Association of Htra1 Gene Polymorphisms with the Risk of Developing AMD in Iranian Population

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Abstract

Background: Half of the cases of vision loss in people under 60 years of age have been attributed to age-related macular degeneration (AMD). This is a multifactorial disease with late onset. It has been demonstrated that many different genetic loci are implicated in the risk of developing AMD in different populations. In the current study, we investigated the association of high-temperature requirement A-1 (HTRA1) gene polymorphisms with the risk of developing AMD in the Iranian population.

Methods: Genomic DNA samples were extracted from 120 patients with AMD and 120 healthy age- and sex-matched controls. A 385 base-pair fragment of the HTRA1 gene promoter region was amplified using the polymerase chain reaction (PCR) technique and sequenced. The frequencies of the alleles were calculated and statistical analysis was performed using SPSS software.

Results: Our study demonstrated that the rate of polymorphisms rs11200638 -625 G>A and rs2672598 -487T>C were significantly greater in AMD patients than in healthy controls from the Iranian population.

Conclusions: The results of our study indicate that HTRA1 gene promoter region polymorphisms are associated with the risk of developing AMD in the Iranian population.

Keywords: HTRA1, Iran, Macular Degeneration, Single Nucleotide Polymorphisms

Introduction

Age-related macular degeneration (AMD) is a neurodegenerative disease that affects vision in the elderly in the developed countries and also causes about half of the cases of vision loss in people under 60 (1). The onset of the disease and its primary stages in adults and the elderly is subclinical and emerges gradually (2). Chronic degeneration of light receptors in the retinal

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Received: Dec 06, 2014; Accepted: Jan 29, 2015
pigment epithelium (RPE) results in vision impairment in these patients (3). The primary stage of the disease, called age-related maculopathy (ARM), is due to drusen (cellular debris) associated with hyperpigmented or depigmented regions. Advanced states of the disease are divided into two main types: dry, also called atrophic or non-exudative AMD, and wet, also called neovascular or exudative AMD (4).

Presence of drusen and/or abnormal pigmentation in the RPE and geographic atrophy (GA) are common characteristics of the dry form, while the presence of serous detachments in the RPE, or chondroidal neovascularization (CNV), and are common characteristics of the wet form. The cause of the wet form of the disease is excessive sub- or intra-RPE ingrowth of blood vessels resulting in hemorrhage and the formation of scar tissue in the neovascularization site. Exudative GA-associated forms of the disease could result in severe vision impairment or loss of vision (4-6).

Epidemiologic studies indicate that about 50 million people suffer from AMD worldwide, with eight million in the United States. The rate of disease is increasing in the elderly (7, 8). There is currently no estimation about the rate of the disease in Iran. Although no distinct causes have been described for developing AMD, studies have demonstrated that numerous genetic and environmental factors, including ageing, are included in the pathogenesis of the disease (9). Familial history of AMD, smoking, alcohol consumption, nutrition, and specific drugs have been implicated in the risk of developing AMD (7, 10-12). Furthermore, numerous studies have discovered the roles of numerous genes in the pathogenesis of the disease. These genes include the factor H complement gene located in the 1q32 region, LOC387715 (ARMS2)/HTRA1 locus in 10q26 region, and genes of the B, C2, and C3 complement systems (6, 13-17). High-temperature requirement A-1 (HTRA1) is a member of heat shock proteins and serine protease family expressed in human and mouse retinas. Ageing is associated with the increase of the expression of this gene in the retina (18, 19). Currently, several polymorphisms on the HTRA1 locus have been identified and their associations with AMD studied. Among these polymorphisms the SNP rs11200638 -625 G>A has been shown to be associated with increased HTRA1 expression and the risk of developing AMD in different populations (15, 20-26). As yet, a potential association between variations in the HTR1 gene and risk of developing AMD has not been studied in the Iranian population. In the current study we analyzed the allele frequency in patients and a control group from the Iranian population and the association of these alleles with the risk of developing AMD.

Materials and Methods

Study design

In this cross-sectional case-control study 120 AMD patients and 120 age- and sex-matched healthy controls were recruited. Patients were from a mixed Iranian population who were admitted to the Fazeli-Sanati Medical Genetics Laboratory in Tehran. All study protocols complied with the Helsinki Declaration for studies including human subjects and were approved by the medical ethics committee of Tehran University of Medical Sciences.

Demographic information of all patients and controls was recorded. Patients of 55 years of age or more who were suffering from exudative or non-exudative AMD in at least one eye and did not suffer from other non-AMD related complications, such as diabetic retinopathies or degenerative myopathy, were included in this study. The control group included age- and sex-matched people who were free of primary or advanced AMD and other ocular diseases, such as cataracts and macular hemorrhages.

To diagnose AMD, patients and controls were examined by an ophthalmologist in the Fazeli-Sanati Medical Genetics Laboratory in Tehran. The examinations included visual acuity measurements, fundus examinations, retinal photographs, and fluorescein angiography. Finally, the disease was diagnosed and classified according to the Age-related Eye Disease Study Research Group (AREDS) (27).

Genotyping

Five ml of venous blood were collected from each subject and DNA was extracted using a GeneJET DNA Extraction kit (Fermentas, Lithuania). DNA samples were stored at -80°C. Primer pairs 5’-ATGCCACCCACAAACAACCTTT-3’ and 5’-CGCGTCTTCTAAACTAATGG-3’
were used to amplify 385 base pairs of the HTRA1 gene promoter region according to previous studies (24). PCR products were sequenced using an ABI 3130 sequencer (Applied Biosystems, USA). Sequencing results were compared with dbSNP database to determine the presence of polymorphisms in subjects.

**Statistical analysis**  
Data were analyzed using SPSS software version 16 (IBM, USA). Descriptive data were represented as mean ± standard deviation and percent. Descriptive statistics, correlation, logistic regression, chi-square and independent t-test were used for data analysis. P-values less than 0.05 were considered significant. The Hardy-Weinberg test was used to examine the frequency of alleles in the study population.

**Result**  
To study the polymorphisms of the HTRA1 gene promoter region, 120 patients 72 ± 8.11 years of age and 120 healthy controls 71.5 ± 6 years of age were recruited. Patients had a higher rate of hypertension (p = 0.01) and more family histories of AMD (p = 0.007) than control group subjects (Table 1).

Table 1. Clinical and demographic data of study population

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>AMD Patients</th>
<th></th>
<th></th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet</td>
<td>Dry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td>88 (73.3%)</td>
<td>32 (26.7%)</td>
<td></td>
<td>120 (100%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Average Age (SD) year</td>
<td>71.6 (8.5)</td>
<td>72.6 (7)</td>
<td></td>
<td>71.6 (6)</td>
<td>0.233</td>
</tr>
<tr>
<td>Male/Female</td>
<td>48/40</td>
<td>23/9</td>
<td></td>
<td>71/49</td>
<td>0.374</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>29 (24.2%)</td>
<td>20 (16.7%)</td>
<td></td>
<td>32 (26.6%)</td>
<td>0.010</td>
</tr>
<tr>
<td>Smoking habit, n (%)</td>
<td>18 (15%)</td>
<td>6 (5%)</td>
<td></td>
<td>16 (13.3%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>6 (5%)</td>
<td>2 (1.6%)</td>
<td></td>
<td>0.0 (0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Linkage disequilibrium of polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A

<table>
<thead>
<tr>
<th>rs11200638 -625 G&gt;A</th>
<th>rs2672598 -487T&gt;C</th>
<th>T&gt;C</th>
<th>Total</th>
<th>p</th>
<th>x2</th>
<th>D</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>58</td>
<td>0</td>
<td>4</td>
<td>62</td>
<td></td>
<td>0.93</td>
<td>0.845</td>
</tr>
<tr>
<td>AG</td>
<td>0</td>
<td>104</td>
<td>4</td>
<td>108</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>3</td>
<td>1</td>
<td>66</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>105</td>
<td>74</td>
<td>240</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Genotyping data showed that allelic frequencies complied with Hardy-Weinberg equilibrium. Polymorphisms rs11200638 -625 G>A (p = 0.001, $\chi^2 = 48.56$) and rs2672598 -487 T>C (p = 0.001, $\chi^2 = 64.17$) were significantly higher in AMD patients than in controls. There was a significant linkage disequilibrium between these two polymorphisms (X2 p < 0.001, D': 0.93, r2: 0.845) (Tables 2 and 3).

Allele A of polymorphism rs11200638 -625 G>A was significantly greater in the patients with wet AMD than controls and associated with the risk of developing AMD (p = 0.001, OR (95% CI) = 2.69 (1.46-4.95). Allele C of polymorphism rs2672598 -487T>C was also significantly higher in patients with wet AMD than controls and associated with increased risk of developing AMD (p = 0.001, OR (95% CI) = 2.68 (1.54-5.30)). Genotype AA of polymorphism rs11200638 -625 G>A (p < 0.001, OR (95% CI) = 10.15(4.47-23.01)) and genotype CC of polymorphism rs2672598 -487T>C (p < 0.001, OR (95% CI) = 8.69 (3.96-19.04)) were associated with greater risk of developing AMD (Table 3).
According to the results of the current study, the frequencies of polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A in the promoter region of the HTRA1 gene were significantly higher in AMD patients than controls. Heterozygotes bearing the AG genotype and AA homozygotes of polymorphism rs11200638 -625 G>A were most frequent among wet AMD patients and showed prevalences of 34.1 and 53.4%, respectively. The frequencies were similar to those with the dry form of the disease, while AG heterozygotes and GG wild-type homozygotes were the dominant genotypes of controls, with 55 and 35% prevalences, respectively.

In the analysis of the allele frequencies in different groups we found that the A allele was significantly higher in patients than controls and increased the risk of developing AMD. We observed a high association of this allele with the wet form of the disease (OR (CI 95%): 2.69 (1.46-4.95)). Due to the tight linkage disequilibrium of these polymorphisms in the subjects ($\chi^2 p < 0.001$, D’:0.93, r$:0.845$), results showed similar frequencies of polymorphism rs2672598 -487T>C in the study population. Also, other studies have noted linkage disequilibrium between polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A (24, 28).

While the T allele was more frequent in patients with dry than with wet AMD, the frequencies of C alleles of polymorphism rs2672598 -487T>C in patients with wet and dry forms of the disease were 75 and 43.7%, respectively. According to the results of the current study, association of the

### Table 3. Frequency of genotypes and alleles of polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AMD cases (n=120)</th>
<th>Controls (n=120)</th>
<th>$\chi^2 (p)$</th>
<th>OR $p$</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet (n=88)</td>
<td>Dry (n=32)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs11200638 -625 G&gt;A</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GG (%)</td>
<td>11 (12.5%)</td>
<td>9 (28.1%)</td>
<td>42 (35%)</td>
<td>---</td>
<td>1.0</td>
</tr>
<tr>
<td>AG (%)</td>
<td>30 (34.1%)</td>
<td>12 (37.5%)</td>
<td>66 (55%)</td>
<td>48.56 (&lt;0.001)</td>
<td>0.388 (1.33 (0.692-2.581)</td>
</tr>
<tr>
<td>AA (%)</td>
<td>47 (53.4%)</td>
<td>11 (34.4%)</td>
<td>12 (10%)</td>
<td>&lt;0.001</td>
<td>10.15 (4.47-23.01)</td>
</tr>
<tr>
<td>$\chi^2$ HW ($p$)</td>
<td>2.88 (0.09)</td>
<td>1.95 (0.16)</td>
<td>3.60 (0.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G (%)</td>
<td>41 (24.8%)</td>
<td>30 (46.9%)</td>
<td>150 (62.5%)</td>
<td>10.52 (0.002)</td>
<td>0.001 (2.69 (1.46-4.95)</td>
</tr>
<tr>
<td>A (%)</td>
<td>124 (75.2%)</td>
<td>34 (53.1%)</td>
<td>90 (37.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2672598 -487T&gt;C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>7 (8%)</td>
<td>12 (37.5%)</td>
<td>42 (35%)</td>
<td>---</td>
<td>1.0</td>
</tr>
<tr>
<td>CT</td>
<td>30 (34%)</td>
<td>12 (37.5%)</td>
<td>63 (52.5%)</td>
<td>55.42 (&lt;0.001)</td>
<td>0.225 (1.47 (0.75- 2.87)</td>
</tr>
<tr>
<td>CC</td>
<td>51 (58%)</td>
<td>8 (25%)</td>
<td>15 (12.5%)</td>
<td>&lt;0.001</td>
<td>8.69 (3.96- 19.04)</td>
</tr>
<tr>
<td>$\chi^2$ HW ($p$)</td>
<td>0.72 (0.393)</td>
<td>1.81 (0.17)</td>
<td>1.34 (0.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (%)</td>
<td>44 (25%)</td>
<td>36 (56.25%)</td>
<td>147 (61.25%)</td>
<td>11.62 (0.001)</td>
<td>0.001 (2.86 (1.54-5.30)</td>
</tr>
<tr>
<td>C (%)</td>
<td>132 (75%)</td>
<td>28 (43.75%)</td>
<td>93 (38.75%)</td>
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</tr>
</tbody>
</table>

**Discussion**

According to the results of the current study, the frequencies of polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A in the promoter region of the HTRA1 gene were significantly higher in AMD patients than controls. Heterozygotes bearing the AG genotype and AA homozygotes of polymorphism rs11200638 -625 G>A were most frequent among wet AMD patients and showed prevalences of 34.1 and 53.4%, respectively. The frequencies were similar to those with the dry form of the disease, while AG heterozygotes and GG wild-type homozygotes were the dominant genotypes of controls, with 55 and 35% prevalences, respectively.

In the analysis of the allele frequencies in different groups we found that the A allele was significantly higher in patients than controls and increased the risk of developing AMD. We observed a high association of this allele with the wet form of the disease (OR (CI 95%): 2.69 (1.46-4.95)). Due to the tight linkage disequilibrium of these polymorphisms in the subjects ($\chi^2 p < 0.001$, D’:0.93, r$:0.845$), results showed similar frequencies of polymorphism rs2672598 -487T>C in the study population. Also, other studies have noted linkage disequilibrium between polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A (24, 28).

While the T allele was more frequent in patients with dry than with wet AMD, the frequencies of C alleles of polymorphism rs2672598 -487T>C in patients with wet and dry forms of the disease were 75 and 43.7%, respectively. According to the results of the current study, association of the
C allele of polymorphism rs2672598 -487T>C with the risk of developing AMD was greater than with polymorphism rs11200638 -625 G>A. Higher prevalences of the A and C mutations of polymorphisms rs11200638 -625 G>A and rs2672598 -487T>C are indicative of associations of these polymorphisms with the risk of developing wet AMD. Numerous studies have shown that polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A in the HTRA1 gene promoter region are associated with increased risk of developing AMD in different populations. According to a study by Yoshida et al., the rs11200638 -625 G>A mutation is associated with an increased risk of developing wet AMD in the Japanese population (29). Other studies in Asian societies found similar results (15, 30, 31). Also, in the case of the rs2672598 -487T>C polymorphism, our results replicated the results from previous studies. These studies have shown that rs2672598 -487T>C is associated with increased risk of developing wet AMD (25, 32).

Our study is the first to show that rs2672598 and rs11200638 are associated with AMD in the Iranian population. In a recent study in Iran, other polymorphic regions, including CFH Y402H and HTRA1 LOC387715, in AMD were studied (33). Studies in Turkey and Egypt found associations between polymorphisms CFH Y402H and LOC387715 A69S and the risk of AMD (34-36). Based on our results, individuals with family histories of AMD were more likely to develop the disease than those without. Similarly, in the family-based studies, the positive family history for AMD has been reported. These results indicate a familial factor in the pathogenesis of AMD (2, 37, 38). No significant relationship between cigarette smoking and AMD was seen (p = 0.374), although patients with the wet form of the disease had higher levels of cigarette smoking than those with the dry form. Other studies have reported a cumulative effect of smoking and polymorphism rs11200638 -625 G>A on the risk of developing AMD (32, 39).

The role of the A allele of polymorphism rs11200638 -625 G>A on the increased risk of developing AMD could be attributed to changes in the transcriptional factor binding sites in the HTRA1 gene promoter region for adaptor related protein complex 2α (AP2α) and serum response factor (SRF) (15). Increased HTRA1 expression can contribute to pathogenesis and augment AMD injuries by increasing the cell death signals through serine protease pathways (40). AMD patients bearing an allele of polymorphism rs11200638 -625 G>A express more HTRA1 than normal individuals, which could be related to the increased risk of developing the disease (22, 31). The rs2672598 -487T>C mutation is also located in the HTRA1 gene promoter region bound by the ELK-1 transcription factor (25); however, no clear relationship between this polymorphism and an elevated risk of developing AMD has been described.

It appears that genetic changes in the HTRA1 gene promoter in the 10q26 chromosomal region are related to the pathogenesis of AMD. Polymorphisms rs2672598 -487T>C and rs11200638 -625 G>A in this region have been demonstrated to be associated with an increased risk of developing AMD in our study and other studies in different populations. This association has also been observed to be related to the more advanced form of wet AMD in the same populations. To further clarify genetic polymorphisms associated with risk of developing AMD other regions of the HTRA1 gene should be investigated. This will be help to identify other genetic risk factors associated with the disease.

Acknowledgments
This study is funded by Cellular and Molecular Research Center (CMRC), Tehran University of Medical Sciences.

We would like to appreciate the head and all staff of CMRC for their support during current project. We also thank Dr. Saeid Kargozar for his useful comments on this manuscript.
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