

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

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Chromodomain helicase DNA-binding protein 4 (CHD4) is an ATP-dependent chromatin remodeler involved in epigenetic regulation of gene transcription, DNA repair, and cell cycle progression. Also known as Mi2 β , CHD4 is an integral subunit of a well-characterized histone deacetylase complex. Here we report five individuals with de novo missense substitutions in *CHD4* identified through whole-exome sequencing and web-based gene matching. These individuals have overlapping phenotypes including developmental delay, intellectual disability, hearing loss, macrocephaly, distinct facial dysmorphisms, palatal abnormalities, ventriculomegaly, and hypogonadism as well as additional findings such as bone fusions. The variants, c.3380G>A (p.Arg1127Gln), c.3443G>T (p.Trp1148Leu), c.3518G>T (p.Arg1173Leu), and c.3008G>A, (p.Gly1003Asp) (GenBank: NM_001273.3), affect evolutionarily highly conserved residues and are predicted to be deleterious. Previous studies in yeast showed the equivalent Arg1127 and Trp1148 residues to be crucial for SNF2 function. Furthermore, mutations in the same positions were reported in malignant tumors, and a de novo missense substitution in an equivalent arginine residue in the C-terminal helicase domain of SMARCA4 is associated with Coffin Siris syndrome. Cell-based studies of the p.Arg1127Gln and p.Arg1173Leu mutants demonstrate normal localization to the nucleus and HDAC1 interaction. Based on these findings, the mutations potentially alter the complex activity but not its formation. This report provides evidence for the role of *CHD4* in human development and expands an increasingly recognized group of Mendelian disorders involving chromatin remodeling and modification.

In the past decade, we have witnessed a dramatic increase in gene discovery of numerous Mendelian disorders associated with intellectual disability. These efforts have shed light on multiple developmental pathways, including the importance of the epigenetic machinery in neuronal development and homeostasis.^{1–3} Chromatin remodeling is an epigenetic mechanism that controls DNA accessibility to transcription, replication, and repair machineries. It is driven by nucleosome remodeling complexes that contain ATP-dependent enzymes able to mobilize nucleosomes and modify DNA packaging.⁴ One of these ATPases is the chromodomain-helicase-DNA-binding protein 4 (CHD4) also known as Mi2- β .^{5–7} CHD4 is a core component of the nucleosome remodeling and deacetylase (NuRD) complex, which possesses both chromatin remodeling and histone deacetylation activities.^{8–11} Both CHD4 and NuRD have been studied extensively for their

role in stem cell differentiation, embryonic development, and oncogenesis.^{9,12} For instance, depletion of CHD4 from certain mammalian embryonic tissues resulted in altered development^{13–17} and somatic mutations in *CHD4* (MIM: 603277) were reported in serous endometrial carcinoma.^{18,19} Here we report five individuals with a form of syndromic intellectual disability that carry de novo missense variants in *CHD4*.

The subjects underwent whole-exome sequencing at four different institutions. They were clinically assessed by experienced clinical geneticists prior to testing and did not have a diagnosis of a known genetic syndrome. Institutional review board-approved consents for whole-exome sequencing were obtained for all subjects. Subject 1 participated in a research project for undiagnosed developmental disorders at the National Human Genome Research Institute (NIH/NHGRI). Subject 2 underwent

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Figure 1. Facial Dysmorphism in Subjects Harboring *CHD4* Mutations

From left to right: pictures of subjects 1, 2, and 5 at the age of 10 years, 12 months, and 18 years (top) and 1 year (bottom), respectively. There are similar subtle dysmorphic features that include macrocephaly, wide-spaced eyes, fullness of eyelids, a squared face, and low-set, small, or cup-shaped ears.

(BWA-MEM v.0.7.5a) and variants were called using the GATK haplotype caller (v.2.7-2). Detected variants were annotated, filtered, and prioritized using the Bench lab NGS v.3.1.2 platform (Cartagenia). For subject 3 the methods are described in Firth et al.²⁴ For subjects 4 and 5, whole-exome sequencing and analysis was performed according to the protocol described in Yang et al.²⁵ In summary, exomes were captured with the Roche

clinical exome sequencing at the University Medical Center Utrecht, the Netherlands,²⁰ and subject 3 participated in the Deciphering Developmental Disorders (DDD) project in the UK.²¹ A de novo missense variant in the C-terminal helicase domain of *CHD4* was independently selected as the leading candidate variant in these three index subjects based on the gene function, sequence conservation, in silico predictions of deleteriousness, and the absence from the Exome Aggregation Consortium (ExAC) database of 60,700 exomes. The three index subjects were matched using GeneMatcher²² and the Decipher website. We then carefully compared their clinical history and physical exams and verified that all individuals had a similar phenotype. Subsequently, we identified subjects 4 and 5 who previously underwent clinical exome sequencing at the Baylor-Miraca Genetics Laboratories (Baylor College of Medicine [BCM]). For each subject, information on additional candidate variants and previous genetic testing is detailed in the [Supplemental Data](#).

For subject 1, whole-exome sequencing was performed at the NIH Intramural Sequencing Center (NISC) using the SeqCap EZ Exome v.3.0 capture kit (Roche NimbleGen) and the Illumina HiSeq2500 platform. Sequencing data were aligned to the human reference genome using Novoa-lign (Novocraft Technologies). Variants were called using the in-house MPG genotype caller. Detected variants were annotated and filtered using VarSifter.²³ Average coverage attained was 65× with on average 95% of targeted bases covered at 10×. For subject 2, exomes were enriched using the SureSelect XT Human All Exon V5 kit (Agilent Technologies) and sequenced in rapid run mode on the HiSeq2500 sequencing system at a mean target depth of 100× and an average 95% of targeted bases covered at 10×. Reads were aligned to hg19 using BWA

NimbleGen VCrome reagent and sequenced using Illumina technology. Reads were aligned using the Mercury pipeline and annotated using the Cassandra software. Average coverage attained was 123× for individual 4 and 160× for individual 5, with on average 97.9% and 98.4% of targeted bases covered at 20×, respectively. The variants we detected were c.3380G>A (p.Arg1127Gln) (seen in subjects 1 and 3), c.3443G>T (p.Trp1148Leu), c.3518G>T (p.Arg1173Leu), and c.3008G>A (p.Gly1003Asp) (GenBank: NM_001273.3). All variants were confirmed as de novo by Sanger sequencing using standard methods and are available upon request.

Frequent findings included a history of developmental delay (5/5), hypotonia (4/5), mild to moderate intellectual disability (4/5), and hearing loss (4/5). The brain MRI demonstrated mild to moderate enlargement of the lateral ventricles in all subjects. Physical exam was significant for macrocephaly (4/5), palatal abnormalities (4/5), and similar facial dysmorphisms (5/5) (e.g., wide-spaced eyes, a square-shaped face, and external ear anomalies) ([Figure 1](#)). In addition, the three male subjects had hypogonadotropic hypogonadism. In subjects 3 and 4, there was a history of short stature, and subject 3 was treated for growth hormone deficiency. Additional congenital anomalies that were seen in two subjects include cervical vertebrae fusions, tarsal coalitions, and heart defects. A summary of the clinical findings is shown in [Table 1](#) and detailed case descriptions are in the [Supplemental Data](#). Overall there were similar facial features and clinical histories, but each of the shared clinical findings were relatively non-specific, making it difficult to make a diagnosis without genotypic data. Furthermore, a few subjects had unusual clinical findings not seen in the others, e.g., congenital stroke and moyamoya disease in subject 1,

Table 1. Clinical Findings in Five Subjects with De Novo Missense Variants in *CHD4*

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
<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, ND	3.7 kg, 37cm	2.99 kg, 35 cm	3.06 kg, ND
Height, OFC at last exam ^a	143 cm (75 th), 56 cm (>98 th)	161 cm (40 th), 62 cm (>98 th)	140 cm ^b (50 th), 56 cm (>98 th)	89.5 cm (<3 rd ; Z score –5), 49 cm (20 th)	167.5 cm (10 th), 52.5 cm at 4 years (90 th)
Developmental delay	+	+	+	+ (severe)	+
Intellectual disability	+	+	+ (mild)	+	+ (mild)
Hearing loss ^c	+	+	+	–	+
Undescended testis, micropenis	+, +	NA	+, +	NA	–, +
Macrocephaly ^d	+	+	+	relative to length	+ ^e
Widely spaced eyes ^f	+	+	+	+	+
Dysmorphic ears ^g	+	+	+	+	+
Palatal anomalies	+ ^h	–	+ ⁱ	+ ⁱ	+ ⁱ
Hypogonadotropic hypogonadism	+	–	+	NT	+
Skeletal survey	advanced bone age by 2–3 years	tarsal coalition, cervical vertebrae fusion	falx calcification	scoliosis, platybasia, fusion of C2-C3, bilateral coxa valga, fusion of the cuboid and the 3 rd cuneiforms bilaterally, brachymesophalangia	diffusely osteopenic bones
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

Abbreviations are as follows: ASD, atrial septal defect; NT, not tested; NA, not applicable; OFC, occipital frontal circumference; PDA, patent ductus arteriosus; VSD, ventricular septal defect.

^aData in parentheses are percentiles.

^bOn growth hormone therapy.

^cConductive and/or sensorineural hearing loss.

^dHead circumference >97th percentile for age and sex.

^eCurrent OFC unavailable, 90th percentile at the age of 4 years.

^fInner canthal distance >97th for age 50.

^gSee a description of ear anomalies in Figure 1.

^hBifid uvula.

ⁱHypernasal speech and or velopharyngeal insufficiency/submucosal cleft palate.

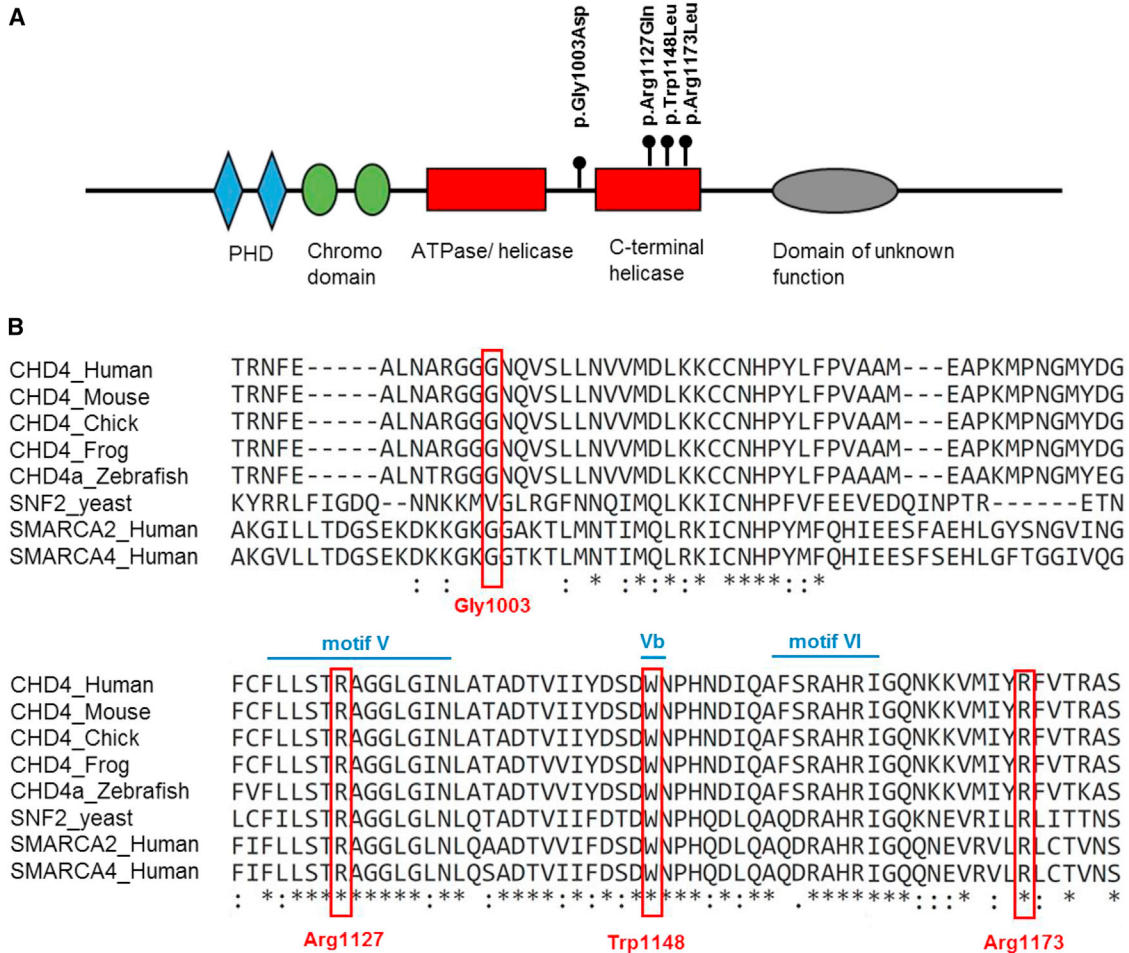


Figure 2. CHD4 Variants and Amino Acid Sequence Conservation

(A) CHD4 protein domains and location of amino acid substitutions. Abbreviation: PHD, plant homeodomain zinc fingers. (B) Protein alignment of CHD4 orthologs across several vertebrate species and yeast. We also aligned with the ATP-dependent helicase SMARCA2 and SMARCA4. The Arg1127, Trp1148, and Arg1173 positions are conserved down to yeast. The p.Arg1127Gln and p.Trp1148Leu variants are located at the helicase motifs V and Vb, respectively.^{6,28}

severe developmental and growth delay in subject 4, and eye abnormalities in subject 5. Increased phenotypic heterogeneity has been reported before in Mendelian disorders of the epigenetic machinery and may be the result of genetic variation in downstream targets.¹

CHD4 belongs to the CHD subfamily II. Similarly to CHD3 and CHD5, CHD4 contains two N-terminal plant homeodomain (PHD) zinc fingers and tandem chromodomains in addition to centrally located ATPase/helicase domains.⁷ The helicase domains provide the energy necessary for nucleosome remodeling and resemble SNF2, the catalytic subunit of the chromatin remodeling SWI/SNF complex in yeast. The PHD and chromodomains are thought to direct CHD4 to its substrates and regulate the remodeling activity.^{26,27} The three variants detected in subjects 1–4 were in the C-terminal helicase domain and subject 5’s variant was between helicase domains (Figure 2A). The involved amino acids are highly conserved across species and other ATP-dependent chromatin remodelers, as shown in Figure 2B. Of note, SNF2

contains conserved motifs that were previously shown to be critical for ATP binding and nucleosome remodeling.^{28,29} Specifically, p.Arg1127Gln and p.Trp1148Leu are within motif V and Vb, respectively, and involve residues shown to be crucial for nucleosome remodeling activity in yeast.²⁸ The Combined Annotation Dependent Depletion (CADD) phred score³⁰ was above 26 for all the variants and they were predicted damaging by Provean and SIFT.^{31,32} Furthermore, Samocha et al. list *CHD4* as one of the top 1,000 genes with excessive constraint to both missense and loss-of-function (LOF) variants.³³

Among the *CHD4* paralogs, *CHD7* (MIM: 608892), *CHD2* (MIM: 615369), and *CHD8* (MIM: 610528) have been associated with neurodevelopmental disorders.^{34–36} Interestingly, there are several similarities between the *CHD4*-associated phenotype and CHARGE syndrome (MIM: 214800), which results from haploinsufficiency of *CHD7*. Those include developmental delay, hearing loss, external ear anomalies, palatal abnormalities, a square-shaped face, and pituitary deficiencies. This correlation

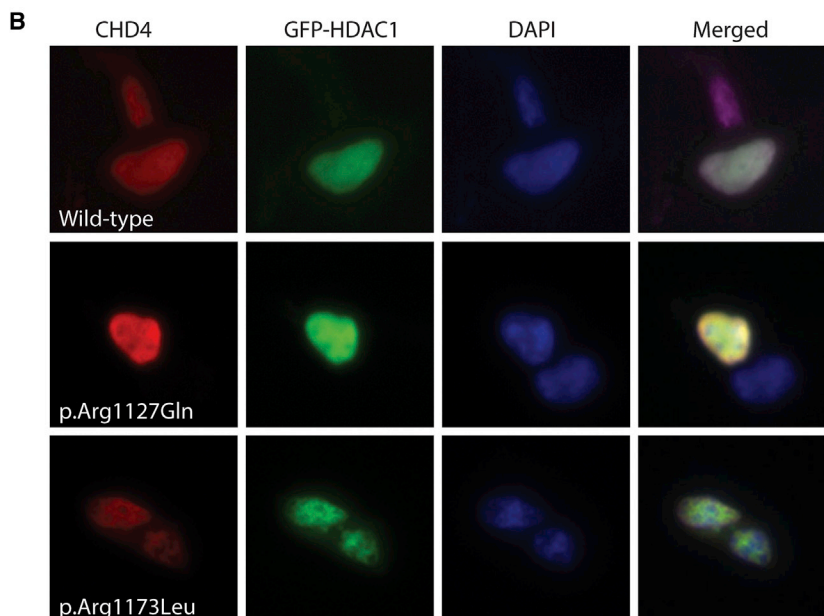
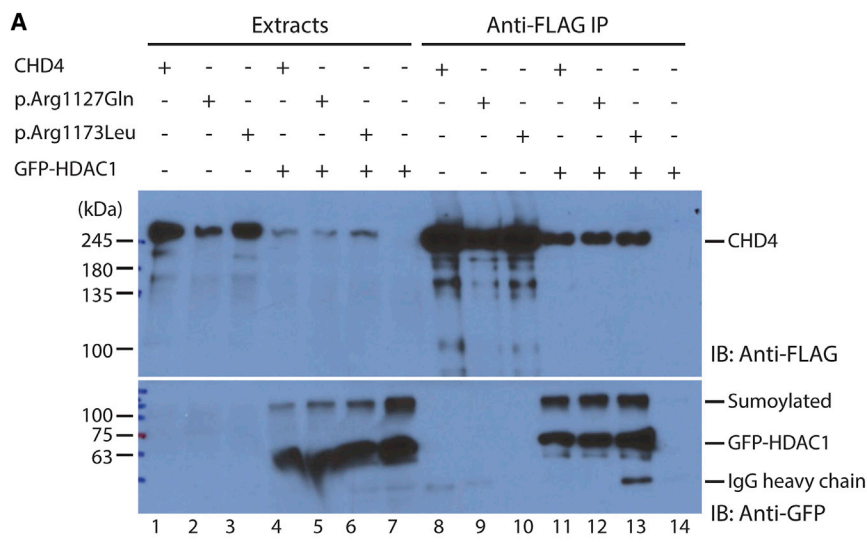


Figure 3. Comparison of Wild-Type and Mutant CHD4 Proteins by Cell-Based Assays

(A) The two mutations do not change HDAC1 interaction. Wild-type CHD4 and the two mutants were transiently expressed in HEK293 cells as FLAG-tagged proteins with or without GFP-HDAC1. Soluble extracts were prepared ~36 hr after transfection for immunoprecipitation (IP) on anti-FLAG antibody conjugated to agarose, and bound proteins were eluted with FLAG peptide for immunoblotting with an anti-FLAG monoclonal antibody. After extensive washing, bound proteins were eluted with FLAG peptide for immunoblotting with anti-FLAG and -GFP antibodies as indicated. HDAC1 is known to be efficiently sumoylated.⁴⁹

(B) Mutations do not affect CHD4 nuclear localization. Wild-type CHD4 and two mutants were expressed in HEK293 cells as FLAG-tagged proteins along with a green fluorescent protein (GFP)-HDAC1 fusion protein. Cells were fixed for indirect immunofluorescence microscopy with the anti-FLAG antibody and a Cy5-conjugated secondary antibody to detect CHD4 and its mutants. Green fluorescence was used as an indicator of HDAC1 levels and nuclear DNA was detected with DAPI staining. The merged images are shown at the right column. HEK293 cell transfection, indirect immunofluorescence microscopy, and immunoprecipitation were carried out as described.⁵⁰

Note: The residual heavy chain on lane 13 is due to some anti-FLAG agarose beads that were incidentally collected when the eluate was transferred out by pipetting.

may suggest common downstream epigenetic targets, such as *TP53*, which is downregulated by both CHD7 and CHD4.^{37,38} In addition to the CHD protein family, there are other proteins with similar ATP-dependent chromatin remodeling activity, such as the ATP-dependent helicase SMARCA2, SMARCA4, and ATRX, which are associated with neurodevelopmental syndromes.^{39–42} The *SMARCA4* (MIM: 603254) missense substitution c.3469C>G (p.Arg1157Gln) (GenBank: NM_003072.3) equivalent to p.Arg1127Gln in *CHD4* has been previously reported in a person with Coffin-Siris syndrome³⁹ (MIM: 614609), providing additional support for the pathogenicity of substitutions in this amino acid residue.

As described above, three of the substitutions are localized to the C-terminal helicase domain of CHD4 (Figure 2A).¹⁰ Co-immunoprecipitation and western blot analysis revealed that the c.3380G>A (p.Arg1127Gln) and c.3518G>T (p.Arg1173Leu) substitutions did not

affect interaction with HDAC1 (Figure 3A), and immunofluorescence staining showed that similar to the wild-type protein, these mutants localized properly to the nucleus along with HDAC1 (Figure 3B). Based on their results, we do not expect these substitutions to directly affect CHD4 complex formation with HDAC1 and HDAC2. Consistent with this, the substitutions are located within the helicase domain (Figure 2A), away from the PHD fingers that are known to mediate HDAC1/2 binding.¹⁰ The substitutions may disrupt the ATPase activity of CHD4, and further experiments will be needed to determine this possibility.

According to the Mouse Gene Expression Database, *Chd4* is broadly expressed in the mouse embryo and highly expressed in the head (brain, ear, and eye), the central nervous system in general, and the genitourinary system. O'Shaughnessy-Kirwan et al. demonstrated that null *Chd4* mouse embryos cannot complete the first lineage step at the blastocyst stage.⁴³ In the developing central nervous system of mice, the lack of *Chd4* resulted in loss of inhibition of astroglial differentiation and impaired

synaptic connectivity.^{13,17} Furthermore, the International Mouse Phenotyping Consortium (IMPC) provides phenotypic information on a *Chd4* knock-out mouse model resulting from a deletion of the critical exons 11 and 12 in the chromodomains region. Mice homozygous for the targeted deletion are embryonic lethal prior to organogenesis. The heterozygous mice are viable and exhibit several abnormalities that overlap with the phenotype seen in humans. There was decreased hearing with abnormal brainstem auditory evoked potentials at 24 kHz, and abnormal locomotor activation with decreased whole arena average speed that may be secondary to developmental delay. In addition, in some of the mutant mice, there was a significant decrease in the lean body mass and body length, abnormal left ventricle morphology, and abnormal lens morphology. Of note, these results are based on the evaluation of 16 mutants (8 females and 8 males). Although QC was completed and p values were significant, further studies are needed to support these findings.

The phenotype seen in the heterozygous knock-out mice might indicate that the phenotype seen in humans resulted from complete *CHD4* loss of function or partial loss of function of the helicase domain. On the other hand, we are not aware of case reports of small microdeletions that include *CHD4* or individuals with truncating mutations. Interestingly, mainly nonsynonymous substitutions in the ATP-dependent helicases *SMARCA4* and *SMARCA2* (MIM: 600014) cause Coffin Siris syndrome and Nicolaides-Baraitser syndrome (MIM: 601358), respectively. The proposed mechanism in those cases is a dominant-negative effect of the abnormal protein on the activity of the SWI/SNF complex.^{3,44} If that is the case in the *CHD4*-related syndrome, we expect to see a different or less severe phenotype in individuals with *CHD4* deletions or truncating mutations. Of note, the ExAC database includes six LOF variants in *CHD4*. These could be explained by sequencing/alignment errors (5/6 are indels) or a mild under-recognized phenotype. As mentioned before, there is significant intolerance to LOF variation relative to the gene's size, but at this time it is not clear whether carriers of truncating mutations will be similarly affected.

CHD4 and NuRD act mainly but not exclusively through transcriptional repression.⁴⁵ Several studies have shown that *CHD4* has a role in DNA damage response and cell cycle progression either independently or as part of the NuRD complex, and it may also function as an oncogene, a tumor suppressor, or both.^{37,46} Le Gallo et al. reported somatic mutations in *CHD4* in 17% of endometrial tumors.¹⁸ Most of the mutations detected resulted in nonsynonymous substitutions, and roughly half of them clustered in the ATPase/C-terminal helicase domain. Interestingly, when they performed alignments with *SMARCA1* (MIM: 606622), *SMARCA4*, and *SMARCA2*, they found that in 2/3 of the cases, the same residues were reported to undergo germline de novo changes causing Schimke immune-osseous dysplasia (MIM: 242900), Coffin-Siris syndrome, or Nicolaides-Baraitser syndrome. This observa-

tion led them to speculate that somatic mutations in the C-terminal helicase domain of *CHD4* are molecular drivers of endometrial cancer progression. Additionally, Zhao et al.¹⁹ reported an increase in the frequency of somatic *CHD4* mutations in endometrial tumors. Interestingly, one of the variants (p.Arg1127Gly) affects the same arginine residue seen in two of our subjects. According to the Cosmic database of genetic variations in tumors, the p.Arg1127Gln variant was identified in gastric tumors and an p.Arg1173Trp mutant was reported in hematologic tumors. The subjects in this study do not have a history of cancer, but we cannot discard the possibility that they will develop malignant tumors later in life. Further reports of individuals with germline mutations in *CHD4* are required to determine the risk of cancer in these individuals. Of note, somatic mutations in *SMARCA2* and *SMARCA4* are seen in different types of cancer.⁴⁷ An increased risk for malignancy in individuals with Coffin-Siris and Nicolaides-Baraitser syndrome has been debated but has not yet been clinically proven.⁴⁸

In summary, we introduce an intellectual disability syndrome associated with macrocephaly, facial dysmorphisms, hearing loss, ventriculomegaly, hypogonadism, and various congenital anomalies including heart defects and bone fusions. This report provides insight on the role of *CHD4* during human development and expands the increasingly recognized group of Mendelian disorders of chromatin remodeling. This is intriguing because *CHD4* is not only a chromatin remodeler but also a critical subunit of a multiprotein histone deacetylase complex, suggesting that alteration in chromatin modeling and histone acetylation may be the culprit. Future descriptions of individuals with this condition will be needed to better understand the phenotypic variability and establish genotype-phenotype correlations. In this study we successfully applied the recently available tool of web-based gene matching and the mouse phenotyping consortium. It provides yet another example of the utility of data sharing in facilitating gene discovery in rare syndromes.

Supplemental Data

Supplemental Data include case reports and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.08.001>.

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Web Resources

1000 Genomes, <http://www.1000genomes.org>

CADD, <http://cadd.gs.washington.edu/>

COSMIC, <http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>

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

ExAC Browser, <http://exac.broadinstitute.org/>

GenBank, <http://www.ncbi.nlm.nih.gov/genbank/>

GeneMatcher, <https://genematcher.org/>

IMPC, <https://www.mousephenotype.org/>

MGI, <http://www.informatics.jax.org/>

OMIM, <http://www.omim.org/>

PROVEAN, <http://provean.jcvi.org>

UCSC Genome Browser, <http://genome.ucsc.edu>

UniProt, <http://www.uniprot.org/>

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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 (85th centile) and the head circumference was 38 cm (90th 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 (75th centile). He was macrocephalic with a head circumference of 56 cm (>97th 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 (10th 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 (40th centile). She was macrocephalic with a head circumference of 62 cm (>97th 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 (70th centile) and head circumference was 37 cm (75th 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 2nd 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 (50th percentile). He was macrocephalic with a head circumference of 56cm (>97th 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 (~25th 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 (10th centile). His last head circumference was from the age of 4 years and measured 52.5 cm (~90th 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*

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
<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 th %ile)/ 56 cm (>98 th %ile)	161 cm (40 th %ile)/ 62 cm (>98 th %ile)	140cm ^a (50 th %ile)/ 56cm (>98 th %ile)	89.5 cm (< 3 rd %ile Zscore -5)/ 49 cm (20 th %ile)	167.5 cm (10 th %ile)/ 52.5cm at 4 years (90 th %ile)
Developmental delay	+	+	+	+ (severe)	+
Hypotonia	-	+	+	+	+
Intellectual disability	+	+	+ (mild)	+	+ (mild)
Hearing loss ^b	+	+	+	-	+
Undescended testis	+ /+	NA	+ / +	NA	- / +

/ micropenis					
Macrocephaly ^c	+	+	+	Relative to length	+ ^d
Widely spaced eyes ^e	+	+	+	+	+
Dysmorphic ears ^f	+	+	+	+	+
Palatal anomalies	+ ^g	-	+ ^h	+ ^h	+ ^h
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

^a On growth hormone therapy

^b Conductive and / or sensorineural hearing loss

^c Head circumference >97th percentile for age and sex

^d Current OFC unavailable, 90th percentile at the age of 4 years

^e Inner canthal distance >97th for age ^{50f} See a description of ear anomalies in figure 1

^g Bifid uvula

^hHypernasal speech and or velopharyngeal insufficiency / submucosal cleft palate