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RESEARCH ARTICLE

A common genetic variant in the Neurexin family member *CNTNAP2* is related to language but not communication skills in youth with Autism Spectrum Disorder

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Abstract

One of the candidate genes related to language variability in individuals with Autism Spectrum Disorder (ASD) is the contactin-associated protein-like 2 gene (*CNTNAP2*), a member of the Neurexin family. However, due to the different assessment tools used, it is unknown whether the polymorphisms of the *CNTNAP2* gene are linked to structural language skills or more general communication abilities. A total of 302 youth aged 7 to 18 years participated in the present study: 131 verbal youth with ASD (62 female), 130 typically developing (TD) youth (64 female), and 41 unaffected siblings (US) of youth with ASD (25 female). Blood samples were collected to obtain genomic DNA and processed by the Rutgers University Cell and Data Repository or using standard protocols (Gentra Puregene Blood DNA extraction kit; Qiagen). Language and verbal communication skills were screened with the Clinical Evaluation of Language Fundamental-4 (CELF-4) and Vineland-II Communication domain, subsequently. The results showed that the polymorphism of *CNTNAP2* (SNP rs2710102) was related to structural language abilities, such that participants carrying the A-allele had lower language skills in comparison to the G-allele homozygotes. No relationship was found between the polymorphism of *CNTNAP2* and

more general communication abilities. Although the study revealed genetic mechanisms that are associated with CELF-4 measures but not Vineland-II in youth with ASD, follow-up studies are needed that will include measures of language and communication that are less correlated to each other as well as will include a group of minimally and/or non-verbal individuals with ASD.

Lay Summary

Language impairment is one of the most common co-occurring conditions in Autism Spectrum Disorder (ASD). However, its genetic mechanisms are still not very well understood. Moreover, male and female autistic individuals have different profiles with respect to language/communication as well as brain functioning. In this study, we reveled, for the first time, in a representative sex-balanced sample of participants that the common variation of *CNTNAP2* gene (SNP rs2710102) was related to language skills but not verbal communication.

KEYWORDS

Autism Spectrum Disorder, communication, language, SNP rs2710102, the polymorphism of *CNTNAP2*

INTRODUCTION

Autism Spectrum Disorder (ASD) is a highly heterogeneous genetically-based neurodevelopmental condition associated with difficulties in social interaction/communication and the presence of stereotyped/repetitive behavior and restricted interests or atypical response to sensor information (American Psychiatric Association, 2013). Often, ASD is accompanied with co-occurring structural language impairments (Arutiunian et al., 2022; Kjelgaard & Tager-Flusberg, 2001; Lindgren et al., 2009), which vary from severely impaired language (minimally verbal or non-verbal ASD) to slightly impaired (Pickles et al., 2014; Tager-Flusberg, 2016; Tager-Flusberg & Kasari, 2013). However, the biological basis of these deficits is still not clearly understood. Given the high variability of language skills in ASD, recent studies have shown that the language profiles in this population can be driven by multiple different molecular and genetic mechanisms in the human genome (Benítez-Burraco et al., 2016; Benítez-Burraco & Murphy, 2016; Uddin et al., 2021).

One of the candidate genes related to language variation in children with ASD is the contactin-associated protein-like 2 gene (*CNTNAP2*), a member of the Neurexin family, spanning over 2 Mb of DNA at chromosome 7q35 (Alarcón et al., 2002, 2005, 2008; Strauss et al., 2006; Uddin et al., 2021; Vernes et al., 2008). For example, Alarcón et al. (2002) showed a relationship between several polymorphisms of *CNTNAP2* and the age of the first word in 152 multiplex autism families. The polymorphism of *CNTNAP2* has also been associated with receptive language in children with ASD without language development delay (Shiota et al., 2022). Specifically, a common genetic variant of *CNTNAP2*, single nucleotide polymorphism (SNP) rs2710102 (and, particularly, the carriers of the A-allele) was most frequently reported as a risk factor for lower language skills and/or delay (Alarcón et al., 2005; Shiota et al., 2022; Vernes et al., 2008) as well as the severity of autistic traits (Arking et al., 2008; Nascimento et al., 2016; Peñagarikano & Geschwind, 2012; Shiota et al., 2021; Steer et al., 2010; Stein et al., 2011). Remarkably, the polymorphisms of *CNTNAP2* were found to be linked to language development and processing not only in the ASD sample but also in the general population (Koeda et al., 2015; Kos et al., 2012; Riva et al., 2018; Whitehouse et al., 2011). Neuroimaging studies (both (f) MRI and EEG/ERP) have contributed to these findings, demonstrating that the variation in *CNTNAP2* crucially influences the structural and functional human brain networks which are associated with language and social abilities (Abrahams et al., 2007; Bai et al., 2019; Li et al., 2021; Scott-Van Zeeland et al., 2010; Whalley et al., 2011). It is important to note, however, that some studies did not find relationships between polymorphisms of *CNTNAP2* and either language (Toma et al., 2013) or the severity of autism (Toma et al., 2018).

Summarizing, most of the previous studies addressing polymorphisms of the *CNTNAP2* gene showed a relationship to both language abilities and social skills in children with ASD. However, due to the different assessment approaches used in the studies (e.g., the age of the first word [Alarcón et al., 2002] and direct assessment of receptive vocabulary [Shiota et al., 2022]), it is unclear whether the variation in rs2710102 is linked to structural language skills (organization of the language system in the brain) or more general communicative abilities (functional aspects of the language system). Moreover, the majority of studies included sex-unbalanced samples and the distribution of the A-allele of rs2710102 was not equal between males and females (e.g., Alarcón et al., 2005; Shiota et al., 2022). Crucially, it has been shown that male and female individuals with ASD can have different profiles with respect to both language abilities (Neuhaus et al., 2022) and the

scores on common measures of autistic traits (Neuhaus et al., 2021; Rea et al., 2023). Therefore, it would be beneficial to investigate the polymorphism of the *CNTNAP2* gene in a sample with equal distribution of the A-allele of rs2710102 between male and female autistic individuals, using tools assessing both structural language skills and communication.

In the present study, we utilized a sex-matched sample of youth with and without ASD to investigate the relationship between rs2710102 A-allele carrier status and participants' language and communication skills. The strengths of the study are threefold. First, we used specific assessment tools to measure separately both structural language skills and verbal communication skills. Second, our sample of participants consisted of an equal number of male and female individuals in general as well as an equal number of male and female individuals for each genotype in each group. Finally, given the highly heritable nature of ASD, we included a group of unaffected siblings (US) of youth with ASD to investigate the possible influence of the variation in rs2710102 on their language and communication abilities in comparison to ASD and typically developing (TD) youth.

METHODS

Participants

A total of 302 youth aged 7 to 18 years were included in the analysis: 131 youth with ASD (62 female), 130 TD youth (64 female), and 41 US of youth with ASD (25 female). Sex is based on parent report of sex assigned at birth. Data were collected from four sites as part of the GENDAAR Autism Center for Excellence network (National Institute of Mental Health Data Archive Data Collection #2021), including Seattle Children's Research Institute, Boston Children's Hospital, the University of California in Los Angeles, and Yale University.

Clinical and behavioral assessment

All children with ASD were diagnosed with the Autism Diagnosis Observation Schedule – Second Edition (ADOS-2) (Lord et al., 2012) and clinical best estimate based on DSM–IV–TR (American Psychiatric Association, 2000). All of them had *either* verbal *or* nonverbal IQ ≥70 based on the Differential Ability Scales – Second Edition (DAS-II) School Aged Cognitive Battery (Elliott, 2007). Exclusion criteria were twin status, co-occurring neurological conditions (e.g., epilepsy), history and/or presence of known chromosomal syndromes/single-gene disorders related to ASD (e.g., Fragile X syndrome, Rett syndrome, etc.), clinically significant visual and auditory impairments, or sensorymotor difficulties that would prevent completion of study procedures. TD children had no first or second degree family members with ASD, and both TD and US participants had no elevation of autism symptoms according to the parent report on the Social Responsiveness Scale – Second Edition (SRS-2) (Constantino, 2012) (T-score <60) or the Social Communication Questionnaire (Rutter et al., 2003) (raw score <11).

Additional characterization was obtained via parental report using the Vineland Adaptive Behavior Scales – Second Edition (Vineland-II) (Sparrow et al., 2005) and the Clinical Evaluation of Language Fundamentals – Fourth Edition (CELF-4) (Semel et al., 2003). The Vineland-II is a parent-clinician interview in which the Communication domain focuses on adaptive communication skills, including receptive, expressive, and written language (e.g., McQuaid et al., 2021). The CELF-4 Core Language Score is usually used to identify language impairments, but it includes different subtests that cover basic structural language skills at different linguistic levels (vocabulary, morphosyntax, semantics, and pragmatics) in both production and comprehension (e.g., Kjelgaard & Tager-Flusberg, 2001). All participants from TD and US group had normal language, communication, and cognitive abilities based on provided measures (Vineland, CELF and DAS scores). Table 1 provides demographic information for all groups of participants as well as descriptive statistics for measures included. See Supplementary Information Table 1 for CELF-4 subtest scores (see also Neuhaus et al., 2022). Greater measure details are provided in Supplementary Information section *Measures*.

CNTNAP2 genotyping

Blood samples were collected to obtain genomic DNA and processed by the Rutgers University Cell and Data Repository (RUCDR) or using standard protocols (Gentra Puregene Blood DNA extraction kit; Qiagen). Subjects were genotyped by the HumanOmni2.5M-8 BeadChip microarray (Illumina). After quality filtering (<5% missing per person/per SNP, >1% minor allele frequency, Hardy–Weinberg equilibrium $p > 10^{-7}$), multidimensional scaling was performed in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) using the default settings with the HapMap 3 reference panel (http://hapmap.ncbi.nlm.nih.gov/). SNP data for rs2710102 was acquired through PLINK for all subjects and extracted for analysis.

The genotype of rs2710102 is GG, GA or AA. Given that previous work suggests that the A-allele is related to increased likelihood of language impairment, we classified the genotypes of rs2710102 into AA/AG (with A-allele) and GG (without A-allele) as previously used (e.g., Bai et al., 2019; Shiota et al., 2021). This approach assumes that having one or two A-alleles has same effect on the tasks (e.g., Shiota et al., 2022). The number of

TABLE 1 Demographic information of participants, M (SD).

Characteristics	Group			Statistics (*t*-tests between groups)		
	ASD ($N = 131$)	TD ($N = 130$)	US ($N = 41$)	ASD vs. TD	ASD vs. US	TD vs. US
Age (months)	148.1 (35.0)	156.2 (34.6)	139.6 (32.9)	$t = -1.8$, $p = 0.06$	$t = 1.4$, $p = 0.16$	$t = 2.8$, $p = \mathbf{0.006}$
Sex (% female)	47%	49%	60%	–	–	–
Ethnicity, *N* (%)						
Not Hispanic or Latino	112 (85.5%)	102 (78.5$)	36 (87.8%)	–	–	–
Hispanic or Latino	17 (13%)	26 (20%)	5 (12.2%)	–	–	–
Not answered	2 (1.5%)	2 (1.5%)	0 (0%)	–	–	–
Race, *N* (%)						
White	99 (75.6%)	96 (73.85%)	34 (83%)	–	–	–
Black or African American	6 (4.6$)	9 (6.92%)	1 (2.4%)	–	–	–
Asian	4 (3.1%)	10 (7.69%)	0 (0%)	–	–	–
Mixed race	18 (13.7%)	14 (10.769%)	6 (14.6%)	–	–	–
Other	2 (1.5%)	0 (0%)	0 (0%)	–	–	–
Not answered	2 (1.5%)	1 (0.769%)	0 (0%)			
CELF-4 (Core Language SS)	91.8 (21.2)	110.6 (11.2)	109.9 (11.5)	$t = -8.9$, $p < \mathbf{0.001}$	$t = -6.9$, $p < \mathbf{0.001}$	$t = 0.3$, $p = 0.71$
SRS-2 (raw total score)	90.4 (28.0)	17.7 (22.1)	26.6 (23.8)	$t = 22.3$, $p < \mathbf{0.001}$	$t = 13.7$, $p < \mathbf{0.001}$	$t = -2.0$, $p = \mathbf{0.04}$
Vineland-2						
Communication SS	75.7 (11.2)	99.2 (14.0)	95.6 (14.3)	$t = -15.0$, $p < \mathbf{0.001}$	$t = -8.1$, $p < \mathbf{0.001}$	$t = 1.4$, $p = 0.16$
Socialization SS	71.2 (11.4)	102.0 (13.0)	101.4 (14.7)	$t = -20.3$, $p < \mathbf{0.001}$	$t = -12.0$, $p < \mathbf{0.001}$	$t = 0.2$, $p = 0.82$
Daily living skills SS	75.7 (13.4)	97.9 (14.1)	97.3 (17.9)	$t = -12.9$, $p < \mathbf{0.001}$	$t = -7.1$, $p < \mathbf{0.001}$	$t = 0.1$, $p = 0.85$
Verbal IQ	99.0 (18.7)	113.5 (16.3)	112.0 (11.3)	$t = -6.6$, $p < \mathbf{0.001}$	$t = -3.9$, $p < \mathbf{0.001}$	$t = 0.6$, $p = 0.50$
Nonverbal IQ	100.0 (17.1)	108.1 (14.8)	111.8 (16.6)	$t = -4.1$, $p < \mathbf{0.001}$	$t = -5.3$, $p < \mathbf{0.001}$	$t = -1.2$, $p = 0.20$
ADOS-2						
CSS Total	6.7 (2.0)	–	–	–	–	–
CSS SA	6.8 (2.1)	–	–	–	–	–
CSS RRB	6.4 (2.9)	–	–	–	–	–

Note: The significance is highlighted in bold ($p < 0.05$).
Abbreviations: CSS, Calibrated Severity Score; RRB, Restrictive and Repetitive Behaviors; SA, Social Affect; SS, Standard Score.

youth in each genotype as well as their sex and chronological age can be seen in Table 2.

Statistical analysis

First, to examine between-group differences in language and communication scores in relation to genotype and assess the influence of social, cognitive, and biological characteristics of youth on language/communication abilities, we fitted two linear models with dependent variables of language score (CELF-4 Core Language Standard Score) or communication score (Vineland-II Communication Standard Score) and the main effects of genotype (intercept corresponded to the GG), sex (intercept corresponded to 'females'), difference between verbal and nonverbal IQ (diff_IQ) (Ankenman et al., 2014), SRS-2 total raw score, genotype × sex interaction,

TABLE 2 Sample characteristics in each genetic group.

Characteristics	Group		
	ASD (62 female, 69 male)	TD (64 female, 66 male)	US (25 female, 16 male)
AA/AG genotype			
N	85	96	28
Age, months (M (SD))	146.7 (34.7)	156.1 (34.6)	144.1 (33.4)
Sex (female/male)	41/44	47/49	16/12
GG genotype			
N	46	34	13
Age, months (M (SD))	150.7 (35.9)	156.6 (35.0)	130.0 (30.7)
Sex (female/male)	21/25	17/17	9/4

genotype × SRS-2 total raw score interaction, genotype × diff_IQ interaction, and group effect nested within each genotype to assess how the language/communication skills of participants differed depending on the genotype. Age was included as a covariate to the models because groups differed in age (see Table 1).

Second, in order to explore the variability of language/communication abilities in relation to genotype within the ASD sample, we fitted two generalized linear models with dependent variable of genotype (coded as 0 for 'genotype 1', 1 for 'genotype 2') and predictors, included language score (CELF Core Language SS) or communication score (Vineland-II Communication SS) as well as ADOS-2 social-affect calibrated severity scores (CSS SA) and ADOS-2 restricted/repetitive behavior calibrated severity scores (CSS RRB), age, and sex to account for the severity of autistic symptoms and biological characteristics of youth.

All models were estimated in R (R Core Team, 2019) with the *lme4* package (Bates et al., 2015), and the data were plotted with *ggplot2* package (Wickham, 2016). A correction for multiple comparisons (false discovery rate, FDR) was applied to the models, and *p*-values for significant predictors were corrected with *p.adjust.method* in R.

Data availability

The codes for statistical analysis and plotting are available in Supplementary Information section *Code*. The behavioral data from the current study are available via the National Institute of Mental Health Data Archive Data Collection #2021. All genetic and biospecimen data from ACE study participants were contributed to the NIMH Repository and Genomics Resource (https://www.nimhgenetics.org) as well as archived through Sampled, Inc. (http://sampled.com), Infinity BiologiX/RUCDR.

RESULTS

Descriptive sample characterization

As shown in Table 1, the ASD group had lower verbal and nonverbal IQ, lower language skills and higher autistic traits in comparison to the non-autistic groups (TD and US groups). TD and US groups did not differ in any of the behavioral measures, except for autism traits; the US group had higher autism traits compared to TD group as assessed via the SRS-2. Also, US youth were younger than TD youth.

Association between language abilities, genotype, and individual characteristics of groups

The results showed a significant main effect of genotype, $\beta = -9.48$, SE = 4.31, $t = -2.20$, $p = 0.029$, $\eta^2 = 0.01$, 95% C.I. [0.00, 0.03], indicating that participants carrying the A-allele had lower language skills in comparison to the G-allele homozygotes. A main effect of sex was identified, $\beta = -4.60$, SE = 2.13, $t = -2.16$, $p = 0.032$, $\eta^2 = 0.01$, 95% C.I. [0.00, 0.03], such that male youth had lower language scores than female youth.

There were significant nested effects of group in relation to the AA/AG genotype, ASD versus TD, $\beta = 22.95$, SE = 4.36, $t = 5.26$, $p < 0.001$, ASD versus US, $\beta = 22.09$, SE = 4.86, $t = 4.55$, $p < 0.001$, $\eta^2 = 0.08$, 95% C.I. [0.03, 0.14]. This nested effect revealed that between-group differences in language abilities were driven by the carriers of the A-allele, but not G-homozygotes, ASD versus TD, $\beta = 8.04$, SE = 6.05, $t = 1.33$, $p = 0.185$, ASD versus US, $\beta = 8.32$, SE = 6.57, $t = 1.27$, $p = 0.206$ (Figure 1a). Other associations were not significant: diff_IQ, $\beta = 0.03$, SE = 0.11, $t = 0.27$, $p = 0.788$; SRS-2 total raw score, $\beta = -0.05$, SE = 0.04, $t = -1.11$, $p = 0.270$; genotype × sex interaction, $\beta = 3.27$, SE = 2.13, $t = 1.53$, $p = 0.126$; genotype × SRS-2 total raw score interaction, $\beta = 0.07$, SE = 0.04, $t = 1.71$, $p = 0.088$; genotype × diff_IQ interaction, $\beta = -0.03$, SE = 0.11, $t = -0.26$, $p = 0.797$; age, $\beta = 0.04$, SE = 0.03, $t = 1.38$, $p = 0.168$ (see Table 3 with the full model outcome).

Association between communication, genotype, and individual characteristics of groups

There was no significant main effect of genotype for communication, $\beta = -4.85$, SE = 3.03, $t = -1.60$, $p = 0.111$. Similarly to the results for language abilities, we identified a significant main effect of sex, $\beta = -3.18$, SE = 1.50, $t = -2.12$, $p = 0.035$, $\eta^2 < 0.01$, 95% C.I. [0.00, 0.02], such that male youth compared to female youth had lower scores and, subsequently, lower communication skills. Also, a significant relationship was found between communication skills and social abilities (SRS-2), so that higher social skills were related to better communication abilities, $\beta = -0.11$, SE = 0.03, $t = -3.80$, $p < 0.001$, $\eta^2 = 0.02$, 95% C.I. [0.00, 0.05]. A significant main effect of age was revealed, $\beta = -0.15$, SE = 0.02, $t = -7.18$, $p < 0.001$, $\eta^2 = 0.08$, 95% C.I. [0.04, 0.13].

The nested effects demonstrated a significant between-group (ASD vs. TD) difference in communication skills for both genotypes: carriers of the A-allele, $\beta = 18.83$, SE = 3.07, $t = 6.13$, $p < 0.001$, G-homozygotes, $\beta = 16.32$, SE = 4.26, $t = 3.83$, $p < 0.001$; as well, between-group (ASD vs. US) difference for AA/AG, $\beta = 15.92$, SE = 3.42, $t = 4.66$, $p < 0.001$, $\eta^2 = 0.09$, 95% C.I. [0.04, 0.13], but no difference for GG, $\beta = 3.41$, SE = 4.62, $t = 0.74$, $p = 0.461$ (see Figure 1b). Other associations were not significant: diff_IQ, $\beta = -0.08$, SE = 0.08, $t = -0.99$, $p = 0.323$; genotype × sex interaction, $\beta = 1.96$, SE = 1.50, $t = 1.31$, $p = 0.193$; genotype × SRS-2 total raw score interaction, $\beta = 0.01$,

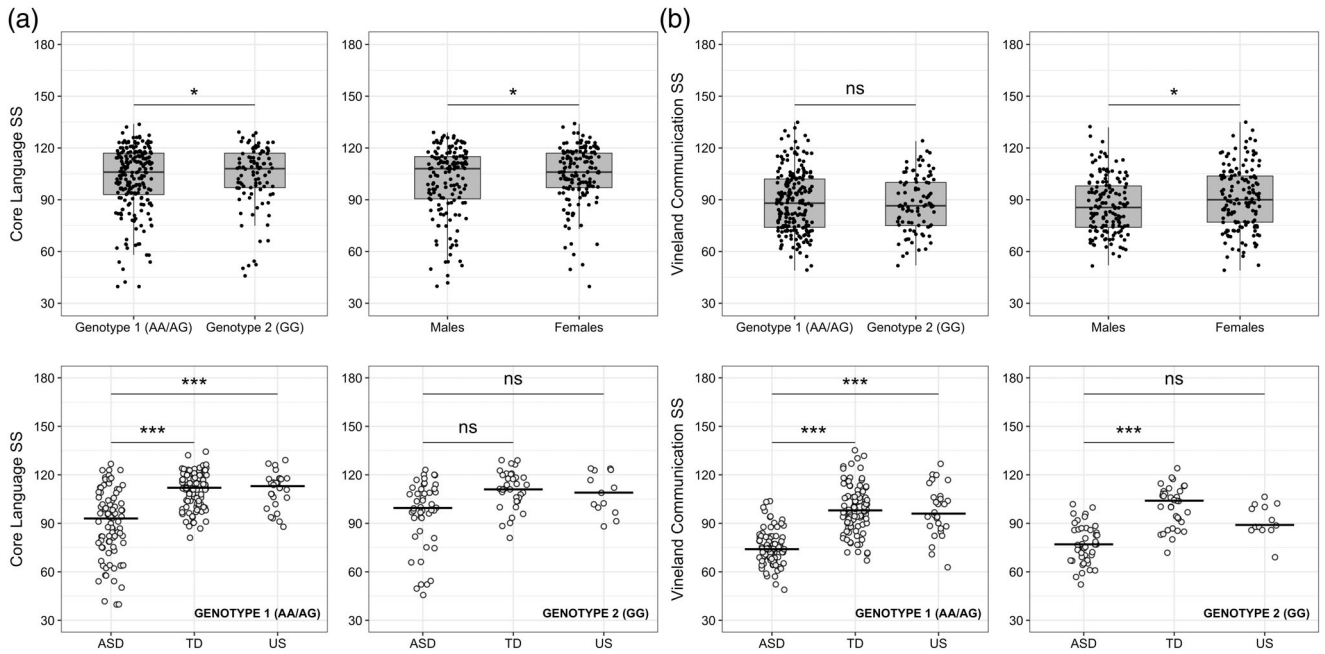

FIGURE 1 The polymorphism of the *CNTNAP2* gene (SNP rs2710102) and its relation to language and communication. Upper panel represents the main effects of genotype and sex in (a) language and (b) communication; lower panel represents between-group comparisons in (a) language and (b) communication skills in each genotype. ASD – Autism Spectrum Disorder, TD – typically developing, US – unaffected siblings of youth with ASD. The significance is labeled with *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = non-significant.

TABLE 3 Association between language skills, genotype, and individual characteristics of groups.

	CELF-4 Core language standard score			
Predictors	**Estimate**	**Standard error**	***t***	***p***
(Intercept)	92.49	5.84	15.85	**<0.001**
Genotype_AA/AG	−9.48	4.31	−2.20	**0.029**
Sex_male	−4.60	2.13	−2.16	**0.032**
Age	0.04	0.03	1.38	0.168
Diff_IQ	0.03	0.11	0.27	0.788
SRS-2 total raw score	−0.05	0.04	−1.11	0.270
Genotype:Sex	3.27	2.13	1.53	0.126
Genotype:SRS-2 total raw score	0.07	0.04	1.71	0.088
Genotype:Diff_IQ	−0.03	0.11	−0.26	0.797
Genotype_AA/AG: Group_TD	22.95	4.36	5.26	**<0.001**
Genotype_GG:Group_TD	8.04	6.05	1.33	0.185
Genotype_AA/AG: Group_US	22.09	4.86	4.55	**<0.001**
Genotype_GG:Group_US	8.32	6.57	1.27	0.206
Observations	278			
R^2/R^2 adjusted	0.302/0.271			

Note: The significance is highlighted in bold ($p < 0.05$).

SE = 0.03, $t = 0.49$, $p = 0.622$; genotype × diff_IQ interaction, β = 0.12, SE = 0.08, $t = 1.42$, $p = 0.157$ (see Table 4 with the full model outcome).

The relationship between language/communication skills and genotype in ASD group

In order to explore the variability of language abilities in relation to genotype within the ASD sample, we divided the youth with ASD in two subgroups, that is, with and without language impairment, using the CELF-4 Core Language SS cut-off value (≤85, representing one standard deviation below the mean). Then we calculated the percent of participants in each language group (average and below-average) with G-homozygotes. As expected, in a subgroup of ASD participants with average language skills, 40% of youth were carriers of the G-allele whereas in a subgroup with below-average language only 25% participants were carriers of the G-allele.

As CELF-4 Core Language SS and Vineland-II Communication SS correlated with each other ($r = 0.49$, $p < 0.001$), two models were fitted separately—the first model included language score and the second one included communication score. Both models also accounted for the severity of autistic symptoms and biological characteristics of youth (see *Methods*). The results of the first model demonstrated that only the CELF-4 Core Language SS was related to genotype, β = 0.02, SE = 0.00, $z = 2.04$, $p = 0.04$, $\eta^2 = 0.04$, 95% C.I. [0.00, 0.10]. Higher language scores were driven by the higher presence of G-homozygotes. There was no association with genotype for the other behavioral measures (CSS SA, β = 0.14, SE = 0.10, $z = 1.47$, $p = 0.14$; CSS RRB, β = 0.04, SE = 0.07, $z = 0.71$, $p = 0.48$) or individual characteristics

TABLE 4 Association between communication, genotype, and individual characteristics of groups.

	Vineland-II communication standard score			
Predictors	**Estimate**	**Standard error**	***t***	***p***
(Intercept)	109.81	4.12	26.67	**<0.001**
Genotype_AA/AG	−4.85	3.03	−1.60	0.111
Sex_male	−3.18	1.50	−2.12	**0.035**
Age	−0.15	0.02	−7.18	**<0.001**
Diff_IQ	−0.08	0.08	−0.99	0.323
SRS-2 total raw score	−0.11	0.03	−3.80	**<0.001**
Genotype:Sex	1.96	1.50	1.31	0.193
Genotype:SRS-2 total raw score	0.01	0.03	0.49	0.622
Genotype:Diff_IQ	0.12	0.08	1.42	0.157
Genotype_AA/AG: Group_TD	18.83	3.07	6.13	**<0.001**
Genotype_GG:Group_TD	16.32	4.26	3.83	**<0.001**
Genotype_AA/AG: Group_US	15.92	3.42	4.66	**<0.001**
Genotype_GG:Group_US	3.41	4.62	0.74	0.461
Observations	277			
R^2/R^2 adjusted	0.581/0.562			

Note: The significance is highlighted in bold ($p < 0.05$).

of youth (age, $\beta = 0.00$, SE $= 0.00$, $z = 0.67$, $p = 0.50$; sex, $\beta = 0.21$, SE $= 0.39$, $z = 0.53$, $p = 0.59$).

The second model using Vineland-II Communication SS did not reveal any significant relationships: $\beta = 0.02$, SE $= 0.01$, $z = 1.38$, $p = 0.17$; CSS SA, $\beta = 0.12$, SE $= 0.09$, $z = 1.28$, $p = 0.20$; CSS RRB, $\beta = 0.02$, SE $= 0.06$, $z = 0.36$, $p = 0.72$; age, $\beta = 0.00$, SE $= 0.00$, $z = 1.03$, $p = 0.30$; sex, $\beta = 0.04$, SE $= 0.39$, $z = 1.01$, $p = 0.91$.

To summarize, rs2710102 A-allele carrier status was related to language abilities in the ASD sample, but we did not find a relationship between rs2710102 A-allele carrier status and communication skills.

DISCUSSION

The goal of the present study was to investigate relationships between *CNTNAP2* rs2710102 A-allele carrier status and distinct language and communication skills in a large sex-matched sample of youth with and without ASD. This work makes a novel contribution to the literature in that it separately assesses the association between genotype and both structural language skills and more general verbal communication skills, using different gold standard assessment tools, and does so in a sample that is sex-balanced within each group (ASD, TD, and US) and genotype.

Similar to previous studies, which showed a relationship between the variation in *CNTNAP2* and language (Alarcón et al., 2002, 2005, 2008; Koeda et al., 2015; Kos et al., 2012; Riva et al., 2018; Shiota et al., 2022; Strauss et al., 2006; Uddin et al., 2021; Vernes et al., 2008; Whitehouse et al., 2011), we observed an association between SNP rs2710102 and language abilities of youth in general. Specifically, a subgroup of participants with A-allele of rs2710102 had lower language scores revealed with CELF-4 than a subgroup of participants with the G-allele homozygotes. This is in line with (Alarcón et al., 2005; Vernes et al., 2008), which demonstrated that lower language skills were driven by the A-allele of rs2710102. Importantly, the common variation in rs2710102 was significantly associated with language (CELF-4 Core Language SS) but not with more general verbal communication skills (Vineland-II Communication SS), pointing to a specific link between the *CNTNAP2* rs2710102 A-allele carrier status and structural language (linguistic) but not communication skills of youth. Between-group comparisons showed that lower language performance in the ASD group was driven by the presence of the A-allele of rs2710102: ASD youth who were A-allele carriers had significantly lower language scores than both TD and US youth who were A-allele carriers. By contrast, no differences in language abilities were found between ASD, TD, or US youth who were G-allele homozygotes.

Between-group comparisons in communication skills did not show a specific relation to genotype. TD youth had higher communication skills than ASD youth regardless of A-allele carrier status. Among A-allele carriers, US youth had higher communication scores in comparison to ASD youth, but no difference was revealed between ASD and US with the GG genotype. This further supports the finding that communicative language functioning is a component of the broader autism phenotype (e.g., Dovgan et al., 2022; Nayar et al., 2022; Pisula & Ziegart-Sadowska, 2015).

Summarizing, the polymorphism of the *CNTNAP2* gene was related to structural language but not communication skills in a total sample, and between-group differences in language scores were associated with the specific genotype (the carriers of the A-allele of SNP rs2710102). However, it is important to note that our measures of structural language skills and verbal communication are highly correlated to each other which makes it impossible to assess their relation to genotype within one model. Additional studies are needed that will use assessment tools that are not correlated. This will help to assess the specific association between rs2710102 and language/communication.

Sex, language, and communication

In accordance with previous findings (Neuhaus et al., 2021, 2022; Payne & Lynn, 2011; Peterson, 2018; Rea et al., 2023; Simpson et al., 2016), we identified the main effects of sex for both language and communication skills, showing that male youth had lower structural

language and general communication skills in comparison to female youth. It is still being debated why male and female individuals (both typically and atypically developing) may have different social and cognitive profiles (Adani & Cepanec, 2019; Baron-Cohen, 2002; Rea et al., 2023; Sturrock et al., 2020). For the ASD population, it is hypothesized that, for example, multiple genetic factors contribute to the likelihood of developing ASD, and females may require a higher genetic load to meet diagnostic criteria (Zhang et al., 2020). In addition, it has been proposed that female autistic behavior may manifest in ways that are not captured by the diagnostic algorithms of ADOS-2: the observable expression of autistic features by many ASD females may be more nuanced, and, thus, difficult for clinicians to assess and diagnose using standardized diagnostic measures (e.g., Beggiato et al., 2017; Estrin et al., 2021; Kirovski et al., 2013; Lai & Baron-Cohen, 2015; Rea et al., 2023; Tsirgiotis et al., 2022). Altogether, although we found sex effects in both language and communication skills, it is important to note that the sex distribution in each genotype was equal and the effect of polymorphism of the *CNTNAP2* gene was not driven by the difference in number of male and female individuals in each genotype.

CNTNAP2 and language

In order to assess whether the polymorphism of *CNTNAP2* the gene can explain variability in language, communication, and social skills of children with ASD, we fitted two models (for language and verbal communication separately) and accounted for biological characteristics of youth (sex and age). The results showed that only structural language skills were associated with genotype, with the carriers of A-allele of rs2710102 demonstrating worse performance on the CELF-4, confirming the previous findings in individuals with ASD (Shiota et al., 2022). Other relationships with the polymorphism of *CNTNAP2* (verbal communication, social skills and demographic characteristics of youth) were not significant.

It is still unclear, however, *why* common genetic variants of *CNTNAP2* drives variation in language abilities of individuals with ASD. A limited number of neuroimaging studies showed that several polymorphisms of *CNTNAP2* affect brain areas that are critically involved in language processing (Koeda et al., 2015; Kos et al., 2012; Riva et al., 2018; Shiota et al., 2022; Whalley et al., 2011; Whitehouse et al., 2011), and both structural and functional alterations of these regions are frequently reported in individuals with ASD (e.g., Berman et al., 2016; Knaus et al., 2010; Richter et al., 2015; Seymour et al., 2020; Sharda et al., 2017; Smith et al., 2016). Future studies would benefit from combining genetic, behavioral, and neuroimaging approaches to reveal how polymorphisms of the *CNTNAP2* gene influence language-related neural networks and, in turn, language behavior in autistic individuals.

LIMITATIONS AND FUTURE DIRECTIONS

The study has some important limitations that should be highlighted. First, all participants from the ASD group had *either* verbal *or* non-verbal IQ ≥70 which means that we focused on a specific subgroup of autistic youth. To generalize the findings, it is necessary to include ASD individuals regardless of their communicative and cognitive abilities. Second, due to the limited sample size and specific aims we analyzed only one of the SNPs of *CNTNAP2* (rs2710102) whereas other SNPs (rs7794745, rs10246256) also have been reported to be related to language skills (e.g., Peñagarikano & Geschwind, 2012). Future studies would benefit from testing the relationship of all these SNPs with language/communication skills and their interaction with the group (ASD vs. TD). Third, we used candidate-gene study design, whereas it has been proposed that the genome-wide association studies are more accurate in psychiatric research (see Duncan et al., 2019). Fourth, some effect sizes are small, which makes the findings not clinically applicable at this point. Fifth, we used CELF-4 and Vineland-II Communication domain to assess structural language skills and verbal communication, but these measures were highly correlated to each other. This makes impossible to include these measures into one model and assess their relation to genotype in combination; thus, the observed effects (especially a possible difference between language and communication in relation to rs2710102) have to be considered only in the framework of this limitation. Finally, we used CELF-4 Core Language SS as a measure of structural language skills and Vineland Communication SS as a measure of more general verbal communication, however, there is a certain overlap between tests as they initially were not design for measuring these abilities but rather to identify language impairments/delay (event though items of the tests cover structural language skills as well as verbal communication). Future studies would benefit from using assessment tools that specifically address structural language skills and communication abilities.

CONCLUSION

The present study provided the first evidence of the relationships between the polymorphism of the *CNTNAP2* gene and structural language skills as well as more general communication abilities in a large group of sex-matched youth with and without ASD (total N of males = 151, total N of females = 151). We showed that the A-allele of rs2710102 is associated with lower structural language skills, but it is not related to more general verbal communication abilities. Importantly, these links between genetics and language behavior were not driven by sex as the samples of participants consisted of equal number of males and females in each genotype.

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CONFLICT OF INTEREST STATEMENT

James C. McPartland consults with Customer Value Partners, Bridgebio, Determined Health, and BlackThorn Therapeutics, has received research funding from Janssen Research and Development, serves on the Scientific Advisory Boards of Pastorus and Modern Clinics, and receives royalties from Guilford Press, Lambert, Oxford, and Springer. The remaining authors reported no biomedical financial interests or potential conflicts of interest.

ETHICS STATEMENT

Ethical oversight was provided by the Yale Institutional Review Board, the UCLA Office of Human Research Protection Program, Boston Children's Hospital Institutional Review Board, USC Office for the Protection of Research Subjects, and the University of Virginia Institutional Review Board for Health Sciences Research. All procedures performed were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all parents of children participating in the study; children provided written assent.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NIMH Repository and Genomics Resource at https://www.nimhgenetics.org.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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