Variants in the 10q26 Gene Cluster (LOC387715 and HTRA1) Exhibit Enhanced Risk of Age-Related Macular Degeneration along with CFH in Indian Patients


PURPOSE. Single nucleotide polymorphisms (SNPs) in the LOC387715 (rs10490924), HTRA1 (rs11200638), and CFH (rs1061170) genes have been implicated in age-related macular degeneration (AMD). The present study was undertaken to determine the involvement of the LOC387715 and HTRA1 in an AMD cohort from India.

METHODS. The coding region of LOC387715 (exon 1) and the promoter of HTRA1 were screened by resequencing in AMD cases and normal controls. Odds ratios were calculated to assess the risk of individual genotypes. Linkage disequilibrium (LD) and haplotype frequencies were estimated with Haploview software. Population attributable risk (PAR %) for the associated SNPs and their combined effects were calculated.

RESULTS. Resequencing revealed seven different SNPs in these genes, of which significant associations were noted with the risk alleles of rs10490924 (T allele; OR = 5.34 × 10^-12) in LOC387715, and rs11200638 (A allele; OR = 4.32 × 10^-15) and rs2672598 (C allele; OR = 3.39 × 10^-11) in HTRA1 among the cases. Correspondingly, the homozygous risk genotypes TT, AA, and CC in these SNPs exhibited higher disease odds and PAR %. rs10490924 and rs11200638 were in tight LD (D' = 0.90; 95% CI, 0.84 – 0.93). G-C-T-A-C was the risk haplotype (P = 8.04 × 10^-15), whereas the G-C-G-T haplotype was protective (P = 2.01 × 10^-4). The combined effect of the CFH (CC) and LOC387715 (TT) risk genotypes exhibited a PAR of 93.7% (OR, 73.89; 95% CI, 8.69 – 628.13).

CONCLUSIONS. The present data provided an independent validation of the association of LOC387715 and HTRA1 SNPs, along with their risk estimates among Indian patients with AMD. These associations underscore their significant involvement in AMD susceptibility, which may be useful for predictive testing.

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A ge-related macular degeneration (AMD; OMIM 603075; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) leads to progressive impairment of central vision and is a major cause of vision loss among the elderly worldwide.1 It is a complex disorder with a multifactorial etiology, and genetic predisposition is a major risk factor in its pathogenesis.2,3 With rapid demographic changes and increased life expectancy worldwide, managing AMD is a major global challenge. The global prevalence of AMD varies across populations but is higher in the age groups 65 to 74 and 75 to 84 years.4 The estimated prevalence of AMD in Indians is similar to that observed in Western populations and increases significantly in the elderly.5,6

Recent genetic association studies conducted on large case-control cohorts have indicated a single nucleotide polymorphism (SNP) in the complement factor-H (CFH; OMIM 134570) gene at 1q32 that regulates innate immunity, in AMD susceptibility. A Tyr402His variant (rs1061170) in CFH has been shown to increase the risk of AMD by several-fold,7–9 and the high-risk allele exhibits similar involvement with the dry and wet stages of AMD.10–12 Although the Tyr402His SNP of CFH has been significantly associated with AMD in most populations worldwide,13 it does not exhibit any major involvement among Japanese patients.12–15

The second AMD locus mapped on 10q26 harbors three important candidate genes: PLEKHA1 (OMIM 607772), LOC387715 (OMIM 611313), and HTRA serine peptidase 1 (HTRA1; OMIM 602194).16 An independent analysis of the 10q26 region indicated a strong association within a 60-kb region of high linkage disequilibrium (LD) that harbored the hypothetical LOC387715 and PLEKHA1 genes. Further analysis has revealed a significant association of the rs10490924 SNP of LOC387715 with AMD in two unrelated German cohorts.17 This association was later replicated in various degrees among Caucasian,18–22 Japanese,23,24 and Russian25 patients with AMD. 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.26–28 The combined effect of the rs10490924 SNP and smoking significantly enhanced the risk of AMD in some populations,20,21,26 but this finding could not be replicated in a large dataset comprising the AREDS (Age-Related Eye Disease Study) and CHS (Cardiovascular Health Study) cohorts.18 The combined additive effect of the rs1061170 (CFH) and rs10490924 SNPs exhibited a high population attributable risk percentage (PAR %) in AMD.20–27

Very recently, another SNP (rs11200638) located 512 bp upstream of the transcription site of HTRA1 in the same 10q26 cluster was implicated in several independent reports on Caucasian,26–30 Chinese,31 and Japanese32–34 AMD subjects. It was also demonstrated that this SNP in the promoter region was in LD with rs10490924 that was a further 6.6 kb upstream of HTRA1.31
and enrollment were based on inclusion and exclusion criteria published in May 2007. As this was an extension of our previous cohort, diagnosis was approved by the institutional review board. The cohort comprised 250 unrelated patients with AMD from seven states of India, along with 250 ethnically matched normal controls presenting at the L. V. Prasad Eye Institute, Hyderabad, India, between August 2004 and May 2007. As this was an extension of our previous cohort, diagnosis and enrollment were based on inclusion and exclusion criteria published earlier.

METHODS

Subjects and Clinical Evaluation

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board. The cohort comprised 250 unrelated patients with AMD from seven states of India, along with 250 ethnically matched normal controls presenting at the L. V. Prasad Eye Institute, Hyderabad, India, between August 2004 and May 2007. As this was an extension of our previous cohort, diagnosis and enrollment were based on inclusion and exclusion criteria published earlier.

Although the rs1061170 SNP (CFH) has been widely replicated across different ethnic groups worldwide, there is a need to replicate the variants in genes in the 10q26 cluster in these populations, to gain a better appreciation of the variants’ role in disease pathogenesis. Earlier, we demonstrated the association of the rs1061170 SNP in an AMD cohort from India and generated haplotypes that indicated similarity in risk and protection to those observed in Caucasians. Herein, we provide an independent validation of associations of the 10q26 SNPs based on extensive screening of an AMD cohort from India. In addition, we provide risk estimates based on the combined effects of these SNPs with the rs1061170 SNP in this cohort. We have also performed a meta-analysis of the associated SNPs across different studies.

Screening of the 10q26 Region

Genomic DNA was extracted from the peripheral blood leukocytes according to standard protocols. The genomic region containing the first coding exon of the hypothetical LOC387715 (Entrez ID: NC_000010.9; chromosomal region, nucleotides 124204133-124204548 nucleotides; www.ncbi.nlm.nih.gov/sites/entrez) and the promoter region of the HTRA1 (Entrez ID: NC_000010.9; chromosomal region, nucleotides 124202111-124210782) genes was PCR amplified using specific primers published earlier. The PCR amplicons were purified with PCR purification columns (MO BIO Laboratories, Inc., Carlsbad, CA) and subjected to resequencing (Genetic Analyzer 3100; Applied Biosystems, Inc. [ABI], Foster City, CA) using dye-termination chemistry (BigDye Terminator; ABI), according to the manufacturer’s protocol. Of the 500 subjects enrolled, the actual number of individuals whose genotype data were available for analysis is indicated in Table 1.

Generation of Haplotypes at the 10q26 Region

A total of six SNPs in the 10q26 region were used to generate haplotypes from the sequence data in patients and controls. The SNPs were in the following order: rs10490923, rs1360911, rs10490924, rs11200638, −502C>T, and rs2672598. The first three SNPs were in exon 1 of the LOC387715 gene and the remaining two in the promoter of HTRA1.

Statistical Analysis

Allele and genotype frequencies were estimated by an allele-counting method. Hardy-Weinberg equilibrium calculations were made, and the
estimated haplotype frequencies were obtained with Haplovew software, that uses the EM algorithm.\textsuperscript{35} LD between the three SNPs was analyzed by using the LD plot function of the software. Odds ratios were computed for estimating the risk of AMD with respect to different genotypes. The combined effects of the LOC387715 and HTRA1 to the CFH genotypes were calculated.

**Meta-analysis**

To understand the significance of the observed associations across the rs10490924 (LOC387715) and rs11200638 (HTRA1) variants in different studies, a meta-analysis was undertaken with the estimated odds ratios under a fixed-effect model. The included studies were based on a literature search in PubMed in October 2007 with the phrases “LOC387715,” “HTRA1,” and “age-related macular degeneration” and their combinations. The articles were restricted to the English language. Only those studies in which the genotype counts (or frequencies) in cases and controls were available, were included in the analysis. Meta-analysis was performed with NCSS-PASS-GESS software (windows XP version) according to the manufacturer’s guidelines.\textsuperscript{36}

**RESULTS**

Among the AMD cases, only 9% had a family history of the disease; the remaining were sporadic cases. There was good interobserver agreement in assignment of AMD status ($\kappa = 0.94 \pm 0.06$). There was an equal distribution of cases of dry (49.7%) and wet (51.3%) AMD. The mean age of patients with risk alleles for the two SNPs of HTRA1 provided in Table 1. There was a significant association of the minor allele of these SNPs along with their genotypes are rs2672598 ($P = 0.94$). A novel change was in complete LD with the rs10490924 SNP, it was stream of rs10490924 was also observed. However, as this change was in complete LD with the rs10490924 SNP, it was excluded from further analysis. Resequencing of the HTRA1 revealed three SNPs that included a novel SNP at $-502C>T$ that lies between the previously reported\textsuperscript{31} SNPs rs11200638 and rs2672598 in the promoter region. Other than these SNPs, no other DNA sequence variants were observed.

There was no significant deviation from Hardy-Weinberg equilibrium among the controls with respect to the six SNPs: $P = 0.622$, rs11200638 ($P = 0.926$), $-502C>T$ ($P = 0.961$), and rs2672598 ($P = 0.319$). The frequency distributions of the six SNPs were largely restricted to rs10490924 $(-502C>T)$, as these three SNPs exhibited very high disease odds and PAR\% for the AMD risk genotypes in our cohort (Table 1).

Subjects homozygous for the risk genotypes in rs10490924 (LOC387715) and rs11200638 and rs2672598 (HTRA1) had a significantly higher risk of AMD, as was evident from their respective disease odds ratios and PAR\% than those carrying a single copy of the risk allele (Table 1), similar to other populations.\textsuperscript{18–24,28–32}

**LD and Haplotype Analysis at the 10q26 Loci**

Pair-wise LD analysis between the five SNPs revealed tight LD between the rs10490924 and rs11200638 SNPs (D\', 0.90; 95% CI, 0.84–0.93). The measure of LD was relatively lower between rs10490924 and rs2672598 (D\', 0.80; 95% CI, 0.74–0.87; Fig. 1).

Four different haplotypes (with haplotype frequencies of >5%) with all six SNPs in the 10q26 region were observed among cases and controls (Table 2). The estimated frequency of the G-C-T-A-C-C haplotype was almost twofold higher in the cases and was deemed a risk haplotype ($P = 8.04 \times 10^{-15}$), whereas the frequency of the G-C-G-G-C-T haplotype was close to twofold higher in the controls and could be protective ($P = 2.01 \times 10^{-4}$). Similarly, the frequency of the A-C-G-G-C-T haplotype was significantly higher in the controls ($P = 0.0066$). The data were reanalyzed with respect to the three major SNPs (rs10490924, rs11200638, and rs2672598) that exhibited significant associations in our cohort (Table 1). It was observed that the frequencies of the risk haplotypes among patients with AMD were consistent, even when two- or three-locus haplotypes comprising the risk alleles of LOC387715 and either or both of the HTRA1 SNPs were considered (data not shown). Similar observations were noted

![Figure 1. Pair-wise LD analysis across the six 10q26 SNPs. Black: LD (high D') between the LOC387715 (rs10490924) and HTRA1 (rs11200638) SNPs. dark and light gray: moderate to low LD; white: almost no significant LD between the SNPs.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932950/)

<table>
<thead>
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<th>Controls</th>
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<td>G-C-T-A-C-C</td>
<td>56.7</td>
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<tr>
<td>G-C-G-G-C-T</td>
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<td>26.3</td>
<td>$2.01 \times 10^{-4}$</td>
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<tr>
<td>A-C-G-G-C-T</td>
<td>6.2</td>
<td>11.6</td>
<td>0.0066*</td>
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<tr>
<td>G-G-G-G-C-C</td>
<td>10.2</td>
<td>13.3</td>
<td>0.1661</td>
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* Significant.
for the protective haplotypes with respect to the wild-type alleles for these loci among the controls.

**Meta-analysis of the Associated SNPs**

Twelve independent studies were identified through the search strategy for the rs10490924 (LOC387715) variant. Of these, four studies did not meet the inclusion criteria because of the unavailability of genotype counts. Thus, the final analysis for rs10490924 was based on eight studies (excluding the present one), which included 3629 cases and 781 controls. Likewise, seven studies were found for the rs11200638 (HTRA1) variant, of which only three studies (Kanda et al., Conley et al., Ross et al.) met the inclusion criteria, providing 994 cases and 4292 controls. Since rs10490924 (LOC387715) and the rs10490924 (CFH) were the most significantly associated SNP in our patient cohort (Table 1), we generated two-locus ORs for rs10490924 along with the previously associated CFH (rs1061170) SNP (published earlier), to obtain their combined effect in AMD pathogenesis. All nine possible genotypes were compared to the baseline wild-type genotypes GG + TT of rs10490924 and rs1061170, respectively (Table 3).

The results reinforced the earlier findings that the rs10490924 (LOC387715) risk genotype TT contributed to an increased risk of AMD (pooled OR, 8.13; 95% CI, 6.82–9.68) compared with a single copy (pooled OR, 2.47; 95% CI, 2.23–2.74) of the risk (T) allele (Fig. 2). The pooled estimate of the odds ratios for the homozygous and heterozygous risk alleles of rs10490924 had very narrow confidence intervals. There was a marked degree of homogeneity, and most of the studies, including the present one, clustered around the pooled estimate. But unlike the rs10490924 SNP, the present study deviated slightly from the pooled estimate of the rs11200638 SNP (Supplementary Fig. S1, online at http://www.iovs.org/cgi/content/full/49/5/1771/DC1). This finding could again be attributable to the tight LD between the rs10490924 and rs11200638 SNPs (Fig. 1). As evident from the table, subjects bearing the homozygous risk genotypes TT (rs10490924) and CC (rs1061170) SNPs were more susceptible to AMD (OR, 73.89; 95% CI, 8.69–628.13) with a PAR of 93.7%. The combined effects of the CC homozygotes (rs1061170) to all the genotypes of rs10490924 exhibited a PAR of 58% to 93.7% (Table 3). The results were almost similar when the combined effects of rs1061170 of CFH and rs11200638 (HTRA1) were calculated (Supplementary Table S1, online at http://www.iovs.org/cgi/content/full/49/5/1771/DC1). This finding could again be attributable to the tight LD between the rs10490924 and rs11200638 SNPs (Fig. 1).

**TABLE 3. Two-Locus Odds Ratios for the rs1061170 (CFH) and the rs10490924 (LOC387715)**

<table>
<thead>
<tr>
<th></th>
<th>rs10490924</th>
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<tr>
<td></td>
<td>GG</td>
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<td>TT</td>
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<tr>
<td>TC</td>
<td>1.61 (0.60–4.35)</td>
<td>2.46 (1.00–6.00)</td>
<td>11.99 (4.50–31.95)</td>
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<tr>
<td>CC</td>
<td>7.78 (1.62–37.30)</td>
<td>23.33 (5.61–97.01)</td>
<td>73.89 (8.69–628.13)</td>
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Odds ratios with 95% CI were calculated to compare all the genotypes with respect to the baseline wild-type genotypes of rs1061170 and rs10490924 (TT and GG).
SNPs, as evident from genotype, haplotype, and meta-analyses. Overall, these results underscore the functional importance of these SNPs in AMD pathogenesis and provide risk estimates in the present cohort that may be useful in predictive testing.

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References


