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

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ABSTRACT

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.

INTRODUCTION

Silver-Russell syndrome (SRS) is a congenital developmental disorder characterized by pre- and post-natal 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. 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.

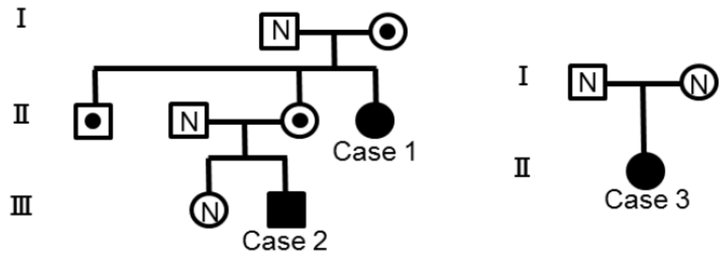
CDKN1C (cyclin-dependent kinase inhibitor 1C) is a maternally expressed gene that resides at the ICR2 just proximal to the ICR1. *CDKN1C* encodes a negative regulator for cell proliferation and, consistent with this, loss-of-function mutations of *CDKN1C* cause Beckwith-Wiedemann syndrome associated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

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. Cases 1–3 satisfied the SRS diagnostic criteria proposed by Netchine et al., although they lacked hemihypotrophy. Oligohydramnios characteristic of SRS 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.

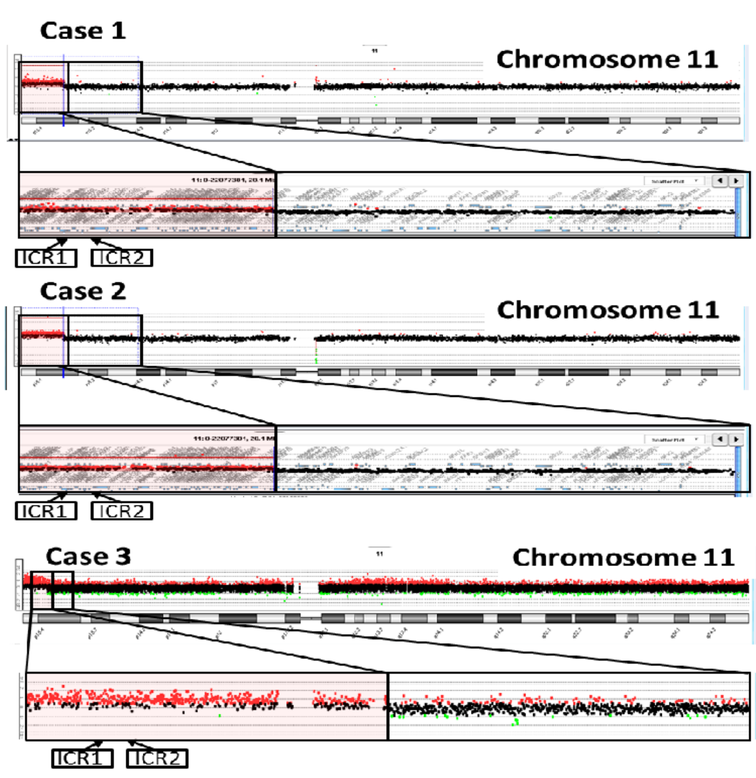


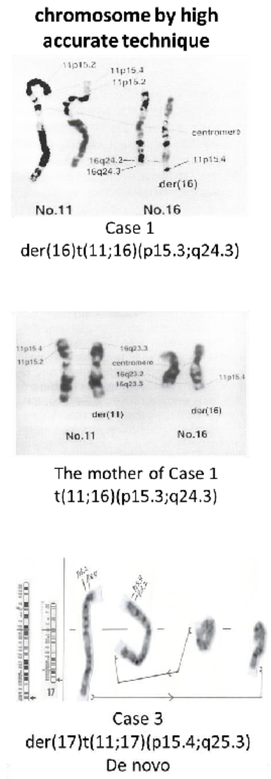
Clinical features of case 1–3 and reported cases with duplications of maternally derived chromosome 11p15 involving *CDKN1C*

	Case 1 Family A Female	Case 2 Family A Male	Case 3 Family B Female	Reported cases (n=16) Ref. 11–19
Silver-Russell syndrome 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.
BW kg (SDS)	1.3 (–5.3)	0.87 (–3.6)	0.82 (–3.7)	N.D.
BOFC cm (SDS)	29.5 (–2.7)	28.3 (–0.6)	27 (–1.2)	N.D.
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.
PW kg (SDS)	37.5 (–1.2)	4.8 (–5.3)	6.8 (–4.0)	N.D.
BMI (kg/m^2) (SDS)	22.0 (0.7)	13.1 (–2.8)	13.6 (–1.5)	N.D.
POFC cm (SDS)	Unknown	45.0 (–0.5)	48.5 (–0.1)	N.D.
Relative macrocephaly at present[b]	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	–	–	–	Not described
Genital abnormality	(Female)	–	(Female)	Not described

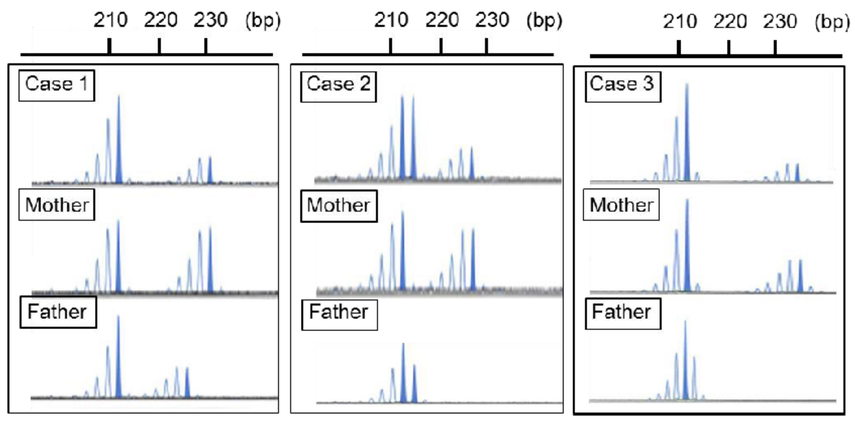
Cytogenetic and molecular studies

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. Then, genomewide oligonucleotide-based array comparative genomic hybridization was carried out using a catalog human array (2×400K format, ID G4448A), 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). 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.

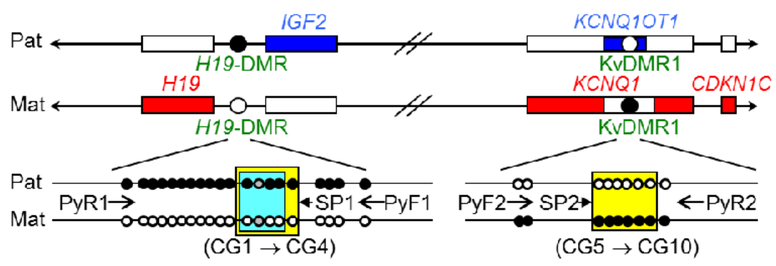




Microsatellite analysis was carried out on the duplicated chromosome 11p, showing the presence of two alleles of maternal origin and a single allele of paternal origin in cases 1–3



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. 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.



	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)

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, and *CDKN1C* gain-of-function mutations have been identified in SRS and IMAGeS, whereas neither *H19* nor *KCNQ1* appears to play a positive role in growth regulation. Indeed, *H19* is regarded as a possible tumor suppressor gene, and *KCNQ1* encoding a voltage-gated potassium channel is involved in cardiac arrhythmias. 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*.

An extra copy of maternally derived chromosome 11p15 involving *CDKN1C* has been identified in 16 patients. Notably, while they frequently show SRS-like phenotype, hemihypotrophy (body asymmetry) has been found only in a single case, 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.

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Growth

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