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

Reza Karkhane*, Aliasghar Ahmadraji, Mohammad Riazi Esfahani, Ramak Roohipour, Zahra Alami Harandi, Alireza Lashay, Mehdi Sharifzadeh Kermani, Reza Roozafzoon, Ahad Khoshzaban

Stem Cell Preparation Unit, Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran

Received 19 September 2015; accepted 15 December 2015

Available online 8 March 2016

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, which 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.

Copyright © 2016, Iranian Society of Ophthalmology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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. Its prevalence is estimated to be 13%—29.7% in people over 55 years. One of the main reasons of visual loss in AMD is choroidal neovascularization (CNV), which occurs in the neovascular form of the disease. Consequently, most available treatment modalities are directed against this advanced neovascular stage of disease.

In addition to well-known risk factors such as aging, smoking, sunlight exposure, and family history, many authors have addressed the role of genetics and special alleles in the pathogenesis of AMD as well as its clinical features. Identification of exact genes and their either offensive or protective role in this disease can clearly alter the therapeutic approaches for AMD.
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≤1 (snellen acuity ≥ 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
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).

The most important SNPs are CFH gene on chromosome 1 and LOC387715/ARMS2/HTRA1 on chromosome 10. CFH gene expression affects the binding affinity of CFH glycoprotein to C-reactive protein and heparin and regulates its anti-inflammatory effects. The exact mechanism of action of the LOC387715 gene product is not clearly understood. Allele frequency of these genes was between 61 and 94% in AMD patients in some studies. 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. 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 10q26 (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.

In the Andreoli cohort study, similar frequencies for both CFH and LOC-387715/ARMS2/HTRA1 SNPs 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 1

| 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 | rs10664136 | 0.966 | 0.773 | 0.342 | 0.514 |
| HTRA1 | rs2672598 | 0.633 | 0.680 | 0.355 | 0.625 |

Table 2

| Gene | SNP | Base change | 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 | 63.65% | 0.5 | 0.357 | 0.142 |
| LOC387715/ARMS2 | rs10664136 | Del AT | 86.36% | 0.352 | 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.
their Chinese AMD patients. In the Iranian population, Babanejad et al. 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 Khana-miri et al.’s case-control study, Y402H and A69S polymorphisms were strongly associated with AMD in a sample of the Iranian population.

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. 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.

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.

Acknowledgments

We would like to express our gratitude and thanks to the nursing, administrative, and secretarial staff of the Retina Department and Clinic at Farabi Eye Hospital, especially to Elmira Tabatabei and Masoumeh Torabi for their contribution to the maintenance of our patient records without which this project would have been impossible.

References

1. Bressler NM, Bressler SB, Congdon NG, et al. Potential public health impact of age-related eye disease study results: AREDS Report No. 11. *Arch Ophthalmol*. 2003;121:1621–1624.
2. Brown GC, Brown MM, Sharma S, et al. Age-Related Eye Disease Study Research Group. The burden of age-related macular degeneration: a value-based analysis. *Curr Opin Ophthalmol*. 2006;17:257–266.
3. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology*. 1992 Jun;99:933–943.
4. Subramanian ML, Ness S, Abedi G, et al. Bevacizumab vs ranibizumab for age-related macular degeneration: early results of a prospective double-masked, randomized clinical trial. *Am J Ophthalmol*. 2009 Dec;148:875–882.

---

**Table 3**

| Year | Author          | Country | Study design     | Mean age (± SD) | Male/female ratio | Earlier age of onset | Better visual acuity (better than 20/2000) | Worse visual acuity | Bilateral involvement | Larger CNV size |
|------|-----------------|---------|------------------|-----------------|-------------------|---------------------|-------------------------------------------|---------------------|----------------------|-----------------|
| 2014 | This study      | Iran    | Case series Retrospective cohort | 74.63 ± 7.55    | 63.6%/36.4%       | Non                 | rs800292 rs11200638 and rs10490924 | Non                 | Non                  | Non             |
| 2009 | Andreoli        | USA     | Retrospective cohort | 72.5 ± 7.8      | 46.4%/53.6%       |                     | rs800292 rs11200638 and rs10490924 | Non                 | Non                  | Non             |
| 2011 | Hiroaki Bessho  | Japan   | Retrospective cohort | 76 ± 6          | 85.4%/14.6%       | Non                 | Non                                       | Non                 | Non                  | Non             |
| 2008 | Leveziel N    | France  | Cohort            | 72.8 ± 8.8      | 32%/68%           | Non                 | rs11200638 and rs10490924 | Non                 | Non                  | Non             |
| 2011 | Brantley MA    | USA     | Case-control       | 79.8            | 36%/64%           | Non                 | rs11200638 and rs10490924 | Non                 | Non                  | Non             |
| 2009 | Shuler RK     | USA     | Case-control       | 76.4            | 33.6%/66.6%       | Non                 | rs11200638 and rs10490924 | Non                 | Non                  | Non             |
| 2011 | Chen H         | USA     | Cohort             | 75.1 ± 9.0      | 46.4%/53.6%       | Non                 | Non                                       | Non                 | Non                  | Non             |
| 2009 | Pai AS         | Australia | Cross-sectional | 73.9 ± 8.3/80.4 ± 7.9 | 34.6%/65.4%       | Non                 | Non                                       | Non                 | Non                  | Non             |
| 2009 | Shuler RK     | USA     | Cross-sectional   | 75.8 ± 8.6      | 44.2%/55.8%       | Non                 | rs10490924 and CFH (T1277C at rs1061170, or Y402H) | Non                 | Non                  | Non             |
| 2010 | Nicolas Leveziel | France | Cohort            | 80.6 ± 5.8      | 33.4%/66.6%       | Non                 | rs10490924 pp/rs10611710 | Non                 | Non                  | Non             |

SNP: single nucleotide polymorphisms, AMD: age related macular degeneration, CNV: choroidal neovascularization, CFH: complement factor H.
5. Brown DM, Michels M, Kaiser PK, et al., ANCHOR Study Group. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: two-year results of the ANCHOR study. *Ophthalmology*. 2009 Jan;116:57–65.e5.

6. Boyer DS, Antoszyk AN, Awh CC, et al., MARINA Study Group. Subgroup analysis of the MARINA study of ranibizumab in neovascular age-related macular degeneration. *Ophthalmology*. 2007 Feb;114:246–252.

7. Chen Wen, Xu Wei, Tao Qiushan, et al. Meta-analysis of the association of the HRTA1 polymorphisms with the risk of age-related macular degeneration. *Exp Eye Res*. 2009;89:292–300.

8. Fisher SA, Abecasis GR, Yashar BM, et al. Meta-analysis of genome scans of age-related macular degeneration. *Hum Mol Genet*. 2005 Aug 1;14:2257–2264.

9. Andreoli MT, Morrison MA, Kim BJ, et al. Comprehensive analysis of complement factor H and loc387715/ARMS2/HTRA1 variants with respect to phenotype in advanced age-related macular degeneration. *Am Ophthalmol*. 2009;148:869–874.

10. DeAngelis MM, Ji F, Kim IK, et al. Cigarette smoking, CFH, APOE, the HRTA1 serine peptidase gene alter the risk of neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2006;47:1215–1220.

11. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in macular degeneration. *Science*. 2005;308:385–389.

12. Edwards AO, Ritter 3rd R, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308:421–424.

13. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419–421.

14. Spencer KL, Hauser MA, Olson LM, et al. Deletion of CFHR3 and CFHR1 genes in age-related macular degeneration. *Hum Mol Genet*. 2008 Apr 1;17:971–977.

15. Zareparasi S, Branham KE, Li M, et al. Strong association of the Y402H variant in complement factor H at lq32 with susceptibility to age-related macular degeneration. *Am J Hum Genet*. 2005;77:149–153.

16. Magnusson KP, Duun S, Sigurdsson H, et al. CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *Plos Med*. 2006;3:e5.

17. Sepp T, Khan JC, Thurlby DA, et al. Complement factor H variant Y402H is a major risk determinant for geographic atrophy and choroidal neovascularization in smokers and nonsmokers. *Invest Ophthalmol Vis Sci*. 2006;47:536–540.

18. DeAngelis MM, Ji F, Adams, et al. Alleles in the HrtA 1 serine peptidase gene alter the risk of neovascular age-related macular degeneration. *Am Acad Ophthalmol*. 2008;115:1209–1215.

19. Leveziel N, Zerbib J, Richard F. Genotype-phenotype correlations for exudative age-related macular degeneration associated with homozygous HTRA1 and CFH genotypes. *Invest Ophthalmol Vis Sci*. 2008;49:3090–3094.

20. Brantley Jr MA, Fang AM, King JM, et al. Association of complement factor H and LOC387715 genotypes with response of exudative age-related macular degeneration to intravitreal bevacizumab. *Ophthalmology*. 2007;114:2168–2173.

21. Brantley Jr MA, Edelstein SL, King JM, et al. Association of complement factor H and LOC387715 genotypes with response of exudative age-related macular degeneration to photodynamic therapy. *Eye*. 2009;23:626–631.

22. Shuler Jr RK, Schmidt S, Gallins P, et al. Phenotype analysis of patients with the risk variant LOC387715 (A69S) in age-related macular degeneration. *Am J Ophthalmol*. 2008;145:303–307.

23. Chen LJ, Liu DT, Tam PO, et al. Association of complement factor H polymorphisms with exudative age-related macular degeneration. *Mol Vis*. 2006;12:1536–1542.

24. Nazari Khamaniri Hossein, Ghasemi Falavarjani Khalil, Sanati Mohammad Hossein, et al. Complement factor H Y402H and LOC387715 A69S polymorphisms in association with age-related macular degeneration in Iran. *J Ophthalmic Vis Res*. 2014;9:181–187.

25. Babanejad Mojgan, Moein Hamidreza, Akbari Mohammad R, et al. Investigating the CFH gene polymorphisms as a risk factor for age-related macular degeneration in an iranian population. *ophthalmic genetics*. 2015;1–6. Early Online.

26. Chen H, Yang Z, Gibbs D, et al. Association of HTRA1 polymorphism and bilaterality in advanced age-related macular degeneration. *Vis Res*. 2008 Feb;48:690–694.

27. Pai Amy Shih-I, Mitchell Paul, Rochtchina Elena, Pai AS, Mitchell P, et al. Complement factor H and the bilaterality of age-related macular degeneration. *Arch Ophthalmol*. 2009 Oct;127:1339–1344.

28. Yücel D, Yilmaz M, Durukan AH, Öztürk RK. Association of CFH Y402H polymorphism with both forms of advanced age-related macular degeneration in Turkish patients. *Ophthalmic Genet*. 2012;33:144–149.

29. Sobrin L, Reynolds R, Yu Y, et al. ARMS2/HTRA1 locus can confer differential susceptibility to the advanced subtypes of age-related macular degeneration. *Am J Ophthalmol*. 2011 Feb;151(2), 345–52.e3.

30. Shuler RK, Hauser MA, Caldwell J, et al. Neovascular age-related macular degeneration and its association with LOC387715 and complement factor H polymorphism. *Arch Ophthalmol*. 2007;125:63–67.

31. Bessho Hiroaki, Honda Shigeru, Kondo Naoshi, Negi Akira. The association of complement factor H at 1q32 with susceptibility to age-related macular degeneration to photodynamic therapy. *Mol Vis*. 2014;9:181–187.

32. Chen Yuhong, Zeng Jiexi, et al. Assessing susceptibility to age-related macular degeneration to photodynamic therapy. 2009;23:626–631.