



Association of Met/Val polymorphism of BDNF gene with Alzheimer's disease in Chinese patients

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ABSTRACT

Alzheimer's is the most common cause of dementia in the elderly. In this disease, genetic and environmental factors are involved. In Alzheimer's, changes of nucleotide 196 (G> A) or valine polymorphism of 66-methionine in the BDNF gene is a risk factor for brain-derived neurogenic factors. In China, this polymorphism has not been studied in Alzheimer's patients and perhaps this study could provide appropriate information on the prognosis and susceptibility of the disease. Therefore, in this case-control study, 73 patients with Alzheimer's disease and 100 patients as a healthy control group were studied. Blood samples were taken from the mentioned individuals and DNA was extracted. After quantitative and qualitative DNA analysis, a PCR-RFLP test was performed and the results of both groups were compared. The results showed that 14 patients and 7 people in the control group had BDNF gene polymorphism. In the patient group, the number of people with normal allele was 59. Heterozygous people were 8 and people with methionine/methionine alleles were 6. In the control group, 93 normal individuals, 5 heterozygous individuals, and 2 people had methionine/methionine alleles. In general, increasing the accumulation of valine/methionine polymorphism of the BDNF gene in Alzheimer's patients compared to control can indicate the role of this polymorphism. Clinically, patients with this polymorphism had a more unfavorable clinical condition compared to patients without it. Therefore, evaluation of the presence of this polymorphism can provide appropriate information about the disease status.

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Introduction

Alzheimer's disease (AD) is a lethal disease that destroys nerve cells and is the most important cause of neurodegenerative disorders in adults (1). The disease manifests itself in the gradual and progressive loss of consciousness and memory and is the most common form of middle-aged and old age insanity. At present, the prevalence of the disease is about 1.5% among middle-aged people in developed countries (2).

The pathological symptoms of AD are associated with the formation of beta-amyloid plaques in various parts of the brain (1). Although AD is generally a disorder of the gray matter of the brain, white matter problems in the brain have also been reported. Numerous genetic factors are involved in the development of Alzheimer's disease. Genes related to amyloid precursor protein (APP) and the genes of presenilin 1 and 2, and apolipoprotein E (APOE) are

known genes, and mutations in these three genes cause the predominant and early form of Alzheimer's disease (2).

Other known genetic factors in Alzheimer's disease include the function of the brain-derived neurotrophic factor (BDNF) gene (3). The BDNF gene is responsible for the production of brain-derived neurogenic factors. This factor belongs to the family of neurotrophins and causes the expansion of the neural network. BDNF plays an important role in the development of neurons and the maintenance of their interaction in the central and peripheral nervous systems (4). This gene is chromosomally located on the short arm of chromosome 11, encoded in the P14.1 region, and has 9 exons. Research has shown that the expression of this gene is an essential factor for axonal growth and its cessation of expression disrupts walking and standing and causes

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uncoordinated movements in mice (5). The mRNA of this gene is found in all areas of the brain, and in Alzheimer's disease, the amount of this mRNA decreases in the temporal lobe and frontal lobe of the cerebral cortex (6, 7).

Several SNPs (single nucleotide polymorphisms) such as val66met and TC 270 have been reported in the BDNF gene, which is associated with AD, and its most important polymorphism is val66met. This polymorphism is caused by nucleotide change (G> A 196) in the BDNF pro-protein region and is associated with a decrease in the production of this neurotrophin and leads to a change in the function of neural communication (8). This nucleotide shift converts the amino acid valine to methionine in a protein derived from BDNF gene expression. The methionine allele inhibits intercellular interactions as well as the secretion of BDNF protein at nerve endings (3). This nucleotide change can be detected by cleavage of the *Hin1II* enzyme (6). Since no study of the polymorphism of this gene in the Alzheimer's population in China has been performed in any study so far, it seems that this study is necessary and can provide physicians with information about the prognosis and susceptibility to the disease. Also, the study of risk factors in predicting Alzheimer's disease in people with a family history is one of the cases that necessitate research.

Materials and methods

In this case-control study, 73 Chinese patients with Alzheimer's disease were studied. Blood samples were taken from patients after consultation and obtaining consent through the patient's supervisor.

The diagnosis of these patients was confirmed by an experienced neurologist based on the diagnostic criteria of DSM-IV-TR (9) and NINCDS-ADRDA (10). These diagnostic criteria briefly include neurological examinations, neurophysiological tests, and brain imaging. A total of 100 people were selected as the control group, all of whom were unrelated and over 65 years old. They had no personal or family history of dementia, including Alzheimer's disease or neurodegenerative disorder. To ensure mental status, after performing a 30-point test, MMSE individuals with a score above 28 were included in the study. In addition, the control group was consistent with the patient group in terms of age and sex.

In addition, the control group was consistent with the patient group in terms of age and sex. Patients and controls were sampled at the laboratory. DNA was extracted from phenol-chloroform by blood samples taken from patients on EDTA anticoagulant. Then, the extracted DNA samples of the patients were quantitatively evaluated by an Eppendorf biophotometer. Also, the quality of DNA was evaluated by agarose gel electrophoresis. PCR_RFLP method was used for polymorphism (G> A) in the BDNF gene. First, suitable primers for amplification of the desired fragment with the polymorphism mentioned in the BDNF gene were selected using previous studies (11) and also using BLAST software available on the NCBI site (Table 1) to be completely specific for this gene (12).

Table 1. Primers used for PCR of BDNF gene

| Gene | Primer Sequence | |
|------|-----------------|-------------------------------|
| BDNF | Forward | 5'- ACTCTGGAGAGCGTGAATGG - 3' |
| | Reverses | 5'- CAGTCTTTTTGTCTGCCGCC - 3' |

Then, to confirm and evaluate the length of the amplified fragments by PCR, the amplified product was electrophoresed on 2% agarose gel. The *Hin1 II* enzyme made by Thermo-Scientific Company (Lot Number 00350199) was used to perform the RFLP test and to investigate the presence of 196G> A polymorphism in the amplified products. First, the *Hin1 II* enzyme was diluted 1: 9 with buffer B19 and then PCR products were adjacent to the mentioned enzyme according to time and temperature in Table 2.

Table 2. Protocol of attaching *Hin1 II* restriction enzyme to PCR product

| Reaction Component | Amount |
|-------------------------------|--------|
| PCR product | 7 µl |
| G Buffer | 2 µl |
| Distilled water | 15 µl |
| Diluted <i>Hin1 II</i> enzyme | 1 µl |
| Total volume | 25 µl |
| Incubation temperature | 37 °C |
| Incubation duration | 1 hour |

Then, based on Tables 3 and 4, the protocol and PCR program were performed. In order to evaluate the cutting status of PCR products, electrophoresis was performed on 2% agarose gel along with DNA Ladder 50bp.

Table 3. PCR protocol for BDNF gene

| PCR Reaction Component | Amount |
|--------------------------|--------------|
| Extracted DNA | 1 μ l |
| Forward Primer | 0.7 μ l |
| Reverse Primer | 0.7 μ l |
| Distilled water | 10.1 μ l |
| Master Mix (2X) | 12.5 μ l |
| Final volume of reaction | 25 μ l |

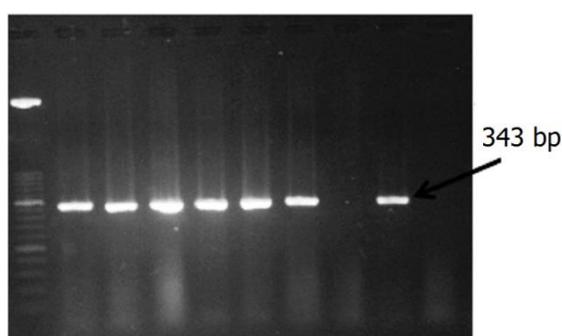
Table 4. Final Program of PCR for BDNF gene amplification

| PCR Stage | Time (second) | Temperature ($^{\circ}$ C) | Number of Cycle |
|--------------------------|---------------|-----------------------------|-----------------|
| Primary DNA Denaturation | 600 | 95 | 1 |
| DNA Denaturation | 30 | 95 | |
| Annealing | 45 | 63.5 | 50 |
| Extension | 60 | 72 | |
| Final Extension | 180 | 72 | 1 |

After analyzing each individual in terms of genotype, the data were analyzed using SPSS software version 23. The frequency of three states of healthy homozygous, heterozygous and mutant homozygous in healthy and control groups for these polymorphisms was evaluated using the chi-square test and the results were compared with a significance level of less than 0.05.

Results and discussion

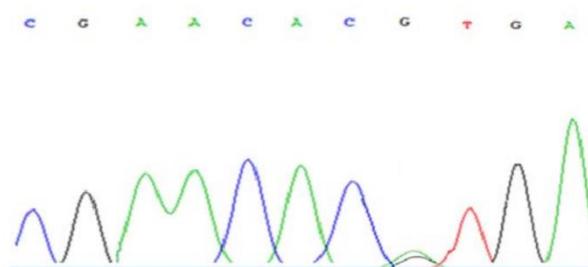
The results showed that the size of the amplified fragment was 343 bp, which was the expected fragment (Figure 1).

**Figure 1.** Length of amplified fragments next to DNA Ladder 50; the length of the PCR product is 343 bp

Using enzymatic digestion, samples with the G allele produced two fragments of 239bp and 94bp, which represents the Val / Val allele (two alleles containing valine). Samples with the A allele produced three fragments of 164bp, 71bp, and 94bp, representing the heterozygous Val / Met allele or the Met / Met homozygous allele. Figure 2 shows it on gel electrophoresis.

**Figure 2.** Agarose gel electrophoresis of PCR product after exposure to Hin1 II enzyme with DNA Ladder 50bp; The G allele has two fragments and the A allele has three fragments.

To isolate individuals with Val/Met and Met/Met alleles, as shown in Figure 3, a sequencing test was used to determine if the individual was heterozygous or homozygous.

**Figure 3.** Alteration of nucleotide 196 and conversion of G to A, which is heterozygous; the Val / Met allele or G/A allele, which is cleaved by the enzyme, is sequenced.

As seen in Table 5, out of the total number of studied patients, 33 were female and 40 were male. The number of patients with Val/Val allele was 59 (27 females and 32 males) and the total number of nucleotide mutants whose DNA was cleaved with the *Hin1II* enzyme was 14. Of these, 8 tetra zygotes with valine/methionine allele were identified, including 4 females and 4 males. Also, the number of homozygous individuals with methionine/methionine allele was 6, including 2 females and 4 males. In the control group, there were a total of 100 people (43 females and 57 males). The number of people with Val/Val allele was 93 and the number of people with Val/Met allele was 5, which included 2 females and 3 males.

The number of people with Met / Met allele in the control group was 2 males. Table 6 shows the comparison of allelic frequency in the control group and patients with $P < 0.05$ and according to the statistical analysis, there is a significant difference between the two groups.

Table 5. Comparison of BDNF gene test results in patients and control group after restricting with *Hin1* II enzyme

| | Gender | number | Val/Val Allel (G/G) | Val/Met Allel (G/A) | Met/Met Allel (A/A) |
|------------------|--------|--------|------------------------|------------------------|------------------------|
| Patient Group | Female | 33 | 27 (81.88%) | 4 (12.12%) | 2 (6%) |
| | Male | 40 | 32 (80%) | 4 (10%) | 4 (10%) |
| Control Group | Female | 43 | 41 (95.35%) | 2 (4.65%) | 0 |
| | Male | 57 | 52 (91.24%) | 3 (5.26%) | 2 (3.5%) |

Table 6. Comparison of BDNF gene test results in patients and control group after cleavage with enzyme in SNP Stat software

| Allel | Patient | | Control | | P-value |
|-------|---------|-----------|---------|-----------|---------|
| | Number | (percent) | Number | (percent) | |
| G | 126 | (86) | 91 | (92) | 0.28 |
| A | 20 | (14) | 9 | (8) | 0.036 |

As can be seen in Table 7, statistical calculations using SNP Stat software show that the allelic distribution of the BDNF gene is subject to Hardy Weinberg's law and, in fact, statistically means that the study population is in Hardy Weinberg equilibrium.

Table 7. Allelic distribution in Hardy Weinberg equilibrium

| | Homozygote (G/G) | Heterozygote (G/A) | Homozygote (A/A) | P value |
|---------|---------------------|-----------------------|---------------------|---------|
| Patient | 59 (80.8%) | 8 (11%) | 6 (8.2%) | 0.014 |
| Control | 93 (93%) | 5 (5%) | 2 (2%) | 0.015 |

According to Table 8, statistical calculations using SNP Stat and SPSS software show that with $P < 0.05$, there is a significant difference between the patient group and the control group in the type of BDNF gene allele. As shown in Table 8, the inheritance model for BDNF gene polymorphisms can be dominant, recessive, or even pronounced. In fact, it indicates that there is a difference between the heterozygous (G / A) state in this gene and the homozygous (G / G).

Table 8. Statistical comparison of patients and control group for BDNF gene

| Inheritance model | Genotype | Patient | Control | P value |
|-------------------|----------|------------|----------|---------|
| Codominant | G/G | 59 (80.8%) | 93 (93%) | 0.044 |
| | G/A | 8 (11%) | 5 (5%) | |
| | A/A | 6 (8.2%) | 2 (2%) | |
| Dominant | G/G | 59 (80.8%) | 93 (93%) | 0.016 |
| | G/A-A/A | 14 (19.2%) | 7 (7%) | |
| Recessive | G/G-G/A | 67 (91.8%) | 98 (98%) | 0.054 |
| | A/A | 6 (8.2%) | 2 (2%) | |

In this study, Val/Met polymorphism was evaluated on 73 Alzheimer's patients along with the control group. The results showed that 14 patients and 7 patients in the control group had these polymorphisms with $P < 0.05$, a significant difference was observed between the two groups. Tessarollo *et al.* (13) and Huang *et al.* (14) demonstrated the vital role of a brain-derived neurotrophic factor in the development of the nervous and cardiovascular systems.

Ventriglia *et al.* (15) identified the role of Val / 66Met polymorphism in Alzheimer's disease. The present study also confirms the role of this gene mutation in Alzheimer's disease. Momose *et al.* (16) noted the importance of this polymorphism in Parkinson's disease. Other extensive studies have considered this polymorphism to be important in neuropsychiatric disorders, especially in memory loss. Accumulation of this polymorphism in patients in comparison with the control group in the present study, in line with other studies, indicates its possible role in Alzheimer's disease. In a study by Bian *et al.* (17), The protective role of G nucleotide in the position of 196 BDNF genes, the Val / Val allele, against Alzheimer's disease in women was studied, while the association of polymorphism was noted Val / Met has been questioned with this disease.

This issue can be easily seen in the present study. So that in the group of patients, 8 people had Val / Met polymorphism and 6 people had Met / Met polymorphism and the clinical examination that was performed on these patients showed that these people had more memory problems than the others. These findings are consistent with a study by Nagata *et al.* (18) that noted the important role of BDNF gene polymorphism in Alzheimer's disease. Also, in a study by Weinstein *et al.* (19), Which looked at serum BDNF levels in different individuals over 10 years and examined different risk factors, the presence of Val / Met polymorphism in the BDNF gene was associated with low serum levels of this neurotrophic factor. He found high levels of serum BDNF to protect against Alzheimer's. Therefore, this mutation can have a significant clinical effect due to its effect on gene expression. Ward *et al.* (20) considered the coexistence of the APOE ϵ 4 alleles and the BDNF gene polymorphism necessary for Alzheimer's disease in adults. The results of this study show that the increased accumulation of Val / Met polymorphism in

the BDNF gene in Alzheimer's patients compared to the control group can indicate the role of this polymorphism and this finding is consistent with the study of Robert *et al.* (21).

There are already reports about the importance of gene polymorphism in the expression of phenotype (23,24). Also, in a study conducted by Cohen-Cory *et al.* (24), the role of Val / Met polymorphism in the BDNF gene in Alzheimer's disease in Asians was introduced. Knowing the genetic basis and molecular basis of diseases will help in many cases to diagnose the disease early and prevent its progression. In Alzheimer's disease, the function of various genes has been evaluated so far.

Conclusions

In the present study as well as other studies, the importance of BDNF gene polymorphisms in Alzheimer's disease has been clarified. Therefore, if acceptable results are obtained in extensive studies and clinical trials, the mentioned nucleotide change can be used in diagnosing the susceptibility to the disease, prognosis and implementation of the treatment protocol, especially drug dose adjustment.

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Interest conflict

The authors declare that they have no conflict of interest.

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