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Missense variants in the chromatin remodeler *CHD1* are associated with neurodevelopmental disability

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Abstract

The list of Mendelian disorders of the epigenetic machinery has expanded rapidly during the last five years. A few missense variants in the chromatin remodeler *CHD1* have been found in several

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Author Contributions:

HTB and HJV conceived study; GOP, HTB and GDB wrote the paper; GOP performed cell culture experiments; LB performed computational analysis; GDB performed structural analysis; HJV, CDA, MTC, CAG, PJB, EB, JMH, LM, IDK, MA, DN, LBH, IMW, BB, MJGS, and HTB provided clinical information regarding patients.

Supplemental Data

Supplemental data include two tables and two figures which can be found with this article online.

Web Resources

DECIPHER, http://decipher.sanger.ac.uk/
1000 Genomes, http://www.1000genomes.org/
ExAC Browser, http://exac.broadinstitute.org
GenBank, http://www.ncbi.nlm.nih.gov/genbank/
GeneMatcher, https://genematcher.org/
OMIM, http://www.omim.org/
UCSC Genome Browser, http://genome.ucsc.edu/
GTEx Portal, http://www.gtexportal.org/home/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
SIFT, http://sift.bii.a-star.edu.sg/

large scale sequencing efforts focused on uncovering the genetic etiology of autism. Here we describe *CHD1* heterozygous missense variants in a cohort of patients with autism, speech apraxia, developmental delay and facial dysmorphic features. Importantly three of these variants occurred *de novo*. We also report on a patient with a *de novo* deletion covering a large fraction of the *CHD1* gene without any obvious neurological phenotype. Our results suggest that variants in *CHD1* can lead to diverse phenotypic outcomes; however, the neurodevelopmental phenotype appears to be limited to patients with missense variants, which is compatible with a dominant negative mechanism of disease.

Keywords

human disease; neurological dysfunction; epigenetic machinery; chromatin; speech apraxia

There has been rapid discovery of the genetic etiologies of intellectual disability with the advent of single nucleotide polymorphism microarray and clinical trio-based exome sequencing in recent years,[1]. Many of the newly discovered genetic variants are found in components of the epigenetic machinery,[2,3]. Patients with these disorders often display variable intellectual disability, growth dysregulation, and facial/limb dysmorphic features, [3]; the variability may be caused by individual variants but also by interaction with genetic or epigenetic variation at target genes,[3,4]. There are approximately 300 known epigenetic factors to date and currently about fifty (17%) have been associated with discernible phenotypes,[3,5–9]. The epigenetic machinery consists of readers, writers, and erasers of epigenetic modifications, as well as remodelers of chromatin, of which the latter three classes are enzymes. Enzymes are a class of proteins which is usually tolerant to the loss of a single allele, however, a deleterious pathogenic variant on a single allele of components of the epigenetic machinery appears to be sufficient to cause a clinical phenotype in a large majority of these diseases. This suggests that dosage may be critically important for enzymatic components of the epigenetic machinery,[3].

A number of well-known disease entities have been found to be associated with dysfunctional chromatin remodelers, including Coffin-Siris syndrome (MIM:135900),[10], CHARGE syndrome (MIM: 214800),[11] and ATR-X syndrome (MIM: 301040),[12]. Among the CHD (chromodomain, helicase, DNA binding) family of ATP-dependent chromatin remodelers, Mendelian disease phenotypes have so far been linked to four genes, *CHD2*, *CHD4*, *CHD7,* and *CHD8*, and common features are intellectual disability, autism, and abnormal head size (Supplemental Table 1). For three of these (*CHD2*, *CHD7* and *CHD8*) the predominant variant type appears to be loss of function. In contrast, only missense variants have been found in *CHD4*. Sequencing of tumors has also revealed variants in the *CHD* gene family in cancer; variants in *CHD5* have been found in neuroblastoma,[13] and variants in the other *CHD* genes (1–4 and 7–9) have been found in tumors of the gut (gastric and colorectal cancers),[14,15].

CHD1 is an ATP-dependent chromatin remodeler,[16] encoded on the long arm of chromosome 5,[17]. CHD1 has two chromodomains,[18,19] that bind to H3K4me3/H3K4me2,[19]. CHD1 regulates the opening of chromatin and contributes to the

pluripotency of embryonic stem cells,[20]. It may play a role in transcript elongation,[21] and help to deposit the H3.3 histone variant,[22]. *CHD1* is expressed in many tissues including in brain, where the highest level of expression is found in the cerebellum and basal ganglia (Supplemental Figure 1).

In our Epigenetics and Chromatin Clinic (https://igm.jhmi.edu/ecc-clinic), we saw two unrelated individuals with missense variants in *CHD1* (Subjects 1 and 2). We submitted an entry into GeneMatcher,[23] and made contact with several clinical groups and the genetic testing company GeneDx. This resulted in six individuals described here with single nucleotide variants, all of which underwent whole exome sequencing at GeneDx which has performed more than 50,000 clinical whole exome sequencing tests to date (January 2017).

In addition to the three new individuals we identified with *de novo* heterozygous missense variants in *CHD1* (Figure 1A Subjects 1, 4, 5), we also found that *de novo* missense (L1016V, R1203Q),[24–26] and nonsense (L1517fs*),[26–27] variants in *CHD1* were previously described in three autistic patients (Figure 1A, gray) identified in large surveys of autistic patients,[24–27]. However, the available phenotypic information is limited, so it is unknown whether these patients show phenotypic overlap beyond autism.

Likely disease causing variants were also identified in three other individuals, though it could not be determined whether the change was *de novo* or not. These included two affected sisters (Subjects 2 and 3) conceived by separate *in vitro* fertilization using eggs from a single, presumably healthy, egg donor for which *CHD1* sequencing is unavailable and a female (Subject 6) for whom parents had not been tested yet (Figure 1A). Phenotypic data of these individuals is described in Table 1, but biological samples are unavailable.

Four of our subjects with *CHD1* mutations carried a diagnosis of speech apraxia. Three of these patients also received a diagnosis of autism, although one (Subject 1) no longer carries this diagnosis. Speech apraxia is a relatively rare (1–2/1000 children) diagnosis in the general population,[28]. However, a recent study suggests that speech apraxia is seen in a large portion of children with autism,[29]. Furthermore, both apraxia and autistic phenotypes have in recent years been linked more heavily to the cerebellum,[30–32] and the *CHD1* chromatin factor is highly expressed in the cerebellum (Supplemental Figure 1). All five patients had developmental delay and hypotonia, all are female and some had epileptiform abnormalities on an EEG (Table 1). Although autism is in general more common in males, the 6 subjects we observe with *CHD1* mutations are all female. Attention should be given in future studies to the sex of patients harboring *CHD1* mutations and if a female skew is observed in larger cohorts, consideration should be given to the mechanism of male intolerance of *CHD1* mutations. A subset of patients had dysmorphic features including a pointed chin, frontal bossing and arched eyebrows (Figure 1B). Although we include information about the *de novo* variant found in subject 5 (D857G), this individual also carried compound heterozygous variants in the *WDR62* gene which were thought to be disease causing. Variants in *WDR62* are the cause of microcephaly 2, primary, autosomal recessive, with or without cortical malformation (MIM: 604317). Therefore no phenotypic information about this patient (Subject 5) is included, as her notable cortical malformation likely accounted for her global developmental delay. Additional phenotypic contributions of

the *de novo* D857G variant in the *CHD1* gene in this patient could not be discerned at 2 years of age.

It was curious that all patients we identified in this study have missense mutations in *CHD1* and we hypothesize that there is a dominant negative mechanism of disease in the case of *CHD1* mutations and their association with neurodevelopmental disability. Five of the six new variants described here involved a loss of an arginine and several are located in structurally important regions. This recurrent loss of arginine may offer a potential clue towards the mechanism of the pathogenicity as even when we take into account the arginine richness of this protein (7% of all amino acids), there is still a statistically significant enrichment of these missense changes involving arginine over what could be expected by chance ($p = 9.3x10^{-5}$). One of these changes, Arg618Gln, substitutes a glutamine for a conserved arginine residue that is adjacent to the Walker B helicase motif which is central for the hydrolysis of ATP. Although the precise function of this arginine is not presently known, its positioning suggests it may help couple DNA binding to ATP hydrolysis (Figure 1C). All of these variants occurred at highly conserved amino acids (Figure 1A) and most were deemed pathogenic by both PolyPhen,[33] (Table 1) and SIFT (data not shown),[34]. No variants have been described at amino acid position 460 and 618 in the ExAC database, [35] of healthy individuals. However, there was a single description of a variant changing the arginine at amino acid position 141 to a serine (frequency of $<1/10^{-5}$) but no variants have been described that change arginine at 141 to a glycine in *CHD1*. The change to glycine at 141 disrupts the conserved basic character at this residue, which may disrupt the protein structure or function. Similarly, there are 23 occurrences of a substitution from arginine to tryptophan at position 1708 (less than 0.03% of the ExAC database) but no observed changes of arginine to glutamine at this position in *CHD1*. Constraint data from the ExAC database also revealed that missense variants are generally poorly tolerated in this gene (z = 3.26, Figure 1D), as are loss of function variants (pLI =1). In three of the unrelated subjects, the variants were found to be *de novo* in the probands, further supporting the potential significance of these variants for the phenotype of our subjects. Additionally, CHD1 has been shown to bind to numerous key factors involved in transcriptional regulation, such as FACT, SPT4–5, RTF1, and components of large complexes such as Mediator and the spliceosome,[36–38]. It is possible that an inactivating mutation, such as many missense mutations, in the CHD1 protein could titrate out important factors or reduce the occupancy of active CHD1 at targeted sites, which could offer an explanation for a dominant negative mechanism of disease.

Moreover, data from DECIPHER, as well as the high predicted intolerance of *CHD1* to loss of function variants (pLI = 1 in ExAC), lend further evidence to the contribution of *CHD1* to disease. For instance, seven deletions overlapping the *CHD1* gene are available from DECIPHER,[39]; six of these ranged from 5.64 to 12.10 Mb and involved a large number of genes. The smallest of these copy number variants was a 2.95 Mb deletion with a loss of six genes in a patient with hypotonia, constipation, and language delay (Supplemental Table 2). However, in addition to *CHD1*, there were other candidate genes within this deletion that could potentially explain the observed phenotype (Supplemental Table 2). Interestingly, there is also a recent description of an individual with isolated talipes equinovarus and a *de novo* deletion of the entire sequence of *RGMB* and the final nine exons of *CHD1* (Hg18,

Chr5:97916544–98250268),[40]. However, we now confirm that this previously described individual, a 9 year old male, has no obvious neurodevelopmental phenotype at this time and that he is generally healthy other than the club foot and asthma. This evidence suggests that deletions of *CHD1* may not cause a consistent neurological phenotype, but missense changes in *CHD1* may, through a dominant negative mechanism. Alternatively, changes in *CHD1* (both deletions and missense changes) could lead to a predisposition towards disease similar to what has been described for *CHD8*, but may not be fully penetrant.

In addition, *Chd1* has been found to be essential for the high transcriptional output needed for rapid growth of the mouse epiblast,[41], and it has also been found to play a role in later murine development,[42]. Despite this, mice with loss of a single *CHD1* allele ($Chd1^{+/-}$) are healthy, fertile and phenotypically normal,[41]. Collectively, these observations highlight that further research is needed to elucidate the consequences of loss of function mutations in *CHD1*. However, we think that together these data are compatible with the hypothesis that the neurodevelopmental phenotype is associated with a dominant negative disease mechanism of missense mutations in*CHD1*.

Since prior studies demonstrate that dysregulation of *CHD1* leads to global changes in chromatin,[20], we explored the functional consequences of one of these variants in fibroblasts from Subject 1. In these fibroblasts, which carry a heterozygous *de novo* variant (Arg618Gln), we observed a global increase of a closed chromatin modification (H3K27me3) compared to fibroblasts from control individuals (Figure 2). These data support the notion that missense changes such as Arg618Gln have functional effects on CHD1 function.

In summary, our data show that missense variants in the chromatin remodeler *CHD1* are associated with a novel neurodevelopmental disorder with intellectual disability, autism, seizures, speech apraxia, and dysmorphic features.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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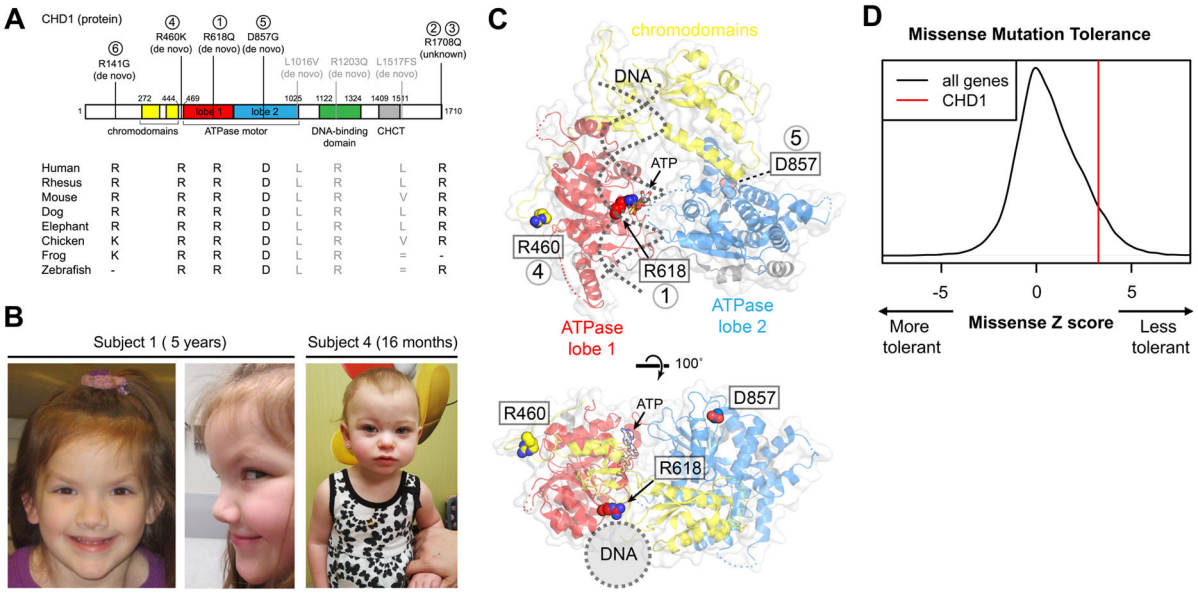

Figure 1. Missense variants in the chromatin remodeler *CHD1* result in a distinct neurological syndrome with dysmorphic features

(A) We have identified five unrelated subjects with missense variants at highly conserved locations within the coding region of the *CHD1* gene. Three of these variants are *de novo*. Three other *de novo* variants previously described in large cohorts of subjects with autism, [24–27] are noted in gray. Two of these variants occur at heavily conserved sites (1016, 1203) and the other (1517) leads to a frameshift at the C-terminal end of the protein. CHCT, which stands for CHD1 helical C-terminal, is an alpha-helical domain of unknown function, [43]. Domain boundaries are based on Chd1 structures,[43–45]. R = Arginine, K = Lysine, D = Aspartic Acid, G = Glycine, Q = Glutamine. (B) Representative images of some of the phenotypic facial features in two of the subjects. (C) Locations of the *de novo* missense sites on the chromodomain-ATPase motor, based on the yeast Chd1 structure,[44]. Two orthogonal views are shown, with the location of the DNA duplex binding highlighted with dotted gray lines. Note that the Arg618Gln variant occurs at the interface between DNA and ATP, and also is expected to pack against ATPase lobe 2 in the active state. (D) This gene does not tolerate missense variation particularly well as it has a relatively high missense Z score compared to all genes. The data here are based on data from the Exome Aggregation Consortium,[35].

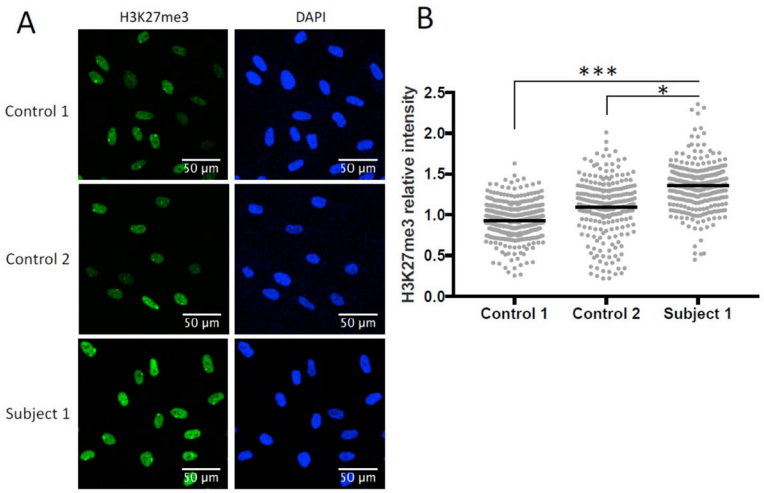

Figure 2. Patient fibroblasts demonstrate increased amounts of a closed chromatin modification (H3K27me3) compared to fibroblasts from control subjects

Since CHD1 is thought to play an active role in the process of opening chromatin we examined the amount of H3K27me3 in fibroblasts from one of our subjects (Subject 1) and fibroblasts from two control individuals. Briefly, samples were cultured in triplicate and stained with antibodies against H3K27me3 (green) and DAPI (blue). Intensity level of H3K27me3 in each nucleus was quantified using ImageJ. (A) Representative images of immunofluorescence staining from Subject 1 and two controls. (B) Quantification of H3K27me3 intensity, normalized to the average control intensity. Gray points represent H3K27me3 intensity in each nucleus and black bars represent the mean intensity of all nuclei measured. Controls were age and sex matched (Karyotypes: 46,XX and 46,XX,del(22)(q11.2q11.2)). * $P<0.05$, ***$P<0.0005$, one-way ANOVA with post hoc Tukey's HSD analysis.

Table 1

Clinical Findings in five subjects with missense variants in *CHD1*

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 6
***CHD1* variant**	c.1853G>A; p.Arg618Gln	c.5123G>A; p.Arg1708Gln	c.5123G>A; p.Arg1708Gln	c.1379G>A; p.Arg460Lys	c.421A>G, p.Arg141Gly
PolyPhen-2	PrD (1.000)	PrD (0.994)	PrD (0.994)	PoD (0.558)	Ben (0.08)
de novo	+	Unknown	Unknown	+	Unknown
Gender, age at last exam	female, 5 years	female, 6 years	female, 10 years	female, 7 months	female, 4 years
Height	103.1 cm (10–25$^{\%ile}$)	99.1 (<3rd$^{\%ile}$)	128 cm (<3$^{\%ile}$)	66.4 cm (63.1$^{\%ile}$)	104 cm (75$^{\%ile}$)
Weight	18.4 kg (50$^{\%ile}$)	18.2 kg (25$^{\%ile}$)	23.4 kg (<3$^{\%ile}$)	7.5 kg (59.7$^{\%ile}$)	17.7 kg (75$^{\%ile}$)
Developmental delay	+	+	regression	+	+
Speech apraxia	+	+	+	–	+
Diagnosis of Autism	+	+	+	–	–
Stereotypies	+	+	+	–	–
Hypotonia	+	+/dyspraxia	+/dyspraxia	+	+
Intellectual disability	–	+	+	n/a	n/a
Macrocephaly	+	+	–	–	–
Immune abnormalities	+	–	+	–	–
Depressed midface	+	+	+	–	–
Postnatal growth retardation	–	+ (on GH)	+ (on GH)	–	–
Translucent skin	+	+	+	–	–
Allergic shiners	+	+	+	–	–
Seizures	absence seizures	on seizure medication	abnormal EEG	+	–
Almond shaped eyes	+	+, long eyelashes	–	–	–
Flaring of eyebrows	+	+	–	–	–
Down-slanting palpebral fissures	+	+	+	–	–
Periorbital fullness	+	+	–	+	–
Fetal finger or toepads	+	–	+	–	–

PrD = probably damaging, PoD = possibly damaging, Ben = benign; Reference transcript for *CHD1* is NM_001270.2. Subject 1 had frequent episodes of fevers and inflammation, with fever ranging up to 104 with swelling of face, and bags under eyes. Immune testing showed incomplete vaccination to pneumococcus but responded to extra booster. Also, had elevated IgE and reduced NK cell function. Subject 2 and 3 both have immune problems which include IgA deficiency and hypogammaglobulinemia. Other features observed in Subject 1 include constipation, flexibility (family history of EDS) and high pain threshold. Although Subject 1 never had growth retardation she is small compared to other family members and had poor growth after being 8 pounds at birth. Subject 1 inherited another variant

of unknown significance from her unaffected mother (*CACNA1H*, c.5608 G>A, p.A1870T; reference transcript: NM_021098.2). Subject 2 has been treated with a ketogenic diet and carries another variant of unknown significance from her unaffected father (*DEPDC5*, c.1355 C>T, p.A452V; reference transcript: NM_001242896.1). Subject 2 and 3 are siblings. Subject 4 was seen again at 16 months and had less delay at that time (5–10 words, waves bye) although was still receiving supportive services (occupational therapy). Her measurements at that point included a height of 77.5 cm (33rd %ile), a weight of 10 kg (56th %ile) and a head circumference of 44.5 cm (16th %ile). One of the parents of Subject 6 also has intellectual disability but has not been available for genetic testing.