Original Article
Association between ACE gene polymorphisms and Alzheimer’s disease in Han population in Hebei Peninsula

Xiaoli Wang¹, Fengchun Zhang¹, Yongjian Cui¹, Lei Zheng², Yan Wei¹

¹Second Department of Neurology, Harrison International Heping Hospital, Hengshui 053000, Hebei, China; ²Central Laboratory of Harrison International Heping Hospital, Hengshui 053000, Hebei, China

Received September 29, 2015; Accepted October 28, 2015; Epub September 1, 2017; Published September 15, 2017

Abstract: Purpose: This study aimed to detect the association between angiotensin I converting enzyme (ACE) gene polymorphisms (rs4343 and rs1800764) and Alzheimer’s disease (AD) in Han population in Hebei Peninsula. Methods: We recruited 113 AD patients and 142 healthy individuals in this case-control study. Differences of genotypes, alleles and haplotypes in two groups were analyzed by chi-square test. Besides, odds ratios (ORs) and 95% confidence intervals (CIs) were used to represent the relative risk of AD. At last, the analyses of linkage disequilibrium and haplotypes were done with HaploView software. Results: In the analyses of genotypes and alleles of ACE polymorphisms (rs4343 and rs1800764) in AD, no obvious association was found between genotypes and alleles of rs4343 with the susceptibility of AD. In rs1800764 polymorphism, only C allele had significant association with AD susceptibility (P=0.035, OR=1.473, 95% CI=1.027-2.111), which suggested that rs1800764 C allele is the susceptible allele of AD. Linkage disequilibrium analysis between rs4343 and rs1800764 polymorphisms indicated there existed 3 haplotypes (A-T, A-C and G-C). A-C haplotype might associate with the susceptibility of AD (P=0.023, OR=2.591, 95% CI=1.111-6.043). Conclusion: Rs4343 polymorphism of ACE gene had no relationship with AD risk. C allele of rs1800764 could increase the susceptibility of AD. A-C haplotype of rs4343 and rs1800764 polymorphisms might increase the risk of AD, and the ORs was 2.591.

Keywords: Angiotensin I converting enzyme (ACE), Alzheimer’s disease (AD), polymorphisms, haplotype

Introduction
Alzheimer’s disease (AD) is a chronic neurodegenerative disease with a concealed onset and progressive development. It is characterized by comprehensive dementia in clinic, such as dysnesia, aphasia, apraxia, agnosia, changes of personality and behavior and etc. With the loss of bodily functions and the neglect of family and society, the AD patients often go to dying. Due to the increase of aging population, the incidence of AD has an growing trend [1, 2]. It is one of the most costly diseases in developed country [3]. AD is a complex disease affected by genetic and environmental factors, but the main cause of the AD risk is believed to be genetic factors [4-10]. However, the pathogenesis of AD has not been clear.

Some studies have pointed out that Angiotensin I converting enzyme (ACE) gene is associated with AD risk [11-15]. ACE gene is located in chromosome 17q23.3, including 26 exons. ACE gene is closely associated with the production and degradation of amyloid-β which relate to the development of AD. Many single nucleotide polymorphisms (SNPs) of ACE gene were involved in various diseases [16-18]. The single Alu insertion/deletion (I/D) is the staple polymorphism of ACE gene [19, 20]. Recent years, two SNPs rs4343 and rs1800764 of ACE gene have been found associated with the occurrence of many diseases including AD [21, 22]. Nevertheless, the association between ACE gene and AD susceptibility is still not clear, and there is scarcely research focused on the two SNPs in Han population in China.

As we all know, the distribution of SNPs genotypes exist region difference. In order to certify the association between ACE gene and AD sus-
Association between ACE polymorphisms and AD

In this study, we carried out the case-control study. In this study, we analyzed the differences of genotypes and alleles of the ACE SNPs (rs4343 and rs1800764) in China Han population. Afterwards, haplotypes of rs4343 and rs1800764 polymorphisms were analyzed.

**Materials and methods**

**Objects**

A total of 113 diagnosed AD patients (aged 52-81 years old, 63 males and 50 females) were enrolled from the outpatients and inpatients of Grade 3 A Class hospitals in five cities in Hebei. 142 healthy subjects (aged 54-79 years old, 77 males and 65 females) who were matched with patients in age, gender and community were recruited as controls. AD patients had been diagnosed by 2 doctors using the combination of clinical data and check means. Diagnoses were in correspondence with the American diagnostic criteria of neurology, aphasia and apoplexy-senile dementia as well as the other criteria of NINCDS-ADRDA. There were no such situations such as memory deterioration, diabetes, diseases of heart and nervous system in controls. This study had been approved by the Ethics Committees of Hebei province, and all the participants had signed informed consent.

**DNA extraction**

5 ml peripheral venous blood from every fasting participant was anticoagulated with EDTA and preserved in -20°C fridge. The blood was undergone the operation of hypotonicity separation for white blood cells. Genome DNA was extracted with potassium iodide method, and dissolved in TE solution.

**Polymerase chain reaction (PCR) amplification**

PCR primers were referenced previous studies [21, 23]. Total volume of PCR system was 50 μl using general constituents. PCR procedures were as the following: 2 min initial denaturation at 94°C, following 30 cycles of 60 s denaturation at 94°C, 45 s annealing at 58°C and 50 s extension at 72°C, and 5 min final extension at 72°C. PCR products were sequenced by Sangon Biotech (Shanghai, China).

**Statistical method**

SPSS 18.0 software was used for statistical calculation. Differences of genotypes and alleles of rs4343 and rs1800764 between case and control groups were examined with χ² test, and had statistical significance when P<0.05. Haplovew software was adopted to analyze linkage disequilibrium and haplotypes. PLINK1.07 was applied to do Hardy-Weinberg equilibrium (HWE) examination. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to represent the relative risk of AD.

| Table 1. Distributions of genotypes and alleles of rs4343 and rs1800764 in AD patient group and control group |
|----------------------------------|------------------|------------------|--------|--------|------------------|------------------|
| SNP                             | Genotype         | Cases            | Controls          | χ²   | P value     | OR (95% CI)     |
| rs4343A/G                       | AA               | 53 (46.9)        | 72 (50.7)         | -    | -           | 1                |
|                                 | AG               | 39 (34.5)        | 53 (37.3)         | 0.000 | 0.999       | 1.000 (0.580-1.724) |
|                                 | GG               | 21 (18.6)        | 17 (12.0)         | 1.945 | 0.163       | 1.678 (0.808-3.487) |
| rs1800764C/T                    | TT               | 39 (34.5)        | 65 (45.8)         | -    | -           | 1                |
|                                 | CT               | 51 (45.1)        | 58 (40.8)         | 1.882 | 0.170       | 1.466 (0.848-2.532) |
|                                 | CC               | 23 (20.4)        | 19 (13.4)         | 3.3649| 0.056       | 2.018 (0.976-4.169) |
| Allele                          | rs4343A/G        | A                | 145 (64.2)        | 197 (69.4) | - | - | 1 |
|                                 | G                | 81 (35.8)        | 87 (30.6)         | 1.545 | 0.214       | 1.265 (0.873-1.833) |
| rs1800764C/T                    | T                | 129 (57.1)       | 188 (66.2)        | -    | -           | 1                |
|                                 | C                | 97 (42.9)        | 96 (33.8)         | 4.448 | 0.035       | 1.473 (1.027-2.111) |

**Table 2. Haplotype analysis of alleles of rs4343 and rs1800764**

| Haplotype | locus1-locus2 | Cases (n=226) | Controls (n=284) | χ²   | P value     | OR (95% CI)     |
|-----------|---------------|---------------|------------------|--------|--------|------------------|------------------|
| A-T       |               | 129 (57.1)    | 188 (66.2)      | -      | -      | 1                |
| A-C       |               | 16 (7.1)      | 9 (3.2)         | 5.154  | 0.023 | 2.591 (1.111-6.043) |
| G-C       |               | 81 (35.8)     | 87 (30.6)       | 2.529  | 0.112 | 1.357 (0.931-1.977) |

Note: locus1, rs4343; locus2, rs1800764.
Association between ACE polymorphisms and AD

Results

Analysis of the objects

Distributions of ages \((t=0.12, P=0.870)\) and genders \(\left(\chi^2=0.63, P=0.963\right)\) in two groups had no significant differences. Genotypes and alleles distributions in control group were corresponding with HWE showed that the goodness of fit was well at each locus \((P>0.05)\), which suggested that the controls were in balanced states and had good representative.

Genotype and allele analyses of rs4343 and rs1800764

Distributions of genotypes and alleles of rs4343 and rs1800764 were listed in Table 1. We found that the genotypes and alleles of rs4343 had no significant relation with AD susceptibility. For rs1800764, only C allele could increase the AD susceptibility \((P=0.035, \text{OR}=1.473, 95\% \text{ CI}=1.027-2.111)\). There were no association between the genotypes of rs1800764 and the susceptibility of AD.

Linkage disequilibrium and haplotype analyses between rs4343 and rs1800764

Linkage disequilibrium between rs4343 and rs1800764 polymorphisms was analyzed by Haploview software. The result indicated that the high linkage disequilibrium was existed between rs4343 and rs1800764 \((D'=1, r^2=0.633)\). Therefore, we analyzed the association of haplotypes with AD susceptibility (Table 2). Compared with A-T haplotype, the A-C haplotype maybe increase the susceptibility of AD \((P=0.023, \text{OR}=2.591, 95\% \text{ CI}=1.111-6.043)\), and G-C haplotype had no correlation with AD. So we suggested that A-C is the susceptible haplotype for AD.

Discussion

As the common chronic neurodegenerative disease which will leads to death, AD is closely associated with age. Incidence of AD is rose obviously after 65 years old [24]. But not all of the old people are suffering the risk of AD, there exist individual difference. The difference was determined by various factors, including genetic and environmental factors. Previous studies indicated that AD is a complex disease. ACE gene is one of the factors which could affect the occurrence of AD [25-28].
Association between ACE polymorphisms and AD

types and the susceptibility of AD was detected. The results manifested that the distribution of A-C haplotype is significantly higher in case group than that in control group, demonstrated a positive association with AD risk. Based on the above results, we suggested that when the linkage disequilibrium was existed between the SNPs, the functions of the single SNPs may be changed.

Although we obtained a meaningful result, there also had many limitations in this study. First, the sample size is small. Second, the results were not adjusted by other factors. It was insufficient to certify the etiology of AD. As such, a well designed study which contained a multiple center and a larger sample size is needed, so as to receive an exact evidence to certify the AD etiology. Then the study could supply useful method for the prevention and treatment of AD, thereby reducing the AD morbidity.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yan Wei, Second Department of Neurology, Harrison International Heping Hospital, Hengshui 053000, Hebei, China. E-mail: jiwuweanig@sina.com

References


Association between ACE polymorphisms and AD


Association between ACE polymorphisms and AD


