1 **SUPPLEMENTARY METHODS**

2 **Ethical approval**

3 This study was approved by the Institutional Review Board Committee at the National
4 Center for Child Health and Development, and was performed after obtaining written
5 informed consent from all individuals. Furthermore, we obtained written informed
6 consent to publish molecular, clinical information and facial photographs of two patients
7 with maternal uniparental disomy of chromosome 16 (UPD(16)mat).

8 **Patients**

9 We summarised the inclusion criteria in online supplementary figure 1. A total of 330
10 patients were referred to us for genetic testing for Silver-Russell syndrome (SRS) by
11 their presenting physicians from 2002 to 2017. Because presenting physicians did not
12 accurately evaluate clinical features related to the Netchine-Harbison clinical scoring
13 system (NH-CSS) before 2017 when an international consensus statement for SRS was
14 published,[1] we did not have enough clinical information of NH-CSS criteria in all of
15 our patients. After excluding patients with loss of methylation on chromosome 11p15
16 (11p15 LOM) (n=90) and with maternal uniparental disomy of chromosome 7
17 (UPD(7)mat) (n=24), we asked the attending physicians of the remaining patients about
18 clinical features related to NH-CSS using questionnaires. Attending physicians were
19 mainly general paediatricians unfamiliar with SRS and some paediatric endocrinologists
20 and paediatric geneticists who were more familiar with SRS. Because of insufficient
21 clinical information, 51 patients were excluded from this study, and 43 patients were
22 also excluded from this study due to low score in NH-CSS. A total of 122 patients
23 without 11p15 LOM and UPD(7)mat satisfied NH-CSS or met three NH-CSS criteria,
24 but were clinically suspected as having SRS. For these patients, we performed
25 methylation analysis for six differentially methylated regions (DMRs), namely,
26 *PLAGL1*:alt-TSS-DMR, *KCNQ1OT1*:TSS-DMR, *MEG3/DLK1*:IG-DMR, *MEG3*:TSS-
27 DMR, *SNURF*:TSS-DMR and *GNAS A/B*:TSS-DMR to detect other imprinting

disorders, and array comparative genomic hybridisation (aCGH) analysis to detect pathogenic CNVs (PCNVs) causing known genetic diseases. After excluding 21 patients with abnormal methylation levels of these DMRs and seven patients with PCNVs, 94 aetiology-unknown patients with SRS phenotype were included in our study. Of the 94 patients, 63 satisfied NH-CSS and the remaining 31 patients met only three NH-CSS criteria, but were clinically suspected as having SRS. We performed aCGH analysis using the SurePrint G3 Human CGH Array Kit 8×60K (catalog number G4450A, Agilent Technologies, Palo Alto, CA, USA).[2] Methylation analysis was performed by combined bisulfite restriction analysis or pyrosequencing as previously reported.[3, 4] All patients were Japanese except for two patients who were from Canada and the USA.

Clinical diagnosis as SRS was based on the NH-CSS, which includes six items: (1) small for gestational age (birth weight and/or birth length ≤−2 SD score (SDS) for gestational age), (2) postnatal growth retardation (height at 24 ± 1 months ≤−2 SDS or height ≤−2 SDS below mid-parental target height), (3) relative macrocephaly at birth (head circumference at birth ≥1.5 SDS above birth weight and/or length SDS), (4) protruding forehead (forehead projecting beyond the facial plane from the side view among toddlers), (5) body asymmetry (leg length discrepancy (LLD) of ≥0.5 cm or arm asymmetry or LLD <0.5 cm with at least two other asymmetrical body parts (one non-face)), (6) feeding difficulties and/or low body mass index (BMI) (BMI ≤−2 SDS at 24 months or current use of a feeding tube or cyproheptadine for appetite stimulation).[1] Patients meeting four or more of these six criteria received a diagnosis of SRS.[1] In addition, patients meeting NH-CSS criteria including both protruding forehead and relative macrocephaly, but normal in all molecular testing, received a diagnosis of clinical SRS.[1] In our patients, 25 out of 63 patients got a diagnosis of clinical SRS. For patients younger than 23 months old, the criterion for postnatal growth retardation was excluded from the NH-CSS criteria.

The diagnosis of SRS can be difficult, as the condition varies widely in

55 severity among affected individuals and many of its features are nonspecific.[1]
56 Furthermore, in our study, many general paediatricians unfamiliar with SRS evaluated
57 the clinical features for their patients. Because triangular face, fifth finger clinodactyly
58 and/or brachydactyly were frequently observed in SRS patients[3] and many general
59 paediatricians suspected their patients as having SRS based on these features before
60 2017 when the consensus statement was published, we adopted these features as clinical
61 features related to continued clinical suspicion of SRS.

62 **Molecular analysis**

63 To detect UPD(16)mat, we first performed methylation analysis with pyrosequencing for
64 the paternally methylated *ZNF597*:TSS-DMR on chromosome 16 using bisulfite-treated
65 genomic DNA (gDNA) from the leucocytes as previously reported.[4, 5] Furthermore,
66 we examined the methylation levels of the maternally methylated *ZNF597*:3′ DMR in the
67 patients with low methylation levels of the *ZNF597*:TSS-DMR. Next, we performed
68 microsatellite analysis for chromosome 16 in patients with abnormal methylation levels
69 of these DMRs using gDNA from the leucocytes of these patients and their parents. In
70 addition, to detect hidden mosaic trisomy 16, we also examined patients' gDNA from
71 buccal cells obtained with Oragene OG-575 (DNA Genotek, Ottawa, Canada). Because
72 the mother of patient 1 had severe short stature, we also performed methylation analysis
73 for nine DMRs related to imprinting disorders and the *ZNF597*:TSS-DMR using gDNA
74 samples from her leucocytes. Sequences of the primer sets for pyrosequencing and
75 microsatellite analyses are shown in online supplementary table 1. Furthermore, we
76 carried out aCGH and SNP array analysis using the SurePrint G3 Human CGH+SNP
77 4×180K Kit (catalog number G4890A, Agilent Technologies, Palo Alto, CA, USA) in
78 patients with the abnormal methylation levels of the *ZNF597*:TSS-DMR and *ZNF597*:3'
79 DMR. When we could not diagnose UPD(16)mat based on the results of the microsatellite
80 and SNP array analyses, we carried out SNP array analysis in the parents of the patients
81 with abnormal methylation levels of these DMRs.

Finally, for the patients with UPD(16)mat, we performed whole-exome sequencing of these patients and their parents to detect gene mutations associated with their phenotypes. Because the mother of patient 1 showed severe short stature, we also searched causative genes for growth failure in her. Enriched libraries generated with SureSelect Human All ExonV6 kit (Agilent Technologies, Santa Clara, CA, USA) were sequenced on a Hiseq 4000 (Illumina, San Diego, CA, USA) operated in a 150 bp paired-end mode (patient 1) and a Hiseq 2500 (Illumina, San Diego, CA, USA) operated in a 101 bp paired-end mode (patient 2). Sequence reads were processed, mapped and analysed as previously reported.[6] In brief, the paired-end reads were mapped against the human reference genome data (hg19/GRCh37) using the Burrows-Wheeler Aligner 0.7.13. The PCR duplicates were removed by Picard 2.1.1. The Genome Analysis Toolkit 3.5 was then used to produce calibrated BAM files for each sample. Subsequently, we excluded common variants on the basis of the 1000 Genomes Project data, Exome Aggregation Consortium, Human Genetic Variation Database and in-house control data. Then, the rare variants were screened for 356 genes related to growth failure[7] and 25 known causative/candidate/susceptible genes for non-syndromic hypospadias[8] (online supplementary table 2). Furthermore, these rare variants were also examined as to whether they would lead to known genetic syndromes or not, based on the Online Mendelian Inheritance in Man.[9]

Statistical analysis

The frequencies of clinical feature differences between patients with UPD(16)mat, 11p15 LOM and UPD(7)mat were analysed by Fisher's exact test using the R environment (http://cran.r-project.org/bin/windows/base/old/2.15.1/). A value of $p < 0.05$ was considered significant.

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