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

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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 from 1.93:1 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 Hong Kong [4]. People in mainland China use simplified Chinese characters, whereas the traditional Chinese is widely used in Taiwan and Hong Kong [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 1204 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, DCCD2, KIAA0319,** and **CNTNAP2** [11–14]. The heritability ($h^2$) of DD was estimated to range from 0.18 to 0.72 [11]. The Colorado twin study ($n = 956$) of reading difficulties found the gender differences in $h^2$ did not reach the significant level (male $h^2 = 0.65$, female $h^2 = 0.54$) [15]. However, a twin study from UK ($n = 3909$) showed males had higher $h^2$ 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) knockout, heterozygous, and wild-type mice. In human studies, a two-stage association study in AGRE (Autism Genetic Resource Exchange) knockouts and sex differences in language delay in autism-spectrum disorder (ASD) appeared more in 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) knockouts and sex differences in language delay in autism-spectrum disorder (ASD) appeared more in 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.

### 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 = $10.09 \pm 1.26$). The male-to-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. ThePRS 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/pidsearchpw.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 $\chi^2$ 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–environment 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 = 0.766, 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 = 0.029; GG vs AA: OR = 0.281, 95%CI = 0.097–0.814, $P_a$ = 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, $P_a$ = 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.

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

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Table 1

| SNP      | Minor/major | MAF in CHB | MAF control | HWE   |
|----------|-------------|------------|-------------|-------|
| rs10240503 | AA          | 0.1893     | 0.1830      | 0.6204|
| rs3779031  | AA          | 0.2913     | 0.3457      | 0.8442|
| rs9648691  | A/G         | 0.3835     | 0.4085      | 0.1458|
| rs987456   | C/A         | 0.3010     | 0.3423      | 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

| SNP      | Model     | Cases | Controls | OR (95% CI) | $P_a$ | $P_b$ |
|----------|-----------|-------|----------|-------------|-------|-------|
| rs10240503 | AA        | 226   | 233      | 1.235 (0.896,1.703) | 0.198 | 0.330 |
| GA       | 121       | 101   |          |             |       |       |
| GG       | 17        | 13    |          |             |       |       |
| Dominant |           |       |          | 1.348 (0.640,2.840) | 0.432 | 0.540 |
| Recessiv |           |       |          | 1.249 (0.917,1.701) | 0.159 | 0.795 |
| Additive |           |       |          | 1.259 (0.802,2.636) | 0.540 | 0.540 |
| rs3779031  | AA        | 178   | 149      | 1.287 (0.643,2.188) | 0.389 | 0.389 |
| GA       | 167       | 160   |          |             |       |       |
| GG       | 25        | 41    |          |             |       |       |
| Dominant |           |       |          | 0.501 (0.297,0.878) | 0.015 | 0.075 |
| Recessiv |           |       |          | 0.799 (0.596,1.073) | 0.136 | 0.170 |
| Additive |           |       |          | 1.205 (0.929,1.564) | 0.161 | 0.403 |
| rs987456   | AA        | 164   | 156      | 1.208 (0.874,1.670) | 0.253 | 0.633 |
| GA       | 169       | 147   |          |             |       |       |
| CC       | 37        | 46    |          |             |       |       |
| Dominant |           |       |          | 0.837 (0.541,1.295) | 0.420 | 0.706 |
| Recessiv |           |       |          | 1.101 (0.810,1.492) | 0.542 | 0.678 |
| Additive |           |       |          | 0.752 (0.507,1.117) | 0.158 | 0.790 |
| rs2462603  | AA        | 158   | 157      | 1.208 (0.874,1.670) | 0.253 | 0.633 |
| GA       | 169       | 162   |          |             |       |       |
| CC       | 40        | 31    |          |             |       |       |
| Dominant |           |       |          | 0.780 (0.464,1.310) | 0.347 | 1.735 |
| Recessiv |           |       |          | 1.258 (0.782,1.190) | 0.738 | 0.738 |
| Additive |           |       |          | 0.965 (0.782,1.190) | 0.738 | 0.738 |

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 $P<0.05$.
no change due to a lack of Cntnap2. 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 3

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

| SNP       | Model | Male (n = 546) | Female (n = 175) | OR (95% CI) | P_a | P_b |
|-----------|-------|----------------|------------------|-------------|-----|-----|
| rs10240503 |       |                |                  |             |     |     |
| AA        | 162   | 180            | 0.480 (0.600)    | 0.640       |     |     |
| GA        | 99    | 76             | 0.820 (0.820)    | 0.834       |     |     |
| GG        | 17    | 9              | 0.143 (0.238)    | 0.209       |     |     |
| Dominant  |       |                | 0.005 (0.005)    | 0.005       |     |     |
| Recressive |      |                | 0.053 (0.053)    | 0.053       |     |     |
| Additive  | 0.014 | 0.070          | 0.014 (0.070)    | 0.014       |     |     |
| rs779031   |       |                |                  |             |     |     |
| AA        | 127   | 118            | 0.769 (0.961)    | 0.961       |     |     |
| GA        | 135   | 119            | 0.154 (0.385)    | 0.385       |     |     |
| GG        | 19    | 28             | 0.875 (0.875)    | 0.875       |     |     |
| Dominant  |       |                | 0.005 (0.005)    | 0.005       |     |     |
| Recressive |      |                | 0.053 (0.053)    | 0.053       |     |     |
| Additive  | 0.411 | 0.685          | 0.411 (0.685)    | 0.411       |     |     |
| rs968691   |       |                |                  |             |     |     |
| AA        | 126   | 120            | 0.957 (0.957)    | 0.957       |     |     |
| GA        | 123   | 117            | 0.759 (3.845)    | 3.845       |     |     |
| CC        | 32    | 28             | 0.767 (1.279)    | 1.279       |     |     |
| Dominant  |       |                | 0.005 (0.005)    | 0.005       |     |     |
| Recressive |      |                | 0.053 (0.053)    | 0.053       |     |     |
| Additive  | 0.790 | 0.988          | 0.790 (0.988)    | 0.988       |     |     |
| rs987456   |       |                |                  |             |     |     |
| AA        | 126   | 120            | 0.957 (0.957)    | 0.957       |     |     |
| GA        | 123   | 117            | 0.759 (3.845)    | 3.845       |     |     |
| CC        | 32    | 28             | 0.767 (1.279)    | 1.279       |     |     |
| Dominant  |       |                | 0.005 (0.005)    | 0.005       |     |     |
| Recressive |      |                | 0.053 (0.053)    | 0.053       |     |     |
| Additive  | 0.790 | 0.988          | 0.790 (0.988)    | 0.988       |     |     |
| rs2462603  |       |                |                  |             |     |     |
| AA        | 122   | 120            | 0.957 (0.957)    | 0.957       |     |     |
| GA        | 124   | 121            | 0.759 (3.845)    | 3.845       |     |     |
| CC        | 32    | 23             | 0.767 (1.279)    | 1.279       |     |     |
| Dominant  |       |                | 0.005 (0.005)    | 0.005       |     |     |
| Recressive |      |                | 0.053 (0.053)    | 0.053       |     |     |
| Additive  | 0.790 | 0.988          | 0.790 (0.988)    | 0.988       |     |     |

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.

### Table 4

The gene-environment interaction in female students.

| rs3779031 | P_a | rs987456 | P_a |
|-----------|-----|----------|-----|
| AA        | GA + GG (OR(95%CI)) | 0.640 (0.100,1.428) | 0.040 |
|          | ref. | 0.111,2.780 | 0.183 |
| Junior high school or below | Senior high School or equivalency | 0.240 (0.053,1.089) | 0.064 |
|          | ref. | 0.999 |
| Mother Education | Senior high school or below | 0.949 (0.293,3.940) | 0.943 |
|          | ref. | 0.435 (0.066,2.893) | 0.389 |
| Junior college or above | 0.431 (0.017,2.639) | 0.363 |
|          | ref. | 0.999 |
| Active learning | None | 0.711 (0.043,1.179) | 0.812 |
|          | ref. | 0.310 (0.056,1.706) | 0.740 |
| Scheduled reading time | NO | 0.531 (0.039,7.195) | 0.634 |
|          | Yes | 0.389 (0.105,1.439) | 0.157 |
| Encourage read | ref. | 0.431 (0.188,0.987) | 0.047 |
|          | ref. | 0.047 |
| times | | 0.047 |
| always | 0.047 |

P_a 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.

References

[1] Mascheretti S, Trezzi V, Giorda R, et al. Complex effects of dyslexia risk factors account for ADHD traits: evidence from two independent samples. J Child Psychol Psychiatry 2017;58(1):75–82. https://doi.org/10.1111/jcpp.12612.
[2] Shao S, Kong K, Zuo L, et al. The roles of genes in the neuronal migration and neurite outgrowth network in developmental dyslexia: single- and multiple-risk genetic variants. Mol Neurobiol 2016;53(6):3967–75. https://doi.org/10.1007/s12035-015-9334-8.
[3] Peterson RL, Pennington BF. Developmental dyslexia. Annu Rev Clin Psychol 2015;11(1):283–307. https://doi.org/10.1146/annurev-clinpsy-032814-112842.
[4] Chan DW, Cih HO, Tsang SM, Sh Lee, Chung KKH. Prevalence, gender ratio and genetic factors in a Chinese population with reading disorder. Sci Rep 2016;6(36697). https://doi.org/10.1038/srep36697.
[5] Liu L, Wang J, Shao S, et al. Descriptive epidemiology of prenatal and perinatal risk factors in a Chinese population with reading disorder. Sci Rep 2016;6(36697).
[6] Flannery KALJ, Daly L, Schultz J. Male prevalence for reading disability is foundin a new findings from four epidemiological studies. JAMA 2004;Vol. 291(16):2007-43. https://doi.org/10.1001/jama.291.16.2007.
[7] Quinn JM, Wagner RK. Gender differences in reading impairment and in the identification of impaired readers: results from a large-scale study of at-risk readers. J Learn Disabil 2015;48(4):431–45. https://doi.org/10.1177/0022219415508323.
[8] Jin H, YH Mo Lei. On the diagnostic tests of Chinese Developmental Dyslexia. J South China Norm Univ 2009;41(5):39–43.
[9] Kore J. The molecular genetics and neurobiology of developmental dyslexia as model of a complex phenotype. Biochem Biophys Res Commun 2014;452(2):236–43. https://doi.org/10.1016/j.jbc.2014.07.102.
[10] {//PlaceHolder:References}{//PlaceHolder:References}{//PlaceHolder:References}
motor activities in a dyslexia family sample. J Neurodev Disord 2011;3(1):39–49. https://doi.org/10.1007/s11689-010-9065-0.

[14] Meng H, Smith SD, Hager K, et al. DCCD2 is associated with reading disability and modulates neuronal development in the brain. PNAS 2005;102(47):17053–8. https://doi.org/10.1073/pnas.0508591102.

[15] Hawke JL, Wadsworth SJ, Defries JC. Genetic influences on reading difficulties in boys and girls: the Colorado twin study. Dyslexia 2006;12(1):21–9.

[16] Harlaar N, Spinath FM, Dale PS, Plomin R. Genetic influences on early word recognition abilities and disabilities: a study of 7-year-old twins. J Child Psychol Psychiatr 2005;46(4):373–84. https://doi.org/10.1111/j.1469-7610.2004.00358.x.

[17] Rodenas-Cuadrado P, Ho J, Vernes SC. Shining a light on CNTNAP2: complex functions to complex disorders. Eur J Hum Genet 2014;22(2):171–8. https://doi.org/10.1038/ejhg.2013.100.

[18] Townsend LB, Smith SL. Genotype- and sex-dependent effects of altered Cntnap2 expression on the function of visual cortical areas. J Neurodev Disord 2017;9:2. https://doi.org/10.1186/s11689-016-9182-5.

[19] Alarcon M, Abrahams BS, Stone JL, et al. Linkage, association, and gene-expression interactions to complex disorders. Eur J Hum Genet 2015;23(1):17–22. https://doi.org/10.1038/ejhg.2014.005.

[20] Iakoubov L, Mossakowska M, Szwed M, Puzianowska-Kuznicka M. A common copy number variation polymorphism in the CNTNAP2 gene: sexual dimorphism in association with healthy aging and disease. Gerontology 2015;61(1):24–31. https://doi.org/10.1159/000363320.

[21] Poot M. Connecting the CNTNAP2 networks with neurodevelopmental disorders. Mol Syndromol 2015;6(1):7–22. https://doi.org/10.1080/15424251.2015.100371594.

[22] St Sauver JLKS, Barbaresi WJ, Colligan RC, Jacobsen SJ. Boy/girl differences in risk for reading disability: potential clues? Am J Epidemiol 2001;154(9):787–93. https://doi.org/10.1093/aje/154.9.787.

[23] Limbrick L, Wheldall K, Madelaine A. Why do more boys than girls have a reading disability? Pediatr Neurol 2017;172:16–21. https://doi.org/10.1016/j.pediatrneurol.2016.02.003.

[24] Innocentino L, Zucman S, Monine C, et al. A common variant of the CNTNAP2 gene is associated with structural variation in the left superior occipital gyrus. Brain Lang 2017;172:16–21. https://doi.org/10.1016/j.bandl.2016.02.003.

[25] Al-Murrani A, Ashton S, George AM, Love DR. Amino-terminal microdeletion within the CNTNAP2 gene associated with variable expressivity of speech delay. Case Rep Genet 2012;2012:172408. https://doi.org/10.1155/2012/172408.

[26] Schachtsin SM, Gagnidze K, Reyes A, et al. Sex-specific gene-environment interactions underlying ASD-like behaviors. Proc Natl Acad Sci 2017;114(6):1383–8. https://doi.org/10.1073/pnas.1619312114.

[27] Hoffman EJ, Turner RJ, Fernandez JM, et al. Estrogens suppress a behavioral phenotype in zebrafish mutants of the autism risk gene, CNTNAP2. Neuron 2016;89(4):725–33. https://doi.org/10.1016/j.neuron.2015.12.039.

[28] Gong J, Tian J, Lou J, et al. A polymorphic MRC response element in KITBD1 influences colorectal cancer risk, especially in interaction with an MYC-regulated SNP rs6983267. Ann Oncol 2018;29(1):632–9. https://doi.org/10.1093/annonc/mdy789.

[29] Evans TM, Flowers DL, Napolioli EM, Eden GF. Sex-specific gray matter volume differences in females with developmental dyslexia. Brain Struct Funct 2014;219(3):1041–54.

[30] St Sauver JLKS, Barbaresi WJ, Colligan RC, Jacobsen SJ. Boy/girl differences in risk for reading disability: potential clues? Am J Epidemiol 2001;154(9):787–93. https://doi.org/10.1093/aje/154.9.787.