ARTICLE

A second cohort of *CHD3* patients expands the molecular mechanisms known to cause Snijders Blok-Campeau syndrome

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Abstract

There has been one previous report of a cohort of patients with variants in Chromodomain Helicase DNA-binding 3 (*CHD3*), now recognized as Snijders Blok-Campeau syndrome. However, with only three previously-reported patients with variants outside the ATPase/helicase domain, it was unclear if variants outside of this domain caused a clinically similar phenotype. We have analyzed 24 new patients with *CHD3* variants, including nine outside the ATPase/helicase domain. All patients were detected with unbiased molecular genetic methods. There is not a significant difference in the clinical or facial features of patients with variants in or outside this domain. These additional patients further expand the clinical and molecular data associated with *CHD3* variants. Importantly we conclude that there is not a significant difference in the phenotypic features of patients with various molecular disruptions, including whole gene deletions and duplications, and missense variants outside the ATPase/helicase domain. This data will aid both clinical geneticists and molecular geneticists in the diagnosis of this emerging syndrome.

Introduction

The Chromodomain Helicase DNA-binding (CHD) proteins constitute a highly conserved protein family seen in species ranging from protists to plants and mammals [1–3]. The family is defined by the presence of N-terminal paired

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Chromatin Organization Modifier (chromo) domains, and two central Sucrose NonFermentable2-like ATP-dependent helicase domains [4]. The CHDs utilize the energy derived from ATP hydrolysis to alter nucleosome positioning and structure, thereby regulating access to DNA and gene transcription [1, 5]. The family has been identified as critical in the regulation of cellular processes such as nucleosome assembly, stem cell quiescence, cell proliferation, and cell fate determination [4, 6]. Nine such CHDs are known to exist in humans, each of which can be broadly categorized into one of three subfamilies [2, 3]. Subfamily I includes human *CHD1* and *CHD2*. Subfamily II, defined by the presence of dual plant homeodomain (PHD) motifs, includes human *CHD3*, *CHD4*, and *CHD5*. Finally,

subfamily III, defined by the presence of Brahma and Kismet domains, includes human *CHD6*, *CHD7*, *CHD8*, and *CHD9*.

Perhaps not surprisingly for a family of genes with such important and conserved cellular functions, genetic perturbation of the CHDs has been linked to a number of human disorders. The first human CHD gene linked to disease was *CHD7*, found to be causative of CHARGE syndrome (MIM 214800) [7], with variants in *CHD1* [8], *CHD2* [9], *CHD4* [10], and *CHD8* [11], identified as causes of Pilarowski-Bjornsson syndrome (MIM 617682), Epileptic encephalopathy (MIM 615369), Sifrim-Hitz-Weiss syndrome (MIM 617159), and autism spectrum disorders (MIM 615032), respectively. In 2018 *CHD3* emerged as the latest CHD gene to be associated with a human syndrome, with variants in *CHD3* identified in 35 patients with a novel neurodevelopmental disorder, Snijders Blok-Campeau syndrome (MIM 618205) [12].

The Snijders Blok-Campeau syndrome is characterized by some degree of intellectual disability, developmental delay (most notably speech delay), and a number of dysmorphic features [12]. Interestingly, most variants identified in the initial cohort of 35 patients appeared to cluster within the protein's highly conserved ATPase/helicase domain, with experimental work providing evidence that both gain-and loss-of function variants in this region can result in the same phenotype [12].

Due to the small number of patients known to have Snijders Blok-Campeau syndrome, many of the core features of the syndrome remain to be more precisely defined, with a clear need to better understand the role of different variant types in the development of the syndrome. Here we present an additional 24 patients with *CHD3* variants and Snijders Blok-Campeau syndrome. With these new patients we are able to better define the Snijders Blok-Campeau phenotype, and confirm and expand the constellation of facial findings that we believe convincingly represent a recognizable pattern of human malformation. Furthermore, our cohort contains 14 patients with *CHD3* variants that fall within the ATPase/helicase domain, but also 10 patients with a variety of other *CHD3* variant types falling outside of this conserved domain (including one microdeletion containing the complete *CHD3* gene, and one microduplication containing the complete *CHD3* gene). Our analysis has shown no difference in phenotype between those patients with missense variants affecting the *CHD3* helicase domain compared with patients with any other *CHD3* variant type, expanding the spectrum of molecular mechanisms that are known to lead to Snijders Blok-Campeau syndrome. Together with the first cohort of patients, our data bring the total number of known Snijders Blok-Campeau patients from 35 to 59, provide further important phenotypic and molecular characterization of the

disorder, and will aid in the diagnosis of the syndrome in future patients.

Materials and methods

Patient cohort and variants identified

The patients we present here were ascertained from numerous clinical and laboratory sites across different countries (Australia, Canada, China, France, Germany, Italy, the Netherlands, and the United States). Altogether, 24 patients were ascertained and included in our cohort. All patients initially presented for evaluation for developmental delay, most with additional findings, and were each found to have a variant in the *CHD3* gene by clinical molecular/ cytogenetic methods, including chromosomal microarray and clinical exome/genome sequencing. In all cases, the treating provider considered the identified *CHD3* variant the most likely underlying genetic etiology for each patient's presentation. Information regarding the inheritance of the *CHD3* variant was known for 22 of the 24 patients. In all but two cases, the *CHD3* variant was found to have arisen de novo. Individual 18 was found to have a maternally-inherited truncating variant in *CHD3*, with her mother noted to have intellectual disability and seizures, and was considered special needs throughout her schooling. In individual 23 the identified *CHD3* duplication was reportedly also found in her similarly-affected brother, and was thought to have likely been inherited from a similarly-affected father, although the father was not available for testing. Patients and/or caregivers were consented by the treating physician for publication, and for each patient for whom a photograph is shown, specific consent for photo publication was obtained by the treating provider.

Clinical information was collected with a standardized questionnaire. Given the previously-reported phenotype of Snijders Blok-Campeau syndrome, specific questions were included regarding head circumference, facial dysmorphisms, structural brain anomalies, congenital heart anomalies, seizures, hernias, developmental delay, autistic features, intellectual disability, and others.

Each subject included in the manuscript has been submitted to the Leiden Open Variation Database, with each entry detailing the *CHD3* variant identified and a brief phenotypic summary. The entries can be found at http://www.lovd.nl/CHD3 (Patient IDs 274276, 274277, 275839–275860).

Statistical analysis

To assess significant phenotypic differences between patients with *CHD3* variants directly affecting the *CHD3*

helicase domain, and those patients with any other *CHD3* variant, two-tailed Fisher's exact tests were performed in RStudio and plotted using the ggplot2 package.

Generation of Snijders Blok-Campeau syndrome composite photos

The deep convolutional neural network architecture provided by Face2Gene (FDNA Inc, USA) [13] was used to create composite photos of individuals with *CHD3* variants, as indicated in the text and figure legends.

Results

CHD3 patient cohort

Our cohort includes 24 patients, all of whom were found to have variants affecting the *CHD3* gene as the most likely explanation for their clinical presentations. The average age of our cohort is 9.3 years (median age of 8), with the youngest patient being 1 year old at the time of last evaluation, and the oldest being 31 years old. The *CHD3* variants identified in our cohort are discussed below, and depicted schematically in Fig. 1a. The clinical features of our patients are discussed below, and can also be found in tabular format in Table 1, and in a more extensive tabular form in Supplemental Table 1. Patient facial characteristics are discussed at length below, with photos of a subset of our patients, divided into groups by variant type, displayed in Fig. 2.

CHD3 variants identified

A number of different *CHD3* variant types were identified in our cohort (Fig. 1a). In the initial Snijders Blok-Campeau syndrome patient cohort, it was noted that *CHD3* missense variants appeared to cluster within the *CHD3* helicase domain [12]. The *CHD3* missense variants identified in our cohort similarly appear to cluster within the *CHD3* helicase domain, which should likely be considered a hotspot for *CHD3* pathogenic variation. Of the nineteen missense/in-frame deletion variants identified, 14 fell within CHD3 amino acid position 886–1262, with the CHD3 helicase domain predicted to span amino acid residues 879–1258 (NP_001005273.1). The remaining four missense variants we identified were found to affect amino acid residues outside of the helicase domain, specifically disrupting amino acids at position 569 (in two individuals, 1–1 and 1–2, monozygotic twins), 1415, and 1955.

In addition, we identified five patients with likely loss-of-function alleles. One patient was found to have a nonsense variant within the *CHD3* gene, two were found to have

frameshift variants, one was found to have a splicing variant (predicted to abolish the splice acceptor site of exon 27, and result in exclusion of exon 27 from the final transcript, generating a frameshift and likely loss-of-function allele), and one was found to have a 0.5 Mb deletion (chr17: g.7394419_7871060del (hg19)) including the complete *CHD3* gene (as well as the OMIM disease genes *MPDU1*, *TP53*, and *WRAP53*). While the deletion of *TP53* in this patient is consistent with an expected diagnosis of Li Fraumeni syndrome, it does not explain the patient's developmental delay or other phenotypic features. The treating physician ultimately felt that the patient's presentation was best explained by the deletion of the *CHD3* gene. It should be noted that, in total, there are 37 additional patients in the DECIPHER database [14] reported as having chromosomal deletions involving the complete *CHD3*, with the patient reported here having the smallest such deletion.

Finally, one patient in our cohort was found to have a large 6.5 Mb duplication (chr17:g.7339633_7902885dup (hg18)) including the complete *CHD3* gene (as well as the OMIM disease genes *CHRNB1*, *MPDU1*, *TP53*, and *WRAP53*, again with none of these other genes thought to be contributing significantly to the patient's phenotype). This patient also had additional testing by next-generation sequencing examining 1162 known intellectual disability genes, which was nondiagnostic of any alternate explanation for the patient's presentation. The DECIPHER database [14] contains an additional 34 patients with chromosomal duplications involving the complete *CHD3* gene, two of which are smaller than the duplication seen in our patient (DECIPHER patients 258312 and 301981). Insufficient clinical information is available for either of these two patients to comment on any potential phenotypic overlap between them and Snijders Blok-Campeau syndrome.

Absence of genotype-phenotype correlation

It was unclear if the 10 patients in our cohort with *CHD3* variants outside of the helicase domain (individuals 1–1, 1–2, and 16 through 23) would present with the Snijders Blok-Campeau syndrome phenotype, or if this phenotype was limited to patients with missense/in-frame deletion variants within the *CHD3* helicase domain. To address this question, we performed an analysis to specifically ascertain any phenotypic differences between the 14 patients in our cohort with variants within/immediately adjacent to the *CHD3* helicase domain, and the 10 patients with any other variant type (Fig. 1b). Overall, no major phenotypic differences were observed. The only clinical feature different between the two groups with a *p* value less than 0.05 (by Fisher's exact test, not surviving Bonferroni correction for multiple testing) was Moderate/Mild-Moderate ID, which was more prevalent in patients with *CHD3* helicase domain
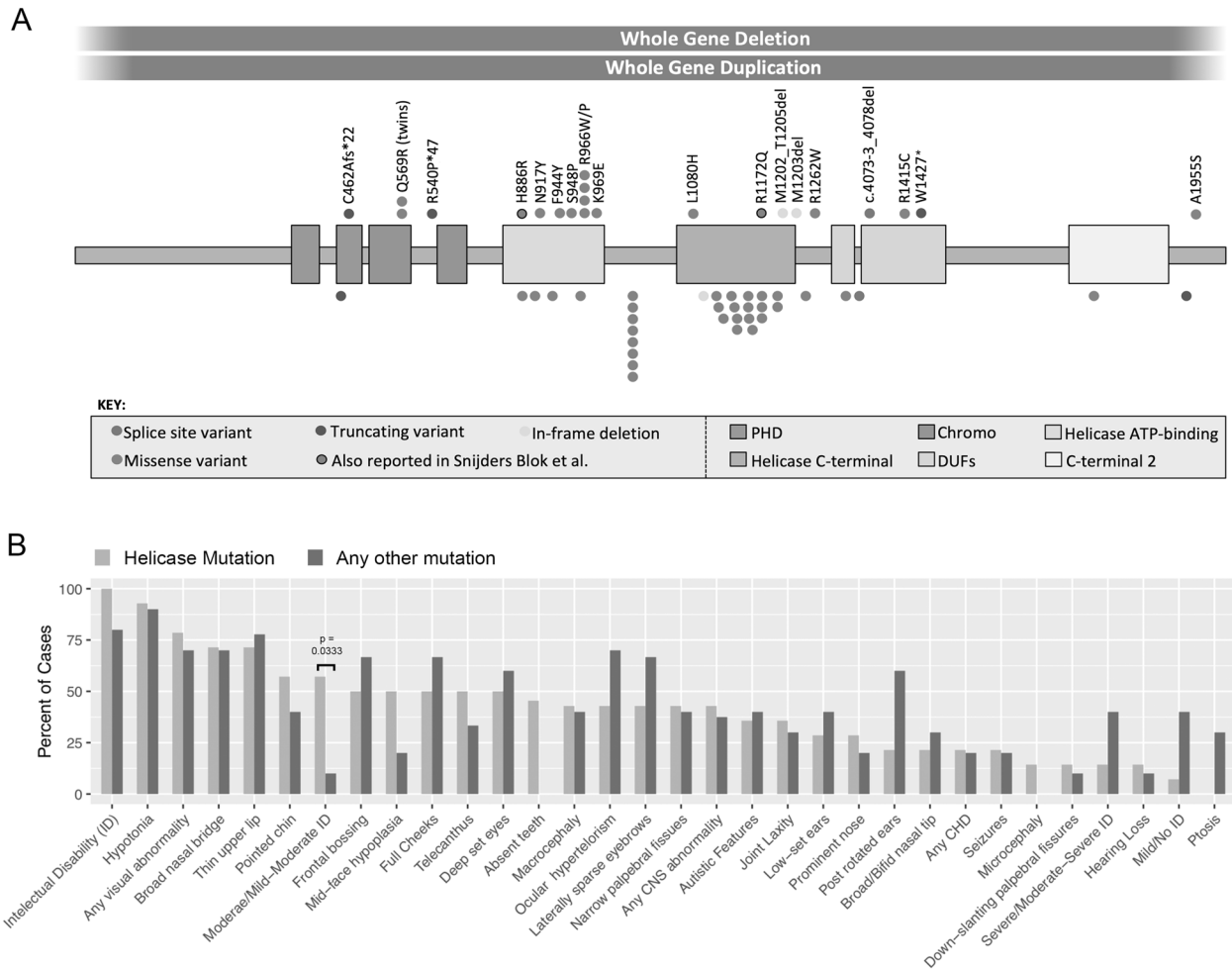
Fig. 1 Phenotypes and *CHD3* variants detected in our patient cohort. a Schematic depicting the CHD3 protein with colored boxes corresponding to the protein domains listed in the included key, based on the amino acid sequence NP_001005273.1. Genetic variants are indicated by large colored bars indicating deletions/duplications, and by dots, colored as per the included key. Variants displayed above the protein schematic are those that were identified in the cohort we present in this paper. Variants displayed below the schematic are those identified in the initial *CHD3* patient cohort [12]. The different domain types displayed are plant homeodomains (PHD), chromodomains (Chromo), a Helicase domain consisting of two parts (Helicase ATP-binding and Helicase C-terminal), Domains of Unknown Function (DUF), and a C-terminal 2 domain. **b** Graph displaying the frequency of various phenotypic features (shown as a percent of the total for each group) for patients with missense/in-frame deletions within or surrounding the *CHD3* helicase domain (individuals 2 through 15, displayed in green, $n = 14$) compared with patients with any other *CHD3* variant type (individuals 1–1, 1–2, 16 through 23, displayed in purple, $n = 10$). Fishers exact test was used to determine if any significant differences existed between the two groups. p values are displayed for all comparisons with a p value < 0.05—all other p values were >0.05.

variants. It should be noted that there were no significant differences between the two groups for intellectual disability overall, or for intellectual disability that was rated as either Severe/Moderate-to-Severe, or Mild/None. Absent teeth, specifically lateral incisors, were only seen in the group with *CHD3* helicase variants, but very few patients were specifically examined for this finding, and thus this difference was not found to be statistically significant (p value of 0.1186 by Fisher's exact test).

A panel of clinical geneticists was not able to identify any striking facial differences between the patients in the two groups (faces displayed in Fig. 2a). In addition, two composite facial masks were generated—one based on

patients with *CHD3* helicase missense variants, and another for patients with *CHD3* missense variants falling outside of the *CHD3* helicase domain, or with non-missense *CHD3* variants (i.e., loss of function, or whole gene deletion)—using photos of patients from our cohort and the original *CHD3* cohort. No obvious differences were apparent between the facial features shown in the two masks.

For all of these reasons, and because of the evidence that Snijders Blok-Campeau syndrome can be caused by both gain-of-function and loss-of-function changes within the *CHD3* gene, we chose to include all 24 patients in our phenotypic analysis. It is likely that the

Table. 1 Phenotypic findings in Snijders Blok-Campeau syndrome patients, both in our cohort and the previously-published cohort.

Development/Intellect	Our cohort		Initial cohort		Combined cohorts	
	Amount	Percentage	Amount	Percentage	Amount	Percentage
Developmental Delay	24/24	100	35/35	100.0	59/59	100
Speech Delay	24/24	100	33/33	100.0	57/57	100
Hypotonia	22/24	92	21/28	75	43/52	83
Autistic Features	9/24	38	9/31	29	18/55	33
Intellectual Disability (ID)	20/21	95	27/35	77	47/56	84
Severe ID	4/20	20	7/27	26	11/47	23
Moderate to Moderate-Severe ID	9/20	45	8/27	30	17/47	36
Mild to Mild-Moderate ID	7/20	35	9/27	33	16/47	34
CNS Anomaly/Seizures						
Seizures	5/24	21	4/34	12	9/55	16
Any Structural CNS abnormality	*9/23*	*39*	*16/28*	*57*	*25/51*	*49*
Prominent extra-axial space	*4/9*	*44*	*10/17*	*59*	*14/26*	*54*
Delayed myelination	*3/9*	*33*	*N/A*	*N/A*	*3/9*	*33*
Congenital Heart Disease (CHD)						
Any CHD	5/24	21	*3/35*	*9*	*8/59*	*14*
Atrial septal defect	3/5	60	1/3	3	4/8	50
Ventricular septal defect	1/5	20	1/3	3	2/8	25
Patent ductus arteriosus	1/5	20	N/A	N/A	1/8	13
Visual abnormality						
Any visual abnormality	18/24	75	23/33	70	41/57	72
Strabismus	6/18	33	10/23	44	16/41	39
Cortical visual impairment	4/18	22	3/23	13	7/41	17
Astigmatism	3/18	17	2/23	9	4/41	10
Hyperopia	3/18	17	11/23	48	14/41	34
Myopia	3/18	17	1/23	4	4/41	10
Head/Face						
Macrocephaly	10/24	42	19/33	58	29/57	51
Microcephaly	2/24	8	1/33	3	3/57	5
Frontal bossing	13/23	57	11/33	33	24/56	43
Full Cheeks	13/23	57	N/A	N/A	13/23	57
Pointed chin	12/24	50	N/A	N/A	12/24	50
Mid-face hypoplasia	9/24	38	N/A	N/A	9/24	38
Eyes						
Ocular hypertelorism	13/24	54	24/31	77	37/55	67
Deep set eyes	13/24	54	N/A	N/A	13/24	54
Laterally sparse eyebrows	12/23	52	N/A	N/A	12/23	52
Narrow palpebral fissues	10/24	42	N/A	N/A	10/24	42
Telecanthus	10/23	44	N/A	N/A	10/23	44
Ears						
Post rotated ears	9/24	38	N/A	N/A	9/24	38
Low-set ears	8/24	33	N/A	N/A	8/24	33
Hearing Loss	3/23	13	N/A	N/A	3/23	13
Nose						
Broad nasal bridge	17/24	71	N/A	N/A	17/24	71
Prominent nose	6/24	25	N/A	N/A	6/24	25
Broad/Bifid nasal tip	6/24	25	N/A	N/A	6/24	25
Mouth						
Thin upper lip	17/23	74	N/A	N/A	17/23	74
Absent teeth	5/15	33	2/30	7	7/45	16

exact underlying molecular pathology differs between patients in different variant groups, but ultimately it appears that all *CHD3* variant types result in a similar phenotypic presentation.

CHD3 recurrent variants

A number of unrelated individuals in the initial Snijders Blok-Campeau syndrome cohort were found to have
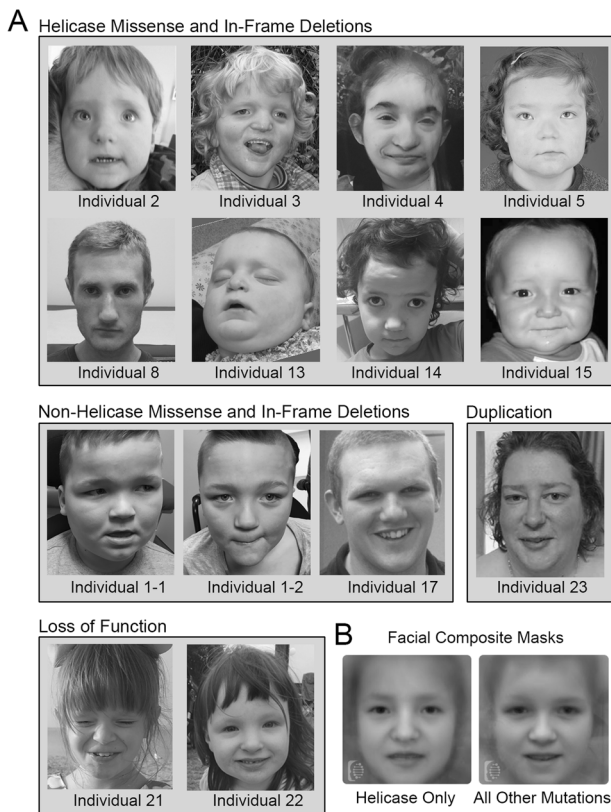
Fig. 2 Facial photographs of *CHD3* patients in our cohort. a Photographs of 14 affected individuals from our cohort, divided by variant type, as indicated. Overall, we note the similar facial appearance between patients in all variant type groups, with a boxy face, prominent forehead, full cheeks, thin upper lip, broad nose and nasal bridge, deep- and widely-set eyes, and pointed chin. We also note that the facial appearance matures over time, with the full cheeks and boxy face becoming less prominent, while the broad forehead, prominent nose, and pointed chin become more defining of the typical facial appearance at older ages. **b** Two composite facial masks were generated. The first, on the left, was generated from 19 photos of patients with missense/in-frame deletion variants within the *CHD3* helicase domain (photographs obtained from both our cohort and the initial CHD3 cohort) [12]. The second, on the right, was generated from 11 photos of patients with any other *CHD3* variant (photographs obtained from both our cohort and the initial CHD3 cohort) [12]. We note a very similar facial appearance between the two masks.

identical variants within the *CHD3* gene, leading to the supposition that certain variants represented recurrent mutations[12]. Of these initially-reported recurrent variants, none were seen in our cohort—in fact, the only previously-reported variants found in our cohort were the p.H886R variant (found in individual 2), and the p.R1172Q variant (found in individual 12). We did, however, identify a recurrent variant within our own cohort, with four unrelated individuals (6, 7, 8, and 9) found to have a *CHD3* variant affecting the arginine residue at amino acid position 966. Again, individuals 1–1 and 1–2, monozygotic twins, were both found to harbor the same *CHD3* variant at amino acid position 569.

Facial features

The facial features observed in the initial cohort of Snijders Blok-Campeau syndrome patients included widely spaced eyes, a broad and bossed forehead, deep-set eyes/periorbital fullness, narrow palpebral fissures, laterally sparse eyebrows, low-set and often simple ears with thick helices, and a pointed chin [12]. Our cohort confirms these observations, finding widely spaced eyes in 13 of 24 patients (54%), prominent/bossed forehead in 13 of 23 patients (57%), deep-set eyes in 13 of 24 patients (54%), narrow palpebral fissures in 10 of 24 patients (42%), laterally sparse eyebrows in 12/23 patients (52%), low set or simple ears in 9 of 24 patients (38%), and a pointed chin in 12 of 24 patients (50%) [12].

In addition to the facial features seen in the initial cohort, other common facial features were apparent in our patient cohort. These include a thin upper lip in 17 of 23 patients (74%), broad nasal bridge in 17 of 24 patients (71%), full cheeks in 13 of 24 patients (57%), and midface hypoplasia in 9 of 24 patients (38%), with a general, but difficult to quantify, square and boxy appearance of the face in most patients, especially at younger ages.

Facial composite masks were generated from all 31 available photos (including the 14 from our cohort, and the photos of patients from the initial cohort)—one for subjects with *CHD3* helicase domain missense variants, and another for all the other subjects.

Structural brain anomalies and macrocephaly

Of the 23 patients in our cohort with any brain imaging, 9 (39%) had structural central nervous system (CNS) anomalies identified. This is similar to the 57% of patients with reported CNS anomalies in the original patient cohort. By far the most common structural CNS anomaly seen in our cohort was widening of the extra-axial spaces, which was present in 4 of the 9 patients with CNS anomalies (44.4%). Again, this is similar to the frequency of this finding in the initial cohort, where 59% of patients with CNS anomalies were found to have some degree of widening of the extra-axial spaces [12].

Interestingly, the presence of prominent extra-axial spaces/ventriculomegaly did not correlate with the presence of macrocephaly in our cohort or in the original cohort. In the initial publication, macrocephaly was a reported finding in 58% of *CHD3* patients. In our cohort, macrocephaly was similarly seen in 10 of 24 patients (42%), while microcephaly was noted in only 2 of 24 patients (8%), with the remaining 50% of patients having head sizes falling within 2 standard deviations of the mean. The mean Z-score for head circumference in our cohort was +0.9 at birth, and +1.3 at the most recent measurement, but with a

standard deviation of 1.5 and 2.7, respectively. Thus, while macrocephaly is a common finding in the Snijders Blok-Campeau syndrome, it is seen in less than 50% of patients in our cohort, with a wide range of head sizes, ranging from microcephalic to macrocephalic.

Following widened extra-axial spaces, the next most common CNS anomaly seen in our cohort was delayed myelination (reported in 3 of the 9 patients with CNS anomalies, 33%). Other CNS anomalies including low white matter volume in 1 patient, cerebral dysgenesis in 1 patient, polycystic structures observed adjacent to the ventricles in 1 patient (seen on head ultrasound, no MRI available), and a short corpus callosum in 1 patient. Again, these findings are in keeping with the diverse set of CNS anomalies seen in the initial cohort.

Vision abnormalities

Strikingly, 18 of 24 patients in our cohort (75%) had some type of vision abnormality. A similar rate of visual impairment was reported in the initial cohort, where 70% of patients were reported as having some type of visual abnormality[12]. Strabismus was seen in 6 of the 18 patients with a reported vision abnormality in our cohort (33%, similar to the 44% reported in the initial cohort), with cortical visual impairment seen in an additional 4 (22%, as compared with 13% of the initial cohort). Astigmatism, myopia, and hyperopia were seen in an additional 3 patients each, with other vision abnormalities included dissociated vertical gaze, exotropia, amblyopia, and esotropia in additional 1 patient each.

Developmental delay and intellectual disability

Similar to the previous cohort, speech delay was seen unequivocally in all 24 of our patients. Intellectual disability was noted in 20 of 21 patients (95%, 3 individuals too young to assess). We also similarly noted that the severity of intellectual disability was very evenly distributed, with 4 patients recorded as having severe intellectual disability, three with moderate to severe, six with moderate, four with mild to moderate, and four with mild. Hypotonia was seen in 22 of 24 of our patients (92%), and autistic features were seen in 9 of 24 patients (38%).

Seizures

Seizures were reported in 5 of 24 patients in our cohort (21%). In one case, seizure activity was confined to a single absence seizure, and in another it was confined to three discreet febrile seizures in infancy. In the other three cases the specifics of seizure frequency/characteristics were not available. Thus, seizure activity appears slightly more prevalent in our group compared with the initial Snijders Blok-Campeau syndrome patient cohort, where 3 of 35 patients (9%) were found to have seizures—one with epilepsy, with an additional two patients reported with neonatal convulsions [12]. There did not seem to be any correlation between the nature/location of the underlying *CHD3* variant and the development of seizures in our cohort. Thus, seizure activity appears slightly more prevalent in our group compared with the initial Snijders Blok-Campeau syndrome patient cohort, where 3 of 35 patients (9%) were found to have seizures—one with epilepsy, with an additional two patients reported with neonatal convulsions [12]. There did not seem to be any correlation between the nature/location of the underlying *CHD3* variant

Congenital heart disease

Congenital heart disease (CHD) was seen in only 5 of 24 (21%) of patients, mostly accounted for by atrial septal defects (seen in 3 patients), followed by patent ductus arteriosus (in 1 patient) and ventricular septal defect (in 1 patient). This is in keeping with the types and frequency of CHD reported in the initial *CHD3* patient cohort [12].

Other findings

Undescended testes and other male genitourinary anomalies, which were present in 37% of males in the initial Snijders Blok-Campeau syndrome patient cohort [12], were reported in only 2 of 10 males (20%) in our cohort, with unilateral undescended testicle reported in two boys. One male was also found to have kidney stones, and one female patient in our cohort was noted to have polycystic ovarian syndrome. Umbilical, inguinal, and hiatal hernias, which were frequently reported in the initial patient cohort (18% of cases) [12], were noted in only one of the patients presented here (4%).

Interestingly, 5 patients (21% of all patients, and 33% of patients who were specifically examined for dental anomalies) were noted to have absent adult teeth, with three noted to have absent lateral incisors, and one patient each with absent upper molars, or single unspecified absent tooth. In the initial cohort, mention is made of two patients with absent lateral incisors, but it is unclear how many patients had been specifically examined for this finding.

Joint laxity was reported in 8 of 24 patients (33%). Hearing loss was uncommon, found in only 3 patients (13%). Dermatologic findings seen in our cohort included, in one patient each, café au lait marks, unusual nuchal skin folds, dry skin, abnormally thick scars, nevus flammeus, deep creases on the soles of the feet, dysplastic toe nails, and a patch of hypopigmented occipital hair.

Discussion

Here we have presented the second large cohort of Snijders Blok-Campeau syndrome patients with variants in the *CHD3* gene, bringing the total number of known patients to 59. This cohort corroborates many of the findings reported in the initial publication by Snijders Blok et al., and also refines and expands the characteristic phenotype and molecular architecture of the disorder.

Overall, with the addition of our patients to the initial cohort, we are better able to define the typical phenotype of the Snijders Blok-Campeau syndrome. A comparison of the findings in our cohort and in the original cohort is shown in Table 1. It is clear that certain features—developmental delay, speech delay, intellectual disability, hypotonia, and abnormalities of vision, for example—are nearly universally present in all patients. In addition, the facial features characteristic of the syndrome are now even more apparent. Altogether, we feel that the constellation of a square/boxy face with a bossed forehead, a thin upper lip, full/prominent cheeks, midface hypoplasia, wide and deeply-set eyes with small palpebral fissures, and laterally sparse eyebrows define a recognizable set of facial features that can aid in the clinical diagnosis of the Snijders Blok-Campeau syndrome. In addition, in our cohort and in the cohort presented in the initial publication, the wide range of ages of patients makes it possible to further define the facial features over time. Patients appear to initially demonstrate many of the above-mentioned cardinal facial features of the syndrome. As the patients age, however, some of these features become less prominent (the boxy face and full cheeks, for instance), while some features, such as the frontal bossing, prominent and wide nose, and in particular the pointed chin, persist and become the defining features of the face. Going forward, we believe that the astute clinician will be able to recognize this facial appearance, helping to aid in the diagnosis of Snijders Blok-Campeau in future patients.

One major question that remained after the publication of the initial Snijders Blok-Campeau syndrome cohort was whether the location or nature of *CHD3* variants correlated with particular phenotypic features. In the initial cohort, of the 35 patients reported, all but 3 were found to have missense variants or single amino acid deletions, with only 1 splicing variant and two truncating variants identified. In addition, the majority of missense variants identified (29 of 31) fell within the helicase domain of the CHD3 protein. These findings begged the question—are helicase domain missense variants the major, or even sole cause of the typical Snijders Blok-Campeau syndrome phenotype? Furthermore, there appeared to be some evidence that particular phenotypic features correlated with particular variant types/locations—for example, the only patient with epilepsy in the initial cohort was noted to be the only patient with a missense variant in the C-terminus of the CHD3 protein.

The findings in our cohort do not support an obvious genotype-phenotype correlation for the Snijders Blok-Campeau syndrome. Furthermore, our report provides evidence that a variety of *CHD3* variants—not only those affecting the helicase domain—result in the typical Snijders Blok-Campeau syndrome phenotype. Of the 24 patients in our cohort, 14 were found to have typical missense/in-frame deletion variants affecting the CHD3 helicase domain, but 10 were found to have different types of *CHD3* variants. These included one complete *CHD3* duplication, one complete *CHD3* deletion, three truncating variants, one splicing variant, and four missense variants outside of the helicase domain. These 10 patients did not stand out in any way from the other 14 patients in our cohort. Even after performing a comprehensive analysis of phenotypic differences between the two groups, only very few differences could be identified. The facial features between these two groups were also not strikingly different, with patients in both groups judged to have the typical facial appearance for the Snijders Blok-Campeau syndrome (as illustrated by the composite facial masks in Fig. 2b).

This finding is particularly interesting in light of the fact that both activating and inactivating variants in the *CHD3* helicase domain (as determined by assays of CHD3 ATPase activity and chromatin remodeling) were shown to result in the typical Snijders Blok-Campeau syndrome phenotype [12]. The data from our cohort, particularly the fact that *CHD3* whole gene deletions and duplications were found in patients with very typical Snijders Blok-Campeau syndrome presentation, adds further evidence to the idea that perturbations of CHD3 function of all kinds might ultimately converge to produce a similar phenotypic output. It must be noted, however, that there is insufficient evidence at this time to make any definitive statement about the pathogenicity of *CHD3* gene duplications in Snijders Blok-Campeau syndrome, as only this single *CHD3* duplication patient has been described thus far. Future clinical and functional work remains to determine if and how both overactivity and underactivity of the *CHD3* gene might result in the same constellation of phenotypic findings.

Taken together, the findings presented in this paper, and those presented in the initial publication on the Snijders Blok-Campeau syndrome [12], solidify the syndrome as a recognizable human neurodevelopmental disorder with stereotypical dysmorphic facial features. We have expanded the list of molecular mechanisms known to cause the syndrome, and show that any perturbation of *CHD3* function appears to result in the same characteristic human phenotype [12].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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