NEURAL REGENERATION RESEARCH
February 2017, Volume 12, Issue 2
www.nrronline.org

RESEARCH ARTICLE

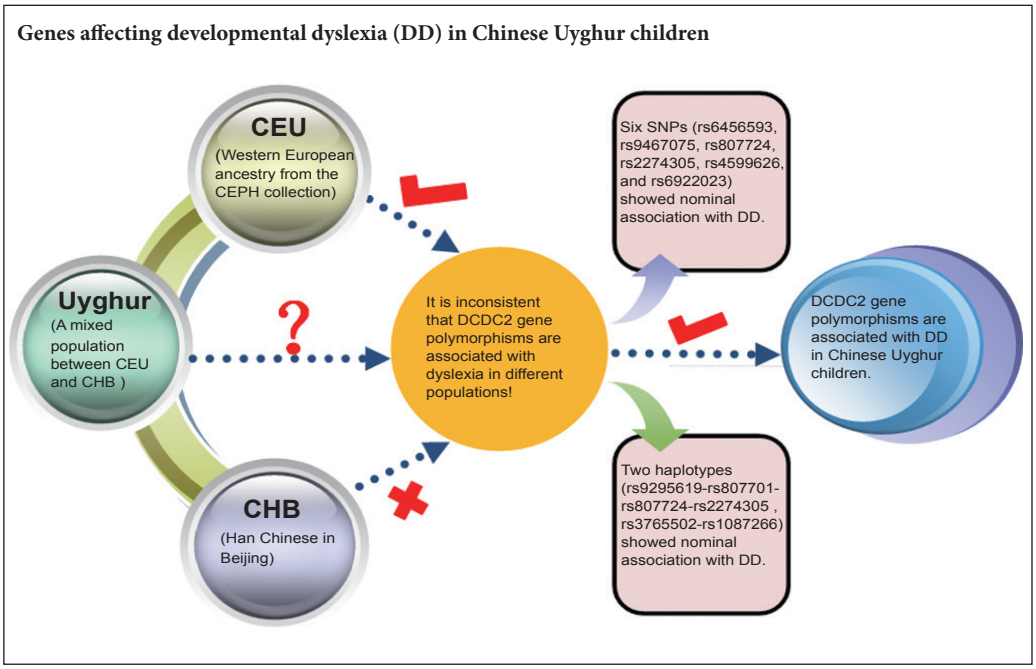
DCDC2 gene polymorphisms are associated with developmental dyslexia in Chinese Uyghur children

Yun Chen[1], **Hua Zhao**[1], **Yi-xin Zhang**[2], **Peng-xiang Zuo**[1,*]
1 Medical College, Shihezi University, Shihezi, Xinjiang Uyghur Autonomous Region, China
2 Special Clinic Department, the 12th Hospital of People's Liberation Army, Kashgar, Xinjiang Uyghur Autonomous Region, China

How to cite this article: *Chen Y, Zhao H, Zhang YX, Zuo PX (2017) DCDC2 gene polymorphisms are associated with developmental dyslexia in Chinese Uyghur children. Neural Regen Res 12(2):259-266.*

Funding: *This study was funded by the National Natural Science Foundation of China, No. 81360434.*

Graphical Abstract



***Correspondence to:**
Peng-xiang Zuo, M.D.,
zuo_pengxiang@sina.com.

orcid:
0000-0001-7881-8644
(Peng-xiang Zuo)

doi: *10.4103/1673-5374.200809*

Accepted: *2016-12-21*

Abstract

Developmental dyslexia is a complex reading and writing disorder with strong genetic components. In previous genetic studies about dyslexia, a number of candidate genes have been identified. These include DCDC2, which has repeatedly been associated with developmental dyslexia in various European and American populations. However, data regarding this relationship are varied according to population. The Uyghur people of China represent a Eurasian population with an interesting genetic profile. Thus, this group may provide useful information about the association between DCDC2 gene polymorphisms and dyslexia. In the current study, we examined genetic data from 392 Uyghur children aged 8–12 years old from the Xinjiang Uyghur Autonomous Region of China. Participants included 196 children with dyslexia and 196 grade-, age-, and gender-matched controls. DNA was isolated from oral mucosal cell samples and fourteen single nucleotide polymorphisms (rs6456593, rs1419228, rs34647318, rs9467075, rs793862, rs9295619, rs807701, rs807724, rs2274305, rs7765678, rs4599626, rs6922023, rs3765502, and rs1087266) in DCDC2 were screened *via* the SNPscan method. We compared SNP frequencies in five models (Codominant, Dominant, Recessive, Heterozygote advantage, and Allele) between the two groups by means of the chi-squared test. A single-locus analysis indicated that, with regard to the allele frequency of these polymorphisms, three SNPs (rs807724, rs2274305, and rs4599626) were associated with dyslexia. rs9467075 and rs2274305 displayed significant associations with developmental dyslexia under the dominant model. rs6456593 and rs6922023 were significantly associated with developmental dyslexia under the dominant model and in the heterozygous genotype. Additionally, we discovered that the T-G-C-T of the four-marker haplotype (rs9295619-rs807701-rs807724-rs2274305) and the T-A of the two-marker haplotype (rs3765502-1087266) were significantly different between cases and controls. Thus, we conclude that DCDC2 gene polymorphisms are associated with developmental dyslexia in Chinese Uyghur children.

Key Words: *nerve regeneration; developmental dyslexia; single nucleotide polymorphisms; Xinjiang Uyghur Autonomous Region; elementary school students; genetics; reading disability; gene polymorphisms; etiology; case-control study; neural regeneration*

Introduction

Developmental dyslexia, which is considered a type of reading disability, is among the most common neurobehavioral learning disorders (Meng et al., 2005). Developmental dyslexia affects between 5% and 17% of school-age children in Western countries (Ludwig et al., 2008; Marino et al., 2011; Matsson et al., 2011; Svensson et al., 2011; Sun et al., 2013) and between 3.9% and 12.6% of those in China (Shao et al., 2015). The condition is characterized by impaired reading and writing abilities in the context of normal intelligence, appropriate motivation, adequate educational opportunities, and sensory acuity (Fisher et al., 2002). Dyslexic children exhibit specific deficiencies in phonological awareness, quick naming, orthographic coding, working memory, and other related psychological processes. One longitudinal study showed that developmental dyslexia is a long-term, persistent deficiency that can adversely affect one's ability to obtain knowledge and improve mental capacity throughout life (Shao et al., 2015). Dyslexia has an enormous negative impact on the intellectual and psychosocial development of children. For instance, individuals with dyslexia experience serious challenges with respect to reading and writing, which, if not addressed, can severely impact social development (Czamara et al., 2011). Elucidating the pathogenesis of developmental dyslexia is crucial for the generation of effective therapeutic strategies and efficient remedial interventions. Therefore, numerous educators, psychologists, and behavioral geneticists are engaged in clarifying the mechanisms underlying the pathogenesis of this condition.

The field of molecular genetics has made great progress in understanding the pathogenesis of developmental dyslexia (Czamara et al., 2011). Twin studies have shown that genetic components exert a substantial influence on reading ability in children: 44–75% of phenotypic variance may be attributed to genetic factors (Plomin and Kovas, 2005; Davis et al., 2014). Linkage and association studies have identified nine regions; DYX1–DYX9, that are implicated in the development of this condition. Further investigations have identified specific candidate genes involved in the development of developmental dyslexia. These include DYX1C1 in DYX1 (Taipale et al., 2003), DCDC2 (Meng et al., 2005) and KIAA0319 (Francks et al., 2004; Cope et al., 2005) in DYX2, and ROBO1 in DYX5 (Hannula-Jouppi et al., 2005). Among these genes, DCDC2 is one of the best studied. The direct association between DCDC2 and developmental dyslexia was originally reported in an American population using high-density genotyping of formerly identified region JA04 (Kaplan et al., 2002). DCDC2 was first identified as a candidate gene for developmental dyslexia based on the association between single nucleotide polymorphisms (SNPs) and a quantitative index of dyslexia degree in a cluster of American families (Meng et al., 2005). Subsequently, a fine-mapping study in a German population reported a significant association between DCDC2 and developmental dyslexia according to the rs793862 polymorphism and two-marker haplotype rs793862-rs807701 (Schumacher et al., 2006). An additional study with a German population reported a significant association between rs807724, rs793862, and rs807701 in DCDC2 and the pathogenesis of developmental dyslexia (Wilcke et al., 2009). Furthermore, a meta-analysis of previous studies indicated that rs807701 may significantly contribute to the risk of developmental dyslexia (Zhong et al., 2013). Additional SNP markers (rs1419228, rs1091047, rs9467075, rs9467076, rs7765678, and rs6922023) in DCDC2 have been associated with dyslexia in an Australian sample (Lind et al., 2010). However, several genetic variations of DCDC2 were found not to exhibit any obvious associations with developmental dyslexia in Indian (Venkatesh et al., 2011, 2013), Brazilian (Svidnicki et al., 2013), and Chinese populations (Zuo et al., 2012). According to previous findings, an association between DCDC2 and developmental dyslexia could not be established across various populations. Despite this, DCDC2 represents an important candidate gene that encodes a doublecortin domain-containing protein 2, which is extensively expressed in the human brain and modulates neuronal migration (Meng et al., 2005). In addition, functional magnetic resonance imaging studies have shown that DCDC2 is associated with brain activation patterns during reading-related assignments (Cope et al., 2012).

Xinjiang, a remote and sparsely populated area in northwest China, spans approximately 1.6 million km^2, and occupies about one-sixth of the country's territory. The region is home to members of numerous ethnic groups such as the Uyghur, Han, Kazakhs, Hui, Kyrgyz, Mongol, Tajik, and Russian people. Among these, the Uyghur population is the largest, reaching 11.27 million in 2014. Because of their specific geographical environment and local customs, Uyghur people rarely intermarry with other nationalities. Therefore, as both population mobility and genetic drift are low, this population carries interesting genetic information, especially in terms of the molecular genetics of various diseases (Lin et al., 2016). The Uyghur population has mixed ancestry and has been deemed to be genetically related to European and East Asian populations according to the following ratios (European: East Asian): 43:57 (Yao et al., 2004), 60:40 (Xu et al., 2008), 47:53 and 52:48 (Xu and Jin, 2008), or 31:69 (Li et al., 2009). In addition, the unique language of this ethnic group, namely Uyghur, is significantly different from Chinese and English in terms of both structure and pronunciation.

In view of the genetic and linguistic differences between global populations, we explored the relationship between DCDC2 polymorphisms and developmental dyslexia in Uyghur people. In the present study, we performed high-density genotyping for several specific SNPs of DCDC2 in Uyghur children from Xinjiang to examine the association between DCDC2 polymorphisms and developmental dyslexia in the Uyghur population.

Subjects and Methods

Participants

We used cluster sampling to recruit a total of 4,251 pupils in 28 Uyghur primary schools in Kashgar and Aksu, Xinjiang, China.

Table 1 Details of polymorphisms analyzed in this study

No.	SNP	Position	Location	Allele	MAF[a]		HWE in controls
					CHB	CEU	
1	rs6456593	24174101	Exon10	C/G	0.489	0.238	0.985
2	rs1419228	24178078	Intron9	A/G	0.029	0.190	0.639
3	rs34647318	24178475	Exon9	A/C	NA	NA	1.000
4	rs9467075	24205008	Exon8	A/G	0.044	0.154	0.902
5	rs793862	24206972	Intron7	A/G	NA	NA	0.336
6	rs9295619	24250283	Intron7	C/T	0.482	0.394	0.095
7	rs807701	24273563	Intron7	C/T	0.278	0.308	0.106
8	rs807724	24278641	Intron6	A/G	0.036	0.190	0.383
9	rs2274305	24290975	Exon5	A/G	0.221	0.330	0.067
10	rs7765678	24330316	Intron2	C/T	0	0.080	0.631
11	rs4599626	24341129	Intron2	A/C	0.208	0.221	0.733
12	rs6922023	24347889	Intron2	A/G	0.310	0.173	0.550
13	rs3765502	24353817	Intron1	A/G	0.357	0.119	0.842
14	rs1087266	24354922	Intron1	C/T	NA	NA	0.378

[a]MAF of CHB and CEU in HapMap release #28 data. MAF: Minor allele frequency; HWE: Hardy-Weinberg equilibrium; CHB: Han Chinese in Beijing, China; CEU: Western European ancestry from the CEPH collection; NA: not required.

Table 2 Participant demographic information

Variable	Case (n = 195)		Control (n = 196)		χ^2/t	P
	n	%	n	%		
Age (year)						
≤ 10	76	38.97	73	37.24	0.124	0.725
≥ 11	119	61.03	123	62.76		
Sex						
Male	125	64.10	120	61.22	0.346	0.556
Female	70	35.90	76	38.78		
Grade						
3	56	28.72	56	28.57		
4	69	35.38	70	35.71	0.005	0.998
5	70	35.90	70	35.71		
IQ score[a]	94.12±12.85		93.80±12.47		0.352	0.295

[a]IQ scores are shown as the mean ± SD and statistical analysis was performed using Student's t-tests. IQ: Intelligence quotient.

Diagnostic criteria for dyslexic children

(1) A score on the Dyslexia Checklist for Uyghur Children of at least 2 standard deviations above the mean score (Wu et al., 2006); (2) a score on the Pupil Rating Scale Revised Screening for Learning Disabilities (PRS) of less than 65 points (Jing et al., 1998).

Inclusion criteria

(1) Between 8 and 12 years of age; (2) a score higher than 80 on the intelligence quotient of the China-Wechsler Intelligence Scale for Children (Gong and Dai, 1988); (3) physically healthy according to school medical history.

Exclusion criteria

(1) History of organic cerebral injury or psychiatric disorders; (2) history of visual or auditory dysfunction.

Finally, 228 Uyghur children were diagnosed with dyslexia, and 196 (126 boys and 70 girls) of these agreed to participate in the current study. The control group consisted of 196 non-dyslexic students recruited from the initial sample. These children were grade-, age-, and gender-matched to the dyslexic children.

All participants were right-handed native speakers of the Uyghur language.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Shihezi University in China. Written informed consent was acquired from the guardians involved in the study.

Selection of DCDC2 polymorphisms

The SNPs were selected using two methods:

(1) Ht SNPs, namely haplotype tag SNPs, were selected using Haploview 4.2 (Mark Daly's lab at the Broad Institute, MA, USA). We filtered the SNPs, reserving those with a minor allele frequency for Western European ancestry (as per the CEPH collection) or Han Chinese in Beijing, China (as per the International HapMap project) at > 5% and set the pairwise r2 threshold at ≥ 0.8.

(2) Hot SNPs, such as rs793862 (Schumacher et al., 2006; Wilcke et al., 2009; Newbury et al., 2011), rs807701 (Wilcke et al., 2009; Zhong et al., 2013), rs807724 (Wilcke et al., 2009; Newbury et al., 2011), and rs2274305 (Venkatesh et al., 2011; Zuo et al., 2012), were identified by browsing the available literature.

This procedure resulted in the selection of the following fourteen SNPs for survey: rs6456593, rs1419228, rs34647318, rs9467075, rs793862, rs9295619, rs807701, rs807724, rs2274305, rs7765678, rs4599626, rs6922023, rs3765502, and rs1087266. For more details regarding these polymorphisms, see **Table 1**.

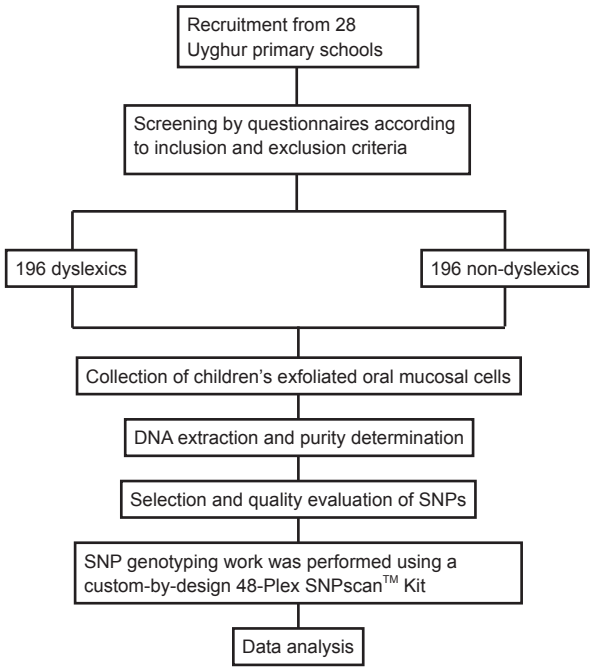


Figure 1 Flow chart showing study procedure.
SNPs: Single nucleotide polymorphisms.

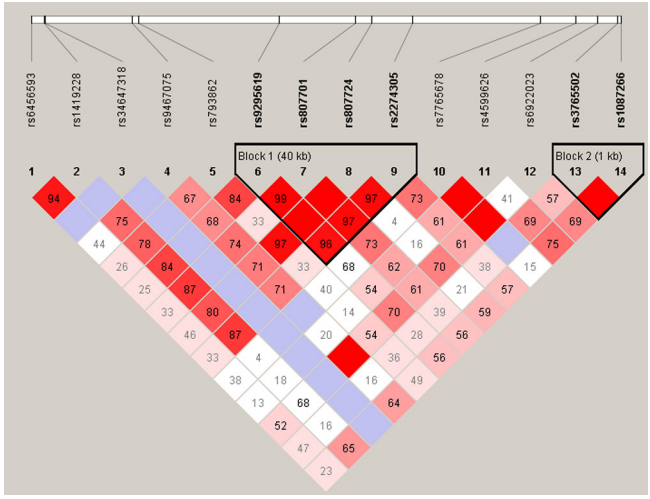


Figure 2 Linkage disequilibrium plot of fourteen single nucleotide polymorphisms in DCDC2.
The results revealed that the T-G-C-T haplotype of the former block (χ^2 = 6.044, P = 0.0140) and the T-A haplotype of the latter block (χ^2 = 4.223, P = 0.0399) were significantly higher in the developmental dyslexia group than in the control group, whereas other haplotypes showed no difference in frequency between the two groups.

Isolation of DNA and genotyping strategies

We extracted genomic DNA from oral mucosal cell samples collected from the 392 participants, according to a previously reported procedure (Zuo et al., 2012). Data were obtained through standard procedures carried out in accordance with the manufacturer's recommendations. SNP genotyping was performed using a custom-by-design 48-Plex SNPscan™ Kit (Genesky Biotechnologies, Shanghai, China), as described previously. The kit was developed by Genesky Biotechnologies Inc. (using patented SNP genotyping technology) based on double ligation and a multiplex fluorescence polymerase chain reaction (Chen et al., 2012). Repeated analyses were conducted for 4% of randomly chosen samples with high DNA quality, for quality control purposes. Raw data were analyzed using GeneMapper V3.0 software (Applied Biosystems, USA).

Statistical analysis

The intelligence quotient (IQ) scores are presented as the mean ± SD. Statistical analyses were performed using Student's *t*-tests. Deviations from the Hardy-Weinberg equilibrium (HWE), as well as differences in the distribution of demographic characteristics, were evaluated using the chi-squared test in control subjects. We calculated the alleles and genotype distributions in four models (Co-dominant, Dominant, Recessive, and Heterozygote advantage) for dyslexic and non-dyslexic children *via* the chi-square test, using SPSS version 19.0 software (IBM, Armonk, NY, USA). The linkage disequilibrium was estimated, haplotype blocks were constructed, and haplotype-based association tests were carried out using Haploview 4.2 software (Barrett et al., 2005). All statistical tests were carried out in a two-sided manner and all P values lower than 0.05 were considered to represent statistical significance without corrections for multiple testing (Chang et al., 2015).

Results

Characteristics of the subjects and HWE result

A DNA sample for one dyslexic subject was not successfully genotyped; therefore, 195 cases and 196 controls were included in the analysis (**Figure 1**). The characteristics of these participants are presented in **Table 2**. The dyslexic and non-dyslexic children were paired by age, gender, education level, and IQ score ($P > 0.05$).

Fourteen SNPs adhered to the HWE in the control group (**Table 1**); therefore, these were all included in the subsequent analyses.

Single marker analysis

We genotyped fourteen SNPs (rs6456593, rs1419228, rs34647318, rs9467075, rs793862, rs9295619, rs807701, rs807724, rs2274305, rs7765678, rs4599626, rs6922023, rs3765502, and rs1087266) in DCDC2 and found a significantly nominal association between six SNPs and dyslexia. The results of six single-site association analyses between the dyslexic and control participants are listed in **Table 3**. Regarding the allele frequency of these polymorphisms, three SNPs (rs807724, P = 0.024; rs2274305, P = 0.033; and rs4599626, P = 0.046) exhibited associations with dyslexia. We then performed association analyses of co-dominant, dominant, recessive, and heterozygote advantage models. As indicated, rs6456593 and rs6922023 were significantly associated with developmental dyslexia under the dominant model (χ^2 = 4.875, P = 0.027; χ^2 = 3.908, P = 0.048) and in the heterozygous genotype (χ^2 = 4.409, P = 0.036; χ^2 = 5.715, P = 0.017), respectively. Additionally, we discovered that rs9467075 (χ^2 = 3.883, P = 0.049) and rs2274305 (χ^2 = 4.300,

Table 3 Association between the candidate polymorphisms and developmental dyslexia risk

SNP	Model	Genotype	Case	Control	χ^2	*P*	SNP	Model	Genotype	Case	Control	χ^2	*P*
rs6456593	Co-dominant	C/C	89	68	5.431	0.066	rs2274305	Co-dominant	C/C	87	108	4.436	0.109
		C/G	74	95					C/T	81	68		
		G/G	32	33					T/T	27	20		
	Dominant	C/C	89	68	4.875	**0.027**		Dominant	C/C	87	108	4.300	**0.038**
		C/G+G/G	106	128					C/T+T/T	108	88		
	Recessive	C/C+C/G	163	163	0.013	0.910		Recessive	C/C+C/T	168	176	1.226	0.268
		G/G	32	33					T/T	27	20		
	Heterozygote advantage	C/C+G/G	121	101	4.409	**0.036**		Heterozygote advantage	C/C+T/T	114	128	1.942	0.163
		C/G	74	95					C/T	81	68		
	Allele	C	252	231	2.677	0.102		Allele	C	255	284	4.555	**0.033**
		G	138	161					T	135	108		
rs9467075	Co-dominant	G/G	134	152	3.885	0.143	rs4599626	Co-dominant	C/C	107	125	4.065	0.131
		G/A	57	41					C/A	75	64		
		A/A	4	3					A/A	13	7		
	Dominant	G/G	134	152	3.883	**0.049**		Dominant	C/C	107	125	3.212	0.073
		G/A+A/A	61	44					C/A+A/A	88	71		
	Recessive	G/G+G/A	191	193	0.151	0.698		Recessive	C/C+C/A	182	189	1.93	0.165
		A/A	4	3					A/A	13	7		
	Heterozygote advantage	G/G+A/A	138	155	3.596	0.058		Heterozygote advantage	C/C+A/A	120	132	1.439	0.230
		G/A	57	41					C/A	75	64		
	Allele	G	325	345	3.485	0.062		Allele	C	289	314	3.987	**0.046**
		A	65	47					A	101	78		
rs807724	Co-dominant	T/T	125	143	4.747	0.093	rs6922023	Co-dominant	G/G	136	118	5.848	0.054
		T/C	57	47					G/A	48	70		
		C/C	13	6					A/A	11	8		
	Dominant	T/T	125	143	3.556	0.060		Dominant	G/G	136	118	3.908	**0.048**
		T/C+C/C	70	53					G/A+A/A	59	78		
	Recessive	T/T+T/C	182	190	2.748	0.097		Recessive	G/G+G/A	184	188	0.514	**0.473**
		C/C	13	6					A/A	11	8		
	Heterozygote advantage	T/T+C/C	138	149	1.381	0.240		Heterozygote advantage	G/G+A/A	147	126	5.715	**0.017**
		T/C	57	47					G/A	48	70		
	Allele	T	307	333	5.108	**0.024**		Allele	G	320	306	1.949	0.163
		C	83	59					A	70	86		

All SNPs were analyzed under co-dominant, dominant, recessive, and heterozygote advantage and allele modes. Statistical analysis was performed using the chi-squared test. Data for which $P < 0.05$ are indicated in bold. SNPs: Single nucleotide polymorphisms.

Table 4 Haplotype analysis for 14 SNPs in children with developmental dyslexia and controls

Block	Haplotype	Frequency	Case [*n*(%)]	Control [*n*(%)]	χ^2	*P*
rs9295619-rs807701-rs807724-rs2274305						
	AAAA	0.514	195.9(50.23)	205.9(52.53)	0.417	0.519
	GGGG	0.179	83.0(21.28)	57.0(14.54)	6.044	0.014
	GAAA	0.169	58.1(14.90)	74.1(18.90)	2.220	0.136
	GGAG	0.125	48.9(12.54)	48.9(12.47)	0	0.983
rs3765502-rs1087266						
	AG	0.455	168.0(43.08)	188.0(47.96)	1.879	0.170
	AA	0.312	135.0(34.62)	109.0(27.81)	4.223	0.040
	GA	0.233	87.0(22.31)	95.0(24.23)	0.407	0.524

Statistical analysis was performe using the chi-square test. Data for which $P < 0.05$ are indicated in bold. SNPs: Single nucleotide polymorphisms.

P = 0.038) displayed significant associations with developmental dyslexia under the dominant model.

Construction and statistical analysis of haplotypes

Based on our data, we constructed haplotype blocks for further analysis of genetic associations between the tested SNPs (**Table 4**); the LD plot is displayed in **Figure 2**. We obtained two blocks, one a four-marker haplotype (rs9295619-rs807701-rs807724-rs2274305) and the other as a two-marker haplotype (rs3765502-1087266). We found that the T-G-C-T haplotype of the former block (χ^2 = 6.044, P = 0.014) and the T-A haplotype of the latter block (χ^2 = 4.223, P = 0.040) were significantly higher in the developmental dyslexia group than in the control group, whereas other haplotypes showed no difference in frequency between the two groups.

Discussion

Genetic association studies enable the efficient identification of possible candidate genes by means of comparing the frequency of SNPs between affected cases and unaffected controls. During our study, we identified six SNPs that varied with group phenotype. These results were conducive to the recognition and identification of dyslexic individuals in the Uyghur population, thus supporting the need for further studies of the genetic susceptibility of various ethnic groups to dyslexia, as well as further elucidation of the role of DCDC2 in the pathogenesis of developmental dyslexia.

This study incorporated SNPs that have been frequently examined in developmental dyslexia studies, such as rs6456593 and rs2274305. These two SNPs have been reported in the Han population of the Xinjiang Uyghur Autonomous Region of China, but were not found to be associated with developmental dyslexia (Zuo et al., 2012). In the present study, we extended this work by enlarging the sample size and examining additional SNPs in the Uyghur population. We found that both rs6456593 and rs2274305 were associated with developmental dyslexia under the dominant model. This may be attributed to several factors, such as genetic and linguistic influences. For instance, the Han Chinese are an ethnic group native to East Asia, whereas the Uyghur people are a hybrid race with Eastern and Western Eurasian genetic and anthropometric traits (Black et al., 2006). One study, which only examined samples from Hotan, reported that Uyghur people had 60% European and 40% East Asian ancestry (Xu et al., 2008). In addition, the Chinese language is ideographic, whereas Uyghur is phonographic. Further, most school-age Han children are Chinese monolinguals, whereas almost all school-age Uyghur children are Uyghur-Chinese bilinguals. A preliminary study of language-related functional brain areas in monolingual and bilingual Uyghur individuals from Xinjiang reported that the scope and volume of activation in language-related areas in the Uyghur population were significantly greater than those in the Han population during tasks that involved speaking the native language (Wang and Tang, 2012).

Functional investigations of DCDC2 are important for elucidating the molecular mechanisms that contribute to developmental dyslexia, as DCDC2 encodes a protein that regulates neuronal migration and is widely expressed in the human brain. Some studies of Western populations have indicated that developmental dyslexia arises due to dysfunction in left temporoparietal brain areas, suggesting that developmental dyslexia has a common biological origin in speakers of different languages. However, other studies of the Han population have indicated that the left middle frontal gyrus is implicated in impaired reading ability for the Chinese language (Siok et al., 2004, 2008). Previous studies have produced inconsistent results with respect to certain functional variants of DCDC2, such as BV677278 (Brkanac et al., 2007; Ludwig et al., 2008; Wilcke et al., 2009; Meng et al., 2011; Marino et al., 2012; Powers et al., 2013), which appears to vary according to population. This indicates that DCDC2 promotes the development of developmental dyslexia *via* differing molecular mechanisms. Therefore, the identification of functional variants, particularly in dyslexic individuals in the Uyghur population, is necessary. In future work, we aim to investigate functional variants within DCDC2 in a larger sole replication cohort. This will enable us to study their role in the pathogenesis of developmental dyslexia.

Numerous studies have examined the DCDC2 gene in the DYX2 region. However, most of these have focused on Western (Schumacher et al., 2006; Wilcke et al., 2009) and Indian populations (Venkatesh et al., 2011, 2013), as well as the Han population in China (Zuo et al., 2012). In the present study, we examined the association between DCDC2 and developmental dyslexia in Uyghur individuals from the Xinjiang Uyghur Autonomous Region, China. To our knowledge, this is the first study to attempt to elucidate the mechanisms by which DCDC2 polymorphisms exert their effect on developmental dyslexia in the Uyghur population. As such, there are several limitations to our study: first, our sample size was small. Second, the associations investigated were statistically significant, but not to a large degree, before correcting for multiple testing. Additionally, the biological functions of the selected SNPs were not assessed. Third, we only evaluated one gene, namely DCDC2, in the present study. To elucidate the genetic basis of developmental dyslexia in the Uyghur population, further studies are required to examine gene-gene interactions between DCDC2 and other candidate genes. Such gene-gene interactions have been identified in other complex diseases, such as asthma (Chan et al., 2008), thrombotic stroke (Liu et al., 2009), breast cancer (Chen et al., 2013), and pulmonary tuberculosis (Collins et al., 2013). In addition, although developmental dyslexia has been reported to be heritable, environmental influences, such as home schooling, are also considered to contribute to the development of developmental dyslexia (Mascheretti et al., 2013). Therefore, future studies should consider the role of gene-environment interactions in the development of developmental dyslexia in the Uyghur population. Further, functional studies of DCDC2 should be conducted to further examine the pathogenesis of developmental dyslexia in the Uyghur population. We

expect that the findings of the present study, in combination with the previous literature, will be valuable in the prevention, diagnosis, intervention, and therapeutic treatment of developmental dyslexia.

Acknowledgments: *The authors thank Dr. Da Ding and Dr. Yan Liu (Genesky Biotechnologies, Shanghai, China) for providing technical support. We also extend our thanks to all the primary students, their parents, teachers, and research staff who participated in this study for their cooperation.*

Author contributions: *PXZ proposed concept, designed technical route, provided technical guidance and financial support, and revised the paper. YC selected the topic, searched literature, chose SNPs of DCDC2, collected samples, analyzed data, and drafted this paper. HZ collected samples, performed the experiments, and helped to prepare the paper. YXZ contributed significantly to the data acquisition. All authors approved the final version of the paper.*

Conflicts of interest: *None declared.*

Plagiarism check: *This paper was screened twice using CrossCheck to verify originality before publication.*

Peer review: *This paper was double-blinded and stringently reviewed by international expert reviewers.*

References

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263-265.

Black ML, Wise CA, Wang W, Bittles AH (2006) Combining genetics and population history in the study of ethnic diversity in the People's Republic of China. Hum Biol 78:277-293.

Brkanac Z, Chapman NH, Matsushita MM, Chun L, Nielsen K, Cochrane E, Berninger VW, Wijsman EM, Raskind WH (2007) Evaluation of candidate genes for DYX1 and DYX2 in families with dyslexia. Am J Med Genet B Neuropsychiatr Genet 144B:556-560.

Chan IH, Tang NL, Leung TF, Huang W, Lam YY, Li CY, Wong CK, Wong GW, Lam CW (2008) Study of gene-gene interactions for endophenotypic quantitative traits in Chinese asthmatic children. Allergy 63:1031-1039.

Chang J, Wei L, Miao X, Yu D, Tan W, Zhang X, Wu C, Lin D (2015) Two novel variants on 13q22.1 are associated with risk of esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 24:1774-1780.

Chen W, Song H, Zhong R, Zhu B, Guo H, Lou J, Shen N, Li J, Chen X, Liu C, Ming J, Huang T, Miao X (2013) Risk of GWAS-identified genetic variants for breast cancer in a Chinese population: a multiple interaction analysis. Breast Cancer Res Treat 142:637-644.

Chen X, Li S, Yang Y, Yang X, Liu Y, Liu Y, Hu W, Jin L, Wang X (2012) Genome-wide association study validation identifies novel loci for atherosclerotic cardiovascular disease. J Thromb Haemost 10:1508-1514.

Collins RL, Hu T, Wejse C, Sirugo G, Williams SM, Moore JH (2013) Multifactor dimensionality reduction reveals a three-locus epistatic interaction associated with susceptibility to pulmonary tuberculosis. BioData Min 6:4.

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, Holmans P, Owen MJ, O'Donovan MC, Williams J (2005) Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet 76:581-591.

Cope N, Eicher JD, Meng H, Gibson CJ, Hager K, Lacadie C, Fulbright RK, Constable RT, Page GP, Gruen JR (2012) Variants in the DYX2 locus are associated with altered brain activation in reading-related brain regions in subjects with reading disability. Neuroimage 63:148-156.

Czamara D, Bruder J, Becker J, Bartling J, Hoffmann P, Ludwig KU, Muller-Myhsok B, Schulte-Korne G (2011) Association of a rare variant with mismatch negativity in a region between KIAA0319 and DCDC2 in dyslexia. Behav Genet 41:110-119.

Davis OS, Band G, Pirinen M, Haworth CM, Meaburn EL, Kovas Y, Harlaar N, Docherty SJ, Hanscombe KB, Trzaskowski M, Curtis CJ, Strange A, Freeman C, Bellenguez C, Su Z, Pearson R, Vukcevic D, Langford C, Deloukas P, Hunt S, et al (2014) The correlation between reading and mathematics ability at age twelve has a substantial genetic component. Nat Commun 5:4204.

Fisher SE, Francks C, Marlow AJ, MacPhie IL, Newbury DF, Cardon LR, Ishikawa-Brush Y, Richardson AJ, Talcott JB, Gayan J, Olson RK, Pennington BF, Smith SD, DeFries JC, Stein JF, Monaco AP (2002) Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia. Nat Genet 30:86-91.

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, Cardon LR, Marlow AJ, MacPhie IL, Walter J, Pennington BF, Fisher SE, Olson RK, DeFries JC, Stein JF, Monaco AP (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. Am J Hum Genet 75:1046-1058.

Gong YX, Dai XY (1988) The test analysis of China–Wechsler Young Children Scale of Intelligence. Psychology 4:364-376.

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, Kaariainen H, Kere J (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1:e50.

Jing J, Hai Y, JF D (1998) The revision and appraisal of the pupil rating scale revised-screening for learning disabilities. Chin J Child Care 6:197-200.

Kaplan DE, Gayan J, Ahn J, Won TW, Pauls D, Olson RK, DeFries JC, Wood F, Pennington BF, Page GP, Smith SD, Gruen JR (2002) Evidence for linkage and association with reading disability on 6p21.3-22. Am J Hum Genet 70:1287-1298.

Li H, Cho K, Kidd JR, Kidd KK (2009) Genetic landscape of Eurasia and "admixture" in Uyghurs. Am J Hum Genet 85:934-939.

Lin GY, Du XL, Shan JJ, Zhang YN, Zhang YQ, Zhang YZ (2016) Molecular genetic analysis of genes from MNS, Duffy and Kell blood groups in the China Xinjiang Uygur population. Zhongguo Zuzhi Gongcheng Yanjiu 20:123-127.

Lind PA, Luciano M, Wright MJ, Montgomery GW, Martin NG, Bates TC (2010) Dyslexia and DCDC2: normal variation in reading and spelling is associated with DCDC2 polymorphisms in an Australian population sample. Eur J Hum Genet 18:668-673.

Liu J, Sun K, Bai Y, Zhang W, Wang X, Wang Y, Wang H, Chen J, Song X, Xin Y, Liu Z, Hui R (2009) Association of three-gene interaction among MTHFR, ALOX5AP and NOTCH3 with thrombotic stroke: a multicenter case-control study. Hum Genet 125:649-656.

Ludwig KU, Roeske D, Schumacher J, Schulte-Korne G, Konig IR, Warnke A, Plume E, Ziegler A, Remschmidt H, Muller-Myhsok B, Nothen MM, Hoffmann P (2008) Investigation of interaction between DCDC2 and KIAA0319 in a large German dyslexia sample. J Neural Transm (Vienna) 115:1587-1589.

Marino C, Meng H, Mascheretti S, Rusconi M, Cope N, Giorda R, Molteni M, Gruen JR (2012) DCDC2 genetic variants and susceptibility to developmental dyslexia. Psychiatr Genet 22:25-30.

Marino C, Mascheretti S, Riva V, Cattaneo F, Rigoletto C, Rusconi M, Gruen JR, Giorda R, Lazazzera C, Molteni M (2011) Pleiotropic effects of DCDC2 and DYX1C1 genes on language and mathematics traits in nuclear families of developmental dyslexia. Behav Genet 41:67-76.

Mascheretti S, Bureau A, Battaglia M, Simone D, Quadrelli E, Croteau J, Cellino MR, Giorda R, Beri S, Maziade M, Marino C (2013) An assessment of gene-by-environment interactions in developmental dyslexia-related phenotypes. Genes Brain Behav 12:47-55.

Matsson H, Tammimies K, Zucchelli M, Anthoni H, Onkamo P, Nopola-Hemmi J, Lyytinen H, Leppanen PH, Neuhoff N, Warnke A, Schulte-Korne G, Schumacher J, Nothen MM, Kere J, Peyrard-Janvid M (2011) SNP variations in the 7q33 region containing DGKI are associated with dyslexia in the Finnish and German populations. Behav Genet 41:134-140.

Meng H, Powers NR, Tang L, Cope NA, Zhang PX, Fuleihan R, Gibson C, Page GP, Gruen JR (2011) A dyslexia-associated variant in DCDC2 changes gene expression. Behav Genet 41:58-66.

Meng H, Smith SD, Hager K, Held M, Liu J, Olson RK, Pennington BF, DeFries JC, Gelernter J, O'Reilly-Pol T, Somlo S, Skudlarski P, Shaywitz SE, Shaywitz BA, Marchione K, Wang Y, Paramasivam M, LoTurco JJ, Page GP, Gruen JR (2005) DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci U S A 102:17053-17058.

Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, Richardson AJ, Walter J, Stein JF, Talcott JB, Monaco AP (2011) Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. Behav Genet 41:90-104.

Plomin R, Kovas Y (2005) Generalist genes and learning disabilities. Psychol Bull 131:592-617.

Powers NR, Eicher JD, Butter F, Kong Y, Miller LL, Ring SM, Mann M, Gruen JR (2013) Alleles of a polymorphic ETV6 binding site in DCDC2 confer risk of reading and language impairment. Am J Hum Genet 93:19-28.

Schumacher J, Anthoni H, Dahdouh F, Konig IR, Hillmer AM, Kluck N, Manthey M, Plume E, Warnke A, Remschmidt H, Hulsmann J, Cichon S, Lindgren CM, Propping P, Zucchelli M, Ziegler A, Peyrard-Janvid M, Schulte-Korne G, Nothen MM, Kere J (2006) Strong genetic evidence of DCDC2 as a susceptibility gene for dyslexia. Am J Hum Genet 78:52-62.

Shao S, Kong R, Zou L, Zhong R, Lou J, Zhou J, Guo S, Wang J, Zhang X, Zhang J, Song R (2015) The roles of genes in the neuronal migration and neurite outgrowth network in developmental dyslexia: single- and multiple-risk genetic variants. Mol Neurobiol 53:3967-3975.

Siok WT, Perfetti CA, Jin Z, Tan LH (2004) Biological abnormality of impaired reading is constrained by culture. Nature 431:71-76.

Siok WT, Niu Z, Jin Z, Perfetti CA, Tan LH (2008) A structural-functional basis for dyslexia in the cortex of Chinese readers. Proc Natl Acad Sci U S A 105:5561-5566.

Sun Z, Zou L, Zhang J, Mo S, Shao S, Zhong R, Ke J, Lu X, Miao X, Song R (2013) Prevalence and associated risk factors of dyslexic children in a middle-sized city of China: a cross-sectional study. PLoS One 8:e56688.

Svensson I, Nilsson S, Wahlstrom J, Jernas M, Carlsson LM, Hjelmquist E (2011) Familial dyslexia in a large Swedish family: a whole genome linkage scan. Behav Genet 41:43-49.

Svidnicki MC, Salgado CA, Lima RF, Ciasca SM, Secolin R, Pomilio MC, Junqueira PA, Pinto MS, Pereira MM, Sartorato EL (2013) Study of candidate genes for dyslexia in Brazilian individuals. Genet Mol Res 12:5356-5364.

Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, Lyytinen H, Muller K, Kaaranen M, Lindsberg PJ, Hannula-Jouppi K, Kere J (2003) A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc Natl Acad Sci U S A 100:11553-11558.

Venkatesh SK, Siddaiah A, Padakannaya P, Ramachandra NB (2011) An examination of candidate gene SNPs for dyslexia in an Indian sample. Behav Genet 41:105-109.

Venkatesh SK, Siddaiah A, Padakannaya P, Ramachandra NB (2013) Analysis of genetic variants of dyslexia candidate genes KIAA0319 and DCDC2 in Indian population. J Hum Genet 58:531-538.

Wang YL, Jia L, Tang WJ (2012) fMRI Study of cerebral functional area in mult linguals. Linchuang Fangshexue Zazhi 3:321-325.

Wilcke A, Weissfuss J, Kirsten H, Wolfram G, Boltze J, Ahnert P (2009) The role of gene DCDC2 in German dyslexics. Ann Dyslexia 59:1-11.

Wu HR, Song RR, Yao B (2006) Preliminary establishment of Dyslexia Checklist for Chinese Children scale. Zhonguo Xuexiao Weisheng 27:189-190.

Xu S, Jin L (2008) A genome-wide analysis of admixture in Uyghurs and a high-density admixture map for disease-gene discovery. Am J Hum Genet 83:322-336.

Xu S, Huang W, Qian J, Jin L (2008) Analysis of genomic admixture in Uyghur and its implication in mapping strategy. Am J Hum Genet 82:883-894.

Yao YG, Kong QP, Wang CY, Zhu CL, Zhang YP (2004) Different matrilineal contributions to genetic structure of ethnic groups in the silk road region in china. Mol Biol Evol 21:2265-2280.

Zhong R, Yang B, Tang H, Zou L, Song R, Zhu LQ, Miao X (2013) Meta-analysis of the association between DCDC2 polymorphisms and risk of dyslexia. Mol Neurobiol 47:435-442.

Zuo PX, Wu HR, Li ZC, Cao XD, Pang LJ, Yang L, Liu F, Zhao F (2012) Association of polymorphisms in the DCDC2 gene with developmental dyslexia in the Han Chinese. Chin Med J (Engl) 125:622-625.

Copyedited by Koke S, Hindle A, Wang J, Li CH, Qiu Y, Song LP, Zhao M