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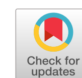
Research Paper

Genetic variants in the *CNTNAP2* gene are associated with gender differences among dyslexic children in China



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A B S T R A C T

Background: It is well known that males have a higher prevalence of developmental dyslexia (DD) than females. Although the mechanism underlying this gender difference remains unknown, the contactin-associated protein-like 2 (*CNTNAP2*) gene, which shows sex-specific patterns in some neurodevelopmental disorders, has attracted extensive attention. This study aimed to explore whether *CNTNAP2* shows a sex-specific association with DD in a Chinese population.
Methods: Using genomic DNA samples of 726 students [372 cases (282 male, 90 female), 354 controls (267 male, 87 female)], we genotyped five SNPs of *CNTNAP2*. Gender-stratified logistic regression models were used to determine the relationships between the *CNTNAP2* variants and DD.
Findings: After adjustment for the false discovery rate (FDR), two SNPs (rs3779031, rs987456) of *CNTNAP2* were associated with DD risk in females but not in males. Female participants carrying the rs3779031 G allele had a lower risk of DD than those with the A genotype [GG vs AA: OR (95%CI) = 0.281 (0.097–0.814)]. The rs987456 CC genotype was associated with a decreased risk of DD in females [CC vs AA+CA: OR (95%CI) = 0.222 (0.078–0.628)]. Furthermore, the interaction between *CNTNAP2* (rs987456) and environmental factors (scheduled reading time) played a protective role in females [OR (95%CI) = 0.431 (0.188–0.987)].
Interpretation: We performed a genetic association study on *CNTNAP2* variants and DD. The sex specificity of *CNTNAP2* in DD, along with the gene-environment interaction may help us to understand gender differences in DD.

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1. Introduction

Developmental dyslexia (DD) also known as reading disability is the most common learning disability [1,2]. Children with dyslexia have difficulties in word recognition, spelling, and decoding, despite adequate intelligence and normal sensory skills [3]. Approximately 3%–12.6% school-aged children have dyslexia in China [4,5].

Many studies have reported that a higher prevalence of dyslexia in males than in females [3]. A large prospective study of white (n = 16,910) and black (n = 15,313) children who were part of the National Collaborative Perinatal Project (NCPP) in the UK showed that the male-female ratio of children with dyslexia was about 2:1, irrespective of severity of disability, race, or exclusion of children with attention deficit hyperactivity disorder (ADHD) [6]. Four epidemiological studies carried out in the UK and New Zealand also reported a higher rate of reading disability in boys than in girls, with a ratio ranging from1.93:1 to 3.29:1 [7]. Evidence from a large sample of second-grade students (n = 491,103) from the state of Florida found that the male-female ratio increased with increasing severity of reading impairment, from 1.6:1 to 2.4:1 [8].

Most studies on gender differences in dyslexia were based on alphabetic languages. Only a few studies have focused on gender differences in dyslexia in China. As an ideographic language, Chinese is entirely different from the alphabetic languages. Chan et al. reported that dyslexia was 1.6 times more common in boys than in girls in Hongkong [4]. People in mainland China use simplified Chinese characters, whereas the traditional Chinese is widely used in Taiwan and Hongkong [9]. We performed an epidemiological study on DD among students (n = 34,748) of 84 primary schools in seven cities of Hubei province in China and identified 1200 dyslexic students. A gender difference in DD was also found in our study, and the male-female ratio was 3:1 [5].

Abbreviations: DD, developmental dyslexia; CNTNAP2, contactin-associated protein-like 2; FDR, false discovery rate; ADHD, attention deficit hyperactivity disorder; ASD, autism-spectrum disorder; CNV, copy number variant; DCCC, The Dyslexia Checklist for Chinese Children; PRS, Pupil Rating Scale-Revised Screening for Learning Disabilities; 5′-UTR, five prime untranslated regions; MAF, minor allele frequency; GMV, gray matter volume; LSOG, the left superior occipital gyrus.

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Although the male predominance of DD is well known, the mechanism underlying the gender difference remains unknown. Developmental dyslexia is among the heritable neurodevelopmental disorders [10]. Some candidate genes contributing to dyslexia have been reported, e.g. *DYX1C1*, *DCDC2*, *KIAA0319*, and *CNTNAP2* [11–14]. The heritability (h_g^2) of DD was estimated to range from 0.18 to 0.72 [1]. The Colorado twin study ($n = 956$) of reading difficulties found the gender differences in h_g^2 did not reach the significant level (male $h_g^2 = 0.65$, female $h_g^2 = 0.54$) [15]. However, a twin study from UK ($n = 3909$) showed males had greater h_g^2 than females in word recognition deficit [16]. These inconsistency results might due to methodological differences, such as the sample size, the measures used, and the age of participants. The high proportion of males with DD indicated that sex-specific genetic factors were involved in the development of dyslexia. One of the candidate genes, *CNTNAP2,* was reported to have sex specificity in many studies. *CNTNAP2* variants were linked to a wide variety of neurodevelopmental disorders, including autism, dyslexia, depression, and Alzheimer's disease [17]. One of the common apparent characteristics of those disorders was the gender-specific difference in prevalence. Evidence from animal studies showed that male mice were more susceptible to the effects of *Cntnap2* mutations than females [18]. Using intrinsic signal optical imaging, Townsend and Smith found that lack of *Cntnap2* expression in adult males (either Cntnap2 knockout or heterozygous) resulted in decreased visually evoked activity in dorsal stream relative to wild-type controls, but in females, dorsal stream responses were similar among *Cntnap2* knockout, heterozygous, and wild-type mice. In human studies, a two-stage association study in AGRE (Autism Genetic Resource Exchange) trios showed that the correlation between *CNTNAP2* (rs2710102) and language delay in autism-spectrum disorder (ASD) appeared to be significant for males only [19]. Iakoubov et al. performed an association study ($n = 1118$) between three copy number variants (CNVs) with an intronic location in *CNTNAP2* and aging. The esv11910 CNV of the *CNTNAP2* gene had the reverse association with healthy aging (no chronic diseases in medical records) in males, but not in females. Male carriers of the *CNTNAP2* esv11910 in. allele had a statistically significant decrease, on average by 71% in the probability of staying healthy at 81–90 years of age, while in females it was statistically insignificant [20]. These studies implied that *CNTNAP2* might have sex-specific patterns in the brain. Additionally, the expression of *CNTNAP2* in certain human brain areas was not identical in males and females (https://www.gtexportal.org/home/gene/CNTNAP2) (supplementary fig. 1).

CNTNAP2 is one of the largest mammalian genes, contains 24 exons, and spans 2.3 Mb on Chromosome 7q35 [21]. The *CNTNAP2* gene encodes CASPR2, which is a member of the neurexin superfamily of proteins that mediates interactions between neurons and glia during nervous system development. In the human cortex, *CNTNAP2* is highly expressed in Broca's area, which is known to be important for speech and language [17]. Considering the importance of *CNTNAP2* as a dyslexia susceptibility gene and the gender difference, we hypothesize that *CNTNAP2* may have a sex-specific effect on dyslexia.

According to previous studies, environmental factors also contribute to gender differences in dyslexia. Evidence from a birth cohort study ($n = 2847$) showed that boys and girls were differentially susceptible to risk factors for dyslexia [22]. Boys were more susceptible than girls to environmental influences, such as paternal age, parental education level, socioeconomic status, teaching methods, and societal pressures [23]. Low birth weight increased the risk for dyslexia in girls but not in boys [24]. Studies from two cities in China reported that learning habits and the home literacy environment were associated with dyslexia [25,26]. It is necessary to determine whether environmental factors affect the gender difference in dyslexia among Chinese children.

Based on a case-control study of a Chinese population, this study aimed to test whether variants in *CNTNAP2* were associated with gender differences in dyslexics and to explore the interaction between *CNTNAP2* and environmental factors.

2. Methods

2.1. Participants

This study was based on an ongoing project named Tongji Reading Environment and Dyslexia (READ) research. Our previous studies have introduced this program [5]. We recruited 726 students (372 dyslexics, 354 non-dyslexics) and obtained oral swabs for DNA genotyping. The cases and controls were matched for gender and age. The age of participants ranged from 6 to 15 (mean age = 10.09 ± 1.26). The male-female ratios were about 3:1 in the dyslexic (282 male, 90 female) and non-dyslexic (267 male, 87 female) group.

2.2. Measuring Tools

The Dyslexia Checklist for Chinese Children (DCCC) and Pupil Rating Scale-Revised Screening for Learning Disabilities (PRS) were used to assess children's reading behaviors. The DCCC is a specific rating scale for dyslexia in Chinese and should be completed by parent/guardian. Higher scores indicate more serious reading difficulty. The PRS is a convenient tool to diagnose learning disability in China, and the scale is filled by teachers. The Higher score means better learning ability. Details of these two scales were available from our previous studies [5].

The dyslexia children should meet the following criteria: (a) the DCCC score was 2 standard deviations above the mean score of students in the same grade; (b) the PRS score was lower than 65 points; (c) the Chinese language exam was below the tenth percentile of all children in the same grade; and (d) children who had suffered from intellectual disability, brain injury, visual and auditory disorders, epilepsy, and other neurological disorders were excluded.

Parent/guardian filled out the questionnaire which contains family SES, home literacy environment, children's learning habits.

2.3. SNP Selection and Genotyping

The procedures of selection for SNPs were as follows.

First, we searched *CNTNAP2* in NCBI-SNP (http://www.ncbi.nlm.nih.gov/snp/), and selected functional SNPs in promoter (5′near gene), five prime untranslated regions (5′-UTR), exon (missense, nonsynonymous), 3′-UTR. Next, using Ensembl (http://asia.ensembl.org/index.html/), we chose SNPs in splice region variant and upstream gene variant. Then all selected SNPs from NCBI-SNP and Ensembl were checked for minor allele frequency (MAF) in 1000 Genomes (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). Those with MAF for Han Chinese in Beijing of China (CHB) >5% were identified for further consideration. After that, we performed linkage disequilibrium (LD) test using SNAP Pairwise LD (http://www.broadinstitute.org/mpg/snap/ldsearchpw.php). As for the redundant SNPs which had strong LD ($R^2 > 0.8$) to each other, only one was retained. Finally, we got five SNPs (rs10240503, rs3779031, rs9648691, rs987456 and rs2462603).

Genomic DNA was extracted from oral swab samples. Genotyping was performed at BIO MIAO BIOLOGICAL Corporation (Beijing, China) with Sequenom MassARRAY platform (San Diego, USA) according to the manufacturer's protocol. As a quality control, we random selected 4% of the samples ($n = 29$) as masked subset, and genotyped them twice. The accordance rate was 100% for all duplicated samples.

2.4. Ethics Statement

The study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. All participants provided written informed consent from their parents.

2.5. Statistical Analyses

We used goodness-of-fit χ^2 test to examine the Hardy–Weinberg equilibrium (HWE) for selected SNPs among controls. We performed the two-sided chi-square test to measure differences in the distribution of demographic characteristics between dyslexics and non-dyslexics. As for the association study, we adopted unconditional univariate logistic regression analysis to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) for the effect of individual SNPs on DD susceptibility, assuming that variant alleles were the risk alleles. We applied the gender-stratified logistic regression models to determine the different relationships between the *CNTNAP2* variants and dyslexia in boys and girls. To adjust the *P* values for multiple tests, we resorted to Benjamini–Hochberg method for controlling false discovery rate (FDR). We also used multivariate logistic regression models to analyze the gene–environmental interaction. All statistical analyses were performed using SPSS 13.0 software.

3. Results

3.1. Association between the CNTNAP2 Gene and DD

Five DNA samples were not successfully genotyped, and the final sample consisted of 370 dyslexics (281 boys and 89 girls) and 351 non-dyslexics (265 boys and 86 girls). The dyslexics and non-dyslexics were matched for gender ($\chi^2 = 0.021$, $P = 0.889$). The genotype distributions of five SNPs were in Hardy-Weinberg equilibrium. The (MAFs) of the five SNPs were similar to those in the HapMap database of Han Chinese in Beijing, China (Table 1). According to the logistic regression analysis, the rs3779031 polymorphism was significantly associated with a reduced risk of DD under the recessive model (OR = 0.546, 95%CI =0.324–0.919, $P = 0.023$) and the additive model (OR = 0.776, 95%CI = 0.617–0.975, $P = 0.029$). After adjustment for the FDR, the additive model reached significance (Table 2).

We conducted further analysis to explore the relationship between five SNPs of *CNTNAP2* and DD by gender (Table 3). Results from the logistic regression analysis showed that three SNPs (rs3779031, rs987456, and rs9648691) were significantly associated with DD in females; the rs10240503 was significantly associated with DD in males. After adjustment for the FDR, the association between two SNPs (rs3779031, rs987456) and DD in females remained statistically significant. Female participants carrying the rs3779031 G allele (GA or GG) had a lower risk of DD than those with the AA genotype (GA vs AA: OR = 0.474, 95%CI = 0.249–0.902, $P_b = 0.029$; GG vs AA: OR = 0.281, 95%CI = 0.097–0.814, $P_b = 0.032$). Additionally, the rs987456 CC genotype was associated with protection from DD in females (CC vs AA: OR = 0.263, 95%OR = 0.088–0.783, $P_b = 0.040$).

3.2. Gene-Environment Interactions

Based on the entire epidemiological study samples, we explored the association between environmental factors and dyslexia. According to the stratification analysis of dyslexia by gender, the shared environmental factors associated with dyslexic boys and girls were active learning, scheduled reading time, parents educational level and encouraging children to read (Table s1).

We analyzed the interactions of the two SNPs (rs3779031, rs987456) and environmental factors in females. As shown in Table 4, we found a significant interaction between the rs987456 polymorphism and scheduled time to read. Individuals with the rs987456 CC genotype who had scheduled reading time had a lower risk of dyslexia (OR = 0.431, 95%CI = 0.188–0.987). Other interactions between two SNPs and environmental factors did not reach significance.

Table 1
Characteristic of 5 SNPs of *CNTNAP2*.

SNP	Minor/major	MAF in CHB	MAF control	HWE
rs10240503	G/A	0.1893	0.1830	0.6204
rs3779031	G/A	0.2913	0.3457	0.8442
rs9648691	A/G	0.3835	0.4085	0.1458
rs987456	C/A	0.3010	0.3425	0.2269
rs2462603	G/A	0.3107	0.3200	0.2344

MAF minor allele frequency, HWE Hardy–Weinberg equilibrium, CHB Han Chinese in Beijing.

Table 2
Distribution and associations of *CNTNAP2* in cases and controls.

SNP	Model	Cases	Controls	OR(95%CI)	P_a	P_b
rs10240503	AA	226	233	1		
	GA	121	101	1.235(0.896,1.703)	0.198	0.330
	GG	17	13	1.348(0.640,2.840)	0.432	0.540
	Dominant			1.249(0.917,1.701)	0.159	0.795
	Recessive			1.259(0.602,2.636)	0.540	0.540
	Additive			1.205(0.929,1.564)	0.161	0.403
rs3779031	AA	178	149	1		
	GA	167	160	0.874(0.643,1.188)	0.389	0.389
	GG	25	41	0.510(0.297,0.878)	0.015	0.075
	Dominant			0.799(0.596,1.073)	0.136	0.170
	Recessive			0.546(0.324,0.919)	**0.023**	0.058
	Additive			0.776(0.617,0.975)	**0.029**	**0.048**
rs9648691	GG	128	129	1		
	GA	187	156	1.208(0.874,1.670)	0.253	0.633
	AA	54	65	0.837(0.541,1.295)	0.425	0.708
	Dominant			1.10(0.810,1.492)	0.542	0.678
	Recessive			0.752(0.507,1.117)	0.158	0.790
	Additive			0.965(0.782,1.190)	0.738	0.738
rs987456	AA	164	156	1		
	CA	169	147	1.094(0.801,1.493)	0.573	0.955
	CC	37	46	0.765(0.471,1.243)	0.279	0.698
	Dominant			1.016(0.757,1.363)	0.919	0.919
	Recessive			0.732(0.462,1.160)	0.182	0.303
	Additive			0.965(0.782,1.190)	0.578	0.723
rs2462603	AA	158	157	1		
	GA	169	162	0.808(0.483,1.355)	0.420	0.700
	GG	40	31	0.780(0.464,1.310)	0.347	1.735
	Dominant			1.077(0.801,1.446)	0.624	0.780
	Recessive			1.258(0.768,2.062)	0.362	0.453
	Additive			0.913(0.728,1.144)	0.428	0.713

P_a Logistic regression analysis for genotype distributions between DD cases and controls.
P_b The *P*-values were FDR adjustment for multiple tests.
OR = Odds Ratio; CI = Confidence Interval.
The results were in bold if $P<0.05$.

4. Discussion

Sex specificity of *CNTNAP2* in dyslexia was observed in this study. Two SNPs (rs3779031, rs987456) were associated with reduced DD risk in females but not in males. The interaction between the *CNTNAP2* gene (rs987456) and environmental factors (scheduled reading time) played a protective role in females. A previous study found that the *CNTNAP2* variants were associated with an increased risk of language impairment, especially for males. In this study, two mutations in non-coding regions of *CNTNAP2* were linked to a decreased risk of dyslexia only in females.

The *CNTNAP2* gene may show a sex-specific effect through structural alteration in the brain or brain activation during language processing [27]. Evidence from neuroimaging studies demonstrated an association of the *CNTNAP2* polymorphism (rs7794745) with the change in gray matter volume (GMV) in the left superior occipital gyrus (LSOG) of the human brain [28]. Furthermore, reduced GMV in the LSOG was found only in female dyslexics, while less GMV in the left inferior parietal cortex (supramarginal/angular gyri) was observed only in male dyslexics [29]. Moreover, altered *CNTNAP2* expression had a sex-dependent effect on some brain regions, such as visual cortical areas. In male mice, decreasing the expression of *Cntnap2* reduced visually evoked activity modulation in the dorsal stream, while females showed

Table 3
Distribution and associations of *CNTNAP2* gene in cases and controls by gender.

Male ($n = 546$)							Female ($n = 175$)				
SNP	Model	Cases	Controls	OR(95%CI)	P_a	P_b	Cases	Controls	OR(95%CI)	P_a	P_b
rs10240503	AA	162	180	1			64	53	1		
	GA	99	76	1.447(1.003,2.088)	0.480	0.600	22	25	0.729(0.370,1.437)	0.361	0.602
	GG	17	9	2.099(0.910,4.839)	0.820	0.820	0	4	0	0.999	0.999
	Dominant			0.540(0.236,1.233)	0.143	0.238			0	0.990	1.238
	Recessive			0.659(0.464,0.937)	**0.021**	0.053			1.592(0.820,3.089)	0.169	0.423
	Additive			0.691(0.514,0.927)	**0.014**	0.070			1.747(0.956,3.192)	0.070	0.350
rs3779031	AA	127	118	1			51	31	1		
	GA	135	119	1.054(0.742,1.498)	0.769	0.961	32	41	0.474(0.249,0.902)	**0.023**	**0.029**
	GG	19	28	0.630(0.334,1.189)	0.154	0.385	6	13	0.281(0.097,0.814)	**0.019**	**0.032**
	Dominant			0.973(0.695,1.364)	0.875	0.875			0.428(0.233,0.787)	**0.006**	**0.025**
	Recessive			0.614(0.334,1.128)	0.116	0.580			0.40(0.145,1.108)	0.078	0.078
	Additive			0.895(0.687,1.166)	0.411	0.685			0.508(0.318,0.811)	**0.005**	**0.015**
rs9648691	GG	99	100	1			29	29	1		
	GA	139	122	1.151(0.796,1.665)	0.456	2.280	48	34	1.412(0.718,2.778)	0.318	0.397
	AA	43	43	1.010(0.609,1.675)	0.969	0.969	11	22	0.500(0.206,1.215)	0.126	0.315
	Dominant			1.114(0.786,1.579)	0.543	1.358			1.054(0.560,1.981)	0.871	0.871
	Recessive			0.933(0.589,1.478)	0.767	1.279			0.409(0.184,0.907)	0.025	0.125
	Additive			1.034(0.811,1.317)	0.790	0.988			0.784(0.514,1.196)	0.259	0.432
rs987456	AA	126	120	1			38	36	1		
	CA	123	116	1.010(0.707,1.442)	0.957	0.957	46	31	1.406(0.738,2.678)	0.300	0.375
	CC	32	28	1.088(0.618,1.916)	0.769	3.845	5	18	0.263(0.088,0.783)	**0.016**	**0.040**
	Dominant			1.025(0.731,1.437)	0.885	1.106			0.986(0.540,1.799)	0.963	0.963
	Recessive			1.083(0.633,1.854)	0.771	1.928			0.222(0.078,0.628)	**0.002**	**0.010**
	Additive			1.032(0.802,1.328)	0.807	1.345			0.712(0.459,1.104)	0.129	0.215
rs2462603	AA	122	120	1			36	37	1		
	GA	124	121	0.737(0.408,1.331)	0.311	0.518	45	41	1.098(0.377,3.192)	0.864	1.440
	GG	32	23	0.731(0.404,1.321)	0.299	0.748	8	8	0.973(0.330,2.871)	0.960	0.960
	Dominant			1.066(0.759,1.495)	0.713	0.713			1.112(0.609,2.028)	0.730	3.650
	Recessive			1.363(0.773,2.397)	0.282	1.410			0.963(0.344,2.692)	0.943	1.179
	Additive			1.107(0.856,1.433)	0.438	0.548			1.058(0.663,1.688)	0.814	2.035

P_a Logistic regression analysis for genotype distributions between DD cases and controls. P_b The *P*-values were FDR adjustment for multiple tests.
OR = Odds Ratio; CI = Confidence Interval. ref. = reference.
The results were in bold if $P<0.05$.

no change due to a lack of *Cntnap2* [18]. Therefore, females are more likely to be neurotypical, even if they carry *CNTNAP2* mutations [30]. An ASD study illustrated the sex specificity of *Cntnap2* via interaction with environmental factors such as prenatal stress-induced MIA (maternal immune activation). The interaction between the *Cntnap2* mutation and MIA increased the expression of corticotropin-releasing hormone receptor 1 (Crhr1) only in male mice, which then led to deficits in social recognition [31]. According to a study by Hoffman et al., estrogens served as modifiers of neural circuits and rescued the mutants in zebrafish, which may throw light on the molecular mechanism of sex specificity of *Cntnap2* [32]. So far, the mechanism underlying the sex specificity of *CNTNAP2* remains elusive and requires further study.

Table 4
The gene-environment interaction in female students.

	rs3779031		P_a	rs987456		P_a
	AA	GA + GG OR(95%CI)		AA + CA	CC OR(95%CI)	
Father education						
Junior high school or below	ref.			ref.		
Senior high School or equivalency		0.642(0.100,4.128)	0.640		0.556(0.111,2.780)	0.143
Junior college or above		0.240(0.053,1.089)	0.064		0	0.999
Mother Education						
Junior high school or below	ref.			ref.		
Senior high School or equivalency		0.949(0.229,3.940)	0.943		0.435(0.066,2.893)	0.389
Junior college or above		0.4310(0.017,2.639)	0.363		0	0.999
Active learning						
None	ref.			ref.		
sometimes		0.711(0.043,11.790)	0.812		0.310(0.056,1.706)	0.740
Always		0.531(0.039,7.195)	0.634		0	0.999
Scheduled reading time						
NO	ref.			ref.		
Yes		0.389(0.105,1.439)	0.157		**0.431(0.188,0.987)**	**0.047**
Encourage read						
seldom	ref			ref		
sometimes		0.083(0.007,1.031)	0.053		0.529(0.084,3.338)	0.498
always		0.106(0.009,1.232)	0.073		0.175(0.028,1.097)	0.063

Pa Logistic regression analysis for genotype distributions between DD cases and controls.
OR = Odds Ratio; CI = Confidence Interval. ref. = reference.
The results were in bold if $P<0.05$.

We found two mutations in non-coding regions of *CNTNAP2* that were associated with dyslexia only in females. There is cumulative evidence indicating that genetic variants in non-coding, especially regulatory, regions are associated with complex diseases or phenotypes [33]. The rs3779031 polymorphism is located in the 19th intron region and plays a role in mRNA splicing (http://rsnp.psych.ac.cn/) and post-transcriptional control. According to the HaploReg v4.1, rs3779031 acts as enhancer histone marks, which may be involved in some epigenetic processes (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php). The rs987456 polymorphism is located in the 3′-UTR, which could influence the translation efficiency, polyadenylation, and stability of the mRNA. The 3′-UTR also contains binding sites that could bind to microRNAs (miRNAs), which could modify gene expression. According to the functional prediction website (https://snpinfo.niehs.nih.gov/), rs987456 is a binding site whose variant A allele binds to has-miR-624-5p and has-miR-556-3p. The rs987456 has effect on the motif change of *FOXP1* (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php). *FOXP1* variants have been linked to language impairment [34]. The mechanism how different SNPs can regulate gender-specific functions need further study.

The interaction between *CNTNAP2* (rs987456) and scheduled reading time was associated with a reduced risk of DD in females. Reading is a complex task which requires the cooperation of many brain areas [35]. Reading ability is associated with the connection strength among reading-related cortical regions [36,37]. Intensive learning contributes to the development of reading networks in childhood and adolescence, which is called learning-induced cortical plasticity [38]. A more economical, integrative and efficient brain network topology depends on efficient reading [37]. If students have scheduled reading time, their reading circuitry will be optimized by the interaction of reading behaviors and genetics. As *CNTNAP2* is involved in the development of cortical circuits [39], we venture that *CNTNAP2* may take part in this learning-induced cortical plasticity. The polymorphism rs987456 may play a role in facilitating the alteration of reading circuitry. Developmental dyslexia is often characterized as a disconnection syndrome, in which functional connections between reading-relevant cortical regions are weakened [35,40]. The sex difference in network connectivity was demonstrated by some magnetic resonance imaging (MRI) studies. The network organization of teenage male brains was more local, more segregated than teenage female brains [41]. Based on connectivity–behavior analysis, proper reading therapy may help individuals with DD to form efficient reading circuitry and improve their reading ability [36,42]. Parents and teachers should help students to develop good learning habits, e.g. scheduled reading time and active learning.

Our study has several limitations. First, only five SNPs in *CNTNAP2* were selected for investigation, additional variants in *CNTNAP2* are need further study. Second, the mechanism how different SNPs can regulate gender-specific functions was not addressed. Third, the sample size was relative small, and the results of this study should be verified in different populations.

We observed sex specificity of *CNTNAP2* in DD. Two mutations in non-coding regions of *CNTNAP2* were associated with a decrease risks in DD in females. The interactions between *CNTNAP2* variants and environmental factors also played protective roles in females. All these results might be helpful to understand gender-based differences in DD.

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Declaration of Interest

None.

Evidence before this study

Developmental dyslexia (DD) is one of the heritable neurodevelopmental disorders. Males show a higher prevalence of DD than females, but the mechanism underlying this gender difference is poorly understood. The contactin-associated protein-like 2 (CNTNAP2) gene shows sex-specific patterns in some neurodevelopmental disorders, which may be one of the potential reasons.

Added value of this study

Using a case-control study in China, we found a sex-specific effect of the candidate gene *CNTNAP2* in children. Two mutations in *CNTNAP2* were linked to a decreased risk of DD only in girls. Our findings might be might be helpful to understand gender-based differences in DD.

Implications of all available evidence

Genetic variants in the *CNTNAP2* gene are associated with gender differences among dyslexic children in China.

Author Contributions

GH and SR designed the study. GH and HF drafted the manuscript. GH, LL, LX, HF, Nkomola P.D., XX and LX collected data and performed experiment. GH, HF and LL analyzed data. SR and Nkomola P.D. reviewed the manuscript. All authors have read the manuscript and approved the submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.07.007.

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