Association between LOC387715/ HTRA1 gene polymorphisms and neovascular age-related macular degeneration in Korean population

인하대학교 대학원
의학과 (안과학 전공)
이수정
의학석사학위 논문
Association between LOC387715/ HTRA1 gene polymorphisms and neovascular age-related macular degeneration in Korean population

지도교수 진희승

이 논문은 석사학위 논문으로 제출함
이 논문을 이수정의 석사학위논문으로 인정함.

2011년 2월

주심____________________

부심____________________

위원____________________
Association between LOC387715/HTRA1 gene Polymorphisms and Neovascular Age-related Macular Degeneration in Korean population

by

Soo-jeong Lee

A THESIS
Submitted to the faculty of
INHA UNIVERSITY
in partial fulfilment of the requirements
for the degree of
MASTER OF MEDICINE

Department of Ophthalmology
February 2011
목차

표 목차 ................................................................. ii

국문 요약 .......................................................... iii

영문 요약 ........................................................... iv

I. 서론 ................................................................. 1

II. 대상 및 방법 .................................................. 3

III. 결과 .............................................................. 5

IV. 고찰 .............................................................. 7

참고문헌 ........................................................... 10
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.</td>
<td>15</td>
</tr>
<tr>
<td>Table 2.</td>
<td>16</td>
</tr>
<tr>
<td>Table 3.</td>
<td>17</td>
</tr>
<tr>
<td>Table 4.</td>
<td>18</td>
</tr>
<tr>
<td>Table 5.</td>
<td>19</td>
</tr>
</tbody>
</table>
국문요약

목적: 한국인에서 LOC387715와 HTRA1 유전자의 변이와 습성 나이관련 황반변성의 관련성을 알아보고자 하였다. 또한 나이관련 황반변성의 위험 인자로 알려진 유전인자와 환경인자를 분석하여 이들 사이의 상호작용을 함께 알아보고자 하였다.

대상과 방법: 형광안저혈관조영술을 통해 습성 나이관련 황반변성을 진단받은 환자군 139명과 안과적으로 특별한 절환이 없는 대조군 187명의 정맥혈을 채혈하여 DNA를 추출하였다. 이를 이용하여 LOC387715 유전자에 위치하는 단일염기다형성 rs10490924와 HTRA1 유전자에 위치하는 rs11200638에 대해 염기서열을 분석하였다.

결과: LOC387715와 HTRA1 두 개의 유전자 모두 습성 나이관련 황반변성과 유의한 관련성이 있었다. (p=0.0001) 두 개의 단일염기다형성을 둔 환자군에서 대조군 보다 유의하게 높았다. (p=0.0001) LOC387715의 위험 대립유전자를 동형접합으로 가지는 경우 나이관련 황반변성의 위험을 3.80배 (p=0.0001), HTRA1의 경우 4.03배 (p=0.0001) 높이는 것으로 나타났다. Complement factor H (CFH) Y402H와 LOC387715, HTRA1 각각의 상호작용을 분석하였을 때, 위험 대립유전자 수가 증가할수록 그에 따라 나이관련 황반변성의 위험도가 증가하였다. 유전자와 환경적 위험인자의 상호작용 분석 결과에서도 흡연이 LOC387715, HTRA1과 상승 작용을 통해 나이관련 황반변성의 위험을 증가시키는 것으로 나타났다.

결론: 한국인에서 LOC387715와 HTRA1 유전자의 변이는 습성 나이관련 황반변성과 유의한 관련성을 보였다. 이는 이전에 다른 인종을 대상으로 한 연구 결과와 일치하는 것이다. 또한 나이관련 황반변성의 위험인자로 알려진 유전인자 사이 혹은 유전인자와 환경인자 사이에 유의한 상호작용을 보이 두 가지의 위험인자가 복합적으로 작용할 경우 황반변성의 위험도를 높이는 것으로 보인다.

핵심되는 말: LOC387715, HTRA1, 나이관련 황반변성, 흡연
Purpose: This study was to investigate the association of two single nucleotide polymorphisms (SNPs) in LOC387715 and HTRA1 with exudative AMD in a Korean population and the gene-gene and gene-environment interactions in the development of AMD.

Methods: We genotyped two SNPs that are located in the LOC387715 locus (rs10490924) and HTRA1 (rs11200638) in 137 cases of exudative AMD and 187 controls.

Results: Both two SNPs were significantly associated with AMD (p=0.0001). Homozygotes for the risk allele at LOC387715 and HTRA1 had a 3.80 and a 4.03-fold increased risk of exudative AMD respectively, compared with homozygotes for the wild-type allele. (p=0.0001) The joint effects for Complement factor H (CFH) Y402H and 10q26 variants indicated an increased risk of exudative AMD. The Odds ratios (ORs) of AMD for individuals carrying one-, two- and three-copy risk alleles of CFH Y402H and LOC387715 were 1.08, 3.49 and 3.64 respectively. Also, the combination effect of the CFH Y402H risk alleles with HTRA1 risk alleles was dose-dependent. The interaction analysis between gene and environmental factors showed that among several factors, smoking synergistically increased the susceptibility of AMD for variants of LOC387715 and HTRA1, with OR 8.33 (3.05-22.74) and OR 8.50 (3.07-23.51) respectively.

Conclusions: This study demonstrated the significant association of the 10q26 SNPs (HTRA1 and LOC387715) in an AMD cohort from Korea and was consistent with previous studies from other populations. Also, a statistically significant interaction between genetic and environmental factors was found.

Key words: LOC387715, HTRA1, Age-related macular degeneration, Smoking
I. Introduction

Age-related macular degeneration (AMD), a leading cause of severe visual impairment and blindness in the elderly (older than 50 years), is a common complex disease derived from both inherited and environmental exposures.\textsuperscript{1-5} Of the environmental risk factors, age and smoking have most consistently been identified as major risks.\textsuperscript{6-7} Recent studies have identified strong genetic associations with advanced AMD, and associations of AMD with certain single nucleotide polymorphisms (SNPs) have been documented.\textsuperscript{5,8-19}

An association between the Y402H polymorphisms of the complement factor H (CFH) gene on chromosome 1 and AMD has been confirmed in several Caucasian populations. In Asians with AMD, the contribution from CFH Y402H was found to be less strong than that in Caucasians.\textsuperscript{20-21,33}

Another important AMD susceptibility locus has been located on chromosome 10q26. Several studies have used refined linkage disequilibrium mapping and case-control association studies to probe the most susceptible alleles, LOC387715 (rs 10490924) and HtrA serine peptidase 1 (HTRA1) (rs 11200638), for AMD.\textsuperscript{22-27} The functional SNP rs11200638, which is located in the promoter region of the HTRA1 gene within the 10q26 locus and showed almost complete linkage disequilibrium with rs10490924, has been identified to be associated with an increased risk for AMD in White, Japanese and Chinese populations.\textsuperscript{23,28-29} It has also been shown that the presence of the rs10490924 SNP, along with an associated history of smoking, strongly modifies the risk of AMD. Indeed, the combined effect of the rs10490924 SNP and smoking significantly enhanced the risk of AMD in some populations.\textsuperscript{30-32}

In previous report, we showed that the Y402H polymorphism was only marginally associated with exudative AMD with low frequency.\textsuperscript{33} Here we investigated whether the LOC387715 (rs10490924) and HTRA1 (rs11200638) variants are associated with AMD in the Korean
population. In addition, we estimated the combined risk and gene-gene and the gene-environment interactions of CFH Y402H, LOC387715, HTRA1 and smoking in the development of AMD.
II. Methods

1. Subjects

We studied 137 Korean patients with exudative AMD and 187 control subjects from three general hospitals (i.e., Inha University Hospital, Severence Hospital, and Wonju Christian Hospital in Korea). This study was approved by the institutional review board (IRB) of Inha University Hospital and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers. Based on history-taking findings, all study subjects resided in the same geographic area and had similar lifestyles in terms of likely exposure to sunlight, alcohol use, and physical exercise. All individuals were enrolled at these three centers using the same protocol. Inclusion criteria were as follows: (1) age > 50 years; (2) a diagnosis of exudative AMD after undergoing comprehensive ophthalmic examinations that included fundus fluorescein angiography; and (3) the presence of a choroidal neovascular membrane in either or both eyes for a diagnosis of exudative disease. Exclusion criteria included the presence of geographic atrophy (GA) or a large drusen in the absence of choroidal neovascularization. Secondary choroidal neovascular diseases, such as, degenerative myopia, angioid streaks, idiopathic choroidal neovascularization, and presumed ocular histoplasmosis syndrome, were excluded based on clinical presentations and angiographic manifestations. Unrelated control subjects were recruited from among patients with no macular pathology or any other eye disease, except for mild senile cataract, and were more than 50 years of age. In addition, the control subjects had no known family history of AMD. Fundus photography was performed routinely on all control subjects.

2. Genotyping

Peripheral venous blood was collected from all subjects into 5-mL EDTA tubes. Genomic DNA
was extracted from leukocytes by using commercially available kits (QIAmpDNA; Qiagen, Valencia, CA), and polymorphic sites were amplified by polymerase chain reaction (PCR) with specific primers. PCR products were used as templates for direct DNA sequencing (Big Dye Terminator cycle sequencing kits; Applied Biosystems, Inc. [ABI], Foster City, CA) on an automated sequencer (model 3730; ABI).

3. Statistics

Allele and genotype frequencies were estimated by the allele counting method. Numerical data were analyzed with Student’s t-test. Associations between several known risk factors and AMD were examined using separate logistic regression models. These known risk factors were treated as categorical variables: age (50–59 years, 0; 60–69 years, 1; and ≥70 years, 2), gender (male, 0; female, 1), smoking status (never, 0; ever, 1), and hypercholesterolemia (<200mg/dl, 0; ≥200mg/dl, 1).

The allele and genotype frequencies of cases and control subjects were compared by χ² analysis. The χ² analysis was used to calculate the unadjusted odds ratios of alleles and genotypes. Conditional logistic regression analysis models were considered for each locus separately, to estimate odds ratios and their corresponding 95% confidence intervals (CIs), after adjusting for other risk factors. All genotypes were set as categorical variables (homozygotes for the risk allele, 2; heterozygotes, 1; no risk allele, 0).

Statistical analysis was performed with commercial software (SPSS for Windows, ver. 12.0; SPSS Inc., Chicago, IL). The criterion for statistical significance was P ≤ 0.05. Joint ORs for pairs of loci (CFH Y402H and LOC387715; CFH Y402H and HTRA1) were calculated for each 2-locus genotype separately, using the non-risk double homozygote genotype.
III. Results

A total of 137 exudative AMD patients and 187 controls were obtained. The demographic features of the study population are given in Table 1. The mean ages (P=0.132) of the patients and control subjects was not significantly different and gender distribution (P=0.053) was marginally insignificant.

Of the putative risk factors, aging (P=0.001) and cigarette smoking (P=0.006) were significantly associated with AMD, whereas no significant association was found for sex, hypercholesterolemia and hypertension.(Table 2).

Association between the Two SNPs (LOC387715 and HTRA1) and AMD

The genotype frequency distributions of patients and control subjects were in Hardy-Weinberg equilibrium.

A strong association with exudative AMD was detected for SNP rs10490924:G>T in LOC387715 (Table 3). The risk T allele frequencies were 60.95% for AMD cases and 44.12% for controls (p=0.0001). The risk allele conferred 1.98-fold (95% CI;1.44-2.71) increase in the likelihood of exudative AMD. Genotype distributions between AMD cases and controls were statistically significantly different (p=0.0001) compared to wild-type GG genotype, the OR for the risk of AMD was 1.31 (95% CI;0.74-2.34) for the heterozygous GT genotype and 3.80 (95% CI;1.98-7.27) for the homozygous TT genotype.

Similarly, the SNP rs11200638;G>A in the promoter of HTRA1 was significantly associated with exudative AMD (Table 3). Frequencies of the risk A allele were 63.14% for AMD cases and 45.45% for controls (P=0.0001). The OR for the risk A allele was 2.06 (95% CI;1.49-2.83). Genotypes of the HTRA1 promoter polymorphism between AMD cases and controls were statistically significantly different. (P=0.0001). The OR was 1.46 (95% CI;0.80-2.66) for the
heterozygous GA genotype and 4.03 (95% CI: 2.08-7.79) for the homozygous AA genotype, compared to the wild-type GG genotype. LOC387715 and HTRA1 were in high LD in cases (D’=0.97)

Two-locus association analysis

We also assessed the joint ORs of CFH Y402H polymorphism, previously shown to marginal association with AMD in the Korean population, and LOC387715 and HTRA1 polymorphisms. Logistic analysis of the combined contribution of the LOC387715 and the Y402H SNP unveiled a synergistic effect between the T allele of the LOC387715 SNP and the C allele of the CFH SNP for AMD susceptibility. (Table 4) The risk of AMD was increased 3.64 times in the individuals carrying 3 of these 4 risk alleles. When genotype combination included double copy risk alleles at Y402H, the odds ratios could not be calculated because no actual frequencies were observed. Similarly, Y402H and HTRA1 increased the risk by 3.87 times when the individuals carry 3 risk alleles at both SNPs together (Table 4). Three-locus association analysis (i.e. CFH Y402H, LOC387715 and HTRA1) was not performed because of the very low frequency of sub-genotype combinations.

Interaction between smoking and the two SNPs

We analyzed the association of genetic polymorphisms and smoking as environmental risk factors for exudative AMD (Table 5). In each genotype category, ever smokers had a higher OR for AMD than never smokers. Individual heterozygotes for the risk allele who also ever smoked had an OR for AMD of 8.33 in LOC387715 and 8.50 in HTRA1, slightly higher than the product of smoking (OR 2.00) and heterogenous genotype (OR 2.53 in LOC387715, OR 3.07 in HTRA1)
IV. Discussion

AMD, a complex disease, is attributed to multiple genes and their interactions with varying magnitudes of effect.\textsuperscript{1-3} As a continuation of our earlier results on the association of CFH with AMD,\textsuperscript{33} we performed the present study to explore the involvement of other AMD-associated variants in the 10q26 region in an extended cohort. Furthermore, we evaluated the interaction between genetic polymorphisms and environmental risk factors with exudative AMD.

The results from the present study indicate strong associations with the LOC387715 (rs10490924) and HTRA1 (rs11200638) SNPs with AMD, consistent with previous studies in Caucasian,\textsuperscript{23,24,36} Japanese\textsuperscript{29,35} and Chinese\textsuperscript{34} populations.

The SNP rs11200638 is located in the promoter region of the HTRA1 gene, approximately 6.1 kb downstream of the LOC387715. The HTRA1 encodes a heat shock serine protease and controls many physiologic and pathologic processes, such as vascular permeability and extracellular matrices (ECMs) remodeling.\textsuperscript{37} Although the function of HTRA1 in ocular tissues is still unclear, active HTRA1 induces cell death in a serine protease-dependent manner and it is overexpressed in the AMD lesion.

LOC387715 encodes a hypothetical protein of unknown function and its expression in the human retina is weak.\textsuperscript{25} Most recently, however, LOC387715 has been suggested to encode a mitochondrial-associated protein found in the retina; the polymorphism results in misfolding of the protein.\textsuperscript{26} HTRA1 was considered to be a major risk factor for exudative AMD,\textsuperscript{23} until another study reported that LOC387715, but not HTRA1, was a major susceptibility variant at 10q26\textsuperscript{26} and was considered to contribute equally to the disease risk in a white population.\textsuperscript{36}

The present data showed almost similar contributions of LOC387715 and HTRA1 to AMD susceptibility. In LOC387715, the individuals carrying double copies of risk allele have a 3.79-
fold increased risk for AMD than those carrying double copies of low-risk allele and 4.03-fold increased risk in HTRA1.

In agreement with previous studies, our results showed strong LD ($D^\prime=0.97$) between these two SNPs. It was also noted that the frequencies of the risk alleles and risk genotypes of rs10490924 and rs11200638 were significantly higher in patients. Our results support that the HTRA1 and LOC387715 gene may be a more important susceptibility locus for exudative AMD than the CFH gene in Asians. Further studies are needed to identify whether HTRA1 and LOC387715 are only in LD or are causative factors for AMD.

The joint effect based on two-locus odds ratios for CFH Y402H and 10q26 variants (LOC387715 and HTRA1) indicated an increased risk of exudative AMD for homozygotes of risk alleles similar to the Caucasian populations. We could not analyze for homozygotes of CFH Y402H because there were no subjects. The number of risk alleles was associated with gradual increased risk of exudative AMD to indicate a dose effect in both LOC387715 and HTRA1. However, the risk association of HTRA1 gene, LOC387715 and joint effects with exudative AMD in present study was lower than that in previous studies. 23-24,34-36,38 The reason for this was not clear, but may have to do with sample size and ethnic variation (for example, extremely lower frequencies of homozygotes of risk alleles in CFH Y402H).

Recent studies from Chinese and Caucasians found an HTRA1-smoking additive effect. 27,39 Also, there were no statistically significant interactions between smoking and CFH Y402H or LOC387715 in some reports. 40-42 In the present study, the joint effect based on gene-environmental analysis suggests that the risk of AMD significantly increased when subjects carried risk alleles at three genes (LOC387715, HTRA1 and CFH Y402H). It is very important to note that although these people can’t alter their genotype, they can modify or reduce the risk of AMD to some extent if they stop smoking.
In conclusion, the present study demonstrated a significant association of the 10q26 SNPs (HTRA1 and LOC387715) in an AMD cohort from Korea and it was consistent with previous studies from other populations. Also, a statistically significant interaction between genetic and environmental factors was found. The impact of genetics on AMD is a major determinant with heritability of up to 71% and several environmental factors may advance the incidence and development of the disease. Therefore, the risk of AMD can be decreased by avoiding modifiable risk factors such as smoking for risk genotype carriers with high susceptibility. Our findings may be helpful for understanding the complicated pathogenesis and for preventing and controlling the disease. Further studies would be needed to clarify biological and functional evidence that could confirm the role of HTRA1 and LOC387715 in the pathogenesis of AMD.
References


19. Hyman L, Schachat AP, He Q, Leske MC. Hypertension, cardiovascular disease, and


Table 1. Baseline Characteristics of the Study Subjects†

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=137)</th>
<th>Controls (n=187)</th>
<th>All Subjects (n=324)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages(y) ‡</td>
<td>67.71±9.29</td>
<td>66.83±7.80</td>
<td>67.11±8.94</td>
<td>0.13</td>
</tr>
<tr>
<td>Sex, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>83(60.58)</td>
<td>94(50.27)</td>
<td>177(54.32)</td>
<td>0.05</td>
</tr>
<tr>
<td>Female</td>
<td>54(39.42)</td>
<td>93(49.73)</td>
<td>147(45.68)</td>
<td></td>
</tr>
</tbody>
</table>

† Cases and controls were matched for age and sex.
‡ Mean ± SD.
Table 2. Description of Variables

<table>
<thead>
<tr>
<th>Age(y)</th>
<th>Cases(M)</th>
<th>Controls</th>
<th>P*</th>
<th>Odds Ratio (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-59</td>
<td>23(16.79)</td>
<td>33(17.65)</td>
<td>1.00</td>
<td>(reference)</td>
</tr>
<tr>
<td>60-69</td>
<td>54(39.42)</td>
<td>72(38.50)</td>
<td>2.37</td>
<td>(1.50-4.79)</td>
</tr>
<tr>
<td>≥70</td>
<td>60(43.80)</td>
<td>82(43.85)</td>
<td>0.001</td>
<td>6.48(3.46-12.15)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>83(60.58)</td>
<td>94(50.27)</td>
<td>0.64</td>
<td>(0.41-1.01)</td>
</tr>
<tr>
<td>Female</td>
<td>54(39.42)</td>
<td>93(49.73)</td>
<td>0.053</td>
<td>(1.00)(reference)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>48(35.04)</td>
<td>79(43.2)</td>
<td>1.00</td>
<td>(reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>89(64.96)</td>
<td>104(56.8)</td>
<td>0.71</td>
<td>(0.45-1.12)</td>
</tr>
<tr>
<td>Cholesterol(mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>40(29.20)</td>
<td>71(38.0)</td>
<td>1.00</td>
<td>(reference)</td>
</tr>
<tr>
<td>≥200</td>
<td>97(70.80)</td>
<td>116(62.0)</td>
<td>1.48</td>
<td>(0.68-2.48)</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>73(53.28)</td>
<td>128(68.5)</td>
<td>1.00</td>
<td>(reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>64(46.72)</td>
<td>59(31.5)</td>
<td>0.006</td>
<td>1.90(1.21-3.00)</td>
</tr>
</tbody>
</table>

* χ2 test for frequency
† ORs and 95% CIs were determined by logistic regression analysis
Table 3. Genotypes and Allele Frequencies and Odds Ratios in Cases and Controls

<table>
<thead>
<tr>
<th>Allele Distribution</th>
<th>Genotype Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases(M) (n=137)</td>
</tr>
<tr>
<td>LOC387 715</td>
<td></td>
</tr>
<tr>
<td>(rs10490924) G</td>
<td>107(39.05)</td>
</tr>
<tr>
<td>HTRA1 (rs11200638) G</td>
<td>101(36.86)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* χ² test for frequency across genotypes and alleles.
† Adjusted for age, gender, hypertension, hypercholesterolemia, and smoking status
‡ Individuals with two copies of the risk allele and individuals with no copy of the risk allele were compared
§ Individuals with one copy of the risk allele and individuals with no copy of the risk allele were compared
Table 4. Joint ORs (95% Confidence Intervals) for CFH Y402H (rs1061170) and two SNPs (LOC387715 and HTRA1)

<table>
<thead>
<tr>
<th></th>
<th>CFH Y402H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
</tr>
<tr>
<td>LOC387715</td>
<td>GG</td>
</tr>
<tr>
<td></td>
<td>GT</td>
</tr>
<tr>
<td></td>
<td>TT</td>
</tr>
<tr>
<td>HTRA1</td>
<td>GG</td>
</tr>
<tr>
<td></td>
<td>GA</td>
</tr>
<tr>
<td></td>
<td>AA</td>
</tr>
</tbody>
</table>
Table 5. Joint ORs (95% Confidence Intervals) for smoking status and two SNPs (LOC387115 and HTRA1)

<table>
<thead>
<tr>
<th></th>
<th>Smoking status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Ever</td>
<td></td>
</tr>
<tr>
<td>LOC387715</td>
<td>GG</td>
<td>1</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>1.39</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>3.78</td>
<td>8.33</td>
</tr>
<tr>
<td>HTRA1</td>
<td>GG</td>
<td>1</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>1.53</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>4.35</td>
<td>8.50</td>
</tr>
</tbody>
</table>

(ORs and 95% CI)