**The *CHD8* overgrowth syndrome: A detailed evaluation of an emerging overgrowth phenotype in 27 patients**

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**Abstract**

*CHD8* has been reported as an autism susceptibility / intellectual disability gene but emerging evidence suggests that it additionally causes an overgrowth phenotype. This study reports 27 unrelated patients with pathogenic or likely pathogenic *CHD8* variants (25 null variants, two missense variants) and a male:female ratio of 21:6 (3.5:1, p<0.01). All patients presented with intellectual disability, with 85% in the mild or moderate range, and 85% had a height and / or head circumference ≥ +2 SD meeting our clinical criteria for overgrowth. Behavioral problems were reported in the majority of patients (78%), with over half (56%) either formally diagnosed with an autistic spectrum disorder or described as having autistic traits. Additional clinical features included neonatal hypotonia (33%), and less frequently seizures, *pes planus*, scoliosis, fifth finger clinodactyly, umbilical hernia, and glabellar hemangioma (≤15% each). These results suggest that, in addition to its established link with autism and intellectual disability, *CHD8* causes an overgrowth phenotype, and should be considered in the differential diagnosis of patients presenting with increased height and / or head circumference in association with intellectual disability.

**Keywords**

*CHD8*

intellectual disability

overgrowth

macrocephaly

**1. Introduction**

Overgrowth intellectual disability (OGID) syndromes are a heterogeneous family of disorders with height and / or head circumference at least two standard deviations above the mean (≥ +2 SD) in association with intellectual disability

*(Tatton-Brown et al., 2017)*. Recently the majority of OGID syndrome genes have been shown to belong to one of two OGID gene families: the epigenetic regulator and PI3K/AKT pathway gene families

*(Tatton-Brown et al., 2017).*

*CHD8* (Chromodomain Helicase Binding Protein-8, OMIM 610528) belongs to the growing family of OGID epigenetic regulator genes

*(Tatton-Brown et al., 2017)*. The gene, at chromosome locus 14q11.2, encodes a 2581 amino acid protein with two chromatin organization modifier domains (“chromodomains”), an SNF2 family N-terminal domain, and a helicase C-terminal domain (Fig. 1A). CHD8 is a member of the chromodomain helicase DNA-binding protein family, and plays a critical role in epigenetic regulation and chromatin remodeling by recruiting histone H1 to target genes of the Wnt/beta-catenin pathway, suppressing their transcription

*(Nishiyama, Skoultchi, & Nakayama, 2012; Thompson, Tremblay, Lin, & Bochar, 2008)*. CHD8 has wide-spread effects in human induced pluripotent stem cells, affecting expression of several thousand genes involved in neurodevelopment, extracellular matrix formation, and skeletal development, with particular enrichment for downstream targets of genes associated with autistic spectrum disorders and schizophrenia

*(Wang et al., 2015)*.

*CHD8* was initially described as a cause of intellectual disability and autistic spectrum disorder when three patients with *de novo* chromosome 14q11.2 microdeletions, with the common critical region containing two candidate genes, *CHD8* and *SUPT16H*, were reported

*(Zahir et al., 2007).* A recent report has detailed the long term follow up of these patients and additionally includes collated phenotypic data from 51 patients with *CHD8* sequence and copy-number variants who had been previously published or deposited in online repositories

(*Yasin et al., 2019; Bernier et al., 2014; Drabova et al., 2015; Kimura et al., 2016; Merner et al., 2016; Neale et al., 2012; O'Roak et al., 2012a; O'Roak et al., 2012b; Prontera et al., 2014; Stolerman et al., 2016*). *CHD8* was first reported as an OGID gene in 2017 with the publication of 12 patients with *CHD8* sequence variants identified though exome-sequencing of patients with OGID (*Tatton-Brown et al., 2017)*. Subsequently, a report of 10 patients with *CHD8* sequence variants suggested that *CHD8* is associated with a Sotos-like phenotype

*(Douzgou et al., 2019).*

In this report, we further investigate the *CHD8*-related phenotype and whether *CHD8* gene variants should be considered one of the primary causes of OGID syndromes through the detailed phenotyping of 27 patients with *CHD8* variants.

**2. Methods**

The study was approved by the UK Research Ethics Committee (10/H0305/83), granted by the Cambridge South Research Ethics Committee and the London Multicenter Ethics Committee (MREC01/02/44 and 05/MRE02/17). Informed consent for participating in the study was obtained for all participants.

27 unrelated patients with 26 different *CHD8* variants were included (Table 1, Fig. 1A), including 17 patients who had previously been reported

*(Douzgou et al., 2019; Tatton-Brown et al., 2017; Yasin et al., 2019)*. The majority (22 patients) were diagnosed through the Deciphering Developmental Disorders (DDD) Study or the Childhood Overgrowth Study, using a trio-based exome sequencing strategy and methods as previously described

*(Firth et al., 2011; Tatton-Brown et al., 2017).* Five patients were diagnosed by exome sequencing in the NHS diagnostic laboratory.

*CHD8* variants were classified as pathogenic or likely pathogenic using the 2015 American College of Medical Genetics and Genomics guidelines

*(Richards et al., 2015)* and 2019 Association for Clinical Genomic Science guidelines

*(Ellard et al., 2019)*. They included ten stop-gain, nine frameshift, five splice-site and two missense variants (Fig. 1A; see Supplementary Table 1 for details of variant interpretation). Null variants were further evaluated using the recommendations of the ClinGen Sequence Variant Interpretation Working Group

*(Tayoun et al., 2018)*. The predicted effect of non-canonical splice-site variants was assessed using Alamut (v.2.11, incorporating NNSplice, MaxEntScan, GeneSplicer, and SpliceSiteFinder-like). The predicted effect of missense variants was assessed using PolyPhen, SIFT, and MutationTaster. All variants are reported relative to the canonical transcript (NM\_001170629.1). Patients with likely pathogenic or pathogenic variants or copy number variants affecting genes other than *CHD8* were excluded, to ensure our phenotypic evaluations were robust and specific to *CHD8*.

Clinical data were obtained through face-to-face review by one of the authors, all experienced dysmorphologists, and a standardized proforma. Intellectual disability was classified by the recruiting clinician as mild, moderate, or severe. For the purposes of our study, mild intellectual disability described an individual with delayed milestones who would attend a mainstream school with some support and live independently, with support, as an adult. Moderate intellectual disability described an individual who required high-level support in a mainstream school or special educational needs schooling and would live with support as an adult. Severe intellectual disability described an individual who required special educational needs schooling, had limited speech, and would not live independently as an adult. More specific individual information regarding language development and behavioral phenotype (including details pertaining to a diagnosis of autistic spectrum disorder) was only available for a minority of patients, and is therefore not reported. Photographs, with accompanying consent to publish, were requested from all and received from 13 families.

Growth parameter Z-scores were calculated with reference to the World Health Organization Child Growth Standards. Correlations between growth parameters and severity of intellectual disability were analyzed using one-way ANOVA with multiple comparisons. The sex distribution of patients was analyzed using a two-tailed Chi square test. Statistical analyses were performed in Prism v.6.0h (GraphPad Software, Inc.).

**3. Results**

Clinical data for the27patients included in the study are summarized in Table 1. The patients were aged between 1 and 27 years at recruitment, with a male to female ratio of 21:6 (3.5:1, p<0.01).

All patients (27/27, 100%) presented with intellectual disability. The majority (23/27, 85%) were classed in the mild (14/27, 52%) or moderate (9/27, 33%) range (Table 1). One patient (Patient 8) had developmental delay but was too young to reliably assess the level of disability (14 months). A total of 21/27 patients (78%) were described as having a behavioral issue, including autistic features or impaired social interaction (15/27, 56%), attention deficit (4/27, 15%), aggressive behavior (4/27, 15%), and/or self-harm (2/27, 7%).

Growth measurements are presented in Table 1, Fig. 2, and Supplementary Fig. 1. Overgrowth, as defined for the purposes of our study by height and/or head circumference at least two SD above the mean (≥ +2 SD), was present in 23/27 (85%) patients.

The mean height Z-score was +2.8 SD, with a range of +0.2 SD to +6.3 SD. 21/27 (78%) patients had a height ≥ +2 SD.

The mean head circumference Z-score was +2.4 SD, with a range of -2.7 SD to +4.8 SD. With the exception of a single outlier with microcephaly (head circumference of –2.7 SD), all patients had a head circumference greater than the age- and gender-adjusted mean (0 SD), with the majority clustering between +1 SD and +4 SD (23/27, 85%). Head circumferences ≥ +4 SD were observed in 3/27 (11%) patients.

There were no significant correlations between head circumference and severity of intellectual disability. Although the mean head circumference Z-score was highest in patients with severe intellectual disability (+3.2 SD, compared to +2.3 SD and +2.1 SD for moderate and mild, respectively), this was based on a small number of patients with severe intellectual disability (n=3) and did not reach statistical significance (p=0.52 by ANOVA, with no significant differences in multiple comparisons).

Birthweights were not significantly increased, with a mean Z-score of +0.8 SD (range, -0.9 to +3.0). Of the 22 patients with a recorded birthweight, only three (14%) had a Z-score ≥ +2 SD. Birth lengths and head circumferences were only recorded for a minority of patients (n=3 each), which precluded meaningful analysis. Mean gestational age was 39.25 weeks (range, 36-42 weeks).

There was a shared but subtle facial gestalt amongst the 27 patients, including frontal bossing, down-slanting palpebral fissures, high hairline and, in some patients, a prominent chin (Fig. 1B).

Additional clinical features, reported in at least two patients, included neonatal hypotonia (9/27 patients, 33%), seizures (4/27 patients, 15% [with one further patient reported as having possible absence seizures]), *pes planus* (4/27 patients, 15%), scoliosis (2/27 patients, 7%), fifth finger clinodactyly (2/27 patients, 7%), umbilical hernia (2/27 patients, 7%), and glabellar hemangioma (2/27 patients, 7%).

**4. Discussion**

We present detailed phenotypic data on 27 patients with 26 different variants in *CHD8*. This study will facilitate the identification and guide the management of this emerging OGID syndrome.

*CHD8* was initially described as an autism susceptibility / intellectual disability gene. Although limited phenotypic data in the autism susceptibility literature precludes an evaluation of these previously reported patients’ growth, our current study provides incontrovertible evidence that *CHD8* is responsible for an OGID phenotype, often in association with autistic spectrum disorder, that we have designated the *CHD8* overgrowth syndrome. Major *CHD8* clinical features (reported in at least 85% of patients) include intellectual disability (most frequently mild) and postnatal overgrowth. Additional clinical associations (reported in at least 15% but fewer than 85% of patients) include autistic features or impaired social interaction, hypotonia, attention deficit, aggressive behavior, seizures, and *pes planus*.

Our study indicates that the *CHD8* overgrowth syndrome phenotype is subtle, non-specific and that there are many overlapping features with other OGID syndromes (notably Sotos syndrome [*NSD1,* MIM 117550], Weaver syndrome [*EZH2,* MIM 277590], and Tatton Brown Rahman syndrome [*DNMT3A*, MIM 615879]), including overgrowth (usually of postnatal rather than prenatal onset), intellectual disability, and autistic spectrum disorder. It is therefore unlikely that a diagnosis of *CHD8* overgrowth syndrome will be made clinically; it is more likely that the diagnosis will be made following an agnostic gene sequencing approach (exome/genome sequencing). We propose, given the frequent association with overgrowth and the differential with the other OGID syndromes listed above, that *CHD8* should be included on targeted gene assays to investigate the overgrowth-intellectual disability syndromes.

It is interesting that there is a significant bias towards males amongst our series of 27 patients (male:female ratio of 21:6 or 3.5:1, p<0.01). One possible explanation for this gender bias is that females present with a milder, potentially sub-clinical phenotype. This hypothesis is corroborated by experiments in *CHD8-*haploinsufficient mice, which suggest a gender-specific effect on transcriptional regulation and phenotype, with male mice more severely affected

*(Jung et al., 2018)*. However, caution is needed to extrapolate these findings to humans. Of note, while the majority of variants in our series were *de novo*, 3/27 (11%) were maternally inherited, with no incidences of paternal inheritance. One male proband’s mother was reported to have mild intellectual disability, while the proband had moderate intellectual disability (Patient 24). While we have limited phenotypic information about the remaining probands’ parents, they were not known to have a significant intellectual disability. This suggests that the cognitive phenotype associated with *CHD8* may be attenuated or even non-penetrant in females. Furthermore, in our series, 62% of male patients (13/21) had autistic features, compared to 33% (2/6) of female patients. While this is in keeping with the higher proportion of males affected with autism in the general population

*(Taylor, Jick, & MacLaughlin, 2013)*, males with autistic spectrum disorder may be more likely to be referred for genetic evaluation thereby introducing an ascertainment bias. However, these observations are based on a relatively small number of patients, and further work is required to investigate whether there is a gender-specific cognitive or behavioral phenotype associated with *CHD8*.

The 24 null variants reported in this study were distributed throughout the gene, and *CHD8* exhibits a high degree of loss-of-function constraint (pLI 1.00, haploinsufficiency index 11.24%), suggesting that null variants are likely acting through haploinsufficiency. We have identified two patients (Patient 10 and Patient 12) with likely pathogenic *CHD8* missense variants, both targeting the SNF2\_N domain (Fig. 1A). Both of these patients had mild intellectual disability. It is interesting that they had small head circumferences, relative to their height (Patient 10: height +3.2 SD, head circumference +1.2 SD; Patient 12: height +0.2 SD, head circumference -2.7 SD). It is therefore possible that *CHD8* missense variants may be associated with a different (potentially non-macrocephalic) phenotype to the null variants, but this observation is only based on two patients, and it is possible that other genetic and/or environmental factors could be contributing to their phenotype. The identification of additional patients with *CHD8* missense variants is required to investigate this further.

In conclusion, through the detailed clinical evaluation of 27 patients, we have delineated an emerging OGID phenotype, designated the *CHD8* overgrowth syndrome, which should be considered in the differential diagnosis of patients with intellectual disability in the presence of macrocephaly and/or tall stature. However, data are still limited. The identification of additional patients with a range of *CHD8* variant types is required to further clarify this emerging disorder, including investigation of the apparent male predominance and *CHD8* genotype-phenotype correlations.

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**Conflicts of Interest**

The authors declare that they have no conflict of interest.

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**Web resources**

*CHD8* reference transcript, <https://www.ncbi.nlm.nih.gov/nuccore/NM_001170629.1>

Online Mendelian Inheritance in Man, <https://omim.org/>

Genome Aggregation Database (gnomAD), <https://gnomad.broadinstitute.org/>

World Health Organization Child Growth Standards, <https://www.who.int/childgrowth/standards/en/>

PolyPhen, <http://genetics.bwh.harvard.edu/pph2/>

SIFT, <https://sift.bii.a-star.edu.sg/>

MutationTaster, <http://www.mutationtaster.org/>

Alamut, <https://www.interactive-biosoftware.com/>

GraphPad Prism, <https://www.graphpad.com/scientific-software/prism/>

**Tables**

**Table 1.** Molecular and clinical details of 27 patients with *CHD8* variants. Patients are numbered in order of variant location (5’ 🡪 3’). Growth measurements are presented as Z-score (SD from the mean expected measurement for age/gender). DECIPHER identifiers are listed for patients with open-access records, in keeping with the DECIPHER publication policy.

**Abbreviations**: PMID, PubMed identifier; -, feature absent; nk, not known; M, male; F, female; ID, intellectual disability; Mod, moderate; Sev, severe; uc, unclassified; ASD, autistic spectrum disorder; Ht, height; Wt, weight; HC, head circumference; SD, standard deviations

**Figure Legends**

**Fig. 1.** **A:** CHD8, its domains and the location of the 26 variants reported in our series of 27 patients. The two missense variants were both in the SNF2\_N domain, and are denoted in blue with dotted arrows. C1/C2, chromodomain 1/2. SNF2\_N, SNF2 family N-terminal domain. Helicase, helicase C-terminal domain. **B:** Photographs of patients showing frontal bossing, down-slanted palpebral fissures, and high hairline. Photo captions include patient number (in bold) and variant details. Pt, patient.

**Fig. 2.** Standardized growth parameters (Z-scores) in *CHD8* patients (n=27). Bars represent mean ± SD. Solid horizontal line indicates the population average for age/gender (Z-score = 0 SD); dashed horizontal lines indicate Z-score cut-offs of ±2 SD. Solid red points indicate parameters with a mean Z-score of ≥ +2 SD (head circumference, height); circles indicate parameters with a mean Z-score of < +2 SD (body-mass index).

**Supplementary information**

**Supplementary Table 1.** Variant classification according to the ACMG criteria. Note patients #3 and #4 were found to have the same variant but were not known to be related. Patient #27 had a frameshift duplication, but this was in the final exon of *CHD8* and predicted to truncate <10% of the protein, and PVS1 was therefore applied at moderate strength

(*Tayoun et al., 2018*).

**Supplementary Fig. 1.** Relationship between age and growth parameters (Z-scores). Solid horizontal line indicates the population average for age/gender (Z-score = 0 SD); dashed horizontal lines indicate Z-score cut-offs of ±2 SD. Note that each data point represents a distinct patient at the age of recruitment, rather than longitudinal growth measurements. There were no significant correlations between age and growth parameters.