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Original research

Complement factor H and LOC387715/ARMS2/HTRA1 variant's frequencies and phenotypic associations in neovascular age-related macular degeneration, a pilot study

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Abstract

***Purpose*:** To evaluate the frequency of 12 single nucleotide polymorphisms (SNPs) of complement factor H (CFH) and LOC387715/ARMS2/HRTA1 and their association with some of the presenting clinical features of neovascular age-related macular degeneration (AMD).
***Methods*:** In this prospective non-comparative case series forty four naïve patients with neovascular AMD were genotyped using sequencing or Sequenom iPLEX technology. Descriptive tests were used for displaying the magnitude of each allele, gender distribution, and age at diagnosis. Fisher exact test was used to evaluate the correlation between visual acuity (VA) and different alleles. Also Kruskal-Wallis test was used for comparison between age at the time of diagnosis and different alleles.
***Results*:** The most frequent SNP among studied patients was rs1061147 with 100% frequency rate. The least common was rs2672598 with a frequency of 52.27%. Only the allele rs800292 of CFH locus on 1q32 was associated with VA better than 20/200 (p value = 0.034). The frequency of this allele was 77.27% (34 patients) in this study. There was no significant association between any of alleles, and VA worse than 20/200($p > 0.05$). Fifteen patients had bilateral exudative AMD (34.09%). There was no significant difference between alleles in bilateral neovascular AMD and unilateral disease. Also bilateral and unilateral patients were not different in terms of age, gender or VA (p value: 0.330, 0.764 and 0.456 respectively). There was also no significant association between any of SNPs and bilaterality of disease.
***Conclusion*:** We designated the frequencies of SNPs of CFH and LOC387715/ARMS2/HRTA1 in neovascular AMD in a sample of Iranian patients. Only the allele rs800292 of CFH locus on chromosome 1q32 was associated with better VA.

Keywords: Complement factor H; Neovascular; Age-related macular degeneration; Single nucleotide polymorphism

Introduction

Age-related macular degeneration (AMD) is the leading cause of severe central visual loss in the elderly.[1,2] Its prevalence is estimated to be 13%–29.7% in people over 55 years.[3] One of the main reasons of visual loss in AMD is choroidal neovascularization (CNV),[4] which occurs in the neovascular form of the disease. Consequently, most available treatment modalities are directed against this advanced neovascular stage of disease.[5,6]

In addition to well-known risk factors such as aging, smoking, sunlight exposure, and family history,[7,8] many authors have addressed the role of genetics and special alleles in the pathogenesis of AMD as well as its clinical features.[9] Identification of exact genes and their either offensive or protective role in this disease can clearly alter the therapeutic approaches for AMD.

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Complement factor H gene (CFH) Y402H variant on 1q32 and several adjacent alleles on 10q26 (loc387715/ARMS2 gene and HtrA serine peptidase 1 gene) have been reported to be strongly associated with neovascular AMD. There are also some conflicting reports about the association of these alleles and some clinical and angiographic features of AMD.[7–19]

In this study, we investigated the frequency of some of the previously reported alleles associated with neovascular AMD as well as the association between these alleles and clinical features of AMD.

Methods

We enrolled 44 patients who were referred to the Retina Service of Farabi Eye Hospital of Tehran University of Medical Sciences (TUMS) between February to April 2014. The study protocol was approved by the review board of Farabi Eye Hospital and the Committee of Medical Ethics of TUMS. Moreover, informed written consent was obtained from all patients.

After recording demographic data (age at the time of diagnosis, gender, family history) and patient medical history, a complete bilateral ophthalmic examination was performed for each patient as follows: examining best corrected visual acuity (BCVA) (using snellen chart and then converting it to logMAR), anterior segment examination, intraocular pressure measurement, and full dilated fundoscopy. The inclusion criteria were presence of neovascular AMD at least in one eye which was defined by having CNV, subretinal hemorrhage, fibrosis, and angiographic documentation of the CNV at the time of diagnosis (using Heidelberg fluorescein angiography) or before entering the study. All the patients with suspicious polypoidal choroidal vasculopathy and retinal angiomatous proliferation were evaluated by indocyanine green (ICG) angiography and excluded from the study if the diagnosis was confirmed. Patients with pathologic myopia, angioid streaks, choroidal rupture, any history of retinal laser treatment, or any disease condition other than AMD which can cause CNV and any history of intravitreal pharmacologic injection treatment were excluded. All patients were treatment naïve and no previous treatment had been performed.

Presence of dry or wet type AMD in the other eye was also recorded. In patients with bilateral neovascular involvement, the eye with a worse clinical state was chosen for statistical analysis. All the patients or their information profile including fluorescein angiography were reviewed at least by 2 retinal sub specialists.

Genetic analysis

15 ml of peripheral blood samples from each 44 of the patients nAMD was collected by antecubital venipuncture into ethylenediaminetetraacetic acid (EDTA)-containing tubes. After adding 10 ml of Red Cell Lysis Buffer and mixing completely, samples were centrifuged for 10 min at 1,300 g (3–30k Refrigerated Centrifuge, Sigma, Germany). After discarding supernatant and adding 10 ml Phosphate Buffered Salts (PBS Tablets; TAKARA BIO INC., Japan), cell pellets were suspended again and centrifuged for 8 min at 1,200 g for washing, twice.

Harvested Cells were used for genomic DNA extraction with a DNA blood kit (QIAamp® DNA Blood Mini kit; Qiagen, Germany) according to the manufacturer's protocol (which was briefly, 20 µl proteinase K was added to the 200 µl of cells plus 200 µl lysis buffer. After adding 200 µl ethanol and vortexing, samples were transferred to the columns and centrifuged at 6000 g for 1 min. Then 500 µl washing buffer was added and centrifuged at 20,000 g for 3 min. Finally, 20 ng of purified DNA was used for genotyping analysis). For genetic analysis Sequenom iPLEX system technology was used (Sequenom, San Diego, CA, USA) to detect AMD related SNPs in the following order: rs203674, rs800292, rs35507625, rs572515, rs1061147, rs7529589, rs1061170, rs12038333, rs2274700, for CFH gene on chromosome 1 and rs10664316, rs11200638, rs2672598 for LOC387715/ARMS2/HTRA1 gene on chromosome 10.

Statistical analysis

Statistical analysis was performed using SPSS 16 software (SPSS, Inc., Chicago,IL). Prescriptive tests were used for displaying the magnitude of each allele, gender distribution, and age (mean ± SD). Visual acuities were converted to the logarithm of the minimal angle of resolution (logMAR) units and were categorized into 2 groups: logMAR$\leq$1 (snellen acuity $\geq$ 20/200) as better visual acuity (VA) and logMAR>1 (snellen visual acuity <20/200) as worse VA. Fisher exact test used to evaluate the correlation between VA and different alleles. Kruskal–Wallis test was also used for comparison between age at the time of diagnosis and different alleles. $p < 0.05$ was considered statistically significant. The association between SNPs and age groups (equal or less than 75 years old versus more than 75 years old), sex, and laterality (disease affecting one eye or both eyes of the patients) have been assessed by using chi square test. The Hardy–Weinberg Equilibrium was calculated for each SNP, and all the SNPs were in Hardy–Weinberg Equilibrium.

Results

44 eligible patients entered the study. 28 patients were male (63.6%), and 16 patients were female (36.4%). The mean age of patients was 74.63 ± 7.55 years (ranged from 58 to 90 years). Mean VA of patients was 1.7 ± 0.8 logMAR. The frequencies of all SNPs among patients are detailed in Table 1. The most frequent SNP among study patients was rs1061147 with 100% frequency. The least common was rs2672598 with a frequency of 52.27%.

Only the allele rs800292 of CFH locus on 1q32 was associated with VA better than 20/200 (p value = 0.034). Mean VA of the patients with this allele was 0.1 ± 0.12 logMAR. The frequency of this allele was 77.27% (34 patients). There was

Table 1
Statistical association of each SNP with age, sex,VA, and laterality.

Gene	SNP	Age (≤75 years vs > 75 years) (p value)	Sex (p value)	Better VA (better than 20/200) (p value)	Laterality (bilateral or unilateral) (p value)
CFH	rs203674	0.994	0.265	0.513	0.330
CFH	rs572515	0.820	0.561	0.773	0.456
CFH	rs800292	0.590	0.886	0.034	0.764
CFH	rs1061147	0.800	0.977	0.444	0.822
CFH	rs1061170	0.378	0.820	0.435	0.424
CFH	rs2274700	0.555	0.197	0.772	0.672
CFH	rs7529589	0.424	0.539	0.591	0.515
CFH	rs12038333	0.185	0.208	0.247	0.522
CFH	rs35507625	0.985	0.892	0.326	0.342
LOC387715/ARMS2	rs11200638	0.672	0.472	0.262	0.635
LOC387715/ARMS2	rs10664316	0.966	0.773	0.342	0.514
HTRA1	rs2672598	0.633	0.680	0.355	0.625

SNP: single nucleotide polymorphisms, VA: visual acuity, CFH: complement factor H, ARMS2: age related maculopathy susceptibility 2 gene, HTRA1: HtrA serine peptidase 1 gene.

no significant correlation between any of alleles and VA worse than 20/200.

Fifteen patients had bilateral neovascular AMD (34.09%). There was no significant difference between allele frequencies between bilateral and unilateral AMD groups.

Discussion

AMD is one of the most common causes of blindness in the elderly worldwide. Multiple risk factors have been proposed in the pathogenesis of this disease. The role of genetic factors in the etiology of AMD is documented, and several predisposing SNPs have been proposed to be associated with AMD (Table 3).[7–30] The most important SNPs are CFH gene on chromosome 1 and LOC387715/ARMS2/HTRA1 on chromosome 10.[11–17,23] CFH gene expression affects the binding affinity of CFH glycoprotein to C-reactive protein and heparin and regulates its anti-inflammatory effects.[17] The exact mechanism of action of the LOC387715 gene product is not clearly understood.[29] Allele frequency of these genes was between 61 and 94% in AMD patients in some studies.[9,12–18,24] However, the frequency is not constant across different ethnic groups. In some reports of Chinese and Turkish population samples, the frequencies are lower than what has been reported in other ethnic groups, especially Caucasians.[23,28] In this study, the frequencies of some of the previously reported SNPs in neovascular AMD patients were evaluated in a sample of an Iranian population.

In our case series, rs1061147 from CFH genes on 1q32 was the most common allele (100% frequency rate), and the least common SNP was rs2672598 from loc387715/ARMS2/HTRA1 on 10 q26 (frequency rate: 52.27%). All the other SNPs' frequencies ranged from 52.27% to 99.9% (Table 2).

These frequencies are consistent with some of the previous studies that reported similar findings in their own ethnic populations.[9,12–18,24] In the Andreoli cohort study, similar frequencies for both CFH and LOC-387715/ARMS2/HRTA1 SNPs[9] were found. However, in some other ethnic populations, the reported frequencies were different. For example, Chen et al. reported a frequency of 5.8% for CFH genes in

Table 2
Single nucleotide polymorphism analyzed in 44 nonvascular AMD patients.

Gene	SNP	Base change[a]	Total frequency (%)	Frequency of homozygous common allele	Frequency of heterozygous allele	Frequency of homozygous rare allele
CFH	rs203674	C→A	90.90%	0.365	0.414	0.219
CFH	rs572515	T→C	93.18%	0.341	0.414	0.243
CFH	rs800292	C→A	77.27%	0.735	0.26	0
CFH	rs1061147	A→C	100%	0.509	0.372	0.117
CFH	rs1061170	C→T	70.45%	0.387	0.483	0.129
CFH	rs2274700	C→T	95.45%	0.547	0.285	0.166
CFH	rs7529589	T→C	88.63%	0.358	0.512	0.128
CFH	rs12038333	G→A	90.90%	0.35	0.45	0.2
CFH	rs35507625	del	93.18%	0.829	0.121	0.04
LOC387715/ARMS2	rs11200638	A→G	63.63%	0.5	0.357	0.142
LOC387715/ARMS2	rs10664316	Del AT	86.36%	0.552	0.342	0.105
HTRA1	rs2672598	G→A	52.27%	0.565	0.434	0

CFH = complement factor H gene, ARMS2 = age-related maculopathy susceptibility 2 gene, HTRA1 = HtrA serine peptidase 1 gene, SNP = single nucleotide polymorphism.

[a] Base change is written common allele > rare allele.

Table 3
Effect of SNPs on clinical findings of AMD patients in different studies.

Year	Author	Countery	Study design	Mean age	Male/female ratio	Earlier age of onset	Better visual acuity (better than 20/200)	Worse visual acuity	Bilateral involvement	Larger CNV size
2014	This study	Iran	Case series	74.63 ± 7.55	63.6%/36.4%	Non	rs800292	Non	Non	Non
2009	Andreoli[9]	USA	Retrospective cohort	72.5 ± 7.8	46.4%/53.6%	rs11200638 and rs10490924	rs10664316 and rs1049331	Non	Non	rs11200638
2011	Hiroaki Bessho[31]	Japan	Retrospective cohort	76 ± 6	85.4%/14.6	Non	Non	Non	Non	rs10490924
2008	Leveziel N[19]	France	Cohort	72.8 ± 8.8	32%/68%	rs11200638 and rs10490924	Non	Non	Non	Non
2007	Brantley MA[20]	USA	Retrospective cohort	79.8	36%/64%	rs11200638 and rs10490924	Non	Non	Non	rs1061170
2009	Brantle MA[21]	USA	Case-control	78.9 ± 8.1	35.6%/64.8%	rs11200638 and rs10490924	Non	Non	Non	Non
2008	Shuler RK[22]	USA	Case-control	76.4	33.6%/66.4%	rs11200638 and rs10490924	Non	Non	Non	Non
2011	Chen H[32]	USA	Cohort	75.1 ± 9.0	46.4%/53.6%	Non	Non	Non	rs11200638/rs 10490924	Non
2009	Pai AS[27]	Australia	Cross-sectional	73.9 ± 8.3/ 80.4 ± 7.9	34.6%/65.4%	Non	Non	Non	CFH CC genes	Non
2007	Shuler RK[30]	USA	Cross-sectional	75.8 ± 8.6	44.2%/55.8%	rs10490924 and CFH (T1277C at rs1061170, or Y402H)	Non	Non	Non	Non
2010	Nicolas Leveziel[33]	France	Cohort	80.6 ± 5.8	33.4%/66.6%	Non	Non	rs10490924 pp/rs10611710 pp	rs10490924 pp/rs10611710 pp	Non

SNP: single nucleotide polymorphisms, AMD: age related macular degeneration, CNV: choroidal neovascularization, CFH: complement factor H.

their Chinese AMD patients.[23] In the Iranian population, Babanejad et al.[25] reported the same frequency for rs800292 C allele, but the reported frequencies for rs2274700 and rs1061170 (either C allele, rare allele and heterozygous allele) was different from our study. Additionally, in Nazari Khanamiri et al.'s case–control study,[24] Y402H and A69S polymorphisms were strongly associated with AMD in a sample of the Iranian population.[24]

Among 12 alleles assessed in this study, only rs800292 of CFH SNPs was associated with VA better than 20/20 (p value 0.034). This is not in accordance with previously published results by Andreoli et al.[9] which found LOC387715/ARMS2 rs10664316 and HTRA1 rs1049331 as the SNPs which were associated with protection from worse visual acuity at the time of diagnosis.

We also did not find any association between assessed alleles and age or gender. Some studies reported rs11200638 and rs10490924 from LOC387715/ARMS2 to be associated with earlier age of onset of AMD.[9,19–23]

In this case series, there was no difference between unilateral or bilateral involvement considering SNP frequencies, but it has been reported in previous trials that CFH genes also rs11200638 cause susceptibility to bilateral AMD involvement [26,27].

This is one of the few studies which has evaluated AMD genetics in an Iranian population.[24, 25] Limitations of this study include a lack of a control group and small sample size.

In conclusion, AMD seems to have a strong genetic pathogenesis which may influence its clinical features. Further studies which include a larger sample size, a control group, and careful follow-up will clarify more details of this multifactorial prevalent disease pathogenesis.

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