



A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome

Salah Azzi, Jennifer Salem, Nathalie Thibaud, Sandra Chantot-Bastaraud, Eli Lieber, Irène Netchine, Madeleine D. Harbison

► To cite this version:

Salah Azzi, Jennifer Salem, Nathalie Thibaud, Sandra Chantot-Bastaraud, Eli Lieber, et al.. A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome. *Journal of Medical Genetics*, 2015, 52 (7), pp.446-453. 10.1136/jmedgenet-2014-102979 . hal-01293084

HAL Id: hal-01293084

<https://hal.sorbonne-universite.fr/hal-01293084v1>

Submitted on 24 Mar 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License



OPEN ACCESS

ORIGINAL ARTICLE

A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome

Salah Azzi,^{1,2,3,4} Jennifer Salem,⁵ Nathalie Thibaud,^{1,2,3} Sandra Chantot-Bastaraud,⁶ Eli Lieber,⁷ Irène Netchine,^{1,2,3} Madeleine D Harbison⁸

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/jmedgenet-2014-102979>).

For numbered affiliations see end of article.

Correspondence to

Dr Irène Netchine, Explorations Fonctionnelles Endocriniennes, Hôpital Armand Trousseau, Pierre & Marie Curie School of Medicine, INSERM UMR-S938, 26 Av du Dr Arnold Netter, Paris 75012, France; irene.netchine@trs.aphp.fr

IN and MDH contributed equally.

Received 30 December 2014

Revised 9 April 2015

Accepted 10 April 2015

Published Online First

7 May 2015

ABSTRACT

Background Multiple clinical scoring systems have been proposed for Silver-Russell syndrome (SRS). Here we aimed to test a clinical scoring system for SRS and to analyse the correlation between (epi)genotype and phenotype.

Subjects and methods Sixty-nine patients were examined by two physicians. Clinical scores were generated for all patients, with a new, six-item scoring system: (1) small for gestational age, birth length and/or weight ≤ -2 SDS, (2) postnatal growth retardation (height ≤ -2 SDS), (3) relative macrocephaly at birth, (4) body asymmetry, (5) feeding difficulties and/or body mass index (BMI) ≤ -2 SDS in toddlers; (6) protruding forehead at the age of 1–3 years. Subjects were considered to have likely SRS if they met at least four of these six criteria. Molecular investigations were performed blind to the clinical data.

Results The 69 patients were classified into two groups (Likely-SRS (n=60), Unlikely-SRS (n=9)). Forty-six Likely-SRS patients (76.7%) displayed either 11p15 ICR1 hypomethylation (n=35; 58.3%) or maternal UPD of chromosome 7 (mUPD7) (n=11; 18.3%). Eight Unlikely-SRS patients had neither ICR1 hypomethylation nor mUPD7, whereas one patient had mUPD7. The clinical score and molecular results yielded four groups that differed significantly overall and for individual scoring system factors. Further molecular screening led identifying chromosomal abnormalities in Likely-SRS-double-negative and Unlikely-SRS groups. Four Likely-SRS-double negative patients carried a *DLK1/GTL2* IG-DMR hypomethylation, a mUPD16; a mUPD20 and a de novo 1q21 microdeletion.

Conclusions This new scoring system is very sensitive (98%) for the detection of patients with SRS with demonstrated molecular abnormalities. Given its clinical and molecular heterogeneity, SRS could be considered as a spectrum.

INTRODUCTION

Silver-Russell syndrome (SRS, OMIM #180860; called also Russell-Silver syndrome, RSS, in the USA) is a clinically and genetically heterogeneous syndrome involving prenatal and postnatal growth retardation first described by Silver *et al*¹ and Russell.² Many other studies have since reported additional features, providing a complex clinical description of SRS.^{3–5} The clinical presentation of SRS is now known to cover a spectrum of signs that are easy to recognise in typical cases but may

be difficult to diagnose clinically in less severely affected individuals. Furthermore, the facial characteristics of SRS tend to become attenuated as the patient grows up, making it difficult to diagnose SRS in older children and adults. Finally, some of the more common typical features of SRS overlap with those of other syndromic intrauterine growth retardation disorders (such as 3M or Mulibrey-nanism syndromes, eg^{6,7}).

There is growing evidence that a definition of SRS based on a compilation of features rather than a checklist of characteristics is required. Unfortunately, there is still no clear consensus about how SRS should be defined. This renders clinical diagnosis difficult, leading to underdiagnosis in some situations and overdiagnosis in others. Five attempts have been made to create a clinical definition of SRS, or an 'SRS scoring system'.^{4,8–11} The various systems developed display some similarities, but also differences. In the paper describing the most recent of these systems, the Birmingham model,¹¹ the four scoring systems that had previously been developed were evaluated, but on retrospective clinical data collected by different physicians, which could not, therefore, necessarily be considered reliable.

Despite the existence of these numerous clinical scoring systems, the identification of appropriate patients for molecular testing remains a challenge, because many of the features of SRS are non-specific or mild, and some may disappear over time.

Two primary molecular causes of SRS have been identified. In about 10% of cases, SRS is due to maternal UPD of chromosome 7 (mUPD7). The major abnormality, present in 50–60% of cases, was only recently identified: hypomethylation of the paternal allele of the 11p15 imprinting centre region 1 (ICR1) regulating the *IGF2/H19* locus.^{9,12,13} Rare genetic or cytogenetic abnormalities have also been identified, but these abnormalities account for less than 2% of cases.¹⁴ The molecular cause of SRS thus remains unknown in about 30–40% of cases.

On the basis of our considerable clinical experience with patients with SRS, we conducted a prospective study in which 69 patients with suspected SRS were assessed clinically and then underwent state-of-the-art molecular investigations. The results obtained were then used to validate a modified scoring system adapted from the original scoring



Open Access
Scan to access more
free content



CrossMark

To cite: Azzi S, Salem J, Thibaud N, *et al*. *J Med Genet* 2015;**52**:446–453.

system developed by Netchine *et al*⁹ in which small for gestational age (SGA) was no longer an obligate factor. We have shown that this new scoring system was highly sensitive for identification of the subjects most likely to test positive for one of the known molecular causes of SRS, and for distinguishing these subjects from those not likely to test positive. This new scoring system is easy to use and flexible enough to be run even if data are missing for one or more factors. The combination of variables in this scoring system may be considered an improvement over those previously published.

METHODS

Study population

The population consisted of 69 patients (37 boys and 32 girls; age range 1.05–20.06 years with a mean age of 6.61 years) clinically diagnosed as SGA or possibly SRS by a local physician. Sixty-three were Caucasians, two were of Asian origin, one was an Afro-American and three were of mixed origin. The patients received information about the study from the MAGIC Foundation, a patient support group for patients with growth disorders with separate divisions for SGA patients and patients with SRS. All the patients attending either the SGA or the SRS division at the MAGIC convention in July 2008 were invited to participate in the study. Each patient was examined by the same two paediatric endocrinologists (IN and MDH) with substantial experience in the field of SRS. Physical characteristics from an extensive list were recorded as present or absent. Photographs of each patient were taken at this time. For patients over the age of 3 years, we also requested additional photos of the child at an age of 1–3 years (face and profile). The parents were also asked to complete a multipage survey and growth records were obtained. Fifteen patients of the 69 have been reported in Azzi *et al*¹⁵ whereas the remaining patients are reported for the first time.

Molecular investigations

Briefly, after DNA extraction and sodium bisulfite treatment, we used TaqMan Allele-Specific Methylated Multiplex Real-Time Quantitative PCR (ASMM RTQ-PCR) to analyse the methylation status at 11p15 ICR1 CBS2, *H19*DMR (*H19* promoter), 11p15 ICR2, *ZAC1* differentially methylated region (DMR), the *GNAS* locus SNRPN and *DLK1/GTL2* IG-DMR locus, as previously described.^{15–18} We ruled out a maternal duplication involving the 11p15 centromeric domain by studying the ICR2 methylation status and mutations at the *CDKN1C* gene as previously described.¹⁹ For more details, see online supplementary data.

SNP microarray: Chromosomal abnormalities were screened using CytoSNP12 microarray (Illumina, San Diego, California). For more details, see online supplementary data.

Clinical score

Our new scoring system, the Netchine-Harbison clinical scoring system (NH-CSS), is based on the original system developed by Netchine *et al*⁹ and no longer includes a requirement for SGA (see online supplementary table S1 and scoring system sheet). Six factors are included in the NH-CSS: (1) prenatal growth retardation (birth weight and/or length ≤ -2 standard deviation score (SDS) for gestational age); (2) postnatal growth retardation (height at 24 ± 1 months ≤ -2 SDS according to the Centers for Disease Control and Prevention (CDC) growth charts (<http://www.cdc.gov/growthcharts/>) or height ≤ -2 SDS from midparental target height); (3) relative macrocephaly at birth (head circumference at birth at least 1.5 SDS above birth weight and/or length

SDS according to Usher and McLean statistical data);²⁰ (4) protruding forehead (defined as a forehead that projects beyond the facial plane on a side view as depicted on [figure 1](#)) as a toddler; (5) body asymmetry (defined as a leg length discrepancy (LLD) of ≥ 0.5 cm or arm asymmetry or LLD < 0.5 cm with at least two other asymmetrical body parts (with one being a non-face part)); (6) feeding difficulties (use of a feeding tube or cyproheptadine for appetite stimulation) and/or low BMI (BMI ≤ -2 SDS at 24 months) according to CDC growth charts (<http://www.cdc.gov/growthcharts/>). Midparental target height was calculated as follows: ((father's height+mother's height)/2)+6.5 cm for boys and –6.5 cm for girls.

A patient was classified as having 'Likely-SRS' if at least four of these six factors were present. A patient was assigned to the 'Unlikely-SRS' group if three or fewer of these factors were present. We demonstrated the reliability of the NH-CSS by also applying the Birmingham¹¹ and Netchine *et al*⁹ scoring systems to our studied population, and comparing the results obtained with the three clinical scoring systems. Sensitivity and specificity were assessed for the identification of a recognised molecular cause of SRS (ie, 11p15 ICR1 hypomethylation or mUPD7). The details of each of the three clinical scoring systems are summarised in online supplementary table S1.

Statistical analysis

See online supplementary data.

RESULTS

Netchine-Harbison clinical scoring system results

The 69 study participants were examined by two paediatric endocrinologists with considerable experience in the diagnosis and care of patients with SRS. These physicians together agreed upon a series of phenotypical characteristics for each patient. The presence or absence of a protruding forehead was determined during the initial examination. For patients over the age of 3 years, the two physicians also evaluated this facial characteristic together, on photos of the child aged 1–3 years (face and profile). This method made it possible to collect homogeneous phenotypical data confirmed by two examiners. Following the review of the photos, each patient was then scored for the new NH-CSS (see online supplementary table S1). Sixty of the 69 patients (35 boys and 25 girls; mean age 6.85 years) included in the study satisfied at least four of the criteria (mean factor frequency=5.29) of the NH-CSS and were consequently classified as 'Likely-SRS'. Nine patients (two boys and seven girls; mean age 4.96 years) were considered as satisfying up to three of these criteria (mean factor frequency=2.89) and were classified as 'Unlikely-SRS'.

We then compared these two groups (Likely-SRS and Unlikely-SRS). We initially studied only those patients for whom we had data for all six criteria ($n=61$). We compared 52 patients from the Likely-SRS group with the 9 patients from the Unlikely-SRS group. As expected, these two groups displayed statistically significant clinical differences (t test; $p=0.000$). We also compared the two groups for each NH-CSS criteria separately. The two groups did not differ significantly in terms of birth weight or length or postnatal growth failure. By contrast, they differed significantly for the other four criteria of the NH-CSS (Pearson's χ^2 ; $p=0.000$) ([table 1](#)).

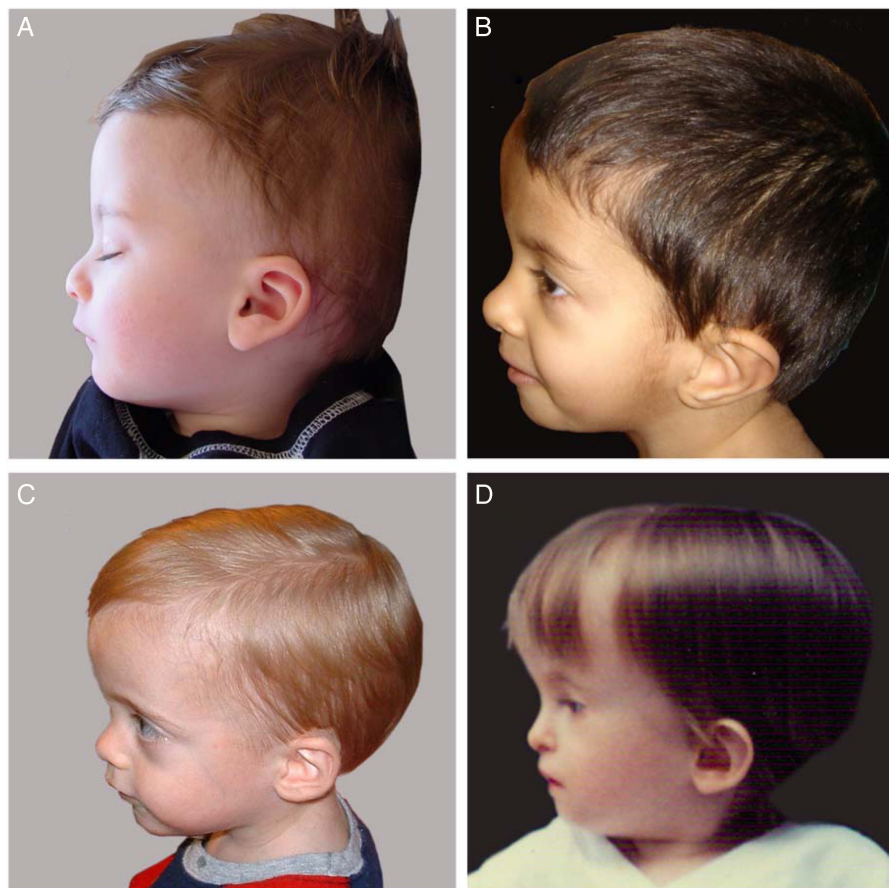
Molecular analysis

11p15 methylation and mUPD7 screening

We assessed 11p15 ICR1 region methylation status and mUPD7 in the 69 patients. We detected 11p15 ICR1 and mUPD7

Genotype-phenotype correlations

Figure 1 Representative pictures of children with a high forehead not protruding (A) or with protruding foreheads of various degrees, mild (B), moderate (C) or marked (D).



abnormalities by carrying out ASMM RTQ-PCR^{16 18} to determine the methylation status of ICR1 CBS2 and *H19*DMR (*H19* promoter) for 11p15 ICR1 and of *PEG/MEST* in the 7p32 region for mUPD7. We identified molecular abnormalities in 46 of the 60 (76.7%) Likely-SRS patients (35 (58.3%) (22 boys and 13 girls; mean age 6.9 years) due to 11p15 ICR1 hypomethylation and 11 (18.3%) (7 boys and 5 girls; mean age 7.69 years) due to mUPD7). Fourteen of the 60 Likely-SRS patients (23.3%) (6 boys and 8 girls; mean age 5.79 years) were negative for both abnormalities and are described hereafter as Likely-SRS double-negatives (L-SRS-dblneg). No molecular abnormality of either the 11p15 ICR1 region or mUPD7 was identified in eight of the nine (88.9%) patients in the Unlikely-SRS group (UL-SRS-dblneg). However, one patient

who was positive for three criteria of the NH-CSS was found to have mUPD7.

Overall, NH-CSS successfully picked up 97.9% of the patients with known molecular abnormalities associated with SRS (100% of the 35 11p15 ICR1 patients and 91.7% of the mUPD7 patients), missing only a single mUPD7 patient.

Multilocus imprinting disturbance in patients with SRS with ICR1 11p15 hypomethylation

Multilocus imprinting disturbance (MLID) is increasingly being reported in human imprinting diseases, including SRS. We therefore screened for MLID in this well clinically characterised cohort of patients with SRS. We investigated the methylation status of 11p15 ICR2, the *DLK1/GTL2* IG-DMR locus on

Table 1 Classification of the SRS population according to the NH-CSS and statistical comparison of the two clinical groups

	Likely-SRS (≥ 4 factors present) Mean or Freq.	Unlikely-SRS (≤ 3 factors present) Mean or Freq.	p Value
Mean of factors recorded 'yes' for each group (subjects with missing data excluded)*†	5.29 (n=52)	2.89 (n=9)	0.000
SGA (birth weight and/or birth length)‡§	55 of 60 (91.67%)	7 of 9 (77.78%)	NS
Postnatal growth failure‡§	55 of 58 (94.83%)	7 of 9 (77.78%)	0.07 (NS)
Relative macrocephaly at birth‡§	46 of 56 (82.14%)	2 of 9 (22.22%)	0.000
Protruding forehead‡§	55 of 58 (94.83%)	5 of 9 (55.56%)	0.000
Body asymmetry‡§	44 of 60 (73.33%)	1 of 9 (11.11%)	0.000
Feeding difficulties and/or BMI ≤ -2 SDS	58 of 59 (98.31%)	3 of 9 (33.33%)	0.000

*†t Test, equal variances not assumed; t=15.279; df 27.619; Sig (2-tailed) 0.000.

†Only subjects with data for all 6 factors were included in this 'overall' top-level analysis, to ensure that group mean factor numbers were comparable.

‡Pearson's χ^2 test.

§The Ns for each scoring system factor can be less than the total of 60, because some subjects were missing data for one or more factors (but still had enough data to qualify for '4 or more factors recorded as yes').

NH-CSS, Netchine-Harblison clinical scoring system; NS, not significant; SGA, small for gestational age; SRS, Silver-Russell syndrome.

chromosome 14q32, *ZAC1* DMR on 6q24, *SNRPN* on 15q11.2 and the *GNAS* locus (*XLα* DMR, *AB* DMR and *NESP55* DMR) on 20q13.32, by ASMM RTQ-PCR. Four of 35 patients (11.4%) were found to display MLID: one with hypomethylation at *ZAC1* DMR (MI=36%; range of normal values 45–57%); one with a methylation profile similar to that found in hypoparathyroidism type 1b (PHP1b) patients (ie, hypomethylation at *XLα* DMR (MI=18%) and *AB* DMR (MI=21%) and hypermethylation at *NESP55* DMR (MI=73%)) and two patients displayed hypomethylation of the *DLK1/GTL2* IG-DMR locus (MIs=17% and 8%).

Further findings

For all patients negative for the conventional molecular aetiologies of SRS,^{21 22} we searched for 11p15 ICR2 duplication, *CDKN1C* mutation or copy number variation (CNV) or imprinting abnormalities in other imprinted regions that might account for the SRS phenotype in the L-SRS-dblneg group or the growth retardation observed in the UL-SRS-dblneg group. We carried out genome-wide screening of DNA with the CytoSNP12 microarray from all the patients of the L-SRS-dblneg and UL-SRS-dblneg groups. Methylation abnormalities in imprinted regions were assessed by ASMM RTQ-PCR. We identified four independent molecular defects in four patients from the L-SRS-dblneg group. Two patients displayed maternal UPD, one of chromosome 16 (mUPD16) and the other of chromosome 20 (mUPD20). A third patient carried a de novo 1q21 3 Mb microdeletion (figure 2). The fourth patient displayed a loss of methylation at the *DLK1/GTL2* IG-DMR locus; and the CytoSNP12 microarray excluded mUPD14, microdeletion or microduplication involving the 14q32 domain.

In the UL-SRS-dblneg group, we identified three independent chromosomal rearrangements in three patients: one patient carried a de novo 20.2 Mb 1q24.3q31.2 microdeletion, the second carried a de novo 5.2 Mb 11p13-p12 microdeletion centromeric to the *WT1* gene and the third carried a paternally transmitted 2.6 Mb 22q11.21 microduplication.

The clinical features of these four L-SRS-dblneg and three UL-SRS-dblneg patients plus the UL-SRS mUPD7 patient are summarised in online supplementary table S7 and supplementary data.

Comparison of NH-CSS criteria between the different molecular groups

We reclassified the Likely-SRS and Unlikely-SRS patients according to the molecular data which results in four groups (11p15 hypomethylation, mUPD7, L-SRS-dblneg and Unlikely-SRS) and then investigated the differences between them in terms of NH-CSS factors. We excluded the mUPD7 false-negative patient from this analysis for the sake of clarity. However, it should be noted that its exclusion had no effect on the results of the statistical analysis (data not shown). Overall scores differed significantly between the four groups (table 2; $p=0.000$). By including only subjects for whom data were available for all six factors, we ensured that comparisons between groups in terms of the overall mean number of NH-CSS positive criteria were carried out correctly. We then compared scoring system factors separately across the four groups. Statistically significant differences between the four groups were found for all the criteria except for one of these factors, postnatal growth failure (χ^2 test; $p=0.06$).

Pairwise comparison analyses showed that the 11p15 ICR1 hypomethylated group was statistically different overall and for all six factors considered separately. The Unlikely-SRS group also differed clinically from the other groups (see online supplementary table S2). Relative macrocephaly was present in a significantly higher percentage of individuals from the SRS 11p15 and SRS mUPD7 groups than in the UL-SRS group (see online supplementary table S3). The percentage of subjects with a protruding forehead was also significantly higher in the SRS 11p15 and SRS mUPD7 groups than in the UL-SRS group (see online supplementary table S4). The percentage of subjects with body asymmetry was also significantly higher in the SRS 11p15 group than in all the other groups but this percentage was significantly higher in the L-SRS-dblneg than in the UL-SRS group (see online supplementary table S5). Finally, feeding difficulties and

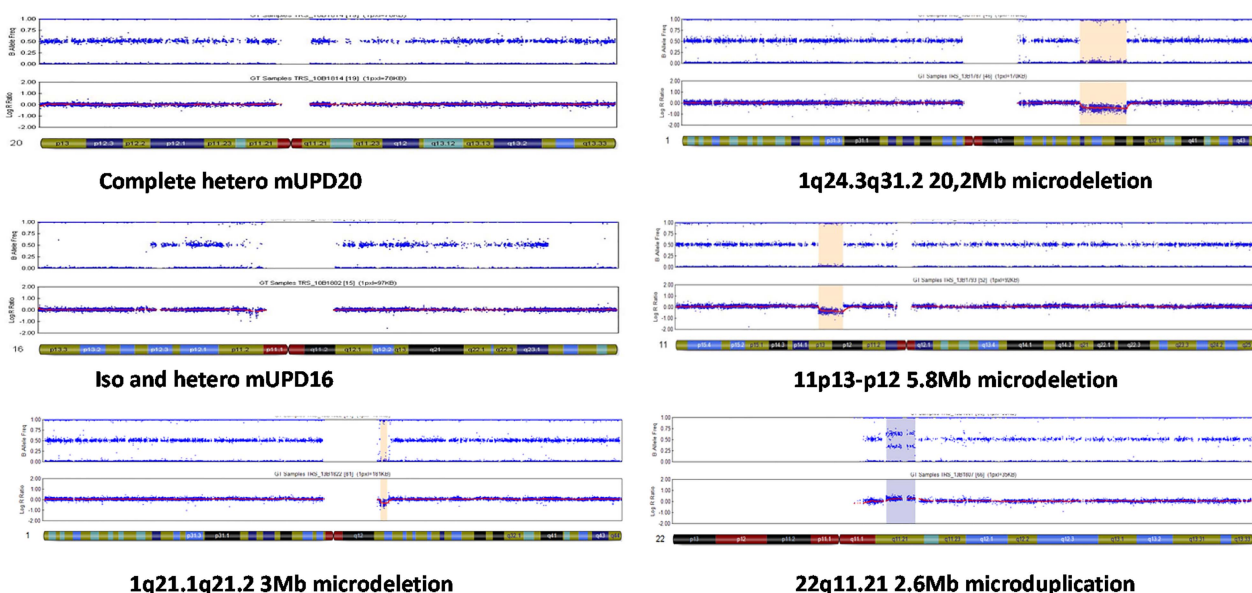


Figure 2 Chromosomal abnormalities found in Likely-Silver-Russell syndrome (SRS) double-negative patients (left panel) and Unlikely-SRS double-negative patients (right panel).

Genotype-phenotype correlations

low BMI were significantly more frequent in the SRS 11p15, SRS mUPD7 and L-SRS-dblneg groups than in the UL-SRS group (see online supplementary table S6).

The NH-CSS failed to identify only one patient for whom subsequent molecular analysis demonstrated SRS mUPD7. Therefore, we sought to identify additional characteristics useful for the clinical diagnosis of SRS for borderline cases with the NH-CSS score of 3–4. We compared the four molecular groups for a number of cognitive and physical variables previously reported or considered by the investigators (IN and MDH) to be common in children with SRS (see online supplementary table S8). The four groups differed significantly in terms of a number of quantifiable physical characteristics; however, incidence calculations showed that the significance of these characteristics was often limited to specific groups (see online supplementary data).

Comparison of the clinical scoring systems for their sensitivity and specificity

We compared our NH-CSS with the scoring system previously reported by Netchine *et al*⁹ and the Birmingham clinical scoring system.¹¹ We applied these three clinical scoring systems to the 69 patients studied here. We considered only the two common molecular aetiologies of SRS, mUPD7 and 11p15 ICR1 hypomethylation, for SRS-positive molecular testing. Our system classified 60 patients as Likely-SRS (table 3), whereas the Netchine *et al*⁹ and Birmingham systems classified only 55 and 47 patients, respectively, as Likely-SRS. The number of Unlikely-SRS patients was therefore higher with these two systems, 14 and 16 patients, respectively than with our new system, 9 patients.

Interestingly, whereas our new scoring system and that of Netchine *et al*⁹ identified 100% of the patients with 11p15 ICR1 hypomethylation, the Birmingham scoring system missed two of the patients (93.7% of identification). In addition, our new scoring system identified all but one of the mUPD7 patients (91.7% identified), whereas the Netchine *et al*⁹ system missed four mUPD7 (66.7% identified) and the Birmingham system missed five mUPD7 (54.6% identified). Overall, the number of false-negative results was reduced in the new scoring system. The Birmingham system uses only four factors making it more prone to the negative effect of missing data points. This resulted in the exclusion of six additional patients due to missing data including three 11p15 ICR1 hypomethylation and one mUPD7

patient. Overall, the Birmingham system failed to identify 11 subjects with molecularly confirmed SRS from our study population.

The new NH-CSS was more sensitive (97.9%) than the Netchine *et al*⁹ (91.5%) and Birmingham (83.7%) clinical scoring systems (table 4). By contrast, our new NH-CSS is less specific (specificity=36%) than the other systems. The three tests had similar positive predictive values, but the NH-CSS had the highest negative predictive value (NPV, 88.9%). Thus, we can have a high degree of confidence that a score of less than four on the NH-CSS screen has a high likelihood of being truly SRS-negative. The Birmingham scoring system had the lowest NPV of the three clinical scoring systems tested (56.3%), due to the number of patients with positive molecular findings who would not have been tested if the decision to test were based on this scoring system.

DISCUSSION

SRS is a clinically and genetically heterogeneous condition. SRS has been extensively studied, but there is still no consensus on the clinical definition of this disorder. Consequently, large numbers of patients not actually meeting typical SRS scoring system criteria, regardless of the scoring system used, are referred to diagnostic laboratories for genetic testing, simply because they may have some of the extensive long list of typical SRS features. This approach is well documented by the low percentage of positive molecular results for both known molecular defects of SRS in molecular diagnostic laboratories which leads to an increase in healthcare costs.²³

Several genotype/phenotype correlation studies have shown that not all patients with SRS are born SGA, and this is particularly true for those with mUPD7.^{24–25} Based on these observations and on our own experience, we revised the Netchine *et al* scoring system and developed a new six-factor NH-CSS. Patients classified as Likely-SRS or Unlikely-SRS with the NH-CSS differed significantly for four of the factors of the scoring system. Furthermore, our new scoring system was found to be highly sensitive and had a strong NPV. Although these values of the NH-CSS might be different in a more heterogeneous population recruited in a growth clinic for short stature, we still could compare for our cohort the sensitivity and the NPV of the NH-CSS with those obtained using other scoring systems previously described. We therefore also applied the Birmingham clinical scoring system¹¹ to our cohort and

Table 2 Statistical comparison between the four molecular groups, overall and individually, for the six factors of the NH-CSS

	11p15 ICR1 hypomethylation	mUPD7*	L-SRS-dblneg	UL-SRS*	p Value
Mean number of factors positive†,‡	5.86 (n=29)	4.73 (n=11)	4.42 (n=12)	2.88 (n=8)	0.000
SGA (birth weight and/or length)§	35 of 35 (100%)	8 of 11 (72.7%)	12 of 14 (85.7%)	7 of 8 (87.5%)	0.034
Postnatal growth failure§	34 of 34 (100%)	10 of 11 (90.9%)	11 of 13 (84.6%)	6 of 8 (75%)	0.060
Relative macrocephaly at birth§	31 of 32 (96.9%)	9 of 11 (81.8%)	6 of 13 (46.2%)	2 of 8 (25%)	0.000
Protruding forehead§	32 of 33 (97.0%)	11 of 11 (100%)	12 of 14 (85.7%)	4 of 8 (50%)	0.001
Body asymmetry§	33 of 35 (94.3%)	3 of 11 (27.3%)	8 of 14 (57.1%)	1 of 8 (12.5%)	0.000
Feeding difficulties and/or BMI <−2SD§§	35 of 35 (100%)	11 of 11 (100.0%)	12 of 13 (92.3%)	3 of 8 (37.5%)	0.000

†One-way ANOVA.

‡ χ^2 test.

*The 'mUPD7 Unlikely SRS' subject was removed from this analysis. Even if this subject was included in the 'Unlikely SRS' group, the significance level of the overall system remained at 0.000 and the level of significance of the factors remained within their overall significance level categories (<0.001, 0.05 or 0.1).

‡Only subjects with data for all 6 factors were included in this 'overall' top-level analysis, to ensure that group mean factor numbers were comparable.

§ANOVA, analysis of variance; L-SRS-dblneg, Likely SRS double-negative; mUPD, maternal UPD of chromosome 7; NH-CSS, Netchine-Harblison clinical scoring system; SGA, small for gestational age; SRS, Silver-Russell syndrome; UL-SRS, Unlikely-SRS.

Table 3 Assessment of the performance of the three clinical scoring systems on our cohort of 69 patients

SCORING SYSTEM	Likely SRS (according to each system)			Unlikely SRS (according to each system)			Excluded Subjects (due to insufficient data)	Number of patients carrying 11p15 ICR1 hypomethylation or mUPD7 classified by each system as "Likely SRS"	Number of patients carrying 11p15 ICR1 hypomethylation or mUPD7 classified by each system as "Unlikely SRS"
	11p15 ICR1 hypomethylation	mUPD7	L-SRS-dblneg	Total Likely-SRS	11p15 ICR1 hypomethylation	mUPD7	UL-SRS-dblneg	Total Unlikely-SRS	
NH CSS	35 (100%)	11 (91.7%)	14	60	0	1 (8.3%)	8	9	46 of 47 (97.9%) 1 of 47 (2.13%)
Netchine <i>et al</i> ⁹	35 (100%)	8 (66.7%)	12	55	0	4 (33.3%)	10	14	43 of 47 (91.5%) 4 of 47 (8.51%)
Birmingham	30 (93.7%)	6 (54.5%)	11	47	2 (6.3%)	5 (45.5%)	9	16	36 of 47 (76.6%) & 4 others were excluded due to missing data

SRS, Silver-Russell syndrome; mUPD, maternal UPD of chromosome 7; NH-CSS, Netchine-Harbison clinical scoring system; SGA, small for gestational age.

demonstrated that this system was less effective than ours. The Birmingham clinical scoring system takes into account only low birth weight and not length, whereas SGA is now defined in terms of low birth weight and/or birth length. Furthermore, previous SRS studies have shown that length is typically more restricted than weight at birth.^{9 26} The failure to include low birth length in the Birmingham clinical scoring system caused the exclusion of 11 patients positive for either mUPD7 or 11p15 ICR1 hypomethylation from our cohort.

In our cohort, only about 20% of the Likely-SRS patients had unknown underlying molecular abnormalities, potentially accounting for the low specificity of the NH-CSS. In the last few years, a number of chromosomal rearrangements/disomies have been identified in patients diagnosed with SRS.^{21 22} We identified six different chromosomal rearrangements present in the combination of the Likely-SRS and Unlikely-SRS groups. The mUPD16, mUPD20 and 1q21 microdeletion abnormalities have all been identified in patients with growth restriction²⁷⁻²⁹ and, recently, in patients with SRS.^{30 31} Various clinical presentations have been reported for subjects with mUPD16 and 1q21 microdeletion, but mUPD20 carriers have a more consistent clinical presentation.^{27 28} *DLK1/GTL2* IG-DMR hypomethylation has been described in patients having Temple syndrome (TS).³² Interestingly, we identified one Likely-SRS patient with *DLK1/GTL2* IG-DMR hypomethylation reminiscent of TS. Kagami *et al* also recently reported two patients with SRS negative for common molecular causes of SRS but with hypomethylation at the *DLK1/GTL2* IG-DMR locus.³³

The identification of *DLK1/GTL2* IG-DMR hypomethylation in patients with a phenotype consistent with SRS may be considered a new finding as hypomethylation at this locus has only been involved in TS, so far. Further studies will allow us to investigate if all patients with TS qualify for the NH-CSS for SRS and if that is the case, should they in the future be part of SRS.

The other chromosomal rearrangements we identified in the three Unlikely-SRS patients have all been reported in patients with the wilms tumour, aniridia, genital anomalies, retardation (WAGR) and Potocki-Shaffer syndromes (11p13-p12 microdeletion),³⁴ 22q11 del/dup syndrome³⁵ or 1q24.3-q31 microdeletion³⁶ but have not been reported in patients considered to have SRS.

The NH-CSS should help physicians to decide if molecular tests for SRS are appropriate for their patients and a chart for

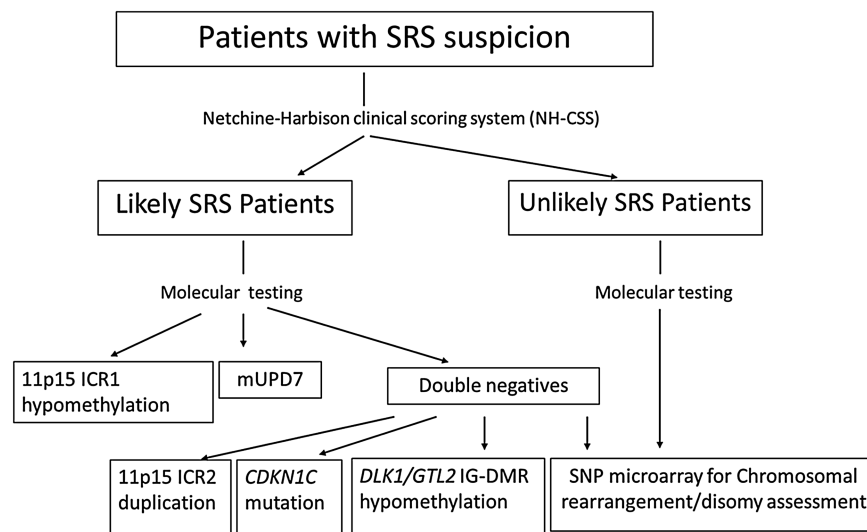
Table 4 Sensitivity and specificity of the three clinical scoring systems calculated for our cohort of patients

	NH CSS (%)	Netchine <i>et al</i> ⁹ (%)	Birmingham (%)
Sensitivity (Birmingham article)	N/A	70.0	82.0
Specificity (Birmingham article)	N/A	81.0	80.0
Sensitivity (Netchine-Harbison data)	97.9	91.5	83.7
Specificity (Netchine-Harbison data)	36.4	45.5	45.0
Positive predictive value	76.7	78.2	76.6
Negative predictive value	88.9	71.4	56.3

The 'Likely-SRS' patients with molecular causes other than mUPD7 or 11p15 ICR1 hypomethylation are considered as false positives for the purposes of this assessment. mUPD, maternal UPD of chromosome 7; NH-CSS, Netchine-Harbison clinical scoring system; SRS, Silver-Russell syndrome.

Genotype-phenotype correlations

Figure 3 Flow chart detailing the molecular testing orientation when a Silver-Russell syndrome (SRS) is clinically suspected.



molecular diagnosis is detailed in figure 3. However, the absence of a positive molecular test result should not rule out the clinical diagnosis of SRS in the patient concerned. This new scoring system missed one patient with genuine SRS who fulfilled only three of the NH-CSS criteria. The heterogeneous clinical presentation of SRS makes it very difficult to diagnose those patients with a mild SRS phenotype, particularly in some mUPD7 cases. Consequently, we looked for additional quantitative variables and typical physical SRS characteristics but did not find any that improve the scoring system but only helped to distinguish mUPD7 and 11p15 hypomethylation groups.

A precise and simple clinical definition of SRS is important in order to establish a prevalence of this rare condition, propose common clinical guidelines and possible common clinical trials for this group of patients and allow the research to progress for the patients with no molecular cause identified. Sixty years after the initial description of SRS,^{1 2} two molecular defects are considered to be common. In the future potentially new molecular aetiologies may be added to this list because they can be identified in patients diagnosed with SRS on our simple clinical scoring system who share enough similar clinical presentations so that they benefit from the same clinical management. This implies that SRS should remain a clinical diagnosis, based on the NH-CSS criteria. Because phenotypical differences exist between the groups of patients with SRS with different molecular aetiologies, and yet the overall clinical SRS group has been found to be statistically different from those unlikely to have SRS, we suggest that SRS should be considered as a spectrum based on positive NH-CSS screening. Similar clinical guidelines for treatment are appropriate for idiopathic SRS, once the differential diagnoses that question the efficacy of growth hormone (GH) therapy (ie, 3M syndrome,^{7 37} IGF1R mutation³⁸) and some that question the safety or preclude GH therapy (ie, Bloom,³⁹ Mulibrey-nanism⁶ and Fanconi⁴⁰ syndromes) have been ruled out.

Author affiliations

¹INSERM, UMR_S 938, CDR Saint-Antoine, Paris, France

²Sorbonne Universités, UPMC Univ Paris 06, UMR_S 938, CDR Saint-Antoine, Paris, France

³Department of Pediatric Endocrinology, APHP, Armand Trousseau Hospital, Paris, France

⁴Epigenetics Programme, The Babraham Institute, Cambridge, UK

⁵MAGIC Foundation, RSS/SGA Research & Education Fund, Oak Park, Illinois, USA

⁶AP-HP, Hôpital Trousseau, Service de Génétique et d'Embryologie Médicales, Paris, France

⁷Department of Psychiatry and Biobehavioral Sciences, Semel Institute, University of California, Los Angeles, California, USA

⁸Department of Pediatrics, Ichan School of Medicine at Mount Sinai, New York, New York, USA

Acknowledgements The authors thank the patients, their families and the physicians for referring patients; the MAGIC Foundation and Dayna Carney for their help with the patients recruited in the study; Frédéric Brioude, Sylvie Rossignol and Yves Le Bouc for helpful discussions; Boris Keren for the SNP arrays analysis and Cristina Das Neves, Marilyne Le Jule and Virginie Steunou for technical assistance.

Contributors SA participated in data analysis, performed the multilocus methylation study and wrote the paper. JS set up the clinical database and managed it, contributed to the statistical analysis and reviewed the paper. EL performed the statistical analysis. NT performed the molecular diagnosis: 11p15 region methylation and mUPD7. SC-B performed the SNP microarray study and reviewed the paper. IN and MDH designed the study, performed the clinical and phenotypical investigations on the entire cohort, analysed the data and reviewed the paper. IN validated the molecular studies.

Funding This work was supported by INSERM, UPMC-Paris6 funding, ANR EPIFEGRO 2010, a Pfizer grant, an Agence de Biomédecine 2010 grant, the MAGIC Foundation, the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 290123. SA was supported by Novonordisk and the INSERM-ANR EPIFEGRO 2010. IN is member of the COST Action BM1208.

Competing interests None declared.

Patient consent Obtained.

Ethics approval IRB I00000204 at the Mount Sinai School of Medicine, NYC, USA.

Provenance and peer review Not commissioned; externally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

REFERENCES

- 1 Silver HK, Kiyasu W, George J, Deamer WC. Syndrome of congenital hemihypertrophy, shortness of stature, and elevated urinary gonadotropins. *Pediatrics* 1953;12:368–76.
- 2 Russell A. A syndrome of intra-uterine dwarfism recognizable at birth with cranio-facial dysostosis, disproportionately short arms, and other anomalies (5 examples). *Proc R Soc Med* 1954;47:1040–4.
- 3 Escobar V, Gleiser S, Weaver DD. Phenotypic and genetic analysis of the silver-Russell syndrome. *Clin Genet* 1978;13:278–88.
- 4 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;36:837–42.

- 5 Anderson J, Viskochil D, O'Gorman M, Gonzales C. Gastrointestinal complications of Russell-Silver syndrome: a pilot study. *Am J Med Genet* 2002;113:15–19.
- 6 Hamalainen RH, Mowat D, Gabbett MT, O'Brien TA, Kallijarvi J, Lehesjoki AE. Wilms' tumor and novel TRIM37 mutations in an Australian patient with mulibrey nanism. *Clin Genet* 2006;70:473–9.
- 7 van der Wal G, Otten BJ, Brunner HG, van der Burgt I. 3-M syndrome: description of six new patients with review of the literature. *Clin Dysmorphol* 2001;10:241–52.
- 8 Lai KY, Skuse D, Stanhope R, Hindmarsh P. Cognitive abilities associated with the Silver-Russell syndrome. *Arch Dis Child* 1994;71:490–6.
- 9 Netchine I, Rossignol S, Dufourg MN, Azzi S, Rousseau A, Perin L, Houang M, Steunou V, Esteve B, Thibaud N, Demay MC, Danton F, Petriczko E, Bertrand AM, Heinrichs C, Carel JC, Loeuille GA, Pinto G, Jacquemont ML, Gicquel C, Cabrol S, Le Bouc Y. 11p15 imprinting center region 1 loss of methylation is a common and specific cause of typical Russell-Silver syndrome: clinical scoring system and epigenetic-phenotypic correlations. *J Clin Endocrinol Metab* 2007;92:3148–54.
- 10 Bartholdi D, Krajewska-Walasek M, Ounap K, Gaspar H, Chrzanowska KH, Ilyana H, Kayserili H, Lurie IW, Schinzel A, Baumer A. Epigenetic mutations of the imprinted IGF2-H19 domain in Silver-Russell syndrome (SRS): results from a large cohort of patients with SRS and SRS-like phenotypes. *J Med Genet* 2009;46:192–7.
- 11 Dias RP, Nightingale P, Hardy C, Kirby G, Tee L, Price S, Macdonald F, Barrett TG, Maher ER. Comparison of the clinical scoring systems in Silver-Russell syndrome and development of modified diagnostic criteria to guide molecular genetic testing. *J Med Genet* 2013;50:635–9.
- 12 Gicquel C, Rossignol S, Cabrol S, Houang M, Steunou V, Barbu V, Danton F, Thibaud N, Le Merrer M, Burglen L, Bertrand AM, Netchine I, Le Bouc Y. Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. *Nat Genet* 2005;37:1003–7.
- 13 Schonherr N, Meyer E, Eggermann K, Ranke MB, Wollmann HA, Eggermann T. (Epi) mutations in 11p15 significantly contribute to Silver-Russell syndrome: but are they generally involved in growth retardation? *Eur J Med Genet* 2006;49:414–18.
- 14 Azzi S, Abi Habib W, Netchine I. Beckwith-Wiedemann and Russell-Silver Syndromes: from new molecular insights to the comprehension of imprinting regulation. *Curr Opin Endocrinol Diabetes Obes* 2014;21:30–8.
- 15 Azzi S, Blaise A, Steunou V, Harbison MD, Salem J, Brioude F, Rossignol S, Habib WA, Thibaud N, Neves CD, Jule ML, Brachet C, Heinrichs C, Bouc YL, Netchine I. Complex tissue-specific epigenotypes in Russell-Silver Syndrome associated with 11p15 ICR1 hypomethylation. *Hum Mutat* 2014;35:1211–20.
- 16 Azzi S, Rossignol S, Steunou V, Sas T, Thibaud N, Danton F, Le Jule M, Heinrichs C, Cabrol S, Gicquel C, Le Bouc Y, Netchine I. Multilocus methylation analysis in a large cohort of 11p15-related foetal growth disorders (Russell Silver and Beckwith Wiedemann syndromes) reveals simultaneous loss of methylation at paternal and maternal imprinted loci. *Hum Mol Genet* 2009;18:4724–33.
- 17 Maupetit-Mehouas S, Azzi S, Steunou V, Sakakini N, Silve C, Reynes C, Perez de Nandares G, Keren B, Chantot S, Barlier A, Linglart A, Netchine I. Simultaneous hyper- and hypomethylation at imprinted loci in a subset of patients with GNAS epimutations underlies a complex and different mechanism of multilocus methylation defect in pseudohypoparathyroidism type 1b. *Hum Mutat* 2013;34:1172–80.
- 18 Azzi S, Steunou V, Rousseau A, Rossignol S, Thibaud N, Danton F, Le Jule M, Gicquel C, Le Bouc Y, Netchine I. Allele-specific methylated multiplex real-time quantitative PCR (ASMM RTQ-PCR), a powerful method for diagnosing loss of imprinting of the 11p15 region in Russell Silver and Beckwith Wiedemann syndromes. *Hum Mutat* 2011;32:249–58.
- 19 Brioude F, Oliver-Petit I, Blaise A, Praz F, Rossignol S, Le Jule M, Thibaud N, Faussat AM, Tauber M, Le Bouc Y, Netchine I. CDKN1C mutation affecting the PCNA-binding domain as a cause of familial Russell Silver syndrome. *J Med Genet* 2013;50:823–30.
- 20 Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 1969;74:901–10.
- 21 Bruce S, Hannula-Jouppi K, Puoskari M, Franssón I, Simola KO, Lipsanen-Nyman M, Kere J. Submicroscopic genomic alterations in Silver-Russell syndrome and Silver-Russell-like patients. *J Med Genet* 2010;47:816–22.
- 22 Fokstuen S, Kotzot D. Chromosomal rearrangements in patients with clinical features of Silver-Russell syndrome. *Am J Med Genet A* 2014;164A:1595–605.
- 23 Eggermann T, Gonzalez D, Spengler S, Arslan-Kirchner M, Binder G, Schonherr N. Broad clinical spectrum in Silver-Russell syndrome and consequences for genetic testing in growth retardation. *Pediatrics* 2009;123:e929–31.
- 24 Binder G, Seidel AK, Martin DD, Schweizer R, Schwarze CP, Wollmann HA, Eggermann T, Ranke MB. The endocrine phenotype in silver-russell syndrome is defined by the underlying epigenetic alteration. *J Clin Endocrinol Metab* 2008;93:1402–7.
- 25 Wakeling EL, Amero SA, Alders M, Bliek J, Forsythe E, Kumar S, Lim DH, MacDonald F, Mackay DJ, Maher ER, Moore GE, Poole RL, Price SM, Tangeraas T, Turner CL, Van Haelst MM, Willoughby C, Temple IK, Cobben JM. Epigenotype-phenotype correlations in Silver-Russell syndrome. *J Med Genet* 2010;47:760–8.
- 26 Fuke T, Mizuno S, Nagai T, Hasegawa T, Horikawa R, Miyoshi Y, Muroya K, Kondoh T, Numakura C, Sato S, Nakabayashi K, Tayama C, Hata K, Sano S, Matsubara K, Kagami M, Yamazawa K, Ogata T. Molecular and clinical studies in 138 Japanese patients with Silver-Russell syndrome. *PLoS ONE* 2013;8:e60105.
- 27 Kotzot D, Utermann G. Uniparental disomy (UPD) other than 15: phenotypes and bibliography updated. *Am J Med Genet A* 2005;136:287–305.
- 28 Brunetti-Pierri N, Berg JS, Scaglia F, Belmont J, Bacino CA, Sahoo T, Lalani SR, Graham B, Lee B, Shinawi M, Shen J, Kang SH, Pursley A, Lotze T, Kennedy G, Lansky-Shafer S, Weaver C, Roeder ER, Grebe TA, Arnold GL, Hutchison T, Reimschisel T, Amato S, Geraghty MT, Innis JW, Obersztyn E, Nowakowska B, Rosengren SS, Bader PI, Grange DK, Naqvi S, Garnica AD, Bernes SM, Fong CT, Summers A, Walters WD, Lupski JR, Stankiewicz P, Cheung SW, Patel A. Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. *Nat Genet* 2008;40:1466–71.
- 29 Mefford HC, Sharp AJ, Baker C, Itsara A, Jiang Z, Buysse K, Huang S, Maloney VK, Crolla JA, Baralle D, Collins A, Mercer C, Norga K, de Ravel T, Devriendt K, Bongers EM, de Leeuw N, Reardon W, Gimelli S, Bena F, Hennekam RC, Male A, Gaunt L, Clayton-Smith J, Simoncic I, Park SM, Mehta SG, Nik-Zainal S, Woods CG, Firth HV, Parkin G, Fichera M, Reitano S, Lo Giudice M, Li KE, Casuga I, Broome A, Conrad B, Schwerzmann M, Raber L, Gallati S, Striano P, Coppola A, Tolmie JL, Tobias ES, Lilley C, Armengol L, Spysschaert Y, Verloo P, De Coene A, Goossens L, Mortier G, Speleman F, van Binsbergen E, Nelen MR, Hochstenbach R, Poot M, Gallagher L, Gill M, McClellan J, King MC, Regan R, Skinner C, Stevenson RE, Antonarakis SE, Chen C, Estivill X, Menten B, Gimelli G, Gribble S, Schwartz S, Sutcliffe JS, Walsh T, Knight SJ, Sebati J, Romano C, Schwartz CE, Veltman JA, de Vries BB, Vermeesch JR, Barber JC, Willatt L, Tassabehji M, Eichler EE. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N Engl J Med* 2008;359:1685–99.
- 30 Poole RL, Docherty LE, Al Sayegh A, Caliebe A, Turner C, Baple E, Wakeling E, Harrison L, Lehmann A, Temple IK, Mackay DJ. Targeted methylation testing of a patient cohort broadens the epigenetic and clinical description of imprinting disorders. *Am J Med Genet A* 2013;161:2174–82.
- 31 Spengler S, Begemann M, Ortiz Bruchle N, Baudis M, Denecke B, Kroisel PM, Oehl-Jaschkowitz B, Schulze B, Raabe-Meyer G, Spaich C, Blumel P, Jauch A, Moog U, Zeres K, Eggermann T. Molecular karyotyping as a relevant diagnostic tool in children with growth retardation with Silver-Russell features. *J Pediatr* 2012;161:933–42.
- 32 Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. *J Med Genet* 2014;51:495–501.
- 33 Kagami M, Mizuno S, Matsubara K, Nakabayashi K, Sano S, Fuke T, Fukami M, Ogata T. Epimutations of the IG-DMR and the MEG3-DMR at the 14q32.2 imprinted region in two patients with Silver-Russell syndrome-compatible phenotype. *Eur J Hum Genet* 2014. Published Online First: 5 Nov 2014. doi: 10.1038/ejhg.2014.234
- 34 Almird GJ, Brondum-Nielsen K, Bangsgaard R, Baekgaard P, Gronskov K. 11p Microdeletion including WT1 but not PAX6, presenting with cataract, mental retardation, genital abnormalities and seizures: a case report. *Mol Cytogenet* 2009;2:6.
- 35 Yobb TM, Somerville MJ, Willatt L, Firth HV, Harrison K, MacKenzie J, Gallo N, Morrow BE, Shaffer LG, Babcock M, Chernos J, Bernier F, Spysak K, Christiansen J, Haase S, Elyas B, Lilley M, Bamforth S, McDermid HE. Microduplication and triplication of 22q11.2: a highly variable syndrome. *Am J Hum Genet* 2005;76:865–76.
- 36 Prontera P, Clerici G, Bernardini L, Schippa M, Capalbo A, Manes I, Giuffrida MG, Barbieri MG, Ardisia C, Dotti E. Prenatal diagnosis and molecular characterization of an interstitial 1q24.3-31.3 deletion: case report and review. *Genet Couns* 2011;22:41–8.
- 37 Clayton PE, Hanson D, Magee L, Murray PG, Saunders E, Abu-Amro SN, Moore GE, Black GC. Exploring the spectrum of 3-M syndrome, a primordial short stature disorder of disrupted ubiquitination. *Clin Endocrinol (Oxf)* 2012;77:335–42.
- 38 Walenkamp MJ, Losekoot M, Wit JM. Molecular IGF-1 and IGF-1 receptor defects: from genetics to clinical management. *Endocr Dev* 2013;24:128–37.
- 39 Renes JS, Willemsen RH, Wagner A, Finken MJ, Hokken-Koelega AC. Bloom syndrome in short children born small for gestational age: a challenging diagnosis. *J Clin Endocrinol Metab* 2013;98:3932–8.
- 40 Chernauek SD. Mendelian genetic causes of the short child born small for gestational age. *J Endocrinol Invest* 2006;29(1 Suppl):16–20.



A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome

Salah Azzi, Jennifer Salem, Nathalie Thibaud, Sandra Chantot-Bastaraud, Eli Lieber, Irène Netchine and Madeleine D Harbison

J Med Genet 2015 52: 446-453 originally published online May 7, 2015
doi: 10.1136/jmedgenet-2014-102979

Updated information and services can be found at:
<http://jmg.bmj.com/content/52/7/446>

These include:

- | | |
|-------------------------------|--|
| Supplementary Material | Supplementary material can be found at:
http://jmg.bmj.com/content/suppl/2015/04/30/jmedgenet-2014-102979.DC1.html |
| References | This article cites 39 articles, 11 of which you can access for free at:
http://jmg.bmj.com/content/52/7/446#BIBL |
| Open Access | This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/ |
| 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 online article. |
-

- | | |
|--------------------------|---|
| Topic Collections | Articles on similar topics can be found in the following collections
Open access (156)
Epidemiology (618) |
|--------------------------|---|
-

Notes

To request permissions go to:
<http://group.bmj.com/group/rights-licensing/permissions>

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

To subscribe to BMJ go to:
<http://group.bmj.com/subscribe/>