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## Supplemental Data

# *De Novo KAT5* Variants Cause a Syndrome with Recognizable Facial Dysmorphisms, Cerebellar Atrophy, Sleep Disturbance, and Epilepsy

**Jonathan Humbert, Smrithi Salian, Periklis Makrythanasis, Gabrielle Lemire, Justine Rousseau, Sophie Ehresmann, Thomas Garcia, Rami Alasiri, Armand Bottani, Sylviane Hanquinet, Erin Beaver, Jennifer Heeley, Ann C.M. Smith, Seth I. Berger, Stylianos E. Antonarakis, Xiang-Jiao Yang, Jacques Côté, and Philippe M. Campeau**

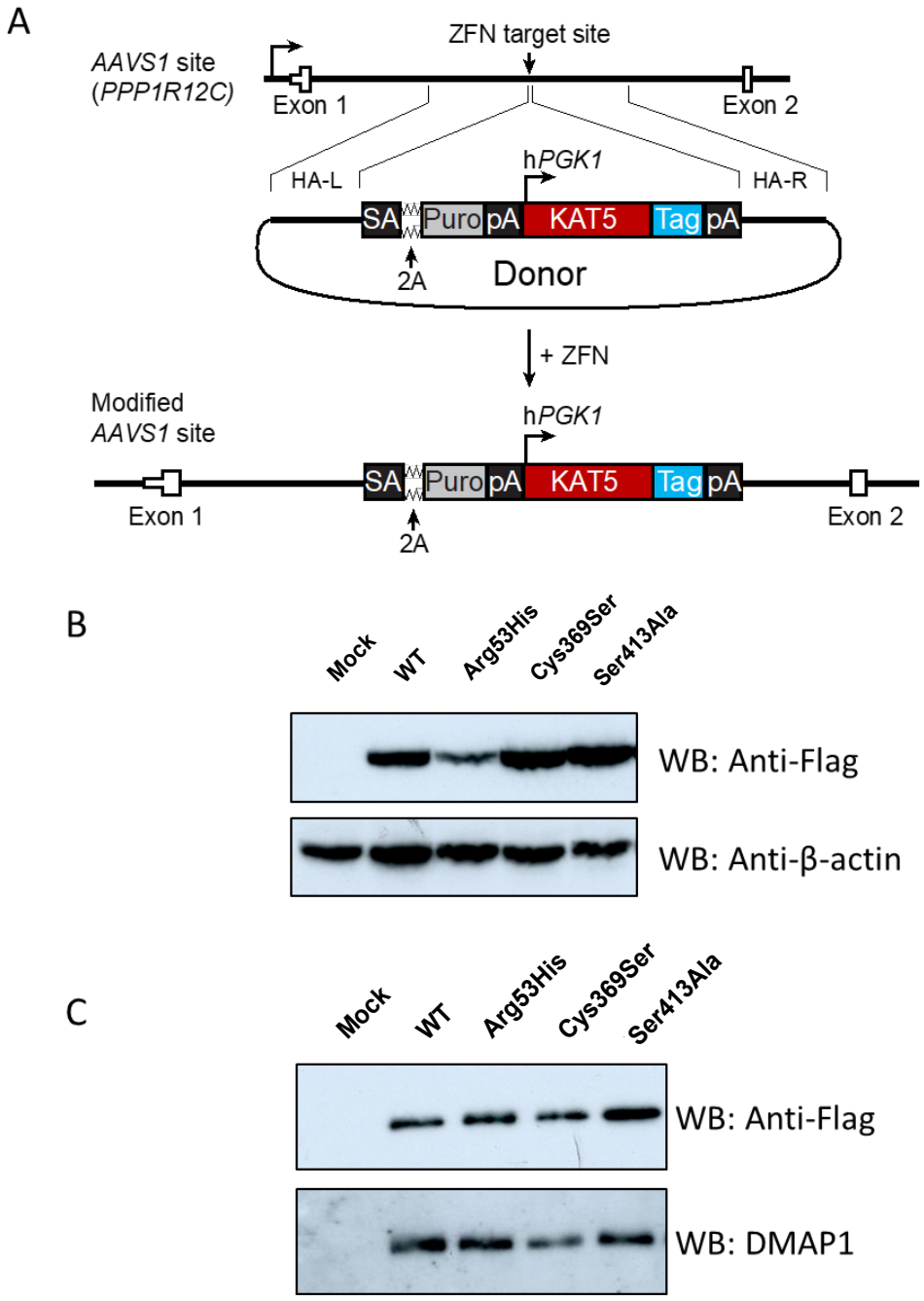


Figure S1. Generation of K562 cell lines stably expressing KAT5 mutants.
A. Schematic of the strategy used to integrate KAT5 cDNA at the AAVS1 locus. The donor construct and the AAVS1 locus following KAT5 cDNA addition are represented. The first two exons of the PPP1R12C gene are shown as open boxes. Also annotated are the locations of the splice acceptor site (SA), 2A self-cleaving peptide sequence (2A), puromycin resistance gene (Puro), polyadenylation sequence(pA), human phosphoglycerate kinase 1 promoter (hPGK1), and 3xFLAG-2xSTREP tandem affinity tag (Tag); homology arms left and right (HA-L, HA-R) are respectively 800 and 840 bp.

B. Western blots showing flag-tagged KAT5 expression in whole cell extracts from selected K562 clones. Actin was used as a loading control.

C. Western blots on purified native NuA4/TIP60 complexes showing the amount of the complex subunits DMAP1 and Flag-tagged KAT5 present in HAT assays shown in Fig. 2.

Figure S1

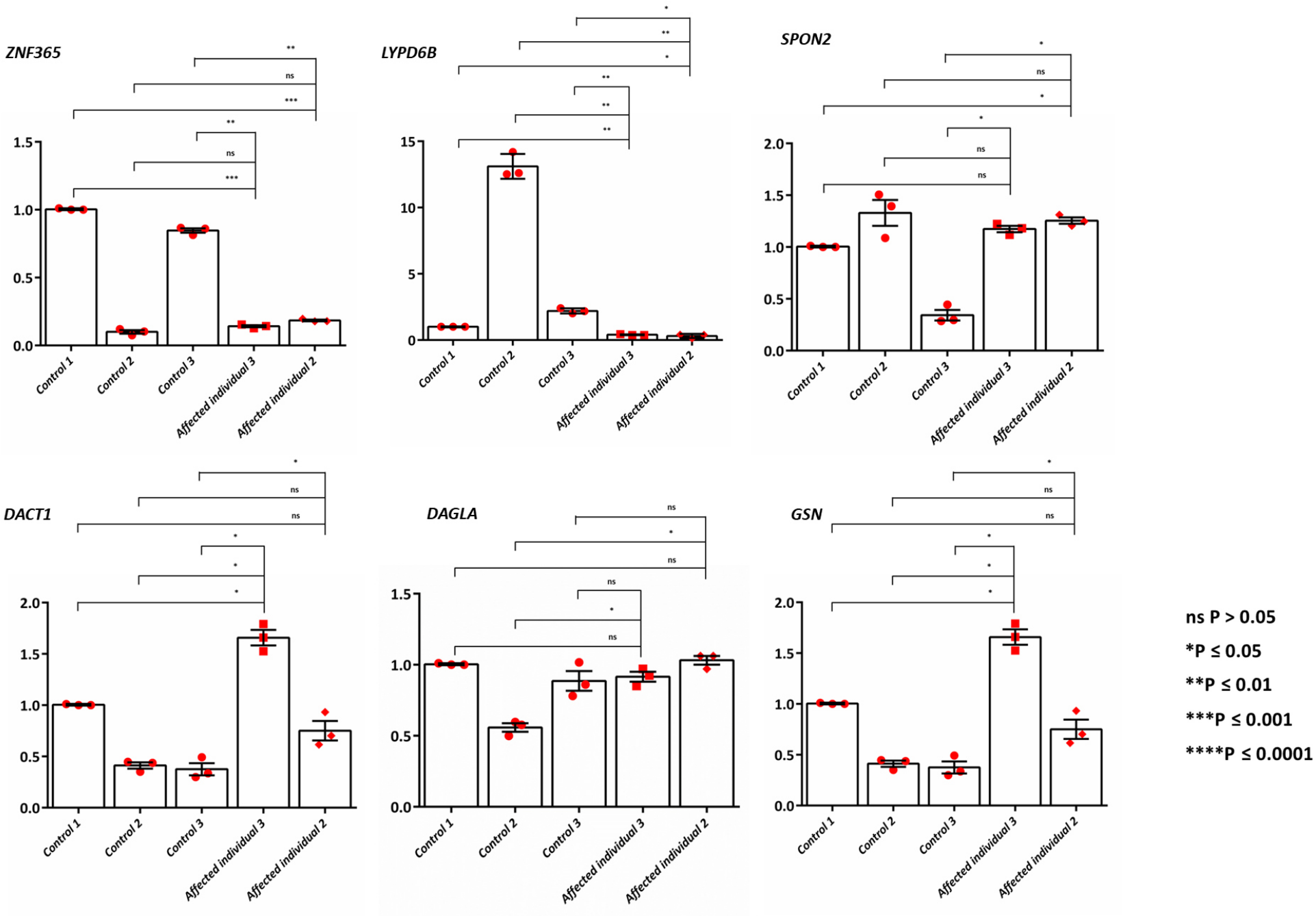


Figure S2. Additional qPCR results. Fibroblast qPCR data for genes identified by RNAseq which did not show consistent and significant up or downregulation. β-actin was used as the reference gene. Triplicates were used. Error bars represent standard deviation. P-values were generated by two-way ANOVA with Dunnett's multiple comparisons test.

Figure S2

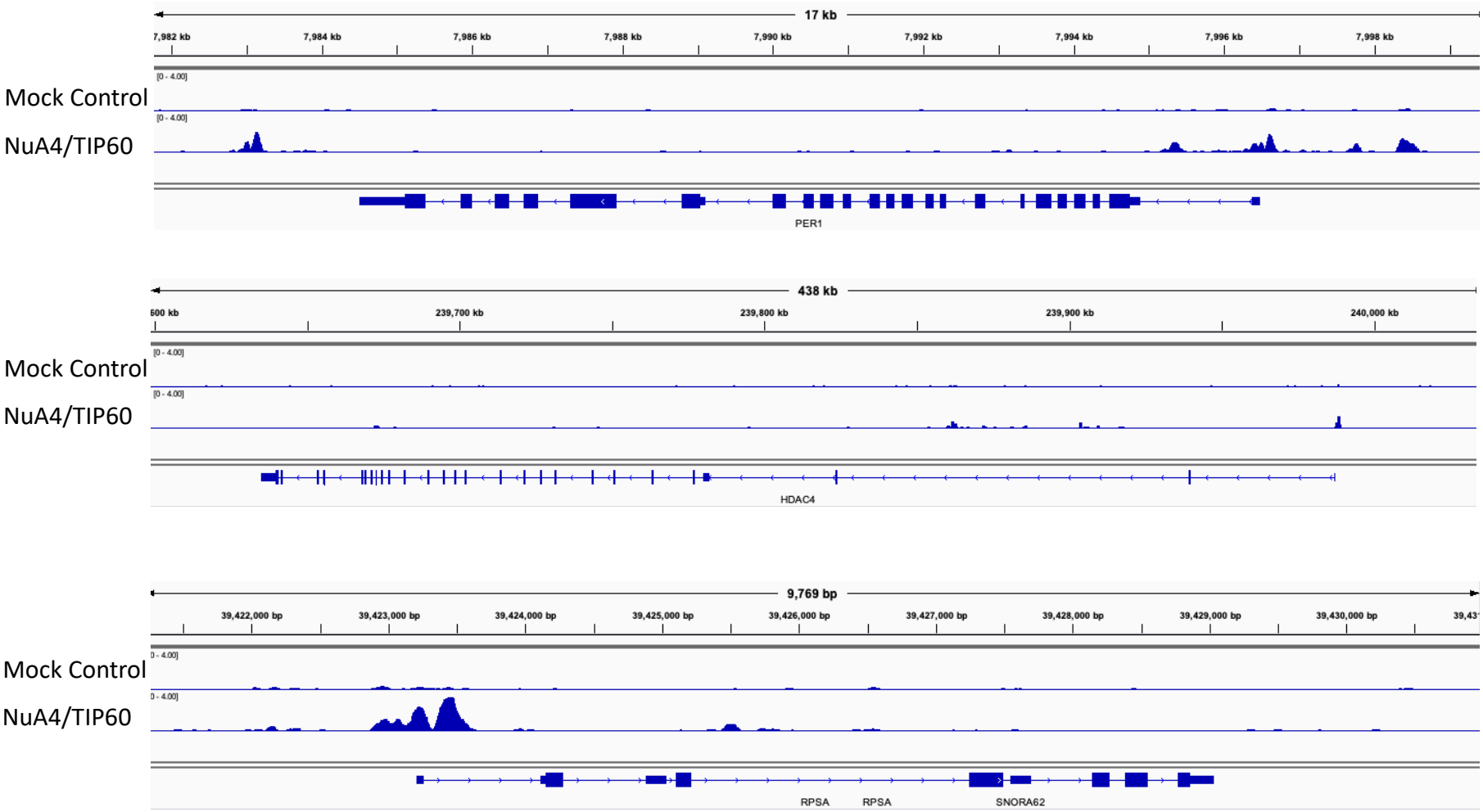


Figure S3. Snapshots of ChIP-seq from Jacquet et al. 2016 for NuA4/TIP60 subunit EPC1 in K562 cells (EPC1(1-584)-3Flag-2Strep integrated at the AAVS1 locus and empty vector control. Profiles were obtained with Integrated Genomics Viewer with reads per million values and the RPSA gene is shown as positive control for NuA4/TIP60 (GEO accession GSE78027).

Figure S3

Table S1. Additional clinical features

| Individual | 1 | 2 | 3 |
|---|---|---|---|
| **Demographic information** | | | |
| Ethnicity | White | White | African-American |
| Gender | F | M | M |
| Age at last evaluation | 29 years | 12 years | 16 months |
| **Family history** | | | |
| Affected family member | No | Cleft lip and palate in cousin once removed | No |
| Unaffected sibs | 1 | 2 maternal half sibs | No |
| Consanguinity | No | No | No |
| **Perinatal history** | | | |
| Perinatal complications | None | None | None |
| At birth: duration gestation (weeks) | 40 | 38 | NA |
| - Weight, g | 3400 ($50^{th}$ centile) | 2740 ($5^{th}$-$10^{th}$ centile) | 2275 ($2^{nd}$ centile) |
| - Length, cm | 48.3 ($10^{th}$ centile) | 47 ($3^{rd}$ centile) | 40.6 (-3.6 SD) |
| - Head circ., cm | NA | 31.5 ($3^{rd}$ centile) | 29.5 (-2.8 SD) |
| Weight at last visit | 80.6 Kg ($92^{nd}$ centile) | 29.7 kg ($3^{rd}$ centile) | 11.2kg ($48^{th}$ centile) |
| Height at last visit | 150.6cm ($3^{rd}$ centile, -1.95 SD) | 133.5 cm ($2^{nd}$ centile, -2.1 SD) | 71.1 cm (<$1^{st}$ centile, -2.9 SD) |
| Head circ at last visit | 55 cm ($73^{rd}$ centile) | 50 cm ($1^{st}$ centile, -2.6 SD) | 44.5 cm ($1^{st}$ centile, -2.2 SD) |
| Comment | Truncal obesity | | |
| **Neurodevelopment** | | | |
| Developmental delay | Yes, severe | Yes, severe | Yes, severe global developmental delay |
| Intellectual disabillity | Yes, FSIQ 40 | Yes, IQ 20-30 | NA |
| Speech | Expressive language at 8-year-old level and receptive language at 4-5-year-old skill level | Absent speech | NA |

| | | | |
|---|---|---|---|
| Feeding problems | No | No | Yes – aspiration of thin liquids on swallow study |
| Hypotonia | Yes | Yes- earlier | No |
| Medications | Valproic acid | Melatonin slow release<br>GH injections | Risperidone and clonidine |
| Seizures | Yes | Yes | Yes |
| Age of first seizure | Adult-onset | infancy | 4 months |
| Type of seizures | Catamenial seizures, grand mal seizures | Variable (partial tonic, late onset infantile spasms, absence) seizures | Generalized tonic clonic and myoclonic seizures |
| Seizure frequency | Every other month | NA | Multiple per day from 4-10 months of age, then infrequently |
| Seizure outcome | Seizure-free since 13 years (2004) post hysterectomy | NA | No seizures since 14 months of age |
| Current anti-epileptic drugs | Valproic acid 1000 mg HS | None | None currently |
| Anti-epileptic drugs ever tried | Phenytoin, valproic acid 500 mg QID | Valproic acid, lamotrigine, vigabatrin | Previously on Onfi and Keppra |
| History of Status Epilepticus | No | No | No |
| History of febrile/complex febrile seizures | No | No | No |
| Behavioral/psychiatric issues | Yes, disruptive behavior, ADHD tantrums, repetitive, aggressive, impulsive and self-injurious behaviors | Yes, multiple stereotypies, hyperactive and disruptive behavior | Yes, behavior difficulties, tantrums, head banging |
| Sleep disorder | Yes, severe | Yes, severe | Yes, severe |
| Neuroradiology | Corpus callosum variant (incomplete development) | Hypoplasia of rostrum and thickening of splenium of corpus callosum; hypoplasia of left caudate nucleus; global progressive cerebellar atrophy (vermis more than hemispheres); small anterior pituitary | Polymicrogyria of right sylvian fissure, cystic dilation of 4th ventricle with inferior cerebellar vermis atrophy, Dandy-Walker variant. |

| | | | |
|---|---|---|---|
| Hearing loss | No, hyperacusis | Mild bilateral asymetric neurosensory hearing deficit | Mild hearing loss with a conductive component |
| Vision impairment | Yes | Yes | Intermittent strabismus, no refractive error |
| If yes, cause | Severe myopia | Divergent strabismus; mild hypermetropia (+2 dioptries) OD; mild myopia (-1.5 dioptries) OS | NA |
| **Malformations and dysmorphisms** | | | |
| Craniofacial features | SMS-like facial dysmorphism. Round face, flat facial profile, prognathism, depressed nasal bridge, downslanting corners of mouth, low-set ears, almond-shaped eyes | Lateral thinning of eyebrows, macrostomia, prognathism, thick lower lip (N.B. repaired cleft lip/palate), bulbous and asymmetric nose | Round face, flat facial profile, downslanting corners of mouth and upturned nose with depressed nasal bridge, prominent brow ridge. Epiblepharon and epicanthal folds |
| Cleft palate | No | Unilateral cleft lip and cleft palate | High arched palate and submucous cleft palate |
| Hand and foot dysmorphisms | Brachydactyly | Bilateral unique palmar creases and bilateral 5th finger clinodactyly. Bilateral pes talus | 5th finger clinodactyly bilaterally |
| Renal anomalies | No | Horseshoe kidney | Not assessed |
| Urogenital anomalies | Recurrent urinary tract infection. | Bilateral vesico-ureteral reflux (stage III R, stage II L) Unilateral cryptorchidism | Hypospadias, cryptorchidism, concealed penis |
| MSK anomalies | Leg length discrepancy due to “dry hip socket” | No | No |
| Cardiac defect | No | No | Perimembranous VSD, dysplastic pulmonary valve with supravalvular and valvular pulmonary stenosis |

| | | | |
|---|---|---|---|
| Gastrointestinal abnormalities | No | gastroesophageal reflux eosinophilic esophagitis<br>severe constipation | Dysphagia, aspiration of thin liquids<br>Umbilical hernia |
| Endocrine anomalies | No | Complete GH deficiency since age 2 years, treated by GH injections | No |
| Surgeries | Hysterectomy to reduce catamenial seizures | - | Ear tubes, hernia repair with ochiopexy |
| Further information | Low immunoglobulins | - | Intermittent rashes on hands and feet |
| **Genetic tests** | | | |
| Chromosomal Microarray | Yes, Normal SNP arrays | Yes, normal oligonucleotides array | Likely benign Yp11.222 duplication, 1.0 Mb |
| Other genetic testing | Nl karyotype<br>Nl methylation study for PWS<br>Nl FISH for SMS<br>Nl sequencing of *RAI1* | Nl karyotype<br>Nl subtelomere FISH<br>Nl subtelomeric MLPA<br>Nl sequencing of *ZFHX1B* | None |
| Biochemical genetics studies | Nl urine organic acids<br>Nl plasma amino acids<br>Nl urine MPS | NA | Creatine kinase 70 U/L (normal) |
| Hematological anomalies | NA | NA | WBC 8.4 K/ul (Nl 5-10), RBC 3.48 M/ul low (Nl 3.89-4.97), Hemoglobin 10.3 g/dl low (Nl 11-13), hematocrit 31.8% (Nl 29-41), platelets 358 K/ul (Nl 250-450) |

Abbreviations: Nl, normal; GH, growth hormone; PWS, Prader-Willi syndrome; SMS, Smith-Magenis syndrome; MPS, mucopolysaccharides; OD, right eye; OS, left eye.

Table S2. Sleep disorder characteristics, based on the Modified Simonds & Parraga Sleep Questionnaire.[1]

| | Individual 1 | Individual 2 | Individual 3 |
|---|---|---|---|
| Bedtime Resistance | No | No | No |
| Sleep Onset Delay | No | Yes | Yes (improved by clonidine) |
| Sleep Anxiety | No | No | No |
| Night-waking | Yes | Yes | Yes |
| Parasomnia (talks, grinds teeth, bangs head, quick movements of extremities, tongue biting, enuresis, sleepwalking) | No | No | No |
| Sleep Disordered Breathing | No | No | No |
| Daytime sleepiness | Yes | No | No |
| Inverted circadian rhythm: | High daytime melatonin level (of 46pg/mL), a sign of inversion. | No | No |

Table S3. List of primers used for RT-qPCR

| **Gene** | **Forward primer (5’ to 3’)** | **Reverse primer (5’ to 3’)** |
|---|---|---|
| *ACTB* | GTTGTCGACGACGAGCG | GCACAGAGCCTCGCCTT |
| *LHX9* | TACTTCAACGGTACGGGCAC | TCTTCTGCGAGGGTGGATAAG |
| *KIRREL3* | TGACGCTACTTTGCGCCAT | GTGGGTAACTTGAGAGGTCCC |
| *GFPT2* | ATGTGCGGAATCTTTGCCTAC | ATCGAGAGCCTTGACTTTCCC |
| *PER1* | GCCAACCAGGAATACTACCAGC | GTGTGTACTCAGACGTGATGTG |
| *HDAC4* | CCTGGGAATGTACGACGCC | CCCGTCTTTCCTGCGTAAC |
| *ZNF365* | ACGGAATCTGAGGAGGAGCT | ATCACGGACAAAGCCAGAGG |
| *LYPD6B* | CTCCTCTCGACCCTACACCA | ATGTGCTTCTTCCGTGGCTG |
| *SPON2* | AAGAACCAGTACGTCAGTAACGG | CACAAACGAGACCAGCGAGT |
| *DACT1* | TTGAACTGTTTGAGGCGAAGAG | ACTGAACACCGAGTTAGAGGAAT |
| *DAGLA* | TGTCACCCTCGGAATGGTTG | GGTTGTAGGTCCGCAGGTTAC |
| *GSN* | AACAGCAATCGGTATGAAAGACT | CTGCACCATTGGAGACCTTGT |

## References

1. Johnson, C.R., Turner, K.S., Foldes, E.L., Malow, B.A., and Wiggs, L. (2012). Comparison of sleep questionnaires in the assessment of sleep disturbances in children with autism spectrum disorders. Sleep Med 13, 795-801.