Microduplication of the ICR2 Domain at Chromosome 11p15 and Familial Silver–Russell Syndrome

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Silver–Russell syndrome (SRS) is characterized by severe intrauterine and postnatal growth retardation in association with a typical small triangular face and other variable features. Genetic and epigenetic disturbances are detected in about 50% of the patients. Most frequently, SRS is caused by altered gene expression on chromosome 11p15 due to hypomethylation of the telomeric imprinting center (ICR1) that is present in at least 40% of the patients. Maternally inherited duplications encompassing ICR1 and ICR2 domains at 11p15 were found in a few patients, and a microduplication restricted to ICR2 was described in a single SRS child. We report on a microduplication of the ICR2 domain encompassing the *KCNQ1*, *KCNQ1OT1*, and *CDKN1C* genes in a three-generation family: there were four instances of paternal transmissions of the microduplication from a single male uniformly resulting in normal offspring, and five maternal transmissions, via two clinically normal sisters, with all the children exhibiting SRS. This report provides confirmatory evidence that a microduplication restricted to the ICR2 domain results in SRS when maternally transmitted.
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INTRODUCTION

Silver–Russell syndrome (SRS; OMIM180860) is clinically characterized by severe intrauterine and postnatal growth retardation and a typical small triangular face. Other frequent features include relative macrocephaly, body asymmetry, and clinodactyly of the fifth fingers. Most cases are sporadic. Imprinted regions on chromosomes 7 and 11 are implicated in the etiology of SRS: altered methylation at 11p15 is the most frequent cause of SRS, being present in at least 40% of patients [Gicquel et al., 2005; Eggermann et al., 2006; Netchine et al., 2007; Eggermann et al., 2008a; Bartholdi et al., 2009], and maternal uniparental disomy of chromosome 7 (mUPD7) is identified in 5–10% of patients [Kotzot et al., 1995; Netchine et al., 2007; Bartholdi et al., 2009].

Human chromosome 11p15 contains a cluster of genes that undergoes parent-of-origin imprinting and plays a crucial role in fetal growth. This cluster includes paternally (*IGF2* and *KCNQ1OT1*) and maternally (*H19*, *KCNQ1*, and *CDKN1C*) expressed genes that are regulated by two imprinting center regions: the more telomeric ICR1 (or H19DMR) controls *H19* and *IGF2* expressions, while the more centromeric ICR2 (or KvDMR1) controls *KCNQ1*, *KCNQ1OT1*, and *CDKN1C* expressions. In normal individuals, the ICR1 is methylated on the paternally derived chromosome resulting in *IGF2* expression, and suppression of *H19*, while on the maternally derived chromosome ICR2 is methylated and results in suppression of *KCNQ1OT1* and expression of *KCNQ1* and *CDKN1C*; gene expression is the reverse on the nonmethylated ICR1 and ICR2, on the maternally and paternally inherited chromosomes, respectively [Reik and Walter, 2001].

Although hypomethylation of the paternal ICR1 is the most frequent alteration in SRS, maternal UPD(11) may occur, thus altering the expression of genes controlled by ICR1 and ICR2 [Bullman et al., 2008]. Further, maternal 11p15 duplications, ranging from 4 to about 14 Mb and encompassing the ICR1 and ICR2 domains were reported in at least eight SRS patients [Fisher et al., 2002; Eggermann et al., 2005; South et al., 2008; Bliek et al.,

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2009; Eggermann et al., 2010]. The finding, in a SRS patient, of a 0.76–1.0 Mb maternally inherited duplication encompassing the ICR2 domain, but not the ICR1, showed that SRS could result from the altered expression of genes controlled by ICR2 [Schönherr et al., 2007]. Opposite (epi)mutations —ICR1 hypermethylation and paternal 11p15 duplications—are present in the overgrowth Beckwith–Wiedemann syndrome (BWS; MIM130650); ICR2 hypomethylation accounts for about 50% of BWS, and other alterations include paternal 11p15 UPD and mutations in the *CDKN1C* gene [Weksberg et al., 2003; Eggermann et al., 2008b].

We describe an ICR2 microduplication that segregates in a three-generation Brazilian family associated with SRS in five children born to two clinically normal sisters, but without overt clinical effect when paternally inherited. These findings provide confirmatory evidence that duplication of just the ICR2 domain is causally associated with SRS when maternally transmitted.

PATIENTS

Five children (III-7-9, 11, and 12, Fig. 1), born to clinically normal sisters (II-4 and 5) presented with clinical features that led to the diagnosis of SRS. The proband (III-7) was born to nonconsanguineous parents, after a pregnancy complicated by oligohydramnios and intrauterine growth restriction (IUGR). He was born by cesarean, at term, with a weight of 1,340 g (<3rd centile) and a length of 38 cm (<3rd centile). His neuromotor development was normal. At the age of 2 11/12 years, he was referred to a genetics clinic due to short stature. He had a small triangular face and clinodactyly of the fifth fingers. Hand X-rays showed delayed bone age. A head MRI and an ophthalmologic evaluation revealed no abnormalities. His G-banded karyotype was normal. Human growth hormone (HGH) therapy was started at age 5 years. At the age of 17 years, still under hormone therapy, his height was 152 cm (<3rd centile), weight, 42.25 kg (<3rd centile), and occipitofrontal head circumference, 53.5 cm (2nd centile).

The proband's sister III-8 presented with IUGR. She was born by cesarean, at term, weighing 1,600 g (<3rd centile) and measuring 42 cm (<3rd centile). Her neuromotor development was normal, but learning disabilities were reported. Clinical examination at the age of 1 5/12 years showed short stature, a triangular face, and a hypochromic macule on the dorsal region. Hand X-rays revealed delayed bone age, and hepatic calcifications were detected by US. Her karyotype was normal. At 14 2/12 years of age, her height was 147.5 cm (<3rd centile), weight 33.2 kg (<3rd centile), and occipitofrontal head circumference 53.5 cm (50th centile). She had undergone HGH therapy for 6 months starting at 8 years of age, but it was discontinued due to complaints of headache and abdominal pain.

The proband's brother III-9 presented IUGR; he was born by cesarean, after a 37-week gestation; his weight was 1,700 g, length 39 cm, and occipitofrontal head circumference 32 cm. At 9 months of age, he had meningitis. He developed with speech and hearing difficulties, which might have been post-meningitis sequelae. At the age of 11 years, his height was 120 cm (<3rd centile), weight 35.3 kg (25th–50th centiles), and occipitofrontal head circumference 54 cm (50th centile). He had a triangular face and slight clinodactyly of the fifth fingers.

The proband's youngest sister (III-10) had insulin-dependent infantile diabetes, with onset at the age of 6 years. She did not present with clinical signs of SRS.

The proband's maternal aunt II-5 had two daughters with the diagnosis of SRS. The oldest girl (III-11) was born after a 30-week pregnancy weighing 790 g. Her neuromotor development was normal. At 6 8/12 years of age, her weight was 17.4 kg (3rd–5th centiles) and height 106 cm (<3rd centile); she had a triangular face. Her younger sister III-12 presented IUGR detected at 20 weeks of
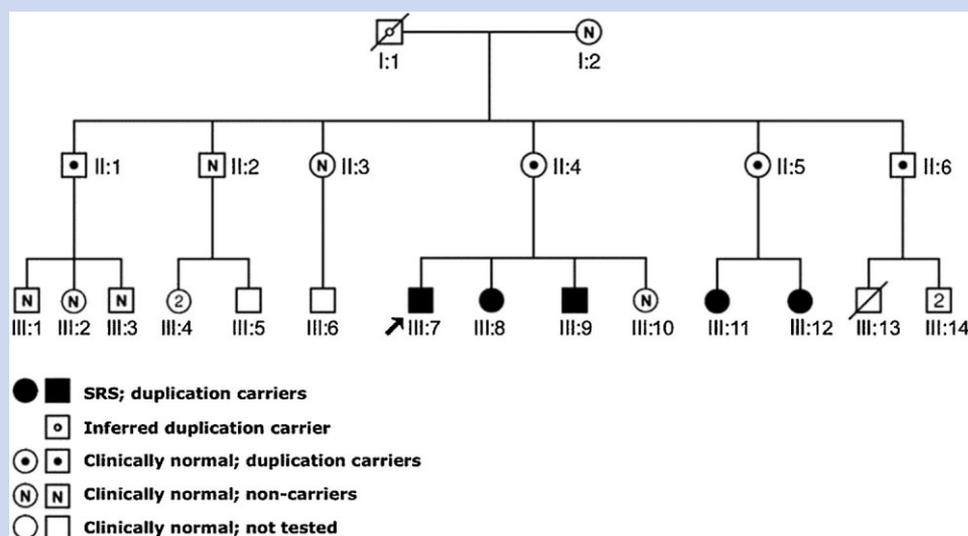

FIG. 1. Segregation of the ICR2 microduplication at 11p15 in a family with five children affected by SRS. When maternally inherited, the microduplication is associated with SRS.

gestation. She was born after a 36-week pregnancy, by cesarean; her birth weight was 1,710 g and length 39 cm. At 12 months of age, her weight was 5.5 kg (<3rd centile), length 61 cm (<3rd centile), and occipitofrontal head circumference 44 cm (25th centile). She had a triangular face, and clinodactyly of fifth fingers. Her neuromotor development was normal.

The maternal grandfather of the affected children, deceased at 64 years of age due to cholecystitis, was reported to have low stature (148 cm; <3rd centile). No information about his siblings was available.

Peripheral blood was obtained from patients and their parents for SRS diagnostic tests. The detection of an ICR2 microduplication in the patients led to the examination of clinically normal members of the family (I-2, II-1-3, 6, and III-1-3, 10) (Fig. 1). The study was approved by the Institutional Ethics Committee (CEP-IBUSP 098/2009), and informed consent was obtained from family members or their legal guardians for the publication of data.

METHODS

DNA was extracted from Peripheral Blood Leucocytes.

Maternal UPD(7) Testing

Maternal UPD(7) was excluded in the proband and in his maternal cousin III-11, by genotyping four microsatellite markers D7S519 (7p13), D7S506 (7p12.1), D7S1870 (7q11.23), and D7S486 (7q31.2).

Methylation-Specific MLPA Analysis

DNA copy number changes and the methylation pattern on chromosome 11p15 were investigated in 16 members of the family by MS-MLPA (Methylation-Specific Multiplex Ligation-dependent Probe Amplification), using SALSA probe-mix ME030 BWS/RSS (2007) or ME030-B1 BWS/RSS (2008) (MRC Holland, Amsterdam/NL). These kits include, respectively, 27 and 26 probes specific for the ICR1 and ICR2 domains, 14 of which contain methylation-sensitive *Hha I* restriction sites. The procedures followed the manufacturer's instructions. Data analysis was performed using the software 'Fragment Profiler' (Amersham Biosciences) and a modified Excel spreadsheet (kindly supplied by T. Eggermann) [Eggermann et al., 2008a].

Array-CGH Analysis

For further mapping of the duplication detected by MLPA, copy number variation of DNA segments (CNV) was investigated in individual III-9, using the SurePrint G3 Human CGH Microarray kit 180 K (Agilent Technologies, Santa Clara, CA). In brief, 0.8 μg of genomic DNA was labeled with Cy3 and Cy5-dUTPs by random priming; purification, hybridization, and washing were carried out as recommended by the manufacturer. Scanned array images were processed and analyzed using the software package Feature Extraction and Agilent Genomic Workbench (both from Agilent Technologies). The statistical algorithm ADM-2 was used with a 6.7 sensitivity threshold, and the minimum number of consecutive aberrant probes was set at 3 to call an abnormality. Accordingly, the minimum size for CNV detection was ∼35–55 kb. The Genome browsers UCSC (http://genome.ucsc.edu) and Ensembl (http://www.ensembl.org) were used to assess the known genes mapped to the duplicated segment.

RESULTS

The MS-MLPA analysis of the ICR domains at 11p15 (genes *H19*, *IGF2*, *KCNQ1*, *KCNQ1OT1*, and *CDKN1C*) revealed a microduplication encompassing the *KCNQ1*, *KCNQ1OT1*, and *CDKN1C* genes (Fig. 2a) in the SRS affected children (III-7-9, 11, and 12, Fig. 1). The *IGF2* and *H19* probes were not duplicated. This microduplication was also detected in the clinically normal mothers (II-4 and 5) and two maternal uncles (II-1 and 6) of the SRS children. Seven of their clinically normal relatives who were examined did not carry the microduplication: the maternal grandmother (I-2), a maternal uncle (II-2), a maternal aunt (II-3), three maternal cousins (III-1-3), and one sibling (III-10). These findings allowed inferring that the deceased grandfather was a carrier of the microduplication. The enhanced methylation of the ICR2 domain observed in the affected children was compatible with a maternal duplication; the ICR1 was not hypomethylated (data not shown).

Array-CGH showed that the microduplication spanned approximately 1.61 Mb (Fig. 2b). The proximal breakpoint was mapped to a nearly 12.5 kb segment between genomic positions chr11:3796585–3809132 (UCSC assembly March 2006, NCBI36/hg18). The location of the distal breakpoint was within a segment of about 10.5 kb between genomic positions chr11:2196379–2206866.

DISCUSSION

We report a maternally inherited microduplication at chromosome 11p15 encompassing the ICR2 domain, in five children affected by SRS, two boys and three girls, born to two clinically normal sisters. This microduplication segregated in a three-generation family, and was associated with SRS whenever maternally transmitted. It was also present in four out of six phenotypically normal sibs in generation II, and since their mother did not carry the duplication, it must have been inherited from their father who was already deceased at the time of the investigation.

The microduplication spans approximately 1.61 Mb and encompasses all known imprinted genes at the ICR2 domain, the maternally expressed *KCNQ1*, *KCNQ1DN*, *CDKN1C*, *SLC22A18* (*TSSC5*), *SLC22A18AS*, and *PHLDA2* (*TSSC3*) genes and the paternally expressed *TRPM5* and *KCNQ1OT1* genes. A maternally inherited microduplication also restricted to the ICR2 domain and associated with SRS in a boy was described previously [Schönherr et al., 2007]. The duplicated segment was 0.76–1.0 Mb long, and encompassed the same imprinted genes as the duplication described herein. Among the duplicated genes, *CDKN1C* is a likely candidate for the SRS phenotype, as suggested previously [Schönherr et al., 2007; Bliek et al., 2009]. *CDKN1C* encodes a cyclin-dependent kinase inhibitor that negatively regulates cell proliferation and growth, and plays a crucial role in human fetal development; its overexpression in mice was shown to arrest cells in G1 [Lee et al., 1995].

FIG. 2. Microduplication at 11p15. A: MLPA analysis: probe dosage in III-10 (gray bars) revealed normal DNA copy numbers; in II-4 (black bars), values higher than 1.3 indicated duplication of *KCNQ1* and *CDKN1C* genes; *KCNQ1OT1* is contained in *KCNQ1* tested segment, and consequently is also duplicated. **B:** Mapping of the microduplication on a 180 K oligonucleotide array: the ~1.61 Mb microduplication at 11p15, in III-9, is represented by the black horizontal line under the idiogram; the previously reported 0.76–1.0 Mb microduplication restricted to the ICR2 [Schönherr et al., 2007] is shown for comparison (gray horizontal line); the dotted black and gray lines represent the possibly duplicated segments. Known genes mapped to the region are shown at the bottom: the genes represented by black bars are active on the maternal chromosome, and those represented by gray bars are active on the paternal chromosome.

SRS patients that inherited larger maternal duplications at 11p15 have been described [Fisher et al., 2002; Eggermann et al., 2005; South et al., 2008; Bliek et al., 2009; Eggermann et al., 2010], and they had two maternal copies of both the ICR1 and ICR2 domains. As in our patients, the overexpression of *CDKN1C* could explain their phenotype. On the other hand, the *IGF2* gene at ICR1, which codes for an insulin-like embryonic growth factor, although duplicated in these patients, should not have an altered expression, since it is inactive on the maternally inherited chromosome.

Paternally-inherited 11p15 duplications are causative of BWS due to the presence of two active *IGF2* alleles [Weksberg et al., 2003]. In the family here reported, individuals carrying a paternally inherited duplication were clinically normal. Since their duplications did not encompass the ICR1 domain, the paternal *IGF2* allele should be normally expressed, and therefore no BWS features would be expected. Being inactive on the paternal chromosome, the duplicated *CDKN1C* alleles would not enhance negative growth regulation, and SRS features would not be present.

One of the common features among SRS patients with ICR1 hypomethylation is body asymmetry [Eggermann et al., 2006; Netchine et al., 2007; Bruce et al., 2009], which has been ascribed to mosaicism for the hypomethylation epimutation present in the majority of patients [Gicquel et al., 2005; Eggermann et al., 2008b]. In line with this conclusion is the absence of body asymmetry in our patients and others [Fisher et al., 2002; Eggermann et al., 2005; Schönherr et al., 2007; South et al., 2008; Bliek et al., 2009; Eggermann et al., 2010], who carry maternally inherited nonmosaic duplications.

It is of interest that in familial SRS, a chromosomal imbalance should be suspected, since the other mechanisms leading to SRS, epimutations, and uniparental disomy, usually represent de novo mutational events.

This report provides confirmation that maternal duplication of the ICR2 domain is causally associated with SRS. Overexpression of the *CDKN1C* gene appears as a candidate for the phenotype through its negative effect on growth.

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