



University of Zurich  
Zurich Open Repository and Archive

Winterthurerstr. 190  
CH-8057 Zurich  
<http://www.zora.uzh.ch>

---

*Year: 2009*

---

## Submicroscopic chromosomal imbalances in idiopathic Silver-Russell syndrome (SRS): the SRS phenotype overlaps with the 12q14 microdeletion syndrome

Spengler, S; Schönherr, N; Binder, G; Wollmann, H; Fricke-Otto, S; Mühlenberg, R; Denecke, B; Baudis, M; Eggermann, T

Spengler, S; Schönherr, N; Binder, G; Wollmann, H; Fricke-Otto, S; Mühlenberg, R; Denecke, B; Baudis, M; Eggermann, T (2009). Submicroscopic chromosomal imbalances in idiopathic Silver-Russell syndrome (SRS): the SRS phenotype overlaps with the 12q14 microdeletion syndrome. *Journal of Medical Genetics*:Epub ahead of print. Postprint available at:  
<http://www.zora.uzh.ch>

Posted at the Zurich Open Repository and Archive, University of Zurich.  
<http://www.zora.uzh.ch>

Originally published at:  
*Journal of Medical Genetics* 2009, :Epub ahead of print.

# Submicroscopic chromosomal imbalances in idiopathic Silver-Russell syndrome (SRS): the SRS phenotype overlaps with the 12q14 microdeletion syndrome

## Abstract

Silver-Russell syndrome (SRS) is a heterogeneous disorder associated with intrauterine and postnatal growth restriction, body asymmetry, a relative macrocephaly, a characteristic triangular face and further dysmorphisms. In about 50% of patients genetic/epigenetic alterations can be detected: >38% of patients show a hypomethylation of the IGF2/H19 imprinting region in 11p15, additional 10% carry a maternal uniparental disomy of chromosome 7. In single cases, cytogenetic aberrations can be detected. Nevertheless, there still remain 50% of SRS patients without known genetic/epigenetic alterations. To find out whether submicroscopic imbalances contribute to the aetiology of SRS we screened 20 idiopathic SRS patients with the Affymetrix GeneChip(R) Human Mapping 500 K array set. Apart from known atherogenic copy number variations (CNVs) we identified one patient with a 12q14 microdeletion. The 12q14 microdeletion syndrome is characterised by dwarfism but it additionally includes mental retardation and osteopoikilosis. The deletion in our patient is smaller than those in the 12q14 microdeletion carriers but it also affects the LEMD3 and the HMGA2 genes. LEMD3 haploinsufficiency and point mutations have been previously associated with osteopoikilosis but radiographs of our patient at the age of 16 years did not reveal any hint for osteopoikilosis lesions. Haploinsufficiency of HMGA2 is probably responsible for aberrant growth in 12q14 microdeletion syndrome. However, we excluded a general role of HMGA2 mutations for SRS by sequencing of 20 idiopathic patients. In conclusion, our results exclude a common cryptic chromosomal imbalance in idiopathic SRS patients but show that chromosomal aberrations are relevant in this disease. Thus molecular karyotyping is indicated in SRS and should be included in the diagnostic algorithm.



## Submicroscopic chromosomal imbalances in idiopathic Silver-Russell syndrome (SRS): the SRS phenotype overlaps with the 12q14 microdeletion syndrome

Sabrina Spengler, Nadine Schönherr, Gerhard Binder, Hartmut Wollmann, Susanne Fricke-Otto, Reinhard Mühlenberg, Bernd Denecke, Michael Baudis and Thomas Eggermann

*J. Med. Genet.* published online 16 Sep 2009;  
doi:10.1136/jmg.2009.070052

---

Updated information and services can be found at:  
<http://jmg.bmj.com/cgi/content/abstract/jmg.2009.070052v1>

---

*These include:*

### Rapid responses

You can respond to this article at:  
<http://jmg.bmj.com/cgi/eletter-submit/jmg.2009.070052v1>

### Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

---

### Notes

---

**Online First** contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

---

To order reprints of this article go to:  
<http://journals.bmj.com/cgi/reprintform>

To subscribe to *Journal of Medical Genetics* go to:  
<http://journals.bmj.com/subscriptions/>

*Letter to the editor:*

**Submicroscopic chromosomal imbalances in idiopathic Silver-Russell  
syndrome (SRS): the SRS phenotype overlaps with the 12q14 microdeletion  
syndrome**

Sabrina Spengler<sup>1</sup>, Nadine Schönherr<sup>1</sup>, Gerhard Binder<sup>2</sup>, Hartmut A. Wollmann<sup>2</sup>, Susanne Fricke-Otto<sup>3</sup>, Reinhard Mühlenberg<sup>3</sup>, Bernd Denecke<sup>4</sup>, Michael Baudis<sup>5</sup>, Thomas Eggermann<sup>1</sup>

<sup>1</sup>Institute of Human Genetics, University Hospital, RWTH Aachen, Germany

<sup>2</sup>University-Children's Hospital, Pediatric Endocrinology and Diabetology, Tübingen, Germany

<sup>3</sup>Children's Hospital, Helios Hospital, Krefeld, Germany

<sup>4</sup>Interdisciplinary Centre for Clinical Research, IZKF "BIOMAT", RWTH Aachen, Germany

<sup>5</sup>Institute of Molecular Biology, University of Zürich, Switzerland

Correspondence should be addressed to:

Thomas Eggermann, Ph.D.  
Institute of Human Genetics  
University Hospital, RWTH Aachen  
Pauwelsstr. 30  
D-52074 Aachen  
Phone: +49 241 8088008  
Fax: +49 241 8082394  
e-mail: [teggermann@ukaachen.de](mailto:teggermann@ukaachen.de)

**Key-words:** Silver-Russell syndrome – 12q14 microdeletion – microarray

## Abstract

Silver-Russell syndrome (SRS) is a heterogeneous disorder associated with intrauterine and postnatal growth restriction, body asymmetry, a relative macrocephaly, a characteristic triangular face and further dysmorphisms. In about 50% of patients genetic/epigenetic alterations can be detected: >38% of patients show a hypomethylation of the IGF2/H19 imprinting region in 11p15, additional 10% carry a maternal uniparental disomy of chromosome 7. In single cases, cytogenetic aberrations can be detected. Nevertheless, there still remain 50% of SRS patients without known genetic/epigenetic alterations. To find out whether submicroscopic imbalances contribute to the aetiology of SRS we screened 20 idiopathic SRS patients with the Affymetrix GeneChip® Human Mapping 500 K array set. Apart from known atherogenic copy number variations (CNVs) we identified one patient with a 12q14 microdeletion. The 12q14 microdeletion syndrome is characterised by dwarfism but it additionally includes mental retardation and osteopoikilosis. The deletion in our patient is smaller than those in the 12q14 microdeletion carriers but it also affects the *LEMD3* and the *HMGA2* genes. *LEMD3* haploinsufficiency and point mutations have been previously associated with osteopoikilosis but radiographs of our patient at the age of 16 years did not reveal any hint for osteopoikilosis lesions. Haploinsufficiency of *HMGA2* is probably responsible for aberrant growth in 12q14 microdeletion syndrome. However, we excluded a general role of *HMGA2* mutations for SRS by sequencing of 20 idiopathic patients. In conclusion, our results exclude a common cryptic chromosomal imbalance in idiopathic SRS patients but show that chromosomal aberrations are relevant in this disease. Thus molecular karyotyping is indicated in SRS and should be included in the diagnostic algorithm.

## Sirs -

Silver-Russell syndrome (SRS, OMIM180860) is a clinically and genetically heterogeneous disorder which is mainly characterised by severe intrauterine and postnatal growth retardation and a characteristic small, triangular face. The disease is associated with failure to thrive and additional dysmorphic features including fifth finger clinodactyly and hemihypoplasia. Although a clinical scoring system to assist the diagnosis has recently been suggested<sup>1</sup>, the accuracy of diagnosis is influenced by the experience of the clinical investigator. Furthermore, the clinical picture of SRS in adulthood is less clear than in early childhood. The clinical heterogeneity is reflected by the heterogeneous genetic/epigenetic findings in SRS patients: in about 10% of cases a maternal uniparental disomy of chromosome 7 (UPD(7)mat) can be detected, whereas >38% carry a methylation defect in the telomeric imprinted region on chromosome 11p15 (for review: <sup>2</sup>). Indeed, the 11p15 epimutation carriers often show the more typical SRS phenotype while UPD(7)mat carriers are milder affected (for review: <sup>3</sup>). Nevertheless, many exceptions have been reported thereby making a strict genotype-phenotype correlation impossible. In addition to these two major disturbances, several SRS patients carry microscopic detectable structural aberrations affecting numerous chromosomes, but only chromosomes 7, 11 and 17 were repeatedly involved in individuals fulfilling strict diagnostic criteria of SRS (fig. 1).

Up to now, chromosomal analysis in SRS has been based on conventional karyotype analysis but a systematic screen for (sub)microscopic disturbances has not yet been performed. With the development of array based genomic screening techniques, a new powerful tool for detection of cryptic imbalances has recently become available. While several new microdeletion syndromes have been identified using genomic array technology (for review: <sup>4</sup>), the search for genomic imbalances has nearly always been focused on patients with mental retardation and facultative clinical features. The need to check SRS patients for submicroscopic chromosomal imbalances was recently illustrated by the identification of two SRS patients carrying 11p15 duplications with a size of 1 Mb and 5.6 Mb respectively (for review: <sup>2</sup>).

We screened a cohort of idiopathic SRS patients for cryptic chromosomal imbalances by single-nucleotide polymorphism oligonucleotide arrays (SNP array) thereby identifying one carrier of a 12q14 microdeletion. Within this region, the gene of the nonhiston chromosomal protein HMGA2 is localised. The identification of a *HMGA2* mutation in the “pygmy” mouse indicated that HMGA2 plays a role in aberrant growth and development.<sup>5</sup> We therefore sequenced the coding region of *HMGA2* in the deletion carrier as well as in 19 further SRS patients without 11p15 epimutation.

The study population consisted of 20 patients with SRS, ascertained as part of ongoing molecular investigations on SRS. The diagnosis of SRS was based on the following criteria: intrauterine growth retardation (birth weight or length below the 3<sup>rd</sup> percentile), lack of postnatal catch-up growth, and at least two of the following criteria: typical face, relative macrocephaly, and skeletal asymmetry. In all patients, 11p15 epimutation and UPD(7)mat had previously been excluded. The study was approved by the ethical committee of the University Hospital Aachen.

Genomic DNA of the probands was isolated from peripheral lymphocyte cells by a simple salting-out procedure.

All samples were investigated by micorarray typing. The used 500 K array (Affymetrix, High Wycombe, UK) consists of two arrays, the *NspI* kit and the *StyI* kit which together include >500,000 SNPs. DNA was processed according to the manufacturers instructions.

Hybridisation and washing was performed according to the manufacturer’s manual. Arrays

were recorded using an Affymetrix GeneChip®Scanner 3000 7G. Data processing including quality assessment was performed using the “R” statistical framework (<http://www.r-project.org>) with dedicated extensions from the “aroma.affymetrix” project.<sup>6</sup> Copy number segmentation results were visualised using tools developed for the Progenetix project (<http://www.progenetix.net>;<sup>7</sup>). The 12q14 microdeletion was then confirmed by quantitative PCR and by typing of the Affymetrix GeneChip®Genome-Wide Human SNP 6.0 array. Direct sequencing of the coding region of the *HMGA2* gene was performed by using the BigDyeTerminatorCycleSequencing System (Applied Biosystems, Weiterstadt, Germany). Primers and PCR conditions are available on request. Samples were electrophoresed on an automatic ABI3130 sequencing system (Applied Biosystems).

Apart from numerous known apathogenic copy number variations (CNVs) in each patient, we identified altogether 7 different so far unregistered copy number alterations (CNAs) in 5 of the 20 patients (table 1). Six affected regions did not harbour genes and were therefore excluded from further analysis. However, the screening for submicroscopic imbalances in 20 idiopathic SRS patients did not provide evidence for a common chromosomal aberration in this syndrome. This finding is not surprising if we consider the extreme clinical but also genetic/epigenetic heterogeneity in this disease. Also, this observation is in line with reports from other genomic screening studies reporting the heterogeneity of pathological CNAs in patients analysed due to one or related phenotypical studies (e.g. studies on mental retardation, epilepsy autism; for review:<sup>4</sup>).

Nevertheless, the identification of one among 20 so far idiopathic SRS patients carrying a microdeletion underlines the necessity to routinely test SRS patients by molecular karyotyping.

In this patient (SR29), we detected a de-novo 1.35 Mb deletion in 12q14, among others the deletion includes the *LEMD3* and the *HMGA2* genes (fig. 2). The girl was the second child of healthy unrelated parents. Her father’s height is 185 cm, her mother’s height is 166 cm. Family history is unremarkable.

Intrauterine growth retardation was noted in the 20<sup>th</sup> week of pregnancy. The child was born at term, length at birth was 46 cm (-2.59 standard deviation (SD)), weight was 2700 g (-1.83 SD). Apgar scores were 10 and 10. Psychomotoric development was unremarkable. Feeding difficulties and a squeaky voice were reported. At an age of 1 year and 9 months, the girl spoke 10 words.

The patient was referred for clinical evaluation at age 1 9/12 years, her height was 70.8 cm (-4.5 SD), weight was 6800 g (-5.4 SD), OFC was 43.7 cm (-3.3 SD) (table 2). In addition to relative macrocephaly she presented a prominent forehead, a slightly triangular face, slightly dysplastic ears, and clinodactyly of the fifth digit. She showed no evidence for lateral asymmetry or skeletal abnormalities.

At the age of 16 years, radiographic evaluation of the right tibia and fibula as well as the right foot did not reveal any lesions. A convex scoliosis was noted. The girl successfully visits a grammar school (“Gymnasium”).

Sequencing of the coding region of the *HMGA2* gene in 12q14 which was deleted in patient SR29 did not reveal any pathogenic mutation neither in the remaining *HMGA2* copy of patient SR29 nor in the 19 additionally sequenced idiopathic SRS patients.

Interestingly, SRS patients share clinical features with one of these recently defined microdeletion disorders, the 12q14 microdeletion syndrome. This entity was firstly reported by Menten et al.<sup>8</sup> and is characterised by failure to thrive in infancy, osteopoikilosis, short stature and mental retardation. In one of these cases the diagnosis of SRS was considered but

then discarded. The three unrelated patients carried deletions ranging from 3.44 Mb to 6 Mb (fig. 2). Among other genes, in all patients the *LEMD3* gene was deleted which was previously shown to be the causal gene for osteopoikilosis (for review: <sup>8</sup>), an uncommon and usually asymptomatic benign sclerosing bone dysplasia. Two further genes in the common deleted regions were *GRIP1* and *HMGA2*. The glutamate receptor interacting protein 1 (GRIP1) is highly expressed in fetal and adult human brain, and involved in glutamergic synaptic transmission, thus a correlation between *GRIP1* haploinsufficiency and learning problems in the 12q14 microdeletion carriers has been proposed. For *HMGA2* an important role in human growth as well as in lipomatosis has been postulated. <sup>8,9</sup> The significance of *HMGA2* for human growth was supported by the identification of a fourth patient with a smaller 12q14 deletion <sup>10</sup>(fig. 2): in this patient the deletion affected six genes, among others *HMGA2* and *GRIP1*, but not *LEMD3*. Indeed, this patient did not show radiological signs of osteopoikilosis but pre- and postnatal growth retardation, failure to thrive and mild developmental delay. Interestingly, a clinical diagnosis of SRS was also considered in this patient.

The findings in our patient underline the clinical overlap between SRS and 12q14 microdeletion syndrome (table 2). However, in contrast to the other four microdeletion carriers reported so far, our patient is not mentally handicapped; this observation is consistent with the finding that the *GRIP1* gene is not affected by the deletion in our patient. Interestingly, at an age of 16 years our patient does not show radiographic features consistent with osteopoikilosis despite the association of *LEMD3* haploinsufficiency with this rare benign sclerosing bone dysplasia. However, the age of osteopoikilosis manifestation is currently unknown, thus we assume that they might occur later in life in our patient. Like in the other microdeletion 12q14 patients, in our patient the *HMGA2* gene is deleted. Point mutations were not detectable in this patient, we therefore agree with Mari et al.<sup>10</sup> that haploinsufficiency of this gene is sufficient to cause growth retardation. However, by screening another 20 SRS patients for point mutations in *HMGA2* we did not obtain evidence for a significant role of this gene for the aetiology of SRS.

Up to now, all screening studies for submicroscopic chromosomal imbalances were focused on patients with mental retardation as the main clinical feature. Our data as well as recently published reports on single growth retarded patients with cryptic imbalances, i.e. in 11p15 <sup>11,12</sup>, show that loss or gain of genomic fragments with a size of several Mb does not automatically cause intellectual incapacities. Indeed, genomic copy number alterations should generally be considered in patients with intrauterine and postnatal growth retardation and minor further anomalies but with normal intelligence. In SRS, molecular karyotyping should be included in the diagnostic algorithm.

## Acknowledgement

We thank all families participating in this study. The project was supported by Pfizer GmbH.

## Databases/Links

[www.ensembl.org](http://www.ensembl.org)  
[www.r-project.org](http://www.r-project.org)  
[www.progenetix.net](http://www.progenetix.net)  
[www.genome.ucsc.edu](http://www.genome.ucsc.edu)  
[projects.tcag.ca/variation](http://projects.tcag.ca/variation)



## References

1. **Netchine I**, Rossignol S, Dufourg MN, Azzi S, Rousseau A, Perin L, Houang M, Seunou V, Esteva B, Thibaud N, Demay M-CR, Danton F, Petricko E, Bertrand A-M, Heirichs C, Carel J-C, Loeuille G-A, Pinto G, Jaquemont M-L, Gicquel C, Cabrol S, Le Bouc Y. 11p15 ICR1 loss of methylation is a common and specific cause of typical Russell-Silver syndrome: clinical scoring system and epigenetic-phenotypic correlations. *J Clin Endocrin Metabol* 2007; **92**:3148-4154.
2. **Eggermann T**, Eggermann K, Schönherr N. Growth retardation versus overgrowth: Silver-Russell syndrome is genetically opposite to Beckwith-Wiedemann syndrome. *Trends Genet* 2008;**24**:195-204.
3. **Kotzot D**. Maternal uniparental disomy 7 and Silver-Russell syndrome – clinical update and comparison with other subgroups. *Eur J Med Genet* 2008;**51**:444-451.
4. **Shaffer LG**, Bejjani BA, Torchia B, Kirkpatrick S, Coppinger J, Ballif BC. The identification of microdeletion syndromes and other chromosome abnormalities: cytogenetic methods of the past, new technologies for the future. *Am J Med Genet* 2007;**145C**:335-345.
5. **Ashar HR**, Schoenberg F, Tkachenko A, Zhou X, Fletcher JA, Weremowicz S, Morton CC, Chada K. Disruption of the architectural factor HMGI-C: DNA-binding AT hook motifs fused in lipomas to distinct transcriptional regulatory domains. *Cell* 1995;**82**:57-65.
6. **Baudis M**, Cleary ML. Progenetix.net: an online repository for molecular cytogenetic aberration data. *Bioinformatics* 2001;**17**:1228-1229.
7. **Bengtsson H**, Irizarry R, Carvalho B, Speed TPEstimation and assessment of raw copy numbers at the single locus level. *Bioinformatics* 2008;**24**:759-767.
8. **Menten B**, Buysse K, Zahir F, Hellemans J, Hamilton SJ, Costa T, Fragerstron C, Anadiotis G, Kingsbury D, McGillivray BC, Marra MA, Friedman JM, Speleman F, Mortier G. Osteopoikilosis, short stature and mental retardation as key features of a new microdeletion syndrome on 12q14. *J Med Genet* 2007;**44**:264-268.
9. **Ligon AH**, Moore SDP, Parisi MA, Mealiffe ME, Harris DJ, Ferguson HL, Quade BJ, Morton CC. Constitutional rearrangement of the architectural factor HMGA2: a novel human phenotype including overgrowth and lipomas. *Am J Hum Genet* 2005; **76**:340-348
10. **Mari F**, Hermanns P, Giovannucci-Uzielli ML, Galluzzi F, Scott D, Lee B, Renieri A, Unger S, Zabel B, Superti-Furga A. Refinement of the 12q14 microdeletion syndrome: primordial dwarfism and developmental delay with or without osteopoikilosis. *Eur J Hum Genet* 2009: epub ahead of print.
11. **Schönherr N**, Meyer E, Schmidt A, Wollmann HA, Eggermann T. The centromeric 11p15 imprinting center is also involved in Silver-Russell syndrome. *J Med Genet* 2007;**44**:59-63.

12. **Eggermann T**, Meyer E, Obermann C, Heil I, Schüler H, Ranke MB, Eggermann K, Wollmann HA. Is maternal duplication 11p15 associated with Silver-Russell syndrome? *J Med Genet* 2005;**42**:e26.
13. **Wollmann HA**, Kirchner T, Enders H, Preece MA, Ranke MB. Growth and symptoms in Silver-Russell syndrome: review on the basis of 386 patients. *Eur J Pediatr* 1995;**154**:958-968.
14. **Price SM**, Stanhope R, Garrett C, Preece MA, Trembath RC The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. *J Med Genet* 1999;**36**:837-842.

patient	chromosome	gain/loss	size	genes affected	Interpretation
SR29	12q14	loss	1.3 Mb	yes (see fig. 2)	CNA
SR115	8q21	gain	710 kb	no	CNV
	16q21	loss	12 kb	no	CNV
SR89	9p23	loss	103 kb	no	CNV
SR95	18q22	loss	51 kb	no	CNV
SR104	6q12	loss	43.1 kb	no	CNV
SR63	14q12	loss	4 kb	no	CNV

**Table 1:** Overview on the 6 patients identified by 500K microarray typing as carriers of so far unreported (de-novo) CNVs/CNAs, and the affected regions.

SRS features	General SRS population <sup>13</sup>	General SRS population (n=50) <sup>14</sup>	12q14 microdeletion carriers* (n=4) <sup>8,10</sup>	our patient*
Birth weight <3 <sup>rd</sup> percentile	94%	86%	2/4	+
Short stature	99%	86%	4/4	+
Hemihypotrophy	51%	34%	0/4	-
Muscular hypotrophy/-tony	45%		1 /4	-
Relative macrocephaly	64%	70%	1 /4	+
Triangular face	79%	62%	1 /4	(+)
Down-slanting corners of the mouth	46%		1 /4	-
Irregular teeth	28%		-	
Ear anomalies	53%		-	-
Clinodactyly V	68%	56%	-	+
Brachydactyly V	48%		-	-
Syndactyly of toes II/III	19%		-	-
Simian crease	25%		-	-
Café-au-lait spots	19%	4%	-	-
Psychomotor retardation	37%	38%	4/4	-
Speech delay		20%	2/2	-
Squeaky voice	22%			-
Feeding difficulties		56%	4/4	-
<b>12q14 microdeletion features</b>				
Mental retardation	-	-	4/4	-
Osteopoikilosis	-	-	¾	Not present at the age of 16 years

**Table 2:** Clinical features of SRS patients, of 12q14 microdeletion carriers and our case. (\* the different sizes of the deletions in these cases have to be considered)

**Figure 1.** Chromosomal aberrations and UPDs in SRS: Review on types, frequencies and affected genomic regions. A complete list of references describing chromosomal aberrations in SRS is available on request. The 12q14 microdeletion in our patient is indicated by an arrow.

**Figure 2.** Local Affymetrix Genotyping 6.0 signal distribution pattern and segmentation result in patient SR29 (SR0029). A deletion in 12q14 can be observed, affecting the whole *HMGA2* and *LEMD3* coding regions and overlapping with the previously reported 12q14 microdeletion syndrome as illustrated by the DECIPHER entries.



