Supporting Information

Novel *SUZ12* mutations in Weaver-like syndrome

Eri Imagawa, Edoarda V. A. Albuquerque, Bertrand Isidor, Satomi Mitsuhashi, Takeshi Mizuguchi, Satoko Miyatake, Atsushi Takata, Noriko Miyake, Margaret C. S. Boguszewski, César L. Boguszewski, Antonio M. Lerario, Mariana A. Funari, Alexander A. L. Jorge, Naomichi Matsumoto

Whole exome sequencing (WES)

WES was performed in two affected individuals (II-3 in family 1, and II-1 in family 2) and the unaffected parents of family 2 as previously described [1, 2]. Genome partitioning was performed using the SureSelect Human All Exon Kit v6 (Agilent Technologies, Santa Clara, CA, USA). The prepared libraries were sequenced on a HiSeq2500 system (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. In family 1, the reads were aligned to the human reference genome (hg19) using BWA-MEM (http://bio-bwa.sourceforge.net). PCR duplicated reads were flagged with the bammarkduplicates tool from biobambam2 (https://github.com/gt1/biobambam2), and variant calling was performed with FreeBayes (https://github.com/ekg/freebayes). In family 2, alignment to hg19, the removal of PCR duplicates and variant calling were processed by Novoalign 3.00 (http://www.novocraft.com), Picard software (http://broadinstitute.github.io/picard/) and the Genome Analysis Toolkit (https://software.broadinstitute.org/gatk/), respectively. Annotation was performed using ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) in both families.

Candidate variant selection

According to the family pedigrees, we suspected that a *de novo* mutation was likely in these patients. Stepwise variant selection is described in Supplementary Tables S1 and S2. Candidate variants in each family were validated by Sanger sequencing. Pathogenic missense variants were determined based on in-silico scores; SIFT: <0.05, PolyPhen-2: >0.9, MutationTaster: Disease causing and CADD score: >25.0.

Clinical reports

Family 1

Individual 1 was a 19-year-old male (II-3 in family 1, Figure 1A) born full term to non-consanguineous Brazilian parents. Gestational weeks were not precisely recorded for Individual 1. His birth head circumference (HC) and prenatal ultrasound data were unrecorded. He showed characteristic facial features as a neonate including round face, hypertelorism, prominent philtrum and down-slanting palpebral fissures (Figure 2A-E), as well as a broad forehead and large ears in early childhood (Figure 2A-C). Developmental milestones were mildly delayed: walking without support at 2 years and speaking meaningful words at 2 years and 5 months. He presented with speech delay and learning impairment, which required special assistance at school. He was able to write and read and graduated from a regular high school with no apparent intellectual disability. His intelligence quotient was unavailable. At 13 years, bilateral cubitus valgus was noted. No nail anomalies were seen, but mild clinodactyly of the bilateral 1st, 2nd and 5th toes were observed (Figure 2F, G). Bone maturation was almost normal: bone age was 14 years at a chronological age of 13 years as assessed by the Greulich–Pyle method. Laboratory tests (blood biochemistry including IGF-1 and growth hormone, complete blood cell counts), electrocardiogram, electroencephalogram, and ophthalmologic examination were all normal at 13 years. At 15 years of age, his height was 200.5 cm (+3.9 SD) and his HC was 62.0 cm (+4.8 SD). Brain magnetic resonance imaging (MRI) at the same age revealed no abnormalities. He is currently 19 years old, and his height, weight and HC are 213.0 cm (+5.8 SD), 150.0 kg (+3.3 SD) and 62.0 cm (+3.5 SD), respectively. He had multiple pigmented nevi distributed over his entire body, but no excessive loose skin. He has had no seizure episodes. No genetic or chromosomal analysis was previously performed. The heights of his father and mother were 185.0 cm and 165.0 cm, respectively. The mother developed rectal

cancer and was surgically treated with chemotherapy at 31 years. However, liver metastasis was found 5 months after the chemotherapy and she passed away at 33 years. His parents had no facial dysmorphism or skeletal abnormalities. His healthy paternal half-brothers (II-1 and II-2 in family 1, Figure 1A) show normal development.

Family 2

Individual 2 (II-1 in family 2, Figure 1A) was a 9-year-old girl born at 38 weeks of gestation to non-consanguineous healthy French parents. Hydramnios was recognized during pregnancy. Her birth length, weight and HC were 52.0 cm (+1.0 SD), 3,400 g (+0.5 SD) and 33.5cm (−0.5 SD), respectively. Back hypertrichosis and thick eyebrows were observed. No characteristic facial features were noticed at birth. Her developmental milestones were normal (walking without support at 15 months and no speech delay). Her muscle tone was normal. Abnormal skeletal features were seen, including bilateral short fifth fingers with mild clinodactyly, and hypoplastic nails specifically in the bilateral fifth toes (Figure 2H, I). Her umbilical hernia was operated on at 4 years. In addition, at 4 years she had the only episode of a non-febrile seizure and she has suffered from chronic constipation since this age. She had mild learning difficulties but was able to read and write and attended a regular school. Her intelligence seems to be normal, although her intelligence quotient has never been evaluated. At 5 years, she was suspected of Coffin-Siris syndrome (CSS), because of mild learning difficulties, hypertrichosis and hypoplastic nails of the fifth toes. However, no CSS-specific craniofacial features were observed including microcephaly, flat nasal bridge, broad nose, wide mouth, macroglossia, thin upper/thick lower lip vermilion, high palate or ptosis [3-5]. She developed hypermetropia and strabismus. At 5 years and 5 months, characteristic facial features (coarse and

round face, broad forehead and large low-set ears) were observed. At that time, her height, weight and HC were 121.0 cm (+3.0 SD), 26.6 kg (+4.0 SD) and 52.5 cm (+1.5 SD), respectively. Currently (9 years and 4 months), her height is 144.0 cm (+2.5 SD), with a weight of 40.0 kg (+3.0 SD), and HC of 55.0 cm (+2.0 SD). Brain MRI was not performed. Her array CGH test was normal. Her parents are healthy with no overgrowth, characteristic facial features or skeletal anomalies.

SUZ12 mutations in control databases

The identified p.Ala282Glnfs*7 and p.Gln599His mutations were not registered in our in-house exome database (healthy Japanese controls, n=575 and Brazilian controls, n=387), dbSNP build 138, the genome Aggregation Database (http://gnomad.broadinstitute.org), the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/), Tohoku Medical Megabank Organization database (https://ijgvd.megabank.tohoku.ac.jp/) and ABraOM (http://abraom.ib.usp.br/).

Supplementary Table S1. Variant filtering in family 1

	Variants
1) All variants	16202
2) Unregistered variants in ExAC, 1000G, ABraOM and in-house Brazilian controls (n=387)†	114
3) Variants remaining after filtering out synonymous coding variants	88
4) Variants prioritizing using the VarElect tool	6
5) Variants located in the coding region and the adjacent 30 bp	5 (*SUZ12, LEMD3, IGF2R, IGFBP2, VCL*)

Abbreviations: ExAC, Exome Aggregation Consortium (http://exac.broadinstitute.org/); 1000G, 1000 Genomes Project (http://www.internationalgenome.org/); ABraOM, Arquivo Brasileiro Online de Mutações: Brazilian genomic variants (http://abraom.ib.usp.br/); VarElect tool (https://ve.genecards.org)
†Brazilian individuals (n=387) with endocrinological, nephrological and neurological diseases, but no overgrowth.

Supplementary Table S2. Variant filtering in family 2

	Variants
1) All variants	7798
2) Unregistered variants in in-house Japanese controls (n=575)†, ESP6500, HGVD, and ExAC	1669
3) Variants remaining after filtering out synonymous coding variants	1248
4) Variants consistent with *de novo* model‡	2
5) Variants in reported overgrowth genes in HGMD§	1 (*SUZ12*)

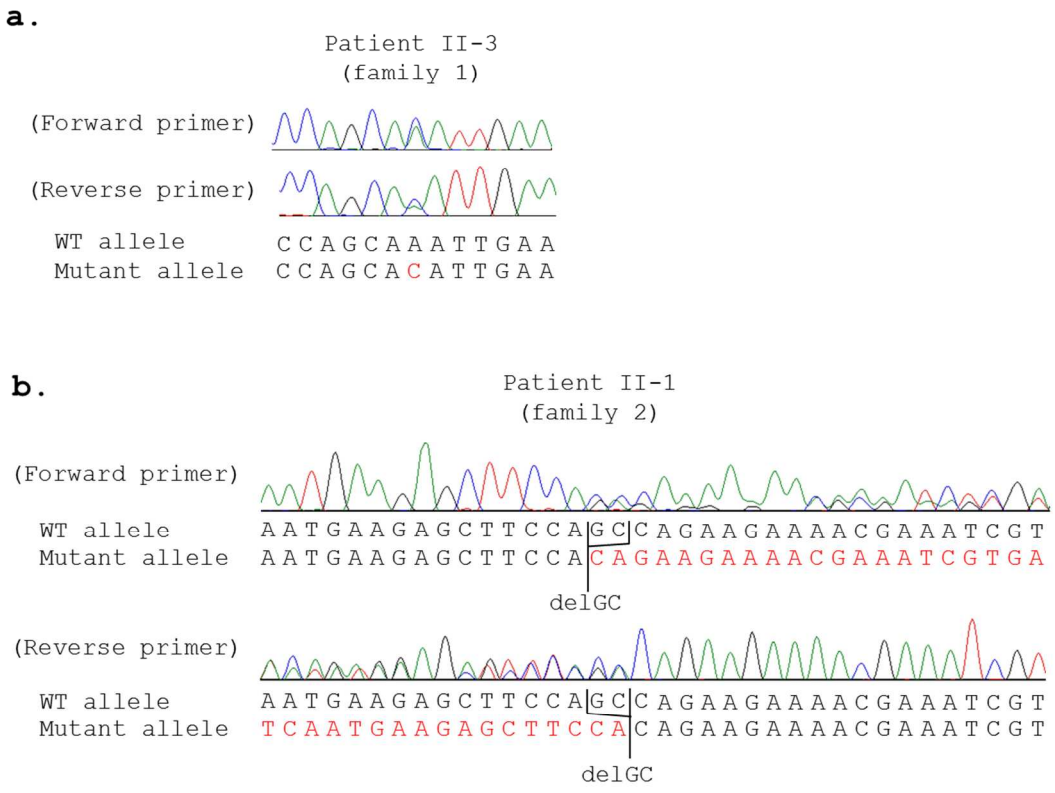
Abbreviations: ESP6500, NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/); HGVD, Human Genome Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/); ExAC, Exome Aggregation Consortium (http://exac.broadinstitute.org/); HGMD, Human Gene Mutation Database Professional release 2017.4 (http://www.hgmd.org).
†Japanese healthy individuals (n=575); we used the available in-house Japanese control data, although the ethnicity is unmatched.
‡ Genotypes correspond to *de novo* model: patient = heterozygote, parents = wild-type.
§ 34 known genes related to Weaver syndrome and other overgrowth syndromes: *AKT1, AKT3, BRWD3, CDKN1B, CHD8, CPQ, DNMT3A, EED, EZH2, FGFRL1, FIBP, GPC3, HERC1, HIST1H1E, IGF1R, LRP4, MTOR, NFIX, NPPC, NSD1, PDGFRB, PIK3CA, PPP2R5B, PPP2R5C, PPP2R5D, PTEN, RNF125, RNF135, SETD2, SIM1, SUZ12, TGFB3, TOP3B* and *YWHAE.*

Supplementary Table S3. Summary of 5 candidate variants in family 1

Gene	Exonic function	Ref accession No.	Nucleotide Change	AA Change[1)]	SIFT	PolyPhen-2	MutationTaster	CADD
SUZ12	Missense	NM_015355.2	c.1797A>C	p.Gln599His	0.02	0.998	Disease causing	26.60
LEMD3	Missense	NM_014319.3	c.596C>G	p.Ala199Gly	0.62	0.001	Polymorphism	11.64
IGF2R	Missense	NM_000876.2	c.2558C>G	p.Ala853Gly	0.03	0.735	Disease causing	20.50
IGFBP2	Missense	NM_000597.2	c.35T>C	p.Leu12Pro	0.02	0.023	Disease causing	22.00
VCL	Missense	NM_003373.3	c.1494G>C	p.Glu498Asp	0.19	0.002	Disease causing	16.27

Abbreviations: AA Change, amino-acid change.

Web resource: SIFT (http://sift.bii.a-star.edu.sg/); PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/); MutationTaster (http://www.mutationtaster.org/); CADD (http://cadd.gs.washington.edu/).



Supplementary Figure S1. Electropherograms of *SUZ12* mutations in two families**. (a)** c.1797A>C and **(b)** c.844_845del mutations in the DNA of peripheral blood leukocytes from the affected individuals. Sequences altered by mutations are shown in red.

Supplementary references

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2. Iwama K, Iwata A, Shiina M et al. A novel mutation in SLC1A3 causes episodic ataxia. *J Hum Genet* 2018;63:207-211.

3. Schrier SA, Bodurtha JN, Burton B et al. The Coffin-Siris syndrome: a proposed diagnostic approach and assessment of 15 overlapping cases. *Am J Med Genet A* 2012;158A:1865-1876.

4. Kosho T, Miyake N, Carey JC. Coffin-Siris syndrome and related disorders involving components of the BAF (mSWI/SNF) complex: historical review and recent advances using next generation sequencing. *Am J Med Genet C Semin Med Genet* 2014;166C:241-251.

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Supplementary Table S4. Clinical features of reported patients with *EZH2* and *EED* mutations.

Gene	*EED*							*EZH2*							
Reference No.	4	3	6	8	10	12	Total	11, 13	7	2	14	5	8	9	Total
Intellectual disability [IQ]	+; Mild [60]	+ ;Mild [52]	+; Moderate	+; Mild [57]	+; Moderate	+ (2 patients); Moderate and severe	7/7 (100%); Mild, 3; Moderate, 3, Severe 1	37/45 (82%); Mild, 21; Moderate, 12; Severe, 2; Unclassified, 2	3/3; Mild	na	na	4/4	+; Severe	na	45/53 (85%); Mild, 24; Moderate, 12; Severe, 3; Unclassified, 6
Excessive postnatal overgrowth	+	+	+	+	+	na	5/5 (100%)	41/45	3/3	+	na	4/4	+	+	51/55 (93%)
Macrocephaly	+	+	+	+	+	na	5/5 (100%)	21/43	3/3	+	na	2/4	+	+	29/53 (55%)
Increased bifrontal diameter	+	-	+	+	na	na	3/4 (75%)	na	3/3	na	na	3/4	na	na	6/7 (86%)
Hypertelorism	+	-	+	+	+	na	4/5 (80%)	na	2/3	+	+	4/4	+	+	10/11 (91%)
Prominent and/or long philtrum	-	-	+	+	+	na	3/5 (60%)	na	3/3	na	na	2/4	+	na	6/8 (75%)
Micrognathia (or retrognathia)	+	+	+	+	+	na	5/5 (100%)	na	3/3	+	+	4/4	+	+	11/11 (100%)
Large ears	+	+	+	+	+	na	5/5 (100%)	na	3/3	na	na	4/4	+	+	9/9 (100%)
Hoarse and low-pitched cry	+	na	+	-	+	na	3/4 (75%)	10/27	3/3	na	na	2/4	+	na	16/35 (46%)
Advanced general and carpal osseous maturation	+	+	+	+	+	na	5/5 (100%)	25/25	3/3	+	na	3/3	+	+	34/34 (100%)
Broad metaphyses	+	+	+	+	na	na	3/4 (75%)	na	1/2	na	na	1/2	na	na	2/4 (50%)
Round face	+	+	+	+	+	na	5/5 (100%); childhood	na	na	+	+	na	+	na	3/3 (100%)
Flat occiput	+	-	na	-	na	na	1/3 (33%)	na	2/3	+	na	4/4	+	na	8/9 (89%)
Low nasal bridge	-	-	+	+	na	na	2/4 (50%)	na	na	na	na	na	+	na	1/1 (100%)
Limb anomalies	+	+	+	+	-	na	4/5 (80%)	na	3/3	na	na	4/4	+	+	9/9 (100%)
Horizontal chin crease	+	+	+	-	na	na	3/4 (75%)	na	na	na	na	na	+	na	1/1 (100%)
Skin pigmented nevi	+	+	na	-	na	na	2/3 (67%)	na	2/3	na	na	1/4	-	na	3/8 (38%)
Scoliosis	+	+	na	-	+	na	3/4 (75%)	6/44	3/3	na	na	1/3	-	na	10/51 (20%)
Hypertonia	+	+	+	-	+	na	4/5 (80%)	11/39	1/3	+	na	3/4	+	na	17/48 (35%)
Hypotonia	-	+	+	-	+	na	3/5 (60%)	18/41	2/3	na	na	3/4	-	na	23/49 (47%)
Excessive loose skin	-	-	+	-	na	na	1/4 (25%)	17/35	2/3	na	na	3/4	+	na	23/43 (53%)
Umbilical hernia	+	+	+	+	-	na	4/5 (80%)	17/40	3/3	+	na	3/4	-	na	24/49 (49%)
Tumorigenesis	-	-	na	-	na	na	0/3 (0%)	2/48	na	na	na	2/4	-	na	4/52 (8%)
Brain MRI abnormalities	-	-	+	-	+	na	2/5 (40%)	na	2/2	+	na	1/3	+	na	5/7 (71%)

na, not assessed; HC, head circumference; MRI, magnetic resonance imaging; +, present; –, not present.