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Association of HTRA1 polymorphism and bilaterality in advanced age-related macular degeneration

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Abstract

Single nucleotide polymorphism (SNP), rs11200638, in the promoter of *HTRA1* has recently been shown to increase the risk for AMD. In order to investigate the association of this *HTRA1* polymorphism and the bilaterality of AMD, we genotyped rs11200638 in control, unilateral, and bilateral advanced AMD patients. The A allele for SNP rs11200638 in *HTRA1*, was significantly more prevalent in bilateral wet AMD and GA patients than in unilateral groups ($p = .02$ and $p = .03$, respectively). The homozygote odds ratios of bilateral wet AMD and GA are significantly greater than those seen in unilateral groups (twofold and threefold increase, respectively). This finding is consistent with the role of HTRA1 in AMD pathogenesis and will help aid in the clinical management and prognosis of AMD patients.

Keywords: Age-related macular degeneration; *HTRA1*; Genetics; Single nucleotide polymorphism

1. Introduction

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in elderly people worldwide, particularly in developed countries (Augood et al., 2006; Friedman et al., 2004; Resnikoff et al., 2004; Wang, Foran, & Mitchell, 2000). Advanced AMD can be divided into two forms—dry and wet. Dry AMD is characterized by geographic atrophy (GA) of the retinal pigmental epithelium (RPE) and overlying choroidal capillaries, whereas wet AMD is characterized by choroidal neovascularization (CNV). AMD can involve one or both eyes and approximately 40% of AMD patients have bilateral disease and 60% have unilateral pathology in cross-section prevalence studies (Augood et al., 2006; Topouzis et al., 2006).

The etiology and pathogenesis of AMD is still poorly understood. Both environmental and genetic factors contribute to the pathogenesis of AMD. Recently, a single nucleotide polymorphism (SNP), rs11200638, in the promoter of High Temperature Requirement A 1 (*HTRA1*) was found to be associated with advanced AMD (Cameron et al., 2007; Dewan et al., 2006; Yang et al., 2006). In this paper, we investigated the individual contribution of the SNP, rs11200638, to bilateral and unilateral AMD using an expanded Utah population. We demonstrate that rs11200638 confers a higher risk toward developing bilateral AMD rather than unilateral AMD, for both wet AMD and GA.

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2. Methods

2.1. Patients

The recruitment and research protocols were reviewed and approved by the Institutional Review Board of the University of Utah. All subjects provided informed consent prior to participation in the study. AMD patients as well as normal age-matched controls, all of which are Caucasian, were recruited in the Moran Eye Center at the University of Utah.

All participants had a standard examination protocol. Slit lamp biomicroscopy of the fundus using a 90 diopter lense was performed by an ophthalmologist. A pair of stereoscopic color fundus photographs (50°) were taken for all participants and fundus fluorescence angiography was performed for all AMD patients using a Topcon fundus camera (Topcon TRV-50VT, Topcon Optical Company, Tokyo, Japan) by trained ophthalmic photographers.

Diagnosis of advanced AMD was made and classified as GA or wet AMD according to the Age-Related Eye Disease Study (AREDS) system for classifying age-related macular degeneration (2001). Unilateral or bilateral AMD was determined at the final visit. AMD patients were classified into four groups: bilateral wet AMD, unilateral wet AMD, bilateral GA, and unilateral GA. Subtype of choroidal neovascularization in each wet AMD eye was classified according to the Macular Photocoagulation Study Group (1991). Normal age-matched controls were defined as individuals aged 60 years or older with no drusen or RPE changes. Other criterion meriting exclusion were ocular conditions that may lead to macular degeneration or subretinal neovascularization such as high myopia, retinal angiomatous proliferation, angioid streaks, juvenile macular degeneration, toxoplasmic retinochoroiditis, and diabetic retinopathy. Ten milliliters of blood was drawn from each participant for genotyping studies.

2.2. Genotyping

A Utah cohort of 774 advanced AMD patients and 294 age and ethnicity matched controls were genotyped for rs11200638. Lab personnel conducting the genotyping experiments were blinded to the phenotype of the participants. Genomic DNA was purified from blood drawn from each participant and the polymerase chain reaction was employed to genotype rs11200638 using oligonucleotide primers 5′-ATGCCACCCACA ACAACTTT-3′ and 5′-CGCGTCCTTCAAACTAATGG-3′ with 5% DMSO. Amplification parameters included denaturing at 95 °C for 3 min, followed by further denaturing at 94 °C for 30 s, annealing at 52 °C for 30 s, extending at 72 °C for 45 s, per cycle for 35 cycles, and finally extending at 72 °C for an additional 10 min. The 385 bp PCR product was digested by restriction endonuclease EagI (New England Biolabs, Ipswich, MA) generating 139 and 246 bp bands representing the normal G allele.

2.3. Statistical analysis

Statistical analysis was carried out using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL) and Microsoft Excel version 2003 (Microsoft, Redmond, WA). Deviation of genotype distributions from Hardy–Weinberg equilibrium was assessed using a χ^2 test in each group. Frequencies of rs11200638 SNP genotype and allele were compared among bilateral wet AMD, unilateral wet AMD, and control groups, and also among bilateral GA, unilateral GA, and control groups by logistic regression to assess association after adjusting for age and sex. Odds ratios (ORs) and 95% confidence intervals were also calculated to estimate risk size for the homozygote and heterozygote risk alleles. Because different angiographic subtypes of choroidal neovascularization may occur in the eyes of 1 patient, the association of rs11200638 SNP genotype and allele with choroidal neovascularization subtypes was carried out by comparing eyes instead of individuals by a χ^2 test. The criterion for statistical significance was set at $p < .05$.

3. Results

In total, there were 774 advanced AMD patients and 294 age and ethnicity matched controls enrolled in this study; 192 had bilateral wet AMD, 278 had unilateral wet AMD, 234 had bilateral GA, and 72 had unilateral GA. No significant difference for age or gender between bilateral and unilateral wet AMD groups or between bilateral and unilateral GA groups existed (Table 1).

All genotyping results were in Hardy–Weinberg equilibrium. Table 2 compares the genotypes and allele frequencies of rs11200638 among different groups. The frequencies of the A allele in both bilateral and unilateral wet AMD groups are significantly higher than controls with p values of 3.72×10^{-10} and 7.67×10^{-5} after adjusting for age and sex. Furthermore, the frequency of the A allele is significantly higher in the bilateral wet AMD group when compared to the unilateral wet AMD group with a p value of .02 after adjusting for age and sex. The AA genotype frequency is highest in the bilateral wet AMD group, second highest in the unilateral wet AMD group and lowest in the control group. The GG frequency is lowest in the bilateral wet AMD group, second lowest in the unilateral wet AMD group, and highest in the control group. Fig. 1 shows the ORs of the AA and AG genotype against the GG genotype among different phenotype groups. The homozygote OR of bilateral wet AMD as compared to controls (10.95, 95%CI 5.26–22.77) was 1.95-fold greater than the OR of unilateral wet AMD (5.62, 95%CI 2.65–11.90) when compared to controls. The same trend, although less significant, was also seen in heterozygotes. The genotype frequency is significantly different between bilateral and unilateral wet AMD in an additive model ($p = .02$, after adjusting for age and sex).

The allele and genotype frequencies in GA follow the same trend as explained for wet AMD. The A allele frequency is significantly higher in bilateral and unilateral GA than in controls ($p = 6.27 \times 10^{-10}$ and $p = .02$, respectively, after adjusting for age and sex, Table 2). The same allele frequency is also significantly higher in bilateral GA than in unilateral GA ($p = .03$, after adjusting for

Table 1
Demographic characters of difference phenotype groups

	Total	Male:Female	Age (mean ± *SD*)
Bilateral wet AMD	192	94:98	81.2 ± 7.9
Unilateral wet AMD	278	146:132	78.9 ± 12.1
Bilateral GA	234	89:128	81.0 ± 10.7
Unilateral GA	72	32:41	78.3 ± 12.7
Control	294	104:190	74 ± 12

Shown are demographic characters of AMD patients and control groups. There is no significant difference of sex ratio ($p = .45$, χ^2 test) or age ($p = .27$, independent t test) between bilateral and unilateral wet AMD groups. There is no significant difference of sex ratio ($p = .67$, χ^2 test) or age ($p = .28$, independent t test) between bilateral and unilateral GA groups. AMD, age-related macular degeneration; *SD*, standard deviation; GA, geographic atrophy.

Table 2
Frequencies of rs11200638 SNP genotypes and allele among bilateral and unilateral AMD patients and control

	Total	Genotype frequency			Allele frequency		Allele frequency logistic regression p value
		AA	AG	GG	A	G	
Bilateral wet AMD	192	40(20.8%)	99(51.6%)	53(27.6%)	179(46.6%)	205(53.4%)	3.72×10^{-10}
Unilateral wet AMD	278	36(12.9%)	146(52.5%)	96(34.5%)	218(39.2%)	338(60.8%)	7.67×10^{-5}
Bilateral GA	234	49(20.9%)	116(49.6%)	69(29.5%)	214(45.7%)	256(54.3%)	6.27×10^{-10}
Unilateral GA	72	6(8.3%)	39(54.2%)	27(37.5%)	51(35.4%)	93(64.6%)	.02
Control	294	10(3.4%)	128(43.5%)	156(53.1%)	148(25.2%)	440(74.8%)	

The p value of logistic regression comparing allele frequency is .02 between bilateral and unilateral wet AMD groups, and .03 between bilateral and unilateral GA groups. AMD, age-related macular degeneration; GA, geographic atrophy.

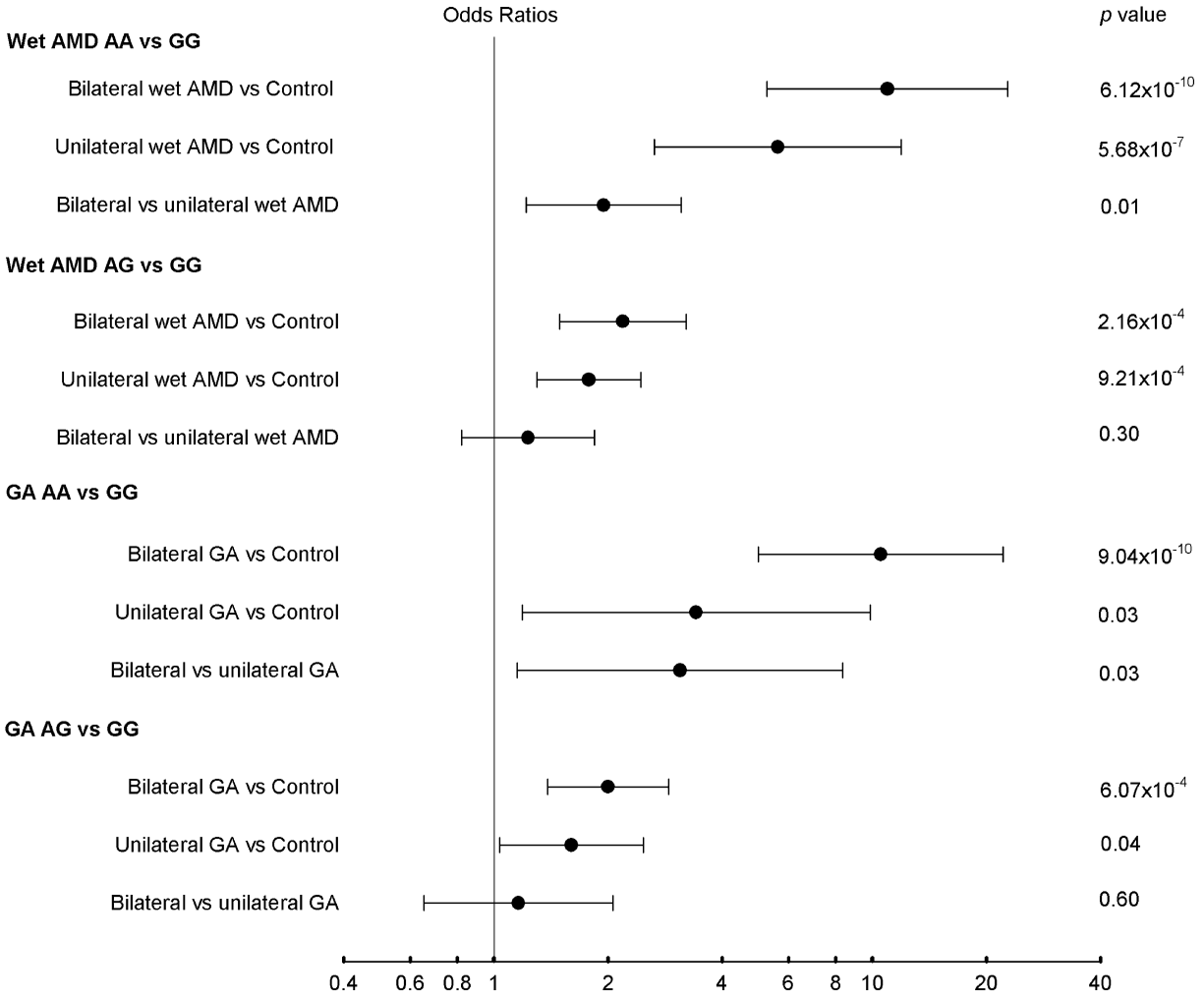

Fig. 1. Odds ratios of genotype frequency. Homozygote odds ratio (OR) of bilateral wet AMD against control (10.95, 95%CI 5.26–22.78) was 1.95-fold of OR of unilateral wet AMD (5.62, 95%CI 2.65–11.90). The same trend was seen in heterozygote with less extent. Homozygote OR of bilateral GA against control (10.51, 95%CI 5.00–22.11) was 3.10-fold of OR of unilateral GA (3.42, 95%CI 1.19–9.86). The same trend was seen in heterozygote with less extent.

age and sex, Table 2). The AA genotype frequency is highest in the bilateral GA group, second highest in the unilateral GA group, and lowest in the control group. The GG frequency is lowest in the bilateral GA group, second lowest in the unilateral GA group, and highest in the control group. The homozygote OR of bilateral GA as compared to controls (10.51, 95%CI 5.00–22.11) was 3.10-fold greater than the OR of unilateral GA (3.42, 95%CI 1.19–9.86). The same trend was seen in heterozygotes, although with less significance (Fig. 1). The genotype frequency is significantly different between bilateral and unilateral GA in an additive model ($p = .03$, after adjusting for age and sex).

There is no significant difference between allele and genotype frequency distribution among of the subtypes of

Table 3
Frequencies of rs11200638 SNP genotypes and allele among subtype of choroidal neovascularization

	Total eyes	Genotype frequency			Allele frequency	
		AA	AG	GG	A	G
Classic	104	13(12.0%)	61(56.5%)	34(31.5%)	87(40.3%)	129(59.7%)
Mix	248	53(21.4%)	119(48.0%)	76(30.6%)	225(45.4%)	271(54.6%)
Occult	137	18(13.1%)	80(58.4%)	39(28.5%)	116(42.3%)	158(57.7%)
Scar	166	32(19.9%)	83(50.0%)	50(30.1%)	149(44.9%)	183(55.1%)

There is no significant difference of allele or genotype frequencies among different subtypes of choroidal neovascularization. The p values are: .58 for allele frequency, .30 for homozygote AA against GG, and .73 for heterozygote AG against GG by χ^2 test.

wet AMD (Table 3). The p value is .58 for allele frequency, .30 for homozygote (AA against GG) frequency, and .73 for heterozygote (AG against GG) frequency distribution among groups as determined by a by χ^2 test.

4. Discussion

AMD may affect one eye or both eyes, yet more than half of AMD patients have unilateral involvement (Augood et al., 2006; Topouzis et al., 2006). Bilateral AMD corresponds to a more severe and debilitating stage of the disease. Association of bilaterality of AMD and the complement factor H (CFH) Y402H polymorphism, the first gene found to be associated with AMD, has been reported (Despriet et al., 2006). The OR of bilateral late AMD (17.93, 95%CI 9.00–35.70) was higher than that of unilateral late AMD (6.58, 95%CI 3.47–12.48) in CFH Y402H homozygotes. The same trend was seen in heterozygotes, but with less significance (Despriet et al., 2006).

SNP rs11200638 in the promoter of *HTRA1* has been reported to be strongly associated with wet AMD in Caucasian and Chinese populations, as well as to GA in Caucasian populations (Cameron et al., 2007; Dewan et al., 2006; Yang et al., 2006). Here we demonstrate that rs11200638 also contributes to the bilaterality of AMD. The A allele and AA genotype of rs11200638 were significantly more prevalent in bilateral wet AMD patients than in unilateral wet AMD patients (both $p = .02$, after adjusting for age and sex). Both were also more prevalent in bilateral GA patients than in unilateral GA patients (both $p = .03$, after adjusting for age and sex). The homozygote OR of bilateral wet AMD was about twofold greater than that of unilateral wet AMD. And the homozygote OR of bilateral GA was approximately threefold greater than that of unilateral GA. Our findings are consistent with the prior report that this SNP is associated with AMD and suggests a role for HTRA1 in the prognosis of AMD.

Bilateral AMD has a much greater impact on one's quality of life compared to unilateral AMD. With unilateral AMD, the affected patient loses stereoscopic vision but still can receive most visual information needed for essential activity in life and work. However, when AMD is bilateral, the affected individual loses all central vision required for reading, driving, cooking, and other activities in daily life. This affect results in despondency, inability to care for self or others, and a state of disability, which is equivalent to that experienced in coronary heart disease and stroke (Warpeha & Chakravarthy, 2003). Commonly, bilateral advanced AMD results in legal blindness, whereas unilateral AMD does not.

Our results show that the AA genotype carrier is 5.62 times more likely to develop unilateral wet AMD than the GG genotype carrier and 10.95 times more likely to develop bilateral wet AMD. An AA genotype carrier is 3.42 times more likely to develop unilateral GA than a GG counterpart and 10.51 times more likely to develop bilateral GA. These results can be used to estimate the risk of developing bilateral AMD patients and provide a guide for follow-up or intervention. For example, knowing that a patient carries the AA genotype and has one eye with advanced AMD, he or she should routinely be examined carefully in the nonaffected eye, which based on our results, is at high risk for developing advanced AMD.

The subtypes of choroidal neovascularization are an important guide for intervention, especially for laser or photodynamic therapy. It has been reported that the CFH 402HH genotype was significantly more prevalent in eyes with classic CNV than mixed or occult CNV (Wegscheider et al., 2007). In this study we compare the allele and genotype frequency of the polymorphism, rs11200638, among different subtypes of CNV and fail to find any significant difference.

In conclusion, our study indicates that a polymorphism in the *HTRA1* promoter confers higher risk to bilateral AMD than unilateral AMD. Our findings support the role of HTRA1 in the pathogenesis of AMD and can be used to estimate the risk of bilaterality and provide valuable prognosis in AMD patients.

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