Journal of Human Genetics (2015) 60, 91–95

www.nature.com/jhg

SHORT COMMUNICATION

Silver–Russell syndrome without body asymmetry in three patients with duplications of maternally derived chromosome 11p15 involving *CDKN1C*

Shinichi Nakashima[1], Fumiko Kato[1], Tomoki Kosho[2], Keisuke Nagasaki[3], Toru Kikuchi[4], Masayo Kagami[5], Maki Fukami[5] and Tsutomu Ogata[1]

We report duplications of maternally derived chromosome 11p15 involving *CDKN1C* encoding a negative regulator for cell proliferation in three Japanese patients (cases 1 and 2 from family A and case 3 from family B) with Silver–Russell syndrome (SRS) phenotype lacking hemihypotrophy. Chromosome analysis showed 46,XX,der(16)t(11;16)(p15.3;q24.3)mat in case 1, 46, XY,der(16)t(11;16)(p15.3;q24.3)mat in case 2 and a *de novo* 46,XX,der(17)t(11;17)(p15.4;q25.3) in case 3. Genomewide oligonucleotide-based array comparative genomic hybridization, microsatellite analysis, pyrosequencing-based methylation analysis and direct sequence analysis revealed the presence of maternally derived extra copies of the distal chromosome 11p involving the wild-type *CDKN1C* (a ~7.98 Mb region in cases 1 and 2 and a ~4.43 Mb region in case 3). The results, in conjunction with the previous findings in patients with similar duplications encompassing *CDKN1C* and in those with intragenic mutations of *CDKN1C*, imply that duplications of *CDKN1C*, as well as relatively mild gain-of-function mutations of *CDKN1C* lead to SRS subtype that usually lack hemihypotrophy.
Journal of Human Genetics (2015) **60**, 91–95; doi:10.1038/jhg.2014.100; published online 27 November 2014

INTRODUCTION

Silver–Russell syndrome (SRS) is a congenital developmental disorder characterized by pre- and postnatal growth failure, relative macrocephaly, hemihypotrophy and fifth-finger clinodactyly.[1] Recent studies have shown that epimutation (hypomethylation) of the paternally derived *H19*-differentially methylated region (DMR) at the imprinting control region 1 (ICR1) on chromosome 11p15.5 and maternal uniparental disomy 7 account for ~45 and ~5% of SRS patients, respectively.[1] Thus, underlying (epi)genetic factors still remain to be clarified in a substantial fraction of SRS patients, although several rare (epi)genetic aberrations have been identified in a small fraction of SRS patients.[1]

CDKN1C (cyclin-dependent kinase inhibitor 1C) is a maternally expressed gene that resides at the ICR2 just proximal to the ICR1.[2] *CDKN1C* encodes a negative regulator for cell proliferation and, consistent with this, loss-of-function mutations of *CDKN1C* cause Beckwith–Wiedemann syndrome associated with overgrowth.[2,3] Furthermore, recent studies have shown that gain-of-function mutations of *CDKN1C* result in IMAGe syndrome (IMAGeS) characterized by intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita and male genital abnormalities,[2] whereas less severe gain-of-function mutations of *CDKN1C* have been identified in a large family with maternally inherited SRS.[4] Thus, it has been suggested that relatively severe and mild *CDKN1C* gain-of-function effects lead to IMAGeS and SRS, respectively.[4,5] Notably, IMAGeS patients satisfy the diagnostic criteria for SRS proposed by Nechine *et al.*[5,6] and IMAGeS and SRS patients with *CDKN1C* mutations invariably lack hemihypotrophy characteristic of SRS.[4–6]

Here, we report three patients with SRS and duplications of maternally derived chromosome 11p15.5 involving *CDKN1C*. The results, in conjunction with previous findings, imply that duplications of *CDKN1C*, as well as relatively mild gain-of-function mutations of *CDKN1C* lead to SRS subtype that usually lack hemihypotrophy.

CASE REPORTS

Patients

We studied three Japanese patients (cases 1–3) from two families (Figure 1). Cases 1–3 satisfied the SRS diagnostic criteria proposed by Netchine *et al.*,[6] although they lacked hemihypotrophy (Table 1, see its footnote for Netchine SRS criteria). Oligohydramnios characteristic of SRS[7] was also noticed during the pregnancies of cases 2 and 3. They exhibited no IMAGeS-like phenotypes such as radiologically discernible skeletal dysplasia, an episode suggestive of adrenal dysfunction or undermasculinized genitalia in male case 2.

[1]Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan; [2]Department of Human Genetics, Shinshu University School of Medicine, Matsumoto, Japan; [3]Department of Pediatrics, Niigata University School of Medicine, Niigata, Japan; [4]Department of Pediatrics, Saitama Medical University, Saitama, Japan and [5]Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan
Correspondence: Professor T Ogata, Department of Pediatrics, Hamamatsu University School of Medicine, 1-20-1, Handayama, Higashi-ku, Hamamatsu, Shizuoka 431-3192, Japan.
E-mail: tomogata@hama-med.ac.jp
Received 10 October 2014; revised 28 October 2014; accepted 31 October 2014; published online 27 November 2014

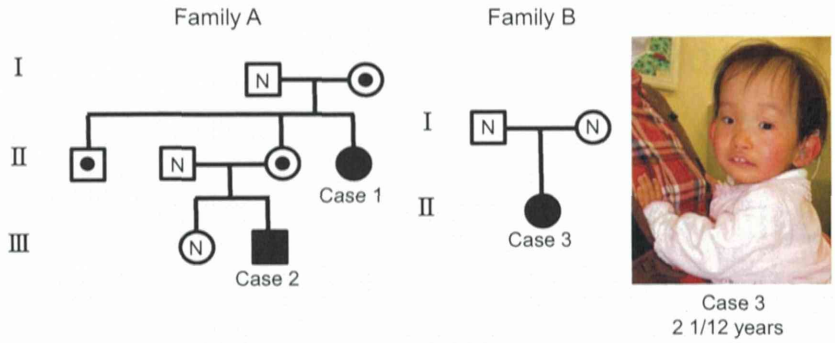
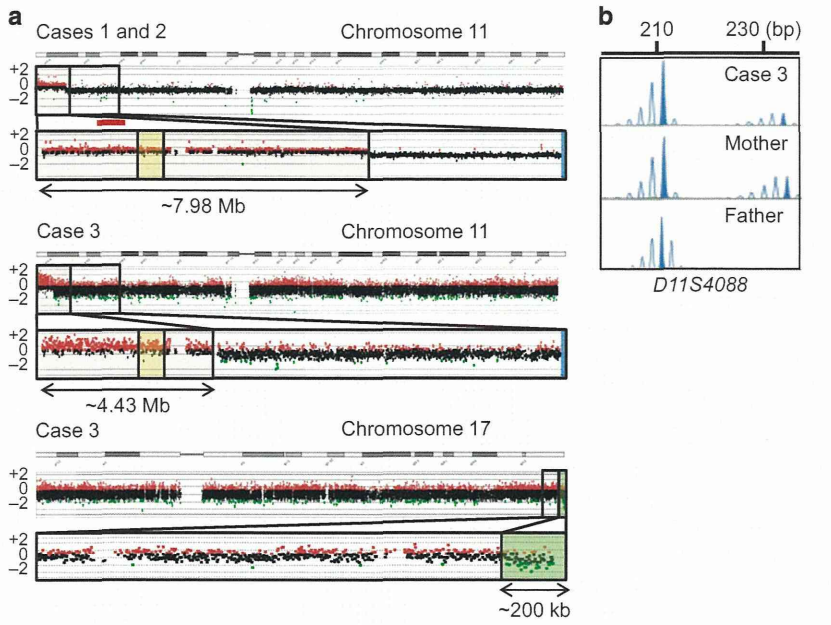


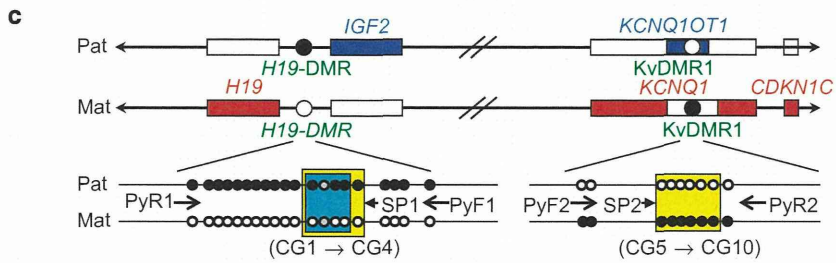
Figure 1 The pedigrees of families A and B and a photograph of case 3. In family A, cases 1 and 2 have an unbalanced translocation involving the distal part of chromosome 11p, the mothers of cases 1 and 2, as well as the brother of case 1 have a balanced translocation involving the distal part of chromosome 11p and the remaining subjects have a normal karyotype. In family B, case 3 has an unbalanced translocation involving the distal part of chromosome 11p and the parents have a normal karyotype. Case 3 exhibits SRS-compatible phenotypes such as prominent forehead, triangular face with relative macrocephaly and micrognathia, ear anomalies and short and curved fifth fingers, but is free from hemihypotrophy.

Table 1 Clinical features of cases 1–3 and reported cases with duplications of maternally derived chromosome 11p15 involving *CDKN1C*

	Case 1 family A female	*Case 2 family A male*	*Case 3 family B female*	*Reported cases (n = 16)*[11–19]
SRS phenotype				
Mandatory criteria for SRS				
BL and/or BW ⩽ −2 SDS	+	+	+	16/16
Scoring system criteria for SRS				
Relative macrocephaly at birth[a]	Unknown	+	+	11/11
PH ⩽ −2 SDS at ⩾2 years	+	Unknown	+	14/14
Prominent forehead	+	+	+	8/9
Body asymmetry	−	−	−	1/15
Feeding difficulties	+	−	Unknown	6/6
Other findings				
Gestational age (weeks)	39	32	32	22–38
Oligohydramnios	Unknown	+	+	Unknown
BL, cm (SDS)	38.0 (−4.9)	34.0 (−3.3)	32.0 (−3.9)	N.D.[b]
BW, kg (SDS)	1.3 (−5.3)	0.87 (−3.6)	0.82 (−3.7)	N.D.[b]
BOFC, cm (SDS)	29.5 (−2.7)	28.3 (−0.6)	27 (−1.2)	N.D.[b]
Present age (years:months)	14:00	1:03	3:03	1–31
PH, cm (SDS)	130.7 (−4.7)	60.8 (−6.4)	70.7 (−6.7)	N.D.[b]
PW, kg (SDS)	37.5 (−1.2)	4.8 (−5.3)	6.8 (−4.0)	N.D.[b]
BMI, kg m^{-2} (SDS)	22.0 (0.7)	13.1 (−2.8)	13.6 (−1.5)	N.D.[b]
POFC, cm (SDS)	Unknown	45.0 (−0.5)	48.5 (−0.1)	N.D.[b]
Relative macrocephaly at present[c]	Unknown	+	+	14/15
Triangular face	−	+	+	12/16
Ear anomalies	−	−	+	8/11
Irregular teeth	Unknown	+	+	1/2
Clinodactyly	+	+	+	10/11
Brachydactyly	+	+	−	4/5
Simian crease	+	+	−	1/2
Muscular hypotonia	Unknown	+	+	4/7
Developmental/speech delay	+	+	+	11/15
IMAGe syndrome phenotype				
IUGR	+	+	+	16/16
Metaphyseal dysplasia	−	−	−	Not described
Adrenal hypoplasia	−	−[d]	−[e]	Not described
Genital abnormality	Female	−	Female	Not described

The diagnosis of Silver–Russell syndrome is made when a patient is positive for the mandatory criteria and at least three of the five scoring system criteria (Netchine *et al*.[5])
Abbreviations: BL, birth length; BMI, body mass index; BOFC, birth occipitofrontal circumference; BW, birth weight; IMAGe, intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita and male genital abnormalities; IUGR, intrauterine growth retardation; N.D., not determined; PH, present height; POFC, present occipitofrontal circumference; PW, present weight; SDS, standard deviation score.
For reported cases, the denominators indicate the number of patients examined for the presence or absence of each feature, and the numerators represent the number of patients assessed to be positive for that feature.
Birth and present body sizes were assessed by the gestational/postnatal age- and sex-matched Japanese reference data from the Ministry of Health, Labor and Welfare and from the Ministry of Education, Science, Sports and Culture.
[a]BL or BW (SDS)—BOFC (SDS) ⩽ −1.5.
[b]N.D. because of various ethnicities of affected individuals and descriptions of height assessment (percentile and SDS).
[c]PH or PW (SDS)—POFC (SDS) ⩽ −1.5.
[d]A rapid adrenocorticotropin stimulation test (0.25 mg m^{-2} bolus i.v.; blood sampling at 0 and 60 min) showed a sufficient cortisol response (14.2 → 26.2 μg dl^{-1}) (reference range >20 μg dl^{-1}).
[e]A growth hormone releasing peptide 2 stimulation test (2 μg kg^{-1} bolus i.v.; blood sampling at 0, 15, 30, 45 and 60 min) yielded a sufficient cortisol response (18.4 → 25.5 μg dl^{-1}).





	Case 1			Case 2			Case 3			Controls (n=50)
	P	F	M	P	F	M	P	F	M	Median (Range)
CG1	30	41	53	34	52	55	29	44	44	45 (36 ~ 55)
CG2	31	42	54	37	54	55	28	43	44	46 (36 ~ 57)
CG3	33	45	56	38	57	60	31	47	46	50 (39 ~ 64)
CG4	33	44	55	39	56	57	31	48	46	48 (37 ~ 60)
CG5	72	56	56	72	56	57	68	56	57	58 (49 ~ 66)
CG6	78	59	59	73	59	60	71	61	58	61 (52 ~ 68)
CG7	67	52	50	66	50	52	61	50	49	48 (41 ~ 54)
CG8	69	55	53	68	53	55	63	52	52	48 (42 ~ 55)
CG9	78	60	61	75	63	61	72	63	59	67 (55 ~ 72)
CG10	74	63	57	79	60	59	77	63	64	64 (55 ~ 71)

Figure 2 Representative molecular findings. (**a**) Array comparative genomic hybridization analysis. The black, red and green dots represent signals indicative of the normal, increased (log2 signal ratio > +0.5) and decreased (log2 signal ratio < −0.8) copy numbers, respectively. The log2 signal ratios of +0.5 and −1.0 indicate the presence of three copies and a single copy of the corresponding regions, respectively. The red and the green rectangles represent increased and decreased copy number regions, respectively. The yellow rectangles denote the regions encompassing the ICR1 and the ICR2. (**b**) Microsatellite analysis for *D11S4088* proximal to the KvDMR1. Unequal amplification of the heterozygous peaks in each subject is consistent with short products being more easily amplified than long products and comparison of area under curves of the 212 bp and the 234 bp alleles between case 3 and the mother indicates the presence of two 212 bp alleles and a single 234 bp allele in case 3. This implies that the maternal 212 and 234 bp alleles and the paternal 212 bp allele have been transmitted to case 3. (**c**) Pyrosequencing-based methylation analysis of the *H19*-DMR at the ICR1 and the KvDMR1 at the ICR2, using bisulfite-treated genomic DNA. The cytosine residues at the CpG dinucleotides within the *H19*-DMR is methylated after paternal transmission (filled circles) and unmethylated after maternal transmission (open circles), whereas those within the KvDMR1 is unmethylated after paternal transmission (open circles) and methylated after maternal transmission (filled circles). Paternally and maternally expressed genes are shown in blue and red, respectively. For the *H19*-DMR, a segment encompassing 21 CpG dinucleotides was PCR amplified with PyF1 and PyR1 primers and a sequence primer (SP1) was hybridized to a single-stranded PCR product. Subsequently, the MIs were obtained for four CpG dinucleotides (CG1–CG4) (indicated with a yellow rectangle). The blue rectangle indicates the CTCF binding site 6. The CpG dinucleotide between CG1 and CG2 was not examined, because it constitutes a C/T SNP (indicated with gray circles). The KvDMR1 was similarly examined using PyF2 and PyR2 primers and SP2 and the MIs were obtained for CG5–CG10. The MIs are summarized in the bottom table. F, father; and M, mother; P, patient.

Cytogenetic and molecular studies

This study was approved by the Institute Review Board Committee at Hamamatsu University School of Medicine and was performed using peripheral leukocyte samples and primers shown in Supplementary Table S1 after obtaining written informed consent. The methods for molecular studies were as reported previously.[7] We also obtained written informed consent to publish the facial photograph of case 3 from the parents.

Chromosome analysis showed 46,XX,der(16)t(11;16)(p15.3;q24.3) mat in case 1, 46,XY,der(16)t(11;16)(p15.3;q24.3)mat in case 2 and a *de novo* 46,XX, der(17)t(11;17)(p15.4;q25.3) in case 3 (Figure 1). Then, genomewide oligonucleotide-based array comparative genomic hybridization was carried out using a catalog human array (2×400 K format, ID G4448A) (Agilent Technologies, Santa Clara, CA, USA), revealing the presence of three copies of the distal parts of chromosome 11p involving the ICR1 and the ICR2 in cases 1–3 (a ~7.98 Mb region in cases 1 and 2 and a ~4.43 Mb region in case 3) (Figure 2a). No discernible deletion was identified on the distal chromosome 16q in cases 1 and 2, indicating the position of the chromosome 16q breakpoint at the very telomeric portion, whereas a ~200 kb deletion was detected in the telomeric portion of chromosome 17q in case 3. There was no other copy number alteration that was not registered in the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home). Microsatellite analysis was carried out for four loci on the duplicated chromosome 11p, showing the presence of two alleles of maternal origin and a single allele of paternal origin in cases 1–3 (Figure 2b and Supplementary Table S2). Subsequently, pyrosequencing-based methylation analysis was performed for four CpG dinucleotides (CG1–CG4) within the *H19*-DMR and six CpG dinucleotides (CG5–CG10) within the KvDMR1 using bisulfite-treated leukocyte genomic DNA samples and methylation index (MI, the ratio of methylated clones) was obtained for each of CG1–CG10 using PyroMark Q24 (Qiagen, Valencia, CA, USA) (Figure 2c). In cases 1–3, the MIs for CG1–CG4 were mildly decreased or around the lower limit of the normal range and those for CG5–CG10 were mildly increased or around the upper limit of the normal range. Direct sequence analysis showed no discernible mutation on the *CDKN1C* coding region.

DISCUSSION

Cases 1–3 had SRS without hemihypotrophy (body asymmetry) in the presence of maternally derived extra copies of the distal chromosome 11p involving the ICR1 and the ICR2. This implies that the SRS phenotype lacking hemihypotrophy in cases 1–3 is primarily caused by two copies of maternally expressed genes on the two ICRs. In this regard, of duplicated maternally expressed genes, *CDKN1C* functions as a negative growth regulator[8] and *CDKN1C* gain-of-function mutations have been identified in SRS and IMAGeS,[2,4,5] whereas neither *H19* nor *KCNQ1* appears to have a positive role in growth regulation. Indeed, *H19* is regarded as a possible tumor suppressor gene[9] and *KCNQ1* encoding a voltage-gated potassium channel is involved in cardiac arrhythmias.[10] Thus, it is likely that SRS phenotype lacking hemihypotrophy in cases 1–3 is primarily caused by the presence of two functional copies of the wild-type *CDKN1C*. It should be pointed out, however, that although the der(16)t(11;16)(p15.3;q24.3) chromosome in cases 1 and 2 had no discernible chromosome 16q deletion, the der(17)t(11;17)(p15.4;q25.3) chromosome in case 3 was missing the ~200 kb telomeric 17q region that harbors several genes. In addition, there are multiple nonimprinted genes on the duplicated chromosome 11p15 regions. Thus, altered dosage of such genes may have exerted a certain effect on growth patterns of cases 1–3.

An extra copy of maternally derived chromosome 11p15 involving *CDKN1C* has been identified in 16 patients (Table 1) (for detailed clinical features of each case, see Supplementary Table S3).[11–19] Notably, although they frequently show SRS-like phenotype, hemihypotrophy (body asymmetry) has been found only in a single case[12] and none of them exhibit IMAGeS-like skeletal, adrenal or genital manifestation. This provides further support for the notion that two copies of maternally derived *CDKN1C*, as well as mild gain-of-function mutations of *CDKN1C* usually lead to SRS subtype lacking hemihypotrophy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid for Scientific Research (A) (25253023) and Research (B) (23390083) from the Japan Society for the Promotion of Science, by Grants for Research on Intractable Diseases (H22-161) from the Ministry of Health, Labor and Welfare (MHLW) and by Grant for National Center for Child Health and Development (25-10).

1 Eggermann, T. Russell-Silver syndrome. *Am. J. Med. Genet. C Semin. Med. Genet.* **154C**, 355–364 (2010).
2 Arboleda, V.A., Lee, H., Parnaik, R., Fleming, A., Banerjee, A. & Ferraz-de-Souza, B. *et al.* Mutations in the PCNA-binding domain of CDKN1C cause IMAGe syndrome. *Nat. Genet.* **44**, 788–792 (2012).
3 Soejima, H. & Higashimoto, K. Epigenetic and genetic alterations of the imprinting disorder Beckwith–Wiedemann syndrome and related disorders. *J. Hum. Genet.* **58**, 402–409 (2013).
4 Brioude, F., Oliver-Petit, I., Blaise, A., Praz, F., Rossignol, S. & Le Jule, M. *et al.* CDKN1C mutation affecting the PCNA-binding domain as a cause of familial Russell Silver syndrome. *J. Med. Genet.* **50**, 823–830 (2013).
5 Kato, F., Hamajima, T., Hasegawa, T., Amano, N., Horikawa, R. & Nishimura, G. *et al.* IMAGe syndrome: clinical and genetic implications based on investigations in three Japanese patients. *Clin. Endocrinol.* **80**, 706–713 (2014).
6 Netchine, I., Rossignol, S., Dufourg, M. N., Azzi, S., Rousseau, A. & Perin, L. *et al.* 11p15 imprinting center region 1 loss of methylation is a common and specific cause of typical Russell-Silver syndrome: clinical scoring system and epigenetic-phenotypic correlations. *J. Clin. Endocrinol. Metab.* **92**, 3148–3154 (2007).
7 Fuke, T., Mizuno, S., Nagai, T., Hasegawa, T., Horikawa, R. & Miyoshi, Y. *et al.* Molecular and clinical studies in 138 Japanese patients with Silver–Russell syndrome. *PLoS ONE* **8**, e60105 (2013).
8 Lee, M. H., Reynisdottir, I. & Massague, J. Cloning of p57(KIP2), a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes Dev.* **9**, 639–649 (1995).
9 Hao, Y., Crenshaw, T., Moulton, T., Newcomb, E. & Tycko, B. Tumour-suppressor activity of H19 RNA. *Nature* **365**, 764–767 (1993).
10 Wang, Q., Curran, M. E., Splawski, I., Burn, T. C., Millholland, J. M. & VanRaay, T. J. *et al.* Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat. Genet.* **12**, 17–23 (1996).
11 Fisher, A. M., Thomas, N. S., Cockwell, A., Stecko, O., Kerr, B. & Temple, I. K. *et al.* Duplications of chromosome 11p15 of maternal origin result in a phenotype that includes growth retardation. *Hum. Genet.* **111**, 290–296 (2002).
12 Eggermann, T., Meyer, E., Obermann, C., Heil, I., Schüler, H. & Ranke, M. B. *et al.* Is maternal duplication of 11p15 associated with Silver–Russell syndrome? *J. Med. Genet.* **42**, e26 (2005).
13 Schönherr, N., Meyer, E., Roos, A., Schmidt, A., Wollmann, H. A. & Eggermann, T. *et al.* The centromeric 11p15 imprinting centre is also involved in Silver–Russell syndrome. *J. Med. Genet.* **44**, 59–63 (2007).
14 South, S. T., Whitby, H., Maxwell, T., Aston, E., Brothman, A. R. & Carey, J. C. Co-occurrence of 4p16.3 deletions with both paternal and maternal duplications of 11p15: modification of the Wolf–Hirschhorn syndrome phenotype by genetic alterations predicted to result in either a Beckwith–Wiedemann or Russell–Silver phenotype. *Am. J. Med. Genet. A* **146A**, 2691–2697 (2008).
15 Bliek, J., Snijder, S., Maas, S. M., Polstra, A., van der Lip, K. & Alders, M. *et al.* Phenotypic discordance upon paternal or maternal transmission of duplications of the 11p15 imprinted regions. *Eur. J. Med. Genet.* **52**, 404–408 (2009).

16 Eggermann, T., Spengler, S., Bachmann, N., Baudis, M., Mau-Holzmann, U. A. & Singer, S. *et al.* Chromosome 11p15 duplication in Silver-Russell syndrome due to a maternally inherited translocation t(11;15). *Am. J. Med. Genet. A* **152A**, 1484–1487 (2010).
17 Cardarelli, L., Sparago, A., De Crescenzo, A., Nalesso, E., Zavan, B. & Cubellis, M. V. *et al.* Silver-Russell syndrome and Beckwith–Wiedemann syndrome phenotypes associated with 11p duplication in a single family. *Pediatr. Dev. Pathol.* **13**, 326–330 (2010).
18 Bonaldi, A., Mazzeu, J. F., Costa, S. S., Honjo, R. S., Bertola, D. R. & Albano, L. M. *et al.* Microduplication of the ICR2 domain at chromosome 11p15 and familial Silver-Russell syndrome. *Am. J. Med. Genet. A* **155A**, 2479–2483 (2011).
19 Chiesa, N., De Crescenzo, A., Mishra, K., Perone, L., Carella, M. & Palumbo, O. *et al.* The KCNQ1OT1 imprinting control region and non-coding RNA: new properties derived from the study of Beckwith-Wiedemann syndrome and Silver–Russell syndrome cases. *Hum. Mol. Genet.* **21**, 10–25 (2012).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

EJHG Open

European Journal of Human Genetics (2015), 1–11

www.nature.com/ejhg

ARTICLE

Comprehensive clinical studies in 34 patients with molecularly defined UPD(14)pat and related conditions (Kagami–Ogata syndrome)

Masayo Kagami[1], Kenji Kurosawa[2], Osamu Miyazaki[3], Fumitoshi Ishino[4], Kentaro Matsuoka[5] and Tsutomu Ogata*[,1,6]

Paternal uniparental disomy 14 (UPD(14)pat) and epimutations and microdeletions affecting the maternally derived 14q32.2 imprinted region lead to a unique constellation of clinical features such as facial abnormalities, small bell-shaped thorax with a coat-hanger appearance of the ribs, abdominal wall defects, placentomegaly, and polyhydramnios. In this study, we performed comprehensive clinical studies in patients with UPD(14)pat ($n=23$), epimutations ($n=5$), and microdeletions ($n=6$), and revealed several notable findings. First, a unique facial appearance with full cheeks and a protruding philtrum and distinctive chest roentgenograms with increased coat-hanger angles to the ribs constituted the pathognomonic features from infancy through childhood. Second, birth size was well preserved, with a median birth length of ±0 SD (range, −1.7 to +3.0 SD) and a median birth weight of +2.3 SD (range, +0.1 to +8.8 SD). Third, developmental delay and/or intellectual disability was invariably present, with a median developmental/intellectual quotient of 55 (range, 29–70). Fourth, hepatoblastoma was identified in three infantile patients (8.8%), and histological examination in two patients showed a poorly differentiated embryonal hepatoblastoma with focal macrotrabecular lesions and well-differentiated hepatoblastoma, respectively. These findings suggest the necessity of an adequate support for developmental delay and periodical screening for hepatoblastoma in the affected patients, and some phenotypic overlap between UPD(14)pat and related conditions and Beckwith–Wiedemann syndrome. On the basis of our previous and present studies that have made a significant contribution to the clarification of underlying (epi)genetic factors and the definition of clinical findings, we propose the name 'Kagami–Ogata syndrome' for UPD(14)pat and related conditions.
European Journal of Human Genetics advance online publication, 18 February 2015; doi:10.1038/ejhg.2015.13

INTRODUCTION

Human chromosome 14q32.2 carries a cluster of imprinted genes including paternally expressed genes (*PEGs*) such as *DLK1* and *RTL1*, and maternally expressed genes (*MEGs*) such as *MEG3* (alias, *GTL2*), *RTL1as* (*RTL1* antisense), *MEG8*, *snoRNAs*, and *microRNAs* (Supplementary Figure S1).[1,2] The parental origin-dependent expression patterns are regulated by the germline-derived primary *DLK1-MEG3* intergenic differentially methylated region (IG-DMR) and the postfertilization-derived secondary *MEG3*-DMR.[2,3] Both DMRs are hypermethylated after paternal transmission and hypomethylated after maternal transmission in the body; in the placenta, the IG-DMR alone remains as a DMR with the same methylation pattern in the body, while the *MEG3*-DMR does not represent a differentially methylated pattern.[2,3] Consistent with such methylation patterns, the hypomethylated IG-DMR and *MEG3*-DMR of maternal origin function as imprinting control centers in the placenta and the body, respectively, and the IG-DMR behaves hierarchically as an upstream regulator for the methylation pattern of the *MEG3*-DMR in the body, but not in the placenta.[3,4]

Paternal uniparental disomy 14 (UPD(14)pat) (OMIM #608149) results in a unique constellation of clinical features such as facial abnormalities, small bell-shaped thorax with coat-hanger appearance of the ribs, abdominal wall defects, placentomegaly, and polyhydramnios.[2,5] These clinical features are also caused by epimutations (hypermethylations) and microdeletions affecting the maternally derived IG-DMR and/or *MEG3*-DMR (Supplementary Figure S1). Such UPD(14)pat and related conditions are rare, with reports of 33 patients with UPD(14)pat, five patients with epimutations, and nine patients with microdeletions (and four new UPD(14)pat patients reported here) (see Supplementary Table S1 for the reference list). For microdeletions, loss of the maternally inherited *MEG3*-DMR alone leads to a typical UPD(14)pat body phenotype and apparently normal placental phenotype,[3,4] whereas loss of the maternally derived IG-DMR alone or both DMRs results in a typical body and placental UPD(14)pat phenotype, consistent with the methylation patterns of the two DMRs.[2,3] Furthermore, correlations between clinical features and deleted segments have indicated the critical role of excessive *RTL1* (but not *DLK1*) expression in phenotypic development.[2,6] Such an excessive *RTL1* expression is primarily due to loss of functional *RTL1as*-encoded *microRNAs* that act as a *trans*-acting repressor for *RTL1* expression.[6] Indeed, the *RTL1* expression level is ~5 times, rather than 2 times, increased in placentas with UPD(14)pat

[1]Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan; [2]Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; [3]Department of Radiology, National Center for Child Health and Development, Tokyo, Japan; [4]Department of Epigenetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [5]Department of Pathology, National Center for Child Health and Development, Tokyo, Japan; [6]Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan
*Correspondence: Dr T Ogata, Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan. Tel: +81 53 435 2310; Fax: +81 53 435 2310; E-mail: tomogata@hama-med.ac.jp
Received 30 August 2014; revised 7 January 2015; accepted 14 January 2015

Table 1 Clinical manifestations in 33 Japanese and one Irish patients with UPD(14)pat and related conditions (Kagami–Ogata syndrome)

	UPD(14)pat	*Epimutations*	*Microdeletions*				*Total*
			Subtype 1	*Subtype 2*	*Subtype 3*	*Subtotal*	
	Pts 1–23 (n = 23)	*Pts 24–28 (n = 5)*	*Pts 29–31 (n = 3)*	*Pt 32 (n = 1)*	*Pts 33–34 (n = 2)*	*Pts 29–34 (n = 6)*	*Pts 1–34 (n = 34)*
Age at the last examination or death (y)	2.9 (0.0–15.0)	2.0 (0.8–5.5)	2.8 (0.8–8.9)	(4 days)	4.5 (3.8–5.1)	3.3 (0.0–8.9)	2.8 (0.0–15.0)
Sex (male:female)	9:14	3:2	1:2	0:1	0:2	1:5	13:21
Molecular findings[a]							
IG-DMR of maternal origin	Absent	Methylated	Deleted	Unmethylated	Deleted		
MEG3-DMR of maternal origin	Absent	Methylated[b]	Deleted/methylated[b]	Deleted	Deleted		
DLK1 expression level	2×	2×	1 or 2×	2× (1×)[c]	1 or 2×		
RTL1 expression level	~5×	~5×	~5×	~5× (1× or ~2.5×)[c]	~2.5×		
MEGs expression level	0×	0×	0×	0× (1× or 0×)[c]	0×		
Pregnancy and delivery							
Polyhydramnios	23/23	5/5	3/3	0/1	2/2	5/6	33/34
Gestational age at Dx (w)	25 (14–30)	27.5 (22–30)	Unknown	—	21	21	25.5 (14–30)
Amnioreduction	18/20	4/5	2/3	0/1	1/2	3/6	25/31
Amnioreduction (>30 w)	18/18	4/4	2/2	—	1/1	3/3	25/25[d]
Placentomegaly[e]	14/17	4/4	3/3	0/1	2/2	5/6	23/27
Prenatal Dx of thoracic abnormality	8/20[f]	2/3	0/1	—	0/1	0/2	10/25
Gestational age at Dx (w)	26 (22–33)	27.5 (25–30)	—	—	—	—	26 (22–33)
Prenatal Dx of abdominal abnormality	6/18	3/3	1/1	—	0/1	1/2	10/23
Gestational age at Dx (w)	26 (22–28)	25	Unknown	Unknown	Unknown	Unknown	25.5 (22–28)
Gestational age (w)	34.5 (24–38)	35 (30–37)	30 (27–33)	28	32.5 (30–35)	30 (27–35)	34 (24–38)
Premature delivery (<37 w)	17/23	4/5	3/3	1/1	2/2	6/6	27/34
Delivery (Cesarean:Vaginal)	15:8	4:1	2:1	0:1	2:0	4:2	23:11
Medically assisted reproduction	1/18	0/1	0/1	Unknown	0/1	0/2	1/21
Growth pattern							
Prenatal growth failure[g]	0/23	0/5	0/3	0/1	0/2	0/6	0/34
Prenatal overgrowth[h]	13/23	3/5	3/3	0/1	1/2	4/6	20/34
Birth length (patient number)	21	5	1	1	2	4	30
SD score, median (range)	+0.3 (−1.7 to +3.0)	−0.5 (−0.9 to +1.4)	0.0	−1.1	+0.7 (−0.1 to +1.5)	−0.1 (−1.1 to +1.5)	±0 (−1.7 to +3.0)
Actual length (cm), median (range)	45.0 (30.6 to 51.0)	43.5 (41.0 to 50.0)	43.0	34.0	43.5 (42.0 to 45.0)	42.5 (34.0 to 45.0)	44.7 (30.6 to 51.0)
Birth weight (patient number)	23	5	3	1	2	6	34
SD score, median (range)	+2.2 (+0.1 +8.8)	+2.2 (+0.5 to +3.7)	+2.8 (+2.4 to +3.7)	+1.5	+1.7 (+0.9 to +2.5)	+2.5 (+0.9 to +3.7)	+2.3 (+0.1 to +8.8)
Actual weight (cm), median (range)	2.79 (1.24 to 3.77)	2.9 (1.61 to 3.28)	2.04 (1.30 to 2.84)	1.32	2.24 (1.55 to 2.94)	1.79 (1.30 to 2.94)	2.79 (1.24 to 3.77)
Postnatal growth failure[i]	7/20	2/5	2/3	—	0/2	2/5	11/30
Postnatal overgrowth[j]	1/20	1/5	0/3	—	0/2	0/5	2/30
Present stature (patient number)	20	5	3	—	1	4	29
SD score, median (range)	−1.6 (−8.7 to +1.1)	−1.8 (−7.1 to +0.9)	−2.2 (−3.3 to −1.3)	—	−1.6	−1.9 (−3.3 to −1.3)	−1.6 (−8.7 to +1.1)
Present weight (patient number)	20	5	3	—	2	5	30
SD score, median (range)	−1.0 (−6.0 to +2.4)	−0.6 (−5.5 to +4.0)	−1.3 (−2.2 to ±0)	—	−1.1 (−1.3 to −0.9)	−1.3 (−2.2 to ±0)	−1.0 (−6.0 to +4.0)

Table 1 (Continued)

	UPD(14)pat	*Epimutations*	*Microdeletions*				*Total*
			Subtype 1	*Subtype 2*	*Subtype 3*	*Subtotal*	
	Pts 1–23 (n = 23)	*Pts 24–28 (n = 5)*	*Pts 29–31 (n = 3)*	*Pt 32 (n = 1)*	*Pts 33–34 (n = 2)*	*Pts 29–34 (n = 6)*	*Pts 1–34 (n = 34)*
Craniofaciocervical features							
Frontal bossing	17/22	4/5	1/3	1/1	2/2	4/6	25/33
Hairy forehead	18/22	1/5	3/3	1/1	0/2	4/6	23/33
Blepharophimosis	18/22	3/5	2/3	0/1	1/2	3/6	24/33
Small ears	8/21	2/5	1/3	1/1	0/2	2/6	12/32
Depressed nasal bridge	23/23	5/5	3/3	0/1	1/2	4/6	32/34
Anteverted nares	19/22	4/5	3/3	0/1	2/2	5/6	28/33
Full cheek	20/21	4/4	2/2	0/1	1/1	3/4	27/29
Protruding philtrum	23/23	5/5	3/3	0/1	2/2	5/6	33/34
Puckered lips	11/21	3/5	3/3	0/1	0/2	3/6	17/32
Micrognathia	20/21	5/5	3/3	1/1	1/2	5/6	30/32
Short webbed neck	22/22	5/5	3/3	1/1	2/2	6/6	33/33
Thoracic abnormality							
Small bell-shaped thorax in infancy[k]	23/23	5/5	3/3	1/1	2/2	6/6	34/34
Coat-hanger appearance in infancy[l]	23/23	5/5	3/3	1/1	2/2	6/6	34/34
Laryngomalacia	8/20	2/5	2/3	—	0/1	2/4	12/29
Tracheostomy	7/21	1/4	0/2	—	2/2	2/4	10/29
Mechanical ventilation	21/23	5/5	3/3	1/1	2/2	6/6	32/34
Duration of ventilation (m)[m]	1.2 (0.1–17)	0.7 (0.1–0.9)	5 (0.23–10)	—	1.5 (1–2)	2 (0.2–10)	1.0 (0.1–17)
Abdominal wall defects							
Omphalocele	7/23	2/5	1/3	1/1	0/2	2/6	11/34
Diastasis recti	16/23	3/5	2/3	0/1	2/2	4/6	23/34
Developmental delay							
Developmental delay	21/21	5/5	3/3	—	2/2	5/5	31/31
Developmental/intellectual quotient	55 (29–70)	52 (48–56)	Unknown	Unknown	Unknown	—	55 (29–70)
Delayed head control (>4 m)[n]	14/16	4/4	1/1	—	1/1	2/2	20/22
Age at head control (m)[o]	7 (3–36)	7 (6–11)	6	—	6	6 (6)	7 (3–36)
Delayed sitting without support (>7 m)[n]	16/16	4/4	2/2	—	1/1	3/3	23/23
Age at sitting without support (m)[o]	12 (8–25)	11.5 (10–20)	22.5 (18–27)	—	18	18 (18–27)	12 (8–27)
Delayed walking without support (>14 m)[n]	17/17	3/3	2/2	—	2/2	4/4	24/24
Age at walking without support (m)[o]	25.5 (20–49)	25 (22–39)	60 (30–90)	—	24	30 (24–90)	25.5 (20–90)
Other features							
Feeding difficulty	20/21	5/5	3/3	—	2/2	5/5	30/31
Duration of tube feeding (m)[p]	6 (0.1–72)	8.5 (0.5–17)	59.5 (30–89)	—	51	51 (30–89)	7.5 (0.1–89)
Joint contractures	14/22	3/5	3/3	0/1	0/2	3/6	20/33
Constipation	12/20	3/4	1/2	—	0/2	1/4	16/28
Kyphoscoliosis	9/21	3/5	1/2	0/1	0/1	1/4	13/30

Table 1 (Continued)

	UPD(14)pat	*Epimutations*	*Microdeletions*				*Total*
			Subtype 1	*Subtype 2*	*Subtype 3*	*Subtotal*	
	Pts 1–23 (n = 23)	*Pts 24–28 (n = 5)*	*Pts 29–31 (n = 3)*	*Pt 32 (n = 1)*	*Pts 33–34 (n = 2)*	*Pts 29–34 (n = 6)*	*Pts 1–34 (n = 34)*
Coxa valga	6/21	1/5	3/3	0/1	1/2	4/6	11/32
Cardiac disease	5/22	1/5	0/3	1/1	1/2	2/6	8/33
Inguinal hernia	5/22	1/5	2/3	0/1	0/2	2/6	8/33
Seizure	1/21	0/5	0/3	0/1	0/2	0/6	1/32
Hepatoblastoma	3/23	0/5	0/3	0/1	0/2	0/6	3/34
Mortality within the first 5 years							
Alive:deceased	18:5	5:0	2:1	0:1	1:1	3:3	26:8
Parents							
Paternal age at childbirth (y)	35 (24–47)	30 (26–36)	37 (34–39)	25	31.5 (27–36)	35 (25–39)	34 (24–47)
Maternal age at childbirth (y)	31 (25–43)	28 (25–35)	31 (27–36)	25	30.5 (28–33)	29.5 (25–36)	31 (25–43)
Advanced childbearing age (⩾35 y)	8/23	1/5	1/3	0/1	0/2	1/6	8/34

Abbreviations: CHA, coat-hanger angle; Dx, diagnosis; m, month; M/W, mid to widest thorax diameter; UPD(14)pat, paternal uniparental disomy 14; w, week; y, year.
Patient #32 is Irish, and the remaining patients are Japanese; the Irish patient has also been examined by Beygo *et al.*[4]
Age data are expressed by median and range.
The denominators indicate the number of patients examined for the presence or absence of each feature, and the numerators represent the number of patient assessed to be positive for that feature; thus, differences between the denominators and numeratorsdenote the number of patients evaluated to be negative for the feature.
[a]For details, see Supplementary Figures S1 and S2.
[b]The *MEG3*-DMR is predicted to be grossly hypomethylated in the placenta.
[c]Expression patterns of the imprinted genes are predicted to be different between the body and the placenta in this patient, while they are predicted to be identical between the body and the placenta in other patients (See Supplementary Figure S1).
[d]Amnioreduction was performed about two times in 23 of the 25 pregnancies.
[e]Placental weight >120% of the gestational age-matched mean placental weight.[34]
[f]The diagnosis of UPD(14)pat has been suspected in two patients (patients #7 and #21).
[g]Birth length and/or birth weight < −2 SD of the gestational age- and sex-matched Japanese reference data (http://jspe.umin.jp/medical/keisan.html).
[h]Birth length and/or birth weight >+2 SD of the gestational age- and sex-matched Japanese reference data (http://jspe.umin.jp/medical/keisan.html).
[i]Present length/height and/or present weight < −2 SD of the age- and sex-matched Japanese reference data (http://jspe.umin.jp/medical/taikaku.html).
[j]Present length/height and/or present weight >+2 SD of the age- and sex-matched Japanese reference data (http://jspe.umin.jp/medical/taikaku.html).
[k]The M/W ratio below nromal range (see Figure 2).
[l]The CHA above the normal range (see Figure 2).
[m]The duration in patients in whom mechanical ventilation could be discontinued.
[n]The age when 90% of infants pass each gross motor developmental milestone (based on Revised Japanese Version of Denver Developmental Screening Test) (http://www.dinf.ne.jp/doc/japanese/prdl/jsrd/norma/n175/img/n175_078i01.gif).
[o]The median (range) of ages in patients who passed each gross motor developmental milestone; patients who have not passed each milestone are not included.
[p]The duration in patients in whom tube feeding could be discontinued.

accompanied by two copies of functional *RTL1* and no functional *RTL1as*.[6] This implies that the *RTL1* expression level is ~2.5 times increased in the absence of functional *RTL1as*-encoded *microRNAs*.

Here, we report comprehensive clinical findings in a series of patients with molecularly confirmed UPD(14)pat and related conditions, and suggest pathognomonic and/or characteristic features and their underlying factors. We also propose the name 'Kagami–Ogata syndrome' for UPD(14)pat and related conditions.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Institute Review Board Committee at the National Center for Child Health and Development, and performed after obtaining written informed consent to publish the clinical and molecular information. We also obtained written informed consent with parental signature to publish facial photographs.

Patients

This study consisted of 33 Japanese patients and one Irish patient (patient #32) with UPD(14)pat and related conditions (13 males and 21 females; 31 patients with normal karyotypes and two patients (#17 and #20) with Robertsonian translocations involving chromosome 14 (karyotyping not performed in patient #1); 30 previously described patients[2,3,7–10] and four new patients) in whom underlying (epi)genetic causes were clarified and detailed clinical findings were obtained (Supplementary Table S2).

The 34 patients were classified into three groups according to the underlying (epi)genetic causes that were determined by methylation analysis for the two DMRs, microsatellite analysis for a total of 24 loci widely dispersed on chromosome 14, fluorescence *in situ* hybridization for the two DMRs, and oligonucleotide array-based comparative genomic hybridization for the 14q32.2 imprinted region, as reported previously:[9] (1) 23 patients with UPD(14)pat (UPD-group); (2) five patients with epimutations (Epi-group); and (3) six patients with microdeletions (Del-group) (Supplementary Figure S2).

Furthermore, the 23 patients of UPD-group were divided into three subtypes in terms of UPD generation mechanisms by microsatellite analysis, as reported previously:[9] (1) 13 patients with monosomy rescue (MR) or postfertilization mitotic error (PE)-mediated UPD(14)pat indicated by full isodisomy (subtype 1) (UPD-S1); (2) a single patient with PE-mediated UPD(14)pat demonstrated by segmental isodisomy (subtype 2) (UPD-S2); and (3) nine patients with trisomy rescue (TR) or gamete complementation (GC)-mediated UPD(14)pat revealed by heterodisomy for at least one locus (subtype 3) (UPD-S3) (Supplementary Figure S2) (it is possible that some patients classified as UPD-S1 may have a cryptic heterodisomic region(s) and actually belong to UPD-S3). Similarly, the six patients of Del-group were divided into three subtypes in terms of the measured/predicted *RTL1* expression level in the body and placenta:[2,3] (1) three patients with ~5 times *RTL1* expression level in both the body and placenta (subtype 1) (Del-S1); (2) a single patient with about five times *RTL1* expression level in the body and normal (1 time) or ~2.5 times *RTL1* expression level in the placenta (subtype 2) (Del-S2); and (3) two patients with ~2.5 times *RTL1* expression level in both the body and placenta (subtype 3) (Del-S3) (Supplementary Figure S2). The measured/predicted expression patterns of the imprinted genes in each group/subtype are illustrated in Supplementary Figure S1.

Clinical studies

We used a comprehensive questionnaire to collect detailed clinical data of all patients from attending physicians. To evaluate chest roentgenographic findings, we obtained the coat-hanger angle (CHA) to the ribs and the ratio of the mid to widest thorax diameter (M/W ratio), as reported previously.[11] We also asked the physicians to report any clinical findings not covered by the questionnaire.

Statistical analysis

Statistical significance of the median among three groups and between two groups/subtypes was examined by the Kruskal–Wallis test and the Mann–Whitney's *U*-test, respectively, and that of the frequency among three groups and between two groups was analyzed by the Fisher's exact probability test, using the R environment (http://cran.r-project.org/bin/windows/base/old/2.15.1/). $P<0.05$ was considered significant. Kaplan–Meier survival curves were constructed using the R environment.

RESULTS

Clinical findings of each group/subtype are summarized in Table 1, and those of each patient are shown in Supplementary Table S2. Phenotypic findings were comparable among UPD-S1, UPD-S2, and UPD-S3, and somewhat different among Del-S1, Del-S2, and Del-S3, as predicted from the expression patterns of the imprinted genes (Supplementary Figure S1). Thus, we showed the data of UPD-group (the sum of UPD-S1, UPD-S2, and UPD-S3) and those of each subtype of Del-group (Del-S1, Del-S2, and Del-S3) in Table 1, and described the data of UPD-S1, UPD-S2, and UPD-S3 in Supplementary Table S3.

We registered the clinical information of each patient in the Leiden Open Variation Database (LOVD) (http://www.lovd.nl/3.0/home; http://databases.lovd.nl/shared/individuals), and the details of each microdeletion in the ClinVar Database (http://www.ncbi.nlm.nih.gov/clinvar/). The LOVD Individual IDs and the ClinVar SCV accession numbers are shown in Supplementary Table S2.

Pregnancy and delivery

Polyhydramnios was observed from ~25 weeks of gestation during the pregnancies of all patients, except for patient #32 of Del-S2 who had deletion of the *MEG3*-DMR and three of the seven *MEG3* exons, and usually required repeated amnioreduction, especially after 30 weeks of gestation. Placentomegaly was usually identified in patients affected with polyhydramnios, but not found in three patients of UPD-group. Thoracic and abdominal abnormalities were found by ultrasound studies in ~40% of patients from ~25 weeks of gestation, and UPD(14)pat was suspected in patients #7 and #21, due to delineation of the bell-shaped thorax with coat-hanger appearance of the ribs. Premature delivery was frequently observed, especially in Del-group. Because of fetal distress and polyhydramnios, ⩾two-thirds of the patients in each group were delivered by Cesarean section. Medically assisted reproduction was reported only in one (patient #8) of 21 patients for whom clinical records on conception were available.

Growth pattern

Prenatal growth was characterized by grossly normal birth length and obviously excessive birth weight. Indeed, birth length ranged from 30.6 to 51.0 cm (−1.7 to +3.0 SD for the gestational age- and sex-matched Japanese reference data) with a median of 44.7 cm (±0 SD), and birth weight ranged from 1.24 to 3.77 kg (+0.1 to +8.8 SD) with a median of 2.79 kg (+2.3 SD). Although birth weight was disproportionately greater than birth length, there was no generalized edema as a possible cause of overweight.

In contrast, postnatal growth was rather compromised, and growth failure (present length/height and/or weight < −2 SD) was observed in about one-third of patients of each group. Postnatal weight was better preserved than postnatal length/height.

Craniofaciocervical features

All patients exhibited strikingly similar craniofaciocervical features (Figure 1). Indeed, >90% of patients had depressed nasal bridge, full cheeks, protruding philtrum, micrognathia, and short webbed neck. In particular, the facial features with full cheeks and protruding philtrum