SUPPORTING INFORMATION

for the article

Weaver syndrome-associated EZH2 protein variants show impaired histone methyltransferase function *in vitro*

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SUPP. TEXT

Proband 5: c.2050C>T (p.Arg684Cys)

This patient was the product of the second pregnancy to non-consanguineous parents. The mother was 38 and the father was 37 at the time of delivery; neither were excessively tall. First trimester aneuploidy screening at 12 weeks gestational age (GA) assessed the background risk for trisomy 21 as 1 in 124, trisomy 18 as 1 in 291 and trisomy 13 as 1 in 917. Further assessment based on nuchal translucency, nasal bone determination, ductus venosus flow, low serum PAPP-A and elevated free beta hCG assessed the posterior risk for trisomy 21 to be 1 in 12, so the screening test was considered positive (1 in 4274 for trisomy 18 and 1 in 7026 for trisomy 13). Amniocentesis was performed at 15 weeks, 6 days GA. The placenta was anterior. Fetal size was consistent with dates, with normal growth and fluid volume. Detailed anatomical ultrasound scan at 24 weeks was read as normal. Karyotype was normal 46,XY. Fetal growth parameters at each ultrasound are described in Supp. Table S3. These remained within normal limits up until 24 weeks GA, though in retrospect, an increase in the percentiles for biparietal diameter and femur length was evident.

Preterm labour began spontaneously at 34 weeks GA. Growth parameters at birth are described in Supp. Table S2. Apgar scores were six at 1 minute and nine at 5 minutes. Ballard score was consistent with a term infant (38-40 weeks' GA). At 2 hours of age he was found to be jittery, and blood glucose testing found hypoglycemia with a glucose level of 1.4 mmol/L; this was treated successfully with IV glucose. He was transferred from the birth hospital to the NICU at BC Children's Hospital at 32 hours of age because of a brief systolic heart murmur, abnormal chest X-ray showing an enlarged heart, persistent need for 25-28% oxygen and dysmorphic

features including micrognathia, a 5 x 10 mm capillary hemangioma on the left side of his chin, and a sacral dimple. He had a high-pitched cry and a brisk startle response. Cranial ultrasound found cysts of varying sizes noted anteriorly within both frontal horns, suggestive of subependymal cysts, consistent with hemorrhage. He was gradually weaned off oxygen support. Echocardiogram found normal cardiac anatomy with mild pulmonary hypertension and aortic insufficiency, which resolved subsequently. Ultrasound study of the head showed a 5 mm subependymal cyst adjacent to the left basal ganglia, and a brain MRI at 5 days of age (Figure 1G) found a hyperintense T1 signal (consistent with blood) posterior to the cerebellum and extending along the posterior tentorium and posterior falx bilaterally. A small T1-hyperintense focus was also seen within the left cerebellar hemisphere and within the periventricular white matter adjacent to the left trigone. There was also asymmetric perisylvian polymicrogyria extending from the mid frontal lobe all the way back to the anterior occipital lobe on the right side, and a similar but smaller lesion (1-2 cm) in the posterior perisylvian region on the left side. He also had a mildly enlarged right and a moderately enlarged left lateral ventricle, with some glial strands crossing the left ventricle. The subependymal cyst was again seen; along with the presence of glial strands, this finding was felt to be consistent with a prenatal bleed. Muscle tone was normal in the neonatal period. Spinal ultrasound was normal. Phototherapy was provided for 24 hours on day 4 of life for transient neonatal jaundice (unconjugated bilirubin 294 micromoles/L). Body weight decreased to 2875 g and increased consistently thereafter. He was transferred from the NICU to the acute care ward at 5 days of life with some ongoing food intake difficulties requiring nasogastric feedings. He was discharged at 5 weeks of life after feeding difficulties resolved.

An Affymetrix 6.0 oligonucleotide microarray identified no large genomic deletions or duplications.

Significant developmental delays were noted, beginning at 3-4 months of age. He developed head control in the prone position at 5 months, was tracking visually by 6 months, and began to roll at 8 months. He was using vowel sounds at one year of age, and by 27 months he had 3 words, which he would use nonspecifically. He was socially interactive and communicated largely with his eyes. At that time he could sit with support, but was not yet crawling or pulling to a stand. In addition, he had gastroesophageal reflux and constipation.

With respect to his physical features, ocular hypertelorism and large ears were noted by 9 months of age (Figure 1A). Head circumference was 44.8 cm (~25th %tile for corrected age of 8 months postnatal). He had a moderate increase in tone on the right side and a mild increase on the left; deep tendon reflexes were brisk and mildly asymmetric (brisker on the left). He had bilateral ankle clonus, more significant on the left than the right. He had one generalized tonic-clonic seizure associated with a febrile illness. EEG studies showed abnormal background rhythm, but no seizure activity. When febrile, he was given clobazam for seizure prophylaxis. He had also developed wheezing, which was treated with fluticasone/salmeterol.

An abdominal ultrasound done at 10 months was normal. Radiographs of the back showed a mild convex right scoliosis centred at the midthoracic region, with a convex left scoliosis centred at T12/L1. There were no vertebral abnormalities. Bone age assessed at 10 and a half months was advanced, consistent with a chronological age of 18 months (Figure 1F).

Subsequently, rapid growth continued. During the first year of life, he experienced recurrent pneumonias, thought to be related to aspiration. A swallowing study showed evidence of swallowing dyscoordination and reflux.

At one year of age, ears were large, measuring 6.5 cm bilaterally (>2 SD above mean) with a posterior earlobe crease on the right side only. He also had long palpebral fissures, with telecanthus (inner canthal distance at 3 cm, +2 SD), but a normal interpupillary distance (4.5 cm, 50th %tile). There was also a supernumerary nipple on the left side, bilateral transverse palm creases (Figure 1B), mild tapering of the fingers with extra creases on the third fingers bilaterally, and small, deep-set nails. He had peripheral hypertonia but truncal hypotonia. Additional features included a short nose, prominent upper lip, slightly coarsened facial features, wide eyebrows, sacral dimple and broad hands. Though cryptorchidism was not apparent at one year, it was apparent at 27 months; testes were located in the inguinal canal.

At 27 months of age, head circumference was 48.6 cm (35th %tile), length 101.1 cm (+3 SD) and weight 20.5 kg (+3-4 SD). Bitemporal narrowing was evident, with some flattening of the occiput. Ocular hypertelorism, downslanting palpebral fissures, epicanthal folds, high and sparse eyebrows and a broad, flat nasal root were all evident (Figure 1C). His ears remained large for his head size, with a posterior earlobe crease on the right side (Figure 1C). He had a high palate with thickened alveolar ridges, and more obvious microretrognathia with a pointed chin and a cleft between the lower lip and the chin (Figure 1C). There was also bilateral cryptorchidism. The scoliosis had resolved. Further, he had prominent fingertip pads with deep-set nails and dimples over the knuckles. He also had bilateral transverse palmar creases with extra distal phalangeal creases on the left third and fourth fingers, and on the right second and fourth fingers. There was mild camptodactyly of the feet with the third toes overlapping the fourth toes. The nails were thin, more so for the toenails than for the fingernails. There was ongoing spasticity, as described above.

At 31 months, he had long eyelashes and there was fine hair all over his lower back. Generous subcutaneous fat was also observed, especially around his neck and on his upper limbs (Figure 1E). He had a deep philtral groove and a "burning red" appearance to his cheeks (Figure 1E). The back of his head was now quite flat. At this point, Weaver syndrome had been confirmed by both research-grade and clinical-grade sequencing. Cost estimates for his investigations are described in Supp. Table S6.

Proband 6: c.398A>G (p.Tyr133Cys)

This patient was the first child of non-consanguineous parents. Family history was unremarkable with the exception of a congenital heart defect in the mother. The pregnancy was complicated only by the finding of a cystic adrenal mass during an ultrasound exam at 8 months. The mom reported normal fetal movements throughout. Delivery was through C-section at 39 weeks (reason not reported). Apgar scores were eight at 1 minute and nine at 5 minutes. Growth parameters at birth are described in Supp. Table S2. In addition, she had distinct facial features including ocular hypertelorism, downslanting palpebral fissures, mild micrognathia and low-set ears. Karyotype was 46, XX. A diagnosis of Weaver syndrome was proposed.

Postnatal ultrasounds revealed the presence of an anechoic mass on the right adrenal gland. After being defined as an undifferentiated neuroblastoma, the mass was surgically removed. There were no complications following the surgery.

Early developmental milestones were achieved with no significant delay, with sitting at 7 months and walking at 15 months. However, initiation of speech was slightly delayed. Bone age was advanced, consistent with 42 months at the chronological age of 20 months.

At 25 months, head circumference was 52.5 cm (> +2 SD), length 102 cm (>97th %tile) and weight 19 kg (>97th %tile). She had a rounded face with broad and tall forehead, ocular hypertelorism with inner canthal distance of 3.25 cm (>97th %tile), bilateral epicanthus, downslanting palpebral fissures, small and bulbous nose with depressed root, long and smooth philtrum, thin upper lip, microstomia, micrognathia, and large ears. Clinodactyly was present in the fifth finger bilaterally with slightly convex nails. A small umbilical hernia was also observed. Her psychomotor skills were assessed by the McCarthy scale; she had significant compromise in her verbal, numerical and memory abilities. She could initiate conversation but did not dominate spontaneous speech. Her speech was more abundant during self-play, but much of it was her own jargon. In addition, she had poor articulation, with some phonological difficulties and poor sentence construction. She had no receptive language difficulties and responded appropriately to simple and complex commands. During her testing, she participated well but had a short attention span. She also became frustrated with failure, and occasionally refusing to continue.

At 31 months of age, the parents described their daughter as having an acceptable psychomotor development stating that it was very close to that expected for her chronological age. At this time, a cognitive stimulation plan, prioritizing the planning of speech therapy, was recommended.

At 17 years of age, the patient was doing well and had completed secondary school. Her parents indicated that she had good memory skills and more difficulties with math and calculus. She had started a course to become a nurse assistant at a continuing education school in a different city, commuting back and forth every day on her own. Parents reported she was very happy and motivated. The patient had no major health problems and had not developed any additional tumours.

Proband 7: c.398A>G (p.Tyr133Cys)

This patient was born at 40 weeks and 3 days GA following an uncomplicated pregnancy. Parents were non-consanguineous; the father was 34 and the mother was 33 years of age, and they had two previous children that were both normal. The proband's growth parameters at birth are described in Supp. Table S2. Apgar score was ten. Due to the presence of a sacral dimple, hip and spinal ultrasounds were carried out but were normal. He had ocular hypertelorism and retrognathia. During the first few weeks of life, he had a tendency to turn his head always towards the same side. His face was described as contorted. There were no feeding or sleep problems.

At 7 months of age, length was at +2 SD, weight + 1.5 SD and head circumference +1.5 SD (values unavailable), which was interpreted as a slowing of the overgrowth noted at birth. He had an easy-going disposition and smiled frequently. He loved to stand but could not roll over. In the prone position, he was hypertonic and lifted his head and lower limbs together. The shape of his skull appeared scaphocephalic when viewed from the back but normal when seen from the front; craniosynostosis was proposed clinically. The previously-noted ocular hypertelorism persisted with narrow palpebral fissures, micrognathia, and a high-arched palate. He had no erupted teeth. His ears were normal. Cardiac and abdominal auscultations were also normal. Other findings included diastasis recti, phimosis of the penis, a small sacral dimple, bilateral single palmar creases and a bilateral plantar sulcus. His feet manifested a slight varus deformity, but were well positioned when standing. Karyotype was normal 46,XY.

At 8 months, head MRI was normal, with no evidence for craniosynostosis. He began walking at 17 months of age. At 30 months he presented with language delay, being able to say only a few

words. He was relatively stubborn but would interact with other children. He was eating significantly more than expected, to the point where his parents had begun to limit his food intake.

Case 15: c.553G>C; p.(Asp185His); paternally inherited

This patient was born at 38 weeks GA. Growth parameters at birth are described in Supp. Table S4. Apgar scores were eight at 1 minute and nine at 5 minutes. He had a small umbilical hernia. Karyotype was normal 46, XY. He is the second child of this couple, their first child was normal. Both parents have a large head circumference (90^{th} %tile) and elevated weight (90-97^{th} %tile) for their height, but both had normal psychomotor development and no learning difficulties. There are no known cases of overgrowth in the family.

Developmental and speech delays were first noted at 18 months; he began speech therapy. Generalized overgrowth was apparent by age 3 years. Electroencephalography (EEG) at age 3 was normal. Brain MRI at age 6 was also normal. Speech had improved with therapy but he still had great difficulties with writing. He was diagnosed with attention deficit hyperactivity disorder.

At 10 years of age, array-CGH was carried out and returned normal. A work-up of growth hormones at age 11 was also normal while his growth parameters remained at a high percentile. He had macrostomia with teeth widely spaced, and a high palate. Padding of the ears was small and irregular. His eyebrows were horizontal and bushy with synophrys. He also had ocular hypertelorism, a flat occipital plate and a horizontal depression of the chin with a flat philtrum. Hands showed flaring of the proximal phalanges and shortened fourth and fifth metacarpals. He had mild scoliosis and required orthotics to correct for flat feet and valgus. At age 11, he was

autonomous but retained cognitive difficulties. Despite the improvements with therapy, his speech was scarce and unclear, and he still struggled with reading and writing. He also had difficulties with fine movements. His sleep was regular and he never had seizures. At age 12 years and 2 months, X-rays of the hands showed advanced bone age (equivalent to 14-15 years). Next-generation sequencing (panel/exome/genome) has not been performed to date in this individual. Given the fact that his father, his sister and his paternal aunt had not presented with similar difficulties but also tested heterozygous for the c.553G>C polymorphism in *EZH2*, a causal inference of this polymorphism on the patient's phenotype seems unlikely, at least in isolation.

Case 40: c.553G>C; p.(Asp185His); paternally inherited

This patient is the only child of healthy, unrelated parents. Growth parameters at birth are described in Supp. Table S4. Apgar score was seven. Delayed development was noted early in life: she held her head up at 4 months, rolled back on her stomach at 7 months, and held toys in her hands at 6-7 months. Cranial ultrasound showed a cystic lesion in the area of the lateral ventricle, with mild ventriculomegaly.

At 9 years of age, the patient reached early puberty and was referred to Medical Genetics in search of a diagnosis for her signs and symptoms of psychomotor retardation, physical overgrowth, abnormal muscle tone, hoarse voice, spastic diplegia, ankle joint contractures and behavioural disorder. A diagnosis of Weaver syndrome was suggested. Ultrasound examination of the internal organs showed diffuse changes in the liver parenchyma and pancreas, with signs of bile duct dyskinesia. Spleen size was increased (+1 cm). Increased intestinal pneumatosis was also noted. Ultrasound examination of the kidneys showed some dysplastic changes and isolated

calyectasis of the right kidney. At 10 years and 2 months, the patient was well and had regular periods.

Next-generation sequencing (panel/exome/genome) has not been performed to date in this individual. Given the fact that her father had not presented with similar difficulties but also tested heterozygous for the c.553G>C polymorphism in *EZH2*, a causal inference of this polymorphism on the patient's phenotype seems unlikely, at least in isolation.

Case 53: c.553G>C; p.(Asp185His); maternally inherited

This patient is the third child of healthy and unrelated parents. She had one paternal aunt with a similar phenotype who died at the age of 9 from an unknown cause, one paternal uncle with mild mental retardation (apparently non-syndromic), one first cousin (the daughter of a healthy paternal uncle) and a distant cousin with mental retardation/developmental delay.

The pregnancy was without complications; she was born vaginally at 38 weeks GA. Growth parameters at birth are described in Supp. Table S4. Apgar scores were eight at 1 minute and ten at 10 minutes. She had hypertrichosis and unusual facial features.

Motor development and language acquisition were delayed. She was initially referred to a paediatric endocrinologist because of overgrowth and excessive appetite. During this evaluation, she was noted to have unusual facial features, hirsutism and intellectual disability, and she was then referred to Medical Genetics. The clinical geneticist described her as a big child (weight and height >95th %tile, head circumference 90th -95th %tile) who had hirsutism on the back and limbs and an umbilical hernia. Her voice was slightly hoarse. Craniofacial features included large bifrontal diameter, large and dysplastic ears, telecanthus, ocular hypertelorism, large and downslanting palpebral fissures, strabismus, bulbous nasal tip, and prominent philtrum and chin

crease. In addition, she had prominent finger pads, camptodactyly of the fingers, broad thumbs and deep-set nails.

Subsequent evaluations included hematological profiling, biochemical, endocrinological and extensive metabolic testing, karyotype and subtelomeric FISH testing, echocardiography, and abdominal and pelvic ultrasounds, all of which were normal. At age 4 years, a wrist radiograph revealed a bone age of 5 years and 9 months. She had mild intellectual disability (IQ 56). Next-generation sequencing (panel/exome/genome) has not been performed to date in this individual. Given the fact that her mother and her sister had not presented with similar difficulties but also tested heterozygous for the c.553G>C polymorphism in *EZH2*, a causal inference of this polymorphism on the patient's phenotype seems unlikely, at least in isolation.

Case 73: c.553G>C; p.(Asp185His); maternally inherited

Because the patient was in the frontal breach position, he was delivered by C-section at 38 weeks GA. Parents are non-consanguineous and there was no family history of overgrowth in first-degree relatives. The father had a history of joint hypermobility and joint pain, thought to be indicative of Ehlers–Danlos syndrome. The mother had significant emesis during pregnancy which was treated with diphenhydramine (Benadryl); the pregnancy was otherwise normal. Growth parameters at birth are described in Supp. Table S4. Apgar scores were nine at 1 minute and ten at 10 minutes. Microarray was normal.

On physical examination it was noted that this patient had quite unusual earlobes with fleshy prominences near the antitragus, a Cupid's bow mouth with downturned corners and a mildly smooth philtrum. His eyes were normally spaced. He had a broad and high-arched palate that was intact. Cardiovascular, abdominal and musculoskeletal examinations were all normal. Other

notable findings included mildly tapered fingers with normal nails and no fetal finger pads hyperextensible fifth fingers bilaterally, linear and streaky hyperpigmentation across his back following the lines of Blaschko, and a few blanchable capillary malformations in his lower groin area.

A head MRI done at 4 years and 9 months of age was normal except for thinning of the inner parietal bones. His head circumference had crossed several percentiles while remaining in proportion with his height. A renal ultrasound at 5 years and 10 months showed small focal linear hyperechoic areas in the right kidney.

At age 7, the patient had bilateral lateral rectus recession surgery for his intermittent exotropia. He also had mild hyperopia. At 9 years 11 months, he was very cooperative, outgoing and friendly. He had central obesity with a weight of 85.6 kg and height of 155 cm, for a BMI of 35.6 kg/m² (>99th %tile, >+3 SD). His head circumference was 56.2 cm (98th %tile, +2.78 SD). He showed some signs of food-seeking behaviour, but his mother had not had to lock food away, and he managed his own portions well in the school setting. Efforts were made to control both portion size and food choices. He had a few scattered pubic hairs; testes were smaller than expected for early Tanner stage II. He had a normal phallus with no hypospadias.

Overall, this patient had global developmental delay, mild intellectual disability, hypotonia, obesity, strabismus, recurrent otitis media and a pattern of linear skin hyperpigmentation across his back. Next-generation sequencing (panel/exome/genome) has not been performed to date in this individual. Given the fact that his mother had not presented with similar difficulties but also tested heterozygous for the c.553G>C polymorphism in *EZH2*, a causal inference of this polymorphism on the patient's phenotype seems unlikely, at least in isolation.

Case 95: c.553G>C; p.(Asp185His); maternally inherited

This patient is the eldest of two siblings of healthy unrelated parents; his younger sister is also healthy. Pregnancy was unremarkable except for recurrent migraines. Spontaneous delivery occurred at 39 weeks GA. Growth parameters at birth are described in Supp. Table S4. Mother reported ear problems in her childhood but no other relevant family history.

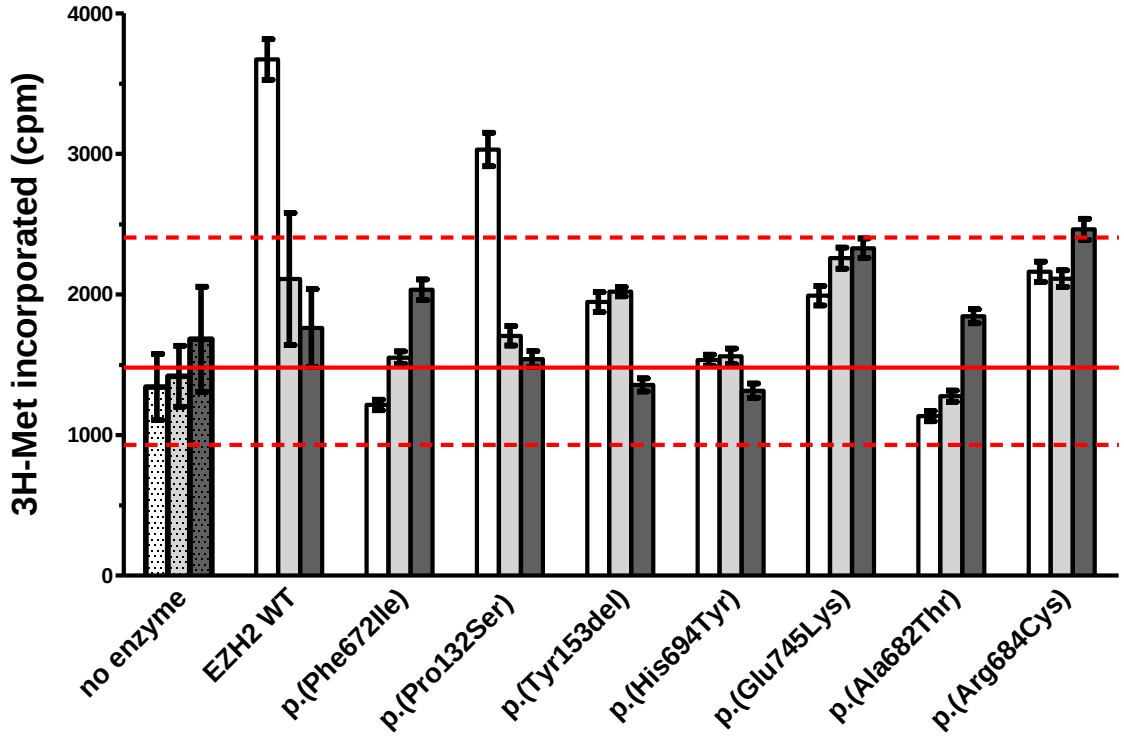
At 3 and a half months, he was admitted due to an infection of unknown cause. Mother said he seemed seriously ill and looked very pale when he was admitted, but he recovered smoothly. At 12 months he had his tonsils removed. At 18 months he had an otitis; hearing was not affected. He was growing fast and had low muscle tone. His development was delayed: he walked at 18 months and had limited speech at 2 years. Parents said it was obvious that he had learning difficulties. He had some dysmorphic features including wide forehead, flattened philtrum, small eyes with downslanting palpebral fissures and a wide nose root. Psychological evaluation ruled out autism spectrum disorder. He seemed to need a lot more sleep than normal, was easily exhausted, and had an excessive appetite. When he was 3 years old, karyotype was 46, XY and he tested normal for Sotos, Prader Willi and Beckwith-Wiedemann syndromes. X-rays at chronologic age of 2 years and 8 months were consistent with a bone age of 3 years and 6 months. He began physiotherapy and attended kindergarden with a special educator.

At 3 years of age, ophthalmological examination was normal. At 3 years and 6 months, brain MRI was also normal. At 4 years and 2 months he required a second adenotomy. At 5 years and 1 month, the patient was doing well. Overgrowth was still significant, with height, weight and head circumference all above the 97th percentile. He was very active during the day (although he napped for 45 minutes) and slept through the night. Appetite had normalized; there were no problems with bowel movements or urination. He could make short sentences, with slightly

slurred speech. He began to play more with other children and making friends at his kindergarden. The dysmorphic traits described earlier became less pronounced except for the wide nose and downslanting palpebral fissures.

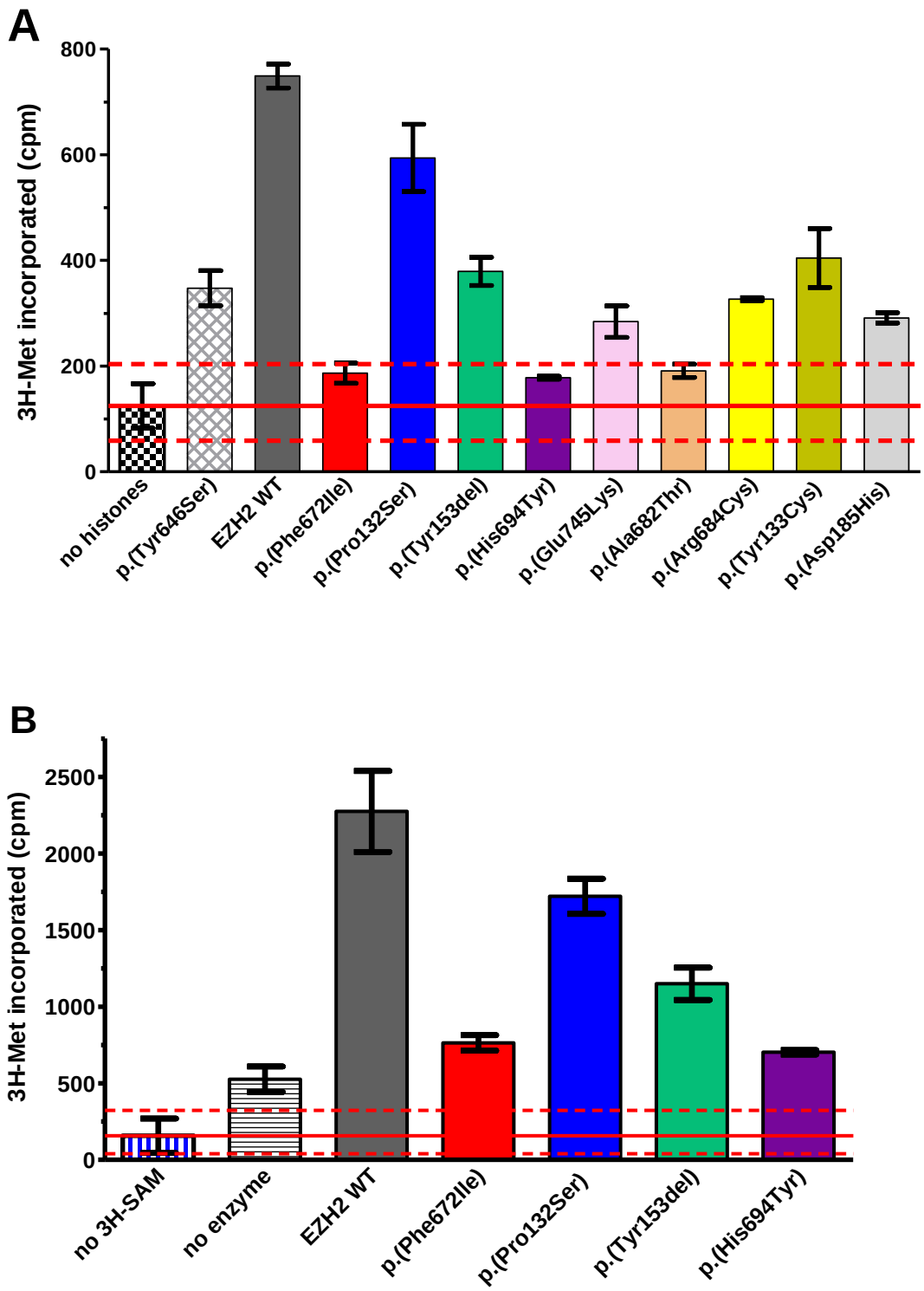
At 5 years and 7 months, he received a tentative diagnosis of Weaver syndrome and testing of *EZH2* was performed locally. Next-generation sequencing (panel/exome/genome) has not been performed to date in this individual. Given the fact that his mother had not presented with similar difficulties but also tested heterozygous for the c.553G>C polymorphism in *EZH2*, a causal inference of this polymorphism on the patient's phenotype seems unlikely, at least in isolation.

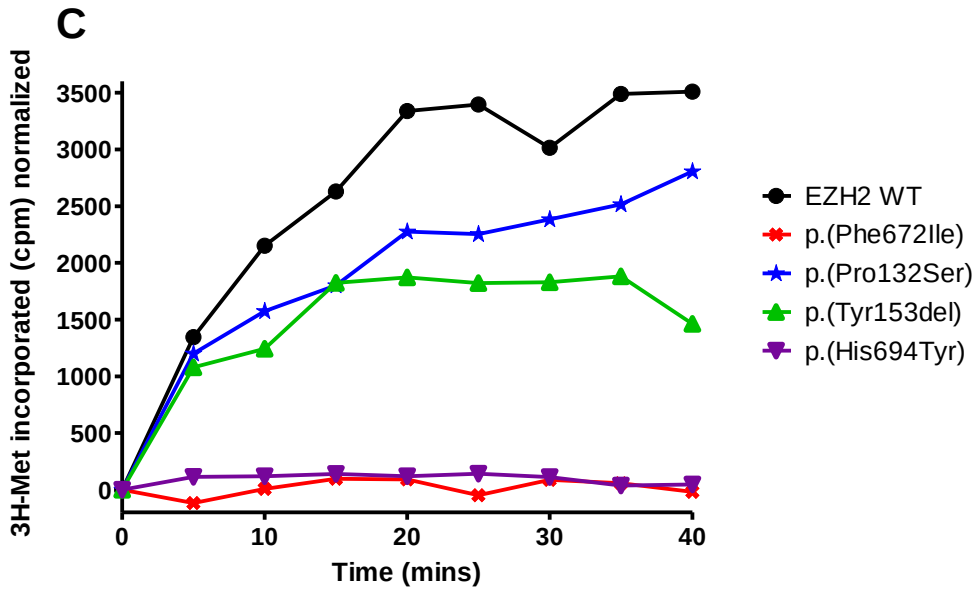
SUPP. FIGURES AND TABLES



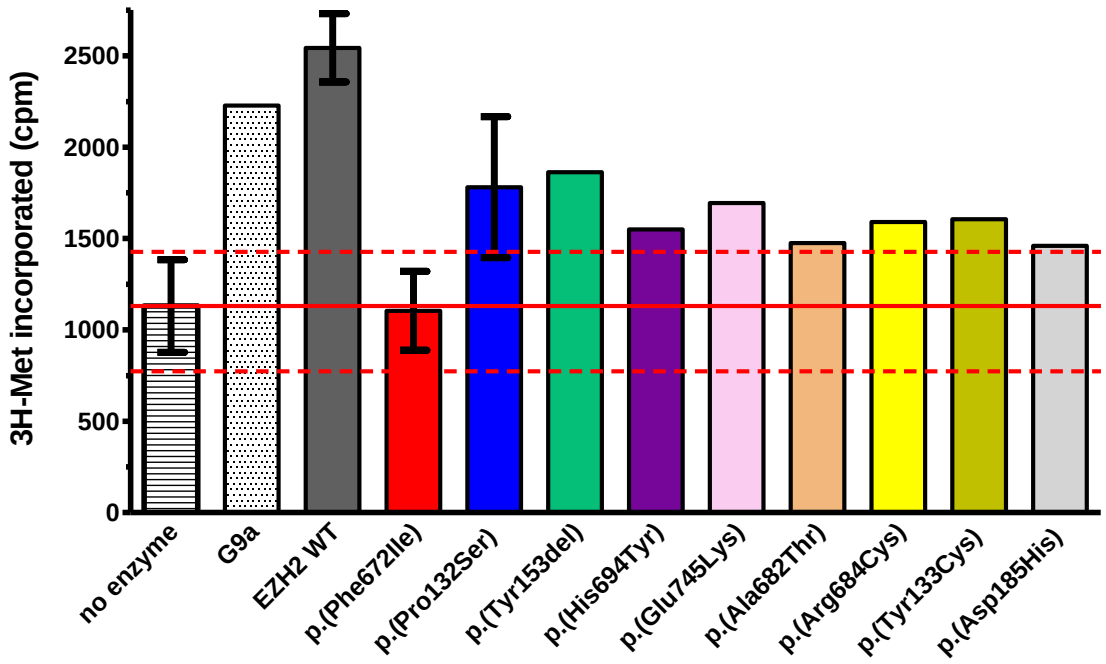
Supp. Figure S1. Histone methyltransferase activity *in vitro* assay using differentially methylated substrates confirmed impaired activity, particularly with reduced ability for monomethylation of H3K27. Histone methyltransferase reactions were performed using 1 µM biotinylated peptide substrate (H3(21-44) mimicking the H3 tail) which had been either unmethylated (H3K27me0, open bars), monomethylated (H3K27me1,light gray bars) or dimethylated (H3K27me2, dark gray bars) at lysine residue 27. The reactions were incubated with 0.67 µM ^{3}H-S-Adenosyl-methionine (^{3}H-SAM) and 250 ng of either wild-type (WT) or a mutant complex (or no enzyme controls). Histone methyltransferase activity was measured based on the incorporation of ^{3}H-labeled methyl groups, represented in scintillation counts per minute (total counts). Error bars represent SD of 2 independent replicates for each mutant complex, and 4 replicates for WT. The red lines represent mean background (solid line), and minimal or maximum background (dotted lines) observed in this experiment. Further statistical data on the background is presented in Supp. Table S5D.

Supp. Figure S2





Supp. Figure S2. Weaver syndrome mutants show impaired histone methyltransferase activity *in vitro*, under variable experimental conditions. Histone methyltransferase reactions were performed with **250 ng** of either wild-type (WT) or a mutant complex. Histone methyltransferase activity was measured based on the incorporation of ^{3}H-labeled methyl groups, represented in scintillation counts per minute. **(A)** Each reaction was incubated with **3 µg** purified core histones and **0.22 µM** ^{3}H-S-Adenosyl-methionine (^{3}H-SAM), for 30 minutes at 30^{0}C. The mutant p.(Tyr646Ser) mutant, commonly observed in cancer [Yap *et al.*, 2011], was added as an additional control. Values represent total counts. Error bars represent SD of 2 independent replicates for each complex. The red lines represent mean background (solid line), and minimal or maximum background (dotted lines) observed in this experiment. Incubation with no histones was also carried out to assess background. Further statistical data on the background is presented in Supp. Table S5B. **(B)** Each reaction was incubated with **2 µg** purified core histones and **1 µM** ^{3}H-SAM, for 30 minutes at 30^{0}C. Values represent total counts. Error bars represent SD of 2 independent replicates for each mutant complex, and 4 replicates for WT. The red lines represent mean background (solid line), and minimal or maximum background (dotted lines) observed in this experiment. Incubation with no ^{3}H-SAM was also carried out to assess background. Further statistical data on the background is presented in Supp. Table S5C. **(C)** Each reaction was incubated with **2 µg** purified core histones and **0.67 µM** ^{3}H-SAM at 30^{0}C. Reactions were stopped at various time points (0 to 40 minutes) by taking an aliquot of the total reaction volume and spotting it onto P81 square paper. Values represented here were normalized by subtracting background counts (i.e. time zero) from the total counts. There was only one replicate for each enzymatic complex at each specific time point.



Supp. Figure S3. Weaver syndrome mutants show impaired histone methyltransferase activity *in vitro*. Histone methyltransferase reactions were performed using 2µg purified core histones and 0.67 µM ^{3}H-S-Adenosyl-methionine (^{3}H-SAM). Each reaction was incubated with 250 ng of either wild-type (WT) or a mutant complex (or no enzyme controls). Active G9a (Millipore) was used as a further positive control. Histone methyltransferase activity was measured based on the incorporation of ^{3}H-labeled methyl groups, represented in scintillation counts per minute (total counts). Error bars represent SD of 4 independent replicates for WT and p.(Phe672Ile), 3 independent replicates for the mutant p.(Pro132Ser), and all 19 no enzyme controls measured. The red lines represent mean background (solid line), and minimal or maximum background (dotted lines) observed in this experiment. Further statistical data on the background is presented in Supp. Table S5A.

Supp. Table S1. Functional prediction scores from PROVEAN v1.1.3 for the two previously undescribed variants in *EZH2*

VARIATION	PROTEIN SEQUENCE CHANGE								PROVEAN PREDICTION				SIFT PREDICTION				ANNOTA-TION
INPUT	PROTEIN ID	LENG-TH	STR-AND	CODON CHANGE	POSI-TION	RESI-DUE REF	RESI-DUE ALT	TYPE	SCORE	PREDIC-TION (cutoff = -2.5)	#SEQ	#CLUS-TER	SCORE	PREDIC-TION (cutoff = 0.05)	MEDIAN INFO	#SEQ	dbSNP ID
7,148526906,T,C	ENSP 00000320147	751	-1	CCT T[A/G]T ATG	133	Y (Tyr)	C (Cys)	Single AA Change	-8.53	Delete-rious	203	30	0.000	Damaging	2.91	170	-
7,148525904,C,G	ENSP 00000320147	751	-1	GAT [G/C]AC GAT	185	D (Asp)	H (His)	Single AA Change	-2.40	Neutral	203	30	0.005	Damaging	3.17	163	rs2302427

Note: For each variant, p.(Tyr133Cys) and p.(Asp185His), only the longest protein isoform is shown (corresponding to NM_004456.4). Coordinates correspond to the GRCh37 (Ensembl 66) human genome assembly. Predictions were carried out by inserting the genomic coordinates and the nucleotide modification equivalent to the sense strand, at: http://provean.jcvi.org/genome_submit_2.php?species=human.

Supp. Table S2. Phenotypic manifestations of Weaver syndrome in patients with *EZH2* mutations

Characteristics	**Proband 4**	**Proband 5**	**Proband 6**	**Proband 7**
EZH2 variant	c.394C>T p.(Pro132Ser)	c.2050C>T p.(Arg684Cys)	c.398A>G p.(Tyr133Cys)	c.398A>G p.(Tyr133Cys)
Inheritance	*de novo*	*de novo*	*de novo*	*de novo*
Growth features				
Gestational age at delivery (weeks)	38	34 (preterm)	39	40 + 3days
Birth weight (kg)	3.59 (75^{th} %tile)	3.08 (>95^{th} %tile)	3.53 (82^{nd} %tile)	4.35 (92^{nd} %tile)
Birth length (cm)	54.5 (95^{th} %tile)	49.5 (94^{th} %tile)	52.4 (96^{th} %tile)	57.5 (>99^{th} %tile)
Birth head circumference (cm)	37 (95^{th} %tile)	33 (81^{st} %tile)	36.2 (97^{th} %tile)	37 (92^{nd} %tile)
Recent weight (kg) [age measured]	16 [13m]	22 [2y7m]	69 [17y]	22 [30m]
Recent height (cm) [age measured]	91.4 [13m]	102.7 [2y7m]	185 [17y]	105 [30m]
Excessive growth of prenatal onset	++	++	++	+++
Accelerated osseous maturation	++	+ (18m at 10y6m)	++ (3y6m at 1y8m)	NK
Neurological features				
Hypertonia	In knees	+ (peripheral)	-	+
Hypotonia	NK	+ (abdominal, left side more prominent)	+	-
Hoarse low-pitched cry	++	-	++	-
Intellectual disability	+	+	+	+
Excessive appetite	NK	+	-	++
Ventriculomegaly	NK	-	-	-
Delayed myelination	NK	-	-	NK
Cerebellar hypoplasia	NK	-	-	-
Seizures [age of onset]	NK	1 GTC febrile-associated [~ 9m]	-	-
Polymicrogyria	-	+++ asymmetric perisylvian	-	-
Pachygyria	-	-	-	-
Poor fine motor coordination	+	+	+	-
Poor balance/ gravitational insecurity	++	+	++	-
Craniofacial				
Macrocephaly	++	-	+++ (>+2SD at 2y1m)	- (+1.5SD at 7m)
Large bifrontal diameter	++	-	++	+

Characteristics	Proband 4	Proband 5	Proband 6	Proband 7
Flat occiput	++	+	+	+
Large ears	++ (with hearing loss)	+++	++	+
Ocular hypertelorism	++	++	++	++
Down slanted palpebral fissures	+	+	+	+
Long philtrum	++	-	+	-
Micrognathia	+	++	+	+
Cardiovascular				
Patent ductus arteriosus	++	-	-	-
Ventricular septal defect	++	-	-	-
Limbs				
Limited elbow and knee extension in early life	+	+	-	-
Limited elbow and knee extension after puberty	NK	NA	-	NA
Widened distal femurs and ulnas	++	-	NK	NK
Hands				
Prominent digit pads	+	-	+	+
Single transverse palmar crease	++	++	-	+
Camptodactyly	NK	-	+	-
Broad thumbs	NK	-	-	-
Thin, deep-set nails	-	++	+	-
Feet				
Clinodactyly, toes	+	-	-	-
Talipes equinovarus	+	-	-	-
Short fourth metatarsals	NK	-	-	-
Hind foot valgus	NK	-	-	-
Skin				
Excessive loose skin	++	+	+	-
Hypoplastic/ supernumerary nipples	+	+	-	-
Thin hair	-	+	-	-
Increased pigmented nevi	+	-	-	-
Connective tissue				
Umbilical hernia	+	-	+	+
Inguinal hernia	NK	-	-	-
Diastasis recti	+	-	-	+
Scoliosis (degrees)	NK	Mild, later resolved	-	-
Kyphosis	+	-	+	-
Endocrine				
Hypothyroidism [age of onset]	NK	-	-	-
Growth hormone deficiency [age of onset]	NK	-	-	-
Hypoglycemia	Perinatal	Mild, resolved	-	-

Characteristics	**Proband 4**	**Proband 5**	**Proband 6**	**Proband 7**
[age of onset]		[birth]		
Neoplasia				
Neuroblastoma [age of onset]	Birth, regressed	-	Prenatal, removed	-
Leukemia [age of onset]	-	-	-	-
Lymphoma [age of onset]	-	-	-	-

Key: + = minimally present; ++ = obviously present; +++ = very prominent; - = assessed and found to be absent; NK = not known; NA = non applicable; y = years; m = months (RefSeq NM_004456.4).

Supp. Table S3. Fetal growth parameters for proband 5

		Gestational age (weeks)*						
Measurement		$8\ ^{3}/_{7}$	$12\ ^{0}/_{7}$	$15\ ^{6}/_{7}$	$18\ ^{4}/_{7}$	$21\ ^{1}/_{7}$	$24\ ^{0}/_{7}$	$34\ ^{0}/_{7}$
Crown-rump length	mm	15	55	-	-	-	-	-
	%tile	-	50th	-	-	-	-	-
Crown-heel length	cm	-	-	-	-	-	-	49.5
	%tile	-	-	-	-	-	-	98th
Biparietal diameter	mm	-	-	32	43	53	62	-
	%tile	-	-	24th	50-90th	50-90th	71st	-
Head Circumference	cm	-	-	-	15.8	19.4	22.4	33
	%tile	-	-	-	50-90th	50-90th	49th	91st
Abdominal Circumference	mm	-	-	-	126	163	204	-
	%tile	-	-	-	10-50th	10-50th	63rd	-
Femur length	mm	-	-	19	27	34	45	-
	%tile	-	-	38th	50-90th	10-50th	71st	-
Estimated Fetal Weight	grams	-	-	-	227	-	752	-
	%tile	-	-	-	-	-	79th	-
Birth Weight	grams	-	-	-	-	-	-	3078
	%tile	-	-	-	-	-	-	95th

* Because gestational age was confirmed by ultrasound at 12 weeks and agreed with menstrual dates at that time, gestational age is reported according to menstrual dates. Percentiles are given for that specific gestational age i.e. birth weight was at the 95th %tile for 34 weeks gestational age, not for a term infant. Centiles are reported as written on the prenatal ultrasound reports.

- = not reported.

Supp. Table S4. Phenotypic description of carriers for the p.(Asp185His) polymorphism identified within our cohort of individuals referred for Weaver-like features

Characteristics	Case 15	Case 40	Case 53	Case 73	Case 95
EZH2 variant	c.553G>C; p.(Asp185His)				
Inheritance	paternal (unaffected)	paternal (unaffected)	maternal (unaffected)	maternal (unaffected)	maternal (unaffected)
Other carriers in the family	sister (unaffected); paternal aunt (unaffected)	NA	sister (unaffected)	NA	NA
Growth features					
Gestational age at delivery (weeks)	38	38	38	38	39
Birth weight (kg)	3.45 (75-90th %tile)	3.55 (75-90th %tile)	4.45 (>95th %tile)	3.05 (10-50th %tile)	3.8 (50-75th %tile)
Birth length (cm)	50 (50-75th %tile)	52 (75-90th %tile)	49 (25-50th %tile)	52 (75-90th %tile)	50 (25-50th %tile)
Birth head circumference (cm)	34 (50-75th %tile)	NK	35 (50-75th %tile)	35 (50-75th %tile)	36 (75-90th %tile)
Recent weight (kg) [age measured]	78 [15y5m]	55 [10y2m]	45 [9y6m]	85.6 [9y11m]	31.7 [5y6m]
Recent height (cm) [age measured]	180 [15y5m]	154 [10y2m]	150 [9y6m]	155 [9y11m]	122.8 [5y6m]
Excessive growth of prenatal onset	-	++	+	-	+
Accelerated osseous maturation	++ (14-15y at 12y2m)	NK	++ (5y9m at 4y)	NK	+ (3y6m at 2y8m)
Neurological features					
Hypertonia	-	+/-	-	-	-
Hypotonia	-	+/-	+	+	+
Hoarse low-pitched cry	-	++	+	-	NK
Intellectual disability	+	+	++	+	+
Excessive appetite	-	NK	++	++	+
Ventriculomegaly	-	+	NK	-	-
Delayed myelination	-	-	NK	-	-
Cerebellar hypoplasia	-	-	NK	-	-
Seizures [age of onset]	-	-	-	-	-
Polymicrogyria	-	-	NK	-	-
Pachygyria	-	-	NK	-	-
Poor fine motor coordination	+++	++	+	-	+
Poor balance/ gravitational insecurity	++	-	-	-	+
Craniofacial					
Macrocephaly	+	-	-	+	+
Large bifrontal diameter	+	-	+	-	-
Flat occiput	++	-	-	-	-

Characteristics	Case 15	Case 40	Case 53	Case 73	Case 95
Large ears	-	-	+	-	-
Ocular hypertelorism	++	-	++	-	+
Down slanted palpebral fissures	-	-	+	-	+
Long philtrum	-	-	+	-	-
Micrognathia	-	+	-	-	+
Cardiovascular					
Patent ductus arteriosus	-	NK	-	NK	NK
Ventricular septal defect	-	NK	-	NK	NK
Limbs					
Limited elbow and knee extension in early life	+	NK	-	-	NK
Limited elbow and knee extension after puberty	+	NK	NA	NA	NA
Widened distal femurs and ulnas	NK	-	-	NK	NK
Hands					
Prominent digit pads	-	-	+	-	-
Single transverse palmar crease	-	NK	-	-	-
Camptodactyly	-	-	+	-	-
Broad thumbs	+	-	+	-	-
Thin, deep-set nails	-	-	+	-	-
Feet					
Clinodactyly, toes	-	+	+	-	-
Talipes equinovarus	-	+	-	-	-
Short fourth metatarsals	+	-	-	-	-
Hind foot valgus	+	+	-	-	-
Skin					
Excessive loose skin	-	-	-	-	-
Hypoplastic/ supernumerary nipples	-	NK	-	-	NK
Thin hair	-	-	-	-	NK
Increased pigmented nevi	-	NK	-	linear skin hyper-pigmentation across back	-
Connective tissue					
Umbilical hernia	+	NK	++	-	NK
Inguinal hernia	-	NK	-	-	NK
Diastasis recti	-	NK	-	-	NK
Scoliosis (degrees)	mild	NK	-	-	NK
Kyphosis	-	-	-	-	-
Endocrine					

Characteristics	**Case 15**	**Case 40**	**Case 53**	**Case 73**	**Case 95**
Hypothyroidism [age of onset]	-	-	-	-	NK
Growth hormone deficiency [age of onset]	-	-	-	-	NK
Hypoglycemia [age of onset]	-	-	-	-	NK
Neoplasia					
Neuroblastoma [age of onset]	-	-	NK	-	-
Leukemia [age of onset]	-	-	-	-	-
Lymphoma [age of onset]	-	-	-	-	-

Key: + = minimally present; ++ = obviously present; +++ = very prominent; - = assessed and found to be absent; +/- = tone reported as abnormal, but hypertonia or hypotonia not specified; NK = not known; NA = non applicable; y = years; m = months (RefSeq NM_004456.4).

Supp. Table S5. Column Statistics on the background reads measured with our *in vitro* histone peptide methyltransferase assay

A

(Column Statistics for Supp. Figure S3)

Number of values	19
Sum	21478
Minimum	773.0
25% Percentile	882.0
Median	1249
75% Percentile	1377
Maximum	1427
10% Percentile	786.0
90% Percentile	1418
Mean	1130
Std. Deviation	253.7
Std. Error	58.21
Lower 95% CI of mean	1008
Upper 95% CI of mean	1253
Coefficient of variation	22.45%

B

(Column Statistics for Supp. Figure S2A)

Number of values	22
Sum	2744
Minimum	59.00
25% Percentile	93.19
Median	111.5
75% Percentile	169.3
Maximum	204.0
10% Percentile	81.50
90% Percentile	196.7
Mean	124.7
Std. Deviation	42.06
Std. Error	8.968
Lower 95% CI of mean	106.1
Upper 95% CI of mean	143.4
Coefficient of variation	33.73%

C

(Column Statistics for Supp. Figure S2B)

Number of values	22
Sum	3457
Minimum	40.00
25% Percentile	65.61
Median	99.00
75% Percentile	293.0
Maximum	322.0
10% Percentile	54.20
90% Percentile	316.1
Mean	157.2
Std. Deviation	112.5
Std. Error	23.99
Lower 95% CI of mean	107.3
Upper 95% CI of mean	207.0
Coefficient of variation	71.60%

D

(Column Statistics for Supp. Figure S1)

	me0 - me1	me1-me2	me2-me3
Number of values	24	30	54
Sum	35960	43960	79920
Minimum	1255	930.0	930.0
25% Percentile	1393	1136	1299
Median	1510	1395	1461
75% Percentile	1600	1655	1615
Maximum	1753	2406	2406
10% Percentile	1279	1061	1104
90% Percentile	1669	2274	1776
Mean	1498	1465	1480
Std. Deviation	138.8	399.3	309.6
Std. Error	28.32	72.89	42.13
Lower 95% CI of mean	1440	1316	1395
Upper 95% CI of mean	1557	1614	1565
Coefficient of variation	9.26%	27.25%	20.92%

Supp. Table S6. Health Economics Estimates - diagnostic workup for proband 5

Disorder	**Genetic/Genomic Test**	**Location Performing**	**Cost ($ USD)**
Aneuploidies	Prenatal FISH for Chromosomes 13, 18, 21, X and Y, with Karyotype	BCCH Cytogenetics Lab	540
Deletion/Duplication Copy Number Variants	Affymetrix 6.0 Microarray	BCCH Cytogenetics Lab	790
Inversions, Translocations	Postnatal Karyotype	BCCH Cytogenetics Lab	425
Fragile X	*FMR1* PCR and Southern Blot	BCCH Molecular Genetics Lab	300
Simpson-Golabi-Behmel Syndrome	MLPA gene dosage testing for Glypican-3 and Glypican-4 and GPC3 sequencing	Hospital for Sick Children, Toronto, ON	2,020
Oral-Facial-Digital Syndrome	*OFD1* sequencing and MLPA deletion-duplication analysis	Prevention Genetics, Marshfield, WI	2,310
X-linked disorders	Maternal X-chromosome inactivation studies	Hospital for Sick Children, Toronto, ON	400
Sotos Syndrome	*NSD1* sequencing and MLPA deletion-duplication analysis	Prevention Genetics, Marshfield, WI	2,580
Amino acidopathies	Plasma Amino Acids	BCCH Biochemical Diseases Lab	80
Peroxisomal Disorders including Zellweger syndrome	Very Long-Chain Fatty Acids	Kennedy Krieger Institute, Baltimore, MD	90
Carbohydrate-Deficient Glycoprotein Syndrome	Transferrin Isoelectric Focusing	BCCH Biochemical Diseases Lab	90
Weaver Syndrome	*EZH2* sequencing	BCCH Molecular Genetics Lab	700
TOTAL			**10,325**