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HSPG2 Gene C/A Polymorphism Does Not Confer Susceptibility to Alzheimer's Disease in Chinese

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Key Words
Late-onset Alzheimer's disease · Apolipoprotein E allele 4 · Perlecan

Abstract
Human HSPG2 participates in the formation of amyloid and tau aggregation in Alzheimer's disease (AD). *HSPG2* gene is located on a susceptibility region to late-onset AD (LOAD), and considered as a candidate gene for LOAD because of its function and location. We performed an association study between the *HSPG2 Bam*H I polymorphism C/A of intron 6 and LOAD on 104 patients and 127 healthy controls of Chinese origin. The C allele was more prominent in LOAD patients than in controls, though the difference was not statistically significant. Likewise with the stratification of *APOE ε4* status, no statistical difference was observed between cases and controls. Our findings suggest that this polymorphism may not represent an additional genetic risk factor for LOAD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative dementia with multifactorial pathogenesis in the elderly. In the brain of patients with AD, deposits of proteins in senile plaques and neurofibrillary tangles lead to the neural dysfunction and the evolution of dementia. In the common late-onset AD (LOAD), only apolipoprotein E allele 4 (*APOE ε4*) is confirmed to increase susceptibility. However, APOE ε4 cannot explain the overall genetic risks for LOAD [1, 2]. The discovery of additional genetic factors is needed for clinical diagnoses and therapy of LOAD.

Some studies have focused on a possible association between LOAD and heparan sulfate proteoglycans 2 (HSPG2, perlecan) on chromosome 1p36.12 [3], a region showing susceptibility to LOAD [4, 5]. *HSPG2* gene encodes a large proteoglycan composed of core protein and covalently attached glycosaminoglycan heparan sulfate side chains [6]. HSPG2 presents in all basement membranes and some tissues including brain [7, 8], and have also been implicated in cell growth and differentiation [9–11]. In patients with AD, HSPG2 was detected in the diffuse amyloid plaques of the hippocampus, a heavily affected area in AD brain, but not of the cerebellum [8, 12]. The high affinity of vascular cell-derived HSPG2 for

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Table 1. *Bam*H I *HSPG2* genotypic and allelic frequencies in LOAD patients and controls

Group	Subjects	Genotype frequency			Allele frequency	
		C/C	C/A	A/A	C	A
LOAD	104	19 (18.3)	55 (52.9)	30 (28.8)	93 (44.7)	115 (55.3)
Controls	127	19 (15.0)	61 (48.0)	47 (37.0)	99 (39.0)	155 (61.0)
		$\chi^2 = 1.79$, d.f. = 2, p = 0.41			$\chi^2 = 1.55$, d.f. = 1, p = 0.21	

Figures in parentheses indicate percentages.

A beta indicated the potential conjunction with A beta in cerebrovascular amyloid deposits in AD brain [13]. Not only in the accumulation, but in the persistence of senile plaques may HSPG2 be involved through binding fibrillar A beta and inhibiting its proteolytic degeneration [14, 15]. Furthermore, HSPG2 may induce the aggregation of tau protein, the major component of neurofibrillary tangles [16]. Based on the above information, *HSPG2* gene may confer additional genetic susceptibility to LOAD.

Recently, the association between a common *Bam*H I polymorphism C/A in intron 6 of *HSPG2* and AD was analyzed by two groups within Finnish and Jewish populations, respectively [17, 18]. *HSPG2* allele A was detected to increase the risk for AD in APOE ε4 carriers with doubled odds ratio (OR) in Finnish subjects [17]. But the study in Jewish populations has failed to find a significant association of the polymorphism with LOAD [18].

To date, the potential role of *HSPG2* in LOAD in populations other than Finnish and Jewish has not been reported. In the present study, we have performed a case-control analysis between the *HSPG2* intronic polymorphism C/A and LOAD in Chinese. To our knowledge, this is the first report on the association analysis between the *Bam*H I polymorphism of *HSPG2* and LOAD in an Asian population.

Materials and Methods

Subjects

Subjects were 104 sporadic patients with LOAD (mean age 79.2 ± 6.3; range 64–97 years; 46% female) and 127 unrelated healthy controls (mean age 68.1 ± 2.8; range 65–77 years; 30% female). They were recruited from the Chinese population of Guangxi. The patients were diagnosed following the DSM-III-R criteria [19] and clinically examined based on the NINCDS-ADRDA criteria [20]. All LOAD patients were evaluated using MRI. Healthy controls were selected by the assessment of a full medical history and a physical examination. Cognitive function was assessed using the Mini-Mental State Examination (MMSE). All participants or their guardians gave informed consent. The study was approved by the Ethics Committee of the Institute of Geriatrics of Beijing Hospital.

Genetic Analysis

Genomic DNA was obtained from peripheral venous blood leukocytes according to standard procedures. The genotyping for APOE was done as previously described [21]. The polymorphic site of *HSPG2* gene (rs 3767140 C/A) was genotyped using the method as reported elsewhere [22] with a minor modification. The region containing the C/A polymorphism was amplified by polymerase chain reaction using the following primer set: 5′-TGTGCTGCTTGCCCTCGTTGTG-3′ and 5′-AGTTCCCAAGAGCCTGCACGG-3′. The polymerase chain reaction generated a fragment of 173 bp. The C/A substitution creates a *Bam*H I recognition sequence with resulting 115 and 58 bp fragments.

Statistics

The allelic and genotypic distributions of *APOE* and *HSPG2* polymorphisms were estimated by allele counting and compared in the LOAD and control groups by the χ^2 test. Logistic regression analysis was performed to examine the effect of *APOE* and *HSPG2* polymorphisms on the risk for LOAD using the Statistical Package for the Social Sciences. The criterion for significance was set at $p < 0.05$.

Results

The APOE ε4 allele frequency was prominent in patients with LOAD compared with controls ($\chi^2 = 9.63$, d.f. = 1, p = 0.002), and the distribution of the allele frequency was in accordance with those reported elsewhere.

The distributions of the *Bam*H I polymorphism C/A in intron 6 of *HSPG2* gene in LOAD subjects and controls are shown in table 1. *HSPG2* genotype counts of patients and controls did not deviate significantly from those expected under Hardy-Weinberg equilibrium (data not shown). Compared with controls, there were higher frequencies of genotype C/C (18.3%) and C/A (52.9%) in patients, but genotypic association with LOAD did not reach significance in our sample ($\chi^2 = 1.79$, d.f. = 2, p = 0.41). Likewise, the allele C frequency in patients (44.7%)

Table 2. *Bam*H I *HSPG2* genotypic and allelic distributions in *APOE ε4* carriers and noncarriers

Group	Subjects	Genotype			Allele	
		C/C	C/A	A/A	C	A
APOE ε4 carriers						
LOAD	28	6 (21.4)	12 (42.9)	10 (35.7)	24 (42.9)	32 (57.1)
Controls	15	2 (13.3)	5 (33.3)	8 (53.4)	9 (30.0)	21 (70.0)
		$\chi^2 = 1.29$, d.f. = 2, p = 0.52			$\chi^2 = 1.37$, d.f. = 1, p = 0.24	
APOE ε4 noncarriers						
LOAD	76	13 (17.1)	43 (56.6)	20 (26.3)	69 (45.4)	83 (54.6)
Controls	112	17 (15.2)	56 (50.0)	39 (34.8)	90 (40.2)	134 (59.8)
		$\chi^2 = 1.52$, d.f. = 2, p = 0.47			$\chi^2 = 1.00$, d.f. = 1, p = 0.32	

Figures in parentheses indicate percentages.

was not significantly different from those in controls (39%; $\chi^2 = 1.55$, d.f. = 1, p = 0.21).

Logistic regression analysis revealed no effect of the interaction between *HSPG2* and *APOE* genotypes on the risk for LOAD ($\chi^2 = 2.82$, d.f. = 1, p = 0.09). All samples stratified by the *APOE ε4* status, genotypic and allelic distributions of *HSPG2* gene in subgroups are shown in table 2. Similarly, no association was obtained on considering *APOE ε4* status ($\chi^2 = 1.29$, d.f. = 2, p = 0.52 for *APOE ε4* carriers, and $\chi^2 = 1.52$, d.f. = 2, p = 0.47 for *APOE ε4* noncarriers), although in both subgroups this polymorphism showed a higher frequency of allele C and genotype C/C and C/A in patients than in controls.

Discussion

The results of this case-control association study demonstrate a tendency towards increased frequency of allele C and genotype C/C and C/A of the *Bam*H I polymorphism of *HSPG2* gene in patients with AD. However, the observed differences of genotype and allele distributions did not reach statistical significance and revealed that this polymorphism was not associated with LOAD in Chinese. This was further supported by the fact that no statistical difference was observed between cases and controls with the stratification of APOE ε4 status. Our finding suggests that *HSPG2* does not represent a genetic risk factor for AD or a modifier gene in our sample.

To our knowledge, this is the first report on the association between the *Bam*H I polymorphism of *HSPG2* and LOAD in Mongoloid populations. The allelic distributions (39% for allele C) in our control sample differed markedly from Finnish and Jewish populations (76 and 15.3%, respectively) and reveal ethnic differences [17, 18]. Despite ethnic differences, our significant negative association of the polymorphism alone with AD is consistent with those two reports [17, 18]. Our finding reinforces the notion that the *HSPG2* polymorphism does not confer major genetic risk for AD in different ethnic groups.

In the present study, we did not find any association between the *Bam*H I polymorphism C/A in intron 6 of *HSPG2* gene alone and LOAD in Chinese. No association was observed either when stratifying our sample by APOE ε4 status. However, it should be noticed that the number of investigated samples in the present study is rather small and not completely matched for sex and age, which may lead to false-negative results. Therefore, further studies in a larger and sex- and age-matched sample as well as different races would be necessary to understand the role of the *Bam*H I polymorphism C/A in intron 6 of *HSPG2* gene in the etiology of LOAD.

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