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

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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, whereas the 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, 20 idiopathic SRS patients were screened with the Affymetrix GeneChip Human Mapping 500 K array set.

Apart from known apathogenic copy number variations, 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, in this study, a general role of *HMGA2* mutations for SRS was excluded 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.

Silver–Russell syndrome (SRS, Online Mendelian Inheritance in Man 180860) is a clinically and genetically heterogeneous disorder that 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,[1] 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[2]). Indeed, the 11p15 epimutation carriers often show the more typical SRS phenotype, while UPD(7)mat carriers are more mildly affected (for review[3]). 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 microscopically detectable structural aberrations affecting numerous chromosomes, but only chromosomes 7, 11 and 17 were repeatedly involved in individuals fulfilling strict diagnostic criteria of SRS (figure 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[4]), 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 and 5.6 Mb, respectively (for review[2]).

We screened a cohort of idiopathic SRS patients for cryptic chromosomal imbalances by single nucleotide polymorphism (SNP) oligonucleotide arrays, thereby identifying one carrier of a 12q14 microdeletion. Within this region, the gene of the non-histone 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.[5] We therefore sequenced the coding region of *HMGA2* in the deletion carrier as well as in 19 further SRS patients without 11p15 epimutation.

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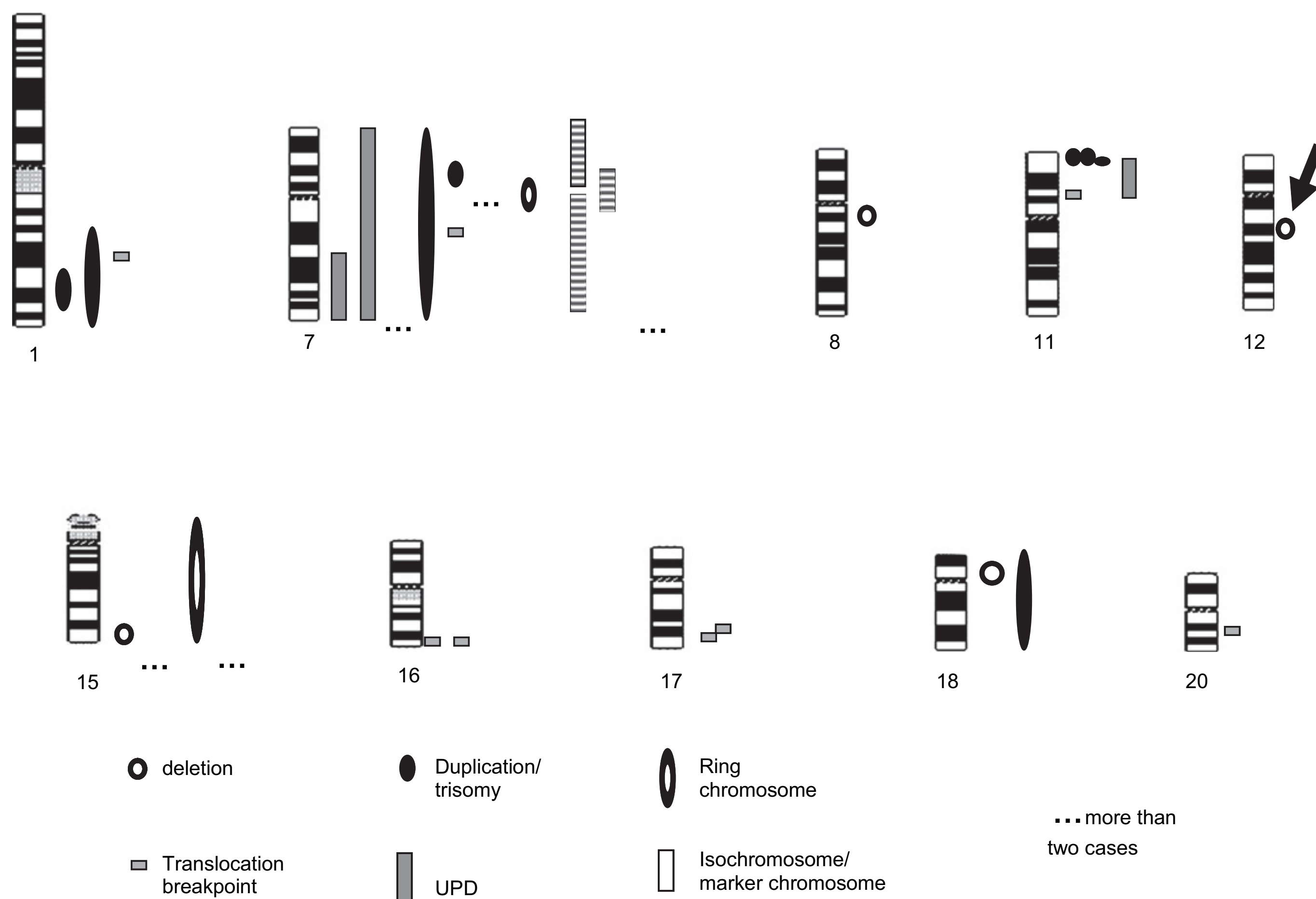

Figure 1 Chromosomal aberrations and uniparental disomies (UPDs) in SRS: review on types, frequencies and affected genomic regions. A complete list of references describing chromosomal aberrations in Silver–Russell syndrome is available on request. The 12q14 microdeletion in our patient is indicated by an arrow.

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 third 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 *Nsp*I kit and the *Sty*I kit, which together include >500 000 SNPs. DNA was processed according to the manufacturer's instructions. Hybridisation and washing were 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.[6] Copy number segmentation results were visualised using tools developed for the Progenetix project (http://www.progenetix.net[7]). 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 BigDye Terminator Cycle Sequencing 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 seven 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 because of one or related phenotypical studies (eg, studies on mental retardation, epilepsy and autism; for review[4]).

Nevertheless, the identification of 1 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, this deletion includes the *LEMD3* and the *HMGA2* genes (fig 2). The girl was the second

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Table 1 Overview on the six patients identified by 500 K microarray typing as carriers of so far unreported (de novo) copy number variations (CNVs)/ copy number alterations (CNAs), and the affected regions

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

child of healthy unrelated parents. Her father's height is 185 cm, while her mother's height is 166 cm. Family history is unremarkable.

Intrauterine growth retardation was noted in the 20th week of pregnancy. The child was born at term, length at birth was 46 cm (−2.59 SD) and weight was 2700 g (−1.83 SD). Apgar scores were 10 and 10. Psychomotor 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 year and 9 months, her height was 70.8 cm (−4.5 SD), her weight was 6800 g (−5.4 SD) and occipitofrontal circumference 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

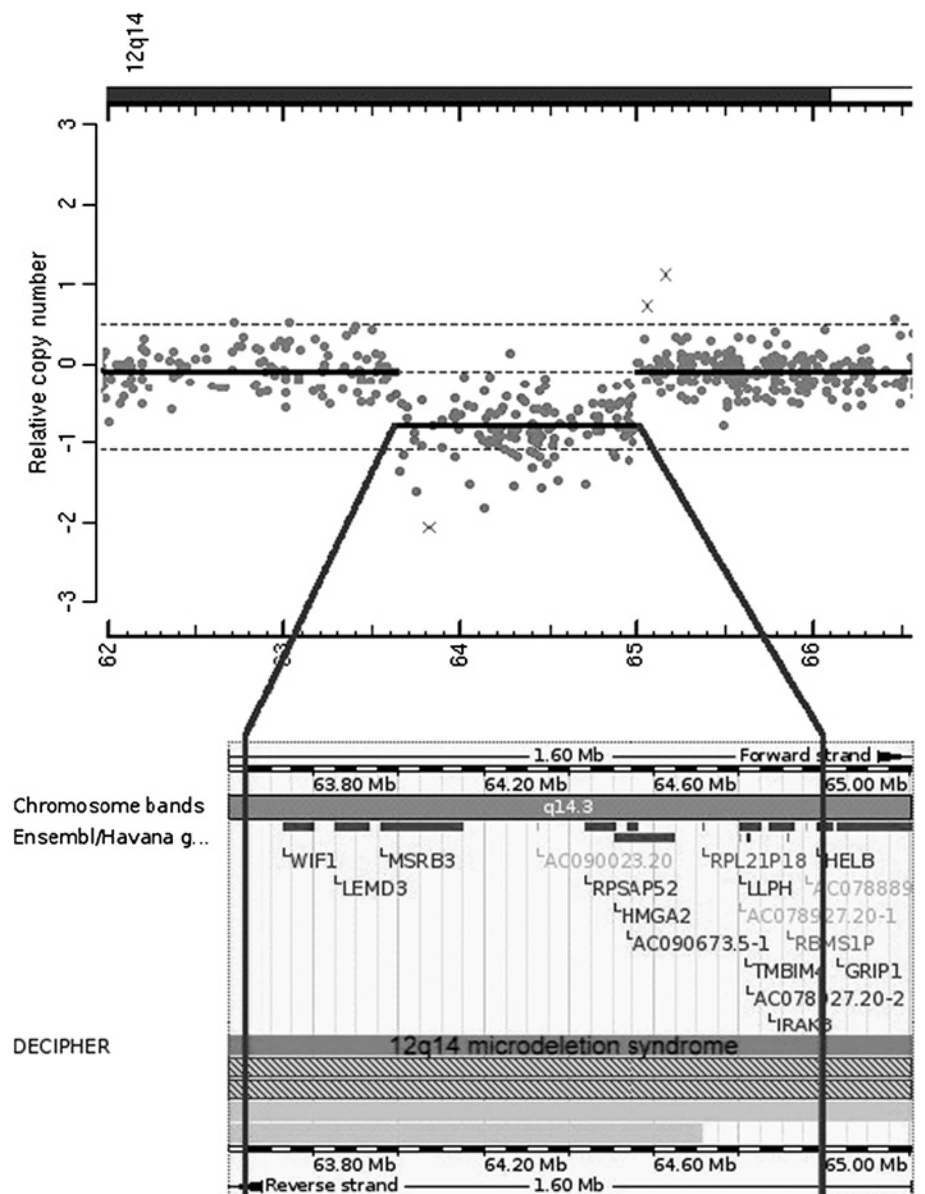

Figure 2 Local Affymetrix Genotyping 6.0 signal distribution pattern and segmentation result in patient SR29 (SR0029). A deletion in 12q14 can be observed, affecting the whole *HMGA2* and *LEMD3* coding regions and overlapping with the previously reported 12q14 microdeletion syndrome as illustrated by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

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Table 2 Clinical features of Silver–Russell syndrome (SRS) patients, of 12q14 microdeletion carriers and our case

SRS features	General SRS population[13] (%)	General SRS population (n=50)[14] (%)	12q14 microdeletion carriers* (n=4)[8 10]	Our patient*
Birth weight below the third percentile	94	86	2/4	+
Short stature	99	86	4/4	+
Hemihypotrophy	51	34	0/4	–
Muscular hypotrophy/hypotony	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	–
12q14 microdeletion features				
Mental retardation	–	–	4/4	–
Osteopoikilosis	–	–	3/4	Not present at the age of 16 years

*The different sizes of the deletions in these cases have to be considered.

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 first reported by Menten *et al*[8] 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 to 6 Mb (figure 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[8]), 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 foetal and adult human brain and involved in glutameric 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.[8 9] The significance of *HMGA2* for human growth was supported by the identification of a fourth patient with a smaller 12q14 deletion[10] (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 prenatal 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*[10] that the 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—that is, in 11p15[11 12]—show that loss or gain of genomic fragments with a size of several megabases does not automatically cause intellectual incapacities. Indeed, genomic CNAs 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.

Databases/links

- http://www.ensemble.org
- http://www.r-project.org
- http://www.progenetix.net
- http://www.genome.ucsc.edu
- http://projects.tcag.ca/variation/

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Ethics approval This study was conducted with the approval of the University Hospital Aachen.

Provenance and peer review Not commissioned; externally peer reviewed.

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