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First Report of a Derivative Chromosome 13 with a Duplicated 11p15 Locus Associated with Silver-Russell syndrome

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Abstract

Silver-Russell Syndrome (SRS) is a disorder that is primarily characterized by intrauterine growth restriction which may occur asymmetrically or in whole, leading to a fetus being small relative to its gestational age. We present a female infant (proband), with severe congenital anomalies. The proband carried a >25Mb duplication of the chromosomal 11p15-11pter locus of chromosome 13; creating a derivative chromosome 13 [der(13)] and was reported as 46,XX,der(13)add(11p15-11pter). A methylation-sensitive assay confirmed a diagnosis of Silver-Russell Syndrome (SRS). Although the prognosis for SRS patients is generally good, our proband presented with a clinically severe phenotype culminating in death at nine months. To the best of our knowledge, this is the first report of a derivative chromosome 13 with a duplicated 11p15 locus being reported in a patient with SRS.

***Keywords*:** Silver-Russell syndrome, growth retardation, imprinting, derivative chromosome.

Introduction

Silver-Russell Syndrome (SRS) is a disorder that is primarily characterized by intrauterine growth restriction which may occur asymmetrically or in whole, leading to a fetus being small relative to its gestational age. Individuals are diagnosed with SRS when they present with growth restriction, relative macrocephaly at birth (head circumference ≥1.5 SD above birth weight and/or length), prominent forehead usually with frontal bossing, triangular facies,micrognathia and feeding difficulties.[1,2] Rarely, SRS patients may also exhibit fifth-finger clinodactyly.[1]

SRS is one of twelve imprinting disorders which are caused by epigenetic (methylation) or genetic abnormalities. Among SRS patients, 35%-50% of cases are due to loss of paternal allele methylation (LOM) at the imprinted control region 1 (ICR1) at 11p15.5 and 7%-10% are due to maternal uniparental disomy (UPD) of chromosome 7.[3,4] In rare cases, somatic mosaicism for maternal UPD(11), duplication of the maternal 11p15.5, inversions and translocations affecting chromosome 11, as well as maternal UPD of chromosomes 14, 16 and 20 have also been reported.[5] In addition to epigenetic and copy number variants (CNVs), mutations in certain genes have also been reported to cause SRS. For example, maternal transmission of gain-of-function mutations in the *CDKN1C* gene[3] or paternal transmission of loss-of-function mutations in the *IGF2* gene[4] has been described in association with SRS. Also, genes which are upstream regulators of *IGF2* such as *HMGA2* or *PLAG1* are also associated with SRS.[4]

Case Report

Our proband was a female infant born in 2018 at a tertiary hospital in Muscat, Oman. She presented with multiple anomalies such as intrauterine growth restriction, macrocephaly, broad fontanelle, feeding difficulty, low set ears and failure to thrive without ventilator support (Figure 1A). The proband was born to unrelated parents (family pedigree shown in Figure 1B). A history of miscarriage at six weeks of pregnancy in the proband's mother prompted the Special Care Baby Unit (SCBU) physician at the referral hospital to request cytogenetic studies in the proband and her parents. Informed consent was obtained from the proband's parents prior to the referral for genetic studies. Peripheral blood samples were obtained from the patient and her parents in both Heparin and EDTA tubes. The proband was referred to our genetic clinic a few days after her birth. However, the infant was in a critical condition and remained in the SCBU on ventilator

until her death at the age of nine months. As a result, a direct clinical assessment of the proband could not be carried out by our genetic clinic. Instead, a description of patient phenotype was communicated to our clinical geneticist by the referring physician over phone.

Chromosomal karyotyping of the proband yielded an abnormal karyotype with a large heterozygous additional chromosomal region on the p-arm of chromosome 13. Genomic DNA was extracted from the whole blood of the proband and used to perform array-based comparative genomic hybridization (CGH) with the Affymetrix Cytoscan HD kit (Thermo Fisher Scientific, USA). Array CGH data analysis using the ChAS software v.3.1.0.15 revealed a heterozygous 25,109kbp (~25 Mb) duplication of the 11p15-11pter chromosomal locus (hg19:chr11:230,615-2,339,766). Hence the karyotype (Figure 2A) was reported as 46,XX,der(13)add(11p15-11pter). To the best of our knowledge, this is the first report of a derivative chromosome 13 with a duplicated 11p15 locus being reported in a patient with SRS.

Given the clinical feature of growth restriction in the proband, the involvement of the 11p locus warranted further testing due to its association with SRS. A methylation-sensitive Multiplex ligation-dependent Probe Amplification (MS-MLPA) assay[6] was conducted on the DNA from the proband using the ME030 (Lot No:C3-0219) kit from MRC Holland (The Netherlands). This kit is a multi-disease assay which tests the 11p15 locus for both BWS and RSS, as well as the 5q35.3 locus (NSD1 gene) for Sotos syndrome. The assay samples were then run on the genome analyzer ABI 3700 and the data generated was analyzed using the Coffalyser (v.210604.1451). The MS-MLPA data (Figure 2B) confirmed the duplication of the 11p15 locus, but also revealed LOM at the ICR1.

Meanwhile, karyotyping both parents of the proband revealed that the der(13) chromosome observed in the patient was maternally inherited (Figure 3). Hence, the diagnosis of SRS due to maternal 11p duplication was established in the proband.

The 25 years old mother (Figure 1, II.3) of the proband was found to be a carrier of a heterozygous balanced non-reciprocal translocation between chromosome 11 and 13: 46,XX,der(13)t(11;13)(p11;p12). This phenotypically normal, but genotypically abnormal

karyotype (Figure 3) was characterized by one of the chromosomes 13 having an additional translocated 11p15-11pter region on its p-arm creating the der(13) chromosome, and one of the chromosomes 11 lacking the region from 11p15-11pter. The father of the proband was observed to have a normal male karyotype.

The parents of the proband had not been amenable to an appointment at our clinic while their child was in the SCBU. After the death of the proband, the parents met with our genetic counselor and the implications of the karyotype and MS-MLPA results were explained to them. During genetic counseling of the proband's parents, it transpired that there was a family history of miscarriages reported in the 52 years old maternal grandmother (Figure 1B, I.1) and a 30 years old maternal aunt of the proband (Figure 1B, II.1). Fertility problems were also reported in a maternal 28 years old uncle (Figure 1B, II.2) of the patient who had a single offspring after treatment for infertility. The maternal grandmother of our proband, I.1 was unable to recall the number of miscarriages she underwent. These individuals were then invited for genetic counseling and offered karyotyping after informed consent. All three tested family members carried karyotypes identical to the proband's mother (balanced non-reciprocal translocation; Figure 3). Another 19 years old maternal uncle of the proband was reported to be diagnosed with unilateral kidney disease (Figure 1B, II.). However, this individual was not willing to undergo genetic counseling or testing.

Informed consent for testing and publication of anonymized data was collected from all patients/guardians involved in this study and appropriate ethical standards were employed in all procedures.

Discussion

This is the first report of a case where SRS is associated with a derivative chromosome 13 carrying a duplicated 11p arm. In light of the fact that translocation events involving chromosome 11p and chromosome 13 have never been reported before except in oncology patients, this finding is quite novel. The der(13) chromosome in the proband, resulted in an extra copy of the maternal 11p12 to 11pter region within the karyotype, with no apparent loss of

chromosome 13 regions according to array CGH analysis The der(13) chromosome was transmitted through at least three generations of a family.

Although rare, maternal duplications of 11p12-11pter which include the 11p15 locus, are estimated to cause the associated SRS phenotype in <1% of SRS patients.[7] The cases of maternal 11p15 duplications reported previously were mostly interstitial duplication events with or without inversions, encompassing the 11p15 locus[8] or rarely, due to unbalanced translocations between chromosome 11 and chromosomes 4, 9, 10, 15, 16 and 17.[9-13] While most of these rearrangements involved ICR1; duplications of the whole ICR2 as well as partial duplication of ICR1 were also rarely reported in association with SRS. However, in all of these cases, the patients survived much longer than our patient, albeit with varying degrees of prognosis[7-15].

SRS patients generally have a good prognosis and can live well into adulthood with occasional complications.[14] However, the severity of clinical presentation in SRS patients with copy number variants (CNV) appears to be dependent on the extent of 11p locus involved in the CNV.[7,14] This is evident in our patient who was unable to survive independently outside of the SCBU facility because the ~25Mb duplicated maternal allele in our proband covered almost the entire 11p15.5 band, which included both the ICR1 and ICR2 regions and was bigger than the majority of the previously reported CNVs involving the 11p15 locus.[7-15] This was accompanied by hypomethylation of the H19 gene. Hence, the classic SRS phenotype of growth restriction in the proband likely reflects an increased expression of the maternally expressed *H19* gene and consequent down-regulation of the IGF2 gene expression.[9-11]

In the case of maternal inheritance, duplication of the 11p15 locus causes the SRS phenotype, whereas a paternally inherited similar duplication would cause the Beckwith Wiedemann syndrome (BWS) phenotype. No instances of BWS were seen within our proband's family, especially since most of the carriers of the der(13) chromosome detected in this family were females. The maximum likelihood of paternal transmission of the der(13) chromosome and risk for BWS is from the maternal uncle (Figure 1, II.2) of the proband, who has one normal offspring.

A key point to be noted in this case is that patients suspected with SRS are usually subjected to molecular genetic tests which can characterize either methylation abnormalities or copy number variants (CNVs) or both; but not chromosomal translocations. However, the clinically severe presentation in our proband and the history of miscarriage in the proband's mother had prompted a referral for cytogenetic studies. This was key to the der(13) translocation-derivative chromosome being detected in multiple members of the family and the provision of accurate genetic counseling to other members of the family who had a history of miscarriages and infertility. The affected couples in the proband's extended family had not suspected a hereditary component to their history of reproductive failures prior to our proband being tested.

The parents of the proband were counseled regarding future risk for affected offspring. However, the mother refused to consider prenatal genetic testing combined with in-vitro fertilization as a reproductive option, since abortion is generally prohibited in Oman (with medical exceptions). The mother decided to have future pregnancies monitored using first trimester ultrasonographic diagnosis.

Conclusions

Although maternal duplications due to 11p15 translocation events are rare, they must be suspected in patients with SRS phenotype who also present with severe failure to thrive. Offering genetic testing to the parents of affected patients may help prevent further recurrences of affected offspring. Determining whether a duplication event is due to the transmission of translocated chromosomes, or due to interstitial duplications or inversions, is also crucial as individuals who carry translocations are at significantly higher risk for infertility, recurrent miscarriages and birth of offspring with moderate to severe disease phenotypes.

Data Availability Statement

Data generated in this study is the sole property of the Royal Hospital, Ministry of Health, Oman. As such, any release of data from this study, outside of journal publications or scientific abstracts, is subject to prior approval of the Scientific Research Committee, Royal Hospital, Oman.

Authors' Contribution

NH carried out molecular genetics analyses and wrote the manuscript; MA conducted clinical sampling; patient counseling and manuscript review; KS carried out cytogenetic analyses and manuscript review and SO conducted patient counseling and manuscript review. All authors approved the final version of the manuscript.

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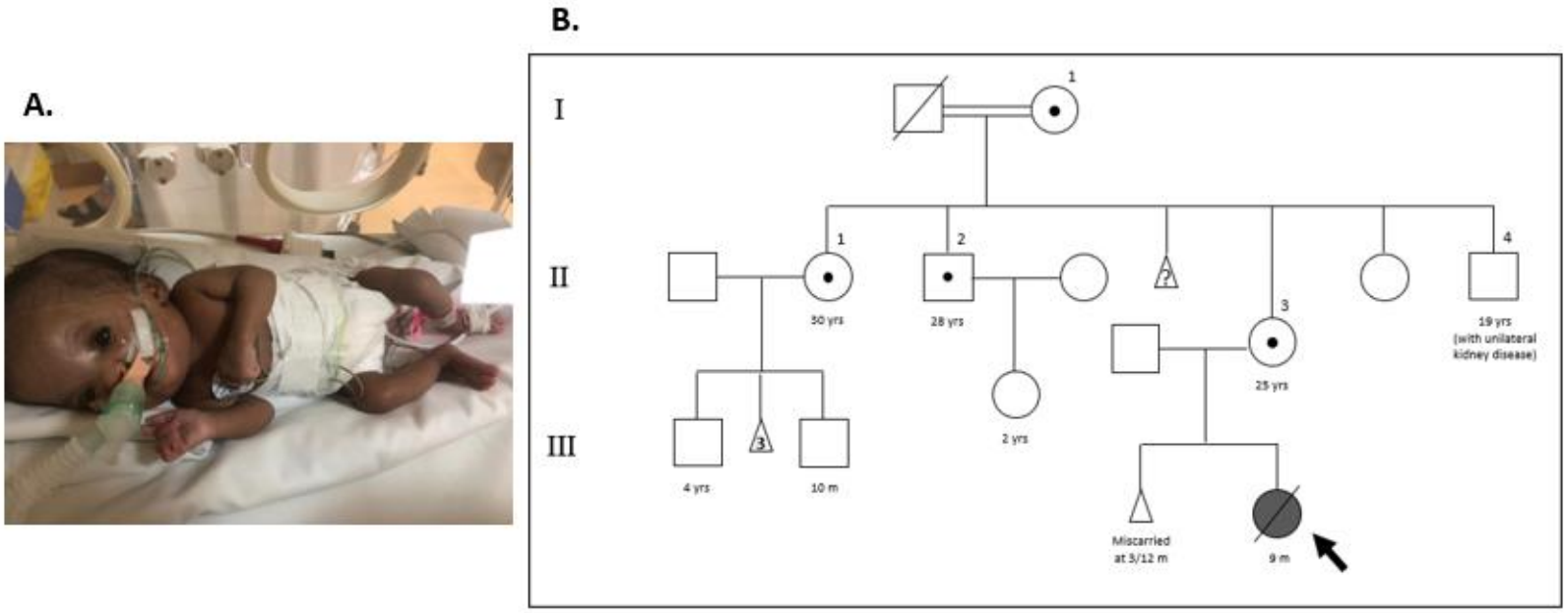
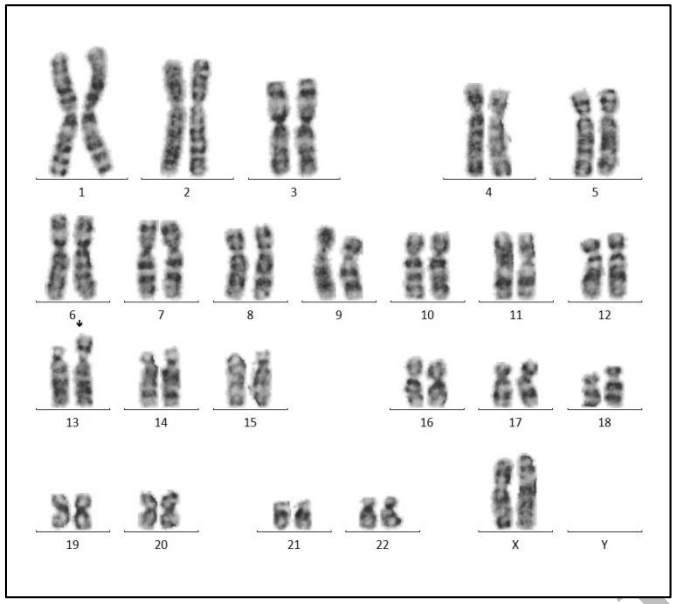


Figure 1: Clinical details of the proband

(A)The female infant was born with macrocephaly, broad fontanelle, low set ears, intrauterine growth restriction and presented with failure to thrive without ventilator support. (B) Family pedigree of the proband

A.



B.

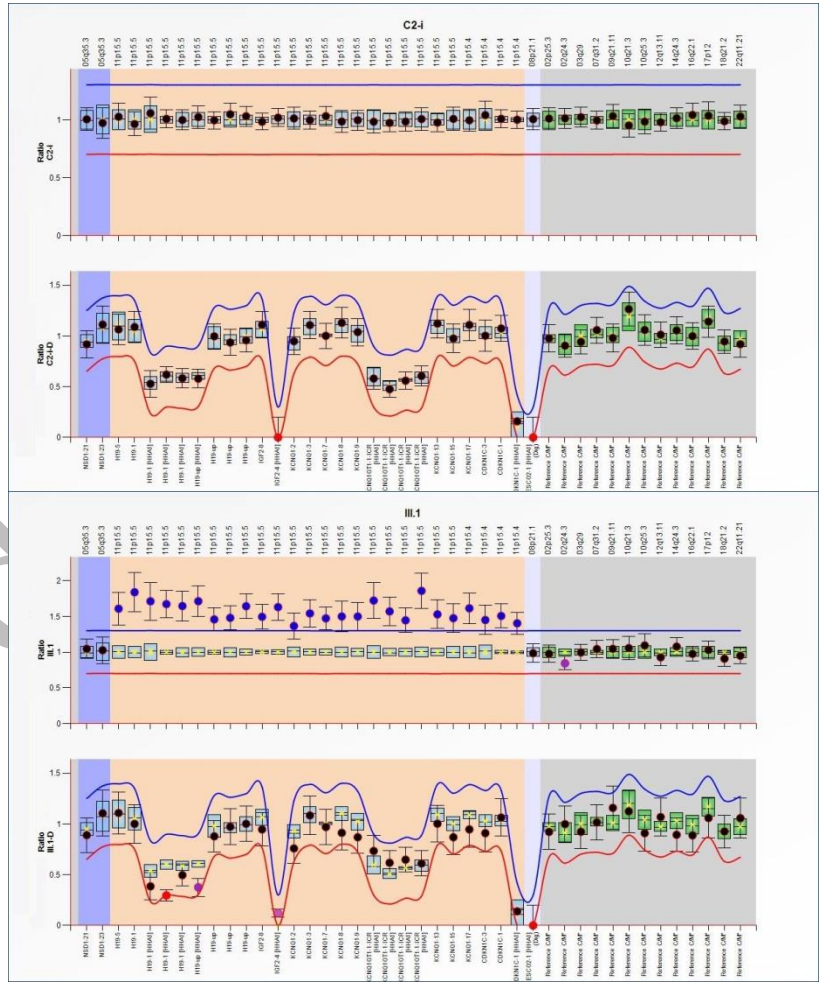


Figure 2: Karyotype and MS-MLPA test results of the proband

(A) The derivative chromosome 13 with a duplicated 11p locus is indicated by a short black arrow in this karyotype of the proband (B) MS-MLPA[6] results: The upper panel shows the CNV analysis (C1) and methylation analysis (C1-D) in a normal control sample. The bottom panel shows the CNV analysis (III.1) and methylation analysis (III.1-D) results for the proband. Black probe signals

277 indicate normalized results in comparison to control samples within the MS-MLPA run. In the CNV panel III.1, the blue signals
278 indicate the duplicated signals (3 copies) from all the probes targeting the 11p15 locus, at an average ratio of 1.5 on the y-axis;
279 whereas the red signal in the panel III.1-D indicates the decrease in methylation of the H19 locus. The orange regions include the
280 probe signals from the 11p15 locus, the grey regions indicate signals from reference probes and the violet regions represent probe
281 signals from the NSD1 gene at the 5q35.3 locus.
282

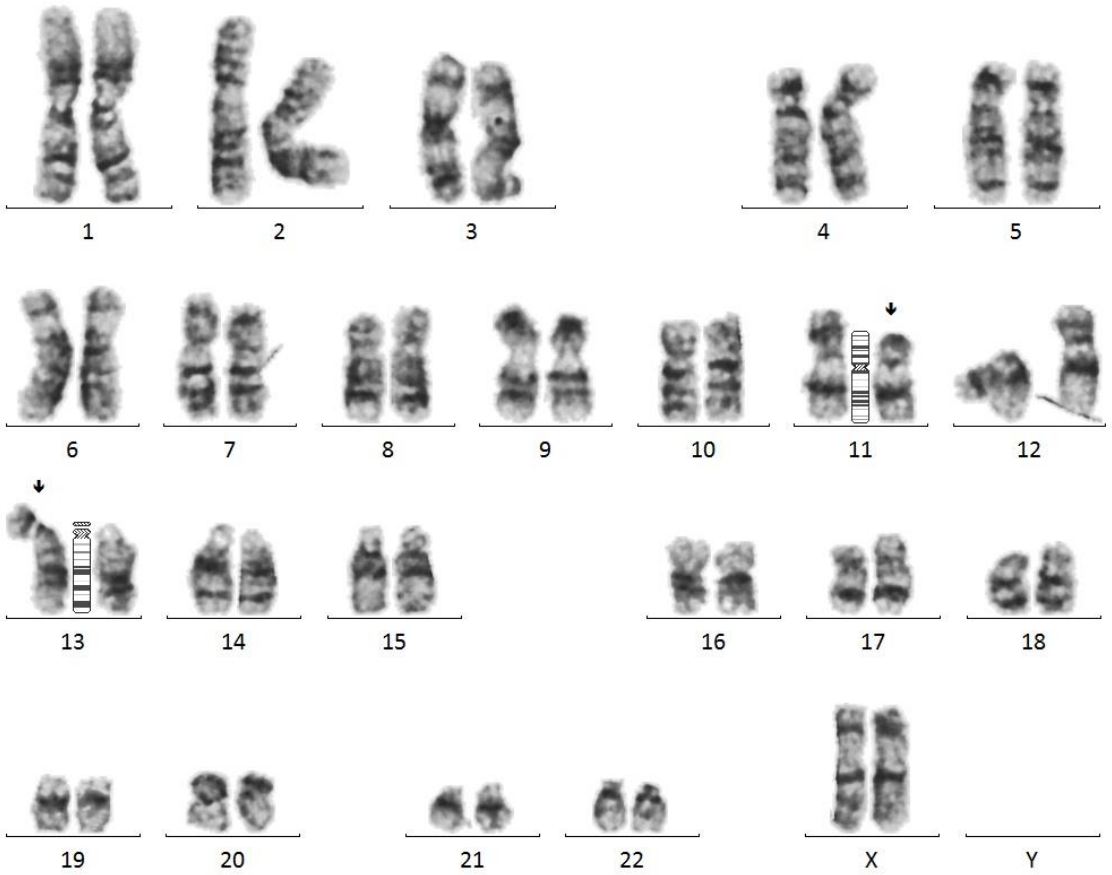


Figure 3: Balanced, non-reciprocal translocation observed in the proband's mother

The deletion at the 11p arm one of the chromosomes 11, and the addition of a 11p15-11pter region on the 13p arm of a chromosome 13 which created a der(13), are both indicated by black arrows. The abnormal chromosomes are compared against representative ideograms.