# First Genetic Screening for Maternal Uniparental Disomy of Chromosome 7 in Turkish Silver-Russell Syndrome Patients

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## Abstract

**Objective:** Silver–Russell syndrome (SRS) is a clinically and genetically heterogeneous syndrome which is characterized by severe intrauterine and postnatal growth retardation, and typical characteristic facial dysmorphisms. It has been associated with maternal uniparental disomy (UPD) for chromosome 7 and hypomethylation of imprinting control region 1 (IGF2/H19) in 11p15. UPD refers to the situation in which both copies of a chromosome pair have originated from one parent. UPD can be presented both as partial heterodisomy and isodisomy. The aim of this study was to determine the maternal UPD7 (matUPD7) in 13 Turkish SRS patients.

**Methods:** Genotyping for matUPD7 was performed with microsatellite markers by polymerase chain reaction.

**Findings:** The maternal UPD7 including the entire chromosome was identified in 1/13 (7.6 %) of individuals within SRS patients. There were no significant differences between clinical features of matUPD7 case and other SRS cases except congenital heart defects.

**Conclusion:** It is often difficult to establish diagnosis of a child with intrauterine growth retardation (IUGR), growth failure and dysmorphic features. Thus, screening for matUPD7 in IUGR children with growth failure and mild SRS features might be a valuable diagnostic tool.

*Iranian Journal of Pediatrics, Volume 22 (Number 4), December 2012, Pages: 445-451*

**Key Words:** Silver–Russell Syndrome; Maternal UPD7; Microsatellite Markers; Intrauterine Growth Retardation

## Introduction

Silver–Russell syndrome (SRS, RSS, OMIM 180860) is a clinically and genetically heterogeneous syndrome which is characterized by severe intrauterine and postnatal growth retardation, feeding difficulties, typical characteristic facial dysmorphic features such as triangular face with a prominent forehead, micrognathia, downturned corners of the mouth and ear anomalies. Further less characteristic findings are cardiac anomalies and genital dysmorphia. Asymmetry of body and limb anomalies present more than 50% of patients[1].

Hypomethylation of the imprinted H19/IGF2 locus in 11p15[1] and matUPD7[2] are two major epigenetic etiologies in SRS. matUPD7 was first reported by Spence et al[3] in a cystic fibrosis patient with SRS like phenotype (short stature and slight asymmetry). After this paper, other cases

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affected by pre and postnatal growth retardation but not SRS phenotype with matUPD7 were published[4-6]. The incidence of SRS is approximately 1/3000 and more than 60 SRS patients with matUPD7 have been reported till the end of 2002[2-16] and matUPD7 has been found in approximately 5-10% of SRS patients with unexplained etiology[17]. After description of matUPD7 SRS cases, both arms of chromosome 7 were searched for candidate genes. Although 7p aberrations of four patients with a SRS phenotype have been described[18], no pathogenic mutations have been identified in several studies focusing on the search for point mutations in 7p-encoded genes so far that might be functionally related to the SRS phenotype. On the other hand, two papers published by Hannula et al[15] and Reboul et al[19] described SRS cases with a segmental maternal UPD 7q showing the importance of human imprinted genes in 7q for human growth and SRS. However, screening studies on mutations in the imprinted genes and transcripts on 7q chromosome region failed to detect any pathogenic variants[20-24].

Ring chromosome 15[25], deletion of 15q[26], deletion of 8q11e13[27], translocations associated with a breakpoint in 17q25[28], deletion of CSH1[29], duplications of 11p15[30], and epimutations of the imprinting center region 1 (ICR1) on 11p15[31] have been reported as the other reasons of SRS. Loss of methylation of ICR1 gene on 11p15, resulting in a down-regulation of IGF-II and H19 genes which were found in 38-64% of cases with SRS, have been reported as the most frequent reason in the etiology of SRS as mentioned before[32,33].

Hypomethylation of matUPD7 has not been studied in Turkish population up to date so far. Therefore, to ascertain the frequency of matUPD7 in the cause of SRS in Turkish population, we decided to screen matUPD7 to perform genotype and phenotype correlation.

## Subjects and Methods

A total of 13 SRS patients aged between 2-9 years, from two genetic subdivisions of the pediatric departments of two University Medical School Hospitals/Turkey were included in this study.

Blood samples were obtained from both parents of patients. Diagnosis of SRS was established in the patients according to the previously described in literature[10].

Chromosomal analysis and subtelomeric fluorescent in situ hybridization (FISH) (TelVysion Probes) (Vysis/Abbott) were performed to identify microscopic and submicroscopic imbalances on all patients. Medical data were reviewed for all patients and evaluated by a clinical geneticist.

Written informed consent was obtained from all families. The study was approved by the Faculty Ethics Committee of the University.

**DNA preparation:** DNA was extracted from 2 mL venous blood taken from all patients and their parents using (DNA Isolation Blood Mini *Kit*, Invitek, Germany) standard procedures.

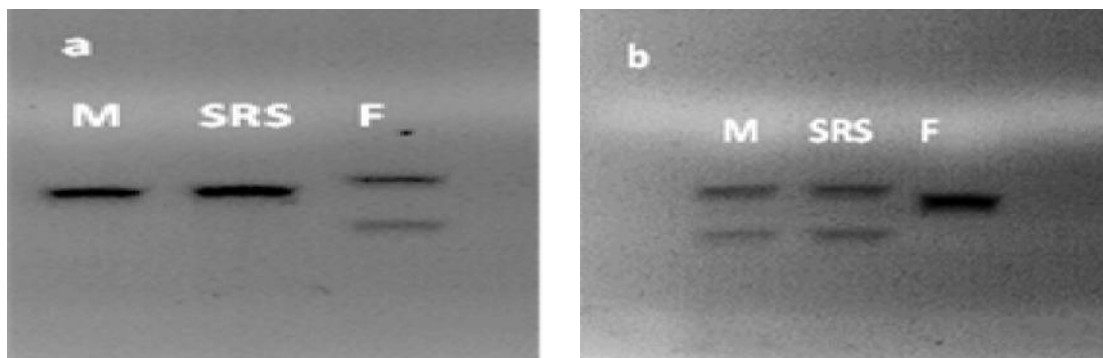**Genetic Analysis:** DNA was isolated from peripheral blood samples by standard procedures.



**Fig 1a–b:** Examples of STR genotyping showing maternal uniparental heterodisomy 7 in SRS patient. **a:** Allelic patterns of the marker D7S821. **b:** Allelic patterns of the marker D7S2195. **M:** Mother, **SRS:** Patient, **F:** Father

Genotyping for matUPD7 in 13 SRS patients was performed on all patients with different chromosome tetra and dinucleotide repeat microsatellite markers (D7S460, D7S821, D7S1808, D7S1818, D7S1804, D7S2195 and D7S2446) by polymerase chain reaction (PCR) (ABI PCR System 9700). PCRs were performed in 25µL reaction containing 50ng DNA, 1xDyNAzyme II buffer, 1.5 mM MgCI2, 300 uM of each dNTP, 0.3 uM of each primer, 1% dimethyl sulfoxide, and 1.25 U Taq DNA polymerase (Fermantase, Leon-Rot, Germany). Amplification was performed with an initial denaturation of 5 minutes at 94°C, followed by 30 to 35 cycles each of 30 s at 94°C, 75 s at 55°C, and 60 s at 72°C with a final extension at 72°C for 10 minutes. Cases of matUPD7 were genotyped for additional chromosome by 7 microsatellite markers, to verify matUPD7 by PCR (Fig. 1). Correct paternity was verified for the matUPD7 patients.

## Findings

Chromosomal analysis and subtelomeric fluorescent in situ hybridization (FISH) analysis were found to be normal in all patients.

One case (7.6%) of matUPD7 was recognized among 13 SRS patients. matUPD7 including the entire chromosome was observed in this case. No cases of segmental matUPD7 were observed.

Clinical features of 13 SRS patients including mat UPD7 patient are given in Table 1. There were no significant differences between clinical features of mat UPD7 case and other SRS cases except congenital heart defects. Echocardiographic studies revealed ventricular septal defect (VSD), patent ductus arteriosus (PDA), and patent foramen ovale (PFO) in the patient with matUPD7.

The matUPD7 case was a 2-year-old girl, the first child of non-consanguineous parents, born 5 weeks before term (33 weeks). Her birth parameters were unknown. There was a history of fetal karyotyping because of intrauterine growth retardation which was found to be normal. On physical examination, her weight was 7500 gr (<3 percentile), height 73 cm (3-10 percentile), and head circumference 48.5 cm (50-75 percentile). She had relative macrocephaly, triangular face, broad prominent forehead, low set ears, downslanted corners of the mouth, micrognathia, clinodactyly of the 5th digit and bracydactyly as dysmorphological features (Fig. 2).

She also had hemihypertrophy of the left side of

**Table 1:** Clinical features of Silver-Russell Syndrome Patients

| | Case | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mat UPD7** | | + | - | - | - | - | - | - | - | - | - | - | - | - | 7.6 |
| **Clinical feature** | IUGR | + | + | + | + | + | - | + | + | - | + | + | + | + | 92 |
| | PNGR | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| | Delayed bone age | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| **Craniofacial features** | Triangular face | + | + | - | + | + | - | - | + | + | + | + | + | + | 76.9 |
| | Relative macrocephaly | + | + | - | + | + | - | - | + | + | + | + | + | + | 76.9 |
| | Frontal bossing | - | - | + | - | - | + | + | - | - | - | - | - | - | 23 |
| | Micrognathia | + | + | - | + | + | - | - | + | + | + | + | + | + | 84.6 |
| | Ear anomalies | - | - | + | - | - | + | + | - | + | + | + | + | + | 61.5 |
| | Downturned mouth corners | + | + | + | - | - | + | + | + | + | + | - | - | - | |
| **Other features** | Skeletal asymmetry | + | + | - | + | - | + | - | + | - | - | - | - | - | 38.4 |
| | Hemihypertrophy | + | + | - | + | + | - | - | + | - | + | - | + | - | 46.1 |
| | Clinodactyly | + | - | + | - | + | + | + | - | + | + | + | + | + | 76.9 |
| | Brachydactyly | + | + | - | + | + | - | - | + | - | + | - | + | - | 46.1 |
| | Syndactyly | - | - | + | - | - | + | + | - | - | - | + | - | + | 38.4 |
| **Development and Behavior** | Delayed psychomotor development | - | + | + | + | + | - | + | - | - | - | + | - | + | 61.5 |
| | Speech difficulties | + | + | - | + | - | - | - | - | + | - | - | - | | 38.4 |
| | Feeding difficulties | + | + | - | - | - | - | - | + | - | + | - | - | - | 30 |
| **Other** | CHD | - | - | - | - | - | - | - | - | - | - | - | - | |

IUGR: Intrauterin Growth Retardation / PNGR: Postnatal Growth Retardation / CHD: Congenital Heart Defect

**Fig. 2:** Dysmorfic features of matUPD7. Facial features including relative macrocephaly, triangular face, broad prominent forehead, down slanted corners of the mouth, micrognathia and hemihypertrophy of the left side of the body are seen.

the body. Feeding by tube was necessary during the first 3 months. Developmental milestones were as follows: sitting without support at age of 8 months, walking alone and speaking several words at age of 14 months. The usual laboratory findings were in the normal range. All metabolic and endocrinologic investigations were found to be normal.

## Discussion

It has been reported that matUPD7 cases cover approximately 10% of all SRS patients[8,9]. In the study presented matUPD7 was detected in 7.6% of SRS patients, which is slightly lower than that in previous reports and this could be caused by the low number of SRS patients in our study. SRS has variable characteristics, however it is easy to recognize with typical symptoms, but diagnosis may be difficult in milder cases. Our matUPD7 patient presented with mild SRS characteristics as the other matUPD7 cases previously described (Table 2). MatUPD7 has been considered to be a subgroup among SRS patients with mild dysmorphic features, severe feeding difficulties and delayed speech[16].

Our patients represented the spectrum of dysmorphic features and developmental difficulties seen typically in patients with SRS (Table 1). The dysmorphic features in matUPD7 patients have been reported to be mild in both SRS[8,16,34] and patients without SRS, and these patients have also been defined as having intrauterine growth retardation (IUGR) and postnatal growth retardation (PNGR) with slight dysmorphic features[35]. It is often difficult to establish a diagnosis of a child with IUGR, growth failure and dysmorphic features. Although growth retardation of unknown cause has been associated with UPD, Hannula et al showed that it is not worthwhile to screen those patients for matUPD7 unless they show characteristic features of SRS and severe growth retardation. The matUPD7 patient described here shows the fulfilling of diagnostic criteria for SRS and supports the suggestion that matUPD7 is not a general cause for growth retardation[35]. Interestingly matUPD7 was also reported in three patients with cystic fibrosis (MIM 219700) and unexpectedly severe short stature[3,5,12]. Among these three cases one had growth hormone (GH) deficiency. Screening for matUPD7 is therefore advisable if abnormally short stature occurs with cystic fibrosis[5] or other recessive disorders mapped to chromosome[7].

Although matUPD and ICR1 hypomethylation patients have overlapping features, asymmetry, fifth finger clinodactyly and congenital anomalies are more commonly seen in patient with ICR1 hypomethylation, whereas learning difficulties and referral for speech therapy are more likely with matUPD7[36]. Our matUPD7 patient had mild

dysmorphic features of SRS that was similar to the patients previously described[8,9,11].

*PEG1/MEST*[37,38] and *COP*35 both at 7q32, and *GRB10*36,37 at 7p11.2-p12,3 are the imprinted genes in SRS that have been identified so far. Paternal mutation or a deletion in the related imprinting gene or genes presents with similar symptoms as in matUPD7 patients. On the other hand, fetal growth regulator genes *EGFR* (epidermal growth factor receptor), *IGFBP1,* and *IGFBP3* (insulin-like growth factor binding protein 1 and 3) are the other candidate imprinting genes that were thought to be responsible in SRS etiology. Congenital heart defects observed in this patient which has not been reported in matUPD7 cases could be the result of one of these or other imprinted gene dysfunctions. Imprinting genes on chromosome 7 may be responsible for this additional anomaly or there may be coincidence.

Methylation analysis indicated that in maternal but not paternal alleles, CpG islands are completely methylated in the promotor region, however, a recent study showed a de novo deletion in 7q32 affecting the paternal imprinted MEST/PEG1 gene copy[39].

One of the other mechanisms in the SRS genetic etiology is matUPD7 mosaicism. It is generally diffucult to exclude mosaicism in an infant with UPD in SRS but it is identified in a patient related with relatively mild SRS-like phenotype[40]. In addition, IUGR and prenatal abnormalities are associated with confined placental mocaicism (CPM). CPM is occasionally found with UPD, where after a meiotic error leads to trisomy, followed by trisomic rescue leads to a UPD cell line in the fetus. Therefore, in cases of UPD originating from CPM, it is difficult to ascertain whether the growth retardation is caused by UPD in the fetus, or the UPD cell line or trisomic cell line in the placenta[41]. We coincidently ruled out CPM, by observing the normal karyotype after chorionic villus sampling with the indication of advanced maternal age.

One of the limitations in our study is the lack of evaluation of the other responsible etiologies, such as hypomethylation of the imprinted H19/IGF2 locus, submicroscopic genomic alterations and mutations in the candidate genes. The second limitation is the low number of patients included in the study.

## Conclusion

matUPD7 was detected in 7.6% of SRS patients firstly in Turkish population, which is slightly lower than that in previous reports. It is also worth to emphasize that congenital cardiopathy of the matUPD7 case has not been described in the literature to date. We suggest testing all patients with intrauterine and severe postnatal growth retardation with only slight signs of SRS.

## Acknowledgment

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