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Risk of macular degeneration affected by polymorphisms in Matrix metalloproteinase-2

A case-control study in Chinese Han population

Jie Cheng, Xiaolin Hao, Zhongchen Zhang*

Abstract

The purpose of this study was to investigate the correlation of single nucleotide polymorphisms (SNPs) in Matrix metalloproteinase -2 (MMP-2) gene and the risk of age-related macular degeneration (AMD) in Chinese Han population.

A total of 126 AMD patients and 141 healthy controls participated in this study. Genotypes of *MMP-2* gene polymorphisms were identified by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). χ^2test was used to detect the differences of genotypes and alleles frequencies between case and control groups. Relative risk of AMD was evaluated by odds ratios (ORs) with 95% confidence intervals (CIs).

Distribution of variant allele carriers (computed tomography + TT genotypes) of *MMP-2* gene rs243865 SNP was significantly different between case and control groups, and might act as protective factors for the onset of AMD ($P = .044$, OR = 0.583, 95% CI = 0.344–0.987). Nevertheless, the T allele might reduce the AMD risk ($P = .030$, OR = 0.611, 95% CI = 0.390–0.956). However, no significant association existed between rs243865 and AMD risk in the subgroup analysis based on age. GA + AA genotypes of rs243866 SNP may associate with a decreased risk of AMD in the age≤65 years subgroup ($P = .028$, OR = 0.399, 95% CI = 0.174–0.915).

MMP-2 gene rs243865 and rs243866 SNPs associated with the risk of AMD. Further studies should be performed to confirm the results.

Abbreviations: AMD = age-related macular degeneration, AREDS = age-related eye disease study, CIs = confidence intervals, ECM = extracellular matrix, ESCC = esophageal squamous cell carcinoma, FFA = fundus fluorescein angiography, HWE = Hardy–Weinberg equilibrium, IA = intracranial aneurysm, LVMI = left ventricular mass index, MMP-2 = matrix metalloproteinase-2, MMPs = matrix metalloproteinases, ORs = odds ratios, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, RA = rheumatoid arthritis, SNPs = single nucleotide polymorphisms.

Keywords: age, AMD, *MMP-2*, polymorphisms

1. Introduction

Age-related macular degeneration (AMD), which leads to progressive vision impairment, is the main reason of the blindness in the elderly people worldwide.[1] Characteristics of AMD are as chronic and progressive degeneration of photoreceptors, retinal pigment epithelium, and Bruch membrane.[2] Neovascular AMD and non-neovascular AMD are the 2 subtypes of AMD.[3] In recent years, epidemiological study found that the morbidity of AMD is increased in China[4] and its incidence in females is higher than that in males in clinical. Moreover, the prevalence of AMD presents trend of increasing along with the age. AMD is one of complex diseases and influenced by the environment and genetic factors. So far, age, smoking, excess alcohol consumption, education level, and diet structure have been identified to be associated with AMD.[5] Genetic factors including a number of genes polymorphisms have been proved as the molecular basis for AMD.[6] Furthermore, many researches also report that the interaction of genetic factors and other risk factors are involved in the pathogenesis of AMD.[7] However, the etiology of AMD is not completely understood nowadays. Angiogenesis is considered one of the important mechanisms for AMD development. Angiogenesis is related to the extracellular matrix (ECM) remodeling which includes various proteolytic systems. Matrix metalloproteinases (MMPs) are 1 kind of the important enzyme in proteolytic systems and involve in many vascular diseases.[8]

MMPs is a zinc-dependent endopeptidases family with the function of resolving ECM proteins. MMP-2, a member of MMPs family also known as gelatinase A, is a membrane-bound protein which can be found in optic nerve head and the retina.[9]*MMP-2* gene, located on chromosome 16 q12.2, includes 17 exons. MMP-2, essential for turnover of ECM,[10] can lead to optic nerve demyelination and other nerve changes.[11,12] It is reported that MMP-2 activity is associated with the collagen deposition and the formation of subretinal deposit.[13,14] It has been identified that the expression level of *MMP-2* is upregulated in choroidal neovascularization in animal

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Department of Ophthalmology, Aerospace Central Hospital, Beijing, China.

* Correspondence: Zhongchen Zhang, Department of Ophthalmology, Aerospace Central Hospital, Beijing 100049, China (e-mail: jkkiwdda@126.com).

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Table 1

Demographic characteristics of the study subjects.

Characteristics	Case (n = 126)	Control (n = 141)	*P* value
Gender (female/male)	42/84	50/91	>.05
Age (mean ± SD)	62.4 ± 9.6	63.8 ± 10.2	>.05
≤65	53	76	
>65	73	65	

Table 2

Primers of *MMP-2* gene polymorphisms.

dbSNP ID	Position	SNP	Primer
rs243865	Chr16	C/T	5'- GCCATTGTCAATGTTCCCTAAAACA-3'
	3726		5'-TGACTTCTGAGCTGAGACCTGAA-3'
rs243866	Chr16	G/A	5'-GTC TGA AGC CCA CTG AGA CC-3'
	3457		5'-CTAGGAAGGGGGCAGATAGG-3'

MMP-2 = matrix metalloproteinase-2, SNP = single nucleotide polymorphism.

model.[15] The study performed by Hussain et al[16] suggested that the total level of pro-MMP-2 was raised in AMD. There are few studies analyzing the influence of *MMP-2* gene polymorphisms on AMD progression.

In this case-control study, we investigated the association between the single nucleotide polymorphisms (SNPs) of *MMP-2* gene and the risk of AMD in Chinese Han population. Our study will provide more theoretical basis for further researches on the pathogenesis of AMD.

2. Materials and methods

2.1. Study subjects

This study was approved by the Ethics Committee of Aerospace Central Hospital. Written informed consent was obtained from all the subjects after informed with the detail and purpose of this study. Processes of sample collection were performed in accordance with the Declaration of Helsinki.

A total of 267 subjects consisting of 126 AMD patients and 141 healthy controls were enrolled in this study (Table 1). The patients with AMD were recruited during August 2013 to September 2015 from Ophthalmology Department of Aerospace Central Hospital. Fundus fluorescein angiography (FFA) and ophthalmic examinations were used to diagnose the AMD patients. Evaluation for AMD was performed according to the Age-Related Eye Disease Study (AREDS).[17] Patients with trauma, cancer, or abnormal liver or renal function tests were not included in this study. One hundred forty-one controls without AMD were recruited randomly from the Health Screening Center of Aerospace Central Hospital between August 2013 and September 2015. Individuals with degenerative myopia, angioid streaks, or drusen were excluded from this study. All of the subjects were Chinese Han population without blood relationship with each other. Frequencies of sex and age between case and control groups were good matched.

2.2. DNA extraction

Five milliliters of blood samples were obtained from all subjects via peripheral venous puncture. Genomic DNA was extracted by DNA extraction kits (QIAGEN, MD) following the instructions of the manufacturer. The genomic DNA was labeled and stored at −70°C until further analysis.

2.3. Genotyping

Region consisting of the *MMP-2* SNPs was amplified using PCR. Primers were designed by Primer Premier 5 software according to the general primer design principles (Table 2). Total reaction system for PCR was 20 μL, which consisted of 3 μL DNA template, 1 μL forward primer, 1 μL reverse primer, 2.5 μL 10 × Buffer, 2 μL $MgC1_2$, 2.5 μL dNTPs, 1 μL Taq enzyme, 7 μL ddH_2O. The reaction system was instantaneously centrifuged to mix well.

PCR conditions for rs243865 were initial denaturation at 94°C for 10 minutes, followed by 35 cycles of 94°C for 40 seconds, 62°C for 30 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 3 minutes. The PCR conditions for rs243866 were initial denaturation at 94°C for 8 minutes followed by 40 cycles of 94°C for 60 seconds, 56°C for 30 seconds, 72°C for 50 seconds, and a final extension at 72°C for 5 minutes. PCR products were preserved at −20°C for standby application.

Restriction enzymes digestion was finished in 20 μL system, including 2 μL restriction enzyme, 8 μL PCR products, 2 μL 10 × Buffer, and 8 μL ddH_2O. Digested products were separated by electrophoresis on 2.0% TBE agarose gel to confirm the size of amplicons.

2.4. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences 18.0 for Windows (Chicago, IL). Hardy–Weinberg equilibrium (HWE) was used to assess the genotype distributions. Genotypic and allelic frequencies of *MMP-2* polymorphisms were calculated by direct counting. Differences of genotype frequencies of *MMP-2* gene polymorphisms between the case and control groups were compared by the χ^2test. Effects of genotypes and alleles on AMD were evaluated by odds ratios (ORs) with 95% confidence intervals (CIs). Statistically significant differences were considered existing with $P < .05$.

3. Result

3.1. HWE test

Genotype distributions of *MMP-2* gene rs243865 and rs243866 SNPs were consistent with the HWE test respectively in cases and controls (Table 3). This result demonstrated that the subjects of present study could represent the general population.

3.2. Correlation of MMP-2 gene polymorphisms and AMD risk

CC, CT, and TT genotype frequencies of *MMP-2* gene rs243865 SNP were 74.60%, 21.43%, 3.97% in AMD patients and 63.12%, 29.79%, 7.09% in healthy controls (Table 3). Variant allele carriers (CT + TT) were frequently observed in the case group, and the CT + TT genotypes might significantly be associated with the risk of AMD ($P = .044$, OR = 0.583, 95% CI = 0.344–0.987). Meanwhile, the T allele might protect against the development of AMD ($P = .030$, OR = 0.611, 95% CI = 0.390–0.956).

Both GA and AA genotypes of rs243866 SNP had higher frequencies in the controls. At the same time, A allele frequencies

Table 3

Correlation of polymorphisms in *MMP-2* gene and the risk of AMD.

	Case (n = 126)		Control (n = 141)				
Genotype/allele	n	%	n	%	χ^2	*P*	OR (95% CI)
rs243865							
CC	94	74.60	89	63.12	–	–	–
CT	27	21.43	42	29.79	3.005	.083	0.609 (0.346–1.070)
TT	5	3.97	10	7.09	1.083	.179	0.473 (0.156–1.439)
CT + TT	32	25.40	52	36.88	4.069	.044	0.583 (0.344–0.987)
C	215	85.32	220	78.01	–	–	–
T	37	14.68	62	21.99	4.700	.030	0.611 (0.390–0.956)
P HWE	0.104		0.118				
rs243866							
GG	104	82.54	107	75.89	–	–	–
GA	21	16.67	31	21.98	1.326	.249	0.697 (0.376–1.291)
AA	1	0.79	3	2.13	0.927	.336	0.343 (0.035–3.350)
GA + AA	22	17.46	34	24.11	1.777	.183	0.666 (0.365–1.213)
G	229	90.87	245	86.88	–	–	–
A	23	9.13	37	13.12	2.128	.145	0.665 (0.383–1.154)
P_{HWE}	0.958		0.672				

AMD = age-related macular degeneration, HWE = Hardy–Weinberg equilibrium, MMP-2 = matrix metalloproteinase -2.

were respectively 9.13% in cases and 13.12% in controls. However, we failed to find any association between rs243866 SNP and the risk of AMD ($P > .05$).

3.3. Subgroup analysis of MMP-2 polymorphisms based on age

To detect the association of *MMP-2* gene polymorphisms with the AMD risk, we performed the subgroup analysis based on age (Table 4). No significant association was observed between rs243865 genotypes and AMD risk both in the age≤65 years and > 65 years subgroups ($P > .05$). GA + AA genotypes of rs243866 polymorphism had a negative association with the AMD risk in the subgroup of age ≤ 65 years old ($P = .028$, OR = 0.399, 95% CI = 0.174–0.915). But rs243866 polymorphism did not relate to the occurrence of AMD in the age > 65 years subgroup ($P > .05$).

4. Discussion

In this case-control study, we found that the *MMP-2* gene rs243865 and rs243866 SNPs might negatively associate with the risk of AMD. For rs243865 polymorphism, compared with the wild homozygote, CT + TT genotypes might associate with 0.583-fold decreased risk of AMD. T allele also reduced the risk of AMD approximately 0.611 fold. But CT and TT genotypes were not related to the risk of AMD respectively, although they had high frequencies in controls. That was in accordance with the previous study performed by Hall et al.[18] Rs243865 SNP CC genotype shows a greater prevalence in AMD patients, and may be a predictor for AMD in a Lithuanian population.[18] This SNP is unlikely to have a major role in AMD onset, but the genotype distributions were significantly different between dry AMD patients and healthy controls.[19] Meanwhile, rs243865 is widely detected in many other diseases. It is revealed that TT genotype reduces the incidence of esophageal squamous cell carcinoma (ESCC).[20] Low et al[21] showed that this SNP marginally related to intracranial aneurysm (IA) in a Japanese population. It also reduces the rheumatoid arthritis (RA) risk in a Chinese population.[22] Lacchini et al[23] found that CC genotype of rs243865 reduced the left ventricular mass index (LVMI) and left ventricular end-diastolic diameter in hypertensive patients. A recent study substantiates that CT + TT genotypes of rs243865 significantly downregulate the protein level of MMP-2.[24] Based on the above results, we suggested that rs243865 was associated with the onset of AMD. However, it was failed to find significant

Table 4

Frequencies of genotypes in rs243865 and rs243866 for the patients with AMD and controls by age.

		Case (n = 126)		Control (n = 141)			
Age	Genotype	n	%	n	%	*P*	OR (95% CI)
	rs243865						
≤65	CC	40	75.47	49	64.47	–	–
	CT + TT	13	24.53	27	35.53	.184	0.590 (0.270–1.290)
>65	CC	54	73.97	40	61.54	–	–
	CT + TT	19	26.03	25	38.46	.118	0.563 (0.273–1.160)
	rs243866						
≤65	GG	43	81.13	48	63.16	–	–
	GA + AA	10	18.87	28	36.84	.028	0.399 (0.174–0.915)
>65	GG	61	83.56	59	90.77	–	–
	GA + AA	12	16.44	6	9.23	.210	1.934 (0.681–5.491)

AMD = age-related macular degeneration, CIs = confidence intervals, ORs = odds ratios.

association between rs243865 and AMD risk in age subgroup analysis.

Rs243866 was another polymorphism of *MMP-2* gene which was assessed in the present study. Variant genotypes were lower in AMD patients, but they did not significantly associate with the risk of AMD. In the people 65 or less than 65 years old, GA + AA genotypes associated with 0.399-fold decreased risk of AMD. This SNP is not detected in AMD in previous studies, but is explored in other diseases. Juiz et al[25] indicated that rs243866 implicated in the ECM remodeling. A previous study shows that A allele carriers relate to decreased risk of systolic heart failure in a Han Chinese population.[26] In s Mexican population, rs243866 A allele might be a predisposing factor for myocardial infarction.[27] Additionally, AA genotype carriers had a down-regulated plasma level of pro-MMP-2, but the difference of pro-MMP-2 levels among the rs243866 genotypes had not statistical significance.[28] Although we obtained an evidence for the association between *MMP-2* gene polymorphisms and AMD risk, the pathogenesis of AMD was still not completely understood. Further studies should be carried out to verify present study in other ethnicity.

To our knowledge, it is the first study to explore the correlation of *MMP-2* gene polymorphisms with the risk of AMD in Chinese Han population. MMPs produced by retinal pigment epithelium cells take part in the homeostasis of components in eye tissues.[29] As a member of the MMPs family, *MMP-2* gene plays a major role in the ECM degradation in angiogenesis, tissue repair, and so on.[30] It is proven that MMP-2 contributes to the development of choroidal neovascularization,[31] while neovascular is one of the direct mechanisms for the occurrence and development of AMD. MMP-2 localizes in the new vessel and envelops Bruch membrane, then contributes to the choroidal neovascular membranes' progressive growth in AMD.[32] Nevertheless, Zeng et al[33] considered that the serum level of MMP-2 is elevated in polypoidal choroidal vasculopathy but not AMD patients. These inconsistent results may be caused by different sample size, study population, genetic background, and the individual difference.

In conclusion, *MMP-2* gene correlates with the risk of AMD, and polymorphisms are conducive to the individual difference for the same disease. Specify results according to AMD type were not performed due to the small sample size that might reduce the statistical power. As one of complex diseases, AMD is affected by the interaction of confounding factors. However, our results cannot rule out the effects of other factors to the AMD. Each population has its specificity, the reports show that the morbidity of AMD in Caucasians is higher than that in Blacks; however, Blacks are easier to develop exudative AMD than Caucasians. The prevalence of AMD in Asians is similar to Caucasians.[34,35] It may derive from different genetic background and environmental factors. Therefore, more researches with larger and different samples should be performed to verify present results in other populations. Even so, we should not ignore the effects of our study, which will provide molecular basic for the study of AMD pathogenesis.

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