EED and EZH2 constitutive variants: a study to expand the Cohen Gibson syndrome phenotype and contrast it with Weaver syndrome

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Abstract

Overgrowth-intellectual disability (OGID) syndromes are characterised by increased growth (height and/or head circumference $\geq 2SD$) in association with an intellectual disability.

Constitutive *EED* variants have previously been reported in five individuals with an OGID syndrome, eponymously designated Cohen-Gibson syndrome and resembling Weaver syndrome. Here we report three additional individuals with constitutive *EED* variants, identified through exome sequencing of an OGID patient series. We compare the *EED* phenotype with that of Weaver syndrome (56 individuals), caused by constitutive *EZH2* variants. We conclude that, whilst there is considerable overlap between the *EED* and *EZH2* phenotypes with both characteristically associated with increased growth and an intellectual disability, individuals with *EED* variants more frequently have cardiac problems and cervical spine abnormalities, boys have cryptorchidism and the facial gestalts can usually be distinguished.

Keywords

Overgrowth, intellectual disability, *EED*, *EZH2*, Cohen-Gibson, Weaver

Introduction

Overgrowth-intellectual disability (OGID) syndromes are characterised by increased growth (height and/or head circumference $\geq +2SD$, UK90 growth data) in association with an intellectual disability (Freeman, 1990; Tatton-Brown, 2013; Tatton-Brown, 2017). Since the advent of multiple parallel sequencing technologies, two predominant OGID syndrome gene families have emerged: the epigenetic regulator gene family and the PI3K/AKT pathway gene family (Tatton-Brown, 2017). *EED*, which encodes a 441 amino acid protein (ENST00000263360) with seven WD40 motifs (figure 1a), is a member of the epigenetic regulator gene family and, together with *EZH2* (Enhancer of Zeste, Drosophila, Homolog 2) and *SUZ12* (Suppressor of Zeste 12, Drosophila, Homolog of), they encode components of the polycomb-repressive complex 2 (PRC2) (Xu, 2010). PRC2 catalyses the transfer of up to three methyl groups to lysine 27 of histone H3 (H3K27), a methylation mark associated with transcriptional repression (Cao, 2002; Cao, 2004; Gall Troselj, 2016; Ketel, 2005; Kuzmichev, 2002).

Constitutive *EZH2* variants cause Weaver syndrome (MIM 277590), an OGID syndrome characterized by tall stature, a variable intellectual disability and recognisable facial features including a broad forehead, hypertelorism, almond-shaped palpebral fissures, a pointed “stuck-on” chin, large ears and retrognathia (Gibson, 2012; Tatton-Brown, 2011; Tatton-Brown, 2013; Weaver, 1974). It has been suggested that Weaver syndrome is also caused by *EED* constitutive variants (Cooney, 2017; Imagawa, 2017; Smiegiel, 2018).

Five individuals with *de novo* constitutive *EED* variants have been reported and the condition has been designated Cohen-Gibson syndrome (MIM 617561) (Cohen, 2015; Cohen, 2016; Cooney, 2017; Imagawa, 2017; Smigiel, 2018). In this report we describe a further three individuals with constitutive *EED* pathogenic variants. In addition, we review the *EED* literature and contrast the *EED* and *EZH2* phenotypes.

Materials and Methods

The study was approved by the London Multicentre Research Ethics Committee (MREC/01/2/44) and written, informed consent was obtained from all study participants and/or their parents.

We undertook exome sequencing on lymphocyte-derived DNA using methods and prioritisation of variants as previously described (Tatton-Brown, 2017). Three individuals with *de novo* *EED* missense variants (reported with reference to the ENST00000263360 transcript) were identified (figure 1a). Two of these variants had previously been reported but detailed phenotypic data were not included in this report (patient one (COG0877) and patient two (COG1738), Tatton-Brown, 2017). All three variants were predicted to be likely pathogenic according to the American College of Medical Genetics/Genomics (ACMG) guidelines, meeting criteria PS2, PM2, PP2 and PP3 (supplementary table 1, Richards, 2015).

We additionally identified 47 individuals with *EZH2* variants considered likely pathogenic or pathogenic according to ACMG guidelines (supplementary table 2). These variants were identified through exome sequencing (12 individuals), Sanger sequencing (33

individuals) and gene panel testing as part of the clinical diagnostic service (two individuals).

Detailed clinical information for 38 of these individuals has previously been described (Tatton-Brown, 2011; Tatton-Brown, 2013) (supplementary table 2).

Clinical data were obtained through a standardised proforma for all individuals and clinic review was undertaken by one of the authors for the three individuals with *EED* variants.

In order to evaluate the facial phenotype associated with *EED* variants and whether it is distinguishable from Weaver syndrome, we presented a series of photographs (of older children and adults) to a panel of ten dysmorphologists from the South West Thames Regional Genetics service. The clinicians were first shown photographs of a reported individual with a pathogenic *EED* variant (Cohen, 2015) and were then asked to assign 22 individuals with a range of OGID phenotypes to one of three groups: 1) **Group 1**, *EED*-associated facial gestalt, Cohen Gibson syndrome (four individuals including the three individuals in the current study) 2) **Group 2**, *EZH2*-associated facial gestalt, Weaver syndrome (nine individuals) and 3) **Group 3**, other OGID related facial gestalt including Sotos syndrome (three individuals) and Tatton-Brown-Rahman syndrome (six individuals). For each individual the majority score was determined.

Results

Patient one, COG0877

Patient one, male, has a *de novo* *EED* c.581A>G; p.(Asn194Ser) likely pathogenic variant. He is the second child of healthy non-consanguineous parents. He was born at term following an uncomplicated pregnancy and delivery with a birth weight of 4.7kg (+2.3SD). There were no postnatal complications.

Patient one has a mild intellectual disability. He lives with his parents but manages daily activities and can read and write.

Patient one first came to medical attention at the age of eight years when he developed seizures. These were initially thought to be epileptic seizures and were treated with sodium valproate but, at 10.2 years of age, the seizures were attributed to hyperinsulinaemic hypoglycaemia for which he was started on ocreotide injections. Additional past medical history included bilateral orchidopexy for cryptorchidism, a cholecystectomy, umbilical hernia and von Willebrand disease.

On examination at the age of 26 years, patient one's height was 199.5cm (+3.1SD), weight was 130kg (+3.7SD) and OFC was 64cm (+3.9SD). He had telecanthus, narrow downslanting palpebral fissures, lateral ptosis, everted lateral lower eyelids, synophrys, large, fleshy ears and pronounced philtral pillars (figure 1b).

Patient two, COG1738

Patient two, male, has a *de novo* *EED* c.706A>G; p.(Arg236Gly) likely pathogenic variant. He is the third child of healthy consanguineous parents. He was born at term by an elective

Caesarean section following an uncomplicated pregnancy with a birth weight of 4.96kg (+2.8SD) and birth length of 57cm (+3.0SD). Postnatally patient two was found to have cryptorchidism, for which he required bilateral orchidopexy, and a small haemodynamically insignificant ventricular septal defect (VSD) which closed spontaneously by two years of age.

Patient two has a severe intellectual disability with limited speech and a short-term memory deficit. His IQ according to the Wechsler scale is 33.

The past medical history was notable for myopic chorioretinal degeneration and cervical spinal canal stenosis with myelopathy at C3. The latter was diagnosed at the age of 11 years following MRI investigation for a progressive deterioration in gait, bilateral internal rotation of the feet, distal hypertonia, hyperreflexia and upgoing plantar reflexes.

On examination, at the age of 11 years, patient two's height was 168cm (+3.7SD), weight was 58.5 kg (+2.4SD) and head circumference was 58.5cm (+2.3SD). He had occipital flattening, broad forehead, short, downward-slanting palpebral fissures, an everted lateral lower eyelid, lateral ptosis, telecanthus, a long philtrum, prognathism and large, fleshy ears. He had large hands and feet (figure 1b).

Patient three, COG1946

Patient three, female, has a *de novo* EED c.710A>G; p.(Asp237Gly) likely pathogenic variant. She is the second of three children born to healthy unrelated parents. The pregnancy was uncomplicated and delivery was at 39 weeks by a normal vaginal delivery. Patient three

weighed 4.08 kg (+1.1SD) and had a length of 55.9cm (+3.0SD) at birth. In the neonatal period she was jaundiced and initially had poor weight gain.

At the age of three weeks, patient three was hospitalised with pneumonia but has been fit and well since other than continuing low muscle tone and scoliosis. She has a mild intellectual disability and attended mainstream school.

Patient three has always been tall with macrocephaly. At the age of ten years, her height was 161.5cm (+3.6SD), her head circumference was 54.5cm (+0.6SD); at the age of 13 years her height was 175cm (+2.8SD) and her weight was 56kg (+1.2SD) and at the age of 24 years her height was 182.1cm (+3.0SD), head circumference was 59.5cm (+2.9SD) and weight was 127kg (+3.6SD). She had a broad forehead, high frontal hairline, heavy brow, narrow palpebral fissures and tall chin with horizontal skin crease (figure 1b).

EED-associated phenotype

We reviewed the literature and evaluated the *EED* phenotype in a total of eight individuals (the current three individuals and five previously reported individuals). The results are summarised in table 1.

All eight individuals had an intellectual disability that was described as mild in four individuals, moderate in three individuals and severe in one individual. In addition, a shared, recognisable facial gestalt was evident. In infancy and early childhood, individuals had a broad forehead, telecanthus (often with hypertelorism), retrognathia and pointed chin with horizontal skin crease. However, at older ages, affected individuals additionally had a

characteristic lateral ptosis often with eversion of the lateral lower eyelid (Cohen, 2015; Cohen, 2016; Cooney, 2017; Imagawa, 2017; Smigiel, 2018).

The mean birth weight amongst the eight individuals was +1.8SD and the mean birth length was +1.9SD. The mean postnatal height was +3.1SD (with a range of +1.7SD to +4.8SD), the mean postnatal weight was +2.9SD (with a range of +1.9SD to +3.6SD) and the mean postnatal head circumference was +2SD (with a range of +0.7SD to +3.9 SD).

A range of additional medical problems were reported in the eight individuals. Medical problems reported in at least three of the eight affected individuals included cardiac anomalies (4/8 individuals including combinations of mitral regurgitation (two individuals), patent ductus arteriosus (two individuals), ventricular septal defect (one individual) and atrial septal defect (one individual)); abnormalities of tone (hypotonia and/or hypertonia, 4/8 individuals); cryptorchidism (4/6 affected males); umbilical hernia (5/8 individuals) and cervical spinal canal abnormalities (stenosis in 2/8 individuals and instability in one individual). Details of these and other medical problems reported in fewer than three individuals are shown in table 1.

EZH2-associated phenotype

We additionally reviewed the clinical features in 56 individuals with likely/pathogenic *EZH2* variants (47 individuals identified through the current study and nine individuals previously reported in the literature (Al-Salem, 2013; Cohen, 2016; Gibson, 2012; Lui, 2018). The

clinical features of these 56 individuals with likely/pathogenic *EZH2* variants are shown in supplementary table 2.

A comparison of the *EED* and *EZH2* associated phenotypes.

Clinical features reported in individuals with *EED* and *EZH2* variants are shown in table 2.

Table 3 summarises the results of the facial gestalt evaluation with the majority of dysmorphologists assigning the reported patient by Imagawa and patients one and two to group 1 (*EED* group, Imagawa, 2017). However, in contrast, patient three was assigned to each of the three groups with no clear decisive majority favouring a single designation. As patient three was older than the other three patients who had been correctly assigned to group 1, this may reflect an evolving facial phenotype with age and a lack of familiarity with the gestalt across the ages.

Discussion

EED pathogenic variants have recently been shown to cause a new OGID syndrome, Cohen Gibson syndrome (MIM 617561). Several reports have suggested that Weaver syndrome and Cohen-Gibson syndrome are clinically indistinguishable (Cooney, 2017; Imagawa, 2017; Smiegiel, 2018).

As a component of a large exome sequencing experiment, we have identified three individuals with *de novo* *EED* likely pathogenic variants bringing the total number of individuals identified with *EED* variants to eight. All eight variants are missense variants, preferentially targeting WD domains 3 and 4 with one hot spot variant at amino acid residue

302 (located two amino acid residues N terminal to the WD5 domain). The significance of this clustering is currently unclear and the predominance of missense rather than protein truncating variants suggests that Cohen-Gibson is unlikely to be the result of simple haploinsufficiency of EED. Functional studies to investigate the mechanism of pathogenesis of Cohen-Gibson syndrome would be of interest but are beyond the scope of the current phenotypic series.

We evaluated the phenotypes of our three patients with *EED* likely pathogenic variants and the additional five previously reported individuals. All eight individuals have an intellectual disability, most frequently in the mild range, a distinctive facial appearance and tall stature. Other frequently associated clinical features include macrocephaly, umbilical hernia, cryptorchidism, camptodactyly, abnormalities of tone, cardiac abnormalities and cervical spine abnormalities. Our current practice is therefore, where a constitutive *EED* variant is identified, to undertake a baseline echocardiogram and to ensure all boys are examined for cryptorchidism. In addition, until further data are available, we have a low threshold for investigating blood glucose levels and imaging for cervical spine abnormalities.

Given that EED and EZH2 partner with SUZ12 to form the Polycomb Repressive Complex 2 (PRC2), several reports have suggested that constitutive *EED* and *EZH2* pathogenic variants cause indistinguishable overgrowth phenotypes (Cooney, 2017; Imagawa, 2017; Smiegiel, 2018). In order to investigate the overlap between the *EED* and *EZH2* phenotypes, we compared the clinical features reported in fifty-six individuals with constitutive *EZH2* variants and the eight individuals with constitutive *EED* likely/pathogenic

variants. Overlapping *EED* and *EZH2* clinical features include an intellectual disability (most frequently mild), increased growth (height with and without macrocephaly), umbilical hernia, kypho/scoliosis, camptodactyly and abnormalities of tone. However, tumours have currently only been reported in association with *EZH2* variants whilst cryptorchidism, cervical spine abnormalities and cardiac abnormalities appear to be more frequent in individuals with *EED* variants. We have additionally demonstrated that the facial gestalt of individuals with constitutive *EED* variants and *EZH2* variants can generally be distinguished at older ages: although both are associated with frontal bossing, a tall chin and horizontal skin crease, individuals with *EED* variants additionally frequently have telecanthus, narrow palpebral fissures with lateral ptosis, a downslant to the lateral margins of the upper eyelid and eversion of the lateral aspects of the lower eyelid.

Although the current study has permitted further refinement of the *EED* phenotype, the small numbers of individuals with *EED* variants preclude robust statistical comparisons. As genomic testing becomes more accessible and more individuals with *EED* variants are identified, it is likely that we will be able better to define the *EED*-associated phenotype, draw more robust comparisons with *EZH2* phenotype and be able to develop *EED* specific evidence-based management guidance.

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Conflicts of Interests

The authors declare that they have no conflict of interest

Web Resources

Ensembl, www.ensembl.org

GnomAD, <http://gnomad.broadinstitute.org/>

Mendelian Inheritance In Man, www.omim.org

Mutation Taster, <http://www.mutationtaster.org/>

SIFT, <http://sift.bii.a-star.edu.sg/>

Uniprot, www.uniprot.org

References

Al-Salem A., Alshammari M.J., Hassan H., Alazami AM., Alkuraya FS. (2013). Weaver syndrome and defective cortical development: A rare association. *Am J Med Genet A*, 161A, 225-7. doi: 10.1002/ajmg.a.35660

Cao, R., Wang L., Wang H., Xia L., Erdjument-Bromage H., Tempst P., Jones RS., Zhang Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science*, 298, 1039-43. doi: 10.1126/science.1076997

Cao, R. & Zhang, Y. (2004). SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED-EZH2 complex. *Mol Cell*, 15, 57-67. doi:10.1016/j.molcel.2004.06.020

Cohen, A.S. & Gibson, W.T. (2016). EED-associated overgrowth in a second male patient. *J Hum Genet*, 61, 831-4. doi: 10.1038/jhg.2016.51

Cohen, A.S., Tuysuz B., Shen Y., Bhalla SK., Jones SJ., Gibson WT. (2015). A novel mutation in EED associated with overgrowth. *J Hum Genet*, 60, 339-42. doi: 10.1038/jhg.2015.26

Cohen, A.S., Yap, D.B., Lewis, S., Chijiwa, C., Ramos-Arroya, M.A., Tkachenko, N., Milano, V., Fradin, M., McKinnon, M.L., Townsend, K.N., et al. (2016). Weaver Syndrome-Associated EZH2 Protein Variants Show Impaired Histone Methyltransferase Function In Vitro. *Hum Mutat*. 2016 Mar; 37: 301–307. Doi: 10.1002/humu.22946

Cooney, E., Bi, W., Schlesinger, A.E., Vinson, S. & Potocki, L. (2017). Novel EED mutation in patient with Weaver syndrome. *Am J Med Genet A*, 173, 541-545. doi: 10.1002/ajmg.a.38055

Freeman J.V., Cole T.J., Chinn S., Jones P.R., White E.M., Preece M.A. (1995). Cross sectional stature and weight reference curves for the UK, 1990. *Arch. Dis. Child*. 73, 17–24

Gall Troselj, K., Novak Kujundzic, R. & Ugarkovic, D. (2016). Polycomb repressive complex's evolutionary conserved function: the role of EZH2 status and cellular background. *Clin Epigenetics*, 8, 55. doi: 10.1186/s13148-016-0226-1

Gibson, W.T., Hood RL., Zhan SH., Bulman DE., Fejes AP., Moore R., Mungall AJ., Eydoux P., Babul-Hirji R., An J., et al. (2012). Mutations in EZH2 cause Weaver syndrome. *Am J Hum Genet*, 90, 110-8. doi: 10.1016/j.ajhg.2011.11.018

Imagawa, E., Higashimoto K., Sakai Y., Numakura C., Okamoto N., Matsunaga S., Ryo A., Sato Y., Sanefuji M., Ihara K., et al. (2017) Mutations in genes encoding polycomb repressive complex 2 subunits cause Weaver syndrome. *Hum Mutat*, 38, 637-648 (2017). doi:

10.1002/humu.23200

Ketel, C.S., Andersen EF., Vargas ML., Suh J., Strome S., Simon JA.. (2005). Subunit contributions to histone methyltransferase activities of fly and worm polycomb group complexes. *Mol Cell Biol*, 25, 6857-68. doi:10.1128/MCB.25.16.6857-6868.2005

Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P. & Reinberg, D. (2002). Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev*, 16, 2893-905. doi:

10.1101/gad.1035902

Lui J. C., Barnes K. M., Dong L., Yue S., Graber E., Rapaport R., Dauber A., Nilsson O. and Baron J. (2018). Ezh2 Mutations Found in the Weaver Overgrowth Syndrome Cause a Partial Loss of H3K27 Histone Methyltransferase Activity. *J Clin Endocrinol Metab*, 103, 1470–1478. doi:10.1210/jc.2017-01948

Richards, S., Aziz, N., Bale, S., Bick, D., Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al; ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 17(5):405-24. doi: 10.1038/gim.2015.30Smigiel, R., Biernacka A.,

Biela M., Murcia-Pienkowski V., Szmida E., Gasperowicz P., Kosinska J., Kostrzewa G., Koppolu AA., Walczak A., et al. (2018). Novel de novo mutation affecting two adjacent aminoacids in the EED gene in a patient with Weaver syndrome. *J Hum Genet*, 63, 517-520.

doi: 10.1038/s10038-017-0391-x

Tatton-Brown, K., Hanks S., Ruark E., Zachariou A., Duarte Sdel V., Ramsay E., Snape K., Murray A., Perdeaux ER., Seal S. et al. (2011). Germline mutations in the oncogene EZH2 cause Weaver syndrome and increased human height. *Oncotarget*, 2, 1127-33. doi:

10.18632/oncotarget.385

Tatton-Brown, K., Loveday C., Yost S., Clarke M., Ramsay E., Zachariou A., Elliott A., Wylie H., Ardisson A., Rittinger O., et al. (2017). Mutations in Epigenetic Regulation Genes Are a Major Cause of Overgrowth with Intellectual Disability. *Am J Hum Genet*, 100, 725-736. doi:

10.1016/j.ajhg.2017.03.010

Tatton-Brown, K. Murray A., Hanks S., Douglas J., Armstrong R., Banka S., Bird LM., Clericuzio CL., Cormier-Daire V., Cushing T., et al. (2013). Weaver syndrome and EZH2 mutations: Clarifying the clinical phenotype. *Am J Med Genet A*, 161A, 2972-80. doi:

10.1002/ajmg.a.36229

Tatton-Brown, K. & Weksberg, R. (2013). Molecular mechanisms of childhood overgrowth. *Am J Med Genet C Semin Med Genet*, 163C, 71-5. doi: 10.1002/ajmg.c.31362

Usemann J., Ernst T., Schäfer V., Lehmborg K., Seeger K. (2016) EZH2 mutation in an adolescent with Weaver syndrome developing acute myeloid leukemia and secondary

hemophagocytic lymphohistiocytosis. *Am J Med Genet*, 170A, 1274-7. doi:

10.1002/ajmg.a.37562

Weaver, D.D., Graham, C.B., Thomas, I.T. & Smith, D.W. (1974). A new overgrowth syndrome with accelerated skeletal maturation, unusual facies, and camptodactyly. *J Pediatr*, 84, 547-52

Xu, C., Bian, C., Yang, W., Galka, M., Ouyang, H., Chen, C., Qiu, W., Liu, H., Jones, A.E., MacKenzie, F. et al. (2010) Binding of different histone marks differentially regulates the activity and specificity of polycomb repressive complex 2 (PRC2). *Proc Natl Acad Sci U S A*, 107:19266-71. doi: 10.1073/pnas.1008937107

Table 1: Clinical features reported in eight individuals with *EED* variants

Table 2: A comparison of the clinical features described in individuals with *EED* and *EZH2* variants.

Table 3: “Blinded” phenotypic assignment of four individuals with *EED* variants. Ten dysmorphologists were asked to assign 22 individuals (including four individuals with *EED* variants) to one of three groups. Group 1 represents individuals with *EED* variants, group 2 represents *EZH2* variants and group 3 represents other OGID syndromes.

Figure titles and legends

Figure 1 a) EED with the seven WD40 repeat domains. Arrows above EED indicate variants identified in the current study, arrows below represent previously reported variants **b)** The facial phenotype in patients one, two and three: individuals have telecanthus; short, narrow palpebral fissures; lateral ptosis, a downslant to the lateral aspect of the upper eyelid and eversion of the lateral aspect of the lower eyelid.

Supplementary file 1

Collaborators

Supplementary table 1: Table detailing evidence and American College of Genomics and Genetics criteria that were used to assign pathogenicity to the three *EED* variants

Supplementary table 2

A table detailing the molecular and clinical information for 52 individuals with *EZH2* variants

Table 1 Clinical features reported in eight individuals with *EED* variants.

Patient details		Current study					
		Patient One (COG0877)	Patient Two (COG1738)	Patient Three (COG1946)	(Cohen, 2015)	(Cohen, 2016)	(Cooney, 2017)
	Sex	Male	Male	Female	Male	Male	Female
Variant details	Age/years	26	11	24	27	30	16
	Nucleotide substitution	c.581A>G	c.706A>G	c.710A>G	c.906A>C	c.772C>T	c.904A>G
	Amino acid substitution	p.(Asn194Ser)	p.(Arg236Gly)	p.(Asp237Gly)	p.(Arg302Ser)	p.(His258Thr)	p.(Arg302Ser)
Growth	Inheritance	De novo	De novo	De novo	De novo	De novo	nk
	Birth weight/SD	2.3	2.8	1.1	1.1	1.6	3.6
	Birth length/SD	nk	3.0	3.0	0.5	1.8	2.4
	Birth HC/SD	nk	nk	nk	nk	1.6	1.7
	Postnatal height/SD	3.1	3.7	3.0	1.7	1.9	1.9
	Postnatal weight/SD	3.7	2.4	3.6	nk	1.9	2.2
	Postnatal HC/SD	3.9	2.3	2.9	1.0	2.2	1.2
Learning	Degree of intellectual disability	Mild	Severe	Mild	Mild	Moderate	Moderate
Additional medical problems	Cardiac anomalies	-	VSD	-	MR	-	PDA, MR, A
	Neurological defects	Seizures	Distal hypertonia;	-	Seizures	Hypotonia	Hypotonia
	Skeletal	-	Cervical spinal canal stenosis	Scoliosis	Scoliosis, C1, C2 instability	-	Camptodactyly, Cervical spine stenosis, kyphoscoliosis, Nephromegaly, duplex kidneys, Umbilical/abdominal/femoral hernia, cleft palate, conductive hearing loss, astigmatism
	Genito-urinary	Cryptorchidism	Cryptorchidism	-	Cryptorchidism	-	-
	Other	Cholecystectomy, hyperinsulinamic hypoglycaemia, umbilical hernia, Von Willebrand disease	Myopic choreoretinitis	-	Umbilical hernia	Umbilical hernia	-

Abbreviations: SD, standard deviations; nk, not known; -, clinical feature is absent; VSD, ventricular septal defect; PDA, patent ductus arteriosus; MR, mitral regurgitation

Table 2: A comparison of the clinical features described in individuals with *EED* and *EZH2*

variants.

Clinical feature		Numbers of affected individuals		Percentage of affected individuals
		EED (n=8)	EZH2 (n=56)	EED
Degree of intellectual disability	None	0	8	0
	Mild	4	29	50
	Moderate	3	12	38
	Severe	1	2	12
	Unclassified	0	3	0
	Not known	0	2	0
Other medical problems	Cryptorchidism	4†	2‡	67
	Umbilical hernia	5	18	63
	Abnormal tone	4	29	50
	Cardiac anomalies	4	6	50
	Kypho/scoliosis	3	12	38
	Cervical spine abnormalities	3	0	38
	Camptodactyly	2	16	25
	Tumours	0	6	0

Notes: †there are six males with *EED* variants, ‡there are 25 males with *EZH2* variants,

Table 3: “Blinded” phenotypic assignment of four individuals with *EED* variants. Ten dysmorphologists were asked to assign 22 patients (including four individuals with *EED* variants) to one of three groups. Group 1 represents individuals with *EED* variants, group 2 represents *EZH2* variants and group 3 represents other OGID syndromes.

Individual reference	Phenotypic groups			Total number of dysmorphologists
	Group 1	Group 2	Group 3	
Patient one	9	1	0	10
Imagawa (2017)	7	0	3	10
Patient two	9	1	0	10
Patient three	2	4	4	10

a.

b.

Patient one

Patient two

Patient three

Table 1 Clinical features reported in eight individuals with *EED* variants.

Patient details		Current study			Reported cases				
		Patient One (COG0877)	Patient Two (COG1738)	Patient Three (COG1946)	(Cohen, 2015)	(Cohen, 2016)	(Cooney, 2017)	(Imagawa, 2017)	(Smigiel, 2018)
	Sex	Male	Male	Female	Male	Male	Female	Male	Male
Variant details	Age/years	26	11	24	27	30	16	5	8
	Nucleotide substitution	c.581A>G	c.706A>G	c.710A>G	c.906A>C	c.772C>T	c.904A>G	c.707G>A	c.907 G>C; c.919A>G
	Amino acid substitution	p.(Asn194Ser)	p.(Arg236Gly)	p.(Asp237Gly)	p.(Arg302Ser)	p.(His258Thr)	p.(Arg302Gly)	p.(Arg236Thr)	p.(Arg306Thr); p.(Arg302Gly)
Growth	Inheritance	De novo	De novo	De novo	De novo	De novo	nk	De novo	De novo
	Birth weight/SD	2.3	2.8	1.1	1.1	1.6	3.6	1.7	Mean
	Birth length/SD	nk	3.0	3.0	0.5	1.8	2.4	1.4	1.5
Learning	Birth HC/SD	nk	nk	nk	nk	1.6	1.7	0.2	-0.1
	Postnatal height/SD	3.1	3.7	3.0	1.7	1.9	1.9	4.4	4.8
	Postnatal weight/SD	3.7	2.4	3.6	nk	1.9	2.2	3.4	3.1
Additional medical problems	Postnatal HC/SD	3.9	2.3	2.9	1.0	2.2	1.2	1.6	0.7
	Degree of intellectual disability	Mild	Severe	Mild	Mild	Moderate	Moderate	Mild	Moderate
	Cardiac anomalies	-	VSD	-	MR	-	PDA, MR, ASD	PDA	-
Additional medical problems	Neurological defects	Seizures	Distal hypertonias;	-	Seizures	Hypotonia	Hypotonia	-	Hypotonia
	Skeletal	-	Cervical spinal canal stenosis	Scoliosis	Scoliosis, C1, C2 instability	-	Camptodactyly, Cervical spine stenosis	-	Camptodactyly; Scoliosis
	Genito-urinary	Cryptorchidism	Cryptorchidism	-	Cryptorchidism	-	kyphoscoliosis Nephromegaly, duplex kidney	-	Cryptorchidism
Additional medical problems	Other	Cholecystectomy, hyperinsulinemic hypoglycaemia, umbilical hernia, Von Willebrand disease	Myopic choreoretinitis	-	Umbilical hernia	Umbilical hernia	Umbilical/inguinal/femoral hernia, cleft palate, conductive hearing loss, astigmatism	Umbilical hernia	Bilateral hypoacusis

Abbreviations: SD, standard deviations; nk, not known; -, clinical feature is absent; VSD, ventricular septal defect; PDA, patent ductus arteriosus; MR, mitral regurgitation

Table 2: A comparison of the clinical features described in individuals with *EED* and *EZH2* variants.

Clinical feature		Numbers of affected individuals		Percentage of affected individuals	
		EED (n=8)	EZH2 (n=56)	EED	EZH2
Degree of intellectual disability	None	0	8	0	14
	Mild	4	29	50	52
	Moderate	3	12	38	21
	Severe	1	2	12	4
	Unclassified	0	3	0	5
	Not known	0	2	0	4
Other medical problems	Cryptorchidism	4 [†]	2 [‡]	67	8 [‡]
	Umbilical hernia	5	18	63	32
	Abnormal tone	4	29	50	52
	Cardiac anomalies	4	6	50	11
	Kypho/scoliosis	3	12	38	21
	Cervical spine abnormalities	3	0	38	0
	Camptodactyly	2	16	25	29
	Tumours	0	6	0	11

Notes: [†]there are six males with *EED* variants, [‡]there are 25 males with *EZH2* variants,

Table 3: “Blinded” phenotypic assignment of four individuals with *EED* variants. Ten dysmorphologists were asked to assign 22 patients (including four individuals with *EED* variants) to one of three groups. Group 1 represents individuals with *EED* variants, group 2 represents *EZH2* variants and group 3 represents other OGID syndromes.

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