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Original Article

The association of FBLN5 polymorphisms with age-related macular degeneration susceptibility in the population of northern China

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Abstract: *Objectives*: This study are intended to explore the effect of *FBLN5* polymorphisms on age-related macular degeneration (AMD) susceptibility and provide evidence for the pathogenesis of AMD. *Methods*: This case-control study was conducted in 138 patients with AMD and 152 healthy persons frequency-match with the former by age and gender. *FBLN5* 1087G>A and 506T>C polymorphisms were genotyped through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The χ^2 test was used to check hardy-weinberg equilibrium (HWE) and calculate odds ratio (OR) with 95% confidence interval (CI) which evaluated the association between polymorphisms and AMD risk. *Results*: HWE test showed that the selection of control group conformed to requirement. As a result, mutant genotypes TC, CC of *FBLN5* 506T>C polymorphism both had the higher frequencies in cases compared with controls (*P*=0.006 and 0.029, respectively). Furthermore its mutant allele C also was associated with the increased risk of AMD (OR=1.725, 95% CI=1.210-2.459). *Conclusions*: *FBLN5* 506T>C polymorphism significantly increased the susceptibility to AMD in population of northern China, but 1087G>A might be not an independent risk factor for AMD development.

Keywords: *FBLN5*, AMD, polymorphism

Introduction

Age-related macular degeneration (AMD) usually occurs in older adults defined as a chronic and progressive disorder with age and is a major cause resulting in blindness [1, 2]. In United State, more than 1.75 million persons have suffered from AMD in 2011, the cases will increase to 3.0 million in 2020 through the estimation of authors owing to the aging population [3]. AMD is becoming the increased prevalent in the global. It is classified as atrophic and exudative senile macular degeneration and both of them are also identified in early, intermediate or late stage with different symptoms [4]. So far, the treatment means of AMD include surgery, drug and nondrug interventions, but the therapeutic efficacy is not ideal [5, 6]. Therefore it is very essential to recognize the susceptibility gene locus for early prevention. Recently, a majority of genes have been identified to participate in the development and progression of AMD, such as *CFH*, *VEGF*, *ARMS2* and *FLT1* genes [7-10].

Fibulin-5 is a member of fibulin family which belongs to the plasma glycoprotein possessed repeated domain structure including seven members, and is encoded by *FBLN5* gene located in human chromosome 14 q31 [11, 12]. It is incorporated into the extracellular matrix and effects cell proliferation and invasion in various diseases, particularly in cancers, such as hepatocellular carcinoma, gastric cancer, nasopharyngeal carcinoma [13-15]. Recently, the development of AMD is associated with changes in extracellular matrix. Thus the mutation of *FBLN5* gene may have an influence on AMD [16]. *FBLN5* 1087G>A, 506T>C polymorphisms both are the missense mutations with the incorrect amino acid instead of the normal one which may change the function of gene. However, the

Table 1. The PCR primer sequences of *FBLN5* polymorphisms

SNP	Primer sequences		Length	Annealing temperature
1087G>A	Forward	5'-GAAGTAGGCACAGCCAGCAC-3'	173bp	54.6°C
	Reverse	5'-AAGATGTCAGCGGGAACG-3'		
506T>C	Forward	5'-TCCGTGACACTCAGTAGG-3'	185bp	51.7°C
	Reverse	5'-CTGGCAGTAACCATAGCG-3'		

studies about the effect of *FBLN5* gene polymorphisms on AMD are very few.

In order to evaluate the association between *FBLN5* 1087G>A, 506T>C polymorphisms and AMD risk and explore its pathogenesis, we conducted this case-control study in a total of 290 participants all from northern areas in China and intended to provide guidance for the precaution and further research of AMD.

Materials and methods

Study subjects

One hundred and thirty-eight patients with AMD pathological diagnosed in the Ophthalmology department of Chinese PLA General Hospital in Shandong, Hebei and Shanxi provinces were enrolled in this case-control study. Their age span was from 55 to 78 with an average of 66.41±5.33, including 57 males and 79 females. The control group was frequency-matched with the cases by age, gender and region and had no other eye or brain diseases or some diseases threaten the body's health seriously. One hundred fifty-two controls contained 62 males and 90 females with a mean age of 67.20±5.08. The study was consistent with the requirement by the Research Ethics Committee in the hospital. All subjects signed informed consents and they were not related to each other by blood.

Sample handling and DNA extraction

In the morning, on an empty stomach, 2 ml venous blood was collected from every participant in the EDTA anticoagulative tube for blood DNA extraction. Next step was conducted using the mean of conventional chloroform-isoamyl alcohol and then DNA was stored at -20°C fridge.

PCR-RFLP

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to conduct the genotyping of *FBLN5* polymorphisms. PCR primers were designed by Primer 5.0 software and synthesized by Sangon Biotech in Shanghai. The detailed sequences were showed in **Table 1**. The PCR reaction solution was a volume of 20 μl, including 1.0 μl genome DNA template, forward and reverse primers for each 0.5 μl, 10.0 μl Master Mix and 8.0 μl deionized water. The PCR program was then conducted according to the following step: initial denaturation at 95°C 5 min, 36 cycles with denaturation at 94°C for 45 s, annealing at 54.6°C, 51.7°C (1087G>A, 506T>C) for 30 s, followed extension at 72°C or 45 s, and finally extension at 72°C for 10 min.

The PCR products were checked by 1.0% agarose gel electrophoresis (AGE) for target fragments and then digested through restriction endonucleases *Pst*I and *Alu*I. The digested products were separated by 2.5% AGE and visualized by EB staining.

Statistical analysis of data

All data were analyzed based on SPSS 18.0 software. The genotype distributions of *FBLN5* polymorphisms in the control group were evaluated by χ^2 whether they were consistent with hardy-weinberg equilibrium (HWE) or not. In order to evaluate the association strength of *FBLN5* gene polymorphisms with AMD risk, odds ratio (OR) with corresponding 95% confidence interval (95% CI) was also calculated by χ^2 test. When *P* was less than 0.05, it was considered the significant difference.

Results

HWE test

The genotype frequencies of *FBLN5* 1087G>A, 506T>C polymorphisms in the control group conformed to HWE (*P*=0.684, 0.203). The result proved that our study was advisable and the selected materials possessed representativeness.

The relationship between FBLN5 polymorphisms and AMD risk

As shown in **Table 2**, the relationship intensity of *FBLN5* gene polymorohsims and AMD was displayed. *FBLN5* 1087G>A polymorphism was

Table 2. The results of association between *FBLN5* polymorphisms and AMD susceptibility

Genotype/allele	Control (n=152, %)	Case (n=138, %)	χ^2	OR	95% CI	*P*
1087G>A						
GG	64 (42.11)	52 (37.68)	-	1.000	Ref.	-
GA	71 (46.71)	63 (45.65)	0.120	1.092	0.663-1.799	0.729
AA	17 (11.18)	23 (16.67)	1.913	1.665	0.806-3.441	0.167
G	199 (65.46)	167 (60.51)	-	1.000	Ref.	-
A	105 (34.54)	109 (39.49)	1.524	1.237	0.882-1.734	0.217
506T>C						
TT	87 (57.24)	54 (39.13)	-	1.000	Ref.	-
TC	52 (34.21)	65 (47.10)	7.664	2.014	1.224-3.315	0.006
CC	13 (8.55)	19 (13.77)	4.750	2.355	1.076-5.152	0.029
T	226 (74.34)	173 (62.68)	-	1.000	Ref.	-
C	78 (25.66)	103 (37.32)	9.163	1.725	1.210-2.459	0.002

not associated with AMD risk remarkably. In the contrary, the frequencies of genotypes TC, CC in 506T>C polymorphism were higher in cases than controls (TC: 47.10%, 34.21%; CC: 13.77%, 8.55%), indicating that they were associated with the significantly increased susceptibility to AMD (TC vs. TT: OR=2.014, 95% CI=1.224-3.315; CC vs. TT: OR=2.355, 95% CI=1.076-5.152). In addition, the frequency of its mutant allele C was 1.725 times higher in the case group than that control group (OR=1.725, 95% CI=1.210-1.459).

Discussion

AMD is a neurodegenerative disease and its development, progression and treatment are considered to be related with immune processes, including dry- and wet-AMD [17-19]. Among of them, dry-AMD accounts for 80% of this disease in medium-term and advanced stages [18]. Gao et al. indicated that AMD was as a multifactorial disease under the control of genetic and environmental factors [20]. Cezario et al. have conducted a case-control study to evaluate the relevance of apolipoprotein E (APOE) polymorphisms and serum lipid profiles with AMD risk, and reported that APOE-*HhaI* polymorphism is not significantly associated with AMD susceptibility, but an increasing level of high-density lipoprotein cholesterol in serum may be as a protective factor against this disease, not considering the genetic variant of *APOE* gene [21]. Zhang et al. conducted a meta-analysis in combination of the previous studies to introduce the association between *ABCA4* polymorphisms and AMD systematically, and showed that G1961E and D2177N polymorphisms in *ABCA4* gene increased the susceptibility to AMD [22]. In order to verify the effect of Omega 3 fatty acids on AMD development and progression, Lawrenson and Evans summarized a mess of studies to find that increasing intake of omega 3 long-chain polyunsaturated fatty acids in diet did not prevent or slow the advanced of AMD [23]. In addition, the occurrences of AMD are also reported to be associated with relative genes with immune system and smoking, ethnicity, and nutrition.

Fibulin5 belongs to Fibulin family and the later contains seven members named as *FBLN1*, *FBLN2*, *FBLN3* (*EFEMP1*), *FBLN4*, *FBLN5*, *FBLN7* and *HMCN1* (Fibulin6), respectively [24]. Fibulins derived from glycoproteins are the components of a fibrillar extracellular matrix and they share an elongated structure and multiple calcium-binding sites. Owing to having some overlapping binding sites, similar functions may exist among them. According to the study of Chen et al. in 2014, the down-regulation of *FBLN3* accelerated the invasion and metastasis of lung cancer cells through activated Wnt/β signaling pathway [25]. Recently, the same group of authors found that *FBLN5* could suppress lung cancer metastasis by inhibiting Wnt/β signaling pathway [26].

Wyatt et al. demonstrated that the CFH 402H/Fibulin3 interaction promoted the development of soft drusen in some patients, which provided a guidance for the treatment of AMD [27]. Stone et al. firstly reported the missense mutations in *FBLN5* were associated with AMD in

American population [28]. However, the effect of *FBLN5* gene polymorphisms on AMD in Asian population has been hardly referred, particularly in Chinese.

In present study, we explored the association of AMD with two polymorphism, 1087G>A and 506T>C, in *FBLN5* gene based on the population of North China. Polymorphism of 1087G>A did not show any relevance with AMD risk. In contrary, the homozygous and heterozygous mutant genotypes of 506T>C were both related with the obviously increased susceptibility to AMD. Even mutant allele also significantly increased the risk of AMD. Therefore, *FBLN5* 506T>C polymorphism may be an independent risk factor in AMD occurrence and advance. This article provides a useful target to explain the pathology and etiology of AMD.

Although we have obtained the available result, some limitations still exist in our study. On the one hand, the occurrence of a disease usually is regulated by multiple factors through the interaction, but we only tack into account of two polymorphisms from one gene. One the other hand, the sample size is relative small and selected population may concentrate on several provinces, which leads to the partial result. So in the future, further studies with well-designed and enough large sample size are required, referring to the interaction of gene-gene, gene-environment meanwhile.

Disclosure of conflict of interest

None.

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