified the coding region (extending from the 2nd exon to the 47th exon) of the *NIPBL* gene using polymerase chain reaction (PCR). An abnormal peak in exon 29 was detected when analyzed using denaturing high-performance liquid

Correspondence: Shinichi Hosokawa, MD, Murodou-chou 840, Izumi City, Osaka 594-1101, Japan. Email: sinhoso@mac.com
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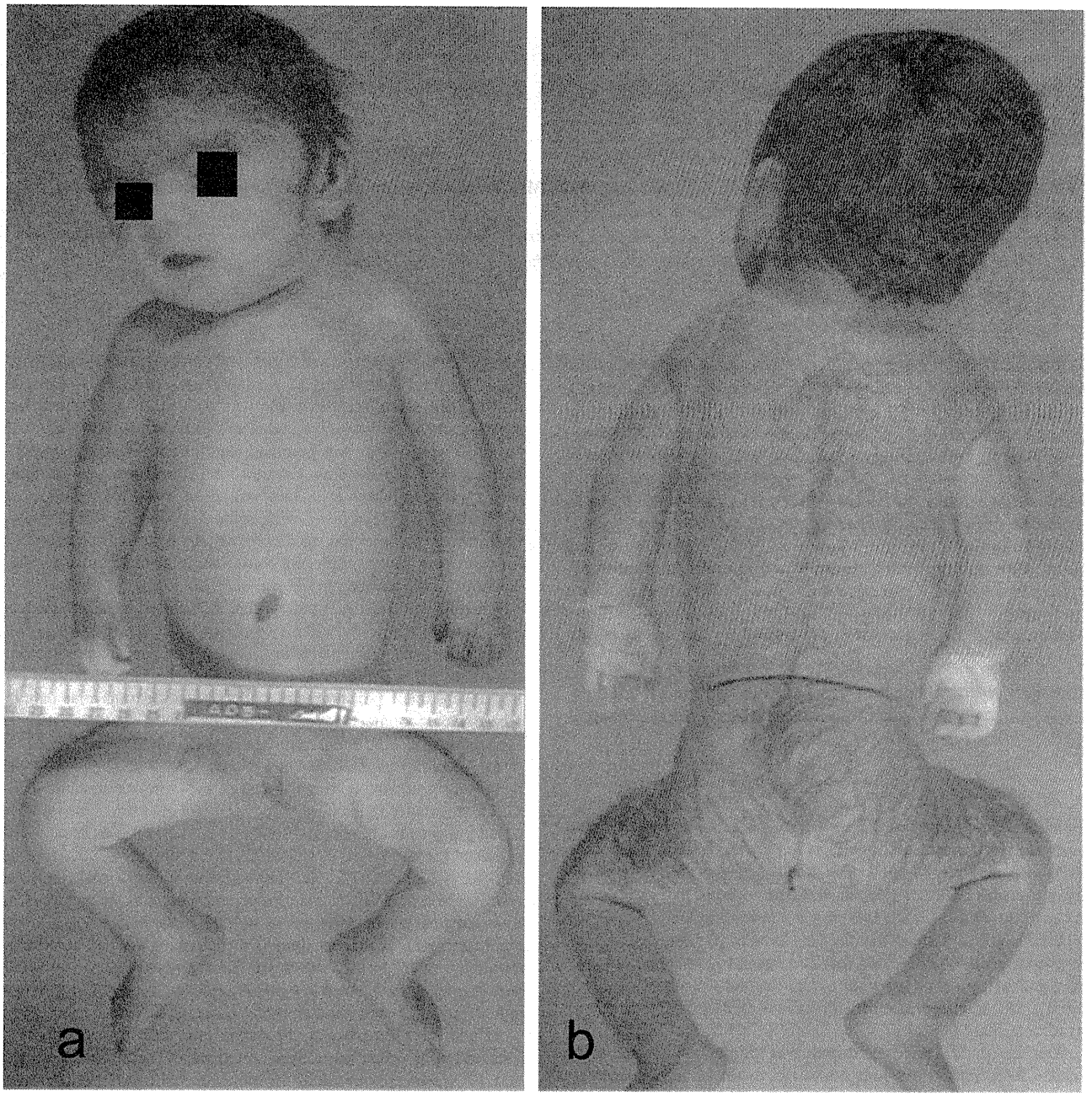

Fig. 1 Photographs highlighting the patient's symptoms (a,b) including hypertrichosis, short extremities, hypoplasia of the nipple and umbilicus, synophrys of the face, short and upturned nose or anteverted nostrils, long philtrum, thin lip, small mandible, short cervix and single bimanual palmar flexion curve without syndactyly or defects of the fingers.

chromatography (Fig. 3). Within the translation area of the *NIPBL* gene, a mutation of cytosine (C) to thymine (T) (nonsense mutation) at the 5524th base was identified using the direct sequence method. This amino acid change formed a stop codon, a result that we hypothesized would influence the complications in this patient.

DISCUSSION

Cornelia de Lange (1933) identified 10 traits, such as mental retardation, low birthweight, dwarfism, microbrachycephaly, heavy eyebrows meeting at the midline, long eyelashes, low-set ears, small hands and feet, proximal placed thumb and syndactyly of the toes in two patients while working at Amsterdam University. Beck (1976) later reported the original diagnostic standards of BDLS (Table 2) and suggested that patients with BDLS could be diagnosed if they exhibited eight of these 10 traits. In the current case, BDLS was not diagnosed in the fetal period, but was diagnosed after birth. The infant demonstrated nine of the traits described by de Lange and five of the traits described in the Beck standards. After confirming our findings with both the de Lange

Table 1 Examination of the umbilical cord and peripheral blood of the present case of Brachmann-de Lange syndrome with congenital diaphragmatic hernia and *NIPBL* gene mutation

	Cord blood	Patient's blood (47 min after birth)
Blood gas analysis		
pH	7.276	6.763
pCO_2	48.9 mmHg	188.0 mmHg
pO_2	20.5 mmHg	3.2 mmHg
Base excess	−4.3 mmol/L	−16.5 mmol/L
Blood cell counts		
White blood count	5900/μL	4900/μL
Platelet count	221 000/μL	93000/μL
Chemistry		
C-reactive protein	<0.05 mg/dL	<0.1 mg/dL
Sodium	140 mmol/L	183 mmol/L
Potassium	4.5 mmol/L	5.3 mmol/L
Calcium	9.5 mg/dL	8.3 mg/dL
Hemoglobin	13.6 g/dL	6.8 g/dL

Table 2 Findings in the present case of Brachmann-de Lange syndrome with congenital diaphragmatic hernia and *NIPBL* gene mutation

	This patient's findings
Cornelia de Lange (1933)	
Mental retardation	?
Low birthweight	+
Dwarfism	?
Microbrachycephaly	+
Heavy eyebrows meeting at the midline	+
Long eyelashes	+
Low ear insertion	−
Small hands and feet	+
Proximally placed thumb	−
Syndactyly of the toes	−
Beck (1976)	
Low hair line on forehead	+
Low hair line on neck	+
Long philtrum	+
Bushy eyebrows	+
Confluent eyebrows	+
Thick eyelashes	+
Antimongoloid eye slanting	−
Anteverted nostrils	+
Crescent-shaped mouth	+
Thin prolabium	+

+, present; ?, not detected due to early death.

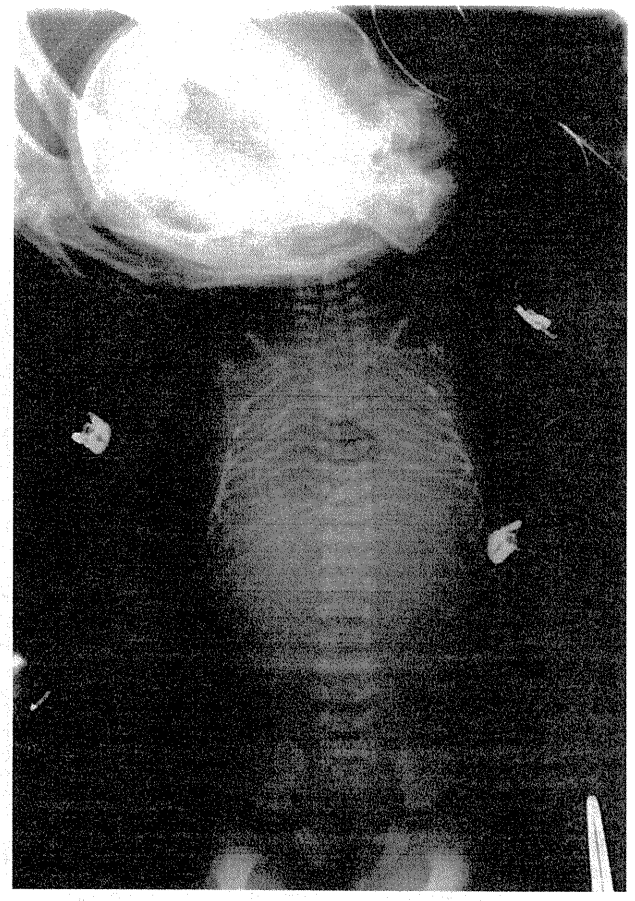

Fig. 2 X-ray of the entire body showing the hanging bell-shaped thoracic cage, low pneumatization in the bilateral lungs and a stomach bubble located in the middle thorax.

and Beck standards, we finally made a diagnosis based on the baby's physical characteristics.

This patient was also diagnosed based on the presence of intrauterine growth retardation and diaphragmatic hernia during the fetal period. Limb shortening was also observed. In the absence of abnormal karyotype or altered bone structures with limb shortening, BDLS is generally considered as a differential diagnosis (Beck and Fenger 1985; Kenneth 1988). Further, the placenta weighed only 190 g, which was low for the gestational period. This finding was consistent with the hypothesis that growth of not only the fetus, but also of the placenta is inadequate in cases of BDLS.

There have been only a few reports of BDLS with congenital diaphragmatic hernia in Japan (Kuroiwa *et al.* 1990; Suzuki *et al.* 1999). A small number of reports (e.g. Cunniff *et al.* (1993), Russel *et al.* (1993) and Marino *et al.* (2002)) have been described in other countries. The reports by these groups suggested that the prognosis was worse when the patient also exhibited congenital diaphragmatic hernia. The precise causes of congenital diaphragmatic hernia remain unknown. BDLS, Fryns syndrome, Goltz syndrome and Smith-Lemli-Opitz syndrome are all associated with congenital diaphragmatic hernia (Tibboel and Gaag 1996; Bianch *et al.* 2000). Recently, gene analysis of these various multiple malformation syndromes has been undertaken (Holder *et al.* 2007). Further gene

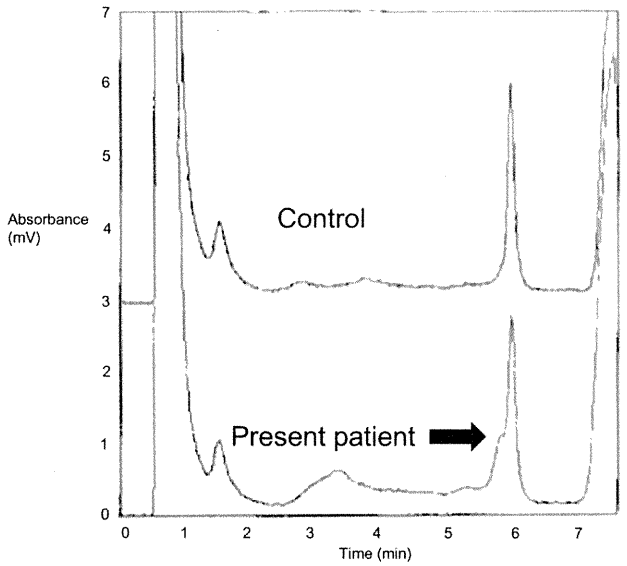

Fig. 3 Denaturing high-performance liquid chromatography of the 29th exon of the *NIPBL* gene (upper panel: control, lower panel: patient). Arrow shows the abnormal peak in the translation area (29th exon) of the *NIPBL* gene.

analyses in the various multiple malformation syndromes specifically associated with congenital diaphragmatic hernia are likely to shed light on which anomalies lead to diaphragmatic hernia.

In the present case, a mutation of C to T (nonsense mutation) at the 5524th base in the translation area of the *NIPBL* gene was identified. As a result, we concluded that this variation was likely to be the cause of the BDLS with diaphragmatic hernia. The *NIPBL* gene is located at 5p13.1 and contains 47 exons, and its transcription is thought to be related to Notch signal transmission. There have been many confirmed gene mutations, including deletion and insertion mutations, that are associated with BDLS (Gillis *et al.* 2004; Bhuiyan *et al.* 2006; Schoumans *et al.* 2007). Further, Musio *et al.* (2006) and Deardorff *et al.* (2007) have presented reports relating BDLS to both *SMC1* and *SMC3* gene mutations.

DNA analysis is important for confirming BDLS diagnosis. Analysis of gene mutations in genes such as *NIPBL* also represents a useful diagnostic method. With the accumulation of cases such as ours, further description of this disease will be possible.

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Co-Occurrence of Prader–Willi and Sotos Syndromes

Nobuhiko Okamoto,[1]* Noriko Akimaru,[1] Keiko Matsuda,[1] Yasuhiro Suzuki,[2] Keiko Shimojima,[3] and Toshiyuki Yamamoto[3]

[1]Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

[2]Department of Pediatric Neurology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

[3]Tokyo Women's Medical University Institute for Integrated Medical Sciences (TIIMS), Tokyo, Japan

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A patient with atypical phenotypes of Prader–Willi syndrome (PWS) was subjected to investigate genomic copy numbers by microarray-based comparative genomic hybridization analysis. Severe developmental delay, relative macrocephaly, protruding forehead, cardiac anomalies, and hydronephrosis were atypical for PWS. Concurrent deletions of 15q11-13 and 5q35 regions were revealed and identified as paternally derived. The sizes and locations of the two deletions were typical for both deletions. Although each deletion independently contributed to the clinical features, developmental disturbance was very severe, suggesting combined effects. This is the first report of co-occurrence of PWS and STS. The co-occurrence of two syndromes is likely incidental. © 2010 Wiley-Liss, Inc.

Key words: Prader–Willi syndrome; Sotos syndrome; aCGH

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INTRODUCTION

Prader–Willi syndrome (PWS; OMIM #176270) is caused by deficiency of paternally expressed imprinted transcripts within chromosome 15q11-q13 [Ledbetter et al., 1981]. It is characterized by obesity, hypotonia, hypogonadism, and behavioral abnormalities [Holm et al., 1993]. Most paternal PWS deletions are bracketed by recurrent breakpoints (BP)1 or BP2 and BP3. Preturbed expression of genes including *SNURF–SNRPN* and multiple small nucleolar RNAs (*snoRNAs*) are associated with the clinical manifestations of PWS, but the specific contributions of individual genes are under investigation. Recent analysis revealed that deficiency of HBII-85 *snoRNAs* causes the key characteristics of the PWS phenotype, although some atypical features suggest that other genes in the region may make more subtle phenotypic contributions [Sahoo et al., 2008].

Sotos syndrome (STS; OMIM#117550) is an overgrowth syndrome characterized by pre- and postnatal overgrowth, macrocephaly, developmental delay, advanced bone age, and a distinctive face including frontal bossing, frontal sparseness of hair, hypertelorism, downslanting palpebral fissures, and pointed chin. Haploinsufficiency of the *NSD1* gene due to 5q35 microdeletions or intragenic mutations causes STS [Kurotaki et al., 2002]. Miyake et al. [2003] observed that microdeletions in STS are mostly of paternal origin. Common deletion breakpoints were located at two flanking low copy repeats (LCR), implying that non-allelic homologous recombination (NAHR) between LCRs is the major mechanism for the common deletion in STS [Kurotaki et al., 2005; Visser et al., 2005]. Central nervous system anomalies, cardiovascular and urogenital symptoms are more frequent in the microdeletion group [Nagai et al., 2003].

In this study, a patient with atypical phenotypes of PWS was subjected to investigate genomic copy numbers by microarray-based comparative genomic hybridization (aCGH) analysis. Concurrent deletions of 15q11-13 and 5q35 regions were detected and identified as paternally derived. Although each deletion independently contributed to the clinical features, growth and developmental disturbance were very severe, suggesting combined effects. This is the first report of co-occurrence of PWS and STS.

CLINICAL REPORT

A 14-year-old male propositus is the first-born child of healthy and non-consanguineous parents. After uncomplicated pregnancy, he was born at 39 weeks of gestation by induced delivery with overgrowth of length with 53 cm (90th centile).

Grant sponsor: Ministry of Health, Labour and Welfare in Japan.
*Correspondence to:
Dr. Nobuhiko Okamoto, Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan. E-mail: okamoto@osaka.email.ne.jp
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His birth weight was within a normal limit as 3,010 g (25th centile). He was the first child of a 26-year-old mother and a 30-year -old father. Since cardiac murmur was found at birth, he was transferred to the neonatal intensive care unit and ventricular septal defect (VSD), atrial septal defect (ASD), and patent ductus arteriosus (PDA) were revealed by echocardiography. Micropenis and bilateral cryptorchidism were noticed. He had severe hypotonia and feeding difficulties in the early infantile period. Until his sucking improved at 6 months old, nasal tube feeding was required. Ultrasonography revealed bilateral vesicoureteral reflux and hydronephrosis. He showed a severe developmental delay with head control at 1 year of age and sitting alone at 6 years of age. He had generalized seizures at age 6 years. Electroencephalography revealed sporadic spikes at that time. Brain MRI showed no significant findings. He developed progressive obesity, as his weight was 10.0 kg (75th centile) at 9 months old of age and 12.4 kg (95th centile) at 1 year old of age. Conventional G-band chromosome analysis showed a normal male karyotype, and subsequent conventional FISH analysis for *SNRPN* revealed a deletion, indicating a diagnosis of PWS. In spite of that, relative macrocephaly, protruding forehead, frontal baldness, and mild overgrowth were atypical for phenotypic features of PWS (Fig. 1A). Although he was interested in food, hyperphagia was not prominent because of his restricted locomotive abilities. Gradually, his height SD scores decreased (Fig. 2). Partial growth hormone deficiency was found by endocrinological studies. When he was 14 years of age his bone age was measured at the 11-year-old level. His parents did not choose GH replacement therapy.

When we examined the patient at the age of 14 years, he showed severe mental retardation without vocalized words, muscular hypotonia, hypopigmentation, scoliosis, and distinctive facial features including protruding forehead; strabismus; hypertelorism; downslanting palpebral fissures; epicanthal folds; full cheeks; microstomia with downturned corners of the mouth; small hands with tapering fingers; and small feet (Fig. 1B). A wheel chair was required for him because his hip joint was unstable and he could not stand alone. His intelligent quotient (IQ) was measured by Kyoto Scale of Psychological Development as below 10. He was a calm and friendly boy. His interest in food became obvious, but self-injurious behaviors such as skin picking were not observed. Behavioral problems associated with STS including autistic spectrum disorder, hyperactivity, and aggression were not present. His weight was 29 kg (<3rd centile), and his length was 132 cm (<3rd centile) (Fig. 2). His head circumference was mean for his age. A comparison of typical features of PWS and STS and their clinical presentation in the patient are shown (Table I).

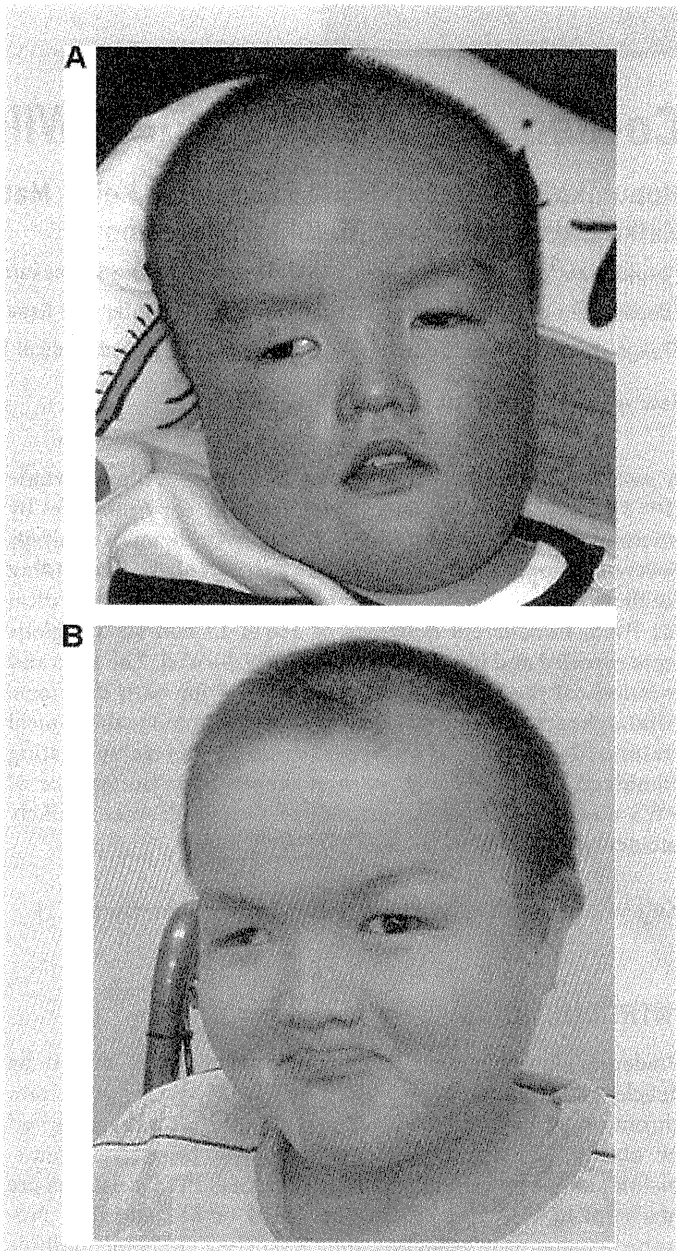

FIG. 1. Facial appearance of the patient at 6 years old (A) and 14 years old (B).

MATERIALS AND METHODS

After obtaining informed consents based on a permission approved by the institution's ethical committee, peripheral blood samples were obtained from the patient and his parents. Genomic DNAs were extracted using the QIAquick DNA extraction kit (QIAgen, Valencia, CA).

Based on the hypothesis that the patient might have an atypically larger deletion of chromosome 15 or have additional chromosomal aberrations, aCGH analysis was performed using the Human Genome CGH Microarray 60K (Agilent Technologies, Santa Clara, CA) as described previously [Shimojima et al., 2009].

Metaphase nuclei were prepared from peripheral blood lymphocytes by mean of standard methods and used for FISH analysis with human BAC clones selected from the UCSC genome browser (http://www.genome.ucsc.edu) as described elsewhere [Shimojima et al., 2009]. Physical positions refer to the March 2006 human reference sequence (NCBI Build 36.1).

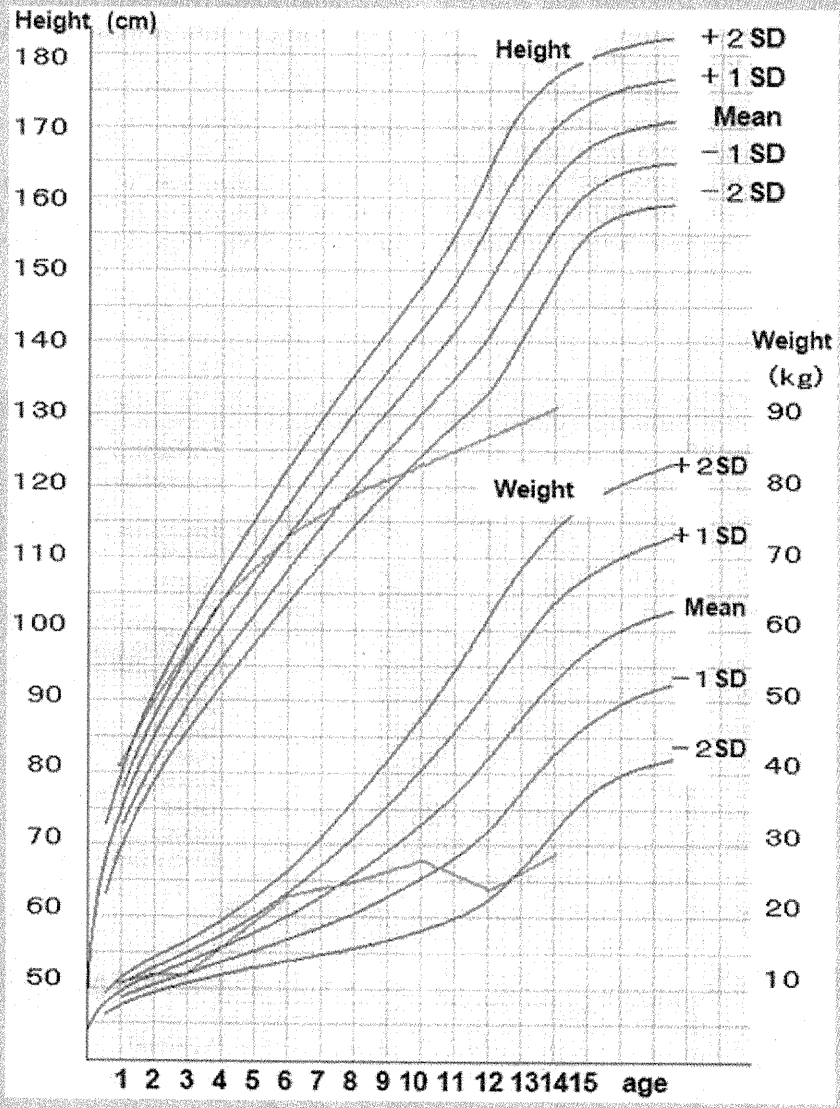

FIG. 2. Growth curve of the patient. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. A Comparison of Typical Features of PWS and STS and Their Clinical Presentation in the Current Patient

	Prader–Willi	Sotos	Current patient
Hypotonia	+	+	++
Mental delay	+	+	++
Hypopigmentation	+	−	+
Prominent forehead	−	+	+
Strabismus	+	+	++
Over growth	−	+	−
Growth delay	+	−	++
Obesity	+	−	+
Epilepsy	−	+	+
Congenital heart disease	−	+	+
Scoliosis	+	+	++
Hydronephrosis	−	+	+
Hypogonadism	+	−	+

+, common features; ++, prominent manifestations.

Microsatellite marker analysis was performed using the ABI Prism Linkage Mapping Set with D15S1002 and analyzed by GeneMapper (Applied Biosystems, Foster City, CA). In the deletion region of STS, no marker was available for the ABI Prism Linkage Mapping Set. Thus, the single-nucleotide polymorphisms (SNP) typing was carried out. From the STS deletion region of 5q35, eight SNPs, IMS-JST038690, IMS-JST087588, IMS-JST087589, IMS-JST183486, IMS-JST172005, IMS-JST073857, IMS-JST087921, and IMS-JST087922, were selected using in silico library, Japanese Single Nucleotide Polymorphisms (JSNP) database (http://snp.ims.u-tokyo.ac.jp/index.html). Allelic types were analyzed by PCR-direct sequencing method using the BigDye terminator (Applied Biosystems, Foster City, CA).

RESULTS

By aCGH analysis, loss of the genomic copy numbers was identified in the region of 15q11.2, which is responsible and typical for PWS (Fig. 3A). The concurrent deletion was

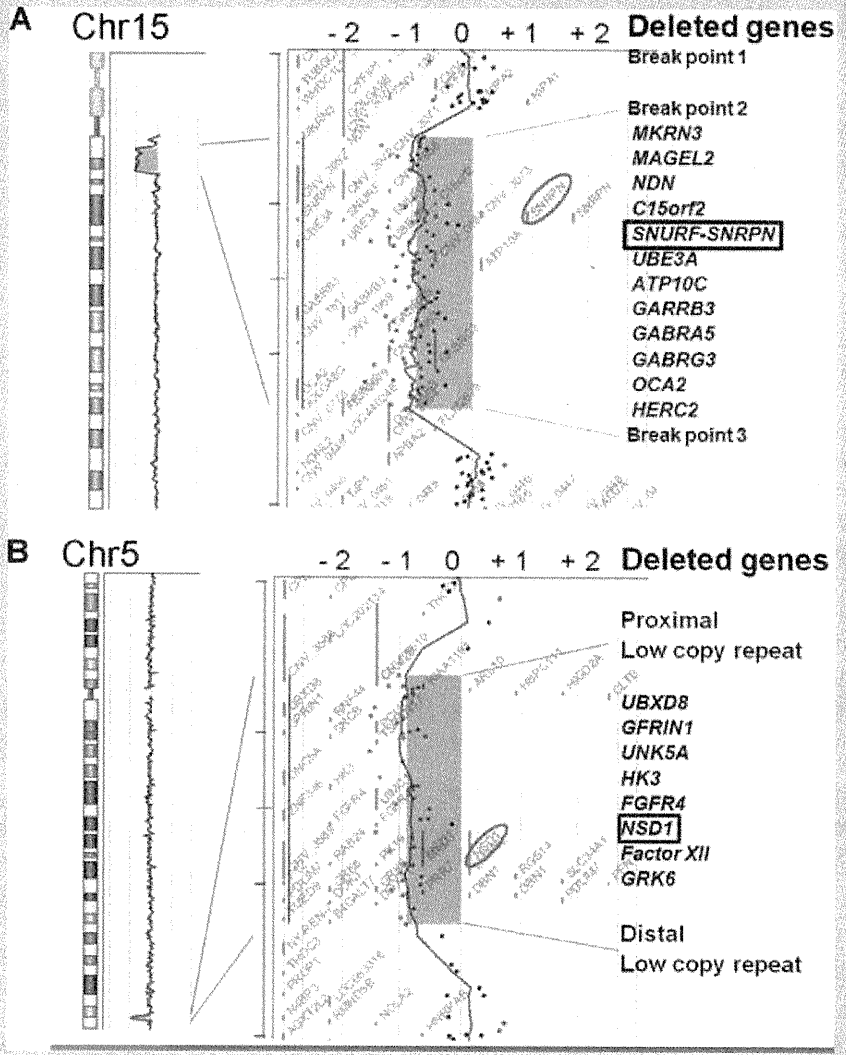

FIG. 3. aCGH profiles of the patient shown by CGH Analytics in Chromosome view (left) and Gene view (right). A: Typical deletion of PWS region including *SNRPN* is shown. B: Typical STS deletion including *NSD1* is indicated. The horizontal axis indicates the log 2 ratio of the genomic copy number. The blue rectangles indicate the regions containing copy number aberrations. The aberration areas are expanded in Gene view (right). The dots indicate the locations and the corresponding log 2 ratios of the probes. The red circles emphasize *SNRPN* and *NSD1*.

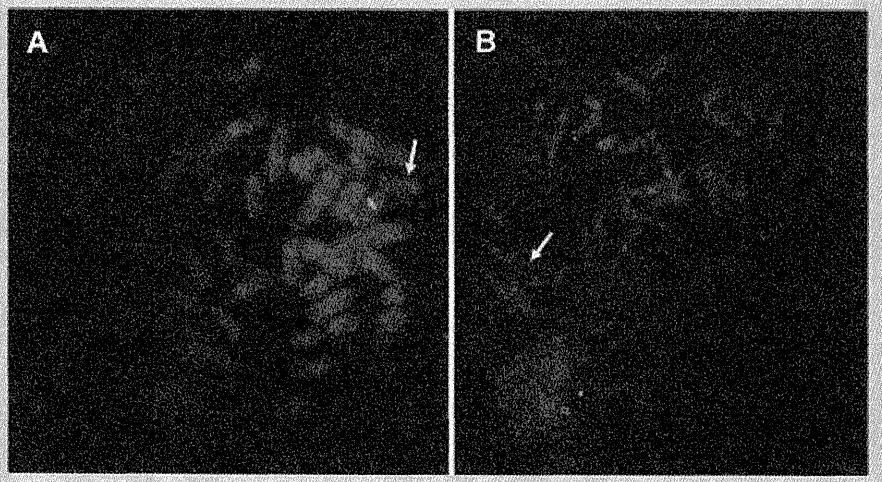

FIG. 4. FISH analysis to confirm the chromosomal deletion. A: One of the green signals covering *SNRPN*, RP11-1071C22 (15q11.2; 22601976–22822028), was deleted. Two red signals are the markers of chr15, RP11-48A4 (15q26.3; 99433829–99587322). B: One of the green signals covering *NSD1*, RP11-99N22 (5q35.2–5q35.3; 176474586–176655375), was deleted, whereas two red labeled RP11-94J21 (5p15.33; 1377471–1540913) signals were confirmed in all cells. Physical positions are referred to NCBI Build 36.1. White arrows indicate abnormal chromosomes in each FISH image.

identified in the region of 5q35, which is also responsible and typical for STS (Fig. 3B). FISH analyses confirmed the deletion of both regions (Fig. 4). There were no deletions of PWS region and STS region in both parents indicating de novo occurrence (data not shown).

To confirm the parental origin of both deletions, polymorphic markers were analyzed in the patient and his parents. Regarding the 15q11.2 region, the patient showed an only allele with 112-bp common to his mother, indicating the deletion of paternal allele (Fig. 5A). Among eight analyzed SNPs, only IMS-JST183486 was informative. The patient showed hemizyogous of T at the SNP position, whereas the father and the mother showed homozygous of A and T, respectively (Fig. 5B). From the result, we concluded that both deletions were derived from the paternal allele.

DISCUSSION

Initially, the patient was diagnosed as PWS due to severe hypotonia, hypopigmentation, hypoplastic genitalia, and small hands and feet. It was supported by hyperphagia and obesity which later developed. However, his facial features including relative macrocephaly, protruding forehead, frontal baldness, strabismus, downslanting palpebral fissures, and pointed chin were atypical for PWS. He also showed congenital cardiac anomalies, hydronephrosis, and epilepsy, which are rare findings in PWS. Severe hypotonia and severe developmental delay were also atypical for PWS. This was the reason why we analyzed genomic copy numbers.

To the best of our knowledge, this is the first report of co-occurrence of PWS and STS. Translocation between chromosomes 5 and 15 was excluded by G-banded analysis. Array CGH demonstrated that the sizes and locations of the two deletions were typical for both syndromes. Both of the deletions were derived from the paternal chromosome. We suspect that co-occurrence of two deletions is incidental.

His growth curve showed an interesting pattern. He showed overgrowth in the infantile period. Gradually, his growth velocity decreased. Now he shows severe growth deficiency. Although we understand that haploinsufficiency of *NSD1* might lead to height gain and patients with STS show advanced bone age, his growth deficiency was worse compared with standard PWS patients and his bone age was delayed [Nagai et al., 2000]. Growth hormone deficiency and severe scoliosis may explain his growth deficiency. We posit that each deletion contributed independently to the features. Severe growth and developmental delay might be explained by the combined effects of PWS and STS.

There are some reports of concurrent chromosomal aberrations in the same patients [Shimojima et al., 2009]. The result of this study indicates that there may be more frequent co-occurrences of two more deletions than what we think. When a patient shows atypical or overlapping features regardless of a previously established diagnosis, we would recommend investigation of whole genomic copy numbers by aCGH.

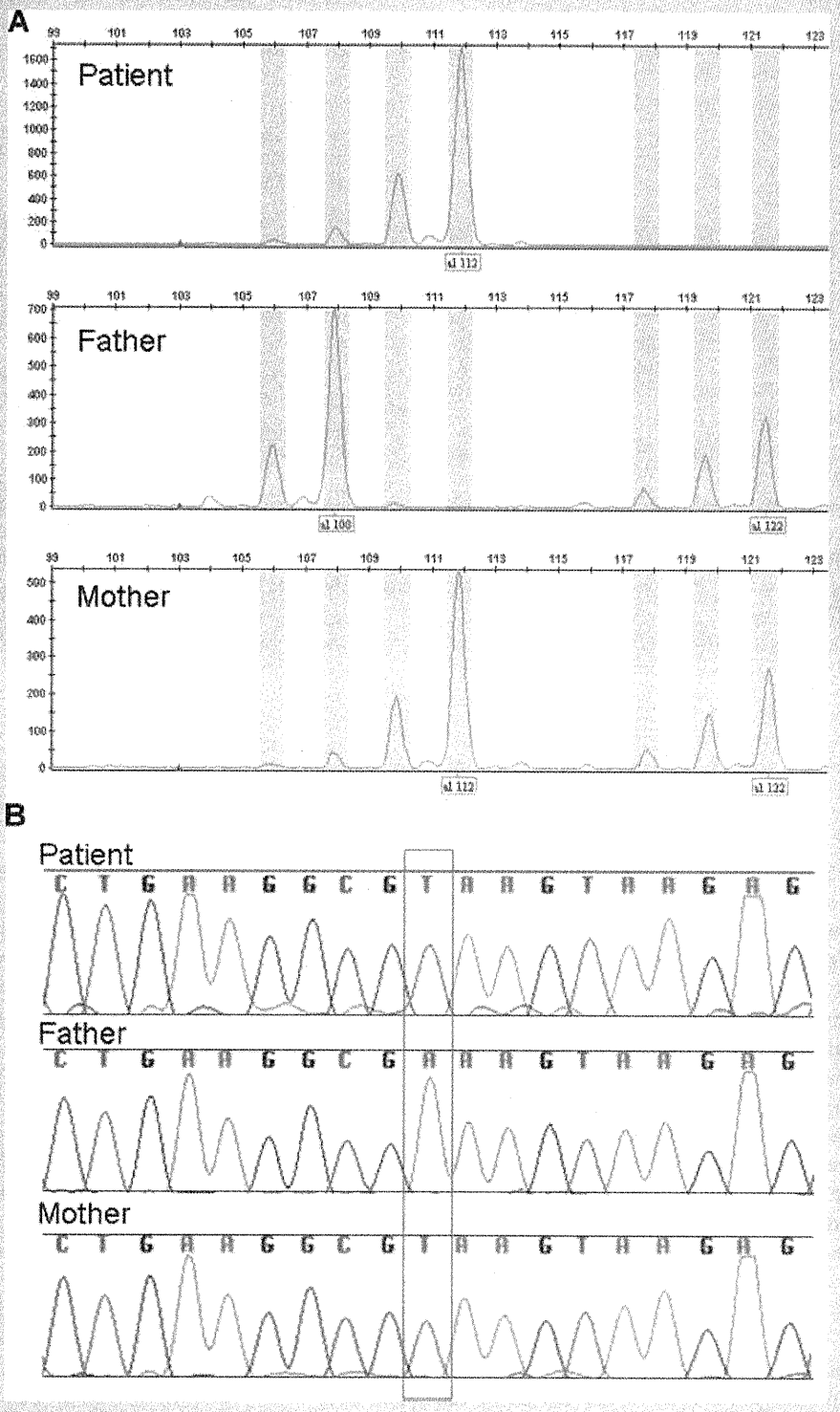

FIG. 5. Molecular analysis of the patient's family. A: GeneMapper analysis using D15S1002. The patient shows only one allele with 112-bp common with his mother, indicating the paternal deletion. B: SNPs analysis of IMS-JST183486. The patient's SNP type as T is only common with his mother, indicating the deletion of paternal allele.

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CASE REPORT

Severe Peters Plus syndrome-like phenotype with anterior eye staphyloma and hypoplastic left heart syndrome: Proposal of a new syndrome

Reiko Shimizu[1], Ryota Saito[2], Kenji Hoshino[2], Kiyoshi Ogawa[2], Takashi Negishi[3], Jiro Nishimura[4], Norimasa Mitsui[5], Makiko Osawa[1], and Hirofumi Ohashi[5]

[1]Department of Pediatrics, School of Medicine, Tokyo Women's Medical University, Tokyo, and Divisions of [2]Cardiology, [3]Ophthalmology, [4]Plastic Surgery, and [5]Medical Genetics, Saitama Children's Medical Center, Saitama, Japan

ABSTRACT **Peters Plus syndrome is a very rare autosomal recessive condition characterized by ocular defects (typically Peters anomaly) and other systemic major/minor anomalies. Mutations in the *B3GALTL* gene encoding β 1,3-glucosyltransferase have been found in virtually all patients with typical Peters Plus syndrome. We report on a female patient with unusually severe manifestations of Peters Plus syndrome, including anterior eye staphyloma, cleft lip and palate, and hypoplastic left heart syndrome (HLHS). Analysis of the *B3GALTL* gene revealed no mutation in the patient. To our knowledge, HLHS has not previously been reported in Peters Plus syndrome so far, and anterior staphyloma, a most severe defect of the anterior eye chamber, is also apparently rare in the syndrome. Our patient might represent a new syndrome of severe Peters Plus syndrome-like phenotype with anterior eye staphyloma and HLHS.**

Key Words: anterior staphyloma, hypoplastic left heart syndrome, Peters anomaly, Peters Plus syndrome

INTRODUCTION

Peters Plus syndrome (MIM 261540) is a rare autosomal recessive disease characterized by ocular anterior chamber defects (typically Peters anomaly), distinctive facies (prominent forehead, hypertelorism, narrow palpebral fissure, long philtrum, cupid bow upper lip and malformed ears), cleft lip and palate, short hands and feet, and growth and developmental delay. Since the first detailed delineation of the syndrome by van Schooneveld *et al.* (1984), who coined the term 'Peters Plus syndrome', on the basis of 11 patients with anterior chamber cleavage defect and other multiple congenital anomalies, more than 60 patients have been reported to date. Recently, the syndrome was found to be caused by mutations in β1,3 glucosyltransferase like gene (*B3GALTL*) which codes for glucosyltransferase, β3Glc-T, suggesting that the syndrome is a glycosylation disorder (Lesnik Oberstein *et al.* 2006; Reis *et al.* 2008).

Congenital heart defects are occasionally found in the syndrome, at a frequency of around 30%, including atrial septal defect, ventricular septal defect, subvalvular aortic stenosis, pulmonary stenosis, and bicuspid pulmonary valve (Maillette de Buy Wenniger-Prick and Hennekam 2002). To our knowledge, however, hypoplastic left heart syndrome (HLHS) has not been previously described in the syndrome. Here we report a Japanese girl with Peters Plus syndrome-like phenotype and HLH. In addition, the patient had anterior eye staphyloma, a most severe developmental defect of the anterior eye chamber.

Correspondence: Reiko Shimizu, MD, PhD, Department of Pediatrics, School of Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 160-0022, Japan. Email: rmuto@ped.twmu.ac.jp
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CLINICAL DETAILS

The patient, a female, was born by normal delivery after an uneventful 37-week pregnancy to a 32-year-old, gravid 3, para 1, abortus 1 mother and a 34-year-old father, both Japanese, healthy and unrelated. No polyhydroamnios was noted in the pregnancy. The patient had a healthy elder brother. Her birth weight was 2390 g (−1.7 SD), length 46.2 cm (−1.4 SD) and occipitofrontal circumference (OFC) 32.0 cm (± 0 SD). Soon after birth, it was noticed that she had bilateral cleft lip and palate, and corneal opacity of both eyes. Other features also noted were a round face, a narrow forehead, hypertelorism, micrognathia, malformed ears, right preauricular pit, short broad hands, short fifth fingers with single flexion crease and hyperextensible finger joints (Fig. 1). No apparent rhizomelic limb shortening was noticed. Ophthalmological examination revealed anterior staphyloma of both eyes, lacking apparent anterior chamber structures (Fig. 2). On B-mode ultrasonography, both eyeballs were found to be small (around 12 mm), while optic nerves were normally observed.

The day after birth, the patient showed tachypnea and cyanosis and was admitted to our hospital on the suspicion of congenital heart defect. On admission, her weight was 2466 g (76 g gain in one day after birth) in spite of poor intake, heart rate 160/min, respiratory rate 70/min, and SpO_2 95% in room air. Cardiothoracic ratio (CTR) was 56% on a chest X-ray. Echocardiography revealed HLHS with aortic and mitral atresia and a large patent ductus arteriosus (Fig. 3). Despite intensive care, including prostaglandin E1 infusion, she died of ductal shock at the age of 15 days. A post-mortem autopsy was not granted and histopathological investigation for eyes was not performed. Although we could not perform thorough imaging studies, such as total skeletal survey and abdominal echography, no vertebral and urogenital anomalies were noted in the patient.

Chromosome analysis on lymphocytes revealed a normal 46, XX karyotype. Polymerase chain reaction (PCR) and direct sequencing analysis of the *B3GALTL* gene using genomic DNA obtained from residual peripheral blood used for chromosome analysis revealed no

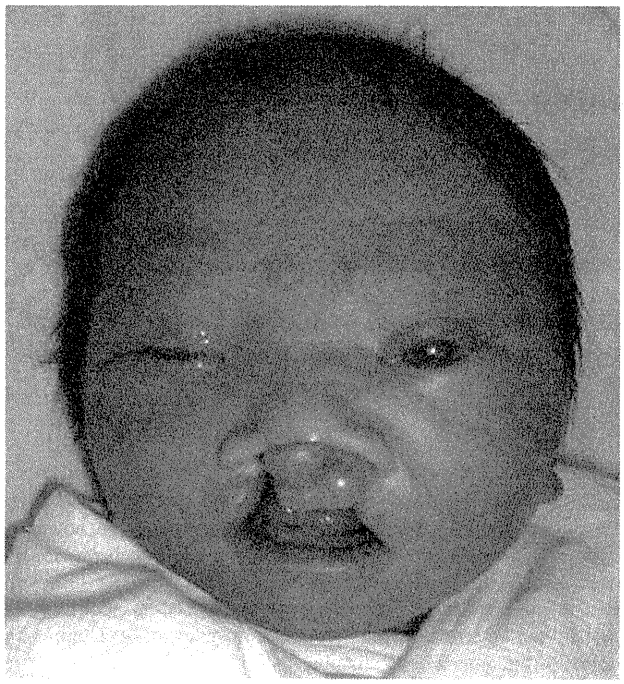

Fig. 1 Craniofacial view of the patient. (Permission for the presentation of this picture was obtained from the patient's parents.)

mutation in any exons or splice sites of the gene in the patient. Unfortunately, we could not perform further studies due to lack of specimen, such as array comparative genomic hybridization and multiplex ligation-dependent probe amplification to evaluate subtle genomic imbalance.

DISCUSSION

HLHS is a serious congenital heart defect characterized by severe underdevelopment of the left side heart structure with a prevalence of 1.4–3.8% among all congenital heart defects. The cause is unknown but is believed to be related to altered blood flow in cardiac embryogenesis leading to obstruction of the left side of the heart (Towbin *et al.* 1999). At least 10% of patients die within one week, and all patients without treatment die in the first year of life. HLHS is known to be occasionally associated with the following genetic entities: (i) Mendelian disorders: Apert syndrome, Holt-Oram syndrome, Ellis-van Creveld syndrome, Smith-Lemi-Opitz syndrome, Beckwith-Wiedemann syndrome, CHARGE syndrome, DiGeorge syndrome, Heterotaxy, Allagile syndrome, Rubinstein-Taybi syndrome, short rib-polydactyly syndrome type 3, PAGOD syndrome (Natowicz *et al.* 1988; Hanauer *et al.* 2002; Robert *et al.* 2007); (ii) Chromosomal abnormalities (including microdeletion syndromes): trisomy 13, trisomy 18, Turner syndrome, 7q35 deletion, Jacobsen syndrome and 16q24 deletion (Grossfeld 1999; Sedmera *et al.* 2005); and (iii) Non-Mendelian disorders: Sirenomelia sequence (Kim *et al.* 2007). However, a search of English-language publications failed to find any cases of Peters Plus syndrome associated with HLHS.

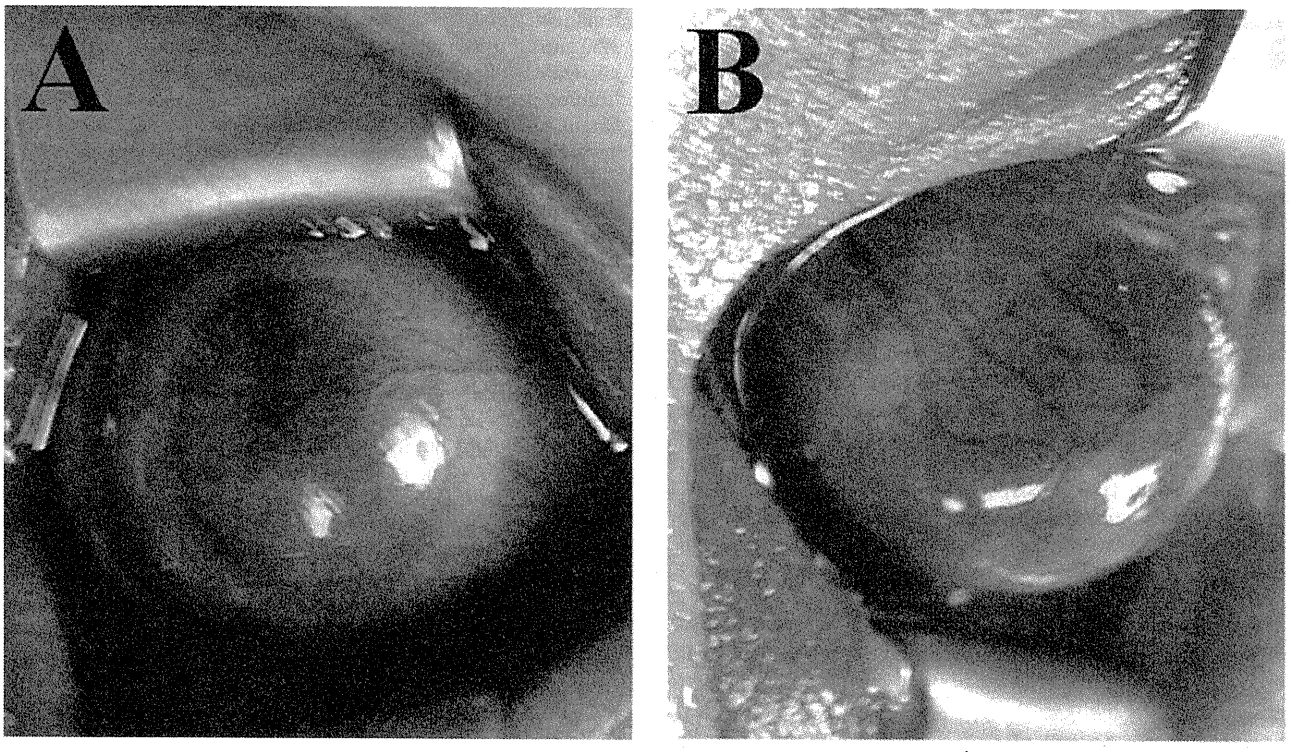

Fig. 2 Anterior staphyloma of both eyes. (A) Right eye. (B) Left eye.

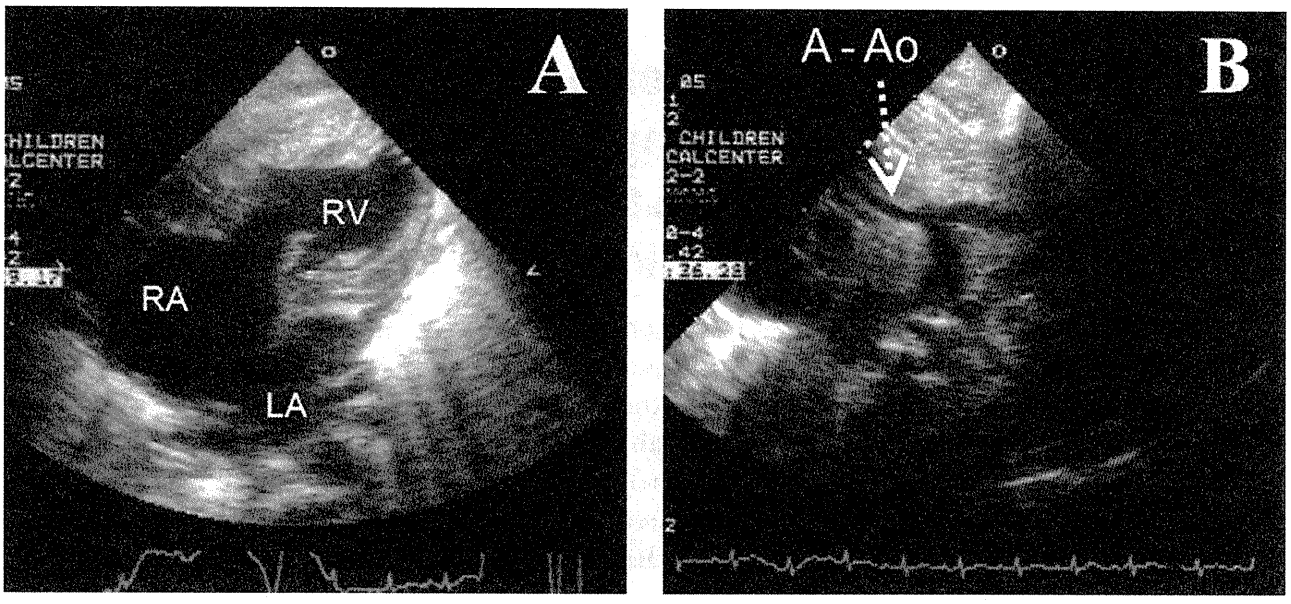

Fig. 3 Echocardiographic image of the patient. (A) Apical four-chamber view. Note hypoplastic left ventricle. (B) Suprasternal sagittal view. Note narrow ascending aorta of less than 1.5 mm in diameter. A-Ao, ascending aorta; LA, left atrium; RA, right atrium; RV, right ventricle.

Analysis of *B3GALTL* gene mutation status in two previous studies revealed the exclusively constant c.660 + 1G > A mutation in exon 8. Of 19 patients (or families) studied, homozygous c.660 + 1G > A mutation was found in 15 patients, and the remaining four patients were compound heterozygotes for the common c.660 + 1G > A mutation and for another mutation, c.230insT, c.347 + 5G > A, c.459 + 1C > A, and entire gene deletion, respectively (Lesnik Oberstein *et al.* 2006; Reis *et al.* 2008). It is noteworthy that mutations were found in all these typical patients.

The most characteristic feature of Peters Plus syndrome is Peters anomaly of the anterior eye chamber characterized by central corneal opacity (leukoma), thinning of the posterior aspect of the cornea, and iridocorneal adhesions, which are observed in 73% of patients (Maillette de Buy Wenniger-Prick and Hennekam 2002). The remaining patients have less severe anterior chamber manifestations, such as posterior embryotoxon. However, anterior staphyloma (ectasia of the cornea usually covered on the posterior surface by remnants of anteriorly displaced iris) present in our patient, a most severe expression of anterior chamber maldevelopment, is apparently rare in the syndrome.

As mentioned above, our patient showed unusual features of Peters Plus syndrome, such as HLHS and anterior staphyloma. Although we failed to identify similar previously published cases, an extensive search of non-English (Japanese) publications revealed a case similar to our patient. Nozaki *et al.* (1996) reported a 25-day -old Japanese boy who had anterior staphyloma in his left eye and HLHS. The cornea in his left eye was protruding and diffusely clouded and no recognizable anterior chamber structures were present, while hyperplasia of the iris stroma was noted in the right eye. Other features also described were congenital teeth, high arched palate, low-set ears and retained testes.

In conclusion, our patient and the patient reported by Nozaki *et al.* (1996) might represent a new syndrome of severe Peters Plus syndrome-like phenotype with anterior eye staphyloma and HLHS. The fact that no *B3GALTL* mutation was found in our patient also suggests this notion.

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RAPID PUBLICATION

AMERICAN JOURNAL OF medical genetics PART A

A New Ehlers–Danlos Syndrome With Craniofacial Characteristics, Multiple Congenital Contractures, Progressive Joint and Skin Laxity, and Multisystem Fragility-Related Manifestations

Tomoki Kosho,[1]* Noriko Miyake,[2] Atsushi Hatamochi,[3] Jun Takahashi,[4] Hiroyuki Kato,[4] Teruyoshi Miyahara,[5] Yasuhiko Igawa,[6] Hiroshi Yasui,[7] Tadao Ishida,[7] Kurahito Ono,[8] Takashi Kosuda,[9] Akihiko Inoue,[10] Mohei Kohyama,[11] Tadashi Hattori,[12] Hirofumi Ohashi,[13] Gen Nishimura,[14] Rie Kawamura,[1] Keiko Wakui,[1] Yoshimitsu Fukushima,[1] and Naomichi Matsumoto[2]

[1]Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan

[2]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan

[3]Department of Dermatology, Dokkyo Medical University, School of Medicine, Mibu, Japan

[4]Department of Orthopedics, Shinshu University School of Medicine, Matsumoto, Japan

[5]Department of Ophthalmology, Shinshu University School of Medicine, Matsumoto, Japan

[6]Department of Urology, Shinshu University School of Medicine, Matsumoto, Japan

[7]First Department of Internal Medicine, Sapporo Medical University, Sapporo, Japan

[8]Department of Orthopedics, Tomioka General Hospital, Tomioka, Japan

[9]Department of Pediatrics, Tomioka General Hospital, Tomioka, Japan

[10]Department of Surgery, Tomioka General Hospital, Tomioka, Japan

[11]Department of Surgery, JA Hiroshima General Hospital, Hiroshima, Japan

[12]Department of Orthopedics, Aichi Children's Health and Medical Center, Oobu, Japan

[13]Division of Medical Genetics, Saitama Children's Medical Center, Saitama, Japan

[14]Department of Radiology, Tokyo Metropolitan Kiyose Children's Hospital, Kiyose, Japan

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We previously described two unrelated patients showing characteristic facial and skeletal features, overlapping with the kyphoscoliosis type Ehlers–Danlos syndrome (EDS) but without lysyl hydroxylase deficiency [Kosho et al. (2005) Am J Med Genet Part A 138A:282–287]. After observations of them over time and encounter with four additional unrelated patients, we have concluded that they represent a new clinically recognizable type of EDS with distinct craniofacial characteristics, multiple congenital contractures, progressive joint and skin laxity, and multisystem fragility-related manifestations. The patients exhibited strikingly similar features according to their age: *craniofacial*, large fontanelle, hypertelorism, short and downslanting palpebral fissures, blue sclerae, short nose with hypoplastic columella, low-set and rotated ears, high palate, long philtrum, thin vermilion of the upper lip, small mouth, and micro-retrognathia in infancy; slender and asymmetric face with protruding jaw from adolescence; *skeletal*, congenital contractures of fingers, wrists, and hips, and talipes equinovarus with anomalous insertions of flexor muscles; progressive joint laxity with recurrent dislocations; slender and/or cylindrical fingers and

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*Correspondence to:
Tomoki Kosho, M.D., Department of Medical Genetics, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan.
E-mail: ktomoki@shinshu-u.ac.jp

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progressive talipes valgus and cavum or planus, with diaphyseal narrowing of phalanges, metacarpals, and metatarsals; pectus deformities; scoliosis or kyphoscoliosis with decreased physiological curvatures of thoracic spines and tall vertebrae; *cutaneous*, progressive hyperextensibility, bruisability, and fragility with atrophic scars; fine palmar creases in childhood to acrogeria -like prominent wrinkles in adulthood, recurrent subcutaneous infections with fistula formation; *cardiovascular*, cardiac valve abnormalities, recurrent large subcutaneous hematomas from childhood; *gastrointestinal*, constipation, diverticula perforation; *respiratory*, (hemo)pneumothorax; and *ophthalmological*, strabismus, glaucoma, refractive errors.

Key words: a new type Ehlers–Danlos syndrome; craniofacial characteristics; multiple congenital contractures; joint laxity; talipes deformities; kyphoscoliosis; skin laxity; multisystem fragility; recurrent subcutaneous hematomas

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INTRODUCTION

The Ehlers–Danlos syndrome (EDS) is a heterogeneous group of heritable connective tissue disorders affecting as many as 1 in 5,000 individuals, characterized by joint and skin laxity, and tissue fragility [Steinmann et al., 2002]. The fundamental mechanisms of EDS are known to consist of dominant-negative effects or haploinsufficiency of mutant procollagen α-chains and deficiency of collagen-processing-enzymes [Mao and Bristow, 2001]. In a revised nosology, Beighton et al. [1998] classified EDS into six major types: (1) classical type (OMIM#130000) (causative gene, *COL5A1* or *COL5A2*; affected protein, α1(V) or α2(V) procollagen), (2) hypermobility type (OMIM#130020) (*TNXB*; tenascin-XB, in a small subset of cases), (3) vascular type (OMIM#130050) (*COL3A1*; α1(III) procollagen), (4) kyphoscoliosis type (OMIM#225400) (*PLOD*; lysyl hydroxylase), (5) arthrochalasia type (OMIM#130060) (*COL1A1* or *COL1A2*; α1(I) or α2(I) procollagen), and (6) dermatospraxis type (OMIM#225410) (*ADAMTS2*; procollagen I N-proteinase). Additional minor variants of EDS have been identified with molecular and biochemical abnormalities: Brittle cornea syndrome (OMIM#229200) (*ZNF469*) [Abu et al., 2008], EDS-like syndrome due to tenascin-XB deficiency (OMIM#606408) (*TNXB*; tenascin-XB) [Schalkwijk et al., 2001], progeroid form (OMIM#130070) (*β4GALT7*; xylosylprotein 4-beta-galactosyltransferase) [Kresse et al., 1987], cardiac valvular form (OMIM#225320) (*COL1A2*; α2(I) procollagen) [Schwarze et al., 2004], and EDS-like spondylocheirodysplasia (OMIM#612350) (*SLC39A13*; a membrane-bound zinc transporter) [Giunta et al., 2008].

We previously described two unrelated patients showing characteristic facial and skeletal features, with similarities to kyphoscoliosis type EDS but without lysyl hydroxylase deficiency [Kosho et al., 2005]. After observations of them over time and encounter with four additional unrelated patients including one reported by Yasui et al. [2003], we have concluded that they represent a new clinically recognizable type of EDS characterized by distinct craniofacial features, multiple congenital contractures, progressive joint and skin laxity, and progressive multisystem complications associated with tissue fragility including recurrent large subcutaneous hematomas. Here, we present detailed clinical courses of the six patients to delineate the disorder.

CLINICAL REPORTS

Patient 1

The patient is a now 16-year-old Japanese girl. Part of her history was described previously [Kosho et al., 2005]. She was the first child of a healthy mother and a healthy non-consanguineous father, both 19 years of age. She was born by normal vaginal delivery at 42 weeks of gestation. Her birth weight was 2,724 g (−1.3 SD), length 50.0 cm (−0.1 SD), and OFC 32.5 cm (−1.0 SD). She was admitted for the treatment of hypoglycemia, hyperbilirubinemia, and left talipes equinovarus (Fig. 1J). Her craniofacial features included a large fontanelle, hypertelorism, short and downslanting palpebral fissures, blue sclerae, a short nose with a hypoplastic columella, low-set and rotated ears, a high palate, a long philtrum, a thin upper lip vermilion, a small mouth, and micro-retrognathia (Fig. 1A). She had arachnodactyly, flexion-adduction contractures of bilateral thumbs, flexion contractures of the metacarpophalangeal (MP) and interphalangeal (IP) joints in the other fingers (Fig. 1E,F), and rigidity of bilateral hip joints. She suckled poorly, and was admitted again for the treatment of dehydration at age 1 month. Talipes equinovarus was treated with serial plaster casts, and was surgically corrected at age 2 years. Anomalous insertions of the flexor muscles were observed at the operation. Gross motor development was delayed: she sat at age 10 months and walked unassisted at age 2 years. Her skin was easily torn, but showed normal hemostasis in open wounds. Her face became longer with bushy and arched eyebrows and a pointed chin (Fig. 1B). At age 4 years, she developed a large subcutaneous hematoma over the occiput after falling, followed by acute hemorrhagic anemia that required admission and transfusion of hemostatic agents and packed red cells. During the admission, she was suspected to have EDS. At age 6 years, she developed a large subcutaneous hematoma over the temporo-occipital region after falling, requiring admission and intravenous administration of hemostatic agents. She had recurrent dislocations of the shoulders, elbows, and knees.

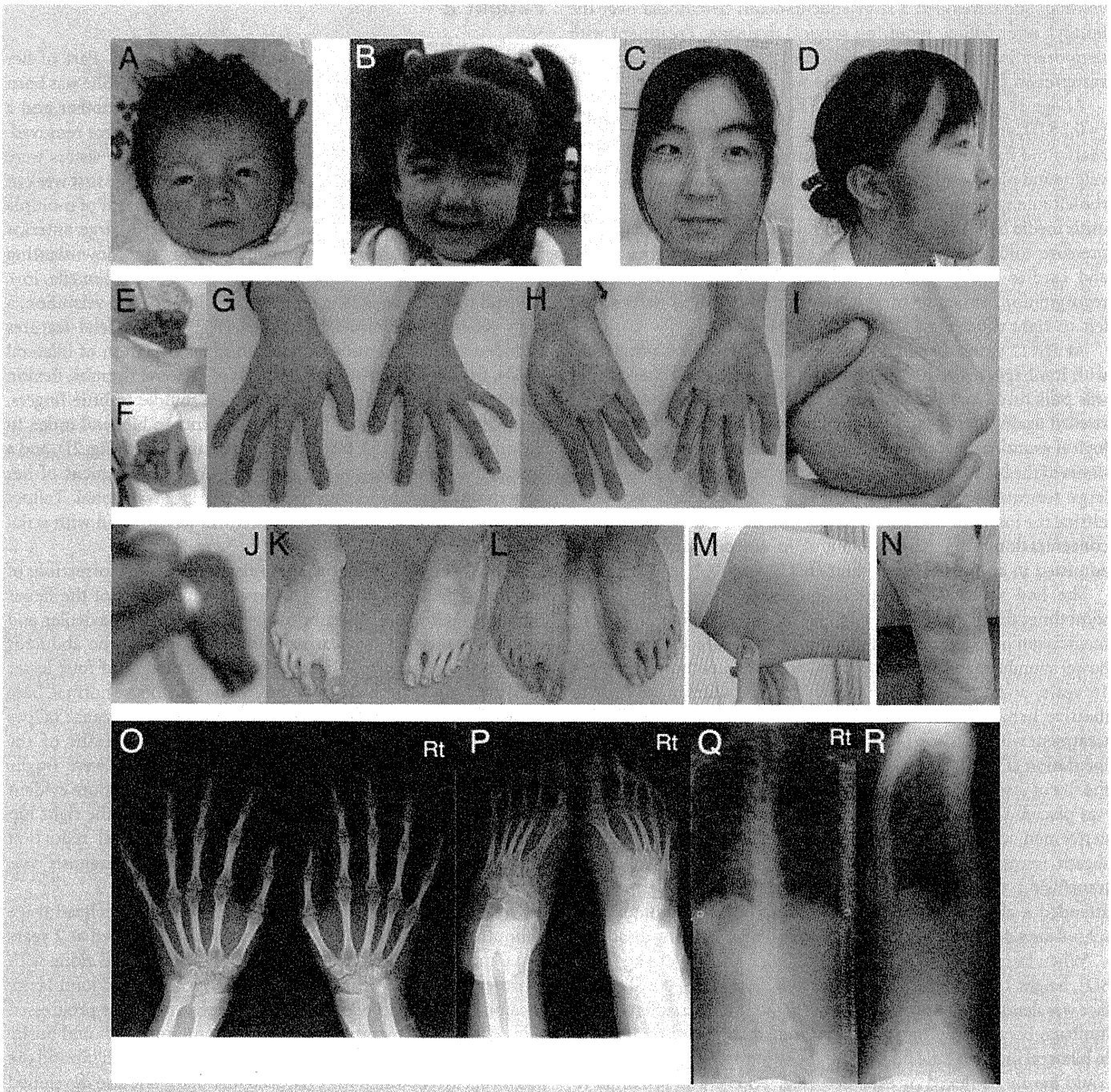

FIG. 1. Patient 1. Clinical photographs of the face at age 23 days (A), 3 years (B), and 16 years (C,D); the left (E) and the right (F) hands at age 23 days; the hands at age 16 years (G,H); the left elbow at age 16 years (I); the feet at birth (J), age 11 years (K), and 16 years (L); the skin on the left upper arm at age 16 years (M); and the left knee at age 16 years (N). Radiographs of the hands (O), the feet (P), and the spine (Q,R) at age 16 years. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

When first seen by us at age 7 years, she weighed 19.2 kg (−1.0 SD), height 123.8 cm (+0.8 SD), and OFC 51.5 cm (±0 SD). She had generalized joint laxity, a straight back with scoliosis, and cylindrical and slender fingers. Her skin was hyperextensible, bruisable, and fragile with multiple atrophic scars. Hyperalgesia to pressure such as measuring blood pressure at the upper arms was noted. Ophthalmological examinations showed microcornea and hyperopia. Otological examinations showed narrow middle ear spaces and hearing impairment of high-pitched sounds. Heart murmurs were not audible, and cardiac ultrasonography showed trivial mitral valve regurgitation. Her bladder was dilated with urinary retention and frequent cystitis, requiring manual pressure