**8q12.1 deletion encompassing PLAG1 as a cause of Silver Russell syndrome.**

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**AUTHOR CONTRIBUTIONS**

J.R.F.F. conceptualized and drafted the initial and final manuscript. C.D.S. presented this case report at the National Spanish Congress of Neonatology (SENEO Congress 02-04 October 2019, Madrid). M.D.H., S.C., K.T., J.M. L.G., M.O.S. and I.N. made substantial contributions to the analysis and interpretation of data, and critically revised the manuscript for important intellectual content. All authors approved the final manuscript as submitted, and agree to be accountable for all aspects of the work.

**ABSTRACT**

Here we describe phenotype and molecular features of a preterm girl diagnosed of Silver-Russell syndrome (SRS), resulting of a de novo heterozygous 8q12.1 deletion encompassing PLAG1 gene. The proband was a 32 weeks preterm girl born asymmetric small for gestational age, after a pregnancy complicated by severe intrauterine growth restriction (IUGR). SRS was clinically diagnosed, but molecular genetic testing for methylation analysis of 11p15.5 and maternal UPD7 yielded normal results. A 60k microarray CGH was also performed simultaneously, showing a 2.1 Mb de novo deletion of 8q12.1 involving PLAG1 gene. To our knowledge, this is the first time that a deletion involving PLAG1 is reported as causative of SRS. This finding could further support the previously reported dominant expression of PLAG1 mutations associated to SRS and could contribute to more accurate diagnosis of affected patients.

**Keywords:** PLAG1, 8q12.1 deletion, Silver-Russell Syndrome.

**INTRODUCTION**

SRS is a clinically and genetically heterogeneous imprinting disorder characterized by assymetric fetal growth restriction, postnatal growth failure, relative macrocephaly at birth, prominent forehead and other clinical features (Azzi et al., 2011). Diagnosis of SRS relies on specific clinical criteria described in the Netchine-Harbison clinical scoring system (NH-CSS) and molecular genetic testing showing hypomethylation on chromosome 11p15.5 or maternal uniparental disomy (UPD) for chromosome 7 in 60% of affected individuals (Azzi et al., 2011), and rarely, showing pathogenic variants in CDKN1C, IGF2, PLAG1, and HMGA2 (Saal, Harbison & Netchine, 2002).

Here, we report an individual with a deletion of 8q12.1 involving PLAG1 gene causing SRS, review the literature and discuss the phenotypic features of PLAG1 related disorders.

**CASE REPORT**

A 32 weeks preterm girl, first daughter from healthy spanish non consanguineous parents, was born after an emergency cesarean section due to altered fetal cardiotocographic register. Pregnancy was complicated by early severe IUGR. Pregnancy infection screening was negative for toxoplasma, rubella, syphilis, herpes, and cytomegalovirus. Apgar score was 7 and 8 at the first and fifth minutes, respectively. Respiratory support with CPAP was started at delivery room. On admission to NICU no evident dysmorphic features were noted. According to Usher and McLean growth chart at birth, weight was 950 g (-3.50 SD), length 34.5 cm (-4.76 SD) and head circumference 26.5 cm (-2.30 SD), being classified as asymmetric small for gestational age.

Hematological and biochemical parameters were unremarkable, with no suggestive signs of congenital infection. No skeletal anomalies were noted. Cardiac and renal ultrasounds were both normal, but brain ultrasound showed a grade I bilateral intraventricular hemorrhage, secondary to prematurity. Ophthalmological anomalies were ruled out and screening for inborn errors of metabolism was normal. Postnatal growth failure was apparent on 35th postnatal day of life at 37 weeks of corrected age, with length and weight both below -3 SD but head circumference in normal range (-1.5 SD). Dysmorphic features were then more apparent at physical exam (figure 1, figure 2 and figure 3) without body asymmetry. Clinical diagnosis of SRS was established according to NH-CSS, accomplishing four of six criteria (small for gestational age, postnatal growth failure, relative macrocephaly at birth and prominent forehead) and molecular genetic testing was requested after informed consent was obtained from the guardians. The SALSA® MS-MLPA® BWS/RSS ME030-C3 and ME032 UPD7-UPD14 probemix kits (MRC-Holland) for methylation analysis of 11p15.5 and maternal UPD7 yielded normal results. A 60k microarray CGH (CGXTM v2, Perkin Elmer) was ordered simultaneously, showing a 2.1 Mb de novo deletion of 8q12.1 (between 56,834,331 and 58,921,491 bp, GRCh37) that encompassed 9 OMIM genes according to DECIPHER (https://decipher.sanger.ac.uk/browser#q/8:56834331-58921491/location/8:55540751-60215071). Two genes were associated on DECIPHER with disease phenotypes: IMPAD1 (MIM 614010) and

PLAG1 (MIM 603026). Homozygous mutation in the IMPAD1 gene causes chondrodysplasia with joint dislocations, gPAPP type (OMIM 614078), an autosomal recessive bone dysplasia that was not concordant with our patient phenotype. We also reviewed on the genome aggregation database (GnomAD v2.1.1 https://gnomad.broadinstitute.org/) the probability that a gene is intolerant to a heterozygous Loss of Function (LoF) mutation (pLI score; according to Lek et al., 2016) for all genes involved in deletion, and we found a low pLI score for IMPAD1 (score = 0.02), suggesting that the probability of intolerance to a heterozygous LoF mutation was very low. Nonetheless, two major genes showed high pLI scores (PLAG1; 1, LYN; 1), suggesting that the probability of intolerance to a heterozygous LoF mutation was maximum. PLAG 1 was fully deleted in our proband and LYN was partially deleted. LYN gene (MIM 165120) is an oncogene from Src family kinases involved in regulation of hematopoietic abnormalities and autoimmune diseases such as asthma and psoriasis, but currently is not associated with a specific human disease neither with SRS phenotype (Ingley, 2012), so we excluded it as a candidate gene for the observed phenotype. On the other hand, PLAG1 is an oncogene that increases Insulin-like growth factor 2 (IGF2) expression in some tumor cells (Zatkova et al., 2004). IGF2 is a growth factor also implicated in pathophysiology of SRS, as hypomethylation of the imprinted 11p15.5 IGF2/H19 domain, leads to downregulation of IGF2 expression (Azzi et al., 2011; Gicquel et al., 2005). Abi Habib et al (2018) have recently reported the implication of PLAG1 in pathophysiology of SRS through the HMGA2–PLAG1–IGF2 pathway, reporting the first mutations of PLAG1 in a familial case with dominant transmission of syndromic IUGR associated with SRS and in a sporadic case of SRS with a de novo mutation. Schinzel, Robinson, Binkert and Fanconi (1994) also reported a girl with SRS like features, who carried an interstitial deletion of proximal 8q (q11-q13) but molecular genetic defect could not be identified due to technical limitations at that moment. Thus, we suggest that the observed phenotype in our patient could be the result of PLAG1 haploinsufficiency, since point mutations of PLAG1 lead to downregulation of IGF2 causing IUGR and SRS with dominant expression (Abi Habib et al., 2018).

Our patient is now 21 months of corrected age and some relevant clinical comorbidities have arisen. Gastroesophageal reflux disease and chronic diarrhea secondary to cow's milk protein allergy, have been troublesome since neonatal period, requiring proton pump inhibitor chronic treatment, elemental enteral formula and a gastrostomy tube that has been placed recently to optimize nutrional support. No relevant hypoglycemic events have been reported since birth to date and nutritional status has shown favorable evolution, with current growth parameters rising above percentil 50 according to specific SRS growth curves. Since discharge from neonatal unit, she has required multiple hospital admissions due to bronchospasm related with rhinovirus infections and gastroesophageal reflux disease. Intermittent stridor and respiratory recurrent infections led us to perform a fibrobronchoscopy which showed a moderate laryngomalacia. Nocturnal non invasive respiratory support (BiPap) at home has been required for treatment of obstructive apnea disorder. Sleep disordered breathing is a recently addressed problem in SRS patients, with obstructive apnea as a prominent manifestation as in our case (Giabicani et al., 2019). Mild neurodevelopmental delay has also been diagnosed in the proband, with progressive improvement after inclusion in early developmental intervention program.

In summary, we report a de novo heterozygous 8q12.1 deletion encompassing PLAG1 gene, proposed as the main candidate gene for the observed phenotype, in a 32 weeks preterm girl with clinical diagnosis of SRS. This finding, in our opinion, could further support the previously reported dominant expression of PLAG1 mutations associated to SRS. However, more studies are needed to better define the role of 8q12.1 deletions in etiology of SRS and refine the phenotype of affected patients. Accordingly, we suggest that PLAG1 haploinsufficiency caused by point mutations or deletions encompassing PLAG1 gene, should be considered in clinically diagnosed SRS patients, when methylation analysis of 11p15.5 and maternal UPD7 yield normal results, suggesting the indication of sequence analysis and/or microarray studies in such cases.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Phenome Central at https://www.phenomecentral.org/P0011261 , reference number P0011261.

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**FIGURE LEGENDS**

Figure 1. Frontal view showing triangular facies, long philtrum, thin lips and mild micrognathia with narrow chin.

Figure 2. Lateral view showing sparse and thin hair, dolichocephaly, long eyelashes and posteriorly rotated and low set ears.

Figure 3. Frontal view showing relative macrocephaly and prominent forehead.