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·Original Article·

Association between NMDA receptor subunit 2b gene polymorphism and Alzheimer's disease in Chinese Han population in Shanghai

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Abstract: Objective N-methyl-D-aspartate (NMDA) receptor has been indicated to be involved in the pathogenesis of Alzheimer's disease (AD). The NMDA receptor subunit 2b (NR2B) has attracted more attention due to its characteristic distribution and selective reduction in AD brain. The present study aimed to explore the association between NMDA gene polymorphism and AD. **Methods** A total of 63 AD patients and 68 normal controls in Shanghai city were employed in this study. Genotype of C2664T variant (rs1806201) in the exon13 of *GRIN2B* gene was determined by gene sequencing. **Results** Among AD patients, 15 (23.6%) subjects were identified as C/C genotype, and 35 (55.6%) were identified as C/T genotype. The left 13 (20.6%) subjects were identified as T/T genotype. In normal controls, 15 (22.1%) subjects were identified as C/C genotype, 39 (57.4%) as C/T genotype and 14 (20.6%) as T/T genotype. The distribution frequency of neither *GRIN2B* C2664T genotype (P=0.895) nor allele (P=0.790) was significantly different between AD patients and normal controls, even when the subjects were stratified by gender and age of disease onset in AD patients. **Conclusion** The results suggest that there is no relation between *GRIN2B* C2664T polymorphism and AD in Chinese Han population of Shanghai City.

Keywords: Alzheimer's disease; N-methyl-D-aspartate receptor; single nucleotide polymorphism

1 Introduction

Alzheimer's disease (AD) is one of the most common causes of dementia that occurs in the elderly and is estimated to affect approximately 5 million people in China[1]. However, the etiology of AD remains still unknown even 100 years after its first identification. All AD patients display progressive memory impairment followed by global cognitive decline, due to the extensive neuron loss in entorhinal cortex, hippocampus and other regions of neocortex[2]. Accumulating evidence suggests that dysfunction of N-methyl-D-aspartate (NMDA) receptor is involved in the pathogenesis of AD[3,4]. NMDA receptor is a ligand-gated ion channel with glutamate receptor concentrated at postsynaptic sites in mammalian brain, the normal function of which is important for excitatory neurotransmission, neuron development and synaptic plasticity in the central neuronal system (CNS)[4]. However, in some pathological conditions, excessive activation of NMDA receptor will cause excitatory neurotoxicity, accelerating the idiopathic neuronal death in many neurodegenerative diseases[3], including AD[5], Parkinson's disease (PD)[6], Huntington's disease (HD)[7] and amyotrophic lateral sclerosis (ALS)[8].

NMDA receptor is a tetramer composed of 2 NR1 subunits and 2 NR2 subunits, and less commonly, a NR3 subunit

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in addition[9]. The NR1 subunit is necessary for functional NMDA receptor channel while the NR2 subunits, identified as NR2A, NR2B, NR2C and NR2D isoforms, function to interact with glutamate and many other important modulating molecules. Of these NR2 subunits, the NR2B has attracted more attention in the study of AD for several reasons. Firstly, NR2B is found to be abundant in hippocampus and neocortex, where the pathological hallmarks of AD concentrate and spread from[10]. In addition, of the NR2 isoforms, NR2B subunit is reduced in AD brain, in comparison with that of normal controls and dementia with lewy body (DLB)[11]. This may be attributed to the selective apoptosis of the neurons expressing NR2B subunit, suggesting that NR2B-containing neurons are particularly sensitive to glutamate-induced neurotoxicity. Secondly, the antagonist of NR2B-containing NMDA receptor has been demonstrated to prevent Aβ oligomer-induced synaptic plasticity disruption *in vivo*[12]. Recently, Rönicke *et al.* have also found that Aβ can mediate early neuronal dysfunction mainly by activation of NR2B-containing NMDA receptor (unpublished data). Thirdly, NR2B subunit-containing NMDA receptor is estimated to be responsible for the long-term potentiation (LTP) in the CA1 region of hippocampus. Mice overexpressing NR2B subunits have been observed to be superior in learning and memory[13], while NR2B deficient rats display impaired spatial memory[14]. Thus, dysfunction of NR2B may contribute, at least partly, to the memory impairment of AD patients. These findings reveal that NR2B subunit is of particular significance for the pathogenesis of AD.

The human NR2B gene (*GRIN2B*) is located to chromosome 12p12[15]. Several single nucleotide polymorphisms (SNPs) of *GRIN2B* have been investigated in many neuropsychiatric disorders, including schizophrenia[16], PD[17] and HD[18]. In the present study, the association between C2664T variant (rs1806201) in exon 13 of *GRIN2B* and AD was investigated in Chinese Han population of Shanghai city.

2 Materials and methods

2.1 Subjects Sixty-three AD patients (19 male and 44 female) and 68 normal controls (17 male and 51 female) were enrolled in the study. The AD patients were recruited from Department of Geriatric Psychiatry, Shanghai Mental Health Center, and were diagnosed of probable AD according to the NINCDS-ADRDA criteria[19]. The mean age at blood collection was (71.10±9.54) years (ranging from 50 to 93 years) and the mean total score of Mini Mental Status Examination (MMSE)[20] was 8.94±6.98 (ranging from 0 to 20). Patients with vascular dementia, major depressive disorder, Vitamin B12 deficiency, hypothyroidism or other diseases that may cause cognitive decline were excluded. Normal controls were recruited from community. Results from MMSE indicated the absence of cognitive decline, and all subjects reported a negative family history of dementia. The normal controls and AD patients were matched for age, gender and educational level. Both groups were from Chinese Han population. Informed consent for participation was obtained from every subject or an appropriate surrogate, and the study was approved by independent Ethics Committee.

2.2 Laboratory methods Genomic DNA was extracted from EDTA-containing venous blood samples using DNA kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. DNA fragment containing C2664T variance site of *GRIN2B* (rs1806201) was amplified by PCR using the following primers: forward: 5'-ACTATTCGCTTCATGCTTTC-3', reverse: 5'-GCGGGTTGTTGTAGGATT-3'. Primers were designed by Primer Premier 3, and the amplified PCR product containing the nucleotide site of interest in the middle region of the fragment was 382 bp in length. PCR was conducted at 95 ºC for 3 min, followed by 33 cycles of denaturation at 94 ºC for 30 s, annealing at 56 ºC for 30 s and extension at 72 ºC for 30 s, with a final extension at 72 ºC for 10 min. The electrophoretic image of PCR products was presented in Fig. 1. Direct sequencing was conducted on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions in Shanghai Genesky BioTech CO., LTD. Sequencing results were presented in Fig. 2.

2.3 Statistical analysis Hardy-Weinberg equilibrium of SNP distribution in both groups was determined by Pearson's χ^2 test. The comparison of genotype and allele frequencies were conducted with χ^2 test and the ages of onset were com-

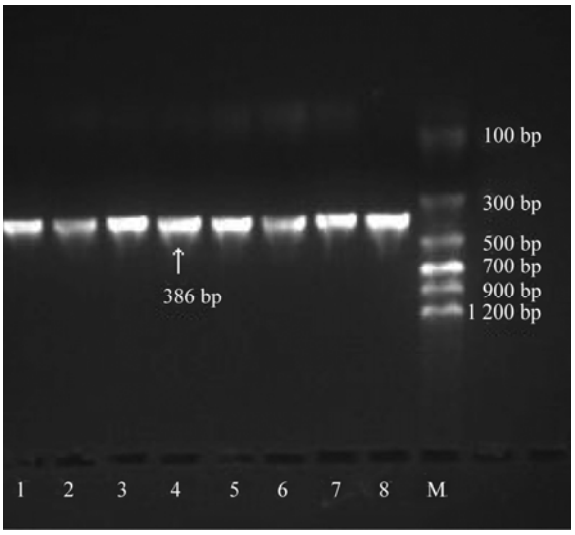

Fig. 1 Electrophoresis of PCR products of the samples. M: DNA marker. Lanes 1-8: PCR products of samples. The size of the target gene was 386 bp.

pared among different genotype groups of AD by using one-way ANOVA. $P < 0.05$ was considered as significantly different.

3 Results

The distributions of *GRIN2B* gene C2664T polymorphism in both AD and normal controls were in Hardy-Weinberg equilibrium. The genotype and allele distributions of *GRIN2B* gene C2664T polymorphism in AD patients and normal controls were presented in Table 1. In both groups, C/T heterozygote was the most common genotype, accounting for 54.0% in AD groups and 57.4% in normal controls. Neither the genotype (P=0.895) nor the allele (P=0.790) frequency differed significantly between the 2 groups, even after the stratification by gender (for genotype frequency, P=0.82 for male and P=0.97 for female; for allele frequency, P= 0.64 for male and P=0.96 for female). AD patients were further grouped into early-onset AD (EOAD) (n=27) and late-onset AD (LOAD) (n=36) according to the age of disease onset (before 65 years or at/after 65 years old)[21]. As shown in Table 1, the frequencies of genotypes and alleles did not differ among age-matched EOAD, LOAD and control subjects. The mean ages of AD onset of different genotypes were presented in

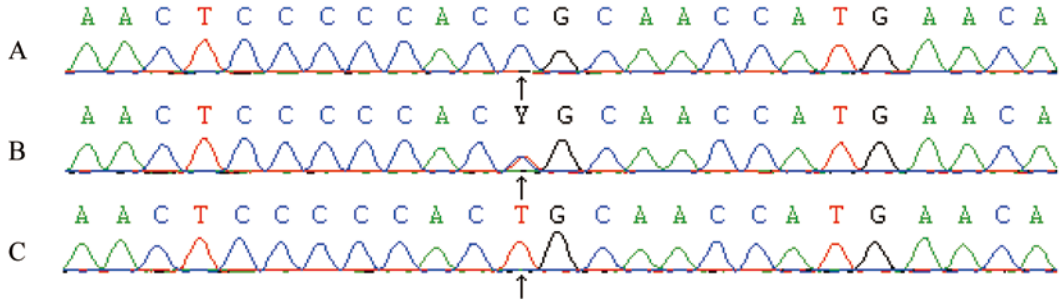

Fig. 2 Sequence results of the *GRIN2B* C2664T polymorphism. A: C/C genotype; B: C/T genotype; C: T/T genotype.

Table 1. Genotype and allele distributions of *GRIN2B* C2664T polymorphism in AD patients and normal controls

Groups	Genotype frequency			*P* value	Allele frequency		*P* value
	C/C (%)	C/T (%)	T/T (%)		C (%)	T (%)	
AD (*n*=63)	16 (25.4)	34 (54.0)	13 (20.6)	0.895	66 (52.4)	60 (47.6)	0.790
Control (*n*=68)	15 (22.1)	39 (57.4)	14 (20.6)		69 (50.7)	67 (49.3)	
EOAD (*n*=27)	8 (29.6)	15 (55.6)	4 (14.8)	0.940	31 (57.4)	23 (42.6)	0.818
Control (*n*=26)	8 (30.8)	15 (57.7)	3 (11.5)		31 (59.6)	21 (40.4)	
LOAD (*n*=36)	8 (22.2)	19 (52.8)	9 (25.0)	0.823	35 (48.6)	37 (51.4)	0.674
Control (*n*=42)	7 (16.7)	24 (57.1)	11 (26.2)		38 (45.2)	46 (54.8)	

Data in parentheses are the percentages of the genotypes.

Table 2. Age at onset for AD patients of different genotypes

	Genotypes			*P* value
	C/C (*n*=16)	C/T (*n*=34)	T/T (*n*=13)	
Age at onset (years)	65.69±8.45	66.94±11.09	71.07±9.26	0.313
Age at onset (years, male)	60.40±4.04	62.56±14.16	75.40±3.84	0.088
Age at onset (years, female)	68.09±8.96	68.58±9.64	68.67±9.18	0.987

Table 2. Results showed that the age of AD onset tended to decrease with the increase of C allele dosage, but the difference of mean age of onset among C/C, C/T and T/T genotypes did not reach the statistical significance (*P*=0.313).

4 Discussion

To the best of our knowledge, this is the first study on the association between *GRIN2B* C2664T polymorphism and AD in Chinese Han population of mainland China. Our result did not reveal any association between this gene variance and AD, which is consistent with the previous studies in Caucasian population[22] and Chinese Han population of Taiwan[23]. Similar results have also been found concerning the association of this polymorphism with PD[17] and schizophrenia[16]. In view of these negative findings from different ethnic populations, combined with the fact that C2664T variant is a silent mutation[24], which does not lead to any change in the sequence of amino acids encoded, we conclude that *GRIN2B* C2664T polymorphism is not associated with disease incidence or age of AD onset.

Although *GRIN2B* C2664T polymophism is a silent mutation, it does not necessarily mean that this variant has no clinical implication. Arning *et al.*[18] have reported that *GRIN2B* C2664T varaint contributes to a difference of 2.8 years for the age of HD onset in different genotypes. Wernicke *et al.*[25] find that C2664T polymorphism is associated with certain types of alcoholism. Our negative findings can not exclude the possibility that this variant may be associated with certain aspects of AD, such as treatment response. Farlow *et al.*[26] have demonstrated that *ApoE* genotypes can affect the response to tacrine treatment in AD patients. Tsai *et al.*[27] have investigated the association of *GRIN2B* C2664T polymorphism with clozapine response in schizophrenia, but the results reveal no significant difference in the therapeutic outcomes among different genotypes. Clozapine is deemed to demonstrate its anti-psychotic effects via interacting with a diversity of receptors in the CNS, and the influence of *GRIN2B* C2664T variant may be beyond the therapeutic effects of clozapine or too minimal to be detected in this sample size. In the aspect of AD treatment, memantine is a non-competitive, partial antagonist of NMDA receptor, and has been demonstrated to be effective in the treatment of moderate or severe AD[3]. Considering the underlying therapeutic mechanism of memantine, it would be of great significance to investigate the association between *GRIN2B* C2664T polymorphism and memantine response in AD.

Accumulating evidence suggests that NMDA receptor dysfunction is involved in the pathogenesis of AD[3,4]. In spite of the negative findings of C2664T polymorphism, we expect that significant results could be obtained on other variants of *GRIN2B*. Recently, Jia *et al.*[28] have reported that -421C/A polymorphism in the promoter region of *GRIN2B* is associated with sporadic AD in ApoE ε4 (-) population. However, some limitations should be realized concerning our study: the small sample size and only one single gene variant for study. Actually, the occurrence of AD is correlated with a diversity of factors, including both genetic and environmental aspects[1]. Even the genetic aspect alone may involve more than a dozen of gene variants[29] and each of these variants contributes to the pathogenesis of AD to a gentle degree that can not be detected in a small size of subjects. Thus, further investigation on the association between *GRIN2B* polymorphism and AD should be carried out in larger-sized samples targeting more gene variants.

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NMDA 受体 NR2B 亚基基因多态性与上海地区汉族人群阿尔茨海默病的关系

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摘要：目的 N- 甲基 -D- 天冬氨酸(N-methyl-D-aspartate, NMDA)受体功能异常可能与阿尔茨海默病(Alzheimer's disease, AD)的发病有关。NMDA 的 NR2B 亚基由于在中枢神经具有特定的分布规律，并被发现在 AD 的脑组织中选择性表达下降，因而备受关注。本研究旨在探讨 NMDA 受体 NR2B 亚基基因(*GRIN2B*) C2664T 多态性与上海地区汉族人群阿尔茨海默病的关系。**方法** 在 63 例 AD 患者和 68 例对照者中，采用基因测序法对 *GRIN2B* 基因第 13 个外显子区的 C2664T 单核苷酸多态性(rs180620)进行检测，在等位基因和基因型水平上进行比较。**结果** AD 组中有 15 名患者基因型为 C/C (23.6%)，35 名基因型为 C/T (55.6%)，13 名为 T/T (20.6%)。在健康对照组中，C/C、C/T 和 T/T 基因型的人数分别为 15 (22.1%)、39 (57.4%)和 14 (20.6%)。在基因型和等位基因水平，NR2B 亚基基因多态性均未显示出与 AD 的相关性。得到的数据经性别和发病年龄分层比较后，仍未显示出相关性。**结论** *GRIN2B* 基因 C2664T 多态性与上海地区汉族人群罹患 AD 无相关性。

关键词：阿尔茨海默病；N- 甲基 -D- 天冬氨酸受体；单核苷酸多态性