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SHORT REPORT

Novel *SUZ12* mutations in Weaver-like syndrome

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SUZ12 is a core component of polycomb repressive complex 2 (PRC2) along with EZH2 and EED. Recently, germline mutations in the *SUZ12*, *EZH2* and *EED* genes have been reported in Weaver syndrome (WS) or Weaver-like syndrome, suggesting a functional link between PRC2 deficits and WS. However, only one case of a *SUZ12* mutation presenting with Weaver-like syndrome has been reported. Here, we report a missense and a frameshift mutation in *SUZ12* (c.1797A>C; p.Gln599His and c.844_845del; p.Ala282Glnfs*7), both of which are novel, in two individuals. Their clinical features included postnatal overgrowth, increased bifrontal diameter, large ears, round face, horizontal chin crease and skeletal anomalies, but did not fulfill the WS diagnostic criteria. These data provide strong evidence that *SUZ12* mutations cause Weaver-like syndrome.

KEYWORDS

SUZ12, Weaver syndrome, Weaver-like syndrome, whole exome sequencing

1 | INTRODUCTION

Weaver syndrome (WS; MIM #277590) is a rare overgrowth disorder. In 2011, Khosravi et al proposed clinical criteria to support the diagnosis of WS.[1] Pathogenic variants in the genes encoding enhancer of zeste homolog 2 (*EZH2*) and embryonic ectoderm development (*EED*)

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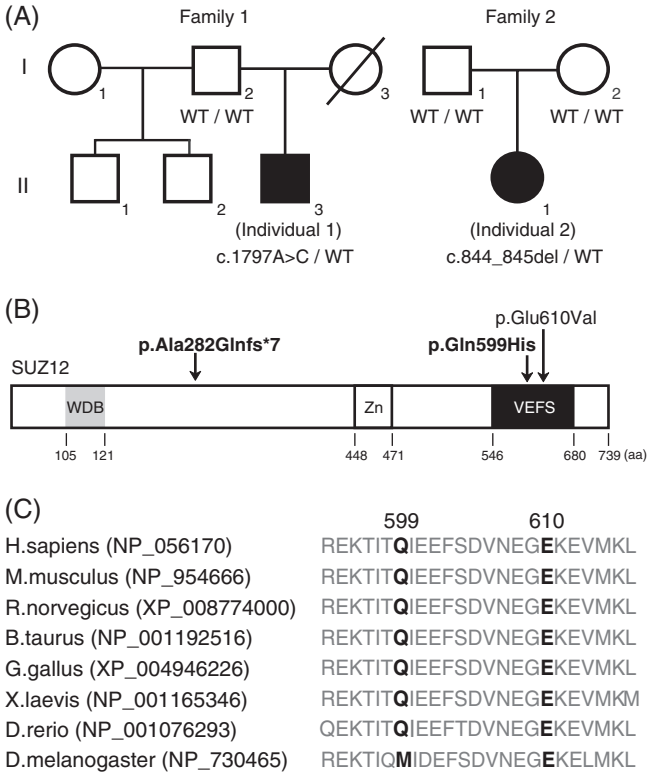

FIGURE 1 Familial pedigrees and *SUZ12* mutations. (A) Familial pedigrees. (B) Human SUZ12 (NP_056170) protein structure and mutations. Novel mutations are shown in bold. p.Glu610Val was previously reported. WDB, WD-40 binding domain; Zn, Zn-finger region; VEFS, VRN2-EMF2-FIS2-SU(Z)12 domain. (C) Evolutionary conservation of p.Gln599 and p.Glu610 in SUZ12 from flies to humans

have been identified in WS or Weaver-like syndrome.[2–14] In particular, *EZH2* was previously established as the causative gene for WS; thus, a molecular diagnostic approach is crucial for overgrowth patients.[11] Nevertheless, individuals with an *EZH2* mutation do not always exhibit the classical manifestations of WS.[13] Recently, we reported a mutation in the gene encoding suppressor of zeste 12 homolog (*SUZ12*) in an individual with Weaver-like syndrome.[8] *SUZ12* as well as *EZH2* and *EED* encode the subunits of the polycomb repressive complex 2 (PRC2).[15] Here, we report two novel *SUZ12* mutations in Brazilian and French families and describe their clinical phenotypes referring to the WS diagnostic criteria.

2 | MATERIALS AND METHODS

Two families (Figure 1A) affected with Weaver-like features were analyzed in this study. This study was approved by the Institutional Review Board of Yokohama City University School of Medicine and University of Sao Paulo School of Medicine. Peripheral blood samples and clinical information were collected after parental consent was provided. DNA was extracted from the peripheral blood leukocytes of families 1 and 2 using QuickGene-610L (Fujifilm, Tokyo, Japan) and salt precipitation methods,[16] respectively. Whole exome sequencing (WES) was performed as previously described[17] using DNA from the two affected probands and the unaffected parents in family 2. WES methods and candidate variant selection are described in Tables S1, S2 and Supporting Information.

2.1 | Clinical reports

The clinical information of the probands (individuals 1 and 2) is shown in Table 1, Figure 2 and Supporting Information.

2.2 | Identification of *SUZ12* mutations

After selecting the variants to investigate, missense (c.1797A>C, p.Gln599His) and frameshift (c.844_845del, p.Ala282Glnfs*7) variants in *SUZ12* (NM_015355.2) were identified in individuals 1 and 2, respectively (Figure S1 and Tables S1 and S2). In individual 1, five missense variants were extracted as candidates (Table S3). When we focused on highly pathogenic variants, only the *SUZ12* mutation remained (Supporting Information). Individual 1 harbored a heterozygous c.1797A>C variant in *SUZ12*, although this variant was absent in his father as determined by Sanger sequencing. Samples from his mother were unavailable for further testing because she had passed away. Therefore, we could not confirm the nature (either *de novo* or inherited) of the mutation in individual 1. The other four candidate variants were also absent in his father as confirmed by Sanger sequencing; thus, their pathogenicity remains undetermined. The other c.844_845del variant occurred *de novo* in individual 2. *In silico* tools predicted that the p.Gln599His variant was deleterious: SIFT (score = 0); PolyPhen-2 (score = 0.998); CADD (score = 26.6); PROVEAN (score = −2.8) and MutationTaster (score = disease causing). The missense variant was located in the VRN2-EMF2-FIS2-SU(Z)12 (VEFS) domain in SUZ12 and the mutated glutamine is evolutionarily conserved from fish to humans (Figure 1B,C). Both variants were not registered in any control databases (Supporting Information).

3 | DISCUSSION

We found two novel *SUZ12* mutations: p.Ala282Glnfs*7 and p.Gln599His in two unrelated individuals with Weaver-like syndrome. SUZ12 is an essential component of PRC2 together with EZH2 and EED. PRC2 has methyltransferase activity for lysine 27 on histone 3 (H3K27), which is catalyzed through the SET (Su(var)3-9, E(z) and Trithorax) domain of EZH2.[18] H3K27 tri-methylation (H3K27me3) is an epigenetic-silence mark involved in the regulation of tissue- or developmental stage-specific gene expression.[19] Heterozygous knock-in mice carrying *Ezh2* mutation (p.Val626Met) showed reduced H3K27me3 levels together with mild overgrowth.[9] Also, heterozygous *Ezh2* knockout mice showed advanced skeletal development.[20] Whereas, interestingly, the *Ezh2*-null mice and the homozygous knock-in mice for p.Val626Met caused early lethality.[9] These results indicate that normal Ezh2 allele products and a partial loss-of-function (LoF) of PRC2 might contribute to the overgrowth phenotype.[9] In our previous report, a *SUZ12* mutation (p.Glu610Val) in a patient with Weaver-like syndrome resulted in decreased levels of H3K27me3 in lymphoblastoid cells, consistent with a LoF of PRC2 activity.[8]

TABLE 1 Clinical features in individuals with *SUZ12* mutations

Reference no.	Present report		8	3, 4, 6, 8, 10, 12	2, 5, 7-9, 11, 13, 14
Subjects	**Individual 1**	**Individual 2**	**Individual 3**	**Reported cases of *EED* mutation (7 patients)**	**Reported cases of *EZH2* mutation (58 patients)**
Current age	19 y	9 y 4 mo	11 y		
Sex	Male	Female	Female		
Ethnicity	Brazilian	French	Japanese		
SUZ12 mutation	c.1797A>C, p.Gln599His	c.844_845del, p.Ala282Glnfs*7	c.1829A>T, p.Glu610Val		
Inheritance	NA (not identified in father)	*de novo*	Inherited from mosaic father		
Diagnosis	Weaver-like syndrome	Weaver-like syndrome	Weaver-like syndrome		
Development					
Gestation	Full term[a]	38 wk	37 wk 3 d		
Birth length	55.0 cm (+2.0SD)	52.0 cm (+1.0SD)	52.2 cm (+1.8SD)		
Birth weight	4500 g (+2.2SD)	3400 g (+0.5SD)	3552 g (+1.4SD)		
Birth HC	NA	33.5 cm (−0.5SD)	34.6 cm (+1.2SD)		
Current height	213.0 cm (+5.8SD)	144.0 cm (+2.5SD)	177.7 cm (+4.5SD)		
Current weight	150.0 kg (+3.3SD)	40.0 kg (+3.0SD)	75.1 kg (+4.6SD)		
Current HC	62.0 cm (+3.5SD)	55.0 cm (+2.0SD)	62.6 cm (+5.5SD)		
Intellectual disability	−	−	+ (moderate)	7/7; 100% (mild, 3; moderate, 3 patients; severe, 1 patient)	45/53; 85% (mild, 24; moderate, 12; severe, 3; unclassified, 6 patients)
Physical phenotypes from WS criteria by Khosravi et al[b]					
Excessive postnatal overgrowth	+	+	+	5/5; 100%	51/55; 93%
Macrocephaly	+	−	+ (plagiocephaly)	5/5; 100%	29/53; 55%
Increased bifrontal diameter	+	+	+	3/4; 75%	6/7; 86%
Hypertelorism	+	−	+	4/5; 80%	10/11; 91%
Prominent and/or long philtrum	+ (prominent)	−	−	3/5; 60%	6/8; 75%
Micrognathia/ retrognathia	−	−	−	5/5; 100%	11/11; 100%
Large ears	+	+	+	5/5; 100%	9/9; 100%
Hoarse and low-pitched cry	−	−	−	3/4; 75%	16/35; 46%
Advanced general and carpal osseous maturation	General; + Carpal; −	NA	General; + Carpal; +	5/5; 100%	34/34; 100%
Broad metaphyses	NA	NA	+	3/4; 75%	2/4; 50%
Other physical phenotypes					
Round face	+	+	+	5/5; 100%	3/3; 100%
Flat occiput	−	NA	+	1/3; 33%	8/9; 89%
Low nasal bridge	−	−	−	2/4; 50%	1/1; 100%
Limb anomalies	Cubitus valgus, clinodactyly of bilateral first, second and fifth toes	Short fifth fingers, clinodactyly and ungual hypoplasia of fifth toes	Flexion disorder of fingers, camptodactyly (mild), ingrown nails of halluces, short second and fourth toes (bilateral)	4/5; 80%	9/9; 100%
Horizontal chin crease	+	+	−	3/4; 75%	1/1; 100%

TABLE 1 (Continued)

Reference no.	Present report		8	3, 4, 6, 8, 10, 12	2, 5, 7-9, 11, 13, 14
Subjects	Individual 1	Individual 2	Individual 3	Reported cases of *EED* mutation (7 patients)	Reported cases of *EZH2* mutation (58 patients)
Skin pigmented nevi	+	NA	−	2/3; 67%	3/8; 38%
Scoliosis	−	−	Mild; <20°	3/4; 75%	10/51; 20%
Hypertonia	−	−	−	4/5; 80%	17/48; 35%
Hypotonia	−	−	+	3/5; 60%	23/49; 47%
Excessive loose skin	−	NA	−	1/4; 25%	23/43; 53%
Umbilical hernia	−	+	+	4/5; 80%	24/49; 49%
Tumorigenesis	−	−	−	0/3; 0%	4/52; 8%
Other findings	Downslanting palpebral fissures	Hypertrichosis, hypermetropia, strabismus, non-febrile seizure, chronic constipation	Abdominal distension at birth, plantar skin defects, knee joints contracture (mild), atrophy of gastrocnemius muscles		
Radiological examination					
Brain MRI	Normal	NA	Enlarged lateral and third ventricles, arachnoid cysts, Chiari malformation type I	2/5; 40% (substantial white matter volume loss, thin corpus callosum and ventriculomegaly)	5/7; 71% (Ventriculomegaly, delayed myelination, cerebellar hypoplasia, polymicrogyria and Chiari malformation)

Abbreviations: HC, head circumference; MRI, magnetic resonance imaging; NA, not assessed. *SUZ12* mutations are based on NM_015355.2. +, present; −, not present (see Table S4).
[a] Precise gestational week of conception was unrecorded for individual 1.
[b] Khosravi et al proposed that WS patients should have at least 8 of 10 features.

SUZ12 has a high probability of loss intolerance (pLI) score of 1.0, indicating that LoF is likely to be involved. In gnomAD (http://gnomad.broadinstitute.org/), nine rare LoF variants were recorded in *SUZ12*. Of these, eight LoF variants were located in the last exon of *SUZ12*; thus, these variants might escape from nonsense-mediated mRNA decay (NMD) because they lack a downstream exon-junction complex that is a primary determinant of NMD.[21] Proteins resulting from these variants retained a functional VEFS domain and might be functionally benign. These are also flagged as LoF variants with low confidence, suggesting somewhat dubious variant annotation or quality. The other variant (p.Lys246Valfs*7) in gnomAD, which was closely located to the variant detected in our proband, is predicted to disrupt the Zn-finger region and VEFS domain. These regions are required for PRC2 binding to a genomic target and stimulation of histone methyltransferase activity.[22] The p.Lys246Valfs*7 and p.Ala282Glnfs*7 variants, located in *SUZ12* exons 7 and 8, respectively, may result in NMD because they are far upstream of the translational stop codon at the end of exon 16. Because the gnomAD data set includes individuals without severe pediatric diseases, an individual harboring the p.Lys246Valfs*7 variant could have mild clinical presentation similar to our case, and might not have been excluded from recruitment to the various gnomAD populations.

The missense mutation p.Gln599His was located near the p.Glu610Val mutation in the VEFS domain. The missense *Z*-score from ExAC (http://exac.broadinstitute.org/) for *SUZ12* was 3.68, indicating that *SUZ12* has a lower number of missense variants than expected in the general population. Therefore, *SUZ12* is considered an intolerant gene for missense variants, although the *Z*-score itself may not directly support the pathogenicity of the variants.

By the diagnostic criteria of Khosravi, six and three features were observed in individuals 1 and 2, respectively (Table 1). The two patients and the previously reported patient (with p.Glu610Val) do not fulfill the criteria for WS. Patients with *EED* or *EZH2* mutations show classic WS phenotypes, and those with a *SUZ12* mutation have common features including postnatal overgrowth, accelerated bone maturation, limb anomalies and umbilical hernia. Craniofacial features are present in patients with *SUZ12*, *EED* and *EZH2* mutations, but these appear differently; micrognathia/retrognathia is prominent in those with *EED* and *EZH2* mutations but was not observed in our three patients with a *SUZ12* mutation. *EZH2* and *EED* mutation-positive individuals generally exhibit specific facial phenotypes at birth or early childhood (<1 year).[2–8,10,11,13,14] In contrast, in the two *SUZ12*-mutated individuals (2 and 3), facial features were noted later at the age of 5 years 5 months and 3 years, respectively. This gap might differentiate *SUZ12*-mutated patients from those with *EZH2* and *EED* mutations. Hoarse and low-pitched cry, hyper/hypotonia and excessive loose skin have not been recognized in patients with *SUZ12* mutations. Individuals with *SUZ12* mutations have varied levels of intellectual impairment as seen in those with *EED* and *EZH2* mutations. Other phenotypes including multiple pigmented nevi, horizontal chin crease and scoliosis, and brain abnormalities are also present at various frequencies in patients with *SUZ12*, *EED* and *EZH2* mutations.

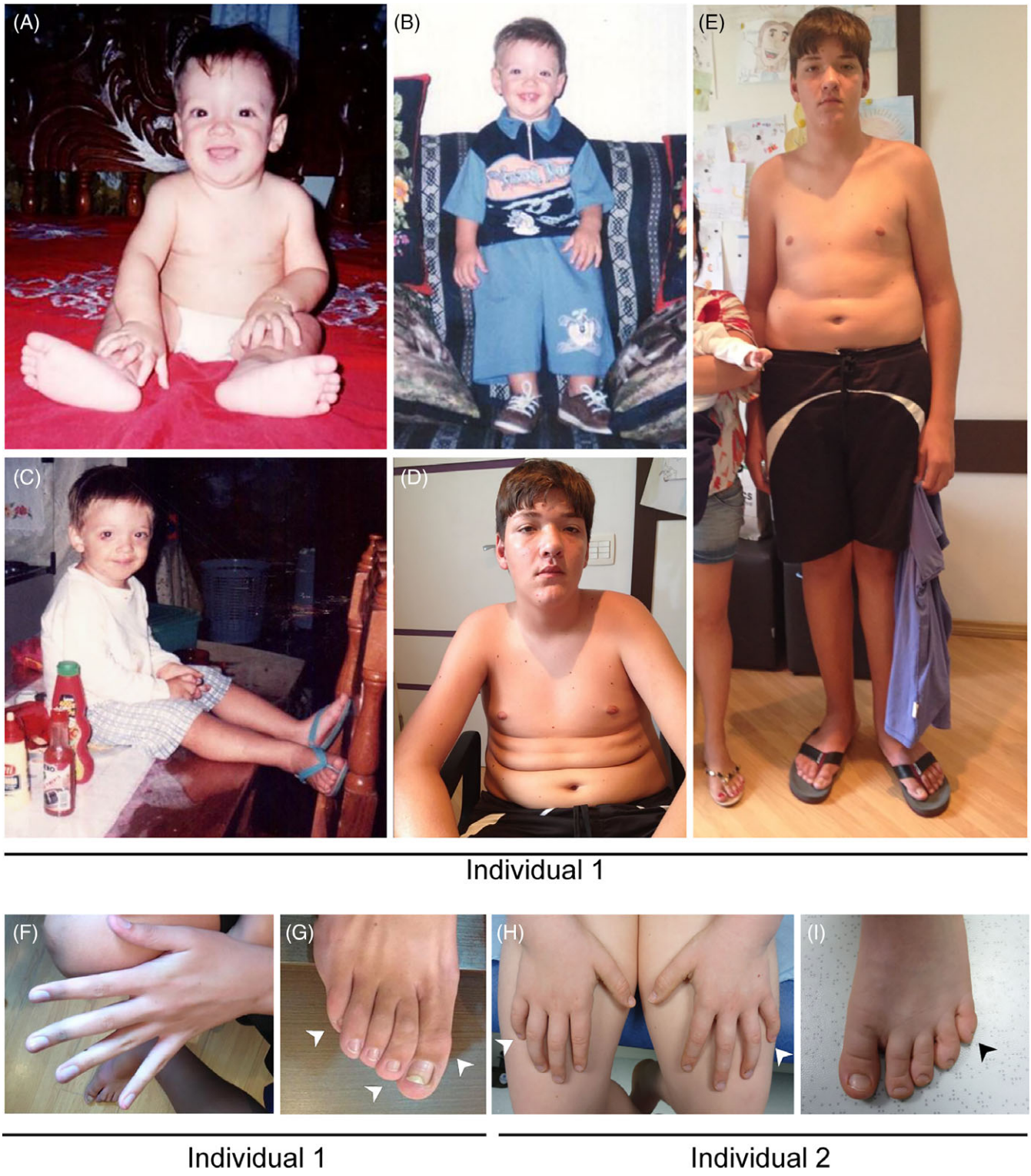

FIGURE 2 Clinical features of the affected individuals. Individual 1 at the age of 8 months (A), 1 year (B), 2 years (C) and 15 years (D, E): Round face, broad forehead, large ears, hypertelorism and large feet were significantly noted in childhood (A-C). In adulthood (D, E), individual 1 had a horizontal chin crease, deep philtrum and multiple pigmented nevi. (F) Hand is relatively large with no nail hypoplasia. (G) Mild clinodactyly of the first, second and fifth toes are shown (white arrow head). Individual 2 at 5 years and 5 months (H, I): Short fifth fingers with mild clinodactyly (white arrow heads) and hypoplastic nail on the fifth toe (black arrow head) are shown [Colour figure can be viewed at wileyonlinelibrary.com]

Therefore, pathogenic variants in *SUZ12* likely cause a Weaver-like syndrome rather than WS, but further case reports are required.

In conclusion, we found two novel *SUZ12* mutations in two patients with Weaver-like syndrome. All three cases described to date have overlapping phenotypes and are from unrelated families of different ethnic backgrounds. Taken together, these data confirm that rare *SUZ12* coding variants cause human overgrowth. Not all rare *SUZ12* coding variants are expected to cause overgrowth and other phenotypes may be associated with genetic variations in *SUZ12*.

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Conflict of interest

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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