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BRIEF COMMUNICATION

Novel de novo mutation affecting two adjacent aminoacids in the *EED* gene in a patient with Weaver syndrome

Robert Smigiel[1] · Anna Biernacka[2,3] · Mateusz Biela[1] · Victor Murcia-Pienkowski[2,3] · Elzbieta Szmida[4] · Piotr Gasperowicz[2] · Joanna Kosinska[2] · Grazyna Kostrzewa[5] · Agnieszka Anna Koppolu[2,3] · Anna Walczak[2] · Dominik Wawrzuta[2] · Malgorzata Rydzanicz[2] · Malgorzata Sasiadek[4] · Rafal Ploski[2]

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Abstract

Overgrowth, macrocephaly, accelerated osseous maturation, variable intellectual disability, and characteristic facial features are the main symptoms of Weaver syndrome, a rare condition caused by mutations in *EZH2* gene. Recently, in four patients with Weaver-like symptoms without mutations in *EZH2* gene, pathogenic variants in *EED* were described. We present another patient clinically diagnosed with Weaver syndrome in whom WES revealed an *EED* de novo mutation affecting two neighboring aminoacids, NM_003797.3:c.917_919delinsCGG/p.(Arg306_Asn307delinsThrAsp) located in one allele (*in cis*). Our observation, together with previous reports suggests that *EED* gene testing is warranted in patients with the overgrowth syndrome features and suspicion of Weaver syndrome with normal results of *EZH2* gene sequencing.

Weaver syndrome (MIM #277590) is a rare condition caused by constitutional mutations in *EZH2* gene which were described in 1974 by Weaver et al. [1]. This classical overgrowth syndrome manifests by increased mass of multiple tissues, prenatal and postnatal overgrowth, often include advanced bone age, facial dysmorphism including hypertelorism, almond-shaped palpebral fissures, a broad forehead, long ears, retrognathia and a pointed, "stuck-on" chin with horizontal skin crease as well as developmental delay and intellectual disability [2]. Recently Cohen et al. identified previously undescribed de novo mutations in *EZH2*'s partner protein EED in 2 patients with Weaver-like syndromes [3, 4]. Furthermore, two new patients with the Weaver-like overgrowth syndrome with *EED* mutation were described by Cooney et al. and by Imagawa et al. [2, 5]. In this report, we present a patient with a clinical diagnosis of Weaver syndrome associated with novel de novo variants in *EED*.

The proband was the second male child of healthy non-consanguineous parents, family history was non-contributory. The boy was born at 38 weeks of gestation by C-section after an uneventful pregnancy with the birth parameters: weight 3550 g (50th percentile), length 54 cm (25–50th percentile), occipito-frontal circumference (OFC) 34 cm (25th percentile), Apgar score 7 points at 1 min. Bilateral cryptorchidism was noticed. Psychomotor development was delayed (siting at 11 month of age, walking at 2 years, first words at 4 years) but somatic development was accelerated. There was bilateral hypoacusis (50 dB). Retrospectively, based on family photos facial features in the infant period were evaluated showing typical features of Weaver syndrome (Fig. 1). Moreover,

Biernacka Anna and Biela Mateusz contributed equally to this work

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✉ Robert Smigiel
robert.smigiel@umed.wroc.pl

✉ Rafal Ploski
rploski@wp.pl

[1] Department of Paediatrics and Rare Disorders, Wroclaw Medical University, Wroclaw, Poland

[2] Department of Medical Genetics, Warsaw Medical University, Warsaw, Poland

[3] Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland

[4] Department of Genetics, Wroclaw Medical University, Wroclaw, Poland

[5] Department of Forensic Medicine, Warsaw Medical University, Warsaw, Poland

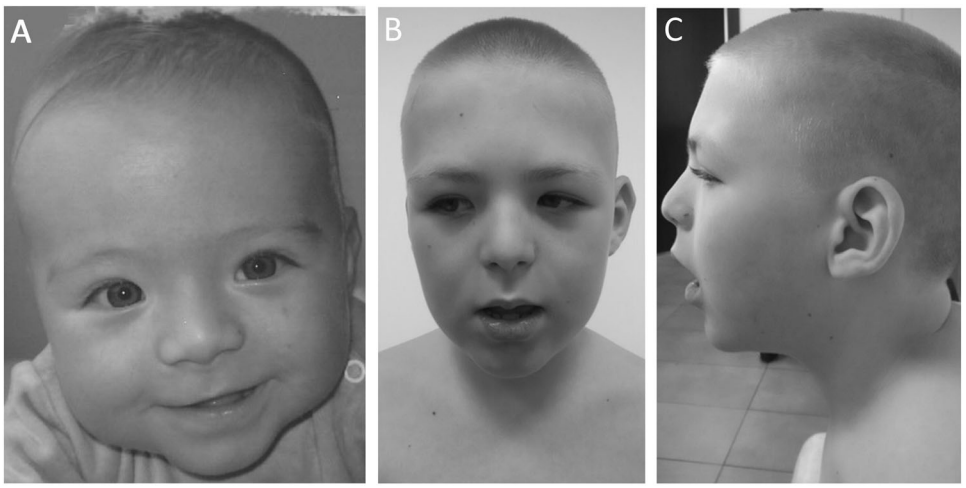

Fig. 1 **a** Proband's facial features in the infant period. Note the typical features of Weaver syndrome with prominent chin. **b**, **c** Proband's facial features at the age of 8 (Color figure online)

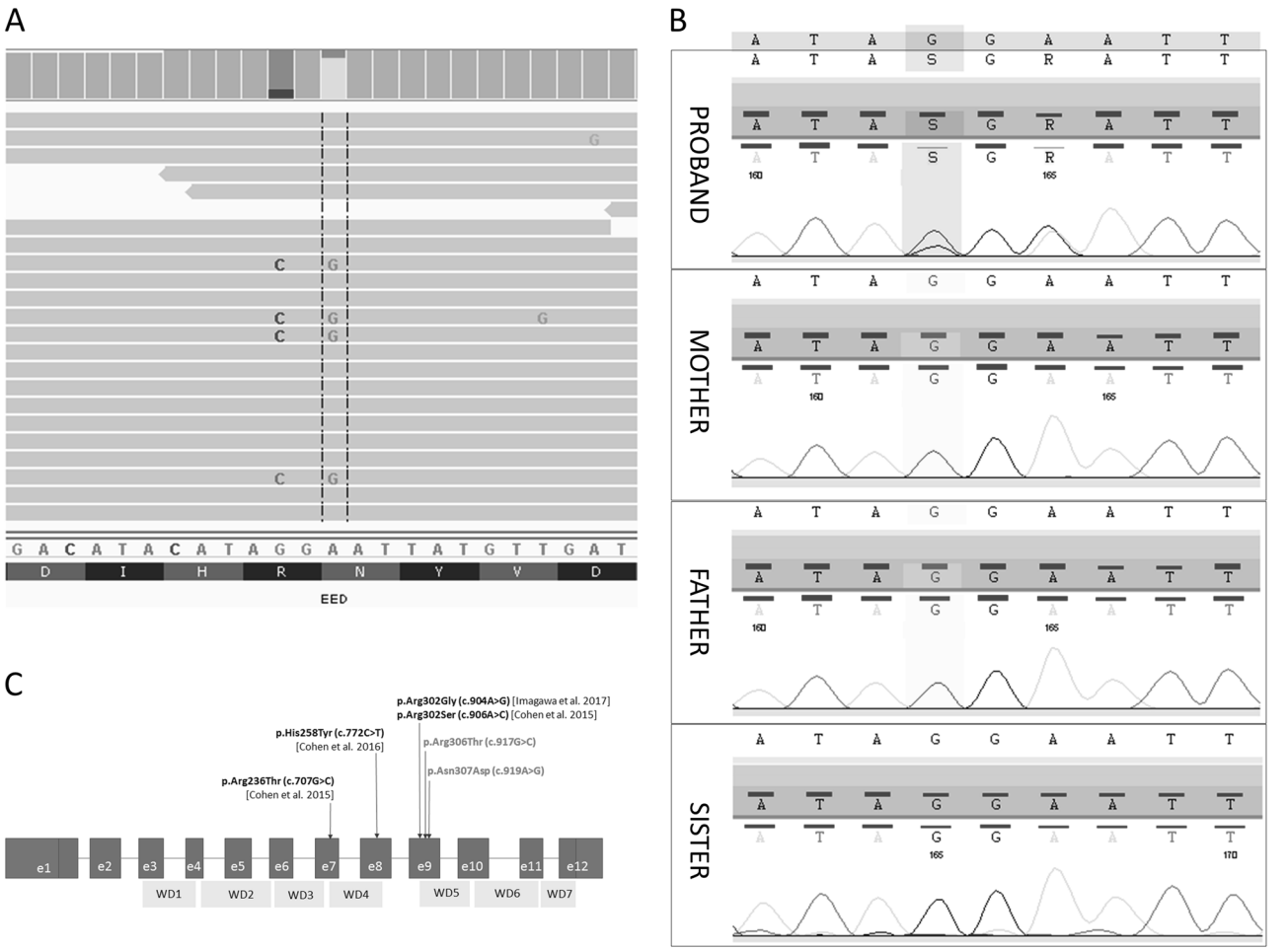

Fig. 2 Genetic results. **a** WES results regarding *EED* gene in proband c.917_919delinsCGG/p.(Arg306_Asn307delinsThrAsp)—view from Integrative Genomic Viewer (IGV); **b** Sanger sequencing results for proband and his parents—view from Variant Reporter 1.1; **c** Schematic presentation of known and novel variants in the *EED* gene. Coding exons are marked with blue rectangles, whereas green rectangles represent non-coding UTRs. Seven WD repeats are marked as yellow rectangles. Mutations described in this report are in red (Color figure online)

advanced bone age on the X-ray of the hand was noted (at the age of 6 years, the bone age was 9 years). MRI of brain showed slightly non-specific enlargement of both ventricles. EEG exam was normal.

On the current physical examination at the age of 8 years, body weight was 46.6 kg (3 kg above 97 percentile), height 154 cm (14 cm above 97 percentile), OFC 55 cm (97 percentile). The child is hypertrophic and hypotonic. The walk is clumsy, scoliosis is observed. Proband has characteristic facial dysmorphic features: fine hair, wide and prominent forehead, large ears, widely spaced eyes, epicanthic folds, almond shaped palpebral fissures, prominent and deep philtrum, open mouth, widely spaced teeth, retrognathia, and a pointed chin (Fig. 1). The hand and feet are large with hyper-mobile fingers. The club and flat feet, and hoarse voice were noted. Speech development was delayed and moderate intellectual disability with friendly personality was observed. There is no history of cancer suspicion in the proband.

After clinical suggestion of Weaver syndrome, *EZH2* gene was sequenced without any pathogenic mutations.

Table 1 Comparison of patients with EED mutation described in literature with our proband

Clinical findings	Patient 1 [3]	Patient 2 [4]	Patient 3 [2]	Patient 4 [5]	Patient 5—Present report
Causative mutation	p. Arg302Ser (c.906 A>C)	p.His258Thr (c.772 C>T)	p.Arg302Gly (c.904 A>G)	p.Arg263Thr (c.707 G>A)	p.Arg306Thr (c.907 G>C) p.Asn307Asp (c.919 A>G)
Sex	Male	Male	Female	Male	Male
Birth weight	4100 g	4336 g	4800 g	4398 g	3550 g
Birth length	52 cm	54.6 cm	53 cm	53.8 cm	54 cm
Birth head circumference	n/a	37.2 cm	35.5 cm	35.5 cm	34 cm
Hypertrophy	+	+	+	+	+
Developmental delay and/or intellectual disability	+	+	+	+	+
Hypotonia	−	+	+	−	+
Advanced bone age	+	+	+	+	+
Hoarse speech	+	n/a	+	−	+
Macrocephaly	+	+	+	+	+
Hypertelorism	+	−	+	+	+
Almond-shaped PF	−	+	+	+	+
Large ears	+	+	+	+	+
Prominent and/or long philtrum	−	−	+	+	+
Pointed chin	+	+	+	−	+
Retrognathia	+	+	+	+	+
Large hands and feet	+	+	+	+	+
Limbs anomalies, including camptodactyly and joint contractures	+	+	+	+	−
Umbilical hernia	+	+	+	+	−
Cleft plate	+	+	+	n/a	−
Ophthalmological abnormalities	+	+	+	n/a	+
Hearing loss	n/a	n/a	+	n/a	+
Epilepsy	+ (first seizure at age 4.5)	−	n/a	n/a	−
Scoliosis	+	+	n/a	−	+
Osteopenia	−	+	+	−	n/a
MRI	normal	normal	Substantial white matter loose and hypoplasia of the corpus callosum	normal	enlargement of both ventricles

n/a not available

Afterward, WES study was performed using SureSelectXT Human All Exon V5 (Agilent) on HiSeq 1500 (Illumina). The mean depth of coverage was 89×, 95% of target sequence was covered min 20× and 99% min 10×.

Bioinformatics analysis of WES was performed as described previously [6] (see also Supplementary Material, Table S1, Fig. S1). It revealed 4 potential pathologic variants: one in *ARHGAP35*, another in *HERC1* and two in *EED* gene (the details of the filtering process are given in supplement). Family study using amplicon deep sequencing (ADS, performed with Nextera XT Library Preparation Kit) showed that the heterozygous *ARHGAP*35 variant was inherited from mother, the heterozygous *HERC1* variant was inherited from father whereas the two heterozygous variants, c.917 G>C (p.Arg306Thr) and c.919 A>G (p.Asn307Asp) in the *EED* gene (NM_003797.3) were absent from parents indicating they were de novo mutations. Inspection of individual NGS reads indicated that these *EED* variants were located in the same allele (*in cis*, Fig. 2). These variants in proband were further confirmed as de novo mutations using Sanger sequencing (Fig. 2). ADS also confirmed the *in cis* configuration (Fig. S2). Given current nomenclature recommendations (http://varnomen.hgvs.org) the detected *EED* variants are described as c.917_919delinsCGG/p.(Arg306_Asn307delinsThrAsp). Both aminoacid changes in the *EED* gene are likely to be damaging with CADD score = 33 for p.Arg306Thr and 25.5 for p.Asn307Asp, moreover pathogenicity estimation by Polyphen2 and MutationTaster predicted both mutations as deleterious. Both variants are absent from ExAC, gnomAD and our in-house database of >500 Polish exomes. Paternity was confirmed analyzing 17 hypervariable STRs (AmpFLSTR™ NGM SElect™ PCR Amplification Kit, ThermoFisher Scientific, odds in favor of paternity > 10^6).

Discussion

We have ascertained an 8 y old boy with clinical symptoms of overgrowth and Weaver syndrome suspicion, in whom two novel mutations affecting two neighboring aminoacids encoded by one allele of the *EED* gene were found: c.917_919delinsCGG/p.(Arg306_Asn307delinsThrAsp).

In WS patients with *EED* mutations the following clinical features were observed: abnormal brain MRI findings (substantial white matter volume loss and moderate to severe thinning of the corpus callosum in one patient), genito-urinary anomalies (2 patients), heart defect (ASD and PDA in 2 patients), umbilical hernia (1 patient), ophthalmological abnormalities (myopia, exotropia, 3 patients), skeletal abnormalities (scoliosis, club foot) as well as hearing loss (1 patient), skin and ligament abnormalities, and rough low voice (Table 1) [2–5].

Patients described in literature with WS phenotype and *EED* mutations don't present leukemia nor embryonic cancers, but the number of patients is still small. In contrast patients with WS caused by mutations in *EZH2* suffer from cancers like AML, ALL, neuroblastoma, lymphoma, sacrococcygeal teratoma [7]. Long-term observation and a bigger group of patients with WS-like phenotype and *EED* mutation are needed to determine if there is a higher risk of cancer among these patients.

In conclusion, we present a patient with a clinical diagnosis of Weaver syndrome and novel de novo sequence variant in *EED*. Our observation together with previous reports [2, 3, 5] suggests that *EED* gene testing is warranted in patients with the overgrowth syndrome features and suspicion of Weaver syndrome with normal results for *EZH2* gene sequencing. Additional patients with pathogenic variants in *EED* gene should be systematically evaluated for better understanding the phenotype and the differences between patients with *EZH2* and *EED* gene mutation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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