Molecular Karyotyping as a Relevant Diagnostic Tool in Children with Growth Retardation with Silver-Russell Features

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Objective To determine the contribution of submicroscopic chromosomal imbalances to the etiology of Silver-Russell syndrome (SRS) and SRS-like phenotypes.

Study design We performed molecular karyotyping in 41 patients with SRS or SRS-like features without known chromosome 7 and 11 defects using the Affymetrix SNP Array 6.0 system (Affymetrix, High Wycombe, United Kingdom).

Results In 8 patients, pathogenic copy number variations with sizes ranging from 672 kb to 9.158 Mb were identified. The deletions in 1q21, 15q26, 17p13, and 22q11 were associated with known microdeletion syndromes with overlapping features with SRS. The duplications in 22q13 and Xq25q27 represent unique novel copy number variations but have an obvious influence on the phenotype. In 5 additional patients, the pathogenetic relevance of the detected variants remained unclear.

Conclusion Pathogenic submicroscopic imbalances were detectable in a significant proportion of patients with short stature and features reminiscent of SRS. Therefore, molecular karyotyping should be implemented in routine diagnostics for growth-retarded patients with even slight dysmorphisms suggestive for SRS. *(J Pediatr 2012;161:933-42)*.

Patients with Silver-Russell syndrome (SRS; Online Mendelian Inheritance in Man 180860) show a severe intrauterine and postnatal growth restriction (<3rd percentile), associated with a variable spectrum of additional features. Classic SRS includes a relative macrocephaly, a triangle-shaped face with a prominent forehead and a small chin, body and limb asymmetry, and fifth finger clinodactyly.[1] Some patients show a mild motor and cognitive delay (learning difficulties and speech delay). By molecular genetic testing, in ~50% of patients with characteristic SRS phenotypes, epigenetic and genomic aberrations can be detected: in 7%-10% of patients, a maternal uniparental disomy of chromosome 7 [upd(7)mat] can be observed, and a hypomethylation of the imprinting control region 1 (ICR1) in 11p15 is present in more than 38% of patients. Additionally, cytogenetic aberrations have been reported in single cases, but uniform patterns were not apparent.[1] However, approximately one-half of patients with SRS remain without molecular diagnosis ("idiopathic").

Because the clinical spectrum of SRS is broad, the diagnosis is challenging and influenced by investigator experience. To assist the clinical diagnosis of SRS, Bartholdi et al[2] developed a detailed scoring system that includes 5 groups of clinical measures (biometry at birth, postnatal course, asymmetry, facial features, other features). According to this system, patients with ICR1 hypomethylation generally show a more classic SRS phenotype than do patients with upd(7)mat or patients with idiopathic SRS. This observation confirms other studies on (epi)genotype-phenotype correlations in the different molecular SRS subgroups.[3-5] Nevertheless, many exceptions exist[6] and molecular testing for SRS should thus also be considered in patients with ambiguous phenotypes.

In the past decade, microarray-based genomic copy-number analysis has become a powerful tool for the detection of submicroscopic chromosomal imbalances. Whereas in conventional cytogenetic methods, the detection of chromosomal aberrations is limited to a resolution of 5-10 Mb, molecular

ADHD	Attention-deficit/hyperactivity disorder
CNV	Copy number variation
FISH	Fluorescence in situ hybridization
ICR1	Imprinting control region 1
IGF	Insulin-like growth factor
PCR	Polymerase chain reaction
RefSeq	Reference Sequence
SRS	Silver-Russell syndrome
upd(7)mat	Maternal uniparental disomy of chromosome 7

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karyotyping enables the detection of considerably smaller imbalances (eg, ≤100 kb, depending on the used array platform). In patients with mental retardation of unknown etiology, the implementation of molecular karyotyping has lead to a >2-fold increase in the detection rate of pathogenic chromosomal anomalies compared with conventional cytogenetic approaches.[7,8] Nevertheless, molecular karyotyping is not commonly implemented in standard genetic diagnostic procedures for disorders lacking mental retardation.

Initial analyses on idiopathic patients with SRS indicate that pathogenetically relevant submicroscopic chromosomal imbalances significantly contribute to the spectrum of (epi) mutations.[9,10] However, the patient cohorts analyzed so far were small and the findings may not be representative. Moreover, novel array systems with higher resolutions are available, potentially resulting in an increased detection rate for cryptic causal aberrations. To corroborate the observation that small chromosomal imbalances significantly contribute to the etiology of SRS(-like) phenotypes, we performed molecular karyotyping in 41 idiopathic patients referred as SRS for routine diagnostic testing.

Methods

The study population consisted of 41 patients with SRS features [without ICR1 (epi)mutations and upd(7)mat] and their parents. All patients were initially referred as SRS for routine diagnostic testing because they had intrauterine and/or postnatal growth retardation and suggestive clinical features. Genomic DNA was isolated from peripheral lymphocyte cells by salting-out.[11] The study was approved by the ethical committee of the University Hospital Aachen. Appropriate informed consent was obtained from all participating patients or legal representatives.

Clinical Scoring System

For a standardized clinical characterization of our idiopathic patients with SRS, we applied the scoring system recently developed by Bartholdi et al[2] (the "Bartholdi score") with some modifications. In brief, the patients' symptoms were assigned to 5 groups: biometric measures at birth, postnatal course of growth, asymmetry, facial features, and other features. For the different groups, at least 3 features were scored and 0-3 points were given when the signs were present or not. In contrast to Bartholdi et al, we defined a maximum score of 14 points because our clinical questionnaire did not include information on genital abnormalities. As we could not obtain complete information on all features of some patients, we calculated a percentage score instead of an absolute score. Patients with a score of ≥53.3% (≥8 of 15 points) were classified as typical SRS according to Bartholdi et al.[2]

Microarray Analyses

For the detection of submicroscopic genomic imbalances (<5 Mb), we typed genomic DNA by using the Affymetrix GeneChip Genome-Wide Human SNP 6.0-Array (Affymetrix, High Wycombe, United Kingdom) including 1.8 M oligonucleotide markers. After polymerase chain reaction (PCR) amplification and labeling of DNA, the samples were hybridized to the arrays according to the manufacturer's instructions. Scanning was performed with an Affymetrix GeneChip Scanner 3000 7G, bioinformatics was done with the Affymetrix Genotyping Console 4.0 and the Chromosome Analysis Suite 1.1 software using annotation files version NA30 (hg18/NCBI build 36) and an in-house reference file consisting of 90 samples. For analysis and interpretation, only copy number variations (CNVs) >100 kb with a mean marker distance <5 kb were considered. To detect uniparental isodisomies, the samples were checked for regions of homozygosity with a minimal size of 1 Mb.

To determine the inheritance of so far unreported CNVs and to confirm the array results, either array analyses of the parents' DNA, short tandem repeat typing, or quantitative PCR was performed. For quantitative PCR analyses, the Power SYBR Green PCR Master Mix (Applied Biosystems, Weiterstadt, Germany) in a final reaction volume of 10 μL was used on a StepOnePlus real-time PCR instrument (Applied Biosystems). Information on primers used for qPCR and microsatellite typing are available in **Appendix 1** (available at www.jpeds.com).

The evaluation of the pathogenic significance of the detected imbalances was based on the assessment algorithm suggested by Miller et al.[8] Imbalances completely covered by CNVs previously identified in healthy controls were regarded as not pathogenic; CNV data were obtained from our own control cohort and from several studies using high-resolution techniques registered in the Database of Genomic Variants (http://projects.tcag.ca/variation/). The remaining CNVs were evaluated in respect to their overlap with common microdeletion/microduplication syndromes and gene coverage using the University of California, Santa Cruz genome browser (http://genome.ucsc.edu/), the Online Mendelian Inheritance in Man database (http://www.ncbi.nlm.nih.gov/omim), and the DECIPHER database (https://decipher.sanger.ac.uk/syndromes). The inheritance of these CNVs was determined by typing of parental DNA samples. In case the CNV was inherited from an unaffected parent, it was classified as a probably apathogenic rare familial variant.

In patients with obviously pathogenic imbalances, conventional cytogenetic and fluorescence in situ hybridization (FISH) analyses of the patients and their parents were suggested to the referring laboratories and physicians.

Results

We detected an average of 8 common CNVs per patient, which were either registered in the Database of Genomic Variants database or had been observed in our own control cohort.

So far, unknown CNVs were detected in 24 of the 41 patients with SRS (**Figure 1**). Eleven of the so far novel CNVs were inherited from an unaffected parent and therefore were more likely to represent nonpathogenic changes. This

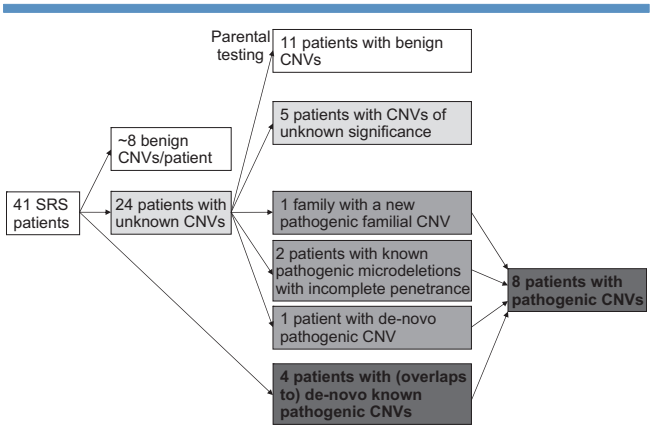


Figure 1. Overall number of CNVs detected in our cohort of growth retarded patients referred as SRS and separation between CNVs with a pathogenic or a benign nature and of unknown clinical significance.

group included a patient with a maternally inherited 15q13 microdeletion, which has recently been reported as a new microdeletion syndrome with incomplete penetrance.[12] The 15q13 microdeletion in our patient could also be observed in her unaffected mother but not in her growth-retarded half-sister. Because the 15q13 microdeletion syndrome is well established and does not include growth retardation as a typical sign, we assumed that the 15q13 microdeletion in our family is not associated with the phenotype.

In 8 patients, the identified imbalances were judged to likely be pathogenic because they fulfilled at least one of the following criteria (**Tables I** and **II**): (1) overlap with common microdeletion/microduplication syndromes associated with growth delay; (2) overlap with CNVs identified in further patients with short stature from public and in-house databases; (3) affecting genes with a role in regulation of growth and development; and (4) cosegregation with growth retardation in the family.

In 5 patients, the pathogenetic relevance of the detected CNVs remained unclear because they have neither been reported in other growth retarded patients nor contained known genes involved in growth and development (**Table I**).

Pathogenic CNVs

The German patient SR5695 carried a heterozygous 1.65-Mb deletion in 1q21, a region that is deleted in patients with distal 1q21-microdeletion syndrome[13] (**Figure 2**, A). The deletion affected 14 Reference Sequence (RefSeq) genes (National Center for Biotechnology Information). It could be excluded in the mother but paternal DNA was not available. The boy was born at term. At the age of 7 years 8 months, the boy had short stature. Further features suggestive of SRS were a triangle-shaped face with a prominent forehead, slightly downturned corners of the mouth, retrognathia, clinodactyly V, café-au-lait spots, and a squeaky voice. Additionally, mitral insufficiency and attention-deficit/hyperactivity disorder (ADHD) were reported. In total, the clinical data in patient SR5695 result in a score of 50.0% and are therefore only slightly suggestive for SRS. Interestingly, we identified a second patient, SR9116 (**Figure 3**, A), with nearly the same aberration in 1q21 (**Figure 2**, A). The girl was born at term and showed prenatal as well as postnatal growth restriction. Speech development was delayed. Craniofacial features included hypertelorism, a broad nasal root, and large posteriorly rotated ears. Clinodactyly of digits V and syndactyly of toes II and III were present. Several nevi were visible. The total score was 46.2%. The deletion was inherited from the unaffected mother (size, 162 ± 0.7 cm [SD]).

In the Turkish girl, SR6882 (**Figure 3**, B), a heterozygous de novo ~5.4-Mb deletion in 15q26 was detected. The imbalance affected 26 RefSeq genes, among them the *IGF1R* (insulin-like growth factor 1 receptor) gene (**Figure 2**, B). FISH analyses of parental samples revealed a normal karyotype without balanced rearrangements involving the deleted region. The patient's conventional cytogenetic karyotype was normal. The girl was born at 42 weeks. Postnatal growth was restricted and at the age of 3 years 8 months, the girl had short stature. No additional SRS features were reported, resulting in a low score of 21.4%. Features not belonging to the SRS spectrum were a broad nasal bridge, thick hair, and small hands and feet. Retardation of motor or mental skills was not reported but speech was slightly delayed.

The German patient SR8464 (**Figure 3**, C) was a carrier of a heterozygous de novo 799-kb deletion in 17p13.3 affecting 20 RefSeq genes (**Figure 2**, C). Conventional karyotyping was performed after birth and showed a normal male karyotype. The deletion was confirmed by FISH. FISH analyses of the parents revealed a normal karyotype. The patient was born at term. Postnatal growth was restricted and the boy had short stature at the age of 2 years 1 month. In addition to relative macrocephaly, the patient had typical SRS features including a triangular face with a prominent forehead, downturned corners of the mouth, and retrognathia and therefore fulfilled the SRS criteria (score: 57.1%). Moreover, a long philtrum, a high hairline, and small hands and feet were reported.

We detected nearly the same heterozygous 17p13.3.de novo deletion in a second patient, SR10177 (**Figure 2**, C). The deletion had a size of 672 kb including 17 RefSeq genes. The German boy was born at term. Conventional karyotyping after birth revealed a normal male karyotype. At the age of 1 year 7 months, the boy had short stature and a relative macrocephaly. Further features were a triangle-shaped face with a prominent forehead and retrognathia resulting in a clinical score of 57.1%, suggestive for SRS.

In patient SR1251/06, a heterozygous de novo 2.5-Mb deletion of the DiGeorge critical region in 22q11 was identified (**Figure 2**, D). The boy was born at term. Growth retardation persisted at the age of 7 years 10 months, and the patient showed a relative macrocephaly. In addition, the triangular face, downturned corners of the mouth, retrognathia, irregular teeth, ear anomalies, and clinodactyly V suggested

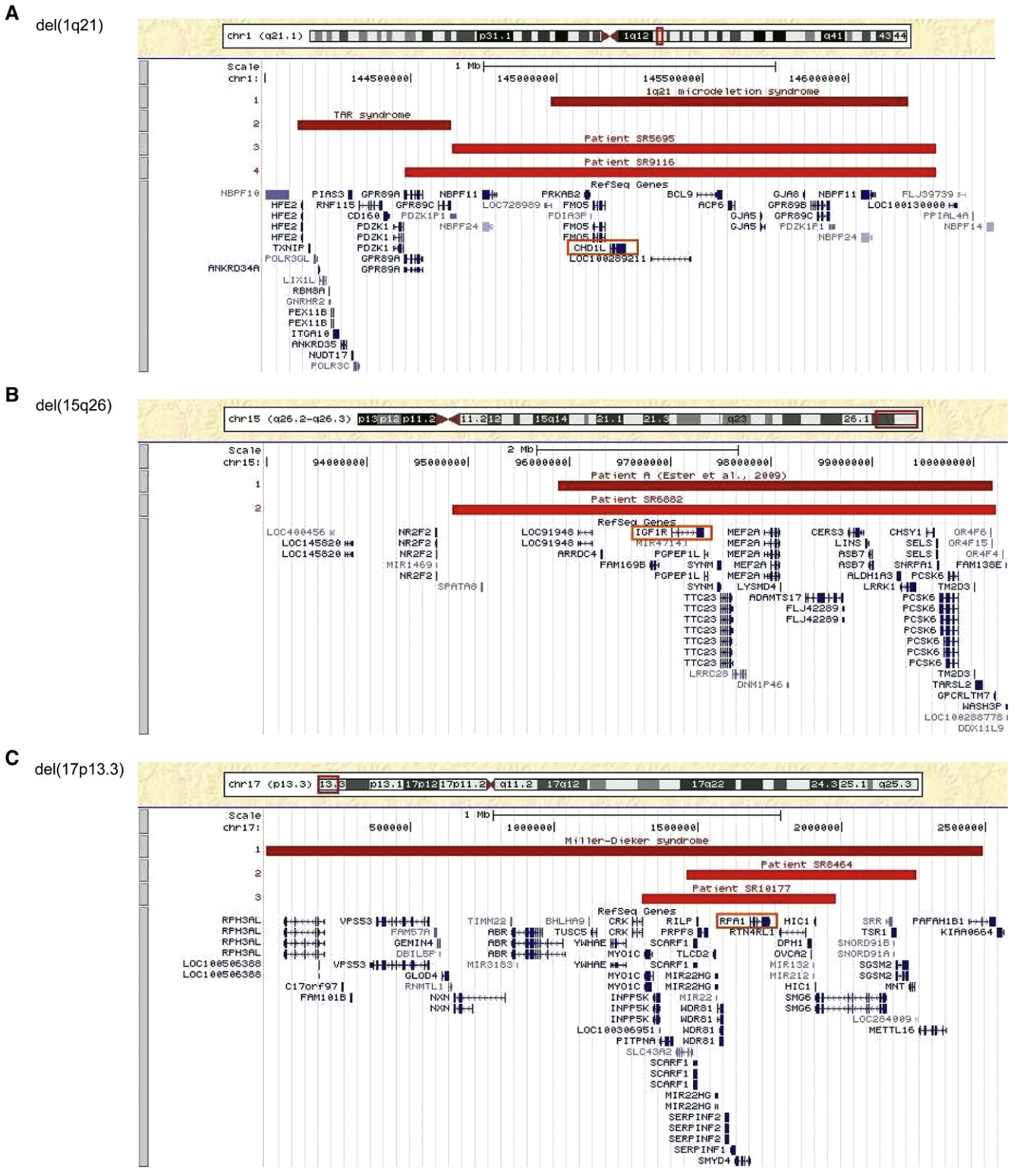
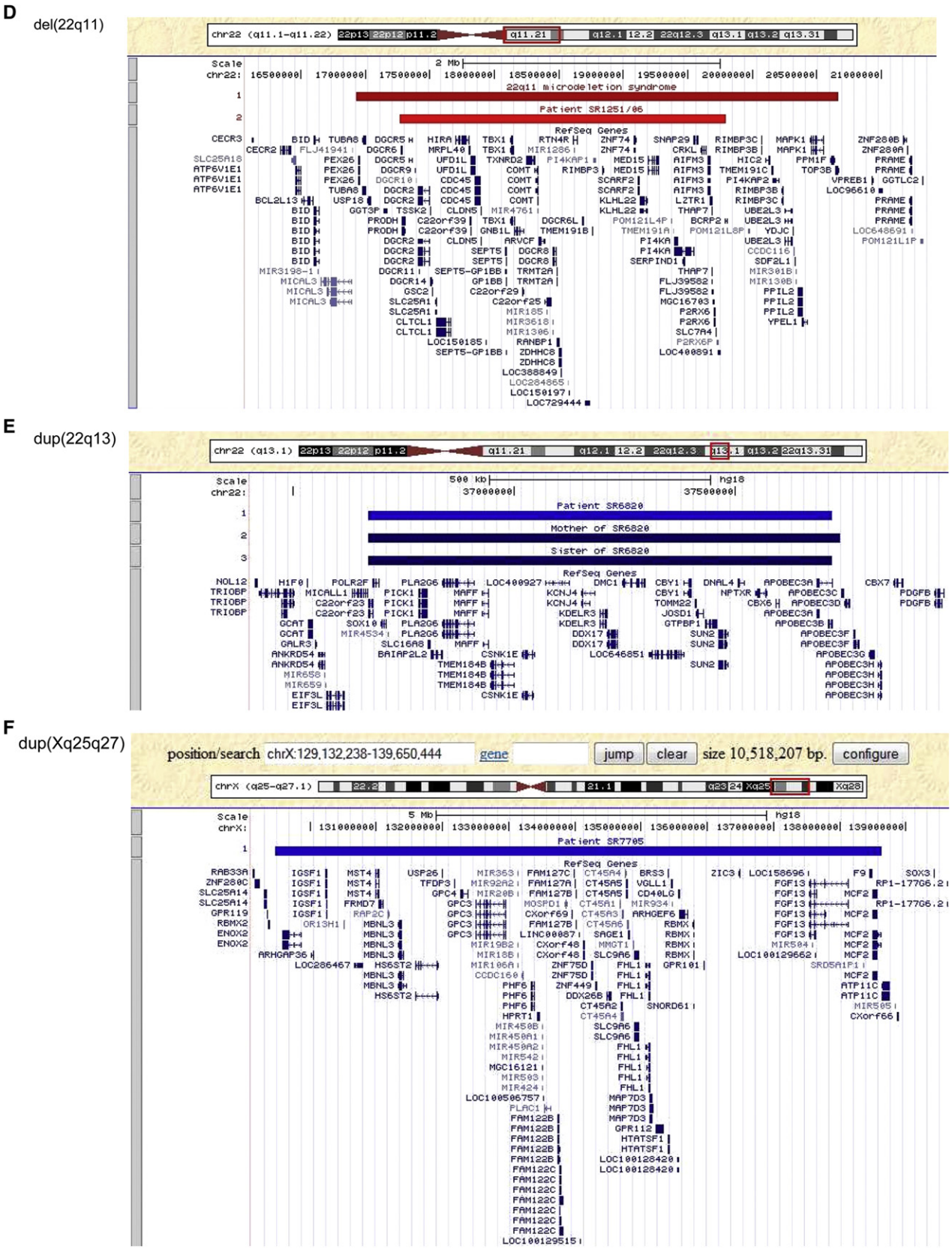


Figure 2. Pathogenic copy number changes in patients referred with the clinical diagnosis of SRS. **A**, 1q21 microdeletion in patients M5695 and M9116. **B**, 15q26 microdeletion in patient M6882. **C**, microdeletion in 17p13.3 in patients M8464 and M10177. **D**, 22q11 microdeletion in M1251/06. **E**, microduplication 22q13 in M6820. **F**, Duplication Xq25q27 in M7705. *Light red bars* indicate deletions, and *green bars* indicate duplications. Regions affected in known microdeletion syndromes are shown in *dark red*. Genes probably associated with the patients' phenotype are highlighted in *orange*. (*Continues*)



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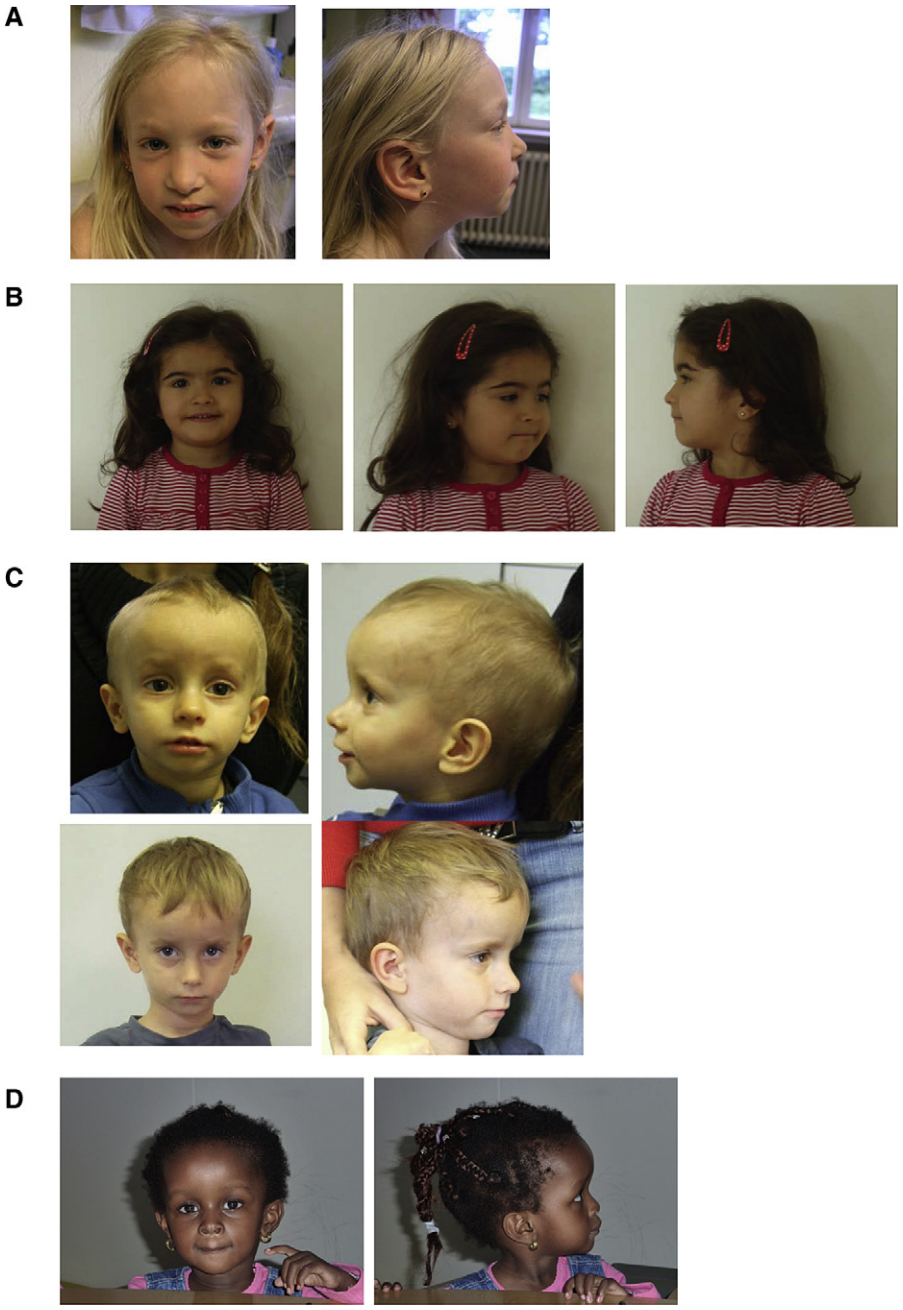

Figure 3. Clinical pictures of children referred as SRS and carrying pathogenic CNVs. **A,** Patient M9116 with a heterozygous 1.65-Mb deletion in 1q21. **B,** Patient M6882 carrying a 5.3-Mb deletion in 15q26 at the age of 4 years 10 months. **C,** Patient M8464 heterozygous for a 799-kb deletion in 17p13 (*left*: 1.5 years, *right*: 3 years). **D,** The 2.5-year-old girl M7705 with a 9.15-Mb duplication in Xq25q27.

SRS, but the clinical score (42.8%) did not fit the initial clinical diagnosis of SRS. Speech delay was reported. No cardiac defects or other features typically associated with the 22q11 microdeletion syndrome were present.

In patient SR6820 and her sister, SR10737, as well as in their mother, a 1-Mb duplication of chromosome 22q13.1 including 25 RefSeq genes was identified (Figure 2, E). All three carriers of this familial duplication were born at term and had intrauterine and postnatal growth restriction. Conventional karyotyping was performed after birth and revealed a normal female karyotype. At an age of 4 years 5 months, the girl had several typical SRS features: besides persisting short stature and a relative macrocephaly, a triangular face with a prominent forehead, retrognathia, clinodactyly V, and body asymmetry were reported (clinical score of 85.7%). Growth hormone therapy was started at an age of 4.3 years with a good response: her height increased from −4.39 to 1.83 SDS on the first 2 years of

Table I. Relevant CNVs detected by molecular karyotyping in growth retarded patients with SRS features

Patient	Copy number	Cytogenetic band	Range (bp, hg18)		Size (kb)	Gene count	Parental origin	Clinical score (%)*	Validation
Pathogenic variants									
SR5695	1	1q21	144,643,812	146,297,795	1.653	14	Not maternal	50.0	qPCR
SR9116	1	1q21	144,481,983	146,297,795	1.816	15	Maternal	46.2	qPCR
SR6882	1	15q26	94,844,379	100,222,647	5.378	26	De novo	21.4	STRs
SR8464	1	17p13	1,459,660	2,259,112	799	20	De novo	57.1	qPCR
SR10177	1	17p13	1,307,036	1,978,951	672	17	De novo	57.1	qPCR
SR1251/06	1	22q11	17,264,837	19,795,835	2.530	56	De novo	42.8	qPCR
SR6820	3	22q13	36,668,860	37,719,685	1.050	25	Maternal	85.7	Maternal
SR7705	3	Xq25q27	129,467,388	138,626,069	9.158	55	De novo	63.6	STRs
CNVs of unknown clinical significance									
SR4178	1	1q21	144,093,480	144,503,409	410	17	De novo	61.5	STRs
SR398/07	3	15q21	53,403,146	53,580,561	177	3	De novo	53.8	STRs
SR6415	3	16p13	14,846,828	16,203,401	1.356	13	Paternal	28.6	Paternal
SR9937	3	16p13	14,742,555	16,202,207	1.459	14	Maternal	46.2	qPCR
SR596/07	1	16q24	87,899,339	88,134,915	236	2	De novo	35.7	qPCR

qPCR, quantitative PCR; *STR*, short tandem repeat.
*Clinical scoring according to Bartholdi et al.[2]

therapy. Her younger sister, M10737, was born with a weight of 2480 g (−2.35 SD) and a length of 44 cm (−3.5 SD). The mother had a birth weight of 2480 g (−2.35 SD), a length of 46 cm (−2.59 SD), and a head circumference of 32 cm (−2.25 SD score). Further symptoms were not reported.

The female patient SR7705 (**Figure 3**, D) from Ethiopia carried a large de novo duplication of chromosome Xq25q27 encompassing 9.1 Mb and including 55 RefSeq genes (**Figure 2**, F). Conventional karyotyping in the patient and her parents revealed a normal karyotype. Microsatellite typing showed that the paternal chromosome was affected; X inactivation studies did not indicate that the affected X chromosome was silenced. Birth measures were not available. At about 3 years of age, the girl had short stature and relative macrocephaly. Further features suggestive of SRS were a triangular face with a prominent forehead and asymmetry of the body and the limbs (clinical score: 63.6%). The girl had a broad nasal bridge, thick hair, and a single palmar crease of both hands. Additionally, a mild mental retardation was reported.

CNVs of Unknown Significance

In 5 patients, CNVs were classified as of unknown significance because a phenotypic effect was not obvious. In the case of patient SR4178, carrying a 1q21 deletion affecting the thrombocytopenia absent radius locus, we assume that the SRS phenotype and the thrombocytopenia absent radius deletion without clinical outcome are coincidental findings; however, a pathophysiological significance cannot be excluded. The variants in 15q21 (SR398/07) and 16q24 (SR596/07) have not yet been reported. Only patients with larger/overlapping imbalances affecting these regions have been described but they did not show phenotypes corresponding to SRS.

In 2 patients (SR6415, SR9937), the evaluation of the familial 16p13 microduplication was challenging because it overlapped with the recently described 16p13 microduplication/microdeletion syndromes. However, the microduplication 16p13 has been rather classified as a benign variant in the population.[14]

To find out whether there is a relationship between the total CNV burden and the clinical severity scores of our patients, we additionally compared the total CNV count in the patient subgroup with a low clinical score not suggestive for SRS and in the patient subgroup with a more typical SRS phenotype. The average CNV count in the low score group was 7.7, and in the high score group, we found 8.1 CNVs on average. Furthermore, we compared the mean clinical severity scores of patients with pathogenic CNVs, CNVs of unknown significance, and apathogenic CNVs that were 53.4%, 45.2%, and 46.5%, respectively (**Appendix 2**; available at www.jpeds.com).

In addition, the samples of the 41 patients were screened for regions of homozygosity suggestive of mitotic recombination events reminiscent of uniparental disomy. No patients with uniparental disomy could be identified.

Discussion

As a uniform and standardized classification system for SRS is lacking, and the clinical spectrum comprises many unspecific features overlapping with other congenital disorders, our cohort consists of a clinically as well as genetically heterogeneous group. Indeed, we have to consider that a significant fraction of our patients was referred with the clinical diagnosis of SRS but showed only slight compatible features. To compare these heterogeneous phenotypes of our patients, we therefore applied the clinical scoring system recently developed by Bartholdi et al.[2] The broad range of the score reflected the clinical heterogeneity in our idiopathic patients with SRS (21.4%-85.7%), and thereby indirectly corresponds to the heterogeneous pattern of genomic imbalances in this cohort. As expected from previous studies reporting on a more typical SRS phenotype in 11p15 hypomethylation carriers in comparison with patients with upd(7)mat and probands with unknown molecular defects,[2,3,5,15] our cohort of

Table II. Clinical overview and scoring of the major SRS symptoms in patients with ICR1 hypomethylation in 11p15, in idiopathic patients, and in 8 carriers of obviously pathogenic CNVs

Clinical features	ICR1 hypomethylation (n = 27*)	Idiopathic SRS (n = 40†)	M5695 del(1q21)	M9116 del(1q21)	M6882 del(15q26)	M8464 del(17p13.3)	M10177 del(17p13.3)	M1251/06 del(22q11)	M6820 dup(22q13)mat	M7705 dup(Xq25q27)
Variables at birth										
Weight ≤10th percentile	88.9% (24/27)	60.5% (23/38)	3050 g (−1.3 SD)	2480 g (−2.06 SD)	3130 g (−1.13 SD)	3280 g (−0.78 SD)	2925 g (−1.59 SD)	3130 g (−1.12 SD)	2510 g (−2.28 SD)	ND
Length ≤10th percentile	92.6% (25/27)	67.6% (25/37)	50 cm (−1.09 SD)	47 cm (−1.86 SD)	48 cm (−1.96 SD)	42 cm (−4.57 SD)	49 cm (−1.52 SD)	49 cm (−1.52 SD)	46 cm (−2.59 SD)	ND
Relative macrocephaly/OFC	77.8% (21/27)	25.8% (8/31)	34 cm (−1.23 SD)	ND	34 cm (−1.08 SD)	35 cm (−0.46 SD)	34 cm (−1.23 SD)	36 cm (0.31 SD)	32 cm (−2.23 SD)	ND
Postnatal course										
Height ≤3rd percentile	96% (24/25)	68.4% (26/38)	114 cm (−2.76 SD)	108.8 cm (−4.06 SD)	83.6 cm (−5.22 SD)	80 cm (−2.08 SD)	76 cm (−2.41 SD)	120 cm (−1.7 SD)	92 cm (−2.96 SD)	83 cm (−3.9 SD)
OFC ≥3rd and ≤97th percentile	80% (20/25)	69% (20/29)	50 cm (−2.03 SD)	49 cm (−2.1 SD)	46.2 cm (−3.08 SD)	48 cm (−1.08 SD)	48 cm (−0.47 SD)	53.2 cm (0.24 SD)	48 cm (−1.69 SD)	50 cm (0.39 SD)
Normal cognitive development‡	59.3% (16/27)	59% (23/39)	–	(+)	+	+	+	(–)	+	(–)
Asymmetry										
Face/body/limbs	66.7% (18/27)	27.5% (11/40)	–	–	–	–	–	–	+	+
Facial features										
Triangle-shaped face	85.2% (23/27)	62.5% (25/40)	+	–	–	+	+	+	+	+
High/bossing forehead	81.5% (22/27)	47.5% (19/40)	+	–	–	+	+	–	+	+
Other: eg, small chin, thin lips, down turned corners of the mouth, ear anomalies, late closure of fontanelle	ND	ND	+	+	–	+	+	+	+	–
Other features										
Clinodactyly 5th finger	63% (17/27)	35.9% (14/39)	+	+	–	–	–	+	+	–
Other: brachymesophalangy, syndactyly toes, inguinal hernia, pigmentary changes	ND	ND	+	+	–	–	–	–	–	–
Score	53.8-92.9% (7/13-13/14)	21.4-85.7% (3/14-12/14)	50% (7/14)	46.2% (6/13)	21.4% (3/14)	57.1% (8/14)	57.1% (8/14)	42.8% (6/14)	85.7% (12/14)	63.6% (7/11)
Median scores (from the own data)	71.8%	47.1%	–	–	–	–	–	–	–	–
Median score from the patients published by[2]	84.7% (13/15; n = 29)	58% (9/15; n = 58)	–	–	–	–	–	–	–	–

ND, not determined; −, not obvious; +, slightly observable.
The modified clinical scoring system of Bartholdi et al[2] was applied.
*Clinical data of the ICR1 hypomethylation carriers were ascertained in an ongoing study on SRS.
†Clinical data were available only from 40 of the 41 idiopathic patients.
‡Normal cognitive development was assumed in case abnormalities were not reported.

idiopathic patients show a lower median clinical score (47.1%) than ICR1 hypomethylation carriers with a mean score of 71.8%.

Among the patients carrying pathogenic CNVs, 4 children showed a clinical score of more than 53.3% corresponding to a characteristic SRS phenotype (ie, the carriers of the deletions in 17p13.3 and of the duplications in 22q13 and Xq25q27). In the other 4 patients with pathogenic CNVs, the clinical scores were lower than 53.3% and only slight features of SRS were reported. Indeed, the latter aberrations are associated with known microdeletion syndromes (1q21, 15q26, 22q11), and our findings rather reflect the nonspecificity of clinical features overlapping between SRS and the detected syndromes.

The distal 1q21 microdeletion that we detected twice (SR5695, SR9116) is associated with a quite variable phenotype including short stature (in 47.6% of carriers), a mild to moderate developmental delay, microcephaly, cardiac abnormalities, and dysmorphic features.[13,16] Additionally, ADHD and further behavioral abnormalities are observed, and ADHD was indeed present in patient SR5695. However, the phenotypic penetrance of the imbalance is incomplete; both affected and unaffected carriers have been identified. Haploinsufficiency of the *CHD1L* gene in 1q21 has been suggested as causative for the growth delay in 1q21 microdeletion patients.[17] CHD1L is implicated in chromatin remodeling and relaxation as well as in DNA damage response[18,19] and decatenation. In cell lines carrying the 1q21 deletion, a defect in chromatin remodeling based on impaired decatenation similar to that observed in cells from Werner syndrome was documented.[17] As the only overlapping clinical feature in both syndromes was short stature, an influence of *CHD1L* haploinsufficiency on growth was postulated. As a result, we assume that the 1q21 microdeletion in our 2 patients explains their short stature. Their initial clinical diagnosis as SRS was mainly based on features reported for both SRS and the 1q21 microdeletion syndrome.

The detection of the 15q26 microdeletion including the *IGF1R* gene in patient M6882 is consistent with data from the literature: Microdeletions affecting *IGF1R* have been reported in 2% of children born small for gestational age with unknown etiology; intragenic deletions and point mutations in *IGF1R* are present in several patients with growth retardation presenting an SRS-like phenotype.[20-22] *IGF1R* is a member of the growth hormone–IGF cascade and is well known to be one of the most important regulators of prenatal growth. In summary, the 15q26 deletion can be classified as causative for the growth retardation in our patient. Indeed, it represents a recurrent pathogenic CNV as recently shown by Bruce et al[9] and accounts for a significant number of patients with growth retardation with SRS features but not for the typical SRS phenotype.

Overlapping de novo deletions in 17p13.3 were detected in 2 patients (M8464 and M10177). The deletions did not affect the genes typically affected in Miller-Dieker syndrome: *YWHAE* and *PAFAH1B*. The smallest region of overlap of both patients spanned 519 kb and contained 16 RefSeq genes, among them the *RPA1* gene, which is involved in ataxia telangiectasia and Rad3-related signaling. A causal relationship between haploinsufficiency of factors regulating ataxia telangiectasia and Rad3-related signaling and the growth retardation, as well as the microcephaly in patients with Miller-Dieker syndrome has been postulated.[23] Patients carrying similar deletions to those identified in our 2 patients and exhibiting intrauterine and postnatal growth retardation were reported by Bruno et al[24] (patients 5, 6a, and 6b). Additionally, several patients with overlapping deletions and growth delay are registered in the DECIPHER database. Considering these patient reports and the rather similar phenotype of our 2 patients (**Table II**), it is well conceivable that small 17p13.3 microdeletions are associated with an SRS-like phenotype.

A further CNV associated with growth retardation and further symptoms compatible with SRS is a deletion in the DiGeorge critical region in 22q11. Investigations on the clinical features in 78 adults with 22q11-microdeletion[25] reported cardiac defects in ~26% and short stature (<3rd percentile) in ~21% of patients. Thus, short stature seems to be a common observation in 22q11 microdeletion carriers.

Moreover, we identified a 1-Mb duplication in 22q13.1 in patient M6820 as well as in her growth-retarded mother and her younger sister, who was born small for gestational age, too. In the literature, only 1 growth-retarded patient with a similar but larger 22q13 (6.9 Mb) duplication has been reported.[26]

Patient SR7705 carries a de novo 9.16-Mb duplication in Xq25q27 affecting 55 RefSeq genes. This imbalance was classified as pathogenic due to its size and gene content. Neither in the literature nor in the DECIPHER patient database are similar patients with clinical data reported.

In addition to these pathogenic variants, we have to consider those imbalances classified as CNVs of unknown significance and the novel 11 benign familial variants in our cohort. It is well conceivable that some of these CNVs turn out to be pathogenic as they either might harbor so far unknown factors involved in growth or they represent pathogenic variants of incomplete penetrance.

Combining our results with those from the literature,[9,10] a total cohort of 73 patients with SRS features but without 11p15 epimutations and upd(7)mat has been analyzed for submicroscopic imbalances by molecular karyotyping. In 16% of patients (n = 12), genomic imbalances with an obvious pathogenic effect were identified. In respect to their pathophysiological significance for SRS and the clinical scoring, we would define 2 groups of imbalances: (1) (recurrent) pathogenic CNVs (ie, in 1q21, 15q26, 17p13, and 22q11). All these aberrations are known to be associated with known microdeletion syndromes with an overlap of features with SRS. In that context, the 12q14 microdeletion syndrome as a further entity characterized by features reminiscent of SRS has to be mentioned (see review[27]); and (2) The second group consists of unique CNVs only reported in

a single patient with SRS but with an obvious influence on the phenotype (ie, in 22q13 and Xq25q27).

This systematic study applied high-resolution techniques in growth-retarded and dysmorphic patients without mental retardation, and our results illustrate the urgent need to apply molecular karyotyping in this group of patients aiming on a satisfactory diagnosis as the basis for an individualized treatment. Finally, these data allow the identification of genes and genomic regions involved in the complex regulation of human growth. ■

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Appendix 1. Microsatellite markers or qPCR primer sets used for confirmation of array results

Patient(s)	CNV	Primer	Sequence (5′ → 3′)
SR5695, SR9116	del(1q21)	CHD1L_F	gccagaggaccttgagaatg
		CHD1L_R	gcagtgcacaatgagagcat
		BCL9_F	ggccataccctaaagcact
		BCL9_R	aaggagtcggcggaaatact
SR6882	del(15q26)	D15S1034_F	tcaaacacgttgtggac
		D15S1034_R	agaagcaatgccttgg
		D15S120_F	tttgtgatggtcttttataggcata
		D15S120_R	ggctcaaagtgtttgcactg
SR8464, SR10177	del(17p13)	PRPF8_F	gagatgcttcaggtccttgc
		PRPF8_R	ctggcagatggattgcagta
		TSR1_F	attcagagtctgccctcgaa
		TSR1_R	ggctaaccagaagcaacagc
SR1251_06	del(22q11)	ARVCF_F	gagctgggacatgaggagag
		ARVCF_R	tcaggggctcataggatgac
		CLTCL1_F	actggagcatggaaggtgac
		CLTCL1_R	aggcttacctgagccgagat
		MED15_F	caaggtctggctctgatggt
		MED15_R	actggttgctctcctgcact
SR7705	dup(Xq25-q27)	DXS994_F	ctgtcctaccctgtactgtcac
		DXS994_R	tattgtcctactgggcatagag
		DXS1211_F	ccctccaatctggcagaa
		DXS1211_R	aagacctgggtttggcct
SR4178	del(1q21)	D1S2344_F	tcatgggactctccatca
		D1S2344_R	aaatactcaggaaatggccta
		D1S442_F	ggtacttagcctcgaaatgaga
		D1S442_R	gtgtcacacaactggttggt
SR398/07	dup(15q21)	D15S1049_F	cactccagcctaaggaacac
		D15S1049_R	tgtcaaagatggcttttattacc
SR596/07	del(16q24)	ANKRD11_F	cacaccgcactcaacagact
		ANKRD11_R	tatgggaggcgtatcctgag
		SPG7_F	ccaagacccatgcctactgt
		SPG7_R	ccaccaactggctaaggtgt
SR9937	dup(16p13)	MPV17L_F	accaacgtgctgctttacg
		MPV17L_R	agttgaagttggcgtggaag
		ABCC6_F	ggcaggagagcaagattctg
		ABCC6_R	tggacatctaggggctgttc

Appendix 2. Correlation between CNV count and clinical score in the study population

Patient	CNV count	Score	Patient	CNV count	Score
Pathogenic CNVs			Low score		
SR5695	5	50.0	SR737/06	12	14.3
SR9116	5	46.2	SR7464	8	16.6
SR6882	13	21.4	SR6882	13	21.4
SR8464	12	57.1	SR3613	8	21.4
SR10177	9	57.1	SR3006	9	21.4
SR1251/06	9	42.8	SR8189	3	25.0
SR6820	8	85.7	SR6846	8	27.3
SR7705	8	63.6	SR736/06	9	28.5
Mean		53.4	SR6415	7	28.6
			SR9148	11	28.6
Unclear CNVs			SR596/07	9	35.7
SR4178	8	61.5	SR2745	8	35.7
SR398/07	7	53.8	SR6208	6	35.7
SR6415	7	28.6	SR277/07	5	38.5
SR9937	8	46.2	SR672/06	8	38.5
SR596/07	9	35.7	SR1251/06	9	42.8
Mean		45.2	SR7875	6	42.9
			SR9116	5	46.2
Apathogenic CNVs			SR9937	8	46.2
SR650/06	13	69.2	SR5695	5	50.0
SR6443	7	64.3	SR3451	9	50.0
SR6835	7	71.4	SR644/06	4	50.0
SR6846	8	27.3	SR8699	7	50.0
SR7875	6	42.9	Mean	7.7	
SR2745	8	35.7			
SR3451	9	50.0	High score		
SR644/06	4	50.0	SR398/07	7	53.8
SR3251	9	84.6	SR7142	7	53.8
SR5138	7	76.9	SR9226	7	53.8
SR6092	7	69.2	SR8639.1	6	54.5
SR7142	7	53.8	SR8464	12	57.1
SR8639.1	6	54.5	SR10177	9	57.1
SR8699	7	50.0	SR4178	8	61.5
SR9148	11	28.6	SR7705	8	63.6
SR3613	8	21.4	SR6443	7	64.3
SR277/07	5	38.5	SR4205	7	64.3
SR3006	9	21.4	SR650/06	13	69.2
SR4205	7	64.3	SR6092	7	69.2
SR6208	6	35.7	SR7883	10	69.2
SR7464	8	16.6	SR6835	7	71.4
SR8189	3	25.0	SR5138	7	76.9
SR7883	10	69.2	SR3251	9	84.6
SR9226	7	53.8	SR6820	8	85.7
SR10265	7	61.5	SR10265	7	61.5
SR736/06	9	28.5	Mean	8.1	
SR737/06	12	14.3			
SR672/06	8	38.5			
Mean		46.5			