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Original Article

Angiotensin-converting enzyme gene polymorphisms and nonarteritic anterior ischemic optic neuropathy risk

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Abstract: Aims: This study was aimed to investigate the association between angiotensin-converting enzyme gene (ACE) polymorphisms (rs4291, rs4309, rs4343, rs4646994) and nonarteritic anterior ischemic optic neuropathy (NAION) risk. Methods: The method of polymerase chain reaction (PCR) was used to perform the genotyping of *ACE* polymorphisms between the case and control groups. The χ^2 test was conduct to detect Hardy-Weinberg equilibrium (HWE) as well as the distribution differences of genotypes, allele, haplotypes in *ACE* polymorphisms between two groups. The linkage disequilibrium (LD) and haplotype were analyzed to identify the combined function of *ACE* polymorphisms. Odds ratio (OR) with 95% confidence interval (95% CI) was calculated to assess the association strength. Results: In four polymorphisms of ACE gene, the distributions of not only genotyes but alleles in rs4309 had the significant differences between the case and control groups (*P*=0.01, 0, respectively). Similarly, rs4646994, a common insertion/deletion mutation in *ACE*, the genotypes and alleles also showed the obvious frequency differences between two study groups (*P*=0.04, 0.01). There was the LD among rs4291, rs4309, rs4343 and A-C-A, T-C-G haplotypes significantly increased the susceptibility of individuals to NAION (OR=1.85, 95% CI=1.05-3.26; OR=4.09, 95% CI=1.83-9.11). Conclusion: ACE rs4309, rs4646994 polymorphisms may be associated with the occurrence of NAION in Chinese Han population and A-C-A, T-C-G haplotypes are also the risk factors for NAION.

Keywords: *ACE*, polymorphisms, nonarteritic anterior ischemic optic neuropathy, haplotype

Introduction

Nonarteritic anterior ischemic optic neuropathy (NAION) is a common acute optic neuropathy in clinic caused by optic nerve head ischemia over the age of 50 [1]. It is characterized by sudden, painless visual loss, monocular and optic disc edema [2]. Its incidence rate is 2-10/100000 annually and the sequelae of most patients are the permanent decrease of visual acuity and even visual field loss [3]. NAION is a multifactorial disease with complex pathogenetic mechanism, meanwhile, it places burdens and stresses on patients and their family in life and economy. Although some risk factors have been presented, such as hypertension, diabetes mellitus, cardiovascular and cerebrovascular diseases, and smoking [4-7], the etiology of NAION still remains poor clearness. Recently, researchers find the significance of genetic factors, particularly single nucleotide polymorphism (SNP) [8, 9].

Angiotensin-converting enzyme (ACE) is one of the key enzymes in renin-angiotensin system (RAS) which is essential to regulate the blood pressure fluid homeostasis [10]. This enzyme is encoded by *ACE* located on chromosome 17q23 [11] and translates angiotensin I into angiotensin II, inactivates bradykinin, modulates vascular tension and affects the susceptibility to cardiovascular disease [12-14]. ACE gene includes multiple SNPs, the insertion/deletion mutation (rs4646994) is a common SNP and is studied in various diseases, such as migraine, cancers, diabetic peripheral neuropathy and even many inflammatory diseases [15-17]. In addition, *ACE* rs4291, rs4309, rs4343 polymorphisms are also focused widely in a variety of diseases, because they are associated with the expression of *ACE* gene. However, the publications about the role of *ACE* SNPs in NAION development are poor, most of studies place the focus of attention on cardiovascular diseases, cancers and inflammatory diseases.

Table 1. Sequences of *ACE* polymorphisms PCR primers

SNP	PCR primer sequences		Length
rs4291	Forward	5'CCCCGGCCTTGTCACTCC3'	184 bp
	Reverse	5'GAAGCTGGAGAAAGGGCCTCCT3'	
rs4309	Forward	5'ATGGACCAGCTCTCCACAGTGC3'	206 bp
	Reverse	5'CCCATACCCGTGTCATTGGTGA3'	
rs4343	Forward	5'CCCCTACCAGATCTGACGAATGTG3'	235 bp
	Reverse	5'CCTAGGCTTGGGGTTTCACAGC3'	
rs4646994	Forward	5'CTGGAGACCACTCCCATCCTTTCT3'	191 bp
	Reverse	5'GATGTGGCCATCACATTCGTCAGAT3'	

Note: SNP: single nucleotide polymorphism; PCR: Polymerase chain reaction.

Therefore, in this study, we genotyped the common four polymoprhisms of ACE gene (rs4291, rs4309, rs4343 and rs4646994) in 96 patients with NAION and 102 healthy persons to explore the effect on the onset of NAION. We hoped to look for the susceptible SNP in ACE of NAION and then provide some evidence for explaining the pathology of this disease through single loci and the interaction studies.

Materials and methods

The selection of study population

In this study, we designed a case-control population, including 96 cases and 102 controls. The patients with NAION in the case group were diagnosed by Liaocheng People's Hospital during May, 2012 and December, 2013, consisting of 59 males and 37 females. The age range was 37-79 years old with the mean age of 62.46±10.62. The control group was healthy people from the Center of Health Examination in the above mentioned hospital at the same period with the cases. The number of males and females in controls was 57, 45 respectively. Their age was from 32 to 76 years old with an average age of 61.89±11.23. In principle, the frequency was matched between the case and control group by age and gender. All subjects were Chinese Han population without any relationship by bloods each other. The design process was confirmed and supported by the Ethics Committee of Liaocheng People's Hospital. At last but not least, the subjects and their family were informed the whole study process and written consents were obtained from every subject.

Sample collecting and DNA extraction

We collected 2 ml fasting venous blood from every subject after acquiring written consent and put it into specified blood collection tube with EDTA-2Na anticoagulation. The DNA sample was extracted using blood genomic DNA extraction Kit from TIANGEN BIOTECH (BEIJING) CO., LTD and then stored at -20°C.

Genotyping

In present study, polymerase chain reaction (PCR) was used to identify the genotypes of *ACE* polymorphisms in every subject. The PCR primer was designed and synthesized by Shanghai Sangon Biotech Co., Ltd and the detailed sequences information was listed in **Table 1**. PCR was conducted in a volume of 25 μl mixture, consisting of 1.0 μl DNA template, each 1.0 μl of forward and reverse primers, 12.5 μl PCR Mix and 9.5 μl ddH_2O. PCR program for rs4291, rs4309, rs4343 was as follows: firstly initial denaturation at 95°C for 3 min, and then 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 45 s, finally extension at 72°C for 7 min. The program of PCR for rs4646994 was initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, finally extension at 72°C for 2 min. The PCR products were detected the quality and concentration by 1.0% agarose gel electrophoresis (AGE) and NanoDrop 2000 NanoVue Plus. And then 10 μl PCR products were respectively sequenced in Shanghai Sangon Biotech Co., Ltd to identify the genotypes of *ACE* polymorphisms in participants.

Data analysis

All data were represented by $\bar{x}\pm s$ or %. The chi-square test was used to detect whether the genotypes distributions of *ACE* polymorphisms in the control group conformed to Hardy-Weinberg equilibrium (HWE) and compare the frequency differences of genotypes, alleles, haplotypes and even others indexes between the case and control groups. The relationship intensity between gene polymorphism and disease was evaluated by odds ratio (OR) with 95% confidence interval (95% CI). The above data analyses were conducted by SPSS 18.0 software and $P<0.05$ represented a significant difference. In addition, in our study, the linkage disequilibrium (LD) was also analyzed.

Table 2. Association analysis of *ACE* polymorphisms and NAION risk based on genotype and allele

SNP	Genotype				Allele				P_{HWE}
		Case (%)	Control (%)	*P*		Case (%)	Control (%)	*P*	
rs4291	AA	37 (38.54)	49 (48.04)	0.30	A	124 (64.58)	140 (68.63)	0.39	0.66
	TA	50 (52.08)	42 (41.18)		T	68 (35.42)	64 (31.37)		
	TT	9 (9.38)	11 (10.78)						
rs4309	TT	37 (38.54)	61 (59.80)	0.01	T	125 (65.10)	160 (78.43)	0	0.31
	TC	51 (53.13)	38 (37.26)		C	67 (34.90)	44 (21.57)		
	CC	8 (8.33)	3 (2.94)						
rs4343	AA	41 (42.71)	53 (51.96)	0.26	A	125 (65.10)	148 (72.55)	0.11	0.73
	AG	43 (44.79)	42 (41.18)		G	67 (34.90)	56 (27.45)		
	GG	12 (12.50)	7 (6.86)						
rs4646994	DD	34 (35.42)	54 (52.94)	0.04	D	111 (57.81)	144 (70.59)	0.01	0.13
	ID	43 (44.79)	36 (35.29)		I	81 (42.19)	60 (29.41)		
	II	19 (19.79)	12 (11.77)						

Note: HWE: Hardy-Weinberg equilibrium.

Table 3. Haplotype analysis among *ACE* polymorphisms in NAION

Haplotype rs4291-rs4309-rs4343	Case/control (n)	OR (95% CI)	*P*
A-T-A	86/113	1.00 (Ref.)	-
A-C-A	38/27	1.85 (1.05-3.26)	0.03
T-T-G	39/47	1.09 (0.66-1.81)	0.74
T-C-G	28/9	4.09 (1.83-9.11)	0
T-C-A	1/8	0.16 (0.02-1.34)	0.06

Results

HWE rest

As was shown in **Table 2**, the genotypes distributions of four polymorphisms in *ACE* were all found to be consistent with HWE in the control group (*P*>0.05), this results indicated that our study population conformed to the standard of group selection and represented a eligible Mendelian population.

The association of ACE polymorphisms with optic neuropathy risk in two study groups

In present study, four polymorphisms of *ACE* gene were selected to explore the association with NAION susceptibility, namely rs4291, rs4309, rs4343 and rs4646994; the relevant results were showed in **Table 2**. However, the genotype distributions of rs4291 and rs4343 were no significant difference between case and control groups (*P*>0.05) and allele frequency differences between two groups were also not significant (*P*>0.05). Referring to rs4309, the frequencies of genotype TT, TC, CC in cases and controls were 38.54%, 53.13%, 8.33% and 59.80%, 37.26%, 2.94%, respectively. CC genotype frequency was obviously higher in case group than that of in control group and TT genotype distribution was reverse (*P*=0.01). What's more, the allele distribution also had a significant difference (*P*=0). Similarly, rs4646994 also showed the association with the onset of NAION. The genotype frequencies (DD, DI, II) were 35.42%, 44.79%, 19.79% in cases and 52.94%, 35.29%, 11.77% in controls and the genotype distribution was obviously different between two groups (*P*=0.04). So was allele distribution (*P*=0.01).

The LD among ACE polymorphisms and haplotype analysis

The LD of *ACE* polymorphisms was analyzed in our study. We found that there was the strong LD among rs4291, rs4309, rs4343 and five haplotypes were detected in our population. The detailed information of these five haplotypes was listed in **Table 3**, including name, number, and role. Haplotypes A-C-A and T-C-G of them significantly increased the susceptibility to NAION, compared with haplotype A-T-A (OR=1.85, 95% CI=1.05-3.26; OR=4.09, 95% CI=1.83-9.11) and they might be the risk factors for NAION occurrence.

Discussion

NAION is a leading cause of acute vision loss in middle-aged people, which is caused by the hypoperfusion or obstruction of optic nerve head resulted from the blood insufficiency of short posterior ciliary arteries in sieve plate district and postzone [18]. In previous studies, the mechanism of NAION includes low blood pressure at night, microcirculation autonomic adjustment disorder and the branch of center vein blocking in retina. They can reduce the blood flow of optic nerve, cause the edema of optic nerve axon, lead to compartment syndrome in optic nerve head, the degeneration of axon and the apoptosis of retinal ganglion [19]. As a multifactorial disease, the onset of NAION is influenced by a variety of high risk factors, such as systematic vessel risk factors, blood flow degeneration and some environmental factors. Among them, gene susceptibility has been proved to be a vital factor for NAION development.

Markoula et al. explore the roles of renin-angiotensin-aldosterone system (RAAS) genes polymorphisms in NAION occurrence, including *ACE* I/D mutation, the angiotensinogen gene M235T polymorphism, angiotensin II type 1 receptor gene A1166C polymorphism, the final results are that the polymorphisms of RAAS genes involved in systematic circulation may be associated with NAION development [20]. Salomon et al. find that a specific platelet polymorphism of variable number of tandem repeats (VNTRs) in glycoprotein Ib alpha gene is associated with NAION occurrence and may be an independent risk factor, meanwhile, the carriage of this risk factor in NAION patients is easier to suffer from second eye involvement [21]. However, the reports which the associations of gene polymorphisms with NAION risk are few so far.

In present study, four polymoprhisms of *ACE* gene were selected to analyze the association with the risk of NAION occurrence. On the one hand, the relationship of the single polymorphism loci and this disease was respectively analyzed. In these polymorphisms, rs4291 and rs4343 were found that there was no dramatically correlation with the generation of NAION. Differently, rs4343 had the significant distribution differences between the case and control groups in genotype and allele, which meant that it may be involve in NAION development. What's more, the insertion/deletion mutation (rs4646994) was also detected a significant correlation with this disease occurrence. But in study of Salomon et al., *ACE*I/D polymorphism doesn't participate in the mechanism of NAION and ACE inhibitors drugs has no significant effect for patients with this disease [22]. The insistent results may derive from the distribution difference of SNP in different races and the complexity of NAION. On the other hand, it was found that there was the strong LD among rs4291, rs4309, rs4343 and A-C-A, T-C-G haplotypes had the high frequencies in cases, compared with that of in controls in our population and they were also the susceptibility factors to NAION.

Renin-angiotensin system (RAS) is an important system to regulate blood vessel and water-electrolyte. RAS has been proved to involve in various diseases, especially hypertension, diabetes mellitus, cardiovascular diseases and inflammatory diseases [23-25] and these diseases were all the risk factors of NAION. ACE is a key catalyzing enzyme in RAS and distributes in circulatory system and some tissues. It can catalyze the process from angiotensin I to angiotensin II and promote water and sodium retention. In the meanwhile, as kininase II, ACE facilitates the digestion of bradykinin and inhibits the vasodilatation caused by bradykinin. In addition, ACE transforms angiotensin 1-7 into activated angiotensin 1-5, so we can inhibit the synthesis of angiotensin II and the digestion of bradykinin and angiotensin 1-7 through controlling ACE to reduce blood press and dilate blood vessel.

All in all, rs4309, rs4646994 polymorphisms in ACE are correlated to the generation of NAION, but rs4291 and rs4343 are not found the similar roles in this disease. The strong LD is also detected in rs4291, rs4309, rs4343 and two haplotypes A-C-A, T-C-G may be the risk factors for the onset of NAION. However, due to the race specificity of NAION [26], the distribution difference of ACE polymorphisms and small sample size, the results are controversial. Therefore, in the future, further researches with well-design and large samples are done to certify the accurate role of ACE polymorphisms in NAION.

Disclosure of conflict of interest

None.

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