as the primary target, followed by the epimutation (hypomethylation) of the *MEG3*-DMR after fertilization; and (2) Loss of the hypermethylated DMRs of paternal origin has no effect on the imprinting status [2,26], so that upd(14)mat-like phenotype is primarily ascribed to the additive effects of loss of functional *DLK1* and *RTL1* from the paternally derived chromosome (the effects of loss of *DIO3* appears to be minor, if any [2,35]). Although the *MEGs* expression dosage is predicted to be normal in Deletion-4 and Deletion-5 and doubled in Epimutation-2 as well as in upd(14)mat, it remains to be determined whether the difference in the *MEGs* expression dosage has major clinical effects or not. (C) Normal and upd(14)pat/mat subjects.
Found at: doi:10.1371/journal.pgen.1000992.s003 (2.72 MB TIF)

Table S1 The results of microsatellite and SNP analyses.
Found at: doi:10.1371/journal.pgen.1000992.s004 (0.19 MB DOC)

Table S2 Clinical features in the mother of patient 1.
Found at: doi:10.1371/journal.pgen.1000992.s005 (0.09 MB DOC)

Table S3 Primers utilized in the present study.
Found at: doi:10.1371/journal.pgen.1000992.s006 (0.14 MB DOC)

Author Contributions

Conceived and designed the experiments: MK ACFS TO. Performed the experiments: MK MF KM FK. Analyzed the data: MK TO. Contributed reagents/materials/analysis tools: MJO AJG YW OA NM KM TO. Wrote the paper: TO.

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Parthenogenetic chimaerism/mosaicism with a Silver-Russell syndrome-like phenotype

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ABSTRACT

Introduction We report a 34-year-old Japanese female with a Silver-Russell syndrome (SRS)-like phenotype and a mosaic Turner syndrome karyotype (45,X/46,XX).

Methods/Results Molecular studies including methylation analysis of 17 differentially methylated regions (DMRs) on the autosomes and the *XIST*-DMR on the X chromosome and genome-wide microsatellite analysis for 96 autosomal loci and 30 X chromosomal loci revealed that the 46,XX cell lineage was accompanied by maternal uniparental isodisomy for all chromosomes (upid(AC)mat), whereas the 45,X cell lineage was associated with biparentally derived autosomes and a maternally derived X chromosome. The frequency of the 46,XX upid(AC)mat cells was calculated as 84% in leukocytes, 56% in salivary cells, and 18% in buccal epithelial cells.

Discussion The results imply that a parthenogenetic activation took place around the time of fertilisation of a sperm missing a sex chromosome, resulting in the generation of the upid(AC)mat 46,XX cell lineage by endoreplication of one blastomere containing a female pronucleus and the 45,X cell lineage by union of male and female pronuclei. It is likely that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of SRS phenotype, but not for the occurrence of other imprinting disorders or recessive Mendelian disorders.

Although a mammal with maternal uniparental disomy for all chromosomes (upd(AC)mat) is incompatible with life because of genomic imprinting,[1] a mammal with a upd(AC)mat cell lineage could be viable in the presence of a co-existing normal cell lineage. In the human, Strain *et al*[2] have reported 46,XX peripheral blood cells with maternal uniparental isodisomy for all chromosomes (upid(AC)mat) in a 1.2-year-old phenotypically male patient with aggressive behaviour, hemifacial hypoplasia and normal birth weight. Because of the 46,XX disorders of sex development, detailed molecular studies were performed, revealing the presence of a normal 46,XY cell lineage in a vast majority of skin fibroblasts and a upid(AC)mat 46,XX cell lineage in nearly all blood cells. In addition, although the data are insufficient to draw a definitive conclusion, Horike *et al*[3] have also identified 46,XX peripheral blood cells with possible upd(AC)mat in a phenotypically male patient through methylation analyses for plural differentially methylated regions (DMRs) in 11 patients with Silver–Russell syndrome (SRS)-like phenotype. This patient was found to have a normal 46,XY cell lineage and a triploid 69,XXY cell lineage in skin fibroblasts.

However, such patients with a upd(AC)mat cell lineage remain extremely rare, and there is no report describing a human with such a cell lineage in the absence of a normal cell lineage. Here, we report a female patient with a upid(AC)mat 46,XX cell lineage and a non-upd 45,X cell lineage who was identified through genetic screenings of 103 patients with SRS-like phenotype.

MATERIALS AND METHODS

Case report

This Japanese female patient was conceived naturally and born at 40 weeks of gestation by a normal vaginal delivery. At birth, her length was 44.0 cm (−3.1 SD), her weight 2.1 kg (−2.9 SD) and her occipitofrontal head circumference (OFC) 30.5 cm (−2.3 SD). The parents and the younger brother were clinically normal (the father died from a traffic accident).

At 2 years of age, she was referred to us because of growth failure. Her height was 77.7 cm (−2.5 SD), her weight 8.45 kg (−2.6 SD) and her OFC 43.5 cm (−2.5 SD). Physical examination revealed several SRS-like somatic features such as triangular face, right hemihypoplasia and bilateral fifth finger clinodactyly. She also had developmental retardation, with a developmental quotient of 56. Endocrine studies for short stature were normal as were radiological studies. Cytogenetic analysis using lymphocytes indicated a low-grade mosaic Turner syndrome (TS) karyotype, 45,X[3]/46,XX[47]. Thus, a screening of TS phenotype[4] was performed, detecting horseshoe kidney but no body surface features or cardiovascular lesion. Chromosome analysis was repeated at 6 and 32 years of age using lymphocytes, revealing a 45,X[8]/46,XX[92] karyotype and a 45,X[12]/46,XX[88] karyotype, respectively. On the last examination at 34 years of age, her height was 125.0 cm (−6.2 SD), her weight 37.5 kg (−2.0 SD) and her OFC 51.2 cm (−2.8 SD). She was engaged in a simple work and was able to get on her daily life for herself.

Sample preparation

This study was approved by the Institutional Review Board Committees at National Center for Child health and Development. After obtaining written informed consent, genomic DNA was extracted from leukocytes of the patient, the mother and the brother and from salivary cells, which comprise ~40% of buccal epithelial cells and ~60% of leukocytes,[5] of the patient. Lymphocyte metaphase spreads and leukocyte RNA were also

obtained from the patient. Leukocytes of healthy adults and patients with imprinting disorders were utilised for controls.

Primers and probes

The primers utilised in this study are summarised in supplementary methods and supplementary tables 1–3.

DMR analyses

We first performed bio-combined bisulfite restriction analysis (COBRA)[6] and bisulfite sequencing of the *H19*-DMR (A) on chromosome 11p15.5 by the previously described methods[7] and methylation-sensitive PCR analysis of the *MEST*-DMR (A) on chromosome 7q32.2 by the previously described methods[8] with minor modifications (the methylated and unmethylated allele-specific primers were designed to yield PCR products of different sizes, and the PCR products were visualised on the 2100 Bioanalyzer (Agilent, Santa Clara, California, USA)). This was because hypomethylation (epimutation) of the normally methylated *H19*-DMR of paternal origin and maternal uniparental disomy 7 are known to account for 35–65% and 5–10% of SRS patients, respectively.[9 10] In addition, fluorescence in situ hybridisation (FISH) analysis was performed with a ~84-kb RP5-998N23 probe containing the *H19*-DMR (BACPAC Resources Center, Oakland, California, USA). We also examined multiple other DMRs by bio-COBRA. The ratio of methylated clones (the methylation index) was calculated using peak heights of digested and undigested fragments on the 2100 Bioanalyzer using 2100 expert software.

Genome-wide microsatellite analysis

Microsatellite analysis was performed for 96 autosomal loci and 30 X chromosomal loci. The segment encompassing each locus was PCR-amplified, and the PCR product size was determined on the ABI PRISM 310 autosequencer using GeneScan software (Applied Biosystems, Foster City, California, USA).

PCR analysis for Y chromosomal loci

Standard PCR was performed for six Y chromosomal loci. The PCR products were electrophoresed using the 2100 Bioanalyzer.

Expression analysis

Quantitative real-time reverse transcriptase PCR analysis was performed for three paternally expressed genes (*IGF2*, *SNRPN* and *ZAC1*) and four maternally expressed genes (*H19*, *MEG3*, *PHLDA2* and *CDKN1C*) that are known to be variably (usually weakly) expressed in leukocytes (UniGene, http://www.ncbi.nlm.nih.gov/sites/entrez?db=unigene), using an ABI Prism 7000 Sequence Detection System (Applied Biosystems). *TBP* and *GAPDH* were utilised as internal controls.

RESULTS

DMR analyses

In leukocytes, the bio-COBRA indicated severely hypomethylated *H19*-DMR, and bisulfite sequencing combined with *rs2251375* SNP typing for 30 clones revealed maternal origin of 29 hypomethylated clones and non-maternal (paternal) origin of a single methylated clone in this patient (figure 1A). Thus, the marked hypomethylation of the *H19*-DMR was caused by predominance of maternally derived clones rather than hypomethylation of the *H19*-DMR of paternal origin. FISH analysis for 100 lymphocyte metaphase spreads excluded an apparent deletion of the paternally derived *H19*-DMR or duplication of the maternally derived *H19*-DMR (Supplementary figure 1). Methylation-sensitive PCR amplification for the *MEST*-DMR delineated a major peak for the methylated allele and a minor peak for the unmethylated allele (figure 1B). This also indicated the predominance of maternally derived clones and the co-existence of a minor portion of paternally derived clones. Furthermore, autosomal DMRs invariably exhibited markedly abnormal methylation patterns consistent with predominance of maternally inherited DMRs, whereas the methylation index of the *XIST*-DMR on the X chromosome remained within the female reference range (figure 1C). The abnormal methylation patterns were less obvious in salivary cells (thus, in buccal epithelial cells) than in leukocytes, except for the methylation index for the *XIST*-DMR that mildly exceeded the female reference range (figure 1A–C).

Microsatellite analysis

Major peaks consistent with maternal uniparental isodisomy and minor peaks of non-maternal (paternal) origin were identified for at least one locus on each autosome, with the minor peaks of non-maternal origin being more obvious in salivary cells than in leukocytes (figure 1D and supplementary table 4). Furthermore, the frequency of the upid(AC)mat cells was calculated as 84% in leukocytes, 56% in salivary cells and 18% in epithelial buccal cells, using the area under curves for the maternally and the non-maternally inherited peaks (supplementary note). Such minor peaks of non-maternal origin were not detected for all the 30 X chromosomal loci examined.

PCR analysis for Y chromosomal loci

PCR amplification failed to detect any trace of Y chromosome-specific bands in leukocytes and salivary cells (Supplementary figure 2).

Expression analysis

Expression analysis using control leukocytes indicated that, of the seven examined genes, *SNRPN* expression alone was strong enough to allow for a precise assessment (Supplementary figure 3). *SNRPN* expression was extremely low in this patient (figure 1E).

DISCUSSION

These results imply that this patient had a upid(AC)mat 46,XX cell lineage and a non-upd 45,X cell lineage. Indeed, methylation patterns of the *XIST*-DMR is explained by assuming that the two X chromosomes in the upid(AC)mat cells undergo random X-inactivation and that 45,X cells with the methylated *XIST*-DMR on a single active X chromosome[11] are relatively prevalent in buccal epithelial cells. Furthermore, lack of non-maternally derived minor peaks for microsatellite loci on the X chromosome is explained by assuming that the two X chromosomes in the upid(AC)mat cells and the single X chromosome in the 45,X cells are derived from a common X chromosome of maternal origin, with no paternally derived sex chromosome. It is likely, therefore, that a parthenogenetic activation took place around the time of fertilisation of a sperm missing a sex chromosome, resulting in the generation of the 46,XX cell lineage with upid (AC)mat by endoreplication (the replication of DNA without the subsequent completion of mitosis) of one blastomere containing a female pronucleus and the 45,X cell lineage with biparentally derived autosomes and a maternally derived X chromosome by union of male and female pronuclei (figure 2), although it is also possible that a paternally derived sex chromosome was present in the sperm but was lost from the normal

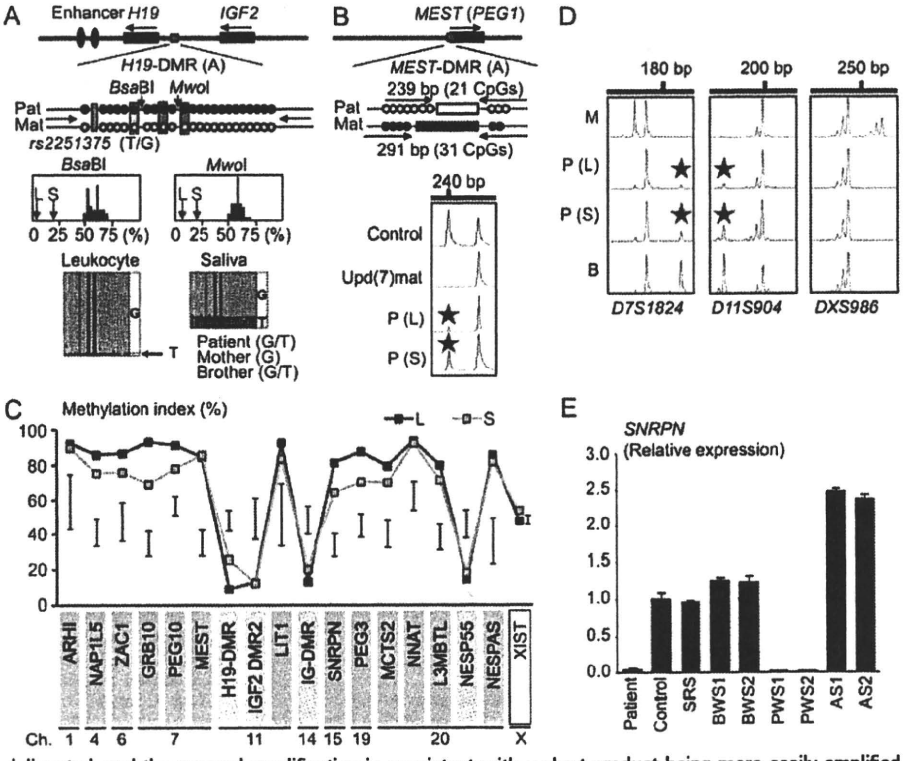

Figure 1 Representative molecular results. Pat, paternally derived allele; Mat, maternally derived allele; P, patient; M, mother; B, brother; L, leukocytes; and S, salivary cells. Filled and open circles in A and B represent methylated and unmethylated cytosine residues at the CpG dinucleotides, respectively. A. Methylation patterns of the *H19*-DMR (A) harbouring 23 CpG dinucleotides and the T/G SNP (*rs2251375*) (a grey box). The PCR products are digested with *Bsa*BI when the cytosine at the sixth CpG dinucleotide (highlighted in yellow) is methylated and with *Mwo*I when the two cytosines at the ninth and the 11th CpG dinucleotides (highlighted in orange) are methylated. For the bio-COBRA data, the black histograms represent the distribution of methylation indices (%) in 50 control participants, and L and S denote the methylation indices for leukocytes and salivary cells of this patient, respectively. For the bisulfite sequencing data, each line indicates a single clone. B. Methylated and unmethylated allele-specific PCR analysis for the *MEST*-DMR (A). In a control participant, the PCR products for methylated and unmethylated alleles are delineated, and the unequal amplification is consistent with a short product being more easily amplified than a long product. In a previously reported patient with upd(7)mat,[8] the methylated allele only is amplified. In this patient, major peaks for the methylated allele and minor peaks for the unmethylated allele (red asterisks) are detected. C. Methylation patterns for the 18 DMRs examined. The DMRs highlighted in blue and pink are methylated after paternal and maternal transmissions, respectively. The black vertical bars indicate the reference data (maximum–minimum) in 20 normal control participants, using leukocyte genomic DNA (for the *XIST*-DMR, 16 female data are shown). D. Representative microsatellite analysis. Minor peaks (red asterisks) have been identified for *D7S1824* and *D11S904* but not for *DXS986* of the patient. Since the peaks for *D7S1824* and *D11S904* are absent in the mother and clearly present in the brother, they are assessed to be of paternal origin. E. Relative expression level (mean ± SD) of *SNRPN* on chromosome 15. The data have been normalised against *TBP*. SRS, an SRS patient with an epimutation (hypomethylation) of the *H19*-DMR; BWS1, a BWS patient with an epimutation (hypermethylation) of the *H19*-DMR; BWS2, a BWS patient with upd(11)pat; PWS1, a PWS patient with upd(15)mat; PWS2, a PWS patient with an epimutation (hypermethylation) of the *SNRPN*-DMR; AS1, an Angelman syndrome (AS) patient with upd(15)pat; and AS2, an AS patient with an epimutation (hypomethylation) of the *SNRPN*-DMR.

cell lineage at the very early developmental stage. Hence, in a strict sense, this patient is neither a chimera resulting from the fusion of two different zygotes nor a mosaic caused by a mitotic error of a single zygote. In this regard, a triploid cell stage is assumed in the generation of a upid(AC)mat cell lineage, and such triploid cells may have been detected in skin fibroblasts of the patient reported by Horike *et al*.[3]

The upid(AC)mat cells accounted for the majority of leukocytes even in adulthood of this patient, despite global negative selective pressure.[12 13] This phenomenon, though intriguing, would not be unexpected in human studies because leukocytes are usually utilised for genetic analyses. Rather, if the upid(AC)mat cells were barely present in leukocytes, they would not have been detected. It is likely, therefore, that upid(AC)mat cells have occupied a relatively large portion of the definitive haematopoietic tissues primarily as a stochastic event. Furthermore, parthenogenetic chimera mouse studies have revealed that parthenogenetic cells are found at a relatively high frequency in some tissues/organs including blood and are barely identified in other tissues/organs such as skeletal muscle and liver.[13] Such a possible tissue-specific selection in favour of the preservation of parthenogenetic cells in the definitive haematopoietic tissues may also be relevant to the predominance of the upid(AC)mat cells in leukocytes. In addition, a reduced growth potential of 45,X cells[14] may also have contributed to the skewed ratio of the two cell lineages.

Clinical features of this patient would be determined by several factors. They include: (1) the ratio of two cell lineages in various tissues/organs, (2) the number of imprinted regions or DMRs relevant to the development of specific imprinting disorders (eg, plural regions/DMRs on chromosomes 7 and 11 for SRS[9 10] and a single region/DMR on chromosome 15 for Prader–Willi syndrome (PWS)),[15] (3) the degree of clinical effects of dysregulated imprinted regions/DMRs (an (epi)dominant effect has been

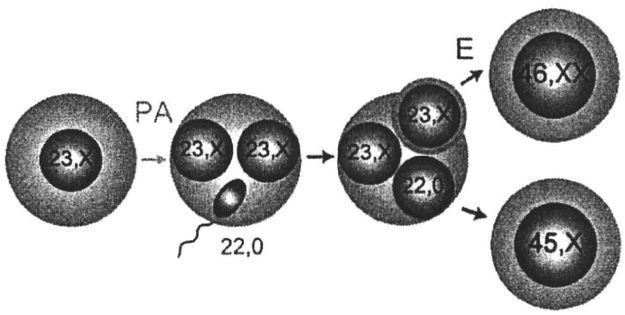

Figure 2 Schematic representation of the generation of the upid(AC)mat 46,XX cell lineage and the non-upd 45,X cell lineage. Polar bodies are not shown. PA, parthenogenetic activation; and E, endoreplication of one blastomere containing a female pronucleus.

assumed for the 11p15.5 imprinted regions including the *IGF2–H19* domain on the basis of SRS or Beckwith–Wiedemann syndrome (BWS) phenotype in patients with multilocus hypomethylation[16] and BWS-like phenotype in patients with a upid (AC)pat cell lineage,[17] a mirror image of a upid(AC)mat cell lineage), (4) expression levels of imprinted genes in upid(AC)mat cells (although *SNRPN* expression of this patient was consistent with upid(AC)mat cells being predominant in leukocytes, complicated expression patterns have been identified for several imprinted genes in androgenetic and parthenogenetic fetal mice, probably because of perturbed *cis*- and *trans*-acting regulatory mechanisms)[18] and (5) unmasking of possible maternally inherited recessive mutation(s) in upid(AC)mat cells.[19] Collectively, it appears that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of SRS phenotype and horseshoe kidney characteristic of TS[4] but remained below the threshold level for the occurrence of other imprinting disorders or recessive Mendelian disorders.

In summary, we identified a upid(AC)mat 46,XX cell lineage in a woman with an SRS-like phenotype and a 45,X cell lineage accompanied by autosomal haploid sets of biparental origin. This report will facilitate further identification of patients with a upid(AC)mat cell lineage and better clarification of the clinical phenotypes in such patients.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Institutional Review Board Committees at National Center for Child health and Development.

Contributors Drs Kazuki Yamazawa (first author) and Kazuhiko Nakabayashi (second author) contributed equally to this work.

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Medical genetics

Familial cases of atypical clinical features genetically diagnosed as LEOPARD syndrome (multiple lentigines syndrome)

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Summary

Five familial cases exhibited ephelides-like multiple lentigines, and we examined three of them, a mother and two sons. All three patients presented with small dark-brown maculae on the face and neck and electrocardiographic abnormalities. These findings sufficed to fulfill the criteria for LEOPARD syndrome (multiple lentigines syndrome), although they lacked five of seven major clinical features. However, the family members presented with a webbed neck and pectus excavatum, which are more frequently seen in Turner or Noonan syndrome. Histological examination of the lentigines revealed slightly elongated rete ridges, a hyperpigmented basal layer, and melanophages in the papillary dermis. Direct sequencing of the patients' genomic DNA revealed that all three had a consistent missense mutation [c.1403C > T (p.T468M)] in the *PTPN11* gene, confirming LEOPARD syndrome with an atypical phenotype. It was suggested that LEOPARD syndrome shows a diverse phenotype but its diagnosis can be verified by mutation analysis.

Introduction

In 1936, Zeisler and Becker[1] reported on a 24-year-old female with multiple lentigines scattered on her body, pectus carinatum, ocular hypertelorism, and mandibular prognathism, which was later named LEOPARD syndrome (LS) by Gorlin *et al.*[2] LEOPARD is an acronym for the major features that characterize the syndrome: multiple *L*entigines, *E*lectrocardiographic conduction defects, *O*cular hypertelorism, *P*ulmonary stenosis, genital *A*bnormality, *R*etardation of growth, and sensorineural *D*eafness. LS is an autosomal dominant disorder that has been presented not only by dermatologists, but also by other specialists,[3–8] and is also called multiple lentigines syndrome.[2,9] The life-threatening problems in LS patients are hypertrophic cardiomyopathy and malignant tumors.[10,11]

Missense mutations in exons 7, 12, and 13 of the protein-tyrosine phosphatase, nonreceptor type 11 (PTPN11) gene, which is located on chromosome 12q24.1 and encodes the protein tyrosine phosphatase SHP2, have been found in LS;[10,12,13] all the mutations are located at the catalytic cleft of the *PTPN11* protein.[14] The SHP2 protein plays an important role in several signal transduction pathways involving several cytokines and hormones, with a particular role in the RAS-mitogen activated protein kinase pathway.[15–17] Thus, although genetic testing is not commonly performed, it is helpful for confirming a diagnosis and differentiating LS from similar diseases, such as Peutz-Jeghers syndrome, Carney syndrome, Noonan syndrome, and Turner syndrome.

We describe a family with members exhibiting multiple lentigines with less-frequent symptoms, such as a webbed neck (pterygium colli) and pectus excavatum (trichterbrust), who were genetically diagnosed as having LS.

Case report

The family consisted of three generations (Fig. 1). In the 1st generation, there were two sisters. The elder sister (70-year-old) had multiple dark-brown lentigines, mainly on the face (similar appearance to ephelides), a webbed

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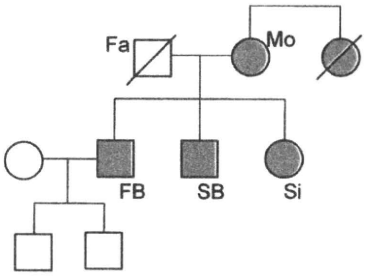

Figure 1 Family pedigree. Two family members in the 1st generation (the mother and mother's younger sister) and all three members in the 2nd generation (two sons and one daughter) presented with multiple lentigines (red). Multiple lentigines were not noted in the father and first brother's sons. Fa, father; Mo, mother; FB, first brother; SB, second brother; Si, sister

neck, and pectus excavatum without a short stature (Fig. 2). She had two sons and one daughter (the 2nd generation) and did not marry consanguineously. Her husband already died of lung cancer at the age of 64. The younger sister (65-year-old) had multiple lentigines and no children before she died.

The second brother of the 2nd generation (41-year-old) presented with small, dark brown, irregularly pigmented maculae 1 to 4 mm in size on the face and neck, including the vermillion, but not involving the oral and orbital mucosa (Fig. 2). The maculae had been present since birth, and new lesions gradually developed until his 20s and darkened with age. He also presented with other features, such as a webbed neck with a lower hairline and pectus excavatum. Electrocardiography indicated arterial fibrillation, ventricular extrasystole, tachycardia, and left anterior hemiblock. Echocardiography showed mild mitral valve regurgitation, tricuspid valve regurgitation, aorta dilation, and left ventricular dilation. Pulmonary stenosis was not found. Gastrointestinal and colon fibroscopy did not detect polyposis or any other abnormalities. Levels of thyroid stimulating hormone, free thyroxine, and free triiodothyronine were normal. Chromosome analysis showed a normal 46, XY karyotype in all the 50 peripheral lymphocytes examined. The first brother (44-year-old) (Fig. 2) and a sister (39-year-old) of the 2nd generation showed almost the same physical findings. Only the second brother had nevus spilus-like maculae on the back and left arm, but neurofibroma did not present in any of the family members. Bilateral blepharoptosis was noted also only by the second brother, although there was no accompanying exophthalmus or ocular hyperterolism.

The first brother of the 2nd generation has two sons (3rd generation), aged 6 and 5 years, with no symptoms suggesting LS, although multiple lentigines may appear in

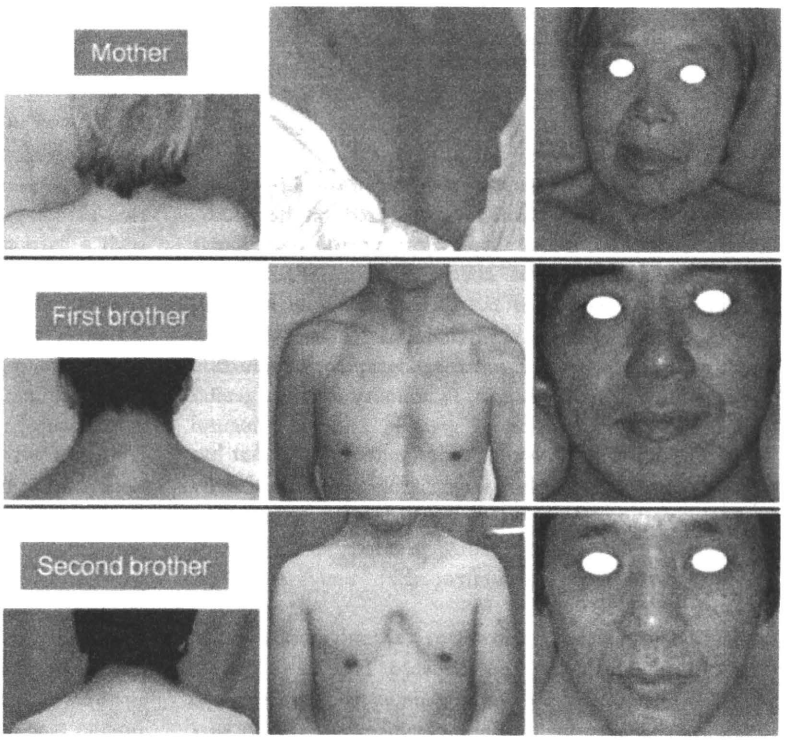

Figure 2 Photographs of three family members. All three members (the mother, the first brother and the second brother) presented with multiple small brown maculae on the face and neck, a webbed neck, and pectus excavatum

Table 1 Summarized clinical manifestations of five family members

Manifestations			Fa	Mo	FB	SB	Si
Genome	Missense mutation in the PTPN11 gene		N/A	+	+	+	N/A
L	Multiple *L*entigines		–	+	+	+	+
E	*E*CG abnormalities		N/A	+	+	+	+
O	*O*cular hypertelorism		–	–	–	–	–
P	*P*ulmonary stenosis		N/A	N/A	N/A	–	N/A
A	*A*bnormal genetalia	Cryptorchidism	–	–	–	–	–
R	*R*etardation of growth		–	–	–	–	–
D	Sensorineural *D*eafness		–	–	–	–	–
Skin	Cafè-au-lait spots		–	+	+	+	N/A
	Neurofibromatosis		–	–	–	–	–
	Curly, coarse hair		–	–	–	–	–
Ear	Low-set ear		+	+	+	+	N/A
Eye (Eyelids)	Light-colored irises		–	–	–	–	N/A
	Blepharoptosis		+	+	+	+	+
	Epicanthal folds		–	–	+	+	N/A
Cardiovascular	Congenital heart defects		N/A	N/A	N/A	+	N/A
	Hypertrophic cardiomyopathy		N/A	N/A	N/A	–	N/A
Skeletal	Short stature		–	–	–	–	–
	Pectus excavatum and/or carinatum		–	+	+	+	+
	Vertebral anomalies	Scoliosis	–	–	–	–	–
	Cubitus valgus		–	–	–	–	–
Hematological	Bleeding diathesis (von Willebrand disease, factors XI and XII deficiency)		–	–	–	–	–
	Thrombocytopenia		–	–	–	–	–
	Leukemia		–	–	–	–	–
Others	Webbed neck with low posterior hairline		–	+	+	+	+
	Malocclusion		–	+	+	+	N/A
	Lymphatic disorder	Lymphedema	–	–	–	–	–
	Triangular facies		–	–	–	–	N/A
	Feeding difficulties		–	–	–	–	–
	Cryptorchidism		–	–	–	–	–
	Mental retardation		–	–	–	–	–
	Sexual infantilism		–	–	–	–	–

ECG, electrocardiogram; Fa, father; Mo, mother; FB, first brother; SB, second brother; Si, sister.

the future. The second brother and a sister do not have any children.

There was no abnormality of the external genitalia or urinary organs in any family members. Intelligence, mental development, and hearing were also normal. The clinical data are summarized in Tables 1 and 2.

Human tissue analyses were performed in compliance with the Declaration of Helsinki Principles. Peripheral blood samples were taken from the mother (1st generation) and both brothers (2nd generation) using an ethics committee-approved protocol for genomic DNA analyses after each patient provided informed consent. Photo release consent was also obtained from each patient. Leukocyte genomic DNA was amplified by PCR for the 15 exons and flanking introns of *PTPN11* and was subjected to direct sequencing from both directions using a CEQ 8000 autosequencer (Beckman Coulter, Fullerton, CA, USA). The primer sequences and PCR conditions were

Table 2 Characteristic manifestations of LEOPARD and Noonan syndrome

Manifestations	Fa	Mo	FB	SB	Si
LEOPARD Multiple Lentigines	–	+	+	+	+
Sensorineural Deafness	–	–	–	–	–
ECG abnormalities Noonan	N/A	+	+	+	+
Facial dysmorphism (e.g. Ocular hypertelorism)	N/A	N/A	N/A	–	N/A
Cardiovascular defects (e.g Pulmonary stenosis)	–	–	–	–	–
Abnormal genetalia (e.g Cryptochidism)	–	–	–	–	–
Retardation of growth (e.g. Short stature)	–	–	–	–	–
Mental retardation	–	–	–	–	–
Webbed neck	–	+	+	+	+
Pectus excavatum	–	+	+	+	+
Hematologic abnormalities (e.g. Leukemia)	–	–	–	–	–

Fa, father; Mo, mother; FB, first brother; SB, second brother; Si, sister.

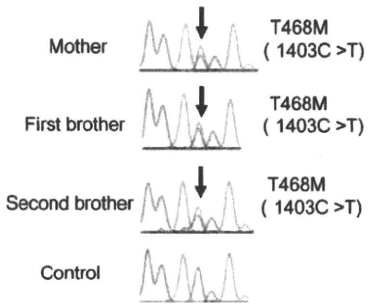

Figure 3 Electrochromatograms for the three family members. The PTPN11 mutation (Thr468Pro, 1403AfiC) was detected in genomic DNA from the leukocytes of the three patients

described previously.[18] To confirm any mutations, three independent PCR products were examined. Mutation analysis indicated a heterozygous C > T substitution at position c.1403 in *PTNP11* exon 12 in all the three subjects, resulting in the missense mutation Thr468Met (Fig. 3), which is one of the known mutations for LS. This mutation is located at the catalytic cleft of the PTP domain and impairs phosphatase activity of SHP2.[19]

A skin biopsy of a pigmented facial lesion was taken from the second brother (2nd generation). The biopsied sample was processed for HE staining and Fontana-Masson ammoniac silver staining. Histological examination of the lentigine specimen (Fig. 4) revealed that epidermal rete ridges were slightly elongated and basal layer of the epidermis were hyperpigmented with increased numbers of melanocytes. No nevus cells were observed. Deposition of melanophages was slightly detected in the top region of the dermal papillae, and we observed moderate infiltration of lymphocytes into the epidermis and hair follicle epithelium.

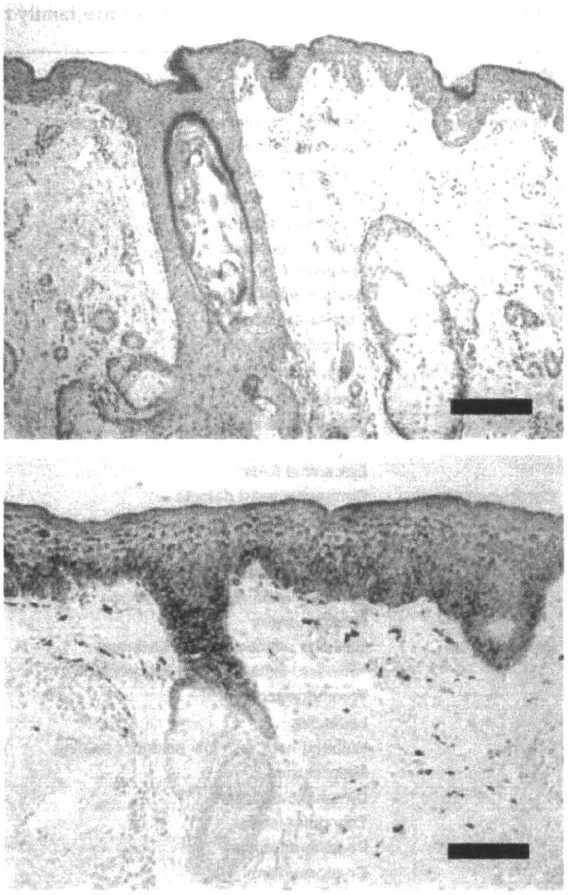

Figure 4 Histological examination of the biopsy specimen from the face of the second brother. Top: Histological examination of a pigmented macule demonstrated slightly elongated rete ridges and epidermal hypermelanosis using (Hematoxylin-Eosin staining. ×100; scale bar = 200 μm). Bottom: Higher magnification of the section revealed a hyperpigmented basal layer, incresed numbers of melanocytes without nest formation, and melanophages in the papillary dermis. (Masson-fontana ammoniac silver staining ×200; scale bar = 100 μm.)

Discussion

There are many reports in the literature of multiple lentigines associated with other symptoms, including Neurofibromatosis–Noonan syndrome,[20] Watson syndrome,[21] centrofacial lentiginosis,[22] inherited patterned lentiginosis,[23] Carney complex,[24] Peutz–Jeghers syndrome,[25] Laugier–Hunziker–Baran syndrome, and Cronkhite–Canada syndrome. In our cases, ephelides-like lentigines were spread predominantly on the face and neck without eruptions on the oral mucosa, and neither neurofibroma nor schwannoma were seen. Intestinal polyposis, myxoma, or endocrine dysfunction was not noted. However, our cases also lacked many major manifestations associated with LS; none of the patients exhibited ocular hypertelorism, pulmonary stenosis, abnormal genitalia, growth retardation, or sensorineural deafness. On the other hand, a webbed neck and pectus excavatum, which are less frequent in LS[9,26] and frequently seen in Noonan syndrome and Turner syndrome,[27] were noted.

LEOPARD syndrome has been reported to present with extremely variable phenotypes. Voron *et al.*[9] grouped the LS features into the following nine categories: cutaneous abnormalities, cardiac abnormalities, genitourinary abnormalities, endocrine findings, neurogenic defects, cephalofacial dysmorphism, short stature, skeletal anomalies, and familial history consistent with an autosomal dominant

mode of inheritance. Voron also proposed minimal diagnostic criteria for LS: at least two other features must be present in cases with multiple lentigines, whereas a diagnosis of LS may be made in cases with family history and three other major features despite an absence of multiple lentigines.[9] In our cases, three other features (cardiac and skeletal abnormalities and family history) were present in addition to multiple lentigines, but only two (multiple lentigines and ECG abnormality) of the seven major clinical manifestations advocated by Gorlin *et al.*[2] were noted. Therefore, careful differentiation from Noonan syndrome is needed because most of the clinical features of LS, such as heart defects, growth retardation, and facial dysmorphism, overlap with those of Noonan syndrome. Noonan syndrome presents as a Turner-like phenotype, such as short stature, cephalofacial dysmorphism, webbed neck, skeletal anomalies, and genitourinary and cardiac abnormalities, particularly pulmonary valve stenosis, although Noonan syndrome has a normal karyotype.[28]

Both LS and Noonan syndrome are known to be caused by heterozygous germline missense mutations in the PTPN11 gene. Approximately 85% of the patients with a definite diagnosis of LS have a missense mutation in the PTPN11 gene,[10] and mutations in the PTPN11 gene are also seen in roughly 50% of Noonan syndrome cases.[27,29] However, it was recently established by analyzing accumulated genetic data of LS and Noonan syndrome that the mutations in LS and Noonan syndrome are almost mutually exclusive.[14,30,31] In Noonan syndrome, PTPN mutations are detected at 33–60%,[27,30] and are recurrent and clustered mostly in exons 3, 7, 8, and 13.[12,27] Noonan syndrome mutations are recognized as gain-of-function mutations, while LS mutations were identified as having dominant negative, not activating, effects.[32] The most frequently (approximately 90%) reported PTPN11 mutations in LS are located in exons 7 (Tyr279Cys) and 12 (Thr468Met),[30] the latter of which was detected in all three family members examined here. In addition, to our knowledge, Thr468Met has never been detected in NS syndrome.[27,33] Taken together with the clinical finding that the three familial patients sufficed Voron's minimal diagnostic criteria for LS, we diagnosed them as LS.

It has been reported that there are typically two histological types of lentigines seen in LS patients:[9,26] melanocytic nevi and lentigo simplex. The biopsy specimen from our case exhibited histological features compatible with the latter, a lack of nevus cells and the presence of epidermal hypermelanosis.

In conclusion, three familial cases presented with ECG abnormalities and multiple lentigines on the face and neck, lacked most of other major features of LS, and exhibited a webbed neck and pectus excavatum. Genetic testing revealed that all of the patients carry a consistent germline missense mutation (Thr468Met, 1403C → T) in the exon 12 of PTPN11 gene, which suggested the diagnoses of LS.

Acknowledgment

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RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics PART A

Prenatal Findings of Paternal Uniparental Disomy 14: Delineation of Further Patient

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TO THE EDITOR:

Human chromosome 14q32.2 carries a cluster of imprinted genes including paternally expressed genes such as *DLK1* and *RTL1* and maternally expressed genes such as *MEG3* (alias *GTL2*) and *RTL1as* (*RTL1* antisense), together with the germline-derived intergenic differentially methylated region (IG-DMR) and the postfertilization-derived *MEG3*-DMR [da Rocha et al., 2008; Kagami et al., 2008a]. Consistent with this, paternal uniparental disomy 14 (upd(14)pat) results in a unique phenotype characterized by facial abnormality, small bell-shaped thorax with coat-hanger appearance of the ribs, abdominal wall defects, placentomegaly, and polyhydramnios [Kagami et al., 2008a,b], and maternal uniparental disomy 14 (upd(14)mat) leads to less-characteristic but clinically discernible features including growth failure [Kotzot, 2004; Kagami et al., 2008a].

For upd(14)pat, this condition has primarily been identified by the pathognomonic chest roentgenographic findings that are obtained immediately after birth because of severe respiratory dysfunction [Kagami et al., 2008a]. However, upd(14)pat has also been suspected prenatally by fetal radiological findings suggestive of small thorax and other characteristic findings [Curtis et al., 2006; Yamanaka et al., 2010]. Here, we report on prenatal findings in a hitherto unreported upd(14)pat patient. The results will serve to the prenatal identification of similarly affected patients and appropriate neonatal care including respiratory management.

A 41-year-old gravida 1, para 0 Japanese woman was referred to Nagoya City University Hospital because of polyhydramnios at 24 weeks of gestation. The polyhydramnios was severe and required repeated amnioreduction (1,600 ml at 26 weeks, 1,800 ml at 29 weeks, 2,000 ml at 32 weeks, and 2,100 ml at 35 weeks). The fetal urine volume was normal (5–12 ml per hr). At 28 weeks of gestation, 3D ultrasound studies were performed, delineating dysmorphic face, anteverted nares, micrognathia and small thorax characteristic of upd(14)pat (Fig. 1), although the differential diagnosis included Beckwith–Wiedemann syndrome and several types of skeletal dysplasia. Thereafter, ultrasound studies were weekly carried out, indicating almost normal fetal growth and normal umbilical artery Doppler.

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At 37 weeks of gestation, a 2,778 g male infant was delivered by cesarean because of fetal distress. The placenta was 1,384 g (gestational age-matched reference, 510 ± 98 g) [Kagami et al., 2008b]. The patient had severe asphyxia, and immediately received appropriate management including mechanical ventilation for 6 days and nasal directional positive airway pressure at the neonatal intensive care unit. At birth, physical examination revealed hairy forehead, blepharophimosis, depressed nasal bridge, anteverted nares, small ears, protruding philtrum, puckered lips, micrognathia, short webbed neck, joint contractures, and diastasis recti, and roentgenograms showed typical bell-shaped thorax with coat-hanger appearance of the ribs (Fig. 2). Coax valga or kyphoscoliosis was uncertain. Discharge from hospital was 35 days after birth. On the last examination at 8 months of age, the patient

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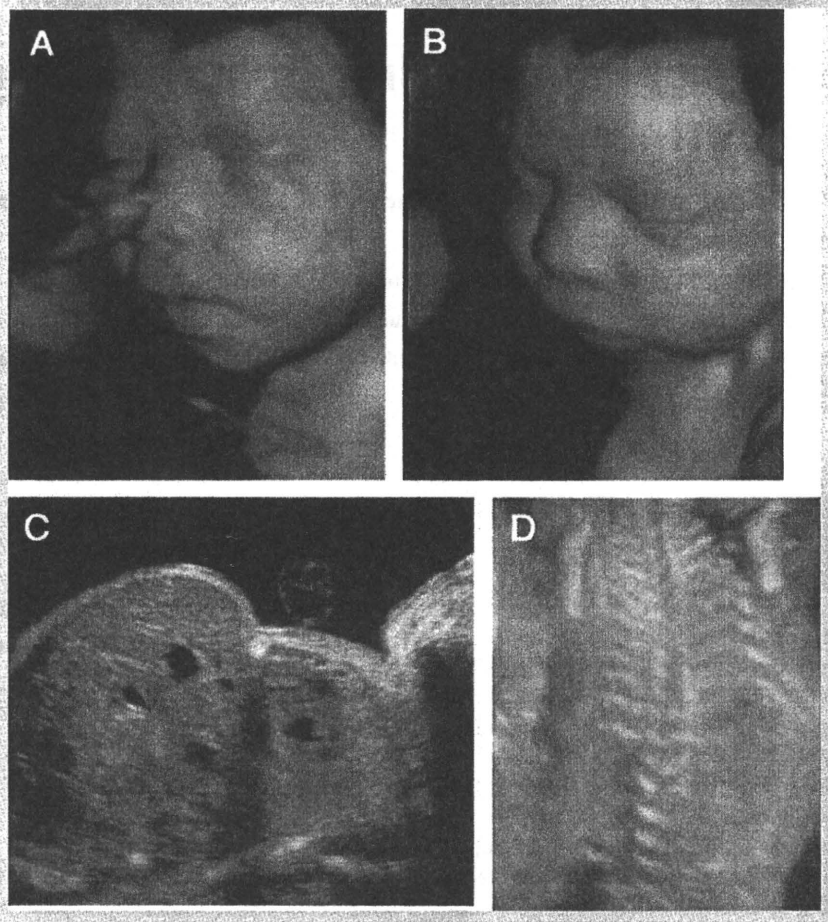

FIG. 1. Prenatal 3D findings at 28 weeks of gestation. A,B: Face appearance with blepharophimosis, depressed nasal bridge, anteverted nares, and micrognathia. C: Small thorax and polyhydramnios. D: Coat-hanger like appearance of the ribs.

required regular oropharyngeal suction and nasogastric tube feeding due to a poor swallowing reflex, and showed developmental delay. At the time of the last evaluation there was no seizure disorder.

To confirm the findings, cytogenetic and molecular studies were performed for the cord blood of the patient by the previously described methods [Kagami et al., 2008a]. This study was approved by the Institutional Review Board Committees at National Center for Child Health and Development and Nagoya City University, and performed after obtaining written informed consent. The karyotype was normal, and metaphase fluorescence in situ hybridization (FISH) analysis with a 202 kb BAC probe containing *DLK1* (RP11-566J3) and a 165 kb BAC probe containing *MG3* and *RTL1/RTL1as* (RP11-123M6) (http://bacpac.-chori.org/) delineated two signals with a similar intensity, respectively. Methylation analysis for bisulfite-treated genomic DNA indicated the presence of paternally derived hypermethylated IG-DMR (CG4 and CG6) and *MEG3*-DMR (CG7) and the absence of maternally derived hypomethylated DMRs. Furthermore, microsatellite analysis was performed using leukocyte genomic DNA of patient and parents, revealing uniparental paternal isodisomy for chromosome 14 (Table I, Fig. 3).

In this patient with molecularly confirmed upd(14)pat, ultrasound studies unequivocally showed typical upd(14)pat phenotypes such as thoracic abnormality and facial dysmorphic features. While this is the first report documenting the facial appearance of the affected fetus, small thorax has been suspected prenatally in five patients with upd(14)pat or epimutations of the IG-DMR and the *MEG3*-DMR, with coat-hanger appearance of the ribs being delineated in one patient [Curtis et al., 2006; Yamanaka et al., 2010]. In this regard, it is notable that polyhydramnios has invariably been identified in upd(14)pat by the second trimester [Kagami et al., 2008a]. It is recommended, therefore, to perform radiological studies for pregnant women with polyhydramnios, to suspect upd(14)pat-compatible clinical features of the fetus. This will permit appropriate counseling and delivery planning at a tertiary

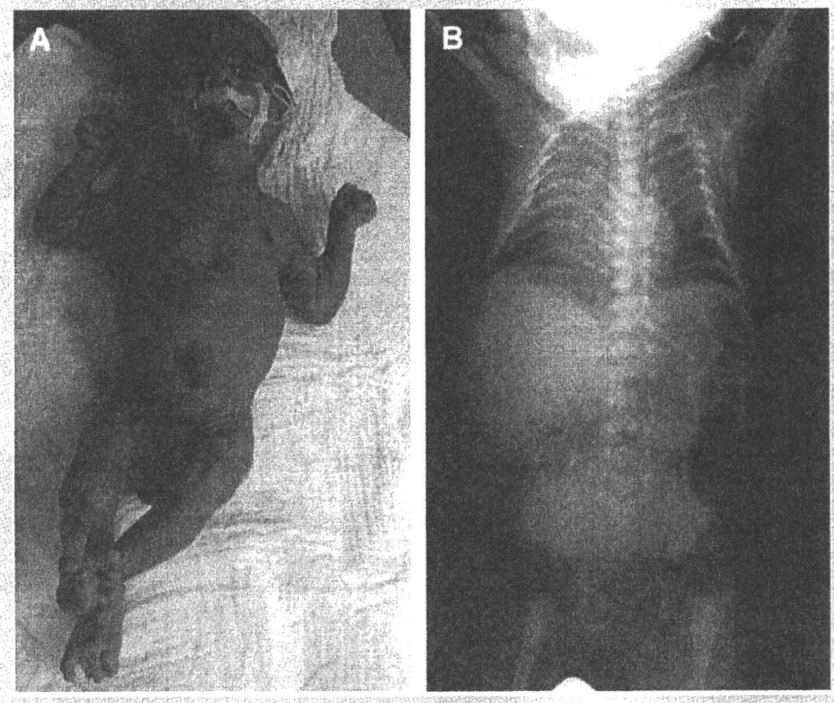

FIG. 2. Postnatal findings at 1 month of age. A: Front view. B: Chest roentgenogram showing bell-shaped thorax with coat-hanger appearance of the ribs.

center with neonatal intensive care as well as pertinent molecular studies using cord blood.

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TABLE I. The Results of Microsatellite Analysis

Locus	Location	Mother	Patient	Father	Assessment
D14S80	14q12	98	98	98	N.I.
D14S608	14q12	200	194	194/210	Isodisomy
D14S588	14q23–24.1	114/126	114	114/122	N.I.
D14S617	14q32.12	139/169	143	143/165	Isodisomy
D14S250	14q32.2	159	159	159/167	N.I.
D14S1006	14q32.2	127/139	127	127/139	N.I.
D14S985	14q32.2	135/137	131	131/133	Isodisomy
D14S1010	14q32.33	134/142	142	142/144	N.I.
D14S1007	14q32.33	119	119	119	N.I.

N.I., not informative.
The Arabic numbers indicate the PCR product sizes in bp.
The imprinted region resides at 14q32.2.
D14S985 is located in the intron of *MEG3*.

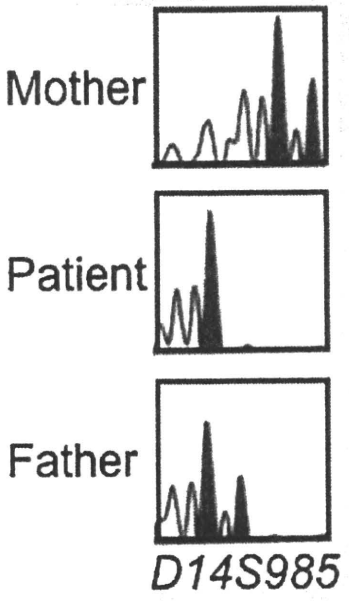

FIG. 3. Microsatellite analysis for *D14S985* residing in the intron of *MEG3*. One of the two peaks in the father is transmitted to the patient, and both of the two peaks in the mother are not inherited by the patient. The PCR fragment size: 135 and 137 bp in the mother, 131 bp in the patient, and 131 and 133 bp in the father. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

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Original Article

1p36 deletion syndrome associated with Prader–Willi-like phenotype

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Abstract ***Background***: 1p36 deletion syndrome is one of the most common subtelomeric deletion syndromes, characterized by moderate to severe mental retardation, characteristic facial appearance, hypotonia, obesity, and seizures. The clinical features often overlap with those of Prader–Willi syndrome (PWS). To elucidate the phenotype–genotype correlation in 1p36 deletion syndrome, two cases involving a PWS-like phenotype were analyzed on molecular cytogenetics.

Methods: Two patients presenting with the PWS-like phenotype but having negative results for PWS underwent fluorescence *in situ* hybridization (FISH). The size of the chromosome 1p36 deletions was characterized using probes of BAC clones based on the University of California, Santa Cruz (UCSC) Genome Browser.

Results: PWS was excluded on FISH and methylation-specific polymerase chain reaction. Subsequent FISH using the probe D1Z2 showed deletion of the 1p36.3 region, confirming the diagnosis of 1p36 deletion syndrome. Further analysis characterized 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.

Conclusion: Patients with 1p36 deletion syndrome exhibit a PWS-like phenotype and are therefore probably underdiagnosed. The possible involvement of the terminal 4 Mb region of chromosome 1p36 in the PWS-like phenotype is hypothesized.

Key words 1p36 deletion syndrome, chromosome, fluorescence *in situ* hybridization, obesity, Prader–Willi-like phenotype.

The terminal deletion of chromosome 1p36 is a newly recognized syndrome with multiple congenital anomalies and mental retardation.[1–4] The prevalence is estimated to range from 1 in 5000 to 1 in 10 000.[1,5] The most frequent clinical findings are moderate–severe mental retardation, facial characteristics including deep-set eyes and pointed chin, hypotonia, and seizures. The deletion size varies in each family and appears to be correlated with the clinical complexity as a result of haploinsufficiency of different genes,[6,7] but most breakpoints cluster at 4.0–4.5 Mb from the telomere (1pter). Some clinical manifestations of the syndrome overlap with those of Prader–Willi syndrome (PWS). Recently, a PWS-like phenotype has been described in patients with monosomy 1p36,[8] maternal uniparental disomy 14 (upd[14]mat),[9,10] and chromosome 6q16 deletion.[11] The common clinical features are global developmental delay, hypotonia, obesity, several craniofacial anomalies, hyperphagia, and behavioral problems.

Here, we describe two cases of 1p36 deletion syndrome in patients who were provisionally diagnosed with PWS, and elucidate the phenotype–genotype correlation in 1p36 deletion syndrome. The study highlights the issues regarding the overlapping clinical findings and manifestations between 1p36 deletion syndrome and PWS.

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Methods

Case reports

Patient 1

Patient 1 was the first child of healthy unrelated parents, with an unremarkable family history. The mother and father were 25 and 29 years of age, respectively, at the time of her birth. She was born at 37 weeks of gestation after an uneventful pregnancy, with a birthweight of 2360 g (–0.71 SD) and length of 46.0 cm (–0.32 SD). The patient had hypotonia and difficulty in sucking, requiring tube feeding, in the neonatal period. At age 3 her cognitive skills and motor development were moderately delayed. She crawled at 10 months, walked at 18 months, and could speak repeated words at 3 years. At age 6 the patient had hyperphagia. On physical examination at the age of 9 years, her weight was 36.9 kg (+1.16 SD), height was 129.4 cm (–0.43 SD), and occipitofrontal circumference (OFC) was 52.8 cm (+1.24 SD). The facial features included deep-set eyes associated with almond-shaped palpebral fissures, straight eyebrows, a prominent forehead, a broad and flat nasal root, and a pointed chin (Fig. 1). She

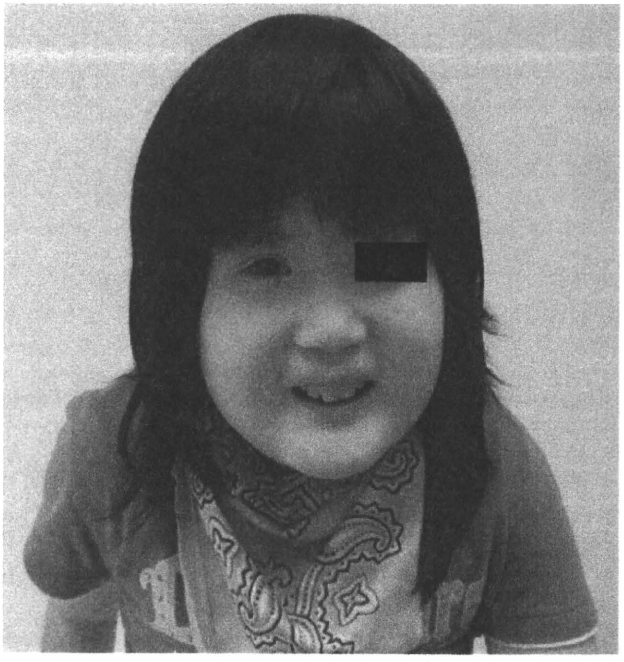

Fig. 1 Patient 1 at age 9. Note the deep-set eyes associated with almond-shaped palpebral fissures, straight eyebrows, prominent forehead, broad and flat nasal root, and pointed chin.

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.

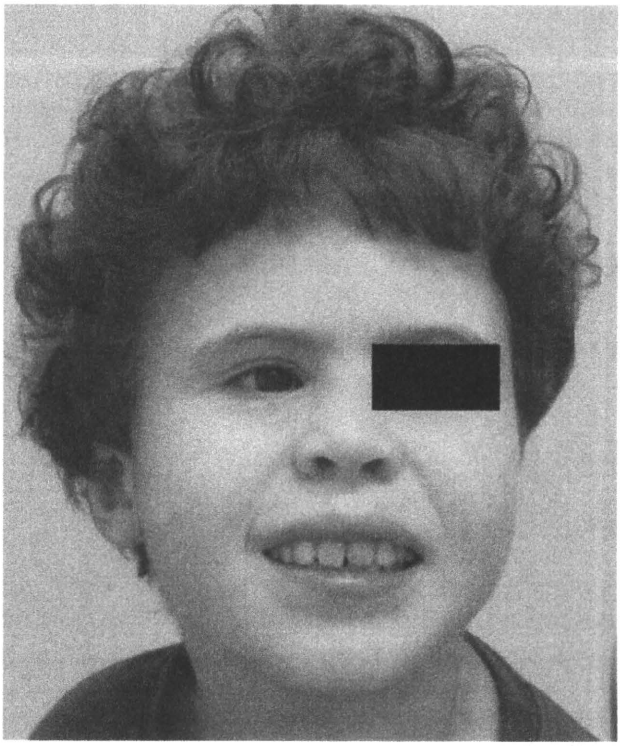

Fig. 2 Patient 2 at 10 years of age.

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

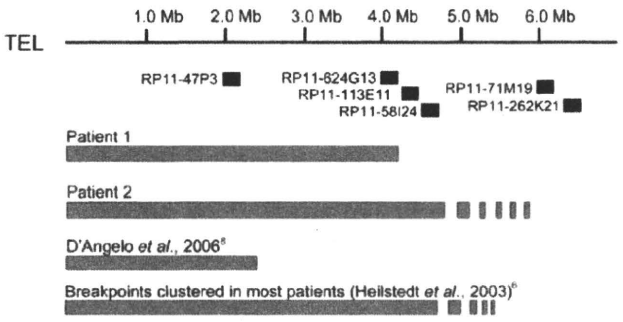

Fig. 3 Characterization of 1p36 deletion. Gray bars, deleted regions. The location of the BAC analyzed are shown according to the University of California, Santa Cruz (UCSC) Genome Browser from the Human March 2006 assembly.

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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神経線維腫症1型における分子細胞遺伝学的スクリーニング

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要旨　神経線維腫症1型large deletion症例のスクリーニングとしてBACクローンによるFISH解析を導入した。37例の定期医療管中の症例のうち、5例で解析を行い、欠失症例は認めなかった。NF1 large deletion症例は、過成長、顔貌異常、より早期からの神経線維腫の出現、学習障害などが特徴と考えられ、比較的容易に行うことができるFISH解析は、本症の医療管理を行う小児専門医療機関では考慮すべき検査と考えられた。

Fluorescence in situ hybridization analysis for detecting a large deletion of *NF1* gene in *NF1* patients.

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Neurofibromatosis type 1 (NF 1) is an autosomal dominant disorder (OMIM. 162200), characterized by cutaneous manifestations including café-au-lait patches and dermal neurofibromas, and multi-organ system disorders. After the identification of NF 1 gene, molecular analysis has been making it possible to understand the pathogenesis of NF 1. The deletion of entire gene of NF 1 is associated with severe phenotype including dysmorphism, mental retardation, and early onset of a large number of neurofibromas. We established the screening system for detecting the large deletion of NF 1 gene region by FISH with probes derived from bacterial artificial chromosome (BAC) clones.

Key words : Fluorescence in situ hybridization (FISH). Neurofibromatosis type 1 (NF 1). Bacterial artificial chromosome (BAC) deletion

はじめに

神経線維腫症1型（Neurofibromatosis type 1, NF1）は、全身の腫瘍性病変と色素斑を特徴とする常染色体優性遺伝病で、原因遺伝子は17q11.2にマップされる*NF1*[1]である。NF1は発生頻度約3000出生に1例で、小児科領域でも比較的経験することが多い疾患である。本症においては根本治療は困難なものの、合併症管理においては年齢ごとの出現しやすい兆候を把握して管理することが重要であり、診断基準や合併症の出現時期や頻度などは、成書でもまとめられている[2]。

*NF1*遺伝子はアミノ酸コードエクソンが58、コードするアミノ酸が2839で、全長が約350kbに及ぶ大きな遺伝子である。変異が起こる割合は他の遺伝子より高く、de novo変異が50%以上を占める理由になっている。多くの変異が遺伝子内欠失や点変異であるが、いわゆるgenotype-phenotypeの相関はあまりないとされている。しかし、*NF1*遺伝子