

Original Article

CX3CR1 polymorphisms and the risk of age-related macular degeneration

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Abstract: Background: Age-related macular degeneration (AMD), a most common eye disease, can lead to irreversible visual impairment. Age, genetic and environmental factors have been implicated in AMD. Chemokine (C-X3-C motif) receptor 1 (CX3CR1) gene polymorphisms could influence the susceptibility of AMD. Methods: We tested the association between AMD and single nucleotide polymorphisms (SNPs) of CX3CR1 gene (rs3732378 and rs3732379) in 102 cases and 115 controls from China. Genotypes were determined by MassArray genotyping assay method. Association between CX3CR1 gene polymorphisms and AMD were examined by χ^2 test and logistic regression. Results: Genotype distribution of CX3CR1 gene polymorphisms were in accordance with HWE examination. No obvious differences were observed in the genotypes of rs3732378 polymorphism between case and control groups ($P>0.05$), but A allele of it could increase the risk of AMD ($P=0.025$, OR=2.391, 95% CI=1.092-5.237). Both TT genotype and T allele of rs3732379 were significantly associated with the susceptibility of AMD ($P=8.663$, OR=8.663, 95% CI=1.044-71.874; $P=0.021$, OR=2.076, 95% CI=1.104-3.903). Age, gender and smoking status were used as common confounders to adjust the association between CX3CR1 gene polymorphism and AMD risk. Then we found that rs3732378 had no obvious association with AMD susceptibility. TT genotype of rs3732379 related to the occurrence of AMD, but the association was not significant ($P=0.050$, OR=8.274, 95% CI=1.002-69.963). T allele of rs3732379 might increase the susceptibility of AMD ($P=0.029$, OR=2.033, 95% CI=1.077-3.838). Conclusion: T allele of rs3732379 might have a positive association with the susceptibility of AMD.

Keywords: Age-related macular degeneration, CX3CR1 gene, polymorphisms

Introduction

Age-related macular degeneration (AMD) is the common eye disease which will lead to decreased vision even blindness in the elderly in developed countries [1, 2]. AMD is a progressive irreversible disorder of the central macular region of the retina, and categorized into early and late two stages. The development of drusen between retinal pigment epithelium (RPE) and underlying choroid is a harbinger of early AMD [3]. In early stage the symptoms of AMD are inconspicuous [4]. Drusen size and quantity have positive correlation with the risk of AMD [5]. AMD has dry and wet two forms. Dry AMD is characterized by geographic atrophy (GA) of RPE, and wet AMD result from abnormal chorioidal neovascularization (CNV) in choriocapillaris. Few personal and environmental risk factors, aside from age have been identified for AMD.

Some studies suggested that family history [6, 7], oxidative stress [8], lifestyle [9, 10], obesity [11], cytokines and their receptors [12, 13] may cause changes in the development of AMD.

Chemokine (C-X3-C motif) receptor 1 (CX3CR1), a receptor of chemokine, might implicate in macular degeneration development [14]. It was reported that CX3CR1 protein level showed a significant alteration in AMD patients [15]. However, the genetic basis and molecular mechanism of CX3CR1 in the occurrence and progression of AMD were unclear. Many efforts have been made to identify the mechanism of CX3CR1 that can be used to forecast the probability of the onset of AMD. Several single nucleotide polymorphisms (SNPs) of CX3CR1 gene have been discovered [16, 17]. However, most of the studies were focused on European descent. Because the distributions of genetic

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Table 1. Features of subjects

	Case n=102	Control n=115	P value
Status			
No AMD		115	
Wet AMD	65	-	
Dry AMD	16	-	
Mixed AMD	21	-	
Age (Mean \pm SD)	66.17 \pm 10.08	68.93 \pm 10.59	0.051
Gender			0.897
Male	55	61	
Female	47	54	
Smoking			0.796
Never	72	83	
Current or past	30	32	

variants exist distinction among ethnic groups, these SNPs of CX3CR1 gene need to be tested in different ethnic groups.

In this study, we recruited AMD patients and healthy controls in Shandong Provincial Qianfushan Hospital and selected the rs3732378 (T280M) and rs3732379 (V249I) SNPs in CX3CR1 gene to detect the association of CX3CR1 gene and the susceptibility of AMD.

Methods

Study participants

This was a case-control designed study, and was approved by the ethic committee of Shandong Provincial Qianfoshan Hospital. Each participator signed the written informed consent form. 102 AMD patients and 115 age and gender-matched healthy controls were recruited from Shandong Provincial Qianfoshan Hospital. Participants were all older than 50 years. All subjects were diagnosed by two ophthalmologists and conducted a full ophthalmic examination. Independent grading of fundus photographs were used to confirm their disease status. Clinical diagnoses and categorization followed the guideline of National Eye Institute Age-Related Eye Disease Study [18]. Individuals had any eye disease were excluded from control group. All procedures were according to the principles of the Declaration of Helsinki. Demographic information of participants was listed in **Table 1**.

Genotyping

Genomic DNA was extracted from 1 ml peripheral blood of each fasting participant using a

TIANamp Blood DNA Kit (TianGen, China). The samples were genotyped by MassARRAY method using a MassARRAY Analyzer 4 system (Sequenom, San Diego, CA, USA), according to the manufacturer's instructions.

Statistic analysis

All of the calculations were conducted by SPSS 18.0. We examined allele distributions of each SNP and Hardy-Weinberg equilibrium (HWE) using Chi square test in cases and controls. Difference of age between case and control groups was assessed by *t* test. Other demographic features were compared by χ^2 test between two groups. All the comparisons were adjusted for age, gender and smoking status by logistic regression. Odds ratios (ORs) and their corresponding 95% confidence intervals (95% CI) were used to evaluate the association strength between CX3CR1 gene and AMD risk. *P* value less than 0.05 was considered to be statistically significant.

Results

Subject features and HWE test

As **Table 1** shows, individual characteristics had no significant difference between case and control groups. In order to evaluate the role of CX3CR1 gene in AMD, we recorded the genotype distribution of CX3CR1 gene polymorphisms (rs3732378 and rs3732379) in case and control groups. The distribution frequencies of CX3CR1 gene polymorphisms did not deviate from HWE in control group.

Association of CX3CR1 gene polymorphisms and AMD risk

Table 2 has showed the genotype distributions of rs3732378 polymorphism had no significant difference between case and control groups. A allele frequency of rs3732378 was significantly higher in cases than that in controls, which might relate to the susceptibility of AMD ($P=0.025$, OR=2.391, 95% CI=1.092-5.237). No significant difference was observed in TC genotype of rs3732379 between case and control groups. Genotype distribution of rs3732379 TT genotype was significantly associated with AMD risk ($P=8.663$, OR=8.663, 95% CI = 1.044-71.874). Meanwhile T allele frequency of

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Table 2. Genotype distributions of rs3732378 and rs3732379 SNPs of CX3CR1 gene

SNP	Case n=102 (%)	Control n=115 (%)	P value	OR (95% CI)	P* value	OR (95% CI)*
Rs3732378						
GG	85 (83.33)	106 (92.17)	-	-	-	-
GA	14 (13.73)	8 (6.96)	0.088	2.182 (0.875-5.445)	0.909	2.293 (0.879-5.980)
AA	3 (2.94)	1 (0.87)	0.225	3.741 (0.382-36.616)	0.344	3.073 (0.301-31.401)
G	184 (90.20)	220 (95.65)	-	-	-	-
A	20 (9.80)	10 (4.35)	0.025	2.391 (1.092-5.237)	0.065	2.157 (0.953-4.880)
Rs3732379						
CC	80 (78.43)	99 (86.09)	-	-	-	-
CT	15 (14.71)	15 (13.04)	0.589	1.238 (0.571-2.683)	0.622	1.217 (0.557-2.658)
TT	7 (6.86)	1 (0.87)	0.018	8.663 (1.044-71.874)	0.050	8.274 (1.002-69.963)
C	175 (85.78)	213 (92.61)	-	-	-	-
T	29 (14.22)	17 (7.39)	0.021	2.076 (1.104-3.903)	0.029	2.033 (1.077-3.838)

Notes: *adjusted by age, gender and smoking status.

rs3732379 also higher in cases, and the allele might increase the risk of AMD ($P=0.021$, $OR=2.076$, $95\% CI=1.104-3.903$).

Adjusted result about the association of CX3CR1 gene polymorphisms and AMD risk

In order to get an accurate result, we took age, gender and smoking status as common confounders in the analysis of the association between CX3CR1 genotype and AMD risk. Adjusted results indicated that the rs3732378 polymorphism not relate to AMD susceptibility ($P>0.05$). Although the TT genotype of rs3732379 may increase the risk of AMD but the relation was not significant ($P=0.05$, $OR=8.274$, $95\% CI=1.002-69.963$). Additionally, T allele of rs3732379 might increase the AMD risk ($P=0.029$, $OR=2.033$, $95\% CI= 1.077-3.838$).

Discussion

AMD is a degenerative change of the eye, usually affect elder adults in developed countries. This disease is a common cause of visual impairment. AMD mainly influence retina, choroid and Bruch's membrane. As a complex disease, AMD derive from genetic and environmental factors [19, 20], and genetic factors play a substantial role [21]. Accumulation of microglial cell (MC) in subretinal space might evoke cardinal characteristics of AMD [17]. No positive CX3CR1 protein was detected in retina in healthy MC [17]. CX3CR1 gene might implicate in the pathogenesis of AMD. CX3CR1 gene

locates in chromosome 3p21.3, and has 5 exons. As the third generation genetic marker, SNP plays an important role in the exploration of the pathogenesis of many disease.

In order to understand the mechanism by which CX3CR1 gene may contribute to the development of AMD, we analyzed the CX3CR1 gene polymorphisms. T280M (rs3732378) and V249I (rs3732379) were widely researched SNPs of CX3CR1 gene. It was reported that the two polymorphisms were associated with the risk of AMD in USA cohort [22]. However, there were very few studies focused on Chinese lineage. The distribution of SNP had a difference among different ethnicity and region. So we genotyped the rs3732378 and rs3732379 polymorphisms of CX3CR1 gene in Chinese Han AMD patients.

In this study, we observed association between CX3CR1 gene polymorphisms (rs3732378 and rs3732379) and AMD. There was no significant difference in the genotype distributions of rs3732378 between case and control groups. But A allele of rs3732378 was obviously increased the risk of AMD with the OR of 2.391. However, the age, gender and smoking status adjusted genotype and allele analysis of rs3732378 had no significant association with the development of AMD. Additionally, TC genotype of rs3732379 did not related to AMD risk. Both TT genotype and T allele of rs3732379 were significantly associated with the susceptibility of AMD, and might 8.663 and 2.076 times increase the AMD risk. After adjusted by age,

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gender and smoking status, TT genotype of rs3732379 was not significantly associated with the risk of AMD. T allele of rs3732379 related to the risk of AMD as before, with the OR of 2.033. The results showed that rs3732379 mutation in *CX3CR1* gene is associated with the susceptibility of AMD, and we failed to find association of rs3732378 SNP of *CX3CR1* gene with AMD risk.

The results of present study are partially in accordance with previous study which suggested that V249I had approximately 2.928 times increased risk of dry AMD in Greek [16]. In another study, it reported that both rs3732378 and rs3732379 were found to be with an increased incidence of AMD in Chinese population [23], the same result also detected in another study in USA [22]. In contrast, a study performed by Combadière et al. indicated that A allele of rs3732378 was associated with an increased risk of AMD, but V249I polymorphism had no significant influence on the occurrence of AMD [17]. However, Debra et al. demonstrated that both rs3732378 and rs3732379 had no significant association with the development of AMD in USA after adjusted multiple factors [24].

In summary, polymorphisms of *CX3CR1* gene had a positive association with the risk of AMD. However, a limitation of this study was notably. Frequencies of minor allele of rs3732378 and 3732379 of *CX3CR1* gene were much lower in Chinese than that in American [25]. So it should be noted that the sample size of this study was not large enough to detect the potential role of variant alleles of *CX3CR1* gene polymorphisms in the development of AMD. Nevertheless, the strengths of our study are as follows: firstly, the participants were selected randomly, therefore the potential selection bias may be diminished; secondly, all of the results were adjusted, so it was more accurate. However, the result was also insufficient to understand the mechanism of *CX3CR1* gene in AMD, and further study was necessary.

Disclosure of conflict of interest

None.

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References

- [1] Margherio RR, Margherio AR and DeSantis ME. Laser treatments with verteporfin therapy and its potential impact on retinal practices. *Retina* 2000; 20: 325-330.
- [2] Jonasson F, Arnarsson A, Eiriksdottir G, Harris TB, Launer LJ, Meuer SM, Klein BE, Klein R, Gudnason V and Cotch MF. Prevalence of age-related macular degeneration in old persons: Age, Gene/environment Susceptibility Reykjavik Study. *Ophthalmology* 2011; 118: 825-830.
- [3] de Jong PT. Age-related macular degeneration. *N Engl J Med* 2006; 355: 1474-1485.
- [4] Hogg RE and Chakravarthy U. Visual function and dysfunction in early and late age-related maculopathy. *Prog Retin Eye Res* 2006; 25: 249-276.
- [5] Klein R, Klein BE, Tomany SC, Meuer SM and Huang GH. Ten-year incidence and progression of age-related maculopathy: The Beaver Dam eye study. *Ophthalmology* 2002; 109: 1767-1779.
- [6] Shahid H, Khan JC, Cipriani V, Sepp T, Matharu BK, Bunce C, Harding SP, Clayton DG, Moore AT and Yates JR. Age-related macular degeneration: the importance of family history as a risk factor. *Br J Ophthalmol* 2012; 96: 427-431.
- [7] Geirsdottir A, Stefansson E, Jonasson F, Helgadóttir G and Sigurdsson H. Age-related macular degeneration in very old individuals with family history. *Am J Ophthalmol* 2007; 143: 889-890.
- [8] Chiras D, Kitsos G, Petersen MB, Skalidakis I and Kroupis C. Oxidative stress in dry age-related macular degeneration and exfoliation syndrome. *Crit Rev Clin Lab Sci* 2015; 52: 12-27.
- [9] Munch IC, Linneberg A and Larsen M. Precursors of age-related macular degeneration: associations with physical activity, obesity, and serum lipids in the inter99 eye study. *Invest Ophthalmol Vis Sci* 2013; 54: 3932-3940.
- [10] Ersoy L, Ristau T, Lechanteur YT, Hahn M, Hoyng CB, Kirchhof B, den Hollander AI and Fauser S. Nutritional risk factors for age-related macular degeneration. *Biomed Res Int* 2014; 2014: 413150.
- [11] Adams MK, Simpson JA, Aung KZ, Makeyeva GA, Giles GG, English DR, Hopper J, Guymer RH, Baird PN and Robman LD. Abdominal obesity and age-related macular degeneration. *Am J Epidemiol* 2011; 173: 1246-1255.

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- [12] Hsu MY, Chen SJ, Chen KH, Hung YC, Tsai HY and Cheng CM. Monitoring VEGF levels with low-volume sampling in major vision-threatening diseases: age-related macular degeneration and diabetic retinopathy. *Lab Chip* 2015; 15: 2357-63.
- [13] Sakurada Y, Nakamura Y, Yoneyama S, Mabuchi F, Gotoh T, Tateno Y, Sugiyama A, Kubota T and Iijima H. Aqueous humor cytokine levels in patients with polypoidal choroidal vasculopathy and neovascular age-related macular degeneration. *Ophthalmic Res* 2015; 53: 2-7.
- [14] Chen M, Hombrebueno JR, Luo C, Penalva R, Zhao J, Colhoun L, Pandi SP, Forrester JV and Xu H. Age- and light-dependent development of localised retinal atrophy in CCL2(-/-) CX3CR1(GFP/GFP) mice. *PLoS One* 2013; 8: e61381.
- [15] Falk MK, Singh A, Faber C, Nissen MH, Hviid T and Sorensen TL. CX3CL1/CX3CR1 and CCL2/CCR2 chemokine/chemokine receptor complex in patients with AMD. *PLoS One* 2014; 9: e112473.
- [16] Anastasopoulos E, Kakoulidou A, Coleman AL, Sinsheimer JS, Wilson MR, Yu F, Salonikiou A, Koskosas A, Pappas T, Founti P, Lambropoulos A and Topouzis F. Association of sequence variation in the CX3CR1 gene with geographic atrophy age-related macular degeneration in a Greek population. *Curr Eye Res* 2012; 37: 1148-1155.
- [17] Combadiere C, Feumi C, Raoul W, Keller N, Rodero M, Pezard A, Lavalette S, Houssier M, Jonet L, Picard E, Debre P, Sirinyan M, Deterre P, Ferroukhi T, Cohen SY, Chauvaud D, Jeanny JC, Chemtob S, Behar-Cohen F and Sennlaub F. CX3CR1-dependent subretinal microglia cell accumulation is associated with cardinal features of age-related macular degeneration. *J Clin Invest* 2007; 117: 2920-2928.
- [18] The Age-Related Eye Disease Study (AREDS): design implications. AREDS report no. 1. *Control Clin Trials* 1999; 20: 573-600.
- [19] Brion M, Sanchez-Salorio M, Corton M, de la Fuente M, Pazos B, Othman M, Swaroop A, Abecasis G, Sobrino B and Carracedo A. Genetic association study of age-related macular degeneration in the Spanish population. *Acta Ophthalmol* 2011; 89: e12-22.
- [20] Cheung LK and Eaton A. Age-related macular degeneration. *Pharmacotherapy* 2013; 33: 838-855.
- [21] Seddon JM, Cote J, Page WF, Aggen SH and Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. *Arch Ophthalmol* 2005; 123: 321-327.
- [22] Tuo J, Smith BC, Bojanowski CM, Meleth AD, Gery I, Csaky KG, Chew EY and Chan CC. The involvement of sequence variation and expression of CX3CR1 in the pathogenesis of age-related macular degeneration. *FASEB J* 2004; 18: 1297-1299.
- [23] Yang X, Hu J, Zhang J and Guan H. Polymorphisms in CFH, HTRA1 and CX3CR1 confer risk to exudative age-related macular degeneration in Han Chinese. *Br J Ophthalmol* 2010; 94: 1211-1214.
- [24] Schaumberg DA, Rose L, DeAngelis MM, Semba RD, Hageman GS and Chasman DI. Prospective study of common variants in CX3CR1 and risk of macular degeneration: pooled analysis from 5 long-term studies. *JAMA Ophthalmol* 2014; 132: 84-95.
- [25] Hu J, Yuan Y, Shen L, Zhang J, Hu N and Guan H. Age-related macular degeneration-susceptibility single nucleotide polymorphisms in a Han Chinese control population. *Ophthalmic Epidemiol* 2011; 18: 137-142.