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Original Article

Relationship between superoxide dismutase 1 and patients with Alzheimer's disease

Yumei Li[1], Chunqing Ma[2], Yan Wei[1]

[1]*Department of Neurology, Harrison International Peace Hospital, Hengshui 053000, China;* [2]*Clinical Laboratory, People's Hospital of Zhangqiu, Jinan 250200, China*

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Abstract: Aims: The purpose of this study was to investigate the association between superoxide dismutase 1 (*SOD1*) rs2070424, rs4998557 polymorphisms and the susceptibility of Alzheimer's Disease (AD) in Chinese Han population. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine genotypes of *SOD1* polymorphisms in 98 patients with AD and 105 healthy persons. The differences of gentoype, allele and haplotype frequencies between cases and controls were detected by the chi-square test, and the relative risk of AD was expressed by odds ratio (OR) and 95% confidence interval (CI). Hardy-Weinberg equilibrium (HWE) in the control group was also checked by χ^2 test. The linkage disequilibrium (LD) and haplotype analyses were carried out with haploview software. Results: For rs2070424, AA genotype had a significantly higher frequency in AD patients than that of the healthy controls (*P*=0.021), and the results showed that people carrying AA genotype were easier to be attacked by AD than GG genotype carriers (OR=2.618, 95% CI=1.212-5.657). The frequency of A allele of rs2070424 in the case group was also significantly higher than the controls and it can increase the onset risk of AD (OR=1.677, 95% CI=1.133-2.484, *P*=0.010). However, no significant differences were found in rs4998557 polymorphism between two groups. $A_{rs2070424}$-$A_{rs4998557}$ and $A_{rs2070424}$-$G_{rs4998557}$ haplotypes significantly associated with the increased risk for AD (*P*=0.009, OR=2.670, 95% CI=1.258-5.664; *P*=0.042, OR=1.530, 95% CI=1.014-2.309). Conclusion: Rs2070424 polymorphism in *SOD1* might be associated with AD susceptibility in Chinese Han population, but rs4998557 was not. Additionally, haplotypes can also affect the occurrence of AD.

Keywords: *SOD1*, polymorphisms, haplotypes, Alzheimer's disease (AD)

Introduction

Alzheimer's disease (AD), also known as senile dementia, is a kind of degenerative disease of the central nervous system characterized by progressive cognitive impairment and memory impairment. Clinical manifestations is that cognitive and memory functions are deteriorating, the ability of daily living is reduced, and there are a variety of mental symptoms and behavior disorders [1]. A clinical evaluation for a community population has showed that, the prevalence rate of AD is estimated to be 10.3% of those over the age of 65 years old and it increases remarkably with the age [2]. The subsequent global research has also indicated that the number of people affected by dementia will double every 20 years and most people with dementia live in developing counties [3]. China is the largest developing country in the world, with its aging population, the prevalence of AD is increasing significantly, which may be affected by sex, education, occupation or age [4]. The prevalence of AD in China has been reported to be comparable with that in Western countries [5]. However, there is not effective treatment although several licensed treatments may alleviate symptoms of AD [6]. More seriously, in developed countries, AD has also become the fourth leading cause of death after heart disease, cancer and stroke. With the increasing of per capita life expectancy, the threat of AD to the health of human life is becoming more and more prominent. Therefore, there is an urgent need to improve our understanding of pathogenesis of AD, and search for methods for effective treatment or diagnosis.

Cu/Zn superoxide dismutase, also known as superoxide dismutase 1 (SOD1), is one of three human superoxide dismutases which has been reported to be implicated in apoptosis and amy-

Table 1. Primer sequences of *SOD1* gene in rs2070424, rs4998557

SNP	Primer sequence		Product length
rs2070424	For.	5'-TTCTTCCCAGAGCATTAG-3'	45 bp
	Rev.	5'-CTTCAAACAAGGCTTCAC-3'	
rs4998557	For.	5'-TGTATGTAGCCACGGAGCA-3'	238 bp
	Rev.	5'-ACAGGCGTAAGCCACCAC-3'	

otrophic lateral sclerosis (ALS) [7, 8]. Its encoding gene, *SOD1* gene, is located on chromosome 21q22. The full length of the *SOD1* gene is 12 Kb, containing 5 exons, encoding 153 amino acids, and forming 33 ku metal enzyme proteins, with the function of scavenging free radicals and antioxidant. What is more, *SOD1* is a 32 kDa homodimer which forms a β-barrel and contains an intramolecular disulfide bond and a binuclear Cu/Zn site in each subunit. This Cu/Zn site holds the copper and a zinc ion and is responsible for catalyzing the disproportionation of superoxide to hydrogen peroxide and dioxygen [9, 10]. Moreover, the SOD1 enzyme is an important constituent in apoptotic signaling and oxidative stress, most notably as part of the mitochondrial death pathway and cardiac myocyte apoptosis signaling [11].

Oxidative stress affects aging, as well as numerous age-related diseases, including AD. Casado A et al. have found impaired antioxidant defense system (ADS) enzymes expression or activity in AD patients, which confirms that oxidative stress plays an important role in the brain damage for AD [12]. SOD1, as an ADS-related enzyme, probably plays a vital role in AD development. However, only few studies have been performed to evaluate the importance of ADS gene polymorphisms for AD risk.

Therefore, in this study, we investigated the association of *SOD1* gene polymorphisms rs2070424, rs4998557 with genetic susceptibility to AD in a Chinese Han population. And through this study, we hoped to provide some theoretical foundations for the mechanism of AD

Materials and methods

The case and the control groups

This study was aimed at testing the correlation between *SOD1* polymorphisms and AD with the method of case-control study. All 98 AD patients of case group were recruited from department of Neurology, Harrison International Peace Hospital from July, 2013 to December, 2014, including 55 males and 43 females. Their age ranged from 45 to 88 years old, with the average age of 71.23±4.15. These cases are in line with "probable AD" diagnostic criteria in National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) [13]. Exclusion criteria: ① Vascular dementia, systemic diseases and dementia caused by material poisoning; ② Pseudo dementia caused by depression; ③ Other neurological diseases and cerebrovascular diseases; ④ The acute phase of patients in the heart, lung, liver, kidney and other major physical disease and patients with malignant tumor.

The healthy control group covered healthy people who had a medical examination also in the same hospital during the same period. There were a total of 105 people, including 59 men and 46 women, aged from 40 to 89 years old, with the average age of 69.58±5.12. The research objects were all Chinese Han population and they had no blood relationship with each other.

Both the patients with AD and healthy controls knew about this research process and signed the informed consent. And the basic information of the objectives was detailedly recorded by specially trained epidemiological investigators. This research was examined and approved by the Ethics Committee of Harrison International Peace Hospital. The process of sample collection complied with the national ethics criteria of human genome research.

DNA extraction

3 ml fasting peripheral blood were extracted from the patients and the healthy controls into anticoagulative tube with ethylene diamine tetra acetie acid (EDTA). The peripheral blood leucocyte genome DNA of all samples was extracted using Beijing TIANGEN biochemical blood genome DNA extraction kit, according to the manufacturer's instructions, and then stored in -20°C refrigerator for standby application.

Table 2. Frequency comparisons of genotypes and alleles in *SOD1* gene polymorphisms

Genotype/allele	Case n=98 (%)	Control n=105 (%)	χ^2	P	OR (95% CI)	P_{HWE}
rs2070424						0.81
GG	22 (22.45)	32 (30.47)	-	-	1.00	
AG	40 (40.82)	53 (50.48)	0.072	0.788	1.098 (0.556-2.168)	
AA	36 (36.73)	20 (19.05)	6.114	0.013	2.618 (1.212-5.657)	
G	84 (42.86)	117 (55.71)	-	-	1.00	
A	112 (57.14)	93 (44.29)	6.704	0.010	1.677 (1.133-2.484)	
rs4998557						0.14
AA	29 (29.59)	36 (34.29)	-	-	1.00	
GA	49 (50.00)	57 (54.29)	0.042	0.837	1.067 (0.574-1.985)	
GG	20 (20.41)	12 (11.42)	2.744	0.098	2.069 (0.870-4.923)	
A	107 (54.59)	129 (61.43)	-	-	1.00	
G	89 (45.41)	81 (38.57)	1.947	0.163	1.325 (0.892-1.967)	

The determination of genotypes in SOD1 polymorphisms

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied for the genotyping of *SOD1* rs2070424, rs4998557 polymorphisms. Primer sequences for *SOD1* rs2070424, rs4998557 polymorphisms were designed by Primer Premier 5.0 software, complying with general primer design principles, and synthesized by Sangon Biotech (Shanghai, China). The detailed sequences were listed in **Table 1**.

PCR amplification was performed in a volume of 25 μl reaction system, including 1.0 μl DNA template, each 2.0 μl of forward and reverse primers, 1.5 μl $MgC1_2$, 1.0 μl dNTPs, 2.0 μl 10×Buffer, 2.0 μl Taq DNA polymerase, and 13.5 μl sterilization ddH_2O. PCR amplification conditions were as the following: 95°C predenaturation for 15 min; followed by 38 cycles of 94°C degeneration for 30 s, 56°C annealing for 60 s, 72°C extension for 60 s, and finally 72°C extension for 10 min. In the end, the products were preserved at 4°C. And the PCR products were checked in 1% agarose gel electrophoresis (AGE).

Enzyme digestion reaction system was a volume of 20 μl solution, including 10.0 μl PCR products, 2.0 μl restriction enzyme (*Hha*I for rs2070424 and *Alu*I for rs4998557), 2.0 μl 10×buffer solution, 6.0 μl double distilled water. The mixture was put in water bath of 37°C for overnight for digestion. The enzyme-digested products were separated by 3% AGE and the results were observed in imaging system.

Statistical analysis

PASW Statistics 18.0 software was used for data analysis. Linkage disequilibrium (LD) and its correlation coefficient (D' value) between rs2070424 and rs4998557 SNPs were showed in Haploview software. And the frequencies of allele, genotype and haplotype in *SOD1* polymorphisms were calculated by direct counting method. All data were represented by $\bar{x}\pm s$ or %. The χ^2 test was used to check whether the genotype distributions matched Hardy-Weinberg equilibrium (HWE) in the control group. The distribution differences of the genotype, allele and haplotype between the two groups were tested by chi-square test, too. The effect of *SOD1* polymorphisms on AD was evaluated with odd ratio (OR) and 95% confidence interval (CI). And $P<0.05$ indicated that the difference had statistical significance.

Results

General conditions of research objects

98 patients with AD and 105 healthy controls were recruited into this study. In the case group, the sex ratio in males and females was 1.28:1.00 with the average age of 71.23±4.15. The controls included 56 males and 44 females with the ratio of 1.28: 1.00 and the average age was 69.58±5.12. There was no significant difference between the two group by gender and age ($P>0.05$). Furthermore, the genotypes

Table 3. Analyses of LD and haplotypes in *SOD1* rs2070424, rs4998557 polymorphisms

Haplotype SNP1-SNP2	Case group 2n=196 (%)	Control group 2n=210 (%)	χ^2	P	OR (95% CI)
GA	84 (42.86)	117 (55.71)	-	-	1.00
AG	89 (45.41)	81 (38.57)	4.128	0.042	1.530 (1.014-2.309)
AA	23 (11.73)	12 (5.72)	6.884	0.009	2.670 (1.258-5.664)

Note: SNP1: rs2070424; SNP2: rs4998557.

distribution of *SOD1* polymorphisms conformed to HWE in the control group (P>0.05), which indicated our study groups had a good matching degree and representativeness (**Table 2**).

The genotype distributions of SOD1 polymorphisms in case and control groups

The results of genotype and allele distributions in *SOD1* rs2070424, rs4998557 SNPs between the case and control groups were displayed in **Table 2**. The frequencies of GG AG and AA genotype in rs2075876 were 22.45%, 40.82%, 36.73% in case group and 30.47%, 50.48%, 19.05% in healthy control group respectively. The G, A allele frequencies were respectively 42.86%, 57.14% and 55.71%, 44.29% in the case and control groups respectively. The frequencies of AA genotype and A allele increased in case group compared with control group, and the differences had statistically significance (P=0.013, P=0.010). It suggested that *SOD1* rs2070424 was associated with AD susceptibility and the mutation of A to G might be protective against the AD onset risk (AA vs. GG, OR=2.618, 95% CI=1.212-5.657; A vs. G, OR=1.677, 95% CI=1.133-2.484).

Referring to rs4998557, the AA, GA, GG genotype frequencies were respectively 29.59%, 50.00%, 20.41% in cases and 34.29%, 54.29%, 11.42% in controls. And A, G allele frequencies were 54.59%, 45.41% and 61.43%, 38.57% in case and control groups, respectively. Although GG genotype and G allele frequencies increased in cases compared with controls, the differences were not statistically significant (P>0.05). These results all demonstrated that rs4998557 was not associated with the susceptibility to AD.

Haplotype analysis between SOD1 rs2070424, rs4998557 polymorphisms

The LD and haplotype analysis of *SOD1* rs2070424 and rs4998557 were performed by haploview software (D'=1.0, r^2=0.981), and three haplotypes were identified in our population formed by rs2070424 and rs4998557, namely G-A, A-G, A-A (**Table 3**). The frequency of G-A, A-G, A-A haplotypes were 42.860%, 45.41%, 11.73% in the case group and 55.71%, 38.57%, 5.72% in the control group. The frequency of A-A haplotype in the case group was markedly higher than that of the control group (P=0.029), which indicated that it could increase the risk of AD occurrence (OR=2.670, 95% CI=1.258-5.664). Meanwhile, the A-G haplotype also significantly associated with the increased AD risk (P=0.042, OR=1.530, 95% CI=1.014-2.309).

Discussion

Alois Alzheimer, an German neuropathologists who is considered to be a founding father of neuropathology, has firstly presented the clinical and neuropathological characteristics of AD in 1906, which is subsequently named after him by Emil Kraepelin [14, 15]. AD is the most common disease of brain degeneration, and has become the third main reasons for the death of the elderly [16]. The general pathological changes include extensive atrophy of the cortex, cerebral groove broadening, and enlargement of the ventricles (memory, language, hippocampus area), microglia and astrocyte reaction, vascular amyloid degeneration and a large number of neuron cell synapse loss [17-19], in which the formation of Amyloid beta (Aβ) deposition into the brain is considered as a crucial step in AD development [20]. Many theories about the pathogenesis of AD have been put forward, such as abnormal protein theory, gene mutation theory, synaptic inactivation theory, mitochondrial dysfunction theory and inflammatory reaction theory and others. But so far, the exact etiology and pathogenesis are not clearly. It is considered as a result of the combined effects of genetic and environmental factors. Up to now, several potential risk genes for AD have been identified, which indicates the important role of genetic factors for the patho-

genesis of the disease. Among these, *ApoE* gene is the most consistently associated risk gene [21-23]. It is important significant to study the genetic susceptibility genes of AD for the prevention of targeted populations, and delay or prevent the occurrence of AD.

The most important endogenous ADS enzymes includes superoxide dismutases (SODs), catalase and glutathione peroxidase 1 (GPx-1). Several studies have found impaired ADS enzymes expression or activity in AD patients [12]. The post-mortem and in-vivo examinations have also showed an accumulation of products of free radical damage in the central nervous system and in the peripheral tissues of individuals with AD, which might be the reason for oxidative and nitrosative stress [24]. What is more, Aβ depositions, the pathologic hallmarks of AD, are thought to be related to oxidative stress [25].

Oxidative damage has been confirmed to be an important factor in the pathogenesis of AD, and SOD1 plays crucial roles in repairing oxidative damage. Human *SOD1* gene is located on chromosome 21, and is one of the three human superoxide dismutases. Spisak K et al. have explored the association of several SNPs of *SOD1* gene, which suggests the risk role of rs2070424 polymorphism of the *SOD1* gene for AD in Polish population [26]. But in china, few studies have been reported.

In present study, we aimed at two polymorphisms rs2070424 and rs4998557 in *SOD1* gene. The results showed that the genotype and allele distributions of *SOD1* rs2070424 polymorphism were significantly different between case and control groups. The data indicated that AA genotype and A allele frequencies of rs2070424 were higher in AD patients than that in the controls, which indicated A allele might be a risk factor for the occurrence of AD. In view of rs4998557, it is the first time to explore its effect on AD, but we didn't find any significant difference between the case and control groups based on either genotypes or alleles frequencies. Yet, we also explored the association between haplotypes formed by *SOD1* rs2070424 and rs4998557 polymorphisms and AD susceptibility. The results demonstrated that $A_{rs2070424}$-$A_{rs4998557}$ and $A_{rs2070424}$-$G_{rs4998557}$ haplotypes obviously increased the risk suffering from AD in old adults. So the outcome of this article proved that *SOD1* gene polymorphisms had a connection with the occurrence of AD. But so far there are not published studies evaluating an association of the rs2070424 SNP of the *SOD1* gene with risk of AD in China. Also genome-wide association studies (GWAS) for AD do not show that rs2070424 SNP or any other region on the 21 chromosome is associated with the disease, and in one study, *SOD1* enzyme activity shows no significant difference in individuals with different genotypes of the rs2070424 SNP [27]. Case-control study is common method for searching genetic factors of diseases. But the results generally can not be replicated in independent populations. Therefore, it is essential to confirm our results replication in different populations.

In conclusion, this study supported the correlation between *SOD1* polymorphisms and AD in Chinese Han population. But the results need to be confirmed by further studies, in order to achieve the aims of precaution, early diagnosis, and timely treatment because there are many limitations in our study, such as small sample size, ignoring the environmental factors.

Disclosure of conflict of interest

None.

Address correspondence to: Yan Wei, Department of Neurology, Harrison International Peace Hospital, Hengshui 053000, China. E-mail: oi3tbvdfkh@sina.com

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