

## SHORT COMMUNICATION

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

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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.

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## INTRODUCTION

Silver–Russell syndrome (SRS) is a congenital developmental disorder characterized by pre- and postnatal growth failure, relative macrocephaly, hemihypotrophy and fifth-finger clinodactyly.<sup>1</sup> 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.<sup>1</sup> 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.<sup>1</sup>

*CDKN1C* (cyclin-dependent kinase inhibitor 1C) is a maternally expressed gene that resides at the ICR2 just proximal to the ICR1.<sup>2</sup> *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.<sup>2,3</sup> 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,<sup>2</sup> whereas less severe gain-of-function mutations of *CDKN1C* have been identified in

a large family with maternally inherited SRS.<sup>4</sup> Thus, it has been suggested that relatively severe and mild *CDKN1C* gain-of-function effects lead to IMAGEs and SRS, respectively.<sup>4,5</sup> Notably, IMAGEs patients satisfy the diagnostic criteria for SRS proposed by Netchine *et al.*<sup>5,6</sup> and IMAGEs and SRS patients with *CDKN1C* mutations invariably lack hemihypotrophy characteristic of SRS.<sup>4–6</sup>

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

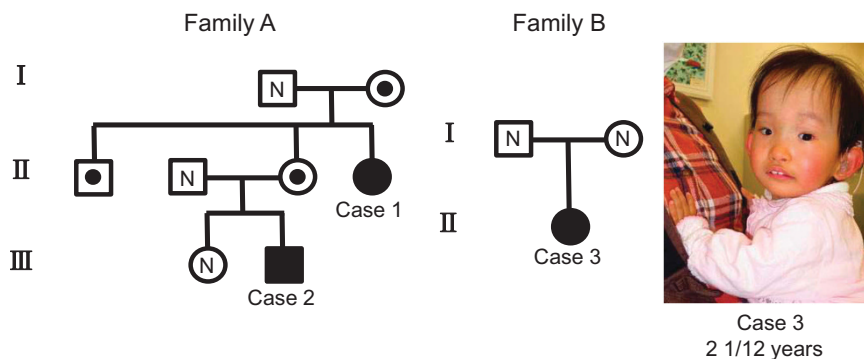
We studied three Japanese patients (cases 1–3) from two families (Figure 1). Cases 1–3 satisfied the SRS diagnostic criteria proposed by Netchine *et al.*,<sup>6</sup> although they lacked hemihypotrophy (Table 1, see its footnote for Netchine SRS criteria). Oligohydramnios characteristic of SRS<sup>7</sup> 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.

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**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) <sup>1–19</sup>
<i>SRS phenotype</i>				
Mandatory criteria for SRS				
BL and/or BW $\leq -2$ SDS	+	+	+	16/16
Scoring system criteria for SRS				
Relative macrocephaly at birth <sup>a</sup>	Unknown	+	+	11/11
PH $\leq -2$ SDS at $\geq 2$ 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. <sup>b</sup>
BW, kg (SDS)	1.3 (–5.3)	0.87 (–3.6)	0.82 (–3.7)	N.D. <sup>b</sup>
BOFC, cm (SDS)	29.5 (–2.7)	28.3 (–0.6)	27 (–1.2)	N.D. <sup>b</sup>
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. <sup>b</sup>
PW, kg (SDS)	37.5 (–1.2)	4.8 (–5.3)	6.8 (–4.0)	N.D. <sup>b</sup>
BMI, kg m <sup>–2</sup> (SDS)	22.0 (0.7)	13.1 (–2.8)	13.6 (–1.5)	N.D. <sup>b</sup>
POFC, cm (SDS)	Unknown	45.0 (–0.5)	48.5 (–0.1)	N.D. <sup>b</sup>
Relative macrocephaly at present <sup>c</sup>	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
<i>IMAGe syndrome phenotype</i>				
IUGR	+	+	+	16/16
Metaphyseal dysplasia	–	–	–	Not described
Adrenal hypoplasia	–	– <sup>d</sup>	– <sup>e</sup>	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.*<sup>5</sup>)

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.

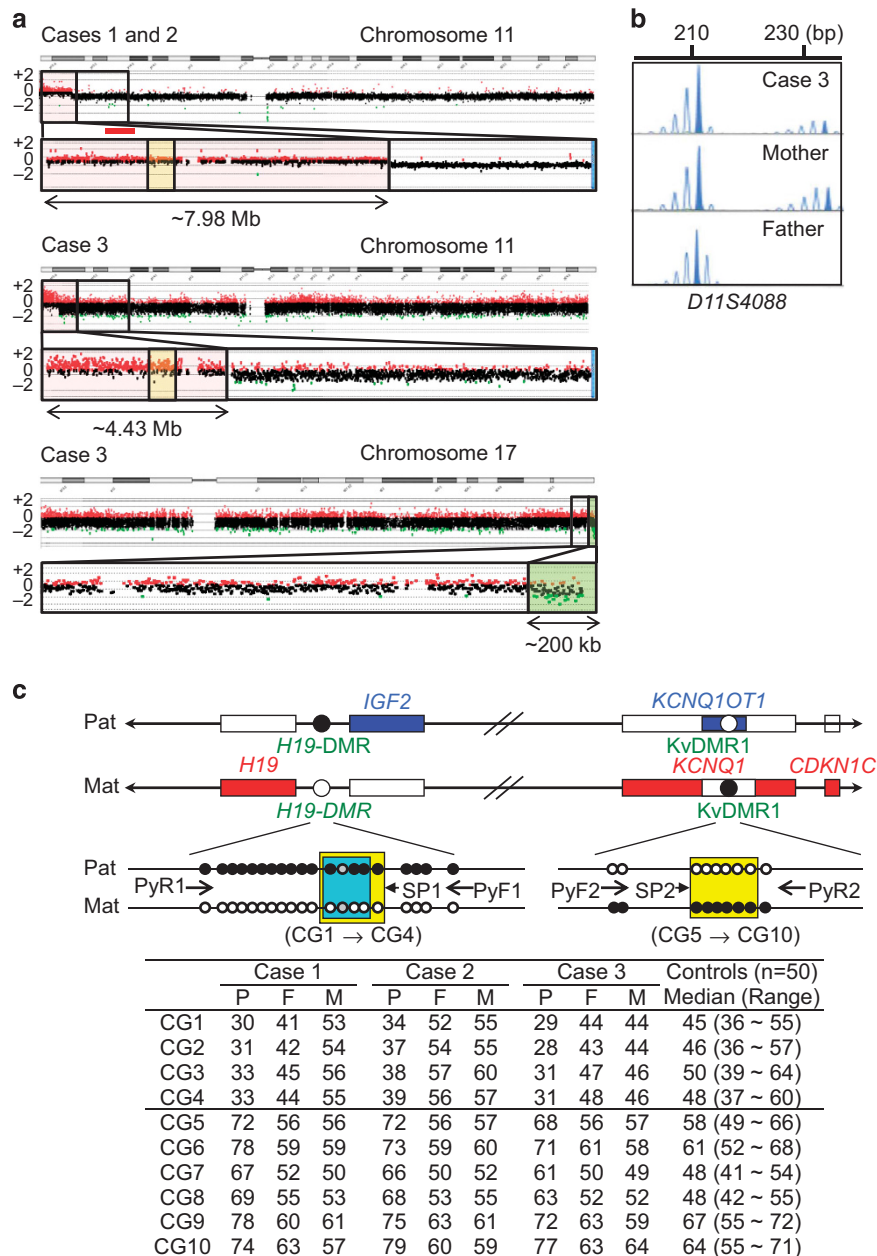
<sup>a</sup>BL or BW (SDS)—BOFC (SDS)  $\leq -1.5$ .

<sup>b</sup>N.D. because of various ethnicities of affected individuals and descriptions of height assessment (percentile and SDS).

<sup>c</sup>PH or PW (SDS)—POFC (SDS)  $\leq -1.5$ .

<sup>d</sup>A rapid adrenocorticotropic stimulation test (0.25 mg m<sup>–2</sup> bolus i.v.; blood sampling at 0 and 60 min) showed a sufficient cortisol response (14.2  $\rightarrow$  26.2  $\mu$ g dl<sup>–1</sup>) (reference range  $>20$   $\mu$ g dl<sup>–1</sup>).

<sup>e</sup>A growth hormone releasing peptide 2 stimulation test (2  $\mu$ g kg<sup>–1</sup> bolus i.v.; blood sampling at 0, 15, 30, 45 and 60 min) yielded a sufficient cortisol response (18.4  $\rightarrow$  25.5  $\mu$ g dl<sup>–1</sup>).



**Figure 2** Representative molecular findings. (a) Array comparative genomic hybridization analysis. The black, red and green dots represent signals indicative of the normal, increased ( $\log_2$  signal ratio  $> +0.5$ ) and decreased ( $\log_2$  signal ratio  $< -0.8$ ) copy numbers, respectively. The  $\log_2$  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.<sup>7</sup> 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<sup>8</sup> and *CDKN1C* gain-of-function mutations have been identified in SRS and IMAGEs,<sup>2,4,5</sup> whereas neither *H19* nor *KCNQ1* appears to have a positive role in growth regulation. Indeed, *H19* is regarded as a possible tumor suppressor gene<sup>9</sup> and *KCNQ1* encoding a voltage-gated potassium channel is involved in cardiac arrhythmias.<sup>10</sup> 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).<sup>11–19</sup> Notably, although they frequently show SRS-like phenotype, hemihypotrophy (body asymmetry) has been found only in a single case<sup>12</sup> 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.

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