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Weaver syndrome and EZH2 mutations: Clarifying the clinical phenotype

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Abstract: Weaver syndrome, first described in 1974, is characterized by tall stature, a typical facial appearance, and variable intellectual disability. In 2011, mutations in the histone methyltransferase, EZH2, were shown to cause Weaver syndrome. To date, we have identified 48 individuals with EZH2 mutations. The mutations were primarily missense mutations occurring throughout the gene, with some clustering in the SET domain (12/48). Truncating mutations were uncommon (4/48) and only identified in the final exon, after the SET domain. Through analyses of clinical data and facial photographs of EZH2 mutation-positive individuals, we have shown that the facial features can be subtle and the clinical diagnosis of Weaver syndrome is thus challenging, especially in older individuals. However, tall stature is very common, reported in >90% of affected individuals. Intellectual disability is also common, present in 80%, but is highly variable and frequently mild. Additional clinical features which may help in stratifying individuals to EZH2 mutation testing include camptodactyly, soft, doughy skin, umbilical hernia, and a low, hoarse cry. Considerable phenotypic overlap between Sotos and Weaver syndromes is also evident. The identification of an EZH2 mutation can therefore provide an objective means of confirming a subtle presentation of Weaver syndrome and/or distinguishing Weaver and Sotos syndromes. As mutation testing becomes increasingly accessible and larger numbers of EZH2 mutation-positive individuals are identified, knowledge of the clinical spectrum and prognostic implications of EZH2 mutations should improve.

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Weaver Syndrome and *EZH2* Mutations: Clarifying the Clinical Phenotype

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Weaver syndrome, first described in 1974, is characterized by tall stature, a typical facial appearance, and variable intellectual disability. In 2011, mutations in the histone methyltransferase, *EZH2*, were shown to cause Weaver syndrome. To date, we have identified 48 individuals with *EZH2* mutations. The mutations were primarily missense mutations occurring throughout the gene, with some clustering in the SET domain (12/48).

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Truncating mutations were uncommon (4/48) and only identified in the final exon, after the SET domain. Through analyses of clinical data and facial photographs of *EZH2* mutation-positive individuals, we have shown that the facial features can be subtle and the clinical diagnosis of Weaver syndrome is thus challenging, especially in older individuals. However, tall stature is very common, reported in >90% of affected individuals. Intellectual disability is also common, present in ~80%, but is highly variable and frequently mild. Additional clinical features which may help in stratifying individuals to *EZH2* mutation testing include camptodactyly, soft, doughy skin, umbilical hernia, and a low, hoarse cry. Considerable phenotypic overlap between Sotos and Weaver syndromes is also evident. The identification of an *EZH2* mutation can therefore provide an objective means of confirming a subtle presentation of Weaver syndrome and/or distinguishing Weaver and Sotos syndromes. As mutation testing becomes increasingly accessible and larger numbers of *EZH2* mutation-positive individuals are identified, knowledge of the clinical spectrum and prognostic implications of *EZH2* mutations should improve. © 2013 Wiley Periodicals, Inc.

Key words: *EZH2*; Weaver syndrome; histone methyl transferases

INTRODUCTION

Weaver et al. [1974] described two boys with accelerated osseous maturation, unusual facies, and camptodactyly and proposed that this constellation of phenotypic features constituted a novel clinical entity. Subsequent to this initial description over 50 individuals with similar and additional clinical features were reported and the condition became eponymously known as Weaver syndrome (OMIM 277590) [Majewski et al., 1981; Meinecke et al., 1983; Roussounis and Crawford, 1983; Tsukahara et al., 1984; Farrell and Hughes, 1985; Ardinger et al., 1986; Thompson et al., 1987; Greenberg et al., 1989; Kondo et al., 1990, 1991; Muhonen and Menezes, 1990; Ramos-Arroyo et al., 1991; Cole et al., 1992; Dumic et al., 1993; Scarano et al., 1996; Fryer et al., 1997; Proud et al., 1998; Derry et al., 1999; Freeman et al., 1999; Sarigul et al., 1999; Kelly et al., 2000; Ozkan and Bereket, 2000; Huffman et al., 2001; Crawford and Rohan, 2005; Coulter et al., 2008; Iatrou et al., 2008; Bansal and Bansal, 2009; Basel-Vanagaite, 2010; Mikalef et al., 2010]. However, because of the subtlety of the Weaver syndrome phenotype and the overlap with other overgrowth syndromes, particularly Sotos syndrome, the clinical diagnosis can be challenging, even for the experienced dysmorphologist. Thus the robustness of the many reported Weaver syndrome clinical associations is unclear.

In 2011, we and another independent group reported that constitutional *EZH2* mutations cause Weaver syndrome [Gibson et al., 2011; Tatton-Brown et al., 2011]. *EZH2* (Enhancer of Zeste homolog 2) is located at 7q36.1 and encodes a histone methyltransferase involved in chromatin modeling and transcriptional repression. In combination with EED (Embryonic Ectoderm Development protein, mouse, homolog of) and SUZ12 (Suppressor

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of Zeste 12, drosophila, homolog of), it forms the polycomb repressive complex 2 (PCR2) which catalyzes the methylation of lysine residue 27 of histone 3 (H3K27) resulting in chromatin compaction and repression of transcription [Cao et al., 2002]. *EZH2* activity is inhibited by AKT (v-akt murine thymoma viral oncogene homolog 1), a key component of the PI3K/mTOR growth regulatory pathway, through the phosphorylation of serine 21, which blocks binding of *EZH2* to histone 3 [Cha et al., 2005].

Somatic activating and inactivating mono- and biallelic *EZH2* mutations have been identified in multiple hematological malignancies [Chase and Cross, 2011]. A recurrent monoallelic activating Tyr646 mutation has been identified in lymphomas, particularly diffuse large cell B-cell type and follicular lymphomas [Morin et al., 2010; Sneeringer et al., 2010]. In contrast, inactivating mono- and biallelic missense and truncating mutations, throughout *EZH2*, are associated with poor prognosis myelodysplastic and myeloproliferative neoplasms [Ernst et al., 2010].

In our initial 2011 paper on *EZH2* and Weaver syndrome we described 19 individuals with *EZH2* mutations [Tatton-Brown et al., 2011]. Subsequent to this study we have extended our molecular and clinical analyses to identify, in total, 48 individuals with *EZH2* mutations from 39 unrelated families. Using data from these cases, we have defined the *EZH2* mutational spectrum and provide the first detailed evaluation of the clinical features associated with *EZH2*-related Weaver syndrome.

PATIENTS AND METHODS

Case Series

The research was approved by the London Multicentre Research Ethics Committee and consent was obtained from participating individuals and/or their parents. Individuals recruited to the Childhood Overgrowth Genetics (COG) study with variable overgrowth phenotypes were screened for mutations in *EZH2*. Forty-eight individuals, 20 males and 28 females, with *EZH2* mutations (termed *EZH2* mutation-positive individuals) were identified. The patients had been recruited from Clinical Genetics

centers worldwide and included 25 from the UK; 10 from the USA; 3 from France; 2 from the Netherlands; 2 from Portugal; 2 from the Reunion Islands, and 1 from each of Austria, Belgium, the Republic of Ireland, and Japan. Clinical information, in the form of a standardized overgrowth questionnaire (routinely sent to recruiting physicians) and an *EZH2*-specific supplementary questionnaire, was requested for all *EZH2* mutation-positive individuals (see Fig. S1). Photographs were additionally received for 43 of the *EZH2* mutation-positive individuals. These were shown to a panel of five experienced dysmorphologists who assigned them to one of three phenotypic categories: (1) “typical Weaver syndrome,” (2) “possible Weaver syndrome,” or (3) “not Weaver syndrome.” If a patient was assigned to the latter category and an alternative diagnosis such as Sotos syndrome was suspected, this was noted. Where possible, a majority score was used. However, if the results from the five panelists were divergent, an average (median) score was calculated and used. The patient’s age when the photograph was taken was recorded as <1, 1–5, 5–10, or >10 years.

Molecular Studies

Four individuals (cases 19, 34, 40, and 46, Table SI) were identified to have *EZH2* mutations through exome sequencing as previously described [Snape et al., 2011; Tatton-Brown et al., 2011]. We subsequently performed Sanger sequencing of the full coding sequence and intron–exon boundaries of *EZH2* in 431 additional individuals from the COG study. We analyzed parental samples for the specific *EZH2* mutation identified in the proband, if samples were available.

Statistical Analyses

Independence of effects of phenotype group and age range was analyzed using a 4×3 contingency table, examining for deviation

from the expected distribution (under the null hypothesis of independence of effects) using Pearson’s chi-squared test (6df).

RESULTS

EZH2 Mutational Spectrum

We identified 48 *EZH2* mutations including 44 missense and 4 truncating variants (Fig. 1a). Variants were considered to be pathogenic if they were de novo, familial, and clearly segregating with the overgrowth phenotype or identical to a variant that had been classified as pathogenic in another individual. Thirty-eight variants fulfilled these criteria (Table SI). DNA was not available from both parents of two individuals with truncating variants; two individuals in whom the same amino acid residue, Arg684 and Glu148, had been substituted by a different amino acid as a de novo event in at least one other individual; one individual with an in-frame duplication and four individuals with missense variants. An additional patient had inherited a His618Tyr missense mutation from his father who did not have a clear overgrowth phenotype (Table SI). However, none of these variants were identified in over 2,000 in-house exomes from controls or breast cancer patients nor in over 6,000 exomes in the Exome variant server (<http://evs.gs.washington.edu/EVS/>) and all the missense variants affected highly conserved residues (Fig. 1b). Therefore, although we cannot be unequivocally certain of pathogenicity because the penetrance/expressivity of the mutations can be variable/mild, we considered it likely that these variants were causative of the overgrowth phenotype.

The 44 missense mutations were distributed throughout the gene with some clustering in the highly conserved SET domain (12/44, Fig. 1a). Two SET domain mutations, Arg684Cys and Ser652Cys, were seen recurrently: the Arg684Cys mutation in five unrelated individuals and the Ser652Cys mutation in two unrelated individuals. Only four truncating mutations were identified, all in the post-SET terminal exon, exon 20.

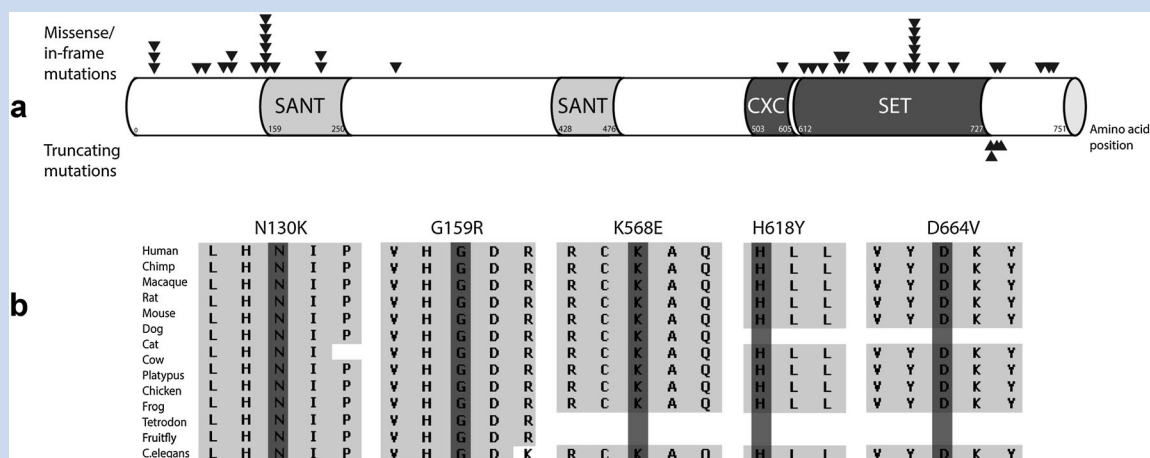


FIG. 1. (a) Distribution of *EZH2* missense and truncating mutations and (b) conservation of mutated residues in five cases with missense mutations (four where DNA from both parents was not available and one where the mutation was inherited from an unaffected father).

Clinical Features Associated With *EZH2* Mutations

The typical Weaver syndrome facial gestalt can be subtle and challenging to recognize. Among the 43 *EZH2* mutation-positive individuals in whom facial photographs were assessed and categorized, 20 were assigned to group 1 (classic Weaver syndrome), 11 to group 2 (possible Weaver syndrome) and 12 to group 3 (not typical for Weaver syndrome). In group 1, 10 individuals were at least 1 year and one individual was >10 years (with five and four individuals 1–5 years and 5–10 years, respectively). In group 2, one individual was <1 year, four were 1–5 years, two were 5–10 years, and four were >10 years and in group 3 there were no individuals who were <1 year, three were 1–5 years, one was 5–10 years, and eight were >10 years (Fig. 2). This distribution of ages and phenotypic group represented a significant excess of younger cases in group 1 and older cases in group 3 ($\chi^2 = 19.6$, $P = 0.003$ [6df]).

Facial features that characterized the 31 *EZH2* mutation-positive individuals considered to have classic and possible Weaver syndrome included ocular hypertelorism, almond shaped palpebral fissures, a broad forehead, and a pointed, “stuck-on” chin with horizontal skin crease and, in early childhood, large, fleshy ears, and retrognathia (Fig. 3). However, while those individuals with classic Weaver syndrome generally had all the listed features, individuals with possible Weaver syndrome had some but not all. Among the third “not Weaver syndrome” category, five individuals were thought to have Sotos syndrome and four were not considered to be dysmorphic. Additional, alternative, diagnoses proposed for category 3 patients included Kabuki syndrome and Peho syndrome, each suggested by one panelist for one affected individual.

The *EZH2*-related growth profile. Information regarding birth weight was available for 39 babies: 22 females and 17 males. Only 38% (15/39, including 11 females and four males) *EZH2* mutation-positive babies had a birth weight greater than two standard deviations above the mean (+2 SDs) with a median birth weight of +1.3 SDs and a range of –1.6 to +4.6 SDs. Birth length is likely to be a better correlate of an *EZH2* mutation with *EZH2* mutation-positive babies frequently described as long and skinny. Unfortunately, however, length is not always measured at birth and, even when it is, parental recall is not as accurate as for weight. However,

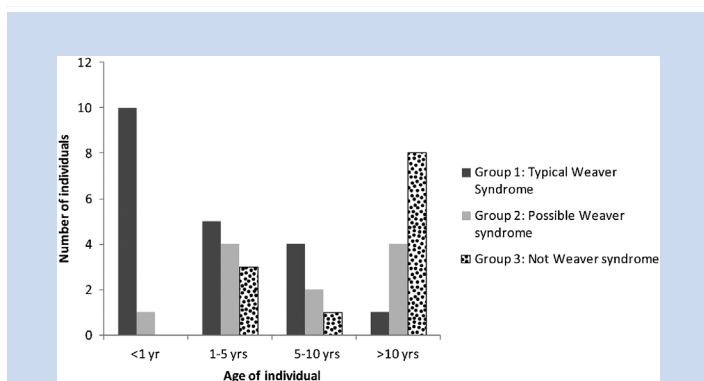


FIG. 2. Distribution of age and [facial] phenotypic groups.

of the 18 babies with *EZH2* mutations and a birth length recorded, 12 had a length greater than 2 SD above the mean with an overall range of –0.5 to +4.9 SDs (Table SI).

Information regarding subsequent growth (height and head circumference) was available for 45 *EZH2* mutation-positive individuals of whom nine were adults (>18 years) and 36 were children (with ages between 0.4 and 16.6 years and a median age of 3.5 years). Thirty-five of the children and six of the adults [in total 41/45 (91%)] had a height at least 2 SD above the mean and in 14 children and 2 adults the height was at least +4 SDs (Fig. 4a, and Table SI). Included amongst the four individuals whose height was less than +2 SDs, were three adults who were reported to be tall as young children: case 4 was overgrown until she developed a severe scoliosis at the age of 18 months for which she subsequently required spinal surgery as a teenager; the height of case 42 was 5.7 SDs above the mean until the age of 3 after which growth velocity decelerated for no apparent reason and case 3 was reported to be tall as a child but no information from childhood was unfortunately not available. In contrast to height, head circumference measurements in 40 *EZH2* mutation-positive children and adults ranged from –0.9 to +5.5 SDs with a median value of +1.8 SDs (Fig. 4b, and Table SI).

Most *EZH2* mutation-positive individuals have a mild intellectual disability. Amongst the 45 *EZH2* mutation-positive individuals where information on intellect was provided, the majority had an intellectual disability (37/45, 82%) but this was most frequently mild (21/37, 57%). Twelve individuals had a moderate intellectual disability and only two had a severe disability. In addition, two individuals were said to have an intellectual disability but the degree of impairment was not provided. Eight individuals were reported to have normal intelligence, two individuals were considered too young to be able to assess the degree of intellectual impairment and no information was available for one individual.

Additional *EZH2*-associated clinical features. Poor coordination, often manifesting as clumsiness, was reported in 80% (28/35) of *EZH2* mutation-positive individuals. Hypotonia and hypertonia were reported in 44% (18/41) and 28% (11/39) of *EZH2* mutation-positive individuals, respectively, with a mixed picture of central hypotonia and peripheral hypertonia reported in three affected individuals. Additional clinical issues frequently reported included soft, doughy skin (49%, 17/35); camptodactyly sometimes in combination with additional joint contractures (45%, 17/38); hoarse, low-pitched cry (37%, 10/27), and umbilical hernia (43%, 17/40, Table SI). It is noteworthy that 8 of the 17 individuals with umbilical hernia required surgical repair. Although not specifically solicited on either the general or *EZH2*-specific questionnaire, flexion of the proximal and hyperextension of the distal interphalangeal joints (analogous to a mild boutonniere deformity) was identified through clinical examination of five affected adults. This deformity was not associated with any detriment to function but may be a useful diagnostic clue in older *EZH2* mutation-positive individuals.

Tumors occur, but not at high frequency, in *EZH2* mutation-positive individuals. Tumors were reported in only two *EZH2* mutation-positive individuals: case 45 with a Glu745Lys missense mutation and a lymphoma diagnosed at the age of 13 years and case 31 with an Ala682Thr mutation, who developed acute lymphoblastic leukemia and neuroblastoma both at 13 months (Table SI). Despite the association between somatic inactivating mutations



FIG. 3. Facial appearance of *EZH2* mutation-positive individuals at (a) less than 1 year of age; (b) ages 1–5 years; (c) 5–10 years of age; (d) teenage/adults.

and chronic hematological malignancies, none of the latter were reported amongst the 48 *EZH2* mutation-positive individuals.

Imaging investigations in *EZH2* mutation-positive Weaver syndrome. Osseous maturation was only assessed in 25 *EZH2* mutation-positive individuals but was advanced in all. Of interest, a discrepancy between the carpal and phalangeal bone maturation, with greater maturation reported for the carpal bones, was recorded for three affected individuals. However, this may be an underestimate of the number of *EZH2* mutation-positive individuals with a carpal/phalangeal bone age discrepancy as details additional to bone and chronological age were not requested on the clinical questionnaires.

Nine *EZH2* mutation-positive individuals, in whom brain imaging had been undertaken, had abnormal studies including four with isolated ventriculomegaly; one individual with ventriculomegaly

and periventricular leukomalacia and four individuals each with either periventricular leukomalacia; pachygyria, and polymicrogyria; a small infarcted area in the cerebellum or a persistent cavum septum pellucidum (Table SI).

Other clinical features. Other clinical features reported in more than one but less than 10 *EZH2* mutation-positive individual are shown in Table I. Until greater numbers of *EZH2* mutation-positive individuals are ascertained, it is not clear which of these features are true *EZH2* associations and which are coincidental, particularly for features that are common in the general population.

DISCUSSION

Through the identification of 48 *EZH2* mutation-positive individuals we have demonstrated that missense mutations are the primary

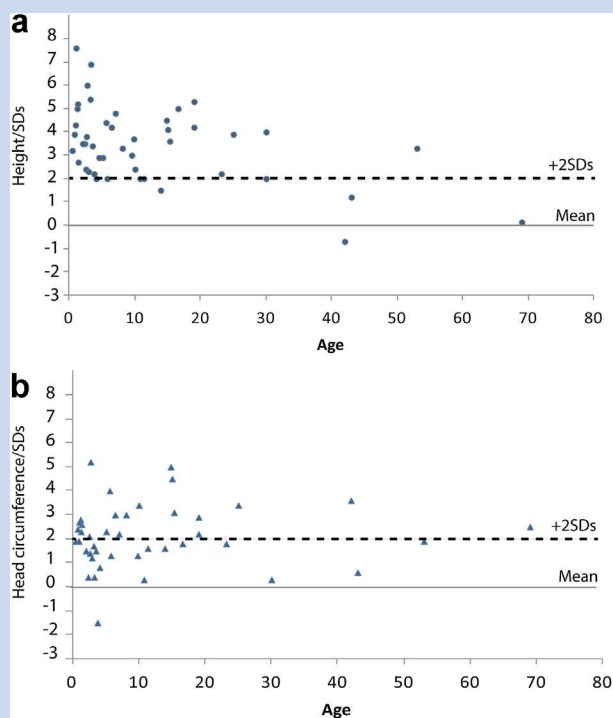


FIG. 4. Distribution of (a) height and (b) head circumference in *EZH2* mutation-positive individuals at varying ages.

EZH2 mutational mechanism in Weaver syndrome, present in over 90% of *EZH2* mutation-positive individuals. To date, only four truncating mutations have been identified, all within the terminal exon, and therefore likely escape nonsense mediated RNA decay and result in a truncated protein that retains the SET domain. The predominance of missense mutations and the terminal location of the truncating mutations suggest that simple *EZH2* haploinsufficiency is unlikely to be the cause of Weaver syndrome. This is corroborated by phenotypic data published for five cases with deletions encompassing *EZH2* where tall stature or overgrowth is not reported although the authors have recently been informed of one case with tall stature and a 1.2 Mb deletion encompassing *EZH2* (<http://decipher.sanger.ac.uk/>). Functional studies to investigate the mechanism of pathogenesis would be of interest, particularly as the *EZH2* mutational spectrum in Weaver syndrome differs from the somatic mutational profile in hematological malignancies.

The clinical features associated with *EZH2* mutations are variable and rather non-specific, and clinical diagnosis is therefore challenging. Less than half (47%) of the *EZH2* mutation-positive individuals were considered to have the classic Weaver syndrome facial gestalt. However, it is noteworthy that the distribution of ages in the group 1 compared with group 3 are very different with over-representation of individuals <1 year of age in group 1 and individuals >10 years of age in group 3. This age distribution suggests that the facial phenotype is more easily recognized in younger childhood, that there is greater familiarity with the Weaver syndrome facial gestalt in this age range, or both. The data also suggest that review of photographs at younger ages may be helpful if

TABLE I. Clinical Features Described in At Least Two But Less Than 10 *EZH2* Mutation-Positive Individuals

Skeletal abnormalities	
Clinodactyly	9
Scoliosis	6
Talipes equinovarus	5
Pectus excavatum/carinatum	4
Behavioral issues	
Temper tantrums	3
Attention deficit	2
Genito-urinary abnormalities	
Hydrocele	2
Congenital cardiac abnormalities	
VSD	2
Ophthalmological abnormalities	
Hypermetropia	3
Strabismus	2
Cutaneous	
Hemangioma	4
Café au lait macule	2
Hearing loss	2
Cleft palate	2
Tumors ^a	2

VSD, ventricular septal defect.

^aTwo *EZH2* mutation-positive individuals but with three tumors: neuroblastoma, ALL, and lymphoma.

a diagnosis of Weaver syndrome is being considered in older children or adults. The presence of additional features may also be helpful. Tall stature, with a height at least 2 SDs above the mean, is the most consistent *EZH2*-related clinical feature, present in ~90% of affected individuals. Intellectual disability is also common, present in ~80%, but can be very mild. Unfortunately both features are non-specific and generally common, which limits their utility as discriminating clinical features of Weaver syndrome.

More specific, but less frequent *EZH2*-related associations of Weaver syndrome, identified in 30–50% of affected individuals, include camptodactyly, hoarse, low cry, and soft, doughy skin. Many of these features are present in the neonatal period/early childhood and later resolve; they may be missed if a detailed neonatal/early childhood history is unavailable.

Five of the 48 individuals in our study were considered to have the facial features of Sotos syndrome, exemplifying the considerable clinical overlap between these two overgrowth conditions (Table II). The facial features associated with *NSD1* mutations are more distinctive than those of *EZH2*, and increased head circumference is more characteristic of *NSD1* mutations than *EZH2* mutations. However, robust clinical differentiation between these two conditions is not possible and thus *NSD1* mutation analysis should also be considered if *EZH2* testing is negative, and vice versa. Multi-gene testing, for example by next-generation sequencing targeted gene or exome assays, will be the optimal analysis allowing both genes to be analyzed.

The identification of constitutional *EZH2* mutations as a cause of Weaver syndrome has provided an objective means of

TABLE II. Molecular and Phenotypic Similarities and Differences Between *NSD1*-Positive Sotos Syndrome and *EZH2*-Positive Weaver Syndrome

Overgrowth syndrome	Causative gene	Growth profile in mutation-positive individuals ^a		Intellectual disability		Facial appearance	Associated clinical features
Weaver syndrome	<i>EZH2</i>	↑ ^b Ht and OFC	40%	None	20%	Frontal bossing, round face, hypertelorism. Large, fleshy ears, retrognathia and "stuck-on chin" in early childhood	Camptodactyly
		↑Ht	50%	Mild	50%		Hoarse cry
		↑OFC	5%	Moderate	25%		Umbilical hernia
		Not overgrown	5%	Severe	5%		Soft, doughy skin
Sotos syndrome ^c	<i>NSD1</i>	↑Ht and OFC	50%	None	5%	Frontal bossing, dolicocephalic, down slanting palpebral fissures and malar flushing in childhood	Congenital heart anomalies
		↑Ht	20%	Mild	30%		Scoliosis
		↑OFC	20%	Moderate	45–50%		Renal abnormalities
		Not overgrown	10%	Severe	20%		Seizures

^aThe numbers are represented as approximate percentages of affected individuals.

^bData from Tatton-Brown et al 2005b.

^cThe arrow represents an increase of at least +2 standard deviations.

Abbreviations, Ht, height; OFC, occipito-frontal circumference; SD, standard deviations.

diagnosing a frequently subtle condition, but there is still much to learn. The molecular mechanism by which *EZH2* mutations result in Weaver syndrome is unknown and the relationship between the clinical manifestations of germline and somatic *EZH2* mutations is intriguing. For example, three missense mutations Gly159Arg, Arg684Cys, and Tyr733*, have been reported somatically in myeloid malignancies and were identified constitutionally in eight unrelated individuals in the current series. None of these individuals has developed a malignancy although, in contrast to our series with constitutional mutations comprised predominantly of affected children, the majority of individuals with myeloid malignancies are typically >60 years. There are currently very little data about the phenotype of individuals with Weaver syndrome in late adulthood, but the available evidence suggests they may be at increased risk of hematological malignancies. Long-term follow-up studies should be able to address this in due course but until these data are available we would recommend clinical vigilance and investigation of any potential tumor related symptoms rather than a specific surveillance program. It would also be of interest to investigate if any of the individuals with hematological malignancies in whom somatic *EZH2* mutations have been identified, in fact have constitutional mutations and undiagnosed Weaver syndrome. Given the mild and subtle phenotype of Weaver syndrome, this is plausible and readily evaluated through analyses of buccal DNA.

Although the current study has allowed characterization of the *EZH2* mutation phenotype and is, to our knowledge, the world's largest cohort, it is a small series. With genetic analysis becoming ever more accessible, the number of individuals identified with an *EZH2* mutation is likely to increase rapidly. This will facilitate understanding of the phenotype at all ages, genotype–phenotype associations and more accurate prognostic information. In turn, this will allow optimal management for individuals with Weaver syndrome.

WEB RESOURCES

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), www.ncbi.nlm.nih.gov/omim

Exome variant server, <http://evs.gs.washington.edu/EVS/>

DECIPHER, <http://decipher.sanger.ac.uk/>

UniProt website, www.uniprot.org

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