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Clinical Phenotypes of Carriers of Mutations in *CHD8* or Its Conserved Target Genes

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ABSTRACT

BACKGROUND: Variants disruptive to *CHD8* (which codes for the protein CHD8 [chromodomain-helicase-DNA-binding protein 8]) are among the most common mutations revealed by exome sequencing in autism spectrum disorder (ASD). Recent work has indicated that *CHD8* plays a role in the regulation of other ASD-risk genes. However, it is unclear whether a possible shared genetic ontology extends to the phenotype.

METHODS: This study (N = 143; 42.7% female participants) investigated clinical and behavioral features of individuals ascertained for the presence of a known disruptive ASD-risk mutation that is 1) *CHD8* (*CHD8* group) (n = 15), 2) a gene targeted by CHD8 (target group) (n = 22), or 3) a gene without confirmed evidence of being targeted by CHD8 (other gene group) (n = 106).

RESULTS: Results indicated shared features between the *CHD8* and target groups that included less severe adaptive deficits in communication skills, similar functional language, more social motivation challenges in those with ASD, larger head circumference, higher weight, and lower seizure prevalence relative to the other gene group.

CONCLUSIONS: These similarities suggest broader genetic ontology accounts for aspects of phenotypic heterogeneity. Improved understanding of the relationships between related disruptive gene events may lead us to improved understanding of shared mechanisms and lead to more focused treatments for individuals with known genetic mutations.

Keywords: Autism spectrum disorder, *CHD8*, Gene regulation, Genetic subtypes, Neurodevelopmental disorder, Precision medicine

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder defined by social communication deficits and the presence of repetitive or restricted behaviors. Although ASD is genetically heterogeneous, rare variants in the form of inherited and de novo copy number variants and largely de novo single-nucleotide variants (SNVs) are estimated to contribute 5% to 10% to the underlying genetic etiology of autism, with no single event accounting for more than ~1% of all cases (1–9). Bridging underlying genetic etiology with ASD symptoms is complex, considering that the ASD phenotype is similarly variable, ranging from severe to mild neurocognitive symptoms. Efforts to identify subtypes based on the behavioral and neural phenotypes have been unsuccessful (10), and the rarity of specific genetic events has rendered subtyping at the copy number variant or single gene mutation level challenging and exceedingly resource intensive. A promising avenue of research instead targets biological pathways shared by multiple ASD-associated risk genes as a means to better explain individual differences in ASD.

One such potential pathway involves the gene that codes for chromodomain-helicase-DNA-binding protein 8 (CHD8) (located at 14q11.2) (8,11). CHD8 is a chromatin modifier (12), and disruptive variants to *CHD8* are among the most common mutations revealed by sequencing efforts in ASD cohorts (8,13,14). Moreover, thousands of CHD8 binding sites have been identified throughout the genome using chromatin immunoprecipitation followed by sequencing in primary human and mouse brain tissue as well as in in vitro models (13–15) enriched for ASD-risk genes (13). A specific human phenotypic profile has been described for *CHD8* mutation carriers, including ASD diagnosis or related symptoms, macrocephaly and brain overgrowth, intellectual disability (ID), specific facial features, gastrointestinal (GI) problems, and sleep disturbances (11,13). However, to date, no study has evaluated the extent to which humans with mutations to genes modified by CHD8 show a similar phenotypic profile.

Given the high rate of ASD among individuals with a mutation to *CHD8* (11) and the regulatory effects of CHD8 on other ASD-risk genes (i.e., target genes) (13,14), the aim of the current study was to evaluate whether mutations to *CHD8* and target genes together constitute an etiological subtype of ASD. Among a sample of 143 individuals, we compared and contrasted the phenotypic presentations of individuals with an ASD-associated risk variant that directly disrupts CHD8, disrupts a conserved CHD8 target gene, or disrupts a gene not identified as a conserved CHD8 target. Specifically, assuming

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a converging biological pathway, we hypothesized that those individuals with disruptive mutations to *CHD8* or CHD8 targets would exhibit a unique phenotype from individuals with disruptive mutations to genes not directly targeted by CHD8.

METHODS AND MATERIALS

Participants

Participants included 143 individuals (range, 44 months of age to 28.3 years of age; 42.7% female, 126 white) with disruptive mutations to ASD-associated risk genes. Consistent with a genetics-first approach, recruitment was not contingent on specific clinical diagnoses (e.g., ASD or no ASD). Participants were ascertained following identification of a disruptive genetic variant through clinical genetic testing (n = 80) or genetic testing conducted during participation in a research study in which participant recontact was possible (n = 63). There were no differences in ascertainment method between gene groups (p = .11). Written and informed consent and/or assent were obtained for all participants and approved by the local ethical review board.

For all participants, presence of a disruptive variant was confirmed through review of the clinical genetic testing lab report or through targeted or exome sequencing conducted as part of the referring study (16). Gene and variant information for participants are listed in Table S2 in Supplement 2. The majority of genetic mutations were found to be de novo (n = 126); however, 4 were inherited (1 from the target group, 3 from the other gene group) and 13 were of unknown inheritance because one or both parents did not complete genetic testing (1 from the target group, 12 from the other gene group). Supplemental analyses were conducted to assess whether inheritance status influenced our results (Supplement 1).

Participants were assigned to one of three mutually exclusive groups. The *CHD8* group (n = 15) included individuals with a disruptive mutation to *CHD8*. The target group (n = 22) included participants with a disruptive mutation to an ASD-risk gene previously identified as a conserved *CHD8*-bound target. Target group designation required positive evidence of CHD8-binding sites and coexpression with CHD8 [per Sugathan *et al.* (14)] as well as expression in the human brain [per Cotney *et al.* (13)], as specified in Table S3 in Supplement 2. Target group genes included *ARID1B*, *CTNNB1*, *PTEN*, *SETBP1*, *TBL1XR1*, and *TRIP12*. The other ASD-associated gene group (other gene group) (n = 106) included participants with a disruptive mutation to an ASD-associated gene (16) that has not been identified as a conserved CHD8 target [i.e., a lack of evidence or lack of consensus between Cotney *et al.* (13) and Sugathan *et al.* (14)]: *ADNP*, *ANK2*, *ASH1L*, *CAPN8*, *CHD1*, *CHD2*, *DSCAM*, *DYRK1A*, *FOXP1*, *GRIN2B*, *KDM6B*, *LARP4B*, *LZTR1*, *MED13L*, *MYH10*, *NCKAP1*, *POGZ*, *SCN2A*, *SETD2*, *STXBP1*, *SUV420H1*, *SYNCRIP*, *TBR1*, *WDFY3*, *WDR33*. The groups did not vary significantly based on age (p = .31) or sex (p = .38) of participants (see Table 1).

Measures

Clinical testing included direct assessment using standardized procedures, with the clinician naïve to the specific genetic mutation until final stages of data analysis. The assessment battery included measures of cognition, adaptive abilities, ASD symptoms, medical diagnoses, and physical measurements.

Clinical Assessment of ASD Symptoms. Research reliable clinicians administered the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), using the module appropriate for the individual based on language ability (17). The Autism Diagnostic Interview-Revised (18) was administered to support ASD diagnostic decisions. To compare group differences in language, the module of the ADOS-2 was noted (module 1 for those with no speech or single words, module 2 for those with phrase speech, modules 3 and 4 for those with fluent speech). ADOS-2–calibrated severity score (CSS) was calculated for the total score as well as for the social affect and restricted and repetitive behavior domains. CSS ranges from 1 to 10, with scores of 8 to 10 in the high range, scores of 5 to 7 in the moderate range, scores of 3 to 4 in the low range, and scores of 1 to 2 in the minimal to no evidence range. Neurodevelopmental diagnoses (e.g., ASD, ID, global developmental delay) were assigned by licensed clinical psychologists per the DSM-5 (19) using information from testing results; clinical observations; and developmental, medical, and psychiatric history. Parents also completed the Social Responsiveness Scale, Second Edition (SRS-2) (20), from which standardized T scores were generated (mean 50 $\pm$ 10) for the total score and 5 subscale scores (i.e., social awareness, social cognition, social communication, social motivation, and autistic mannerism).

Cognitive, Language, and Adaptive Functioning. Participants 4 to 17 years of age were administered the Differential Abilities Scales, Second Edition (21). Participants 18 years of age and older were administered the Wechsler Abbreviated Scales of Intelligence, Second Edition (22). For both instruments, standardized deviation scores (mean 100 $\pm$ 15) were generated, except when the participant's level of functioning did not allow for calculation of a deviation score (i.e., performance below the floor); in those cases, ratio scores were calculated by dividing mental age (defined as normative group-referenced age equivalencies) by the participant's chronological age. In 9 cases, valid test administration could not be conducted owing to functioning level below the floor of the test. When this occurred, the floor deviation score was used to estimate an IQ value for the participant (i.e., Differential Abilities Scales-Second Edition scores of 31 for full-scale IQ or 30 for verbal and nonverbal IQ). To assess adaptive functioning, parents were administered the survey interview form of the Vineland Adaptive Behavior Scales, Second Edition (Vineland-II) (23). An overall score, the adaptive behavior composite, as well as domain scores in communication, daily living skills, and socialization, were generated (mean 100 $\pm$ 15). The Achenbach System of Empirically Based Assessment was used to assess internalizing and externalizing behavior challenges, specifically, using the Child Behavior Checklist (24) or Adult Behavior Checklist (25), where appropriate.

Medical Diagnoses. The medical history was collected via structured interview adapted from the Simons Simplex Collection (26) and involved characterization of comorbid medical issues. Review of past medical records and past

Table 1. Clinical and Behavioral Characterization

	CHD8 Group			Target Group			Other Gene Group			Group Difference	*p*
Participants											
n	15			22			106				
Female:Male, *n*	6:9			12:10			43:63			$\chi^2_2 = 1.96$	.375
Age, Months, Mean ± SD	138.67 ± 67.7			108.05 ± 47.8			128.25 ± 65.7			$\chi^2_2 = 2.33$	.311
Age Range, Months	56–260			44–221			47–340				
	%	Prev[a]	*n*	%	Prev	*n*	%	Prev	*n*		
Ascertainment											
Research study	66.7	10	15	31.8	7	22	43.4	46	106	$\chi^2_2 = 4.51$	.105
Clinical report	33.3	5	15	68.2	15	22	56.6	60	106		
ASD Symptoms											
ASD diagnosis	100.0	15	15	70.6	12	17	77.2	71	92	$\chi^2_2 = 4.90$	.086
ADOS-2 Module											
Module 1	21.4	3	14	35.3	6	17	50.0	43	86	$\chi^2_6 = 24.89$	<.001
Module 2	42.9	6	14	58.8	10	17	12.8	11	86		
Module 3	35.7	5	14	0.0	0	17	33.7	29	86		
Module 4	0.0	0	14	5.9	1	17	3.5	3	86		
Cognitive and Adaptive Functioning											
ID diagnosis	53.3	8	15	86.4	19	22	75.7	78	103	$\chi^2_2 = 5.04$	.081
Adaptive deficit[b]	60.0	9	15	77.3	17	22	86.0	86	100	$\chi^2_2 = 5.42$	.066
Medical Problems											
Sleep issues	100	15	15	81.8	18	22	90.3	93	103	$\chi^2_2 = 4.52$	.103
Seizure activity	13.3	2	15	18.2	4	22	44.0	44	100	$\chi^2_2 = 9.98$	.007
Gastrointestinal problems	80.0	12	15	77.3	17	22	81.0	81	100	$\chi^2_2 = 0.16$	.925
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*		
ASD Symptoms											
ADOS-2 CSS	7.93	1.69	14	5.88	2.34	17	6.47	2.38	86	$\chi^2_2 = 6.06$	.048
Social affect CSS	7.36	2.06	14	5.76	2.36	17	6.44	2.54	86	$\chi^2_2 = 3.54$	.170
RRB CSS	9.07	1.21	14	6.82	2.43	17	7.07	2.39	86	$\chi^2_2 = 12.3$	.002
SRS-2 Total T Scores	82.27	9.12	15	76.21	11.98	19	76.31	11.2	98	$F_{2,129} = 1.92$	.150
Social Motivation	76.13	12.57	15	64.95	11.21	19	64.84	12.68	98	$F_{2,129} = 5.44$	.005
Awareness	76.07	8.12	15	73.53	8.64	19	73.45	10.66	98	$F_{2,129} = 0.44$	.646
Cognitive	78.67	11.17	15	73.47	11.24	19	75.17	9.74	98	$F_{2,129} = 1.15$	.321
Communication	79.47	8.21	15	75.84	13.7	19	76.43	11.49	98	$F_{2,129} = 0.51$	.600
Mannerisms	81.47	10.84	15	76.74	16.79	19	74.71	13.58	98	$F_{2,129} = 1.61$	.204
Cognitive and Adaptive Functioning											
Full-scale IQ	62.0	26.39	12	49.88	17.98	17	50.49	27.51	90	$\chi^2_2 = 2.93$	.231
Verbal IQ	63.75	28.99	12	55.59	24.4	17	50.3	28.34	80	$\chi^2_2 = 3.89$	.143
Nonverbal IQ	60.64	28.55	14	47.76	17.63	17	51.64	28.63	80	$\chi^2_2 = 1.86$	.395
VABS-II Composite	63.4	20.02	15	61.68	11.21	22	54.81	15.06	100	$F_{2,134} = 3.43$	.035
Communication	68.67	22. 36	15	65.5	14.64	22	55.33	16.48	100	$F_{2,134} = 6.34$	.002
Socialization	63	18.28	15	64.68	13.98	22	58.7	14.08	100	$F_{2,134} = 1.85$	.161
Daily Living	64.53	19.76	15	62.27	10.93	22	56.33	16.87	100	$F_{2,134} = 2.44$	.091
Physical Features (*z* Scores)											
Head circumference	1.88	1.61	12	0.64	2.59	17	−0.49	2.14	81	$F_{2,107} = 7.29$	.001
Height	1.93	1.62	12	0.02	1.62	15	−0.41	1.3	79	$F_{2,103} = 14.97$	<.0001
Weight	1.13	0.81	11	0.57	1.17	15	−0.24	1.62	75	$F_{2,98} = 5.13$	.008
Behavior Problems											
CBCL internalizing	61.21	7.07	14	57.41	8.43	17	59.26	11.06	84	$F_{2,102} = 0.53$	.592
CBCL externalizing	51.21	9.29	14	54.24	9.58	17	57.69	11.18	84	$F_{2,102} = 4.04$	.02

ADOS-2, Autism Diagnostic Observation Schedule, Second Edition; ASD, autism spectrum disorder; CBCL, Child Behavior Checklist; CSS, calibrated severity score; Prev, prevalence; RRB, restricted and repetitive behaviors; SRS-2, Social Responsiveness Scale, Second Edition; VABS-II, Vineland Adaptive Behavior Scales, Second Edition.

[a]For categorical variables, prevalence is reported as the total number in each group with the condition.

[b]Defined as having an adaptive behavior composite score at or below 70 on the VABS-II.

psychological and educational testing was also conducted to confirm parent report of diagnoses. Standardized medical exams were conducted by a licensed medical geneticist and included assessment of dysmorphic features, physical exam, and review of systems. Continuous measurements of occipital frontal head circumference, body height, and body weight were transformed to age- and sex-standardized *z* scores. Standard scores for head circumference were calculated using population norms based on white children (27), given that ethnicity-specific growth charts did not have publicly available norms (i.e., Hispanic children [$n = 7$ in our study]) and were not appropriate for mixed race/ethnicity ($n = 7$), and specific race designations (e.g., South Asian vs. Chinese) were not available ($n = 3$). Standard scores for height and weight were generated using norms from the National Health and Nutrition Examination Survey (28) of a representative sample of children in the United States. Dichotomous variables were derived to indicate whether individuals had medical problems related to the following: GI issues, seizure activity, and sleep disturbances.

Statistical Analyses

Analyses were conducted in SPSS version 19 (IBM Corp., Armonk, NY), with a focus on targeting effects of genetic group using a series of analyses of variance. Kruskal-Wallis and Mann-Whitney tests were used when distributions violated the assumption of normality for the following variables: age of participants, IQ, and CSS. Binary logistic regression was used to compare groups for dichotomous variables (e.g., medical complaints), with one exception: 100% of the *CHD8* group had an ASD diagnosis; thus, pairwise χ^2 tests were conducted. χ^2 test was used to compare groups on language ability. Omnibus results, means, and standard deviations are presented for variables in Table 1. Bonferroni correction was applied by SPSS for all pairwise comparisons to account for conducting 3 comparisons in each analysis following analysis of variance; *p* values reported in Table 1 are adjusted and should be interpreted with $p < .05$.

RESULTS

Autism Symptoms

Consistent with the *CHD8* phenotype (11), all children with *CHD8* mutations in this cohort were diagnosed with ASD. Importantly, the prevalence rate of ASD was higher in the *CHD8* group relative to both the target group (70.6%) ($\chi^2_1 = 5.23$, $p = .022$) and the other gene group (78%) ($\chi^2_1 = 4.26$, $p = .039$). No differences were found between prevalence of ASD between the target and other gene groups ($p = .56$). Group differences in ADOS-2 CSS (Figure 1) indicated increased severity of ASD symptoms in the *CHD8* group relative to both other groups (*p* values $<.037$). A similar pattern was found on the repetitive and restricted behaviors subscale but not the social affect subscale. Results were consistent when only those individuals with ASD were included in the analyses. The lack of social differences between groups was consistent with caregiver report on the SRS-2, with the exception of the social motivation subscale. As illustrated in Figure 1, results indicated that the *CHD8* group exhibited more problems with social motivation than both the other gene group ($p = .004$) and the target group ($p = .032$). There were no differences between the target and other gene groups ($p = 1.0$). However, within the subset of participants with an ASD diagnosis, the target group had similarly elevated social motivation problems as did the *CHD8* group ($p = .090$), unlike the other gene group ($p = .005$).

Cognitive, Language, and Adaptive Functioning

Groups had comparable rates of ID, and there were no group differences in cognition across any of the continuous measures ($\chi^2_2 < 3.89$, *p* values $> .09$). However, normality tests indicated a bimodal distribution present for the other gene (*p* values $< .0001$) but not the *CHD8* or target groups (*p* values $> .15$) (see Figure S1 in Supplement 1).

Groups differed on language ability based on the module of the ADOS-2 administered ($\chi^2_6 = 24.89$, $p < .001$). There was no difference between the *CHD8* group and target group ($\chi^2_3 = 7.78$, $p = .051$), and both the *CHD8* group ($\chi^2_3 = 9.04$, $p = .029$) and target group ($\chi^2_3 = 21.34$, $p < .001$) differed from the other gene group.

Group differences in overall adaptive functioning were significant at an omnibus level, but pairwise comparisons did not survive correction for multiple comparisons (*p* values $> .13$, Bonferroni) (see Figure 2). Consistent with functional language differences found on ADOS-2 module, a pattern of less severe deficits in the *CHD8* and target groups relative to the other gene group was observed specifically for the communication domain of the Vineland-II ($p = .002$), such that *CHD8* ($p = .015$) and target ($p = .035$) groups had substantially less severe communication challenges than the other gene group. There were no group differences in socialization or daily living skills.

Groups differed on parent ratings of externalizing behavior problems ($p = .020$) but not internalizing problems ($p = .592$), such that the *CHD8* group had similar scores to the target group ($p > .999$) and had fewer problems than the other gene group ($p = .036$). There was no difference in ratings of externalizing behavior between the target and other gene groups ($p = .294$).

Medical Conditions

Aligned with prior work associating larger head sizes with the *CHD8* phenotype (11), head circumference differed across groups (Figure 3). Specifically, the *CHD8* group had comparable head size to the target group ($p = .405$) but larger head size than the other gene group ($p = .002$). The target and other gene groups did not differ ($p = .156$). Similarly, the *CHD8* group had similar body weight (i.e., *z* score body weight) compared to the target group ($p = 1.000$), but participants in the *CHD8* group were heavier than the other gene group ($p = .017$). The target and other gene group did not differ ($p = .183$). *CHD8* carriers alone were taller (i.e., *z* score body height) than participants in both comparison groups ($F_{2,82} = 11.95$, $p < .001$).

Seizure activity was more prevalent in the other gene group compared with the *CHD8* group ($p = .038$). There was no difference between the *CHD8* and target groups ($p = .70$). There were no significant group differences in prevalence of sleep problems or GI concerns.

Supplemental analyses found consistent results across medical conditions, even when considering the potential influence of a microcephaly-associated gene [i.e., *DYRK1A* (29)]

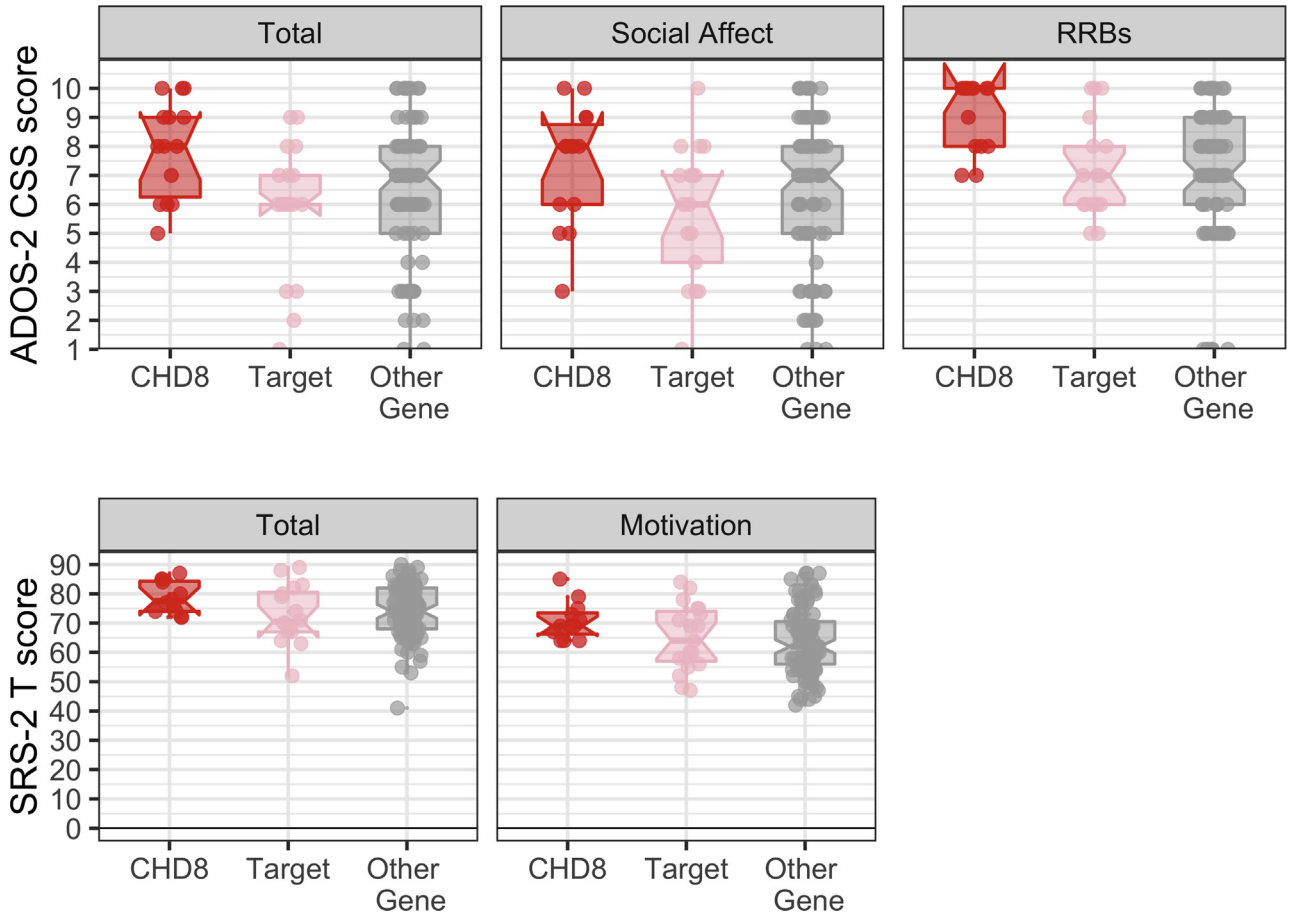

Figure 1. Autism symptoms and social responsiveness problems between groups. Autism symptoms (top row) are measured via Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) calibrated severity score (CSS) and illustrated for total symptoms as well as for social affect and restricted and repetitive behaviors (RRB) subscales. The *CHD8* group demonstrated significantly more RRBs compared to the other groups. Social responsiveness problems (bottom row) are illustrated for the Social Responsiveness Scale, Second Edition (SRS-2) overall T scores and social motivation subscale. Error bars represent SEM. Horizontal bars reflect significant group differences with Bonferroni correction applied. The *CHD8* group had significantly higher scores on this subscale compared with the other gene group, whereas they did not differ significantly from the target group.

(Supplement 1) or a seizure-associated gene [i.e., *SCN2A* (30)] (Supplement 1) and the potential influence of ethnicity on growth parameters (Supplement 1).

DISCUSSION

In this study, we characterized phenotypic profiles of groups of individuals with genetic mutations associated with ASD to evaluate a possible converging biological pathway associated with disruptive mutations to *CHD8* and genes directly regulated by CHD8. Overall, phenotypic comparisons supported our hypothesis that the *CHD8* and target groups were more similar to each other than to the other gene group. Specifically, we found that the *CHD8* and target groups were characterized by increased social motivation problems in those diagnosed with ASD, similar functional language, less severe adaptive deficits in the area of communication skills, less severe externalizing symptoms, large head circumference, higher weight, and a lower incidence of seizures compared with the other gene group. Although rates of sleep and GI problems have previously been reported as common in the *CHD8* group, our analyses indicated that these symptoms are equally prevalent across groups.

Increased head circumference among individuals with *CHD8* mutations is the most replicated finding across human and animal studies (11,31). Neuroimaging of *Chd8* haploinsufficient mice (32,33), measurement of *Chd8* zebrafish interorbital distance (11), postmortem examination of children with or without ASD (34), and tabulation of shared ontological properties of *CHD8* and its targets are all strongly indicative of a model wherein brain overgrowth in early development explains head enlargement. CHD8 target genes have been independently associated with macrocephaly, including *PTEN* and *ARID1B* (35,36). Thus, one hypothesis is that the rates of macrocephaly in individuals with *CHD8* mutations may be secondary to disrupted downstream modulation of these targets. Alternately, each ASD-associated disruptive gene event may be independently affecting head growth, which contributes to the overall heterogeneity in head size among individuals with ASD. Given higher rates of ASD in our *CHD8* and

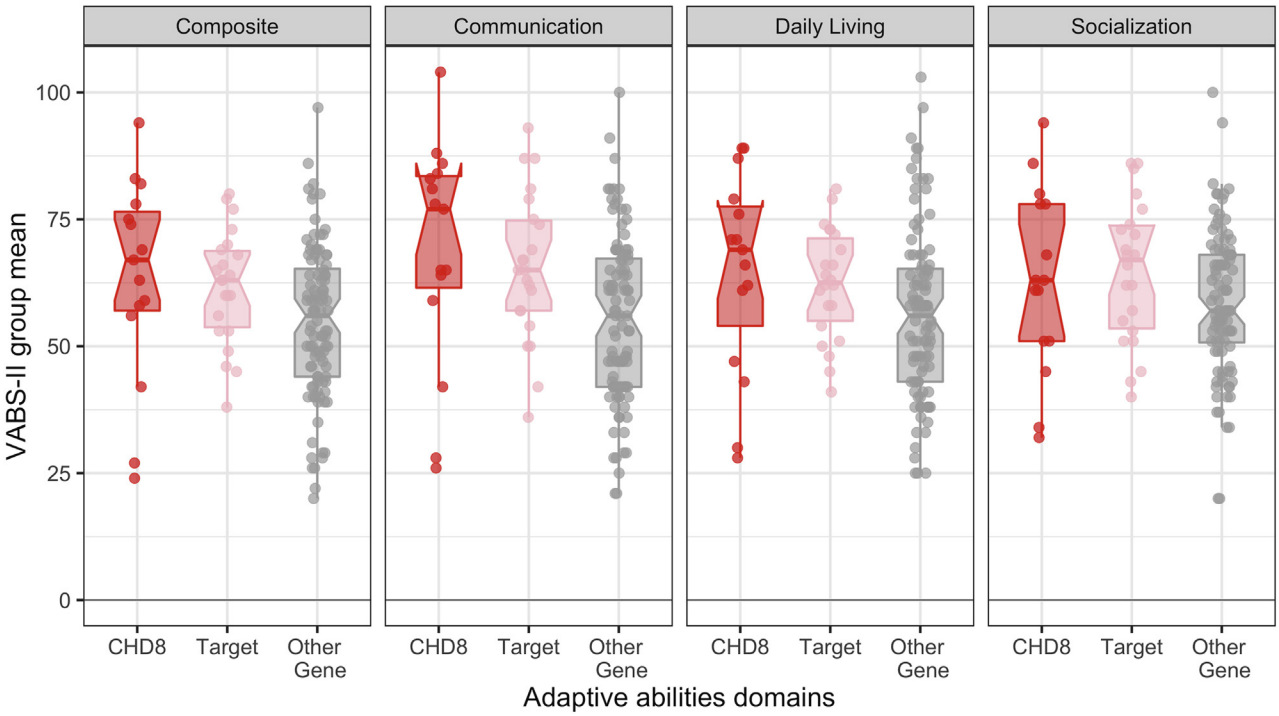

Figure 2. Vineland Adaptive Behavior Scales, Second Edition (VABS-II) standard scores. Adaptive abilities as measured by the VABS-II. Composite and domain standard scores are illustrated for *CHD8* (red, left), target (pink, middle), and other gene (gray, right) groups. Error bars represent SEM. Horizontal bars reflect significant (solid) or trending (dashed) group differences with Bonferroni correction applied. The *CHD8* group did not differ from the target group on any of the domains. The other gene group had significantly greater adaptive deficits compared with the *CHD8* group on the composite score as well as in the communication domain; the other gene group had a trend (*p* values <.089) toward greater adaptive deficits compared with the target group on the composite score as well as in the daily living skills domain.

target groups, we cannot rule out that this shared pattern of larger head circumference may be due to an unknown third factor, explaining higher rates of macrocephaly in ASD more broadly (37–47). Considering that in addition to increased head circumference in the *CHD8* and target groups these two groups also evince larger body weights than the other gene group, overgrowth may be a key phenotypic commonality between individuals with a mutation to *CHD8* and those with a mutation to a gene that is regulated by CHD8. Overall, with the *CHD8* group also measuring significantly taller than the other two groups, results are consistent with prior research that describes individuals with *CHD8* mutations as tall and lean (11).

Rates of ASD were highest in the *CHD8* group, and this group also demonstrated more severe restricted and repetitive behaviors. Because the pattern of higher CSS for the restricted and repetitive behavior domain held even when participants without ASD were excluded from the analyses, it is possible that the repetitive qualities are specific to the *CHD8* presentation. Platt *et al.* (48) found disruption to *Chd8* in mice results in impairments in the ventral striatum. In humans, the striatum, and specifically, the caudate nucleus, have been implicated in the ontogeny of restricted and repetitive behavior (48–50). Taken together, these results may help link converging biological explanations of ASD, such that the potential shared genetic pathways associated with CHD8 may have consequences for the striatal system that, in turn, yield a specific constellation of elevated restricted and repetitive behaviors.

In contrast, all genetic groups scored similarly in the social affect domain of the ADOS-2, as well as on the SRS-2, with the exception of the social motivation subscale, in which the *CHD8* group showed more problems than the other two groups. Because all groups evince similarly high amounts of social difficulty overall, it may be that these measures are tapping into an overall deficit rather than social problems consistent with ASD. Hus *et al.* (51) found that SRS scores may reflect overall impairment in groups that have significant deficits in language or cognition, or when there are significant behavior problems. In the present study, it is also possible that the elevated externalizing behavior problems for children in the other gene group may inflate challenges as reported by parents on the SRS-2. Of note, when considering only those individuals with ASD, the *CHD8* and target groups had similar elevated scores on the social motivation subscale, suggesting the possibility of a shared etiological subtype of ASD associated with poorer social motivation and resulting from or consistent with a shared mechanism. Paired with other work evaluating how potential autism subgroups are linked to genetic etiologies for ASD-specific traits (52), these results may facilitate a deeper understanding of the genetic underpinnings of the social problems in ASD.

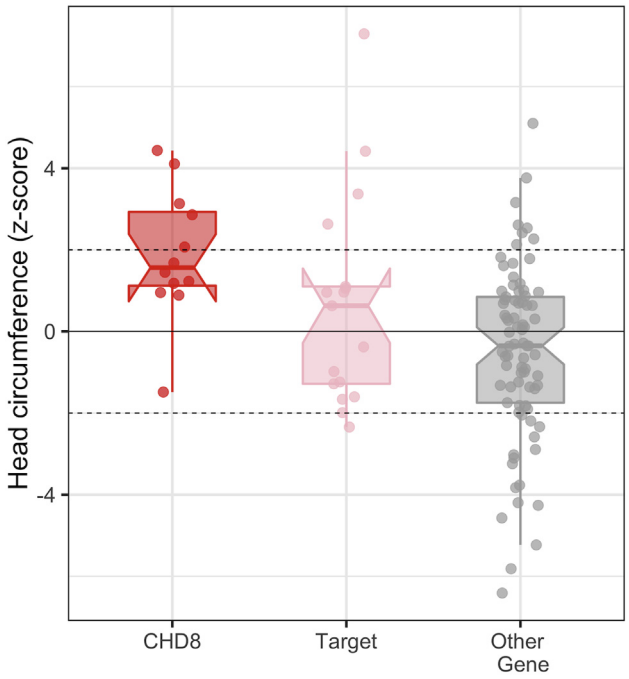

Figure 3. Head circumference distribution. Horizontal bars reflect significant group differences between *CHD8* (red, left), target (pink, middle), and other gene (gray, right) groups. Distribution of head circumference *z* scores indicates larger head sizes for the *CHD8* and target groups relative to the other gene group. Horizontal dashed lines indicate clinical criterion for macrocephaly (*z* score = 2) and microcephaly (*z* score = −2). Solid horizontal lines indicate significant group differences with Bonferroni correction applied.

Overall functioning level did not differ among groups in terms of presence of ID or IQ scores, and adaptive skill differences were limited to the area of communication with significant differences in gross indicators of language abilities as measured by the communication domain of the Vineland-II and differences in the module of the ADOS-2 that was administered. Within a larger sample, particularly within the *CHD8* and target groups, it is possible that we may see more nuanced differences in terms of global functioning between groups. The bimodal distribution observed within other gene group may indicate a potential subgroup within our designated group, such that better specifying functioning ability may better elucidate classification between groups. It is also possible that language skills are more sensitive to our level of measurement.

Contrary to our hypothesis, we found similar rates of GI and sleep problems across groups. Elevated GI problems have previously been reported in individuals with disruptive mutations to *CHD8* and were recapitulated in an animal model (11); however, the current study reports similarly high rates of GI problems associated with other disruptive genetic events. Similarly, while sleep issues are known to be more prevalent and more severe in ASD (53,54), the extent to which genetic etiology dictates sleep mechanisms is unclear. Chromatin modification is linked to the maintenance of circadian rhythms (55), which may explain high rates of sleep disruptions in children with *CHD8* mutations (11). However, both the target and other gene groups exhibited similarly high prevalence of sleep issues to *CHD8* carriers, potentially driven by a different kind of shared disruption [e.g., dysregulation of fundamental neurotransmitters (56)], though other alternatives beyond neurotransmission may also be implicated [e.g., medical and behavioral conditions (57)].

It may be worth considering moderating or mediating factors that may impact overall functioning for individuals with mutations to ASD-risk genes. For one, CHD8 predominantly downregulates genes involved in cell adhesion, axonal guidance, and calcium signaling pathways (31) that are functions critical for cortical development. However, there may be differential phenotypic patterns based on the regulating mechanism. Future work would benefit from careful evaluation of specific variants to identify alternative pathways and developmental processes, as well as explore other potential mechanisms (e.g., protein-protein interactions).

There are several limitations to the current study. The sizes of the *CHD8* and target groups relative to the other gene group are small, potentially making it difficult to elucidate differences statistically owing to power limitations. Future work will be necessary to replicate and more deeply evaluate subtle aspects of the phenotype, considering that the implication of the significant findings here should be tempered, given our sample size and statistical limitations (i.e., Bonferroni correction). In addition, although the group designations were generated based on conservative expectations of molecular pathway given criteria per Cotney *et al.* (13) and Sugathan *et al.* (14), it is possible that these groups are improperly specified. The conservative approach may have resulted in genes being included in the other gene group that are, in fact, regulated by CHD8. In addition, this strategy does not account for other indirect regulatory relationships. Further, it is important to note possibilities of ascertainment bias, such that participants were recruited from previous studies on ASD, specifically. Last, there is a need for more nuanced measures of social functioning to enable clearer phenotypic characterization of social deficits associated with etiological subtypes of ASD. There are potential concerns regarding using the SRS-2 in severely affected populations (58), and it is possible that the social features measured in the current study (e.g., CSS of the ADOS-2, socialization domain of the Vineland-II) are not sufficient to adequately describe the specific deficit areas. Future work should evaluate other implicit measurements that may better capture social characteristics that are impacted in individuals with these gene events with and without ASD, especially in a globally impaired sample.

Conclusions

Whereas recent efforts have focused on phenotypic characterization of small groups of individuals with mutations to a specific gene or genomic region, in this study we hypothesized a neurodevelopmental subtype defined by gene-gene interactions and shared genetic ontology. We found support for a pathogenic effect of early, atypical neurogenesis shared by individuals with disruptions to *CHD8* or CHD8 targets. Functional genetic clustering is a promising step toward development of precision medicine approaches to ASD and associated neurodevelopmental disorders.

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