

ELECTRONIC LETTER

Is maternal duplication of 11p15 associated with Silver-Russell syndrome?

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Background: Silver-Russell syndrome (SRS) is a heterogeneous malformation syndrome characterised by intrauterine and postnatal growth retardation (IUGR, PGR) and dysmorphisms. The basic causes are unknown, however in approximately 10% of patients a maternal uniparental disomy (UPD) of chromosome 7 or chromosomal aberrations can be detected. Four growth retarded children, two with SRS-like features, associated with maternal duplications of 11p15 have been described. Considering the involvement of this genomic region in Beckwith-Wiedemann overgrowth syndrome (BWS), we postulated that some cases of SRS—with an opposite phenotype to BWS—might also be caused by genomic disturbances in 11p15.

Methods: A total of 46 SRS patients were screened for genomic rearrangements in 11p15 by STR typing and FISH analysis.

Results: Two SRS patients with duplications of maternal 11p15 material in our study population (n=46) were detected. In patient SR46, the duplicated region covered at least 9 Mb; FISH analysis revealed a translocation of 11p15 onto 10q. In patient SR90, additional 11p15 material (approximately 5 Mb) was translocated to the short arm of chromosome 15.

Conclusions: We suggest that diagnostic testing for duplication in 11p15 should be offered to patients with severe IUGR and PGR with clinical signs reminiscent of SRS. SRS is a genetically heterogeneous condition and patients with a maternal duplication of 11p15.5 may form an important subgroup.

Silver-Russell syndrome (SRS) is a heterogeneous syndrome mainly characterised by severe intrauterine and postnatal growth retardation (<3rd percentile, P3) (IUGR, PGR), typical craniofacial features such as a prominent forehead and a triangular face, hemihypotrophy, and clinodactyly (table 1). Several reports on SRS families as well as on chromosomal disturbances indicate the disease has a genetic background. As chromosomal aberrations affecting the short arm of chromosome 7 and the long arm of chromosome 17 have been identified several times, studies on the genetic basis of SRS have focused on these regions (for a review, see Hitchins *et al*¹). Furthermore, in 7–10% of SRS patients, maternal uniparental disomy (UPD) of chromosome 7 can be detected.

Recently two reports suggested a putative role of factors in 11p15 in the aetiology of SRS. In 2000, Kosaki and coworkers² reported on a patient with an SRS-like phenotype and a maternally derived duplication in 11p15. Three further cases with a duplication of maternal 11p15 material were described by Fisher *et al*³; all patients showed IUGR and PGR and one patient was initially diagnosed to have SRS features.

Interestingly, paternally derived rearrangements in 11p15 and paternal UPD11 are associated with the overgrowth

disease Beckwith-Wiedemann syndrome (BWS). In the case of BWS, several genomic disturbances within 11p15 have been shown to be involved in the aetiology of the disease (for a review, see Weksberg *et al*⁴).

To assess whether duplications/deletions or UPD of 11p15 are present in SRS patients, we analysed 46 German SRS families by short tandem repeat (STR) typing. While UPD of 11p15 was not detected, STR patterns in two patients point to a duplication of maternal 11p15 material. These patients were therefore further analysed by molecular cytogenetic approaches.

METHODS

Study population

All 46 patients showed SRS features corresponding to those reported by Wollmann *et al*.⁵ UPD of chromosomes 2, 7, 9, 14, 16, and 20 as well as endocrinological abnormalities were excluded in previous studies. The study was approved by the ethical committees of the universities of Tübingen and Aachen.

DNA studies

The search for unbalanced rearrangements in chromosome 11p15 was initially performed by STR typing. The markers used were D11S4046 and D11S1984 proximal to the *IGF2* gene, and D11S4177 distal to this region. In case of uninformativity, the markers D11S922, D11S1318, and D11S1758 were typed. In families with unusual inheritance of these markers, further STRs were analysed (table 2). After electrophoresis on a denaturing sequencing gel, the alleles were visualised by an automated ABI377 sequencing system (ABI, Darmstadt, Germany). Information on primers and the physical order of the markers was obtained from the Genome Database (www.gdb.org) and from the UCSC browser (www.genome.ucsc.edu).

Cytogenetic and FISH studies

Chromosome preparations were obtained from peripheral lymphocytes and G banding was performed according to standard protocols. Fluorescence in situ hybridisation on peripheral lymphocytes of both patients was performed using (a) a whole chromosome painting probe (WCP) for chromosome 11 (Quantum/Applicgene, Illkirch, France) and (b) BAC clones from the RP11 library mapping to 11p15 (RP11-89F15, RP11-222J5, RP11-645I8). The latter were provided by the BACPAC Resource Centre (Buffalo, NY, USA). BAC-DNA was labelled by nick translation. In case of SR46, additional FISH analyses included hybridisations with a WCP for chromosome 10, a 10q telomeric probe (D10S2290; Qbiogene,

Abbreviations: BWS, Beckwith-Wiedemann syndrome; IUGR, intrauterine growth retardation; PGR, postnatal growth retardation; rhGH, recombinant human growth hormone; SRS, Silver-Russell syndrome; STR, short tandem repeat; UPD, uniparental disomy; WCP, whole chromosome painting probe

Table 1 Frequency of clinical features in the general SRS population compared to maternal UPD7 cases and to those with maternal duplication of 11p15 and available clinical data

Symptom	Frequency (n = 143)*	Mat UPD7 (n = 35)†	Fisher 1 [‡]	Fisher 2 [‡]	SR46	SR90
Partial karyotypes			inv dup del(11)	t(11;20)	t(10;11)	t(11;15)
Gender			F	F	M	F
Birth weight <3rd percentile	94%	97%	+	+	+	+
Short stature	99%	100%	+	+	+	+
Hemihypotrophy	51%	34%	-	-	+	-
Relative macrocephaly	64%	70%	-	-	-	+
Triangular face	79%	62%	-	+	-	-
Down slanting corners of the mouth	46%	0%	-	+	-	-
Irregular teeth	28%		/	/	+	-
Ear anomalies	53%		/	/	+	+
Clinodactyly V	68%	56%	+	+	+	+
Brachydactyly V	48%		/	/	+	+
Syndactyly	19%		+	/	-	-
Simian crease	25%		/	/	(+)	-
Café au lait spots	19%	4%	/	/	-	-
Psychomotor retardation	37%	38%	+	-	+	(+)
Muscular hypotrophy/tony	45%		/	-	+	-
Squeaky voice	22%		/	/	+	+
Early puberty	8%		+	Too young	-	-
Precocious puberty	5%		-	Too young	-	-

*Wollmann *et al*.; †for a review, see Hitchins *et al*.; ‡diagnosis of SRS was discussed; /, not reported; -, not present.

Heidelberg, Germany), and a BAC clone in 10q26 (RP11-35C24). FISH was performed according to standard protocols. Slides were counterstained with DAPI vector shield and metaphase images were captured and enhanced with Isis software (Metasystems, Altlußheim, Germany).

RESULTS

Screening for duplications, deletions, or UPD of the chromosomal region 11p15 in 46 SRS families, we did not identify any case of UPD or deletion but did identify two patients with a duplication of maternal 11p15 material.

In family SR46, the affected region consisted of at least 9 Mb in 11p15 bordered by D11S2071 at the tip of 11p and D11S4188 (table 2). In the second family, SR90, the

duplicated segment included at least 5 Mb in the same region, spanning from D11S2071 to D11S1760 (fig 1, table 2).

Since conventional cytogenetic analyses in external laboratories did not reveal any suggestion of chromosomal aberrations, more detailed karyotyping was repeated in both families. In family SR46, a translocation of 11p15 onto 10qter was visible by GTG banding, resulting in a partial trisomy 11p15 (karyotype: 46,XY,der(10)t(10;11)(q26.3;p15.3)) (fig 2A).

More detailed cytogenetic investigation of patient SR90 showed an apparently normal female karyotype. Karyotypes of the parents of both patients were normal.

The STR results were then confirmed by FISH. Hybridisation with a chromosome 11 WCP probe revealed

Table 2 Results of STR typing in SRS families SR46 and SR90

Marker	Mb from 11pter*	SR46			SR90		
		Father	Mother	Child	Father	Mother	Child
D11S2071	0.95	2-3	1-4	1†-3	1-2	3-4	1-3†
D11S4177	1.45	1-3	1-2	1†-3	-	-	1
D11S1984	1.53	1-2	1	1	2-3	1-2	1†-3
D11S922	1.57	1-4	2-3	3†-4	1	2-3	1-3†
D11S4046	1.92	1-2	1	1†-2	1-2	3	1-3†
IGF2	2.11						
D11S1318	2.29	2-4	1-3	3†-4	1-2	3	1-3†
D11S4088	2.71	2	1	1†-2	1-4	2-3	3†-4
D11S1758	4.70	2	1-3	1†-2	-	-	1
D11S4181	4.73	1	2-3	1-2†	-	-	1
D11S1760	5.34	1-3	1-2	2†-3	3-4	1-2	1†-4
D11S4124	5.52	1	1-2	1-2†	3-4	1-2	1-4
D11S1338	5.95	1-2	2	1†-2	1-2	1-2	1
D11S1323	6.24	1-3	2	1-2†	1-3	1-2	1-2
D11S1331	7.26	1-2	1	1	1-2	2	1-2
D11S932	8.36	3-4	1-2	1†-3	1-2	1-3	1-3
D11S909	8.74	1-3	2-3	1-3†	2	1-2	2
D11S4188	9.03	2	1	1†-2	-	-	-
D11S4149	9.09	1-2	2	2	2	1-2	2
D11S1904	10.35	1-2	3	2-3	2-3	1-3	2-3
D11S1329	10.68	1-3	2-3	3	1-3	2-3	1-3
D11S875	10.99	1-2	1-2	1-2	1-2	2	1-2
D11S4189	11.56	1	2-3	1-2	1	2	1-2
D11S1349	11.72	1-3	2-3	3	2-3	1-2	1-2
D11S1334	12.92	1-3	2-3	2-3	1-3	2	2-3
D11S4170	14.47	2-3	1-3	1-3	1-2	1-3	1-3
D11S4121	15.34	2-3	1-2	1-2	1-2	1	1-2
D11S4125	133.98	1-2	3-4	1-3	1-4	2-3	1-2

*Physical data were obtained from the UCSC genome browser; †intensities were increased, indicating two copies of the same allele; -, not typed.

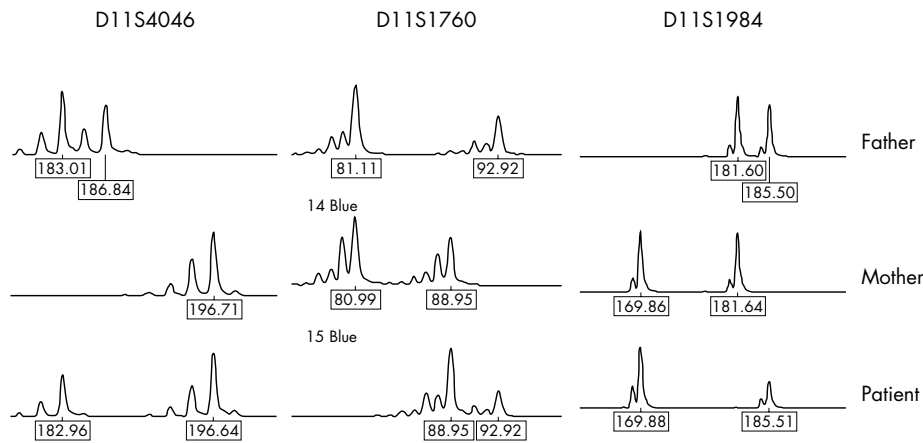


Figure 1 Inheritance of alleles of patient SR90 and her parents for three STR markers within the duplicated region in 11p15. In comparison to the paternally inherited alleles, the patient's DNA showed increased copy numbers of the maternally inherited allele 196.64 at locus D11S4046, allele 88.95 at D11S1760, and allele 169.88 at D11S1984.

translocations of 11p15 material in both patients: apart from the two homologous chromosomes 11, the 11p15 segments translocated on 10q (SR46) and 15p (SR90) both showed hybridisation signals. FISH with BAC probes specific for 11p15 confirmed the translocations.

In case of SR46, FISH with a WCP of chromosome 10 did not display additional signals on chromosomes other than chromosome 10. The 10q telomeric probe D10S2290 showed hybridisation to only one chromosome 10, while the BAC probe in 10q26 revealed regular hybridisation patterns, thus indicating that the 10q deletion is restricted to the telomeric region.

In SR90, the FISH results and DAPI staining allowed the identification of a de novo cryptic translocation of 11p15 onto 15p (karyotype: 46,XX,der(15)t(11;15)(p15;p12).ish der(15)(wcp11+,RP11-89F15+,RP11-222J5+) (fig 2B).

Clinically, our two patients with duplications of 11p15 showed SRS features as described by Wollmann *et al*⁵ (table 1).

The male patient SR46 was born at term with a birth length of 42 cm, a weight of 1650 g, and a occipitofrontal circumference (OFC) of 32 cm (all <P3). The severe growth restriction persisted in later life: at 16 years of age, his height was 142 cm (SDS -4.6), weight was 25 kg, and OFC was 48 cm (SDS -5.4). His parents were of normal height (father: 180 cm, mother 165 cm). Further clinical signs included micrognathia, irregularities of teeth, ear anomalies, clino- and brachydactyly V, and a simian crease. His voice was squeaky. The boy suffered from muscular hypotonia and showed delayed psychomotor development.

Patient SR90 is the second child of healthy parents. After a 38 week uneventful pregnancy, the girl's length at birth was 44 cm (<P3), weight was 2050 g (<P3), and OFC was 34.5 cm (>P94). Her father's height is 178 cm and her mother's height is 166 cm; menarche occurred at age 14.

Dysmorphic features at birth included macrocephaly with frontal bossing, slight epicanthus, microphthalmia, narrow lower jaw, and dysplastic ears; the craniofacial aspects were reminiscent of SRS. Additionally, clino-brachydactyly V and a low set thumb were diagnosed, but hemihypotrophy of the limbs was not present. MRI of the brain was normal with broad subarachnoid spaces and a widened interhemisphere distance. Early psychomotor development was delayed. The child attends regular primary school.

The girl presented at endocrine clinics at the age of 4 with severe short stature (height SDS -4.0). The patient showed a retarded bone age, and her voice was squeaky. Treatment with recombinant human growth hormone (rhGH) was started at the age of 5.4 years. At the age of 9 years, after 3.6 years of continuous treatment with rhGH, height (133.1 cm; SDS -0.71) was completely normalised. The phenotype of the girl, which had been rather typical for a

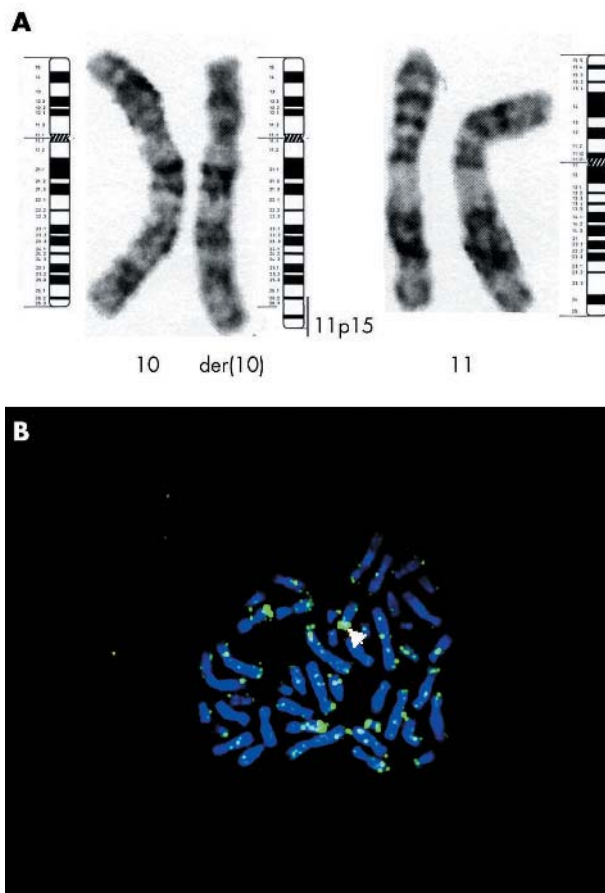


Figure 2 (A) Partial karyotype of patient SR46 illustrating the translocation of 11p15 material onto 10q. (B) FISH result in patient SR90 using the BAC derived probe RP1-89F15. The probe shows regular hybridisation to the tips of the unaffected chromosomes 11 as well as hybridisation to 15p (marked by an arrow).



Figure 3 Patient SR90 at 9 years. (The authors have written consent from the legal guardian of patient SR90 for publication of the images in print and online.)

child with SRS in infancy and early childhood, was still dysmorphic, but less striking than before (fig 3). For clinicians this is well known: the facial SRS phenotype is most striking in infancy and early childhood, whereas most patients would not be diagnosed as having SRS during adolescence.

DISCUSSION

Typing of STRs in 11p15 in a cohort of SRS patients allowed us to identify two patients with maternal duplications of this chromosomal region. Both patients were referred for molecular analysis because of clinical signs corresponding to SRS (table 1); clinical findings reminiscent of BWS were not present. In fact, the patients share several clinical features with patients with maternal duplication of 11p15 in the literature (table 1), in particular IUGR and PGR. Additionally, features resembling those of SRS were present in our patients as well as in two patients from the literature.^{2,3} It is therefore conceivable that patients with maternal 11p15 duplications have a common phenotype corresponding to SRS.

Whether the cryptic deletion at the tip of 10q influences the phenotype in patient SR46 is unclear at the moment. Several patients carrying (cryptic) 10q deletions have been reported,^{6,7} but a uniform clinical picture could not be defined. On the other hand, Martin and coworkers⁸ described a 10q telomere duplication in a handicapped boy and his phenotypically normal mother.

The finding that in all reported growth retarded patients with duplication of 11p15 the maternal copy is duplicated is interesting since the presence of two paternally derived copies of the same region result in the overgrowth syndrome BWS. Thus, growth retardation in patients with maternal duplication of 11p15 might have causes similar to those of BWS and possibly represents the opposite phenotype. In addition, Constanca *et al*⁹ recently demonstrated the

profound contribution of *Igf2* to fetal growth in mice; its human homologue *IGF2* is localised in 11p15. We, therefore, decided to screen three obvious candidate genes/transcripts in 11p15, *IGF2*, *CDKN1C*, and *KCNQ1OT1*, for mutations in SRS patients but failed to detect any pathogenic variant.^{10,11} Of course, further studies are needed to characterise the duplicated regions in 11p15 for alterations responsible for growth retardation and associated phenotypes.

Due to the heterogeneity of SRS, the disease might represent a common phenotype caused by any of the members of the *insulin-like growth factor 1 receptor (IGF1R)* axis such as the ligand *IGF2*, its receptor *IGF1R*, and the *IGF* signalling modulator *GRB10*. Interestingly, carriers of deletions in 15q26-qter who are hemizygous for *IGF1R* as well as carriers of duplications in 7p including the *GRB10* gene, are known to present with features compatible with the diagnosis of SRS; *IGF1R* mutations have been shown to be involved in IUGR and PGR.¹² However, *IGF1R* as well as *GRB10* has been extensively studied in SRS patients but both have been excluded as playing a major role in the aetiology of the disease (for a review, see Hitchins *et al*¹).

Searching for duplications in 11p15 in a cohort of 46 SRS patients revealed two cases with maternal duplications of this region, corresponding to a frequency of more than 4%. The results in these patients as well as in those SRS patients with rearrangements in chromosomes 7 and 17, show that (high resolution) conventional cytogenetic analysis is indicated in growth retarded patients with SRS-like features.

In conclusion, the search for maternal duplication of 11p15 will shed more light on the aetiology of SRS and growth disturbances in general. Since SRS patients show a broad phenotypic spectrum and SRS is therefore a clinically heterogeneous condition, it is conceivable that carriers of 11p15 disturbances belong to a subpopulation of SRS patients as suggested for maternal UPD7 carriers.¹³ It will be interesting to see whether 11p15 rearrangements will be detectable in other patient cohorts characterised by growth disturbances. However, diagnostic testing for duplication in 11p15 should be offered to patients with severe IUGR and PGR in combination with clinical signs reminiscent of SRS.

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ELECTRONIC-DATABASE INFORMATION



The Genome Database can be found at www.gdb.org and the UCSC browser at www.genome.ucsc.edu.

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