# Association study on the *DLG4* gene and schizophrenia in the Chinese Han population

Xing-wang Li[a,c], Bao-cheng Liu[a,c], Yang Wang[a,c], Qing-zhu Zhao[a,c], Qi Shen[a,c], Tao Yu[a,c], Shi-qing Chen[a,c], Feng-ping Yang[a,c], Wei-dong Li[a,c], Ling-han Gao[a,c], Yi-feng Xu[d], Guo-yin Feng[d], Lin He[a,b,c] and Guang He[a,c]

***Background*** **Abnormal expressions of the *N*-methyl-D-aspartate receptor and its interacting postsynaptic density (PSD) molecules have been hypothesized to be involved in the pathophysiology of schizophrenia. Few studies have carried out association studies with *DLG4* gene (coding PSD-95 protein) and sought to validate the results with Asian schizophrenia patients.**

***Patients and methods*** **To further investigate the significance of *DLG4* in Asian schizophrenic patients, we examined seven single-nucleotide polymorphisms (SNPs) within this gene in 1504 unrelated Chinese mainland individuals (893 patients and 611 controls).**

***Results*** **No association was found between these seven SNPs and schizophrenia within our sample. No significant differences in allele or genotype frequencies between schizophrenic paranoid patients and controls were found.**

***Conclusion*** **Although no allelic or genotypic variances of this gene were observed, the possibility that SNPs within *DLG4* represent a positive schizophrenia risk gene cannot be excluded. Our research provided a reference for further research into this gene in other populations. *Psychiatr Genet* 23:247–250 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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**Keywords: association study, case–control, *DLG4*, PSD-95, schizophrenia**

[a]Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Bio-X Institutes, Shanghai Jiao Tong University, [b]Institutes of Biomedical Sciences, Fudan University, [c]Institute for Nutritional Sciences, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences and [d]Shanghai Institute of Mental Health, Shanghai, China

Correspondence to Guang He, MD, PhD, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Bio-X Institutes, Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, China
Tel/fax: +86 021 62822491; e-mail: heguang@sjtu.edu.cn

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## Introduction

Schizophrenia (MIM 181500) is a common psychiatric disorder, with a prevalence of ~1% in the world population, and is characterized by psychotic symptoms such as hallucinations, delusions and thought disturbances, etc. Heritability measurements vary from 68 to 89% in different ethnic populations (Freedman, 2003). The search for specific nosogenesis chromosomal loci and genes has been slow and frustrating as a number of genetic and environmental factors, either alone or interacting with one another, influence patient susceptibility to this disease (Tsuang, 2000; Harrison and Owen, 2003). Identification of susceptibility genes for schizophrenia will provide greater insight into its etiological mechanism and ultimately facilitate the development of more effective prevention and treatments, including personalized medicine regimes.

Both preclinical models and therapeutic studies in schizophrenic patients have shown that dysfunctions of the glutamate synapse and the glutamatergic receptor may play an important role in the pathophysiology of schizophrenia and is a promising target for drug development (Goff and Coyle, 2001; Javitt, 2010). Among glutamate receptors, compelling scientific evidence suggests that *N*-methyl-D-aspartate (NMDA) receptor (NMDAR)-mediated signal transduction is implicated in the pathophysiology of the disease (Coyle and Tsai, 2004; Stahl, 2007; Shim *et al.*, 2008). *DLG4* (discs, large homolog 4), encoding postsynaptic density 95 (PSD-95) protein, binds to NMDAR 2A/2B subunits (Garner *et al.*, 2000). PSD-95 protein contains three PDZ-binding domains, which enable the anchoring of NMDARs through the C-terminal of NMDA2 subunits (Kornau *et al.*, 1995).

*DLG4* is considered to be central in organizing the NMDAR signaling complex (Husi *et al.*, 2000), and a study by Ohnuma *et al.* (2000) found the expression of *DLG4* to be significantly decreased in the Brodmann area of the prefrontal cortex of schizophrenic patients. Few association studies have been carried out to test the association of this gene with schizophrenia and have yielded inconsistent results (Kawashima *et al.*, 2006; Tsai *et al.*, 2007; Cheng *et al.*, 2010). To further evaluate the importance of *DLG4* in Asian schizophrenic patients, we carried out an association study on the polymorphisms within this gene in relatively larger Chinese samples.

## Patients and methods

### Patients

A total of 1504 unrelated Chinese mainland individuals were recruited for this case–control study, consisting of 893 schizophrenic patients (389 women and 504 men;

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**Table 1 Distributions of genotypes and alleles for the seven single-nucleotide polymorphisms within *DLG4***

| SNPs | *n* | Genotype | | | $\chi^2$ | *P* value | Allele | | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| rs507506 | | AA | AG | GG | | | A | G | |
| Control | 605 | 164 (0.271) | 302 (0.499) | 139 (0.230) | 4.28572 | 0.1174 | 630 (0.521) | 580 (0.479) | 0.04598 |
| Patients | 867 | 197 (0.227) | 444 (0.512) | 226 (0.261) | | | 838 (0.483) | 896 (0.517) | |
| rs1875673 | | GG | GT | TT | | | G | T | |
| Control | 602 | 234 (0.389) | 288 (0.478) | 80 (0.133) | 2.16915 | 0.3381 | 756 (0.628) | 448 (0.372) | 0.15058 |
| Patients | 865 | 368 (0.425) | 395 (0.457) | 102 (0.118) | | | 1311 (0.654) | 599 (0.346) | |
| rs929229 | | TT | TC | CC | | | T | C | |
| Control | 574 | 171 (0.298) | 270 (0.470) | 133 (0.232) | 5.3805 | 0.068 | 612 (0.533) | 536 (0.467) | 0.03298 |
| Patients | 827 | 201 (0.243) | 412 (0.498) | 214 (0.259) | | | 814 (0.492) | 840 (0.508) | |
| rs17203281 | | AA | AG | GG | | | A | G | |
| Control | 587 | 64 (0.109) | 239 (0.407) | 284 (0.484) | 1.72567 | 0.422 | 367 (0.313) | 807 (0.687) | 0.75783 |
| Patients | 830 | 81 (0.098) | 366 (0.441) | 383 (0.461) | | | 528 (0.318) | 1132 (0.682) | |
| rs2242449 | | CC | CT | TT | | | C | T | |
| Control | 600 | 208 (0.347) | 288 (0.480) | 104 (0.173) | 3.38432 | 0.1842 | 704 (0.587) | 496 (0.413) | 0.21063 |
| Patients | 882 | 267 (0.303) | 460 (0.522) | 155 (0.176) | | | 994 (0.563) | 770 (0.437) | |
| rs314253 | | CC | CT | TT | | | C | T | |
| Control | 594 | 141 (0.237) | 284 (0.478) | 169 (0.285) | 0.08314 | 0.9593 | 566 (0.476) | 622 (0.524) | 0.82583 |
| Patients | 850 | 203 (0.239) | 411 (0.484) | 236 (0.278) | | | 817 (0.481) | 883 (0.519) | |
| rs11650232 | | AA | AG | GG | | | A | G | |
| Control | 611 | 606 (0.992) | 5 (0.008) | 0 (0) | 0.36244 | 0.5472 | 1217 (0.996) | 5 (0.004) | 0.54785 |
| Patients | 891 | 886 (0.994) | 5 (0.006) | 0 (0) | | | 1777 (0.997) | 5 (0.003) | |

SNP, single-nucleotide polymorphism.

mean ± SD age, 50.4 ± 13.4 years; mean ± SD age of onset, 26.7 ± 8.9 years) and 611 controls (278 women and 333 men; mean ± SD age, 42.0 ± 9.9 years). More detailed information on the patients can be found in our previous paper (Shen *et al.*, 2011). Patients were chosen on the basis of two criteria: (a) they had been diagnosed with schizophrenia by at least two independent experienced psychiatrists who conducted clinical interviews on the basis of the criteria of the *Diagnostic and Statistical Manual of Mental Disorder, 4th ed.* (DSM-IV) (American Psychiatric Association, 1994). (b) They had no physical disease, history of traumatic brain injury, or other psychiatric disease besides schizophrenia. All of the control participants were directly interviewed to exclude psychiatric disorders by a 10-item questionnaire according to Mini-International Neuropsychiatric Interview (version 5.0.0), and free from present and past individual and family history of psychiatric illness, traumatic brain injury, or substance abuse. Written informed consent was obtained from either participants or their legal representatives after the research aims and procedures had been fully explained. The study protocol was reviewed and approved by the Shanghai Ethical Committee of Human Genetic Resources.

### Genotyping and single-nucleotide polymorphism selection

A total of seven single-nucleotide polymorphisms (SNPs) spanning *DLG4* gene were screened. Three Tag SNPs (rs507506, rs17203281, and rs2242449) were selected on the basis of their ability to tag surrounding variants in the Chinese Beijing (CHB) population from the international HapMap project website (*http://www.hapmap.org*). We also enrolled some polymorphisms tested previously by other research papers, such as rs507506 and rs2242449 (Kawashima *et al.*, 2006; Lou *et al.*, 2007) and rs17203281 (Tsai *et al.*, 2007), rs314253 (Kawashima *et al.*, 2006). Details of selected SNPs are presented in Table 1.

Genomic DNA was prepared from venous blood using standard phenol chloroform extraction. All SNPs were genotyped on the ABI 7900 DNA detection system (Applied Biosystems, Foster City, California, USA) using TaqMan technology. All probes were designed by the Applied Biosystems. The standard 5 μl PCR reaction was carried out using TaqMan Universal PCR Master Mix reagent kits following the protocols provided.

### Statistical analysis

Genotype frequencies were calculated using SPSS for windows (version 19.0; IBM, Armonk, New York, USA). Genepop 3.4 software (Rousset, 2008) was used to test for deviations from Hardy–Weinberg equilibrium for each polymorphism between the patient and the control groups (Rousset and Raymond, 1995). Genotypic association was also analyzed for these SNPs under both dominant and recessive genetic models using the $\chi^2$-test. Pairwise linkage disequilibrium (LD) within the *DLG4* and permutation analysis were carried out on Haploview software (Haploview 4.1) using the standard summary statistic $D'$ (Barrett *et al.*, 2005). Haplotype blocks were assigned using the $D'$ confidence interval algorithm (Gabriel *et al.*, 2002). Power calculations for the sample were performed using the G* power version 3.1 (Faul *et al.*, 2007).

To substantiate the results, the online software program SHEsis *http://202.120.31.177/myanalysis.php* (Shi and He, 2005) was used to recalculate allele and genotype association, odds ratio, and haplotypes.

## Results

All genotype results were called blind to clinical antipsychotic status. Positive and negative quality control

sample match rates were achieved 100%. All seven SNPs were in Hardy–Weinberg equilibrium. Statistical analysis of the data for all seven markers within *DLG4* is presented in Table 1. We found that allelic frequencies of rs507506 ($P = 0.045977$) and rs929229 ($P = 0.032981$) were marginally differently distributed between patients and controls and there were no other significant differences in allele or genotype frequencies between schizophrenic patients and controls at any of the genetic polymorphisms. LD within the SNPs is shown in Fig. 1. Our seven SNPs could cover 86.9% genetic variation in *DLG4* gene on the basis of their LD block in our samples. In the haplotype analysis, no overall positive association was found with schizophrenia. None of the positive associations survived after 100 000 permutations (best permutation $\chi^2 = 23.532$, the smallest $P = 0.2325$, other data not shown). Power calculation for all seven SNPs was 0.99 with effect size 0.1.

Different subtypes of schizophrenia may have varied genetic etiologies of disease progression (Fanous and Kendler, 2008), and we therefore tested the genetic association of those polymorphism markers in our paranoid subtypes (481 patients representing 53.9% of the total). No significant differences in allele or genotype frequencies between schizophrenic paranoid patients and controls were found at any of the genetic polymorphisms. Neither of the positive findings was found to be related to age of onset within our patients.

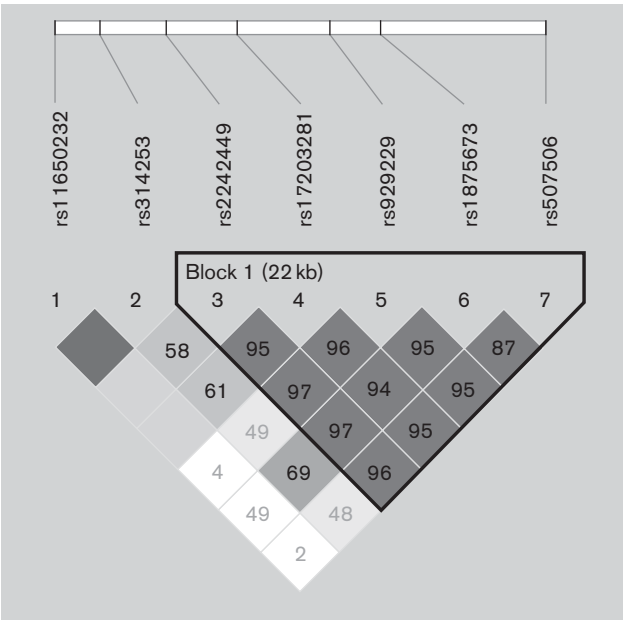
## Discussion

The 723-amino-acid PSD-95 protein orchestrates protein–protein interactions and receptor stabilization at excitatory synapses (Kim and Sheng, 2004). Moreover, the expression of *DLG4* is known to serve as a modulator in schizophrenia and mood disorder brain samples (Ohnuma *et al.*, 2000; Toro and Deakin, 2005), suggesting that this gene may play a role in the etiology of these psychiatric diseases. The present study aimed to examine whether the *DLG4* gene confers genetic susceptibility to schizophrenia by investigating the association of polymorphisms within this gene with susceptibility to the disease in 1504 unrelated Chinese Han samples. We found a marginally positive association in our sample, with allelic frequencies having *P* values of 0.045977 and 0.032981 for rs507506 and rs929229, respectively. 100 000 permutations were performed to avoid type I error, but none of the associations survived (best permutation $\chi^2 = 23.532$, the smallest $P = 0.2325$, other data not shown). Our results are supported by one Japanese study, which also found no association for *DLG4* in their schizophrenic patients (Kawashima *et al.*, 2006). Haplotypes composed of two to seven SNPs showed no significant distribution between our patients and controls.

Altered gene/protein expression of *DLG4* has been found in the prefrontal cortex region of schizophrenic patients (Ohnuma *et al.*, 2000). Mice lacking DLG4 protein show long-time potentiation and learning deficits, which are clinical features of schizophrenia (Migaud *et al.*, 1998). A recent animal model found that *DLG4* gene disruption in mice produces a complex range of behavioral and molecular abnormalities relevant to William's syndrome and autism spectrum disorders (Feyder *et al.*, 2010). Although proteomics and animal model studies have shown that *DLG4* may underlie or contribute toward the pathophysiology of schizophrenia, given the complexity and variety of psychiatric disorders, it is not unusual to find that transcript/protein levels of certain genes in brain regions are not consistent with their genetic association study result. Variations in genetic levels do not account for post-transcriptional changes that might affect protein translation, trafficking, and protein stability (Kristiansen *et al.*, 2006). One of the common limitations of case–control studies is the complexity and variety of genetic background implicit in the ethnical LD pattern (Hoggart *et al.*, 2003). Currently, there are only three *DLG4* gene association studies within an Asian population; other racial data are needed to validate our results. Although our current research does not support the association of *DLG4* with schizophrenia, it does not exclude the possibility of an association between other SNPs within this gene and the disease both in Chinese and in other populations.

In summary, we carried out a detailed study of an association between *DLG4* polymorphisms and susceptibility to schizophrenia in a relatively large Chinese Han sample. Although no allelic or genotypic variances of this gene were observed, the possibility that SNPs within *DLG4* represent a positive schizophrenia risk gene cannot be excluded. Our research provided a reference for further research into this gene in other populations and

**Fig. 1**




Linkage disequilibrium between single-nucleotide polymorphisms within *DLG4*.

suggests that more SNP probes and meta-analysis across different studies are required.

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### Conflicts of interest

There are no conflicts of interest.

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