SUPPLEMENTARY METHODS

DNA extraction

Blood samples were collected from all 69 patients, and genomic DNA was extracted from the leukocytes of each patient by a previously described salting-out procedure [1], at the Molecular Biology Laboratory of Mount Sinai School of Medicine, New York City, USA. All further molecular investigations were carried out at the Molecular Biology Laboratory of Armand Trousseau Hospital, France.

Sodium bisulfite treatment of DNA

Genomic DNA (1 µg) was treated with sodium bisulfite, with the EZ DNA methylation kit (Zymo Research, Orange, CA), according to the manufacturer's instructions, and was then eluted in 50 µl RNase-free H_2O.

TaqMan Allele-Specific Methylated Multiplex Real-Time Quantitative PCR (ASMM RTQ-PCR)

ASMM RTQ-PCR was performed as previously described [2], with the 7900HT Fast Real-Time PCR System. Briefly, multiplex amplification was performed on a 96-well plate, in a reaction volume of 20 µl, containing 10 ng of sodium bisulfite-treated genomic DNA (3 µl per well), 9 µl TaqMan Master Mix, 900 nM of each primer (Sigma Aldrich, Saint-Quentin Fallavier, France) and 200 nM of each TaqMan-MGB probe (Life Technologies, Saint Aubin, France). The analysis was performed with SDS 2.4 software (Life Technologies, Saint Aubin, France). The methylation index (MI) at each locus was determined by calculating the ratio of methylated to unmethylated alleles as follows: (number of methylated alleles / sum of methylated and unmethylated alleles) x 100. For each locus, we considered the MI to be normal if it was within two standard deviations of the mean MI value for the control population.

The MI was considered abnormal if outside this range. The primers and probes are available upon request.

SNP microarray

We used Illumina CytoSNP-12 arrays (Illumina, San Diego, California) to screen for copy number variation (CNV) and uniparental disomy (UPD), uniparental isodisomy (UPiD) as well as uniparental heterodisomy (UPhD). Briefly, samples were processed for the Infinium® assay and the results were analyzed with Illumina GenomeStudio® software. References were built with the clustering algorithm Illumina Gentrain 2.0 with SNP profiles from the 96 samples processed in the same run. DNA samples from patients and their parents, when indicate, were processed in the same run.

Statistical analysis

Data from 69 patients were available for analysis. The characteristics of the population are described as percentages for qualitative variables or as SDS, mean, and/or range for continuous variables. Relationships between categorical and continuous variables were analyzed by standard one-way ANOVA, with statistical significance assessed in *F* tests. If a significant *F* value was obtained, we carried out pairwise comparisons, using Tukey's HSD. Relationships between two categorical variables were assessed in Pearson's χ^2 tests. If the χ^2 value obtained was significant, and where indicated, pairwise comparisons were carried out, with the Z-test for equality between two proportions. A *P*-value ≤ 0.05 was considered to indicate statistical significance. All tests were two-tailed. We used SPSS Statistics V17 (SPSS Inc. Chicago, IL) for statistical analysis.

SUPPLEMENTARY RESULTS

Additional characteristics useful for the clinical diagnosis of SRS

The presence of shoulder dimples and low muscle mass differed significantly between the four groups. Both occurred in higher incidence rates in the 11p15 and mUPD7 groups and moderately higher rates in the L-SRS-dblneg group than in the Unlikely-SRS group. The frequency of a prominent heel also differed significantly between the four groups (p=0.000), with the mUPD7 group having a 100% incidence rate, while the other three groups had similar low incidence rates. Likewise, autism traits/PDD (pervasive developmental disorders**)** and diagnosed cognitive disabilities were far more frequent in the mUPD7 group than in the other three groups. Thus, additional characteristics can help the physician to decide whether or not to test for mUPD7 or 11p15 hypomethylation. But in general, these characteristics will not be helpful in distinguishing between Likely- and Unlikely-SRS.

The clinical features of the patients carrying the new molecular etiologies

Each of these four L–SRS-dblneg patients was positive for four or five criteria of the NH-CSS and displayed many clinical characteristics commonly found in SRS patients. All presented with postnatal growth failure and had a protruding forehead. Three of the four were small for gestational age, displayed body asymmetry and had feeding difficulties. Interestingly, three of these four patients did not have relative macrocephaly at birth. No single additional physical characteristic was common to all four subjects but none of these four patients had autism/PDD.

The three Unlikely-SRS patients with molecular rearrangements, each of whom was positive for only three of the NH-CSS criteria, were similar for SGA, feeding difficulties and lack of body asymmetry. For the additional physical characteristics, there appeared to be little consistency across the three subjects for any single

characteristic other than the presence of clinodactyly and downturned mouth in all three and absence of shoulder dimples.

SUPPLEMENTARY REFERENCES

1. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;**16**(3):1215
2. Azzi S, Steunou V, Rousseau A, et al. 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**(2):249-58 doi: 10.1002/humu.21403[published Online First: Epub Date]|.