The Impact of *EGFR* Gene Polymorphisms on the Risk of Alzheimer's Disease in a Chinese Han Population: A Case-Controlled Study

Authors' Contribution:
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Background: The aim of this study was to investigate the association between polymorphisms of the epidermal growth factor receptor (EGFR) gene with the risk of Alzheimer's disease (AD) in a Chinese Han population.

Material/Methods: A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was used to genotype 139 patients with AD and 152 healthy control individuals. The Hardy-Weinberg equilibrium (HWE) was analyzed using the chi-squared (χ^2) test, and genotype and allele frequencies were compared between the two population groups, using the χ^2 test. The odds ratios (ORs) and corresponding 95% confidence intervals (CI) were calculated to express the degree of risk of AD resulting from polymorphisms in the *EGFR* gene. Linkage disequilibrium among *EGFR* polymorphisms was analyzed using the Haploview bioinformatics software.

Results: The CC genotype and C allele frequencies of rs730437 were significantly lower in patients with AD compared with the controls (P=0.037), indicating that rs730437 was associated with a reduced risk of AD (CC *vs.* AA: OR=0.446, 95% CI=0.207–0.960) (C *vs.* A: OR=0.702, 95% CI=0.502–0.980). The presence of the TT genotype of rs1468727 significantly reduced the risk of AD (P=0.003; OR=0.333, 95% CI=0.160–0.691), and T allele carriers of rs1468727 had a 0.605-fold increased risk of AD. Haplotype A-C-C was significantly correlated with an increased risk of AD (OR=1.922, 95% CI=1.130–3.269).

Conclusions: In a Han Chinese population, *EGFR* gene polymorphisms, rs730437 and rs1468727 and haplotype A-C-C were shown to be possible protective factors for the development of AD.

MeSH Keywords: **Alzheimer Disease • Haplotypes • Polymorphism, Genetic • Receptor, Epidermal Growth Factor**

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Background

Alzheimer's disease (AD) is one of the most common causes of dementia in the elderly, accounting for between 60–80% of all cases of dementia [1–3]. The prevalence of AD increases with age, and increases from 3% in people aged between 65–74 years, to almost 50% in people who are more than 80 years of age [4]. The incidence of AD in women is slightly greater than that in men. Worldwide, with the increasingly aging population, AD has resulted in an increasing burden on families and society [5]. According to previous studies, AD is a multiple-factor disease influenced by genetic and environmental factors [6]. The known environmental factors associated with AD also include a history of infection, brain trauma, drug and other toxicities, and poor nutrition [7,8]. Heredity has an important role in the pathogenesis of AD, and recent studies have identified several genes in the etiology of AD, including *APOE*, *PICALM*, *BIN1*, and *TREM2* [9]. However, the genetic mechanisms that are involved in the pathogenesis of AD remain poorly understood.

Epidermal growth factor receptor (EGFR), also known as ErbB-1 or HER1 in humans, is an important transmembrane receptor with tyrosine kinases activity belonging to ErbB family [10]. The *EGFR* gene has been shown to regulate signaling pathways that include the PI3K-PKC-κB and RAS-RAF-MEK-MAPK pathways, and activate the factors involved in the processes of transcription and translation [11]. Expression of the *EGFR g*ene may participate in several cellular processes, including cell proliferation, survival, differentiation, invasion, adhesion, apoptosis, and repair of cell damage [12,13]. The key ligands of the EGFR protein, epidermal growth factors (EGFs) can control brain development, and neuronal survival and function [14]. Therefore, EGFR and the expression of the *EGFR* gene and *EGFR* gene polymorphisms may have a role in neurometabolic disorders, including AD [12,15].

It has previously been confirmed that the encoding gene for EGFR is located on chromosome 7p12.1-12.3 and consists of 30 exons and 29 introns, and several single nucleotide polymorphisms (SNPs) [16]. However, few recently previously published studies have been undertaken on the role of EGFR SNPs and the risk of the development of AD.

The aim of this study was to investigate the association between polymorphisms of the *EGFR* gene with the risk of AD in a Chinese Han population, and three common *EGFR* gene SNPs were selected, rs730437, rs3752651, and rs1468727.

Material and Methods

Patients with Alzheimer's disease (AD) and controls

This study was designed as a case-controlled study and included 139 patients with Alzheimer's disease (AD) and 152 healthy individuals in the control group. All patients with AD were diagnosed by clinical symptoms and by neuroimaging, in the Department of Neurology of the Inner Mongolia International Mongolian Hospital, according to the diagnostic criteria of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA). The 139 patients with AD included 62 men and 77 women, with an age range of between 59–87 years. The 152 healthy controls were selected by physical examination at the same department and hospital, and included 63 men and 89 women, with an age range of between 56–84 years. The controls were frequency-matched with the patients with AD in age and gender. All the subjects in the study were from the Chinese Han population and were unrelated, non-family members.

This study was supported by the Research Ethics Committee of the Inner Mongolia International Mongolian Hospital. All subjects and their families were informed of the objectives and design of the study. Before blood samples were collected, written informed consent was signed by each subject, or on their behalf by a family member.

DNA extraction

Each subject in the study provided 2 ml of peripheral venous blood, in the early morning, using vacuum tubes that contained the preservative and anticoagulant, ethylenediaminetetraacetic acid (EDTA). The genomic DNA was extracted from whole blood specimens using TIANamp Genomic DNA Kit (Tiangen Biotech, Bejing, China), according to the manufacturer's instructions. The quality and concentration of genomic DNA were determined using 1.0% agarose gel electrophoresis (AGE) and the NanoVue NanoDrop Plus 2000c (Thermo Scientific). Extracted DNA was stored at –20°C for further studies.

Genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to genotype each polymorphism in the *EGFR* gene. PCR primers were designed by Primer Premier 5.0 software and synthesized by the Shanghai Sangon Biotech Co., Ltd. The detailed information of PCR primer sequences is shown in Table 1.

The PCR system included a volume of 25.0 µl of the mixture, including a DNA template, forward and reverse primers, PCR

Table 1. The detailed information of PCR primers sequences.

Position			Primer sequence	Annealing temperature	Restriction enzyme
rs730437	Intron4	For.	5'GTCCCTGTCCCATTCC3'	55°C	*Aco*I
		Rev.	5'ATCCAAAGCCCTGTAGTT3'		
rs3752651	Intron13	For.	5'TTATTCTTTGCCCTTGGCTTTTG3'	56°C	*Hinf*I
		Rev.	5'TGGCTAGATGAACCATTGATGAC3'		
rs1468727	Intron13	For.	5'GATCCAGAAATATTTAGGAGC3'	56°C	*Mac*I
		Rev.	5'TTTCATCACCTTGCCTCT3'		

Table 2. The basic characteristics of subjects in the case and control groups.

Index		Case, n=139	Control, n=152	*P*
Gender	Male/Female	62/77	63/89	0.587
Age/years	The range	59–87	56–84	
	Mean age	74.6±6.8	75.2±6.3	0.436
Body mass index (kg/m²)		23.1±3.4	23.6±3.7	0.283
Smoking status/%		57/41.0	48/31.6	0.212
Alcohol consumption/%		46/33.1	33/21.7	0.029
Diabetes/%		35/25.2	21/13.8	0.014
Hypertension/%		46/33.1	32/20.1	0.021
Hypercholesterolemia/%		29/20.9	18/11.8	0.037

Master Mix and ddH$_2$O (sterile, ultra-pure water). The PCR procedure included an initial denaturation at 95°C for 5min, followed by 35 cycles of 94°C denaturation for 30s, annealing for 30s at 55°C or 56°C (Table 1) and extension at 72°C for 30s, and a final extension at 72°C for 7min. PCR products were checked by 1.0% AGE.

The PCR products were digested by restriction enzymes, AcoI, Hinfl and MacI, which were used for rs730437, rs3752651, and rs1468727 respectively. Enzyme-digested products were separated by 2.0% AGE to determine the genotype of each polymorphism in the *EGFR* gene.

Statistical analysis

The genotype frequencies were obtained by direct counting. The genotype distribution of each polymorphism in the patients with AD and the control group were analyzed according to the Hardy-Weinberg equilibrium (HWE) and analyzed using the chi-squared (χ^2) test. The differences in genotype and allele frequencies in polymorphism were compared between the Patients with AD and the controls by the chi-squared (χ^2) test, as well as haplotype and basic indices. In this study, the risk intensity of developing AD was expressed by the odds ratio (OR) with a corresponding 95% confidence interval (CI). The above data processing was conducted by SPSS version18.0 software. Linkage disequilibrium among *EGFR* polymorphisms was analyzed using the Haploview bioinformatics software. P<0.05 represented a statistically significant difference.

Results

Clinical information and clinical risk factors for patients with Alzheimer's disease (AD) compared with the normal case-controlled subjects in the study

The basic characteristics of subjects in the two study groups are summarized in Table 2. The mean ages of the patients with Alzheimer's disease (AD) and the controls were 74.6±6.8 years and 75.2±6.3 years, respectively. There was no significant difference between the two groups in age or gender (P>0.05). The body mass index (BMI) between the two groups was not significantly different (P=0.283), and smoking history was not a risk factor for AD. Alcohol consumption was significantly associated with the occurrence of AD (P=0.029). In patients with

Table 3. The genotype and allele distribution difference of *EGFR* polymorphisms between the case and control groups.

	Case, n=139/%	Control, n=152/%	OR (95%CI)	P	P_{HWE}
rs730437					0.135
AA	54/38.85	43/28.29	Ref.	–	
AC	71/51.08	84/55.26	0.673 (0.404, 1.121)	0.128	
CC	14/10.07	25/16.45	0.446 (0.207, 0.960)	0.037	
A	179/64.39	170/55.92	Ref.	–	
C	99/35.61	134/44.08	0.702 (0.502, 0.980)	0.037	
rs3752651					0.209
TT	102/73.38	124/81.58	Ref.	–	
CT	32/23.02	25/16.45	1.556 (0.867, 2.793)	0.137	
CC	5/3.60	3/1.97	2.026 (0.473, 8.682)	0.333	
T	236/84.89	273/89.80	Ref.	–	
C	42/15.11	31/10.20	1.567 (0.955, 2.573)	0.074	
rs1468727					0.719
CC	56/40.29	41/26.97	Ref.	–	
CT	68/48.92	78/51.32	0.638 (0.380, 1.071)	0.088	
TT	15/10.79	33/21.71	0.333 (0.160, 0.691)	0.003	
C	180/64.75	160/52.63	Ref.	–	
T	98/35.25	144/47.37	0.605 (0.433, 0.844)	0.003	

HWE – Hardy-Weinberg equilibrium; Ref. – Reference; '–' – indicated no available data.

AD, 25.5% of patients suffered from diabetes mellitus, while the prevalence of diabetes mellitus was 13.8% in healthy controls (P=0.014). Hypertension in patients in AD was also significantly increased compared with the controls (33.1% *vs.* 20.1%) (P=0.021), and hypercholesterolemia was also significantly associated with AD (P=0.037).

The Hardy-Weinberg equilibrium (HWE) in the control population

The status of the Hardy-Weinberg equilibrium (HWE) was tested in the control group and the results showed that the genotype distribution of each polymorphism of the *EGFR* gene, rs730437, rs3752651, and rs1468727, in the control group all conformed to HWE (P=0.135, 0.209, and 0.719, respectively) in the present study, indicating that this population was a representative group.

The genotype and allele distributions of *EGFR* gene polymorphisms in the patients with AD and the control group

The genotype and allele frequencies of *EGFR* gene polymorphisms in the patients with AD compared with the control

groups are shown in Table 3. The CC genotype and C allele frequencies of rs730437 were significantly lower in patients with AD compared with the controls (P=0.037), and compared with AA genotype and A allele, respectively, indicating that rs730437 was significantly associated with a reduced risk of AD (CC *vs.* AA: OR=0.446, 95% CI=0.207–0.960) (C *vs.* A: OR=0.702, 95% CI=0.502–0.980). The TT genotype and T allele frequencies of rs1468727 were also significantly different between the two groups (P=0.003), indicating that rs1468727 significantly reduced the susceptibility of AD in the studied population (TT *vs.* CC: OR=0.333, 95% CI=0.160–0.691) (T *vs.* C: OR=0.605, 95% CI=0.433–0.844). However, there was no significant association between the rs3752651 polymorphism and risk of AD (P>0.05).

The haplotype analysis of *EGFR* gene polymorphisms and the occurrence of AD

In the current study, the linkage disequilibrium of *EGFR* polymorphisms was also analyzed, and strong linkage disequilibrium was found. A total of three haplotypes were identified and analyzed for their effect on the risk of AD; other haplotypes were excluded due to their low frequency. The three haplotypes were A-T-T, A-C-C, and C-T-C, and their frequencies

Table 4. The haplotype analysis of *EGFR* polymorphisms in the occurrence of AD.

Haplotype Site1-site2-site3	Case, 2n=278/%	Control, 2n=304/%	OR (95%CI)	*P*
A-T-T	98/35.25	139/45.72	Ref.	–
A-C-C	42/15.11	31/10.20	1.922 (1.130, 3.269)	0.015
C-T-C	99/35.61	129/42.43	1.089 (0.753, 1.573)	0.651

Site1 – rs730437; site2 – rs3752651; site3 – rs1468727.

are shown in Table 4. The A-C-C haplotype had a significantly increased frequency in the group of patients with AD compared with the control group (15.11% *vs.* 10.20%) (P=0.015), compared with haplotype A-T-T (35.25% *vs.* 45.72%), indicating that the A-C-C haplotype was a risk factor for the development of AD (OR=1.922, 95% CI=1.130–3.269).

Discussion

Alzheimer's disease (AD) is a common and irreversible neurodegenerative disease that is characterized by progressive cognitive impairment, resulting in an increasing degree of stress and economic burden to patients and their families. The pathogenesis of AD is likely to involve the interaction between genetic and environmental factors, and these factors are recently becoming recognized. In 2016, Killin and colleagues undertook a systematic review of published studies on environmental factors and the risk of developing dementia, including AD, and found six environmental factors associated with the development of AD, including air quality, toxicity from heavy metals and other metals, lack of trace elements, occupational-related exposure to neurotoxins, and miscellaneous environmental factors [17]. Also, lifestyle and poor diet were identified as risk factors for AD [18]. Baumgaertel and colleagues showed that levels of education and the development of the hippocampus reduced the risk of developing AD [19]. Recently, genetic factors have also been discovered to be associated with AD, including expression of the *APOE*, *PSEN1*, *PSEN2*, and *APP* genes [20]. However, the identification of the specific environmental and genetic factors associated with the development of AD remains unclear. Therefore, the aim of this study was to investigate the association between polymorphisms of the epidermal growth factor receptor (EGFR) gene with the risk of AD in a Chinese Han population.

The *EGFR* gene encodes a transmembrane glycoprotein that is one of four members in HER/ErbB family which belongs to tyrosine kinases receptors and activates multiple signaling molecules and pathways that participate in a range of cellular processes, including cell proliferation, differentiation, survival, adhesion, and apoptosis [21]. Activated *EGFR* combines

with ligands to initiate intracellular signal transduction, and mitogen-activated protein kinase (MAPK), an important downstream pathway that has been shown to be involved in the pathogenesis of AD [22]. In 2016, Lee and colleagues showed that the activation or downregulation of *EGFR* signaling pathways was associated with several human diseases, including AD [23]. The expression of presenilin 1 (PS-1) has been shown to be related with early-onset AD, and has an influence on the expression and function of EGFR, which can regulate the development, survival and function of neurons in the brain, as PS-1-null mice have been shown to die of neuronal abnormalities at birth, indicating that PS1 and EGFR may have roles in the development and function of the brain [14].

Recently, studies on the role of single nucleotide polymorphisms (SNPs) have become an important means of exploring individual susceptibility to disease. Several SNPs have been previously reported to be associated with an increased risk AD, but few reports have focused on the effects of *EGFR* SNPs in AD. Also, several SNPs have been identified in the *EGFR* gene, including rs1050171, rs730437 and rs11506105, but they have not previously been studied in AD [24,25]. Rs730437 is a mutation in intron 4 of the *EGFR* gene with the alteration of A/C, which has been shown to be involved in the occurrence of glioma in several studies that have included different populations [24,25]. Rs3752651 is located in intron 13 of the *EGFR* gene and affects the development of multiple diseases, including glioma, colon cancer, and rectal cancer [25,26]. Rs1468727 occurs as a mutation in intron 13 of the *EGFR* gene as well, but is not likely to influence the function of *EGFR*, but takes part in disease initiation through its close linkage with other functional polymorphisms [16].

In the present study, the genetic association of *EGFR* rs730437, rs3752651, and rs1468727 polymorphisms were studied in association with the risk of AD in a Chinese Han population. Alcohol consumption was a risk factor for developing AD in this population, but a history of smoking and body mass index (BMI) were not. Diabetes, hypertension, and hypercholesterolemia were risk factors for AD when patients with AD were compared with healthy people. For polymorphisms in the *EGFR* gene, rs730437 was significantly associated with a decreased

risk of developing AD in both the mutant genotype and allele. Similarly, the mutant genotype or allele of rs1468727 carriers had a significantly lower risk of AD compared with individuals with the homozygous wild-type genotype and allele. However, the rs3752651 *EGFR* polymorphism was not a factor in the susceptibility to AD in the population in this study. Also, carriers of the A-C-C haplotype had an increased risk of AD when compared with carriers of haplotype A-T-T. This study is the first to show the genetic effects of *EGFR* gene polymorphisms on the development of AD development in a Chinese Han population.

In spite of the encouraging results, this study had several limitations. Firstly, the sample size was relatively small. Second, the single study population of Han Chinese could not show the reasons for any regional diversity of the occurrence of AD occurrence and the diversity of occurrence of the *EGFR* gene polymorphisms associated with AD. Third, an interaction analysis was not included in the present study, and the molecular mechanisms for the functional roles of the *EGFR* gene in the etiology of AD was not explored in this study. Of note, it has previously been reported that the regulatory sequences of the *EGFR* gene were located within the 5-flanking region and intron regions [27]. In this study, all of the selected SNPs were located in intron regions of the *EGFR* gene. However, whether these further gene polymorphisms could influence transcriptional activity of the *EGFR* gene or relevant signaling pathways on the etiology of AD remain poorly understood. Therefore, further controlled studies are required that have a more detailed molecular approach in their design, are on a larger scale, involve multiple centers and populations to verify the results of this study and to explore further the molecular mechanisms involved in AD.

Conclusions

In this study, in a population of Han Chinese, the rs730437 and rs1468727 polymorphisms of the *EGFR* gene were shown to be protective factors for Alzheimer's disease (AD). Carriers of the A-C-C haplotype had an increased risk of AD. Further large-scale, multicenter, controlled clinical studies are required to explore these preliminary findings.

Conflict of interest

None.

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