

Original Article

Association between *APOE* polymorphisms and glioma risk

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Received November 3, 2015; Accepted January 1, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Aims: This study aimed to investigate the association of apolipoprotein E (*APOE*) polymorphisms with the risk of glioma. Methods: Genotyping of *APOE* polymorphisms was performed on 132 patients with glioma and 130 healthy people with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The control group was matched with the cases by age and gender. The chi-square test was used to analyze the frequency of genotypes and alleles. The correlation between *APOE* polymorphisms and glioma was evaluated by odds ratios (OR) and 95% confidence intervals (CI). Results: The genotype frequencies of *APOE* polymorphism in the control group was in accordance with Hardy-Weinberg Equilibrium (HWE). Among *APOE* polymorphisms, E4/4 genotype had obviously higher frequency in cases than the control group and was associated with the increased risk of glioma (OR=2.598, 95% CI=1.039-6.493). Similarly, ϵ 4 allele might be also a risk factor (OR=1.679, 95% CI=1.103-2.554). However, genotype E3/3 and allele ϵ 3 played the protective role in the development of glioma (E3/3: OR=0.605, 95% CI=0.370-0.990; ϵ 3: OR=0.558, 95% CI=0.386-0.807). Conclusions: Allele ϵ 4 in *APOE* polymorphisms might be a risk factor for the occurrence of glioma, but ϵ 3 was a protective factor to be against glioma in Chinese study population.

Keywords: Apolipoprotein E (*APOE*), polymorphism, glioma

Introduction

Glioma is a type tumor occurring in the brain or spine and the incidence is increasing every year around the world [1, 2]. In terms of the pathogenesis of tumors, there is no essential difference between glioma and the tumors in other parts of the body [3]. According to China Health Statistics Yearbook, in recent years, the incidence rate of glioma is about five to ten Chinese people per hundred thousand, showing a steady growth every year [4]. So far, a large number of scholars have devoted to the field of glioma and required several achievements. Studies show that DHPM-fatty acids and caudatin may be as the new antitumor drugs for against glioma cell growth [5, 6]. In the other hand, several reports have explored the effect of gene polymorphism on glioma, such as *NOTCH3*, *RTEL1*, *XRCC1*, *BCL2* [7-10]. However, the exact pathogenesis of glioma is still not clear.

Apolipoprotein E (*APOE*) regulates the lipoprotein metabolism of the plasma and is newly reported to play a far more significant role in nervous system. As one of the main apolipoproteins in plasma, *APOE* consists of 299 single-chain polypeptides formed by amino acids [11, 12]. *APOE* is mainly synthesized by liver cells, next is brain tissue cells, and it derives from glial cells. Human *APOE* gene is located on the long arm of chromosome 19 (19 q13.2) and consists of four exons and three introns including multiple single nucleotide polymorphisms. Among of them, three common alleles ϵ 2, ϵ 3 and ϵ 4 compile three major isomers: E2, E3 and E4 respectively, which produces six different phenotypes, namely three homozygotes E2/2, E3/3, E4/4 and three heterozygotes E2/3, E2/4, E3/4 [13, 14]. *APOE* polymorphisms refer to interchanges between cystine (Cys) and arginine (Arg) at positions 112 and 158, and E2 represents Cys residue on these two polymorphisms yet Arg residue on the 158th locus [15, 16]. What's more, some

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Table 1. Primer sequences of APOE

SNP	Primer	Primer sequence	Length
APOE exon 4 containing sequences encoding residues 112 and 158	Forward	5'-ACAGAATTCGCCCGGCCTGGTACAC-3'	295 bp
	Reverse	5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'	

reports indicated that APOE had high expressions in tumor tissues such as glioma and meningioma.

Therefore, we explored the preliminary relationship between APOE polymorphisms and glioma susceptibility through 132 patients with glioma and 130 healthy controls.

Materials and methods

Clinical data

A case-control design in present study included 132 cases (72 males and 60 females) aged from 16 to 76 years with an average age of 46.5 ± 16.3 . They were pathologically diagnosed with glioma and divided into two groups according to pathological grade, 79 cases in grades I-II and 53 cases in grades III-IV. The patients did not undergo radiotherapy and chemotherapy before sample collection. 130 healthy controls frequency-matched with cases by sex and age, including 70 males and 60 females, were aged from 18 to 80 with a median age of 44.4 ± 10.8 . All controls were recruited from the physical examinations center of hospital without family history of tumors or other nervous system diseases. All the subjects were unrelated by blood from Han population. The samples were collected according to the national ethics guidelines for human genome research. Our study obtained the approval from the Ethics Committee of The Second Hospital of Hebei Medical University and written informed consent from each participant.

DNA extraction

5 ml fasting venous blood from every subject was collected and conducted anticoagulation with EDTA-Na₂ and reserved at -80°C for detecting after plasmapheresis. Leucocyte genome DNA was extracted with Nal method for the genotyping of APOE polymorphisms.

The genotypes detection of APOE polymorphisms

APOE polymorphisms genotypes were tested by polymerase chain reaction-restriction frag-

ment length polymorphism (PCR-RFLP) method. The PCR primers were designed by Primer 5.0 program and synthesized by Shanghai Sangon Biotechnology Co., LTD. The primer sequences are shown in **Table 1**. The total reaction volume was 30 μl solution, including 1 μl genomic DNA, 200 $\mu\text{mol/L}$ dNTP, 30 pmol/L primers, 1 U Taq enzyme, 10% dimethyl alum and 1.5 mmol/L MgCl_2 . The PCR conditions were: 97°C initial denaturation for 5 min; followed by 30 cycles of 95°C degeneration for 40 s, 65°C annealing for 30s and 72°C extension for 30 s; and finally 72°C extension for 5 min.

The PCR products were tested by 2% agarose gel electrophoresis, and digested with 5 U restrictive enzymes *HhaI* at 37°C for 4 h, the PCR amplified products were observed under ultraviolet lamp after the separation of enzyme-digested products by electrophoresis in 12% polyacrylamide gel and ethidium bromide staining. The genotyping of APOE polymorphisms product, a length of 299 bp formed six combinations of various fragments and represent the following six genotypes: E2/E2 with 91 bp, 83 bp and 61 bp; E2/E3 with 91 bp, 83 bp, 61 bp and 48 bp; E2/E4 with 91 bp, 83 bp, 72 bp, 61 bp and 48 bp; E3/E3 with 91 bp, 61 bp and 48 bp; E3/E4 with 91 bp, 72 bp, 61 bp and 48 bp; E4/E4 with 72 bp, 61 bp and 48 bp.

Statistical methods

Results were expressed by mean \pm standard deviation ($\bar{x} \pm s$) or rate (%). SPSS 18.0 software was used for statistical analysis and t test for the comparison of the differences in age between the two groups. The χ^2 test was used to analyze the genotype and allele distributions in two groups and detect whether the genotype distribution of the controls deviated from Hardy-Weinberg Equilibrium (HWE). The odds ratio (OR) and 95% confidence interval (95% CI) were calculated to assess the relevance strength of APOE polymorphisms and glioma risk. $P < 0.05$ was considered as the statistical significance.

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Table 2. General data of the study objects

Characteristic	Cases n=132 (%)	Controls n=130 (%)	χ^2	P
Age (y)			0.051	0.863
<29	19 (14.39)	20 (15.38)		
30-49	45 (34.09)	38 (29.23)		
>50	68 (51.52)	72 (55.39)		
Gender			0.013	1.000
Male	72 (54.55)	70 (53.85)		
Female	60 (45.45)	60 (46.15)		
Smoking			2.257	0.159
No	89 (67.42)	76 (58.46)		
Yes	43 (32.58)	54 (41.54)		

Table 3. Genotype and allele distribution of APOE in the case and control groups

Genotype/ Allele	Cases n=132 (%)	Controls n=130 (%)	χ^2	P	OR (95% CI)
APOE					
E2/2	1 (0.76)	0 (0)	0.989	0.320	0.992 (0.978-1.007)
E2/3	12 (9.09)	10 (7.69)	0.167	0.683	1.200 (0.499-2.883)
E2/4	21 (15.91)	14 (10.77)	1.495	0.221	1.568 (0.760-3.235)
E3/3	66 (50.00)	81 (62.31)	4.029	0.045	0.605 (0.370-0.990)
E3/4	15 (11.36)	18 (13.85)	0.367	0.545	0.798 (0.383-1.660)
E4/4	17 (12.88)	7 (5.38)	4.421	0.036	2.598 (1.039-6.493)
ϵ 2	35 (13.26)	24 (9.23)	2.126	0.145	1.503 (0.867-2.606)
ϵ 3	159 (60.23)	190 (73.08)	9.724	0.002	0.558 (0.386-0.807)
ϵ 4	70 (26.51)	46 (17.69)	5.916	0.015	1.679 (1.103-2.554)

Results

General characteristics of the objects

This study totally had 132 cases of glioma and 132 healthy controls. The distributions of age, gender, and smoking status for the two groups were shown in **Table 2**. The results indicated that there was no significant difference between two groups ($P>0.05$). Two groups were demographically representative ($P>0.05$).

Distribution of APOE genotypes and alleles in the case and control groups

As shown in **Table 3**, E4/4 genotype frequency of APOE polymorphisms in cases was significantly higher than the control group (12.88% and 5.38%) and the comparative result showed in **Table 3** that it was associated with the remarkably increased susceptibility to glioma (OR=2.598, 95% CI=1.039-6.493) and ϵ 4 allele might be also an independent factor associat-

ed with the increased risk of glioma (OR=1.679, 95% CI=1.103-2.554).

In contrary, genotype E3/3 had an significantly lower frequency in the case group compared with the controls (50.00% and 62.31%) and it could decreased the risk of glioma (E3/3: OR=0.605, 95% CI=0.370-0.990). What's more, allele ϵ 3 played a protective role for people to be against the occurrence of glioma (OR=0.558, 95% CI=0.386-0.807). However, there no relevance existed in the other genotypes of APOE polymorphisms independently.

Discussion

Glioma, one of the most common primary tumors in central nervous system, accounts for about 45%-60% of intracranial tumors in adults. Because of its special pathological nature and infiltrative growth, glioma

is really hard to be cured by surgery, chemotherapy and radiotherapy [17]. The two-year survival rate after surgery is generally about 52.9%, but the five-year survival rate is only 5.5% [18, 19]. Maybe exploring the relative genes polymorphisms to find the population carried susceptibility genotype is a good choice, which can diagnose early and timely treat glioma. So far, the effects of DNA repair genes polymorphisms on glioma risk are paid more attention by researchers. Cui et al. conducts a meta-analysis to explore the association of ERCC1 and ERCC2 polymorphisms with glioma in Chinese and Caucasian populations, the results suggest that they both are the important risk factors for glioma development in Chinese population, but not in Caucasian population [20]. A study based on Xu et al. showed that XRCC1 polymorphism may a genetic risk factor for the development of glioma, especially in Asian population [21]. Even so, it is not enough to uncover the pathogenesis of glioma,

what's more, the major genes influenced glioma occurrence are still not verified.

Human *APOE* is primarily synthesized by liver cells and brain tissues, and other synthesizing tissues include mononuclear cells (including macrophages), adrenal glands and ovarian granulosa cells [22]. The expression level of *APOE* mRNA in brain is one third of that in liver, and astrocytes are the main synthesizing sites. Generated *APOE* may play roles in redistributing intracellular lipid to balance the cholesterol in brain. *APOE* has been found to have high concentration in brain tumors, which infers that it may be a sign of brain glioma [23, 24]. In addition, of the cerebrospinal fluid, *APOE* accounts for more than a half and influences lipids distribution and tissue reconstruction in nervous system through mediating receptor-dependent pathways. It is also the most important apolipoprotein in nervous system [25]. Study has found that *APOE* expression is higher in glioma patients [26]. But the report about the effect of *APOE* polymorphisms on glioma development is rare nowadays.

Therefore, in present study, we evaluate the relevance between *APOE* polymorphisms and glioma susceptibility in a case-control design. Among all genotypes and alleles, E4/4 genotype had an obviously high frequencies in cases compared with controls and was associated with the significantly increased susceptibility to glioma. Parallel result was found in *APOE* ε4 allele that it may be also a negative factor for glioma. Unlike with ε4, allele ε3 is a protective factor for against the occurrence of glioma, furthermore, homozygous genotype E3/3 also plays the protective role for carriers to resist glioma. The other genotypes or alleles may effect glioma development through the interaction of gene-environment, gene-gene or the different polymorphisms from one gene.

In a word, as a potential tumor marker, *APOE* gene polymorphism may have great influence on the diagnosis and prognosis of tumors. The present study only covered a small range of samples limited to Chinese Han population; therefore it is necessary to carry out in-depth studies with large-scale sample in multiple races to verify the results of this study so as to provide a new way for predicting, preventing and treating glioma.

Disclosure of conflict of interest

None.

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