

Silver–Russell Syndrome Due to Paternal *H19/IGF2* Hypomethylation in a Twin Girl Born After In Vitro Fertilization

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Silver–Russell syndrome (SRS) is a clinically and genetically heterogeneous syndrome characterized by severe intrauterine and postnatal growth retardation, facial dysmorphism and body asymmetry. One of the main molecular mechanisms leading to the syndrome involves methylation abnormalities of chromosome 11p15. In the last decades, an increase of imprinting disorders have been reported in children born from assisted reproductive technology (ART); however there is currently little evidence linking SRS and ART. Only few infants with SRS born using ART, supported by molecular analysis, have been described. We report on a twin-girl conceived using intracytoplasmic sperm injection (ICSI) diagnosed with SRS. Molecular studies revealed a hypomethylation of the paternal *H19/IGF2* Imprinting Control Region. Her twin sister had a normal prenatal and postnatal growth and a normal methylation pattern of the chromosome 11p15. This is the second reported case of a twin infant with SRS conceived using ART with hypomethylation of *H19/IGF2*; it provides additional evidence of a possible relationship between ART procedures and methylation defects observed in SRS. Given the clinical heterogeneity of SRS, and the increased risk of multiple and preterm births in the ART-conceived children, it is possible that a number of cases of SRS remains undiagnosed in this population. Future studies should investigate the possible link between ART and SRS, in order to better understand the causes of epimutations in ART pregnancies, and to help clinicians to adequately counsel parents who approach to ART and to assess the opportunity of a long-term follow-up of children conceived using ART. © 2013 Wiley Periodicals, Inc.

Key words: Silver–Russell syndrome; in vitro fertilization; imprinting disorders

INTRODUCTION

Silver–Russell syndrome (SRS) is a heterogeneous syndrome mainly characterized by severe intrauterine and postnatal growth retardation (<3rd centile); additional features include a triangular facial appearance with a prominent forehead, body asymmetry and fifth finger clinodactyly [Price et al., 1999].

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Most cases of SRS and SRS-like phenotype are sporadic, but some families with apparent autosomal dominant inheritance, as well as with autosomal recessive and X-linked inheritance have been reported. The features associated with the syndrome have been described in association with many genetic abnormalities; however the main molecular mechanisms which seem to be involved are the maternal uniparental disomy for chromosome 7 (mUPD 7) and methylation abnormalities of chromosome 11p15 [Gicquel et al., 2005; Abu-Amro et al., 2008]. DNA methylation, together with histone modifications, are major mechanisms for genetic imprinting, which is a dynamic process of chemical modifications of nucleotides in which only one allele of specific genes is functioning and the other allele is silenced based on the parent-of-origin.

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Abbreviations: ART, assisted reproductive technology; GA, gestational age; ICSI, intracytoplasmic sperm injection; IRC, Imprinting Control Region; IVF, in vitro fertilization; mUPD 7, maternal uniparental disomy for chromosome 7; OFC, occipital–frontal circumference; SRS, Silver–Russell syndrome.

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In the last decades, an increase of imprinting-associated syndromes has been reported in infants born from assisted reproductive technology (ART). Evidence of an association with ART has been shown for Beckwith–Wiedemann syndrome, Angelman syndrome and syndrome of hypomethylation at multiple loci [Rossignol et al., 2006; Boonen et al., 2008; Manipalviratn et al., 2009]. However a number of infants with other imprinting-related conditions, such as SRS, retinoblastoma, Prader–Willi syndrome, conceived with ART has been reported [Amor and Halliday, 2008].

To our knowledge, to date, 12 cases of SRS following in vitro fertilization have been described (Table I) [Svensson et al., 2005; Bliiek et al., 2006; Kagami et al., 2007; Galli-Tsinopoulou et al., 2008; Douzgou et al., 2008; Chopra et al., 2012; Hiura et al., 2012]. We report a twin girl conceived with use of ART with SRS syndrome and loss of methylation at the paternal *H19/IGF2* locus.

CLINICAL REPORT

The twin girls were conceived with use of intracytoplasmic sperm injection (ICSI) and delivered by selective cesarean section at

35 weeks and 3/7 of gestational age (GA). Maternal oocytes were taken after gonadotropin stimulation; paternal sperm was collected using a condom. The parents, with negative family and personal history, were not consanguineous; the father's height was cm 170, and the mother's height was 148 cm. Short height was reported on the mother's side.

A growth discrepancy between the twins was noted since the 29th week of GA at ultrasound scan, with one fetus growing above the 5th centile and the other growing on the 50th centile.

At birth, the neonatal weight of the proband was 1,380 g (<3rd centile), the occipital–frontal circumference (OFC) was 32 cm (10th–50th centile) and the length was 34 cm (<3rd centile). The weight of the twin was 2,400 g (≈50th centile), the OFC was 33 cm (50th–75th centile) and the length was 49 cm (75th–90th centile).

In the postnatal period the infant presented with feeding problems, which resolved during the first month of life. Physical examination of the infant showed: small and triangular face, prominent forehead, micrognathia, downturned mouth, thin lips, left hemihypotrophy of the lower limbs, clinodactyly and brachydactyly of the fifth finger of the hands. These findings

TABLE I. Reported Cases of Silver–Russell Syndrome Following In Vitro Fertilization

Patient	Gender	Pregnancy	Conception	Phenotype	Molecular data
Svensson et al. [2005] – Patient 1	F	Singleton	IVF/ICSI	Classic SRS	Not available
Svensson et al. [2005]—Patient 2	F	Singleton	IVF/ICSI	Classic SRS	Not available
Bliiek et al. [2006]	F	Singleton	IVF/ICSI	SRS-like phenotype, pre and postnatal growth failure	Hypomethylation of paternally derived <i>H19/IGF2</i>
Kagami et al. [2007]	F	Twin	IVF	Classic SRS	Partial hypermethylation of <i>MEST</i>
Galli-Tsinopoulou et al. [2008]	F	Singleton	IVF	Classic SRS plus mesocardia and clitoral enlargement	Not available
Douzgou et al. [2008]	M	Singleton	IVF	Classic SRS	Hypomethylation of paternally derived <i>H19/IGF2</i>
Chopra et al. [2012]	F	Twin	IVF/ICSI	Classic SRS	Hypomethylation of paternally derived <i>H19/IGF2</i>
Hiura et al. [2012]—Patient 1	Not available	Not available	IVF	Classic SRS	Hypermethylation of paternally derived PEG1, PEG10, GRB10; hypomethylation of paternally derived <i>H19/IGF2</i> ; hypomethylation of maternally derived ZNF597
Hiura et al. [2012]—Patient 2	Not available	Not available	IVF	Classic SRS	Hypomethylation of paternally derived <i>H19/IGF2</i>
Hiura et al. [2012]—Patient 3	Not available	Not available	IVF	Classic SRS	Hypomethylation of paternally derived <i>H19/IGF2</i> ; hypermethylation of paternally derived PEG1
Hiura et al. [2012]—Patient 4	Not available	Not available	IVF	Classic SRS	Hypomethylation of paternally derived <i>H19/IGF2</i> ; hypermethylation of paternally derived GRB10
Hiura et al. [2012]—Patient 5	Not available	Not available	IVF	Classic SRS	Hypomethylation of paternally derived <i>H19/IGF2</i> ; hypermethylation of paternally derived INPP5F
Current patient	F	Twin	IVF/ICSI	Classic SRS	Hypomethylation of paternally derived <i>H19/IGF2</i>

F, female; M, male; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; SRS, Silver–Russell syndrome.

became better defined during subsequent assessments. Genitalia and skin were normal. Abdominal and cerebral ultrasounds, and the electrocardiogram were all normal.

The infant was enrolled in a follow-up program, with several measurements performed to assess her height and weight growth, also in comparison with the development of the twin sister. The weight and height of the infant remained steadily smaller than the third centile; at the age of 6 months her weight was 5,155 kg (\ll 3rd centile), the height was 57 cm (\ll 3rd centile), with preservation of OFC (44.6 cm: 50th–90th centile). Her twin sister continued to maintain a weight and a height growth within the age norm; at the age of 6 months her weight was 7,870 kg ($>$ 50th centile), the OFC was 43.4 (10th–50th centile) and her height was 68 cm (50th–90th centile).

No cardiac, gastrointestinal or genital anomalies were detected. The psychomotor development of the girl always appears to be fitting to her age.

A clinical diagnosis of SRS was made. Molecular analysis was conducted at the age of 7 months after informed written consent obtained from the infant's parents. The presence of chromosomal abnormalities was excluded by FISH analysis. Analysis of DNA methylation of the chromosome 11p15 imprinted genes by bisulfite treatment coupled with restriction enzyme digestion (combined bisulfite restriction analysis, COBRA) [Chiesa et al., 2012] on DNAs derived from blood and buccal brush revealed the presence of hypomethylation at 11p15.5 *H19/IGF2* ICR. The pattern of methylation was normal at the *KCNQ1OT1* ICR (*KvDMR1*). Molecular tests were performed also in the parents and in the twin sister of the proband, and did not detect any methylation abnormality at 11p15.5 *KvDMR1* and *H19* loci. Analysis of microsatellite markers in the proband and her parents demonstrated biparental inheritance at 11p15.5 thus excluding pUPD 11 as cause of the DNA methylation abnormality. The presence of mUPD 7 was excluded. Methylation was also analyzed by COBRA at imprinted loci other than 11p15.5, including *GTL2-IG* (14q32), *MEST* (7q32.2), *IGF2R* (6q25.3), *GNAS-1A* (20q13.32), *PEG3* (19q13) and *PLAGL1* (6q24), as described [Blik et al., 2009]. At all these loci, methylation levels were found normal. At 6 years of age, the infant weight was 14.150 kg ($<$ 3rd centile), the height 97 cm tall ($<$ 3rd centile) and the OFC was 52.2 cm (\approx 50th centile).

DISCUSSION

This is the second reported infant conceived by ICSI born to a twin pregnancy, diagnosed with SRS due to hypomethylation of paternal *H19/IGF2* locus. As in a previously described case, only one twin girl was affected; the other twin girl had a normal pre- and post-natal growth pattern and a normal DNA methylation [Chopra et al., 2012].

Including the current patient, there have been 13 reported with SRS or SRS-like phenotype who were conceived using ART. Molecular data are not available in three of these cases [Svensson et al., 2005; Blik et al., 2006; Kagami et al., 2007; Galli-Tsinopoulou et al., 2008; Douzgou et al., 2008; Chopra et al., 2012; Hiura et al., 2012].

Significantly, 9/10 of the other cases had methylation defects at the *IGF/H19* locus and in some cases at the other loci. The first

reported ICSI-conceived infant with a SRS-like phenotype was found to have hypomethylation of the *H19* promoter; however this patient did not fulfill the diagnostic criteria for SRS proposed by Price et al. [Price et al., 1999; Blik et al., 2006]. One year later a twin girl conceived using IVF diagnosed with SRS was reported: molecular analysis showed a hypermethylation of the paternal *MEST* (7q DMR), but whether this finding may be involved in SRS remains uncertain, since the patient's father, with normal clinical features had the same methylation pattern of the affected child [Kagami et al., 2007]. The third infant, conceived by IVF, was diagnosed with SRS and was found to have a partial loss of methylation at the paternal *H19/IGF2* locus [Douzgou et al., 2008]. Another similar case, with diagnosis of SRS and hypomethylation within the paternal *H19* DMR, was reported in a twin girl conceived using ICSI [Chopra et al., 2012]. Hiura et al. [2012] described five patients conceived using IVF with classic SRS phenotype: one of these five infants had hypomethylation of paternally derived *H19/IGF2*, while in the other four cases, DNA methylation errors were not restricted to the *H19/IGF2*, and were present at both maternally and paternally methylated gametic DMRs. In this study, a 10 fold increased frequency of BWS and SRS associated with ART was found [Hiura et al., 2012]. This figure is consistent with the incidence of ART (2/18) we found in our cohort of SRS children with *H19* DMR hypomethylation born between 2000 and 2009 (unpublished data).

The increased frequency of genomic imprinting disorders among children born using ART should be of major concern, given that it is estimated that worldwide $>$ 1 million children have born after pregnancy conceived using ART, and that this number will probably continue to increase [Schultz and Williams, 2002; Ceelen et al., 2008]. Pregnancy after ART has been reported to be at increased risk for adverse perinatal outcome, including preterm birth, low birth weight, being small for gestational age, perinatal mortality, multiple births. A number of factors have been suggested to be involved, including causes of infertility that necessitated ART, and aspects of the ART procedure itself. ART involves manipulation of many steps of conception: the use of hormones to stimulate ovary for oocyte production; in vitro maturation of oocytes; the use of immature sperm; the use of ICSI with direct injection of sperm; the in vitro culture of pre-implantation embryos; and the cryopreservation of gametes or embryos. Any of these steps may alter the normal imprinting process, mainly by interfering with the DNA methylation process. Deregulation of imprinted genes has profound effects on fetal growth and development, varying from embryonic death to excessive, defective or impaired growth and causes several genetic syndromes [Manipalviratn et al., 2009].

Even if currently there is little evidence linking SRS with ART, sporadic reports continue to appear in literature. Our patient provides additional evidence of a possible relationship between ART procedures and methylation defects observed in SRS. Given the clinical heterogeneity of SRS, and the increased risk of multiple and preterm births in the ART-conceived children, it is possible that a number of cases of SRS remains undiagnosed in this population. Future studies should further investigate the possible link between ART and imprinting defects, focusing on syndromes where a significant proportion of cases are caused by epimutations, such

as SRS. Such studies might lead to a better understanding of the causes and of the incidence of epimutations in ART pregnancies and might also help the clinicians to adequately counsel parents who approach to ART and to assess the opportunity of a long-term follow-up of children conceived using ART.

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