Original Article
Correlation of *ABCA4* polymorphisms with age-related macular degeneration

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Abstract: Aims: This research aimed to explore the correlation of ATP-binding cassette, sub-family A, member 4 (*ABCA4*) gene polymorphisms (rs560426 and 6389T>A) with the occurrence of age-related macular degeneration (AMD). Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the genotypes of rs560426 and 6389T>A polymorphisms in 110 AMD patients and 125 healthy controls. Cases and controls were matched with each other by age and gender. Genotypic frequencies between cases and controls were compared by chi-square test. Relative risk of AMD was expressed by odds ratios (ORs) and 95% confidence intervals (CIs). Results: Variant homozygote and variant allele of rs560426 were frequently detected in cases. But the difference had no statistical significance ($P > 0.05$), indicating no significant association existed between rs560426 polymorphism and AMD risk. Similar results were observed in TA and AA genotypes of 6389T>A polymorphism ($P > 0.05$). However, significant correlation existed between 6389A allele and AMD susceptibility ($P = 0.016$, OR = 1.677, 95% CI = 1.098-2.564). Conclusion: *ABCA4* gene rs560426 polymorphism had no obvious association with the occurrence of AMD, 6389A allele might associate with the onset of AMD.

Keywords: ATP-binding cassette, sub-family A, member 4 (*ABCA4*) gene, age-related macular degeneration (AMD), polymorphisms

Introduction

Age-related macular degeneration (AMD), also called senile macular degeneration or aging macular degeneration, is an elderly eye disease caused by chronic degenerative disease in fundus macular. This disease will lead to loss of central vision. Main features of AMD is the decline of ingest ability of retinal pigment epitheliums for rod outer segments. This decline results in the retention of membrane disk residues in basal cell protoplasm and deposits in bruch membrane by line, and finally causing pachynsis of bruch membrane and formation of drusen. Owing to the particularities of macular in structures and functions, this kind of change is the most obvious one. AMD is the main reason of blindness in populations over the age of 55 from western developed countries [1]. Some studies have found that the incidence and prevalence of AMD are obviously different in different races [2-10]. In China, the prevalence of

AMD is relatively lower. However, with the rising of aging populations, there is an upward trend in the AMD incidence. It has become one of the major blinding eye diseases, which greatly diminishes the quality of life of the elderly. So it was necessary to explore the pathogenesis of AMD, which will provide a theoretical foundation for the therapy of AMD.

ATP-binding cassette (ABC), sub-family A, member 4 (ABCA4, also called ABCR), is a retinal-specific member of the ABC family. ABCA4 take part in the functions of photoreceptor outer segments as a flipase of all-trans retinal. Allikmets and his colleagues proved that the distemperedness of this gene can lead to macular disorders [11, 12]. Other research found that they are the same ABC transporter through comparing *ABCA4* with the sequences of radiation protein RmP (a kind of membrane protein with high molecular weight in photoreceptor) [13]. It's been turned out that the mutation of

Table 1. Primer sequences of *ABCA4* gene rs560426 and 6389T>A polymorphisms

Locus	Primer sequence	
Rs560426	Forward	5'-CTCCTGTGGGAGAATCTTGC-3'
	Reverse	5'-TTACTCACAGCCAGGTGCAG-3'
6389T>A	Forward	5'-CATCCCACAGGCAAGAGATT-3'
	Reverse	5'-GCAGCAGGACTCTTCCAAGT-3'

Table 2. Subject features

		Cases n = 110	Control n = 125	P
Age (mean ± SD)		60±5.4	61.5±4.8	> 0.05
Gender	Male	65	71	> 0.05
	Female	45	54	
Family history	Yes	87	98	> 0.05
	No	23	25	

ABCA4 may bring about the occurrences of a series of retinal diseases, such as AMD, retinitis pigmentosa, and rod-cone dystrophy [14].

Variations in the *ABCA4* gene have been confirmed to be related to Stargardt disease (STGD) [15, 16]. STGD is the most common form of hereditary recessive retinal disease and is characterized by central visual impairment, perimacualr yellow deposits, and atrophy of the retinal pigment epithelium [17]. The similarities between STGD and AMD have led to increased investigations of whether variants in *ABCA4* cause not only STGD but also AMD [18-22]. However the association of *ABCA4* gene polymorphisms with AMD occurrence was still unclear.

So we carried out this study to discuss the correlation between *ABCA4* gene rs560426 and 6389T>A polymorphisms and the onset of AMD in unrelated Han Chinese.

Materials and methods

Cases and controls

All research objects in this study were unrelated Han population from southern China. They were all involved in this study with informed consents. Later, specially trained investigators recorded relevant information and gathered blood samples. This study was authorized by the Ethics Committee of The First Affiliated Hospital of Harbin Medical University. Further-

more, the process of sample collection was conducted on the provisions of Helsinki declaration.

110 AMD patients who were diagnosed in treatment in The First Affiliated Hospital of Harbin Medical University from March, 2013 to March, 2015 were enrolled into the case group. Case group consisted of 65 males and 45 females with the age range from 51 to 69 (mean = 60±5.4). AMD patients were diagnosed by the same ophthalmologist following the results of contact lens slit-lamp examination, optical coherence tomography (OCT) test, fundus photography and fund us fluorescence angiography inspection. Clinical datum of the cases, including their sex, age, disease symptoms, sign, and family history were detailed collected, recorded and input into Excel forms.

125 healthy individuals, 71 males and 54 females, with age range from 53 to 70 years old (mean = 61.5±4.8) were recruited as healthy controls from physical examination center of The First Affiliated Hospital of Harbin Medical University during the same period. All of the controls had no blood diseases, renal insufficiency, diabetes, tumors and other eye diseases. Controls were matched with the cases in terms of age and gender.

DNA extraction

5 ml peripheral blood was collected from every participant and anti-coagulated by EDTA. Genomic DNA was extracted and purified by a GenElute™ Blood Genomic DNA Kit (Sigma, Germany) and stored in -20°C refrigerator for standby application.

Polymerase chain reaction (PCR) reaction system and amplification process

According to *ABCA4* gene sequences, PCR primers of rs560426 and 6389T>A polymorphisms were designed by Primer Premier 5.0, and synthesis by Sangon Biotech (Shanghai, China). Primer sequences were listed in **Table 1**.

PCR reaction system was carried out in 50 μl total volume, containing 5 μl DNA templates, 4 μl dNTPs, 1 μl forward primer, 1 μl reward primer, 1 μl TaqDNA polymerase, 10 μl 10× buffer by sterile water completed. Amplification pro-

Table 3. Genotype and allele distribution comparison of *ABCA4* gene rs560426 and 6389T>A polymorphisms between case and control groups

Genotype/ Allele	Case n = 110 (%)	Control n = 125 (%)	χ^2	*P*	OR (95% CI)
Rs560426					
AA	27 (24.55)	36 (28.80)	-	-	1
AG	41 (37.27)	57 (45.60)	0.016	0.898	0.959 (0.506-1.820)
GG	42 (38.18)	32 (25.60)	2.630	0.105	1.750 (0.888-3.449)
A	95 (43.18)	129 (51.60)	-	-	1
G	125 (56.82)	121 (48.40)	3.324	0.068	1.403 (0.975-2.019)
6389T>A					
TT	59 (53.64)	83 (66.40)	-	-	1
TA	37 (33.64)	34 (27.20)	2.133	0.144	1.531 (0.863-2.715)
AA	14 (12.73)	8 (6.40)	3.762	0.052	2.462 (0.971-6.243)
T	155 (70.45)	200 (80.00)	-	-	1
A	65 (29.55)	50 (20.00)	5.769	0.016	1.677 (1.098-2.564)

Table 2), which stated good matched-degree of selected study objects.

Distributions of ABCA4 polymorphisms in case and control groups

Genotype and allele distributions of *ABCA4* gene rs560426 and 6389T>A polymorphisms were displayed in **Table 3**. Genotype distributions of *ABCA4* gene rs560426 and 6389T>A polymorphisms were not diverged from HWE in control group (*P* > 0.05).

cess was as follows: 94°C predegeneration for 5 min, and then followed by 30 cycles of 94°C degeneration for 45 sec, 57°C annealing for 45 sec, 72°C extension for 75 sec, and 72°C finally extension 10 min. PCR products were digested by restriction endonuclease, and then the digested products were tested by 3% agarose gel electrophoresis (AGE).

Statistical analysis

Representativeness of the cases and controls were evaluated by Hardy-Weinberg equilibrium (HWE). Clinical characteristics between cases and controls were compared by t test or Chi-square test. Direct counting was used to calculate the genotype and allele frequencies. χ^2 test was used to compare the distribution differences of genotype and allele between case and control groups. Statistical analysis was accomplished through PASW statistics 18.0 statistical software. Moreover, the relative association of AMD with the *ABCA4* gene polymorphisms were evaluated by odds ratios (ORs) and 95% confidence intervals (CIs). *P* < 0.05 stood for statistically significant difference.

Results

Clinical characteristics of case and control groups

All the clinical characteristics such as age, gender, and family history between two groups had no statistically significant differences (*P* > 0.05,

GG, GA, AA genotype frequencies of rs560426 polymorphism were 24.55%, 37.27%, 38.18% in the case group and 28.80%, 45.60%, 25.60% in the control group, respectively. The variant GG genotype and G allele frequencies were highly in cases than that in controls, but they had no significant association with the risk of AMD (*P* > 0.05). It also turned out that 6389TA and 6389AA genotypes were frequently observed in case group (33.64% for TA and 12.73% for AA) than control group (27.20% for TA and 6.40% for AA). The data showed that no significant association between these genotypes and AMD occurrence. However, the variant 6389A allele was significantly associated with the onset of AMD (*P* = 0.016, OR = 1.677, 95% CI = 1.098-2.564).

Discussion

AMD is considered to be a complex lesion involving retinal photoreceptor, retinal pigment epithelium layer bruch membrane, and multi-organizations of choroid lining blood vessel [23, 24]. AMD present as a series of pathological process in clinic. In addition, degenerative changes in the macular area and neovascularization can finally bring about patients with a loss of central vision. In developed countries, AMD is the most common cause of irreversible visual impairment in people over the age of 50, and even results in a loss of vision in the center of the visual field because of damage to the retina. AMD is the leading cause of being unable to heal the visual impairment for the

elder [2, 25, 26]. In the early stage of AMD, patients feel no self-conscious symptom. When it was diagnosed, it might at an advanced stage. AMD can make it difficult or impossible to read, although enough peripheral vision remains to allow other activities of daily life. That will cause inconvenience to the daily life of patients. At present, with the aging of the population, the morbidity of AMD is increasing year by year. Therefore, the exploration of the AMD etiology is necessary. That will furnish theoretical foundation for the diagnosis and therapy methods of AMD. Epidemiological studies have displayed that the risk factors associated with AMD consist of age, family history, smoking, the white race, shallow iris, ultraviolet radiation, diet, and nutrition states [27-30]. Among these risk factors, genetic factor is the crucial factor for the onset of AMD.

Multiple researches indicate that *ABCA4* gene play a potential role in the development of STGD [15, 16]. STGD has many similarities with AMD, both of them are the diseases in macular region. ABCA4 belongs to the super family of ABC transporter proteins. *ABCA4* is first cloned and characterized in 1997 as a gene which leads to Stargardt disease [11]. Soon after, it is found that *ABCA4* gene relate to the incidence of AMD [12]. This gene encodes a large retina-specific protein with two transmembrane domains (TMD), two glycosylated extracellular domains (ECD), and two nucleotide-binding domains (NBD). *ABCA4* gene almost exclusively expressed in the outer segment disk edges of rods and cones. Biswas et al. [31] demonstrate that *ABCA4* mediates the inside and outside transshipment of photoreceptor cell molecules or ions. This transshipment function is likely to affect the exchange of substance between retinal pigment epithelium and photoreceptor cell [32]. Mutations in *ABCA4* gene might alter the expression and function of the protein, and then lead to many retinopathies [33-36].

Many polymorphisms were found in *ABCA4* gene, but there only a few of them were studied in the occurrence of AMD. Meanwhile, most of the widely researched polymorphisms were had low MAF in Han Chinese (MAF < 0.05), such as G1961E and D2177N [18]. So, in this study, we investigated the role of a single nucleotide polymorphism (SNP) which with high MAF (rs560426, intron6) and a newly discovered SNP (6389T>A, exon47) in the development of AMD.

Mainly finding of this study was that the rs560426 SNP had no significant association with the occurrence of AMD, 6389A allele significantly associated with AMD risk. No previous study focused on the association of rs-560426 and AMD risk. The results of 6389T>A polymorphism was partly consistent with previous study [36]. Although the controls had representativeness, the result was insufficient to certify the AMD pathogenesis. There existed some limitations in this study. Firstly, only one ethnicity in this study. Secondly, the sample size was small. Thirdly, the results were not adjusted by confounding factors. Besides, the gene-gene and gene-environment interactions were not involved in this study. Therefore the results should be confirmed by further studies.

Disclosure of conflict of interest

None.

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