

Association of *CFH* and *MAP1LC3B* gene polymorphisms with age-related macular degeneration in a high-altitude population

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Abstract

• **AIM:** To evaluate the association of complement factor H (*CFH*) and microtubule-associated protein 1 light chain 3 beta (*MAP1LC3B*) gene polymorphisms with the risk of age-related macular degeneration (AMD) in a high-altitude population.

• **METHODS:** The study group consisted of 172 participants with symptoms of AMD who were examined and diagnosed between January 2019 and June 2020. The control group was composed of 120 healthy individuals. Each participant was required to provide two milliliters of peripheral blood for DNA extraction. Two single nucleotide polymorphisms (SNPs) of *CFH* (rs1061170 and rs800292) and two SNPs of *MAP1LC3B* (rs8044820 and rs9903) were genotyped. The genotypes and allele frequencies of the SNPs in the study and control groups were further compared using Chi-square and Fisher's exact tests.

• **RESULTS:** In a high-altitude population, the nominally significant differences of rs800292 and rs9903's genotype AG frequencies were observed in the AMD group ($P=0.034$ and 0.004 , respectively). The frequencies of allele G of rs800292 and allele A of rs9903 were also significantly different in the AMD group compared to the control [$(P=0.034, OR=0.70, 95\%CI: 0.50-0.98)$ and $(P=0.004, OR=1.60, 95\%CI: 1.15-2.22)$, respectively]. No significant differences in the genotype distributions ($P=0.16$ and 0.40 , respectively) and allele frequencies ($P>0.05$) of rs1061170 and rs8044820 were observed in the AMD group.

• **CONCLUSION:** Genotype AG of rs800292 may be a protective factor for AMD. Conversely, rs9903 seems to be a risk factor for AMD. Therefore, allele G of rs800292 may be a protective factor, and allele A of rs9903, a risk factor for AMD in Qinghai high-altitude population.

• **KEYWORDS:** age-related macular degeneration; complement factor H; microtubule-associated protein 1 light chain 3 beta; single nucleotide polymorphisms; pathogenesis

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INTRODUCTION

Age-related macular degeneration (AMD) is one of the most common irreversible blinding diseases of the eye that severely affects the life quality of tens of millions of patients worldwide^[1]. Additionally, and each year, hundreds of thousands of patients are newly diagnosed^[1]. The occurrence and development of AMD are associated with several factors, including sunlight exposure, obesity, eating habits, ethnicity, sex, and age^[2]. However, the specific causes remain unclear. The occurrence and development of AMD are associated with several factors, including sunlight exposure, obesity, eating habits, ethnicity, sex, and age; however, the specific causes remain unclear^[2]. Recently, studies have confirmed that the occurrence and development of AMD are predominantly associated with genetic factors, and AMD heritability may approach 71%, indicating that genetics are a crucial factor^[3]. Complement factor H (CFH) is a major inhibitor of the complement replacement pathway and an essential protective factor in the retina^[4]; however, the gene variant *CFH* Y402H increases retinal damage caused by oxidative stress^[5]. In addition, autophagy is significantly involved in AMD development^[6], and microtubule-associated protein 1 light chain 3 beta (*MAP1LC3B*) can increase autophagy^[7].

Nevertheless, potential correlations between *MAP1LC3B* and AMD risk have not been examined to date. In this study, two single nucleotide polymorphisms (SNPs) in *CFH* (rs1061170 and rs800292) and two SNPs in *MAP1LC3B* (rs8044820 and rs9903) were selected to evaluate the associations of *CFH* and *MAP1LC3B* with AMD risk.

SUBJECTS AND METHODS

Ethical Approval This study was performed in accordance with the Tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Qinghai Provincial People's Hospital (No.2017-21). All the patients enrolled in this study signed the written informed consent before the start of the study.

Study Participants The total sample size is 292 persons in this study, including 172 patients with AMD and 120 control participants. From December 2016 to December 2019, patients with symptoms were diagnosed with AMD by routine eye examination, slit lamp, fundus photography, fundus fluorescein angiography, and optical coherence tomography. Healthy individuals who were examined at the physical examination center of the Qinghai Provincial People's Hospital were selected as controls according to the following criteria: age > 40y; AMD excluded through fundus examination. The patients with severe systemic diseases, including benign and malignant tumors, blood diseases, renal insufficiency, diabetes, and hypertension, were excluded. Furthermore, patients with glaucoma, high myopia, and other fundus lesions were also excluded from this study.

Inclusion, Exclusion, and Diagnostic Criteria The AMD patients who were more than 40 years old and residents at altitudes higher than 2000 m for no less than 20y were included in this study. The diagnostic criteria were employed in the AMD diagnosis.

All patients with hypertensive retinopathy, diabetic retinopathy, retinal vascular occlusion, Stargardt disease, choroidal neovascularization with high myopia, late vitelliform macular degeneration, and other eye diseases were excluded from this study.

The AMD^[8] and the diagnostic criteria of the "Chinese clinical diagnosis and treatment pathway for AMD"^[9] were employed. AMD is a macular lesion that can be diagnosed when it has one or more of the following characteristics: retinal hemangioma hyperplasia, polypoid choroidal vasculopathy, choroidal neovascularization (exudative), retinal pigment epithelium map atrophy, pigment metaplasia, migration, and proliferation, hypopigmentation, and medium-sized hyaline warts (>63 μm in diameter).

Extraction of DNA For further experiments, blood samples (2 mL) were collected, treated with an anticoagulant, and stored at -80°C. Genomic DNA was subsequently extracted for analysis

of gene polymorphisms. Anticoagulated whole blood [warmed to room temperature (RT) before use] was homogenized, and 200 μL was aspirated and placed in a centrifuge tube with 600 μL of red blood cell lysate. The tube was then inverted 6-8 times for homogenization. After 10-minute of incubation with several-time invert and mix, and 1-minute centrifugation (12 000 rpm at RT), the red supernatant was removed, and the residual blood at the orifice of the tube was aspirated, after which 300 μL of red cell lysate was added. The mixture was pipetted up and down several times for homogenization. Then the samples were centrifuged at 12 000 rpm for 1min to remove the supernatant. Three hundred milliliters of nuclear lysate heated to 50°C was carefully added and beaten 10-15 times to break up the cell mass with care to avoid destroying genomic DNA; foam was not removed, and the reaction was incubated at 60°C-65°C for 45min until the solution was clear. Next, 150 μL of protein precipitation solution was added, and the reaction was vortex-mixed for 25s. The reaction was then centrifuged at 12 000 rpm and at RT for 5min. The supernatant (about 450 μL) was carefully aspirated and placed in a new centrifuge tube. An equal volume of isopropanol (450 μL) was added, and the tube was gently inverted 30 times to ensure sufficient mixing. After centrifugation at 12 000 rpm and at RT for 5min, white DNA precipitates occurred at the bottom of the tube, and the supernatant was discarded. Next, ethanol was added (500 μL, 70%), and the mixture was pipetted up and down several times to dissolve the DNA, after which the mixture was again centrifuged at 12 000 rpm and at RT for 3min. The supernatant was discarded with care not to disturb the DNA precipitate. This ethanol washing step was repeated once. At RT, the mixture was centrifuged again at 12 000 rpm for 30s, the residual liquid was removed, and the opened tube was incubated at RT for 1min. ddH₂O (100 μL) was added to elute the DNA.

DNA Quality Assessment A NanoDrop 2000 spectrophotometer (Thermo Fisher) was employed to examine the quality of the extracted DNA samples^[10].

SNP Genotyping A MassARRAY system (Sequenom, San Diego, USA) was employed to determine the genotype of SNPs. The primers used in this study are listed in Table 1.

Statistical Analysis This study's analyses were conducted using SPSS V25.0 software (IBM, Armonk, NY, USA). The comparisons between control and AMD groups were performed using the Student's *t*-test. The Chi-squared test was used to analyze the categorical data, and the results were presented as *n* (%). Chi-squared test was employed to test the Hardy-Weinberg equilibrium of the genotype distributions. We also conducted the Fisher's exact test or Chi-squared test to compare and analyze genotype and allele frequencies between control and case groups. Moreover, we defined the significant

Table 1 Polymerase chain reaction primer sequences used in this study

SNP	Forword primer	Reverse primer	Amplicono (bp)
rs1061170	5'-ATTTATCATTGTTATGGTCTTAGG-3'	5'-AGAAAGAGATGAACAGCAGGATT-3'	244
rs800292	5'-ACTGTGACTGTCTAGGCTTTT-3'	5'-GGCAATAGTGATATATTAGGCA-3'	387
rs8044820	5'-GTAAATGGCACAGGCTTGATC-3'	5'-GAGCTGTAAGGGCCTTCTTTG-3'	327
rs9903	5'-TGCAATAAAATAGACCTCAAGTT-3'	5'-GCGTTTGTGCCAACTTGT-3'	249

SNP: Single nucleotide polymorphism.

difference between indicated groups as $P < 0.05$ and conducted the Genetic Power Calculator to perform Power analysis^[11]. All the numerical values obtained from the analysis in this study were presented as mean±standard deviation.

RESULTS

Demographic and Clinical Features of AMD Patients and Controls The mean age of the AMD and healthy control group were 61.48±11.68 and 62.67±11.40y, and 46.5% (80) and 53.3% (64) of the participants in the AMD and healthy control group were males. No statistically significant differences were observed between the AMD and control groups regarding alcohol consumption, smoking, lipid profile, diabetes mellitus, hypertension, age, and sex ($P > 0.05$; Table 2).

Genotype Distributions of the CFH and MAP1LC3B Gene Polymorphisms of AMD Patients and Controls As shown in Table 3, the genotype distribution of four SNPs was consistent with the Hardy-Weinberg equilibrium in the healthy control group ($P > 0.05$; Table 3). Compared to the healthy control group, we observed a significant difference in the genotype frequencies for rs800292 and rs9903 in the AMD group ($P = 0.034$ and 0.004 , respectively; Table 4). However, there was no significant difference between the two groups in the genotype distribution of rs1061170 and rs8044820 ($P = 0.16$ and 0.40 ; Table 4).

Allele Distributions of CFH and MAP1LC3B Gene Polymorphisms of AMD Patients and Controls We observed a significant difference of the rs800292's allele G frequencies ($P = 0.034$, OR=0.70, 95%CI: 0.50-0.98) and rs9903's allele A frequencies ($P = 0.004$, OR=1.60, 95%CI: 1.15-2.22) in the AMD patients compared to those healthy controls (Table 5). However, there was no significant difference between rs1061170 and rs8044820's allele frequencies in the AMD group ($P > 0.05$; Table 5) compared to the healthy control group.

DISCUSSION

AMD is a considerably complex affliction caused by environmental and genetic factors. In 2008, genome-wide association analysis of 146 participants found that the *CFH*, *ARMS2*, and *HTRA1* genes were significantly associated with AMD risk^[12]. Yang *et al*^[13] replicated the correlation of *CFH* and *HTRA1* SNPs and found that SNPs from different genes did not interact with each other. They concluded that these SNPs might independently affect the risk of AMD and

Table 2 Demographic and clinical features of AMD and controls

Feature	AMD patients	Controls	<i>P</i>
Gender (M/F)	80/92	64/56	0.251
Age (y)	61.48±11.68	62.67±11.40	0.397
Hypertension (yes/no)	36/136	24/96	0.847
Diabetes (yes/no)	27/145	25/95	0.259
Hyperlipidemia (yes/no)	37/135	36/84	0.099
Smoking (yes/no)	25/147	16/104	0.771
Drinking (yes/no)	89/83	67/53	0.490

AMD: Age-related macular degeneration; M: Male; F: Female.

first reported an association between *CX3CR1* and exudative AMD in the Chinese Han population. Some susceptibility genes have been suggested, including *C2*, *CFB*, *C3*, *CFI*, *CETP*, and *VEGFA*^[14]. However, the genetic makeup of AMD patients has not been sufficiently elucidated to date; therefore, more research should be conducted to identify additional susceptibility genes. Herein, we evaluated the connection of AMD in a high-altitude population and the *CFH* and *MAP1LC3B* gene by comparing the *CFH* and *MAP1LC3B* gene polymorphisms' frequencies between AMD and healthy control groups.

Four SNPs were examined in the current study, revealing that the genotype frequency of the A/G of SNP rs800292 in *CFH* and SNP rs9903 in *MAP1LC3B* significantly differed in the AMD group when compared to the control group ($P < 0.05$; Table 4). Furthermore, the differences of the rs800292's allele G and rs9903's allele A frequencies were significant between the AMD and control groups ($P < 0.05$; Table 5). These data indicate that genotype AG of rs800292 and rs9903 were negatively and positively correlated with the risk of AMD development, respectively. However, the specific protective mechanism of the rs800292 A allele in AMD needs to be further explored.

CFH is located in the region of 1q25-31 and expresses a 155-kDa plasma protein that is significantly involved in inhibiting the complement replacement pathway. The suppressive effect of *CFH* on the activity and formation of C3 invertase, formation of c5b9 membrane attack complex, and anaphylactic toxins C3a and C5a were observed previously^[4]. However, the neutralization of *CFH* on oxidized lipids was attenuated, and the toxicity and pro-inflammatory functions were enhanced by

Table 3 Hardy-Weinberg equilibrium test for genotype distribution of four SNP

SNP	Allele	Group	Genotype observed (expected)			HWp
			1/1	1/2	2/2	
rs800292	A/G	AMD	62 (0.36)	84 (0.49)	26 (0.15)	0.87
		Controls	32 (0.27)	60 (0.50)	28 (0.23)	1
rs1061170	C/T	AMD	60 (0.35)	82 (0.48)	30 (0.17)	0.88
		Controls	51 (0.43)	53 (0.44)	16 (0.13)	0.69
rs8044820	A/G	AMD	55 (0.32)	83 (0.48)	37 (0.20)	0.88
		Controls	37 (0.31)	52 (0.43)	31 (0.26)	0.15
rs9903	A/G	AMD	53 (0.31)	75 (0.44)	44 (0.26)	0.095
		Controls	52 (0.43)	52 (0.43)	16 (0.43)	0.69

SNP: Single nucleotide polymorphism; AMD: Age-related macular degeneration.

Table 4 Distribution of single nucleotide polymorphism genotypes in AMD and controls

SNP	Allele (1/2)	Group	Genotype, n (%)			χ^2	P
			1/1	1/2	2/2		
rs800292	A/G	AMD	62 (0.36)	84 (0.49)	26 (0.15)	4.523	0.034
		Control	32 (0.27)	60 (0.50)	28 (0.23)		
rs1061170	A/G	AMD	60 (0.35)	82 (0.48)	30 (0.17)	0.91	0.16
		Control	51 (0.43)	53 (0.44)	16 (0.13)		
rs8044820	A/G	AMD	55 (0.32)	83 (0.48)	37 (0.20)	0.691	0.41
		Control	37 (0.31)	52 (0.43)	31 (0.26)		
rs9903	A/G	AMD	53 (0.31)	75 (0.44)	44 (0.26)	8.18	0.004
		Control	52 (0.43)	52 (0.43)	16 (0.43)		

SNP: Single nucleotide polymorphism; AMD: Age-related macular degeneration.

Table 5 Distribution and odds ratio estimations of different single nucleotide polymorphism alleles in AMD patients and controls

SNP	Allele	AMD, n (%)	Controls, n (%)	P	OR	95%CI
rs800292	A	136 (0.4)	116 (0.48)	0.034	0.70	0.50-0.98
	G	208 (0.6)	124 (0.52)			
rs1061170	A	202 (0.59)	155 (0.65)	0.16	1.27	0.91-1.79
	G	142 (0.41)	85 (0.35)			
rs8044820	A	193 (0.56)	156 (0.52)	0.40	0.87	0.63-1.20
	G	151 (0.44)	84 (0.48)			
rs9903	A	181 (0.53)	156 (0.65)	0.004	1.60	1.15-2.22
	G	163 (0.47)	84 (0.35)			

AMD: Age-related macular degeneration; CI: Confidence interval; OR: Odds ratio; SNP: Single nucleotide polymorphism.

CFH variant Y402H, suggesting the significant regulatory roles of CFH Y402H for oxidative stress^[5]. In turn, oxidative stress is a multifactorial and complex pathogenic factor, and previous studies have shown that CFH can protect retinal pigment epithelial cells from hydrogen peroxide damage; however, the specific protective mechanisms remain unclear^[13].

Furthermore, autophagy is a catabolic process that plays an essential role in AMD occurrence and development^[12]. It can cause lysosomes to degrade cytoplasmic contents such as abnormal proteins and damaged organelles^[6,14]. Amino acids and fatty acids produced by protein hydrolysis and lipolysis are

further catabolized to produce cellular energy. Autophagy is activated under different conditions, and continuous autophagy requires a transcription mechanism^[15-16]. MAP1LC3B is necessary for autophagy-induced cycle prolongation^[17-18]. However, it remains to be investigated whether MAP1LC3B affects AMD development. Our study shows that the distribution of G alleles of *MAP1LC3B* rs9903 is higher in the AMD group, which may be significantly related to the occurrence of AMD. This may be related to the damage of retinal pigment epithelial cells caused by autophagy; nevertheless, the specific mechanism is not yet known.

This study also has several limitations. First, it lacks long-term clinical data and fails to correlate the clinical data with SNP typing. Second, there are many AMD phenotypes in humans due to genetic background, different lifestyles, and environmental effects. This study does not compare the populations in different environments and lifestyles so that they may be underrepresented in the broader range. Third, the healthy control participants are younger than the AMD patients because most patients were diagnosed at age 60y or older. Therefore, a selection bias might be introduced in this study by the age cut-off, leading to a lower power to detect real associations.

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REFERENCES

- Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet* 2018;392(10153):1147-1159.
- Heesterbeek TJ, Lorés-Motta L, Hoyng CB, Lechanteur YTE, den Hollander AI. Risk factors for progression of age-related macular degeneration. *Ophthalmic Physiol Opt* 2020;40(2):140-170.
- DeAngelis MM, Owen LA, Morrison MA, Morgan DJ, Li MY, Shakoor A, Vitale A, Iyengar S, Stambolian D, Kim IK, Farrer LA. Genetics of age-related macular degeneration (AMD). *Hum Mol Genet* 2017;26(R1):R45-R50.
- Vavvas DG, Small KW, Awh CC, Zanke BW, Tibshirani RJ, Kustra R. CFH and ARMS2 genetic risk determines progression to neovascular age-related macular degeneration after antioxidant and zinc supplementation. *Proc Natl Acad Sci U S A* 2018;115(4):E696-E704.
- Hong N, Shen Y, Yu CY, Wang SQ, Tong JP. Association of the polymorphism Y402H in the CFH gene with response to anti-VEGF treatment in age-related macular degeneration: a systematic review and meta-analysis. *Acta Ophthalmol* 2016;94(4):334-345.
- Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011;147(4):728-741.
- Cerulli RA, Shehaj L, Brown H, Pace J, Mei Y, Kritzer JA. Stapled peptide inhibitors of autophagy adapter LC3B. *Chembiochem* 2020;21(19):2777-2785.
- Flaxel CJ, Adelman RA, Bailey ST, Fawzi A, Lim JJ, Vemulakonda GA, Ying GS. Age-related macular degeneration preferred practice pattern®. *Ophthalmology* 2020;127(1):P1-P65.
- Ocular Fundus Diseases Society, Chinese Ophthalmological Society, Chinese Medical Association. Clinical pathway of age-related macular degeneration in China. *China J Ocul Fundus Dis* 2013;29(04):343-355.
- Yan X, Kang ZF, Li L, Guan RJ. Association between NF-κB polymorphism and age-related macular degeneration in a high-altitude population. *PLoS One* 2021;16(6):e0251931.
- Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003;19(1):149-150.
- Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;308(5720):385-389.
- Yang X, Hu J, Zhang J, Guan H. Polymorphisms in CFH, HTRA1 and CX3CR1 confer risk to exudative age-related macular degeneration in Han Chinese. *Br J Ophthalmol* 2010;94(9):1211-1214.
- Lauwen S, Jong EKD, Lefeber DJ, den Hollander AL. Omics biomarkers in ophthalmology. *Invest Ophthalmol Vis Sci* 2017;58(6):BIO88-BIO98.
- Zhang ZY, Bao XL, Cong YY, Fan B, Li GY. Autophagy in age-related macular degeneration: a regulatory mechanism of oxidative stress. *Oxid Med Cell Longev* 2020;2020:2896036.
- Wang SB, Wang XR, Cheng YQ, Ouyang WJ, Sang X, Liu JH, Su YR, Liu Y, Li CY, Yang L, Jin L, Wang ZC. Autophagy dysfunction, cellular senescence, and abnormal immune-inflammatory responses in AMD: from mechanisms to therapeutic potential. *Oxid Med Cell Longev* 2019;2019:3632169.
- Schaaf MBE, Keulers TG, Vooijs MA, Rouschop KMA. LC3/GABARAP family proteins: autophagy-(un)related functions. *FASEB J* 2016;30(12):3961-3978.
- Kang HM, Noh KH, Chang TK, Park D, Cho HS, Lim JH, Jung CR. Ubiquitination of MAP1LC3B by pVHL is associated with autophagy and cell death in renal cell carcinoma. *Cell Death Dis* 2019;10:279.