

**Supplemental Data**

**De Novo Mutations in *CHD4*, an ATP-Dependent  
Chromatin Remodeler Gene, Cause an Intellectual  
Disability Syndrome with Distinctive Dysmorphisms**

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## Clinical descriptions of five subjects with *de novo* *CHD4* mutations

**Subject 1** was born after a normal pregnancy at 41 weeks gestation. The birth weight was 4000 gr (85<sup>th</sup> centile) and the head circumference was 38 cm (90<sup>th</sup> centile). His newborn exam was notable for left hemiparesis. He underwent brain imaging that demonstrated a stroke in the right middle cerebral artery region. On angiography there was evidence of moyamoya phenomenon. In addition, he had undescended testis and a micropenis. The testosterone and the LH and FSH levels were low on repeated tests (0.9 nmol/L, 0.2 IU/L and 0.8 IU/L respectively). There was global developmental delay, he walked at 20 months and his first words were at 30 months. He attended a special education class. He had left hearing loss and wore a hearing aid. He had a normal skeletal survey but the bone age was advanced by 2-3 years at the age of 10 years. The brain MRI showed non-specific changes in periventricular white matter and mild enlargement of the lateral ventricles. At the age of 10 years his height was 143 cm (75<sup>th</sup> centile). He was macrocephalic with a head circumference of 56 cm (>97<sup>th</sup> centile). Additional findings included; wide spaced eyes, a squared face, cupped ears, dental crowding, a bifid uvula and tapered fingers. Chromosomal microarray and fragile X testing were normal. Whole exome sequencing was done as a trio. In addition to the *CHD4* variant there was a *de novo* predicted deleterious missense change in *APOBEC1* (NM\_001644: c.268T>G).

**Subject 2** was born after a normal pregnancy at 40 weeks gestation. The birth weight was 2800 gr (10<sup>th</sup> centile). She had recurrent vomiting as a newborn that resolved. There was hypotonia and global developmental delay. She walked at 3.5 years and said her first words after the age of 3 years. She attended special education classes. She had hearing loss for which she used hearing aids. The brain MRI showed dilated lateral ventricles and a Chiari 1 malformation. On skeletal survey she had bilateral tarsal coalitions and fused vertebrae C2-C6. She had her first menstruation at 16 years and it has been short and irregular. There was evidence of polycystic ovaries on a pelvis ultrasound and the LSH and LH were 5.6 and 4.4 U/l. At the age of 16 years

her height was 161 cm (40<sup>th</sup> centile). She was macrocephalic with a head circumference of 62 cm (>97<sup>th</sup> centile). Additional findings included; hypertelorism, small ears with overlapping helices, high palate, significant hyperlaxity of the fingers and mild scoliosis. Chromosomal microarray, fragile X testing, and molecular analysis of the *PTEN* gene were normal. Whole exome sequencing was done as a trio. Except for *CHD4* there were no additional candidate genes.

**Subject 3** was born after a normal pregnancy at 40 weeks gestation. Birth weight was 3690 gr (70<sup>th</sup> centile) and head circumference was 37 cm (75<sup>th</sup> centile). He had bilateral undescended testicles and a micropenis, and was started on testosterone treatment. There was global developmental delay. He walked at 2 years. His first words were at 10 months but he did not progress as expected. He received continued help with reading and writing at school. He had hearing loss that required hearing aids. He had an abnormal gait and underwent a guided growth osteotomy for genu varum. He received growth hormone (GH) treatment for history of short stature (length below 2<sup>nd</sup> percentile). The skeletal survey demonstrated falx calcification on the skull X ray. The brain MRI at 10 months showed enlarged ventricles and increased white matter volume. At the age of 10 years his height was 140cm (50<sup>th</sup> percentile). He was macrocephalic with a head circumference of 56cm (>97<sup>th</sup> centile). Additional findings included; hypertelorism, low set and dysmorphic helices, and a high palate with a hyper-nasal voice. Chromosomal microarray, fragile X, and molecular testing for Gorlin syndrome were normal. Whole exome sequencing was done as a trio. Except for *CHD4* there were no additional candidate genes.

**Subject 4** The prenatal history was remarkable for hydronephrosis noted at 36 weeks gestation. She was born at 39 weeks by cesarean section due to breech presentation. Her gestational age- adjusted birth weight was weight 2990g (30% centile), length 45cm (3% centile), and head circumference 35cm (73% centile). In the perinatal period an ECHO showed two ventricular

septal defects (VSD), a patent ductus arteriosus (PDA), and an atrial septal defect (ASD). A kidney US showed bilateral renal pelviectasis and the initial brain MRI revealed choroid plexus cysts. Facial dysmorphisms were also noted and described as telecanthus posterior nuchal redundancy, and variant palmar creases. She remained in the NICU for 20 days due to feeding difficulties requiring NG tube feedings. Her PDA was closed surgically at 4 months of age. At this time she was diagnosed with chronic renal insufficiency. US showed hydroureter, and a VCUg detected bilateral severe (grade 4) reflux. Formal developmental assessment showed global developmental delay, predominantly of language and visual motor abilities, short stature, and hypotonia (truncal and distal). She first rolled over at 8 months and sat at 2 years 10 months. At five years she is currently working on taking steps with assistance. Her first word was said at 1 year, and she currently uses less than 10 words. Her course clinical was also marked by frequent hospitalizations due to respiratory failure in the setting of upper respiratory infections. A sleep study demonstrated hypoventilation and severe OSA (AHI 30). Her brain MRI at age three revealed fusion of the cervical spine vertebrae, abnormal appearance of the base of the skull with a tight foramen magnum, and mild ventriculomegaly. A skeletal survey showed cervical spine and basal skull abnormalities consistent with Klippel-Feil anomaly, bilateral coxa valga, fusion of the cuboid and the 3rd cuneiforms in the bilateral feet, right brachymesophalangy II and IV and II and V on left. At the age of 5 years her length was 89.5 cm (< 3rd % centile Z score -5) and the head circumference was 49 cm (20th % centile). Her facial features included hypertelorism, low set ears, submucous cleft palate, proptosis, midface hypoplasia, epicanthal folds and a flat philtrum. Prior to whole exome sequencing genetic testing included normal karyotype, chromosome array, very long chain fatty acids, and RASopathy panel. Whole exome sequencing was done as a singleton. In addition to the *CHD4* variant there was a pathogenic variant in the Joubert syndrome gene *C5orf42* (NM\_023073:c.8710C>T), a VUS in *KANSL1* (NM\_001193466:c.665T>C) and a VUS in *SETBP1* (NM\_015550:c.2868C>G), all of which were inherited from an unaffected parent upon Sanger sequencing.

**Subject 5** was born full term with a birth weight of 3.06 kg (~25<sup>th</sup> centile). At the age of 8 months he underwent surgical repair for an ASD and PDA. He also had a VSD that closed spontaneously and a bicuspid aortic valve currently with mild aortic insufficiency. He had developmental delay with delayed speech and learning difficulties in school. He completed high school with an individualized education plan, and went on to work in a part time job in a special environment for individuals with intellectual disabilities. He had a history of glaucoma and sensorineural hearing loss. A submucous cleft palate was diagnosed and repaired at the age of 4 years. He had a micropenis and delayed sexual development with decreased gonadotropins. In addition, there was a history of short stature but no evidence of GH deficiency. He was also diagnosed with moderate obstructive sleep apnea. The skeletal survey demonstrated osteopenia. The brain MRI showed enlarged lateral and third ventricles. At the age of 17 years his height was 167.5 cm (10<sup>th</sup> centile). His last head circumference was from the age of 4 years and measured 52.5 cm (~90<sup>th</sup> centile). Additional findings included; hypertelorism, a squared face, widow's peak, short palpebral fissures with microcornea small ears with dysmorphic helices and significant joint hyperlaxity. Previous testing included a chromosomal microarray that demonstrated a subtelomeric duplication of 8p of unknown significance. He had normal testing for 22q11 deletion syndrome, Stickler and Weill Marchesani syndrome. Whole exome sequencing was done as a singleton. Except for *CHD4* there were no additional candidate genes.

**Supplemental table: Clinical findings in 5 subjects with *de novo* missense variants in *CHD4***

	<b>Subject 1</b>	<b>Subject 2</b>	<b>Subject 3</b>	<b>Subject 4</b>	<b>Subject 5</b>
<i>CHD4</i> variant	c.3380G>A, p.Arg1127Gln	c.3518G>T, p.Arg1173Leu	c.3380G>A, p.Arg1127Gln	c.3443G>T, p.Trp1148Leu	c.3008G>A, p.Gly1003Asp
Gender / age at last exam	male / 10 years	female / 16 years	male / 10 years	Female / 5 years	Male / 18 years
Birth weight / OFC	4 kg / 38 cm	2.8 kg / -	3.7 kg / 37cm	2.99 kg / 35 cm	3.06 kg / -
Height / OFC at last exam	143 cm (75 <sup>th</sup> %ile)/ 56 cm (>98 <sup>th</sup> %ile)	161 cm (40 <sup>th</sup> %ile)/ 62 cm (>98 <sup>th</sup> %ile)	140cm <sup>a</sup> (50 <sup>th</sup> %ile)/ 56cm (>98 <sup>th</sup> %ile)	89.5 cm (< 3 <sup>rd</sup> %ile Zscore -5)/ 49 cm (20 <sup>th</sup> %ile)	167.5 cm (10 <sup>th</sup> %ile)/ 52.5cm at 4 years (90 <sup>th</sup> %ile)
Developmental delay	+	+	+	+ (severe)	+
Hypotonia	-	+	+	+	+
Intellectual disability	+	+	+ (mild)	+	+ (mild)
Hearing loss <sup>b</sup>	+	+	+	-	+
Undescended testis	+ /+	NA	+ / +	NA	- / +

/ micropenis					
Macrocephaly <sup>c</sup>	+	+	+	Relative to length	+ <sup>d</sup>
Widely spaced eyes <sup>e</sup>	+	+	+	+	+
Dysmorphic ears <sup>f</sup>	+	+	+	+	+
Palatal anomalies	+ <sup>g</sup>	-	+ <sup>h</sup>	+ <sup>h</sup>	+ <sup>h</sup>
Other dysmorphic features				Proptosis, midface hypoplasia, epicanthal folds, flat philtrum	Short palpebral fissures and microcornea
Hypogonadotropic hypogonadism	+	-	+	NT	+
Growth hormone deficiency	-	-	+	NT	-
Skeletal survey	Advanced bone age by 2-3 years	Tarsal coalition Cervical vertebrae fusion	Falx calcification	Scoliosis, platybasia, fusion of C2-C3, bilateral	Diffusely osteopenic bones

				coxa valga, fusion of the cuboid and the 3rd cuneiforms bilaterally, brachymesophalangia	
Brain MRI	Enlarged lateral ventricles Congenital stroke with moyamoya disease	Enlarged lateral ventricles Chiari 1 malformation	Enlarged lateral ventricles	Enlarged ventricles (mild), basilar, invagination and narrow foramen magnum	Enlarged lateral and third ventricles
Heart				congenital heart defect (PDA s/p ligation, PFO, ASD, and VSD)	ASD, PDA s/p repair, VSD, bicuspid aortic valve, mild dilatation of aortic root
Other		Joint hyperlaxity		Obstructive sleep apnea	Joint hyperlaxity, Obstructive sleep



				Stage II/III chronic kidney disease secondary to bilateral vesicoureteral reflux s/p vesicostomy	apnea History of infantile hypoglycemia, Glaucoma, s/p strabismus surgery, Subtelomeric 8p duplication
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ASD – atrial septal defect, NT - not tested, NA – not applicable, OFC - occipital frontal circumference, PDA – patent ductus arteriosus

VSD- ventricular septal defect

<sup>a</sup> On growth hormone therapy

<sup>b</sup> Conductive and / or sensorineural hearing loss

<sup>c</sup> Head circumference >97<sup>th</sup> percentile for age and sex

<sup>d</sup> Current OFC unavailable, 90<sup>th</sup> percentile at the age of 4 years

<sup>e</sup> Inner canthal distance >97<sup>th</sup> for age <sup>50f</sup> See a description of ear anomalies in figure 1

<sup>g</sup> Bifid uvula

<sup>h</sup>Hypernasal speech and or velopharyngeal insufficiency / submucosal cleft palate