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SHORT COMMUNICATION

A novel mutation in *EED* associated with overgrowth

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In a patient suspected clinically to have Weaver syndrome, we ruled out mutations in *EZH2* and *NSD1*, then identified a previously undescribed *de novo* mutation in EZH2's partner protein EED. Both proteins are members of the Polycomb Repressive Complex 2 that maintains gene silencing. On the basis of the similarities of the patient's phenotype to Weaver syndrome, which is caused by *de novo* mutations in *EZH2*, and on other lines of evidence including mouse *Eed* hypomorphs, we characterize this mutation as probably pathogenic for a Weaver-like overgrowth syndrome. This is the first report of overgrowth and related phenotypes associated with a constitutional mutation in human *EED*.
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INTRODUCTION

Overgrowth syndromes manifest increased mass of multiple tissues and often include advanced bone age, facial dysmorphism and intellectual disability. Mutations in epigenetic regulators frequently affect many aspects of growth and metabolism, causing either short or tall stature. 'Classical' overgrowth syndromes include Sotos (MIM #117550) and Weaver (MIM #277590), caused by germline mutations in *NSD1*[1] and *EZH2*[2,3] respectively. Recently, *de novo* mutations in *DNMT3A* and *SETD2*, two other epigenetic regulators, have been shown to cause distinct overgrowth syndromes.[4,5] Somatic mutations in all of these genes have also been implicated in various cancers, particularly hematological malignancies. On the basis of this evidence, we hypothesized that rare *de novo* mutations in other epigenetic regulators, particularly other members of the Polycomb Repressive Complex (PRC) 2, might explain unsolved overgrowth cases. Here we report on a Turkish man with overgrowth, facial dysmorphism and intellectual disability, and identify a probably pathogenic mutation in *EED*, another PRC2 member.

CASE REPORT

Our proband was born full term to non-consanguineous Turkish parents. Birth weight was 4100 g (standard deviation score (SDS) +1.09) and birth length 52 cm (SDS +0.38). Birth head circumference was not recorded. Dysmorphic features in childhood included macrocephaly, large bifrontal diameter, ocular hypertelorism and large ears (Figures 1a and b). Mild intellectual disability (IQ: 60) with speech delay (first words at age 2 years, first sentences at age 5 years) and poor fine motor skills were present. Brain magnetic resonance imaging was normal. He developed epilepsy, with his first seizure occurring at 4.5 years. Electroencephalogram showed a generalized irregular wave pattern, and phenytoin and primidone reduced seizure frequency to 1–2 per year (with no seizures from age 27 years onwards). He also developed moderate myopia (prescription lenses −5.5 and −6.5 diopters). Echocardiogram found mild mitral regurgitation. He developed tall stature in childhood: height was 87 cm (SDS +4.2) at 13 months, 138 cm (SDS +5.1) at 5 years 7 months and 158 cm (SDS +4.6) at 8 years 8 months (WHO growth charts). Blood hormone levels including thyroid-stimulating hormone (1.4 IU ml^{-1}), T4 (1.22 ng ml^{-1}), insulin-like growth factor 1 (201 ng ml^{-1}) and fasting glucose (98 mg dl^{-1} or 5.4 mmol l^{-1}) were all normal. He also had an umbilical hernia, and required surgical correction for cryptorchidism and a post-traumatic patellar dislocation at age 9 years. Following his knee surgery, circulatory failure led to amputation of his right leg over the knee at age 11 years. X-rays at age 4 years revealed dense physeal bands within the proximal femurs, and at 14.5 years bone age was consistent with 15 years. Additional X-rays at various ages (data not shown) revealed significant scoliosis, abnormal flaring of the distal clavicles, distal ribs, and metaphyses of the distal radius, distal ulna, distal femur and proximal tibia. The metaphyses were also abnormally lucent. He had flattened glenoid fossae and humeral heads, as well as a flattened left acetabulum and femoral head. At the most recent examination at age 27 years, his final height was 190 cm (SDS +1.85) and head circumference 59 cm (SDS +1.46). He has since undergone bilateral cataract surgery (age 30 years 10 months). Photographs of the patient confirm dysmorphic features including hypertelorism (adult interpupillary distance 8 cm), downslanting palpebral fissures and retrognathia with a prominent crease between the lower lip and the chin (Figures 1a to 1h, Supplementary Table 1). He also has kyphoscoliosis, widely spaced nipples and several pigmented nevi (Figures 1e and f), as well as large hands with

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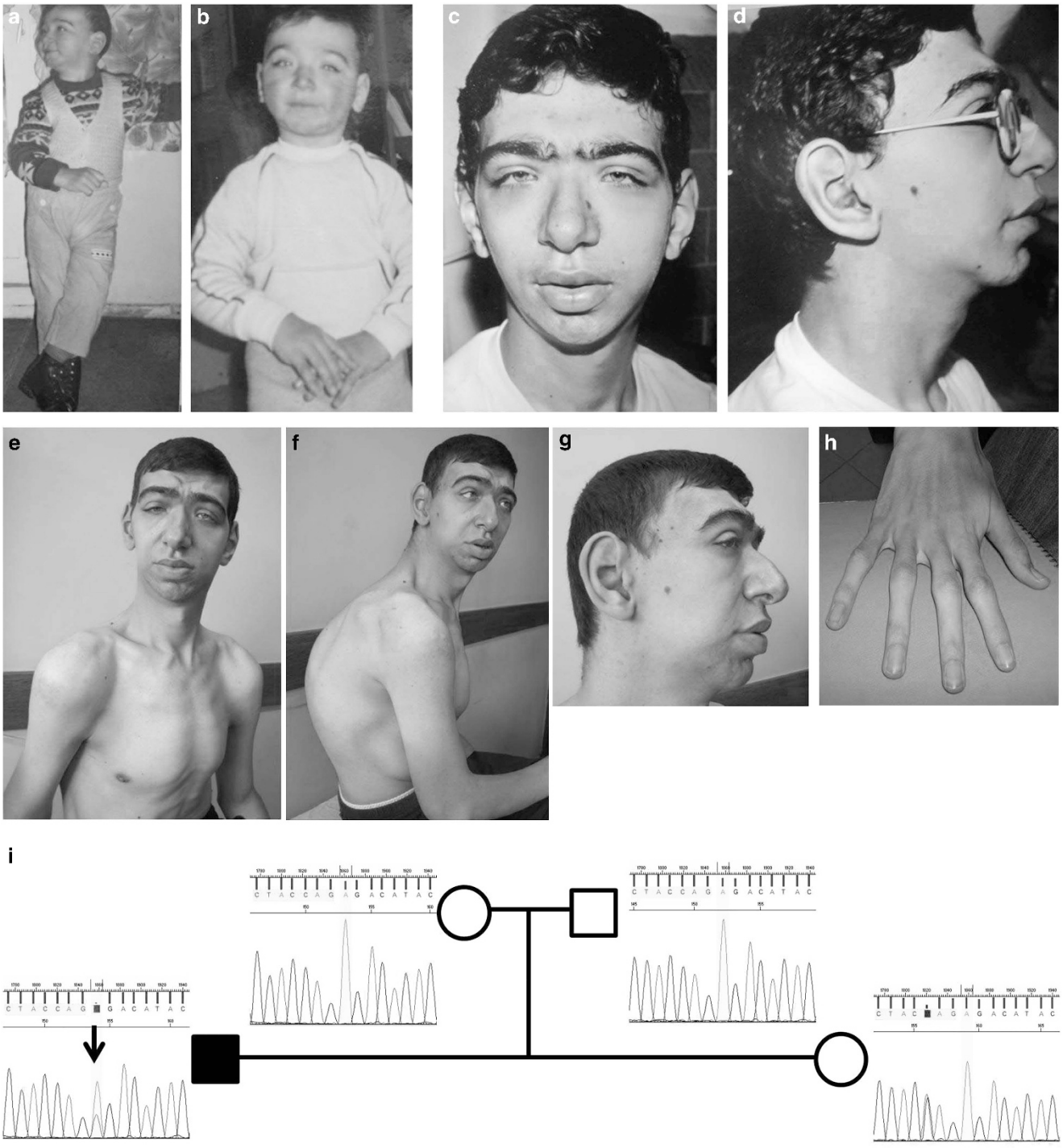

Figure 1 Characterization of patient with *EED* mutation. (**a**–**h**) Photographs of the proband show the features described in the paper at various ages: 3 years (**a**, **b**), 14 years (**c**, **d**) and 30 years (**e**–**g**); proband's right hand is shown at 27 years of age (**h**). (**i**) Pedigree with Sanger confirmation that the patient carries a *de novo* c.1372A>C (p.R302S) mutation in *EED*. This mutation is absent in the sister who carries a variant in the adjacent residue; this variant is synonymous (p.T301=) and thus consistent with her lack of overgrowth and dysmorphic features. Sanger traces were analyzed using Sequence Scanner v1.0 (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

camptodactyly (Figure 1h). To date, he has had no malignant or premalignant phenotypes and his blood cell counts remain normal (red cells 4.9×10^{12} per liter, white cells 6.5×10^{9} per liter and platelets 250×10^{9} per liter). He has a younger sister who does not have any overgrowth or dysmorphic features.

WHOLE-EXOME SEQUENCING AND RESULTS

Parents gave informed consent for participation in our study. *EZH2* and *NSD1* screening in the proband by Sanger sequencing were negative, so we constructed an Agilent (Santa Clara, CA, USA) All Exon V5+UTR capture library from his DNA before exome

sequencing using Illumina (San Diego, CA, USA) HiSeq 2500. Read alignment and variant calling, filtering and annotation were carried out using our in-house pipeline. Variants unique to our patient (defined as not previously observed in public repositories or the in-house human variation database[6]) were prioritized. Variants consistent with the phenotype were analyzed further; top candidates were validated by Sanger sequencing in the quartet.

We identified a novel c.1372A>C missense mutation in *EED* (NM_003797.3), confirmed as *de novo* by Sanger sequencing (Figure 1i), present only in the proband. This variant is predicted to convert arginine residue 302 to serine, scored as damaging by Polyphen (http://genetics.bwh.harvard.edu/pph2/) although not by SIFT (http://sift.jcvi.org/) (Table 1). We also found and Sanger-validated two novel variants in *MEGF8* (NM_001410.2): c.4517C>A inherited from the mother and c.3567G>A from the father, both absent in the sister (Supplementary Figure 1). Although this gene has been associated with an autosomal recessive subtype of Carpenter syndrome (MIM #614976),[7] the proband had no major features of this syndrome (lacking polydactyly, heterotaxia or obvious craniosynostosis) and both variants were predicted to be benign (Table 1).

DISCUSSION

We believe the *de novo EED* mutation is the most likely cause of the patient's excessive height in childhood (max SDS +5.1). We found no other coding variants that could plausibly explain these features. Carpenter syndrome, suggested by the two rare variants in *MEGF8*,[7] does feature some of the phenotypic traits observed in our patient (e.g. intellectual disability, cryptorchidism and camptodactyly). However, he had none of the cardinal manifestations of this latter syndrome (Supplementary Table 1).

EED is required for proper methyltransferase activity of *EZH2*.[8] It is highly conserved in mammals with 100% amino-acid identity between mouse Eed and human EED, despite significant coding nucleotide differences between the *Eed* and *EED* genes.[9] This form of conservation suggests that disruption of any given residue is likely to affect protein function. EED contains seven WD40 domains,[10,11] of which five appear functionally necessary[12] with potentially specific roles.[13] Our patient's mutation (c.1372A>C, encoding p.Arg302Ser or R302S) falls within a conserved nucleotide region[9] that affects all EED isoforms.[12] It localizes within a WD40 domain that is required for H3K27 methylation[12] and is possibly necessary for the EED–EZH2 interaction,[12] which is in turn required for *EZH2*'s methyltransferase activity. Consistent with this hypothesis, mutations in one of the

Table 1 Summary of candidate variants

Gene	*Chr*	*Position (build 37)*	*Mutation*	*Amino-acid change*	*Inheritance*	*Polyphen/SIFT prediction*	*Public database reference*[a]	*In-house databases*[b]
EED	11	85979543	c.1372A>C	p.Arg302Ser	*De novo*	Probably damaging/tolerated	Not described	Not described
MEGF8	19	42856391	c.3567G>A	p.Gly978Ser	Paternal	Benign/tolerated	Not described	Not described
MEGF8	19	42858811	c.4517C>A	p.Ser1294Arg	Maternal	Benign/tolerated	Not described	Not described

[a]Public databases checked include: dbNSP, COSMIC, Exome Variant Server, Ensembl variant database, NHLBI exome-sequencing data and LOVD.
[b]In-house databases refer to Canada's Michael Smith Genome Sciences Centre's human variation database and a Turkish-specific population database consisting of 587 exomes from the TÜBİTAK Advanced Genomics and Bioinformatics Research infrastructure (http://www.igbam.bilgem.tubitak.gov.tr/en/index.html).

Table 2 Genotype/phenotype correlations of the *EED* gene (11q14.2) and orthologues

Organism	*Gene*	*Gene disruption*	*Observations*	*Database entry*	*Reference*
Human	*EED*	p.R302G (somatic)	Myeloproliferative neoplasm	COSMIC COSM3720451	Nangalia *et al.*[17]
Human	*EED*	Copy number loss 11q14.1-22.3	Developmental delay and/or other significant developmental or morphological phenotypes	ClinVar: SCV000080064; dbVar: nsv531427	Kaminsky *et al.*[18]
Human	*EED*	Copy number loss 11q14.1-22.2	Abnormal facial shape, Muscular hypotonia	ClinVar: SCV000080065; dbVar: nsv531428	Kaminsky *et al.*[18]
Human & Mouse	*EED*	Null allele (c.T1040C) and hypomorphic allele (c.T1031A) in the same WD domain	Mutations in construct encoding human EED disrupt direct interaction with mouse Ezh2 *in vitro*	Non applicable	Denisenko *et al.*[14]
Mouse	*eed*	Hypomorphic allele *I7Rn5*^{1989SB} (c.T1031A, p.N193I) in second WD domain	Heterozygous mutant skeletons display intermediate phenotype: non-lethal posterior transformations along the anterior-posterior axis.	Non applicable	Shumacher *et al.*[19]
Drosophila	*esc*	Site-directed mutants RDE216AAA, GG210AA and double mutant RED216AAA DFST278AFAA	Mutant esc proteins that affect residues on the surface of the protein and that showed reduced binding to e(z) *in vitro* are capable of associating in complex with e(z) but have impaired function *in vivo*.	Non applicable	Ng *et al.*[20]
Drosophila	*esc*	Various mutants within WD domains including esc^5 (p.Q171STOP), *esc*^{9} (p.M236K) and *esc*^{2} (frameshift insertion and deletion at V_{404})	De-repression of homeotic genes resulting in transformation of body segments, for all mutants, despite some alterations being in 'non-conserved' residues, suggesting that individual WD repeats are all important and may have specialized roles.	Non applicable	Sathe *et al.*[13]

WD40 repeats in Eed block its interaction with Ezh2.[14] The region surrounding residue 302 is thought to be involved in binding of EED to PRC2 and also to PRC1.[15] This region appears to have a more important role in binding directly to H3K27, through a small pocket on the surface of EED formed by hydrophobic residues including C324, Y364 and nearby residue Y308.[11,16] Of interest, a mutation at Y358, six amino acids away from Y364 (the same distance as R302 to Y308), reduced EED binding to histone peptides by twofold.[11]

Further lines of evidence supporting the hypothesis that the R302S mutation disrupts EED function are summarized in Table 2. Hypothesis-testing experiments have shown that hypomorphic mutations in *Eed* cause developmental defects in mice. In addition, rare structural variants (deletions) including human *EED* co-occur with developmental defects that manifest in childhood. Rare somatic mutations in *EED* have also been observed in human cancers. In particular, p.R302G (a mutation affecting the same amino acid) has been reported in a myeloproliferative neoplasm[17] (a type of tumor that has recurrent mutations in other overgrowth genes such as *EZH2* and *DNMT3A*), reinforcing the concept that this particular residue is likely important for normal EED function.

The fact that common amino-acid polymorphisms in *EED* have not been observed across multiple healthy controls strongly suggests that sequence variation at the protein level is not compatible with good health, at least at the whole organism level. Given the known effects on growth of coding mutations in *EZH2*, we posited a high prior probability that functional mutations in other members of the PRC2 complex, such as *EED*, would likely cause overgrowth. Having now observed overgrowth in a patient with a *de novo* coding mutation in *EED*, we conclude that this patient's overgrowth is attributable to this mutation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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