Case Report

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Neonatal Silver-Russell syndrome assumed to result from maternal uniparental heterodisomy of chromosome 7

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Silver-Russell syndrome (SRS) is a rare genetic disorder characterized by intrauterine growth restriction, poor postnatal growth, relative macrocephaly, a triangular face, body asymmetry, and feeding difficulties. It is primarily diagnosed according to a clinical scoring system; however, the clinical diagnosis is confirmed with molecular testing, and the disease is stratified into the specific molecular subtypes. SRS is a genetically heterogeneous condition. The major molecular changes are hypomethylation of imprinting control region 1 in 11p15.5 and maternal uniparental disomy of chromosome 7 (UPD(7)mat). Therefore, first-line molecular testing should include methylation-specific approaches for these regions. Here, we report an extremely low birth weight (ELBW) infant with intrauterine growth retardation, postnatal growth retardation, and dysmorphic facial appearance—characteristics consistent with the clinical diagnostic criteria of SRS. Methylation-specific molecular genetic analysis revealed UPD(7)mat, while the loss of heterozygosity was not detected on chromosomal microarray analysis. We present a case of SRS with suspected uniparental heterodisomy of chromosome 7 in an ELBW infant.

Key words: Silver-Russell syndrome, Uniparental disomy, Genomic imprinting, Infant, Extremely low birth weight infant.

Introduction

Silver-Russel syndrome (SRS) is a rare but well-recognized imprinting disorder mainly characterized by idiopathic intrauterine growth retardation (IUGR) and postnatal growth failure. Children with SRS can be distinguished from those with idiopathic IUGR and postnatal growth failure but without SRS by the presence of other characteristic features, including relative macrocephaly, a prominent forehead, body asymmetry, and feeding difficulties. Furthermore, the less consistent clinical features include a triangular face, micrognathia, low muscle mass, and fifth-finger clinodactyly. The incidence of this disorder ranges from 1 in 3,000 to 1 in 100,000 individuals [1]. A clinical diagnosis of SRS is primarily established on the basis of a combination of characteristic features as reflected in the Netchine-Harbison clinical scoring system (NH-CSS) [2]. However, the diagnosis of SRS can be difficult owing to the variations in the manifestation of the condition among the affected individuals and several features of SRS are nonspecific.

Molecular testing can confirm the diagnosis of SRS in approximately 60% of the cases, and the molecular subtype can be defined, which may aid in selection of the appropriate manage-

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ment strategies. SRS is a genetically heterogeneous disorder; the loss of methylation (LOM) at imprinting control region (ICR) 1 in 11p15.5 is detected in up to 40% of the cases and maternal uniparental disomy of chromosome 7 (UPD[7]mat) accounts for 7-10% of the cases. Other rare molecular genetic or cytogenetic abnormalities have also been identified [2]. Methylation-specific approaches for chromosomes 7 and 11 are recommended as the first-line tests for the genetic diagnosis of SRS. In particular, methylation-specific polymerase chain reaction (MS-PCR) for chromosome 7 and methylation-specific multiplex ligation probe-dependent analysis (MS-MLPA) for chromosome 11 have been suggested [3,4]. For infants with IUGR, an early genetic diagnosis can improve the medical outcomes through the implementation of specialized management strategies, such as administration of growth hormone therapy and early interventions for neurocognitive problems [1]. Here, we report a case of SRS with suspected uniparental heterodisomy of chromosome 7 in an extremely low birth weight (ELBW) infant with IUGR.

Case

A preterm female infant was born at a gestational age of 27 weeks and 4 days via an emergency cesarean section performed because of severe maternal pregnancy-induced hypertension. She was a naturally conceived singleton whose parents were nonconsanguineous Koreans. Her mother was a 34-year-old multigravida with a history of two artificial abortions and one spontaneous abortion. The findings of antenatal ultrasound at a gestational age of 27 weeks and 2 days were suggestive of IUGR and fetal anomalies, including cardiomegaly, pyelectasis, and focal bowel dilatation. Maternal placental biopsy did not reveal signs of inflammation, infarction, or structural abnormalities (Fig. 1). The parents reported no family history of genetic syndromes. The infant's birth weight, length, and head circumference were 720 g (–1.12 standard deviation [SD]), 30 cm (–2.19 SD), and 23.5 cm (–0.86 SD), respectively (Fig. 2) [5]. Physical examination showed dysmorphic facial features, including a broad forehead, downward slanted palpebral fissures, small pointed chin, and large low-set ears. The Apgar scores were 1 at 1 minutes and 2 at 5 minutes. She was immediately intubated and received a surfactant after birth. Thereafter, she was admitted to the neonatal intensive care unit (NICU). She was maintained on mechanical ventilator support for 35 days, followed by oxygen supplementation via a nasal cannula and hood for an additional 43 days. She was treated for bronchopulmonary dysplasia and secondary pulmonary hypertension. She also had other medical issues, including patent ductus arteriosus, atrial septal defect, retinopathy of prematurity, congenital hypothyroidism, sepsis, meningitis, and unilateral inguinal hernia.

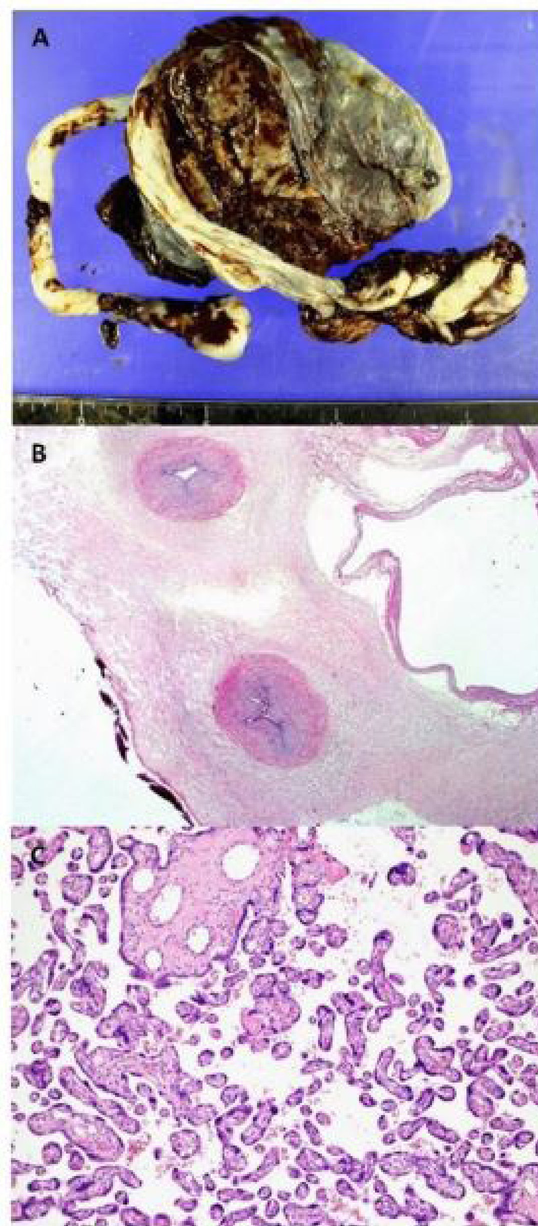

Fig. 1. Maternal placental biopsy of the patient. (A) Gross morphology showing no gross abnormality of the maternal placenta. (B) Microscopic view of the umbilical cord showing normal vascular structure without signs of inflammation (hematoxylin-eosin [H&E] staining; magnification, ×12.5). (C) Microscopic view of the placental parenchyma showing no vasculopathy or infarction (H&E staining; magnification, ×100).

Owing to feeding difficulties because of poor appetite and oromotor issues, the infant was fed via a nasogastric tube and was administered oromotor stimulation therapy daily. Owing to poor weight gain, we coordinated with the nutrition sup-

port team on a weekly basis to optimize her growth. She was fed a high-calorie fortified formula, and her daily caloric intake was closely monitored. However, at 40+0 weeks of postmenstrual age, she continued to show postnatal growth failure; her weight, length, and head circumference were 1,920 g (−3.74 SD), 49 cm (−2.69 SD), and 31 cm (−0.63 SD), respectively (Fig. 2) [5].

We assessed the possibility of a genetic cause of IUGR, postnatal growth failure, feeding difficulties, and facial dysmorphic features. As these four features correspond to the characteristic phenotype of SRS, we conducted the following first-line molecular tests for SRS: MS-MLPA for chromosome 11 and MS-PCR restriction fragment length polymorphism (RFLP) analysis for chromosome 7. MS-MLPA was performed at the *LIT1*-differentially methylated region (DMR) and *H19*-DMR of chromosome 11. The methylation patterns for the 7q.21.3 region (*SGCE* gene) were analyzed using PCR products of bisulfite-modified SGCE. MS-MLPA showed normal methylation patterns of 11p15; however, MS-PCR RFLP amplified only the maternal allele, suggesting UPD(7)mat (Fig. 3A, B). Chromosomal microarray (CMA) analysis was also performed to confirm the molecular diagnosis of SRS and identify other possible genetic disorders and chromosomal aberrations. CytoScan Dx assay (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used, which combines array-based comparative genomic hybridization and single nucleotide polymorphism (SNP) genotyping. CMA analysis did not reveal the copy number variation in the 11p15 and 7q21 regions or loss of heterozygosity in the 7q21 region. A duplication of Xp22.2p22.13 was detected on CMA analysis (Fig. 3C). The

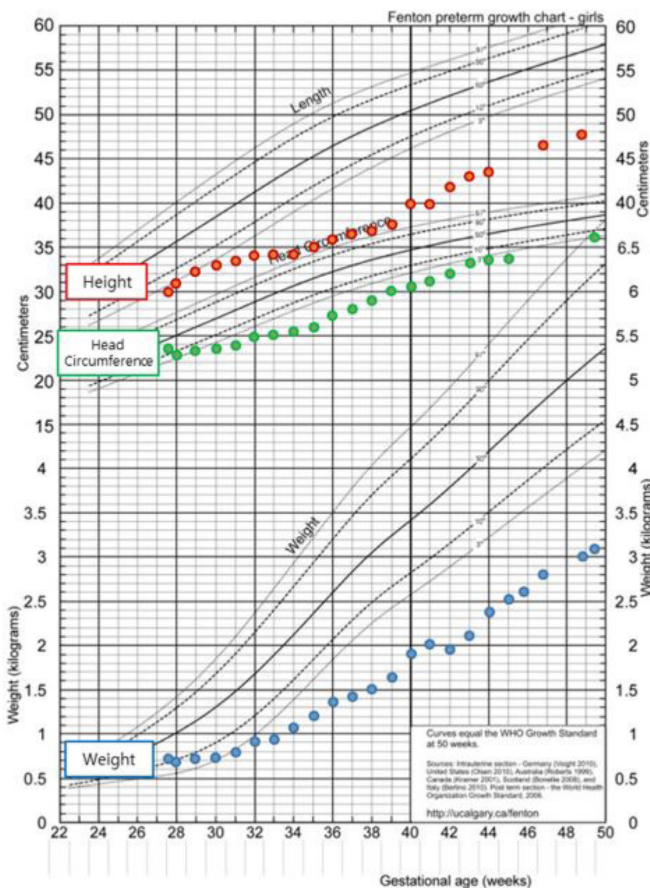

Fig. 2. Growth curve of the patient. The growth curve of the patient shows intrauterine growth retardation and postnatal growth failure without catch-up [5].

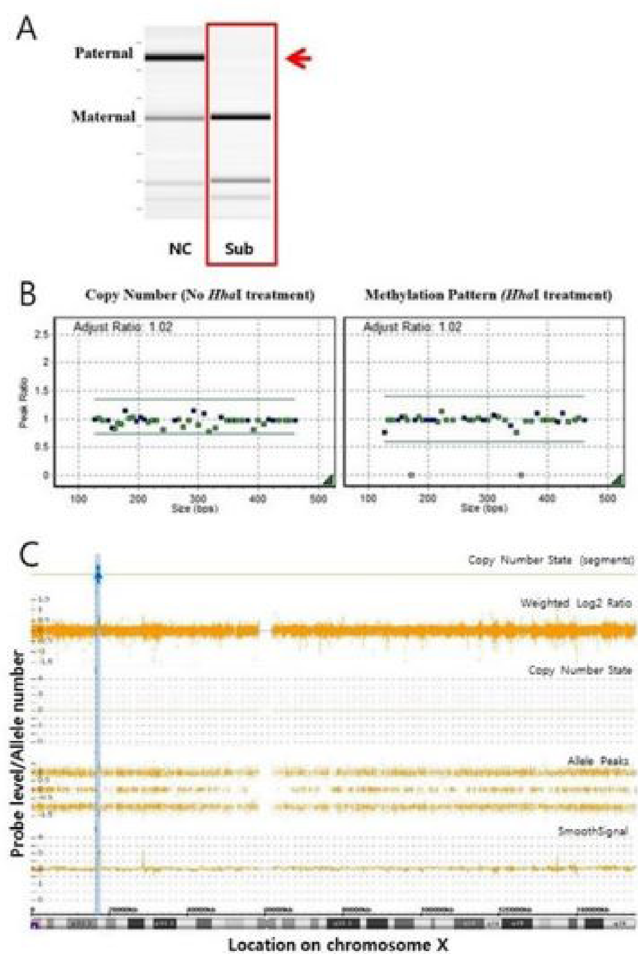

Fig. 3. Genetic testing of the patient. (A) MS-PCR RFLP analysis of the 7q.21.3 region (*SGCE*) revealed UPD(7)mat in the patient. The arrow indicates the missing paternal section. (B) MS-MLPA analysis of 11p15 showed a normal methylation pattern and the absence of copy number variation. (C) Copy number gain at Xp22.2p22.13 (16,985,921-17,731,098 kb) was detected on chromosomal microarray analysis (the duplicated region is indicated by the blue arrow with light blue outline). The X-axis presents the probe index on chromosome X, and the Y-axis presents the signal log 2 ratio of the probe. MS-PCR RFLP, methylation-specific polymerase chain reaction restriction fragment length polymorphism; UPD(7)mat, maternal uniparental disomy of chromosome 7; MS-MLPA, methylation-specific multiplex ligation probe-dependent analysis; Sub, subject; NC, normal control.

duplication was classified as a variant of uncertain significance (VOUS).

After the diagnosis, we conducted a surveillance medical examination and provided education and genetic counseling to the patients. We also established management plans for the short stature and low body mass index (BMI) of the infant; the plans included provision of adequate nutritional support and initiation of growth hormone therapy at the optimal time. We educated the mother regarding the increased risk for hypoglycemia, precocious puberty, developmental delay, and learning difficulties noted in children with SRS with UPD(7)mat. The patient was discharged from the NICU at 165 days after birth at the postmenstrual age of 46+0 weeks. She required nasogastric tube feeding at times, but her general medical condition was well otherwise. At the age of 7 months, her height and weight were 55.7 cm (below −3 SD) and 4.5 kg (below −3 SD), respectively. Her serum insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 levels at the age of 7 months were 112.7 ng/mL (reference range: 5.1-186.5 ng/mL) and 2,560 ng/mL (reference range: 950-2,120 ng/mL), respectively [6]. We plan to assess her growth and neurocognitive development in the upcoming visits to the hospital to ensure that appropriate interventions are provided.

Discussion

IUGR is defined as the rate of fetal growth being less than the normal growth rate based on the growth potential of a specific infant. The diagnosis is based on at least two ultrasonography measurements performed at least 2 weeks apart, with the fetal weight below the 10th percentile for the gestational age [1]. The etiology of IUGR is extremely heterogeneous and may involve maternal, placental, and fetal factors [7]. Genetic anomalies in the fetus account for 5-20% of the IUGR cases. These anomalies include chromosomal aberrations, imprinting disorders, and other genetic syndromes such as SRS, Bloom syndrome, Cornelia de Lange syndrome, Mulibrey nanism syndrome, and Rubenstein-Taybi syndrome [8,9]. Although most genetic syndromes with chromosomal aberrations can be detected with CMA analysis, some imprinting disorders, such as SRS, may yield normal results on CMA testing, and specific molecular testing is required for the diagnosis of these disorders.

SRS is characterized by IUGR, poor postnatal growth failure, relative macrocephaly, a triangular face, body asymmetry, and feeding difficulties. The syndrome was first described by Silver et al. [10] and Russell [11], who independently described a group of children with low birth weight, postnatal short stature, characteristic facial features, and body asymmetry. In 2015, Azzi et al. [2] proposed the NH-CSS for SRS. The clinical diagnosis of SRS can be established in an individual who meets at least four of the six NH-CSS clinical criteria, including prominent forehead/frontal bossing and relative macrocephaly at birth and two additional findings, and in whom other disorders have been ruled out. The other criteria are small for gestational age/IUGR, postnatal growth failure, body asymmetry, and feeding difficulties/low BMI. The supporting clinical findings of SRS include delayed closure of the anterior fontanelle (43%), triangular face (94%), micrognathia (62%), dental crowding (37%), down-turned corner of the mouth (48%), high-pitched voice (45%), decreased muscle mass (56%), skeletal abnormalities, developmental delay, and genitourinary anomalies [1]. Patients with SRS also develop endocrine issues, including fasting hypoglycemia, premature adrenarche, early puberty, and insulin resistance. The possible skeletal abnormalities of SRS include asymmetry with respect to the limb length, fifth-finger clinodactyly, and scoliosis. In our case, the premature infant presented with IUGR, postnatal growth failure, feeding difficulties, and a triangular face with a broad forehead and micrognathia. Asymmetry in the leg length was clinically noted (discrepancy of 0.5 cm) but not confirmed on radiography. However, the infant showed no signs of scoliosis, clinodactyly, or genitourinary anomalies.

Considering the subjective nature of the clinical diagnosis, molecular genetic identification of SRS is particularly important [12]. Molecular testing also defines the genetic subtype of SRS, which can guide appropriate management of patients with different genotypes. The major molecular changes are LOM of 11p15 and UPD(7)mat. Other rare etiologies include copy number variations on chromosome 7 or 11, or an intragenic pathogenic variant in *CDKN1C*, *IGF2*, *PLAG2*, or *HMGA2* [13]. Methylation-specific analysis for both chromosomes 7 and 11 should be considered in patients whose phenotype is consistent with SRS. MS-PCR for UPD(7)mat and MS-MLPA for 11p15 LOM have been recommended as first-line testing [3,4]. The methylation defects of *H19*-DMR and *LITI*-DMR (previously known as ICR 1 and 2) can be detected with MS-MLPA of 11p15 [1,3]. Hypomethylation of the *H19*-DMR in 11p15 results in reduced paternal *IGF2* expression and increased maternal *H19* expression, leading to growth restriction [1].

UPD is the presence of a chromosome pair derived only from one parent in a disomic cell line [14]. The phenotype of SRS patients with UPD(7)mat is believed to result from altered expressions of the imprinted growth and developmental regulatory

genes. *GRB10* and *MEST* have been suggested as the candidate genes on chromosome 7 accounting for the development of SRS [4]. Patients with UPD(7)mat generally show a milder phenotype than patients with LOM of 11p15. Micrognathia, low set ears, learning difficulties, and speech disorders have been more frequently reported in patients with UPD(7)mat [15] However, pathognomonic features distinguishing between the UPD(7)mat genotype and other genotypes have not been reported [3,16,17]. In cases of UPD(7)mat identified with MS-PCR, the diagnosis can be confirmed by performing microsatellite analysis with the DNA from at least one parent. We identified UPD(7)mat in the patient; however, we could not perform microsatellite analysis, as the parents refused parental testing. Although abnormal methylation patterns may not be detected with CMA analysis in some cases, CMA analysis can still provide useful information for the classification of UPD [18]. Maternal UPD can be detected using CMA with SNP array analyses only in cases of isodisomy [19]. Additionally, in one-third of the cases, UPD develops in association with or as a result of a chromosomal rearrangement [14]. In our case, CMA with SNP array analysis did not reveal copy number variation or loss of heterozygosity in the 7q21 region, which implies that UPD(7)mat developed in the infant because of heterodisomy. In this case, a 745-kb duplication at Xp22.2p22.13 (16,985,921-17,731,098) was detected on CMA analysis. The duplicated region involves some of the exons in the *NHS* gene. Mutation in the *NHS* gene can lead to the development of Nance-Horan syndrome through loss of gene function. Nance-Horan syndrome is a rare X-linked recessive disease characterized by severe congenital dense nuclear cataracts and developmental delay in hemizygous male patients. Carrier females can display milder symptoms [20]. The infant in our case was a girl with duplication, whose lenses appeared normal on ophthalmologic examination. Thus, the variation was suspected to be VOUS, which would have been confirmed if parental testing was performed.

In 2017, Wakeling et al. [1] published the first international consensus statement on the diagnosis and management of SRS, which emphasized adequate nutritional status with the avoidance of rapid and excessive weight gain. Early initiation of growth hormone therapy benefits patients by improving the body composition, motor development, and appetite, thus, optimizing linear growth and reducing the risk of hypoglycemia. Patients should be monitored for signs of premature adrenarche, early and rapid central puberty, and insulin resistance. Gonadotropin-releasing hormone analogues can delay the progression of central puberty and preserve the potential to achieve the optimal adult height in the future. For our patient, we established a specific management plan and provided educated the parent regarding nutritional support, recurrence risk, and possible medical issues, including neurocognitive problems, endocrine problems, surgical/anesthesia issues, orthopedic problems, and maxillofacial abnormalities. Owing to early diagnosis, we were able to provide appropriate management and parental education for each subtype of SRS. The parents were informed that the recurrence risk was low because of the *de novo* development of UPD(7)mat [1]. Three weeks after discharge, the infant was readmitted to the hospital for surgical repair of an inguinal hernia. Before being sent to the operating room, she was intubated by the NICU team owing to difficulties associated with visualizing the airway and intubating SRS patients with small mandibles.

Identification of the underlying molecular subtype can influence the management strategies with respect to the specific risk factors associated with each subtype; however, this can be challenging. We report a case of SRS with suspected uniparental heterodisomy of chromosome 7 in an ELBW infant. SRS is a rare genetic cause of IUGR, and ELBW infants have several risk factors that may contribute towards feeding difficulties and postnatal growth failure; however, when relative macrocephaly, prominent forehead, or body asymmetry is noted in patients with IUGR, clinicians should suspect SRS. Once SRS is suspected, appropriate methylation-specific genetic testing should be conducted, the abnormal methylation patterns may not be detected on performing CMA analysis or other molecular genetic tests. With this report we aim to improve the understanding of the complex molecular etiology of SRS and aid clinicians in establishing accurate diagnoses early on, thereby, promoting better outcomes in patients with SRS.

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