Analysis of Six Genetic Risk Factors Highly Associated with AMD in the Region Surrounding ARMS2 and HTRA1 on Chromosome 10, Region q26

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PURPOSE. To determine the relationship of six genetic variants (rs10490924, rs3750848, del443ins54, rs3793917, rs11200638, and rs952275) localized to the ARMS2-HTRA1 region of chromosome 10, region q26, as risk factors for age-related macular degeneration (AMD), to define the haplotype structure of these six loci, and to confirm their genetic association with the disease.

METHODS. Caucasian patients (n = 482) were stratified into categories based on AREDS (Age-Related Eye Disease Study) grading criteria (groups 0 and 1 served as the control, groups 3 and 4 contained subjects with AMD, and group 2 was excluded from the analysis). The six genetic variants in the ARMS2-HTRA1 region were genotyped and analyzed both independently and as a joint haplotype for association in subjects with disease (n = 291) compared with the control (n = 191).

RESULTS. The six high-risk alleles all showed a statistically significant association with AMD (the most significant SNP was rs10490924 [P = 3.31 × 10−5, OR = 1.86]; the least significant SNP was rs952275 [P = 9.15 × 10−5, OR = 1.78]). Multi-marker analysis revealed that all six markers were in strong linkage disequilibrium with each other, and the two major haplotypes that captured >98% of the genetic variation in the region were both significantly associated with the disease: One increased the risk of AMD and contained only risk alleles (P ≤ 2.20 × 10−7), and the other haplotype decreased the risk of AMD and contained only wild-type alleles (P ≤ 6.81 × 10−5). Furthermore, 36 individuals comprising both cases and controls were identified outside of these two major haplotypes, with at least one discordant marker.

CONCLUSIONS. The results replicate the previously reported association between the high-risk alleles and AMD and independently confirm, for the first time, an association with AMD and the del(443ins54) polymorphism in a Caucasian population. Two major haplotypes that are associated with AMD and many minor novel haplotypes were identified. The novel haplotypes, identified from 36 cases and controls with discordant alleles spanning the ARMS2-HTRA1 region provide unique opportunities to gauge the relative phenotypic contributions of each of these genetic risk factors. With the identification of more discordant patients in the future, it may be possible to resolve the ongoing controversy as to which of the risk alleles and genes (ARMS2 vs. HTRA1) has the greatest impact on disease susceptibility. Future work should include the analysis of larger and more diverse populations, to further define the linkage structure of the region with a focus on phenotypic effects on AMD of the various haplotypes involving 10q26, as well as a functional analysis of the normal ARMS2 protein. (Invest Ophthalmol Vis Sci. 2010;51:2191–2196) DOI:10.1167/iovs.09-3798

Age-related macular degeneration (AMD) is the most common cause of visual impairment in individuals older than 55 years in developed countries and has caused more than 30 million people to become blind worldwide. The major risk factors for AMD include age, smoking, and family history, with age being the strongest risk factor. Multiple population-based studies have confirmed the influence of age. Pooled data from two population-based studies reveal an estimated prevalence of advanced AMD of ~0.2% in persons age 55 to 64 years, with a sharp increase to 13% in those older than 85 years. Of importance, the number of individuals with AMD is expected to increase worldwide as the longevity of the population increases. Smoking has been shown to increase the risk of AMD twofold. Of note, a smoking dose-effect that increases the risk of AMD with the increase in number of cigarettes smoked has also been reported. Finally, familial studies have confirmed the existence of a genetic component in AMD. In addition, twin studies have confirmed that genetic background accounts for 46% to 71% of the variation in the overall severity of AMD.

Perhaps the most significant advancement in our understanding of the genetics of AMD came in early 2005 with the identification of a strong association between disease and variants associated with the complement factor H (CFH) gene. This breakthrough association has now been validated in numerous studies worldwide and has been confirmed in multiple ethnic populations. The association between the 10q26 locus and AMD was originally identified through family linkage studies and fine mapping and confirmed in several studies, including a genome-wide association study. At least three potential

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candidate genes reside in the 10q26 region. Currently, the identity of the gene that may induce disease susceptibility has been controversial, as there is strong linkage disequilibrium (LD) across the region. The controversy has involved two nearby genes: ARMS2 (age-related maculopathy susceptibility 2, also known as LOC387715) and HTRA1 (high-temperature requirement factor A). There are six risk alleles involving the region between ARMS2 and HTRA1 (rs10490924, rs3750848, delAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA...
The risk allele frequencies at the six loci ranged from 35% to 36% in the cases, relative to 23% to 24% in the controls, a statistically significant difference at each locus (Table 1). By odds ratios, the most strongly associated marker was rs10490924, an SNP encoding an nonsynonymous alanine-to-serine substitution in the ARMS2 proximal exon (P = 5.31 × 10⁻⁵, OR = 1.86). The next most strongly associated marker was the del443ins54 indel in the 3’ UTR of ARMS2 (P = 5.46 × 10⁻⁵, OR = 1.80), followed by rs3750848 in the only intron of ARMS2 (P = 6.41 × 10⁻⁵, OR = 1.83). The SNP, rs11200638, in the promoter of HTRA1 was strongly associated (P = 6.41 × 10⁻⁵, OR = 1.80), as was rs3793917 in the intergenic region between ARMS2 and HTRA1 (P = 7.82 × 10⁻⁵, OR = 1.80) and rs932275 in an internal intron of HTRA1 (P = 9.15 × 10⁻⁵, OR = 1.78).

Multimarker analysis of the six markers showed that all the markers were in strong LD with each other, and haplotypes inferred from the six markers yielded three major haplotypes. The next most common haplotype, GT1CGG (1 represents no indel), was found in 76% of the control subjects and represents a wild-type haplotype with wild-type alleles at each locus. The second most common haplotype, TG2GAA (2 represents indel), was found in 22% of the control subjects and represents a risk haplotype composed of risk alleles at each locus. The next most common haplotype, GTIGG, was found in 2% of the subjects analyzed. Haplotype analysis showed that only the two major haplotypes (i.e., the risk haplotype and the wild-type haplotype) were significantly associated with AMD (Table 2). The risk haplotype (TG2GAA) significantly increased the risk of AMD in 35% of the cases versus 22% of the controls (P = 2.20 × 10⁻⁵), and the wild-type haplotype (GTIGG) significantly decreased the risk of AMD in 63% of the cases versus 76% of the controls (6.81 × 10⁻⁵).

There were 36 individuals who had neither complete risk nor wild-type haplotypes (Supplementary Table S1, http://www iovs org/cgi/content/full/51/4/2191/DC1), and 14 of these individuals were discordant at the three loci most likely to be available at www.ncbi.nlm.nih.gov/locuslink/refseq/). Pairwise comparisons of these three loci revealed that eight individuals had discordant genotypes at A69S and the indel. Seven of these eight individuals were cases, and four of them had more risk alleles at the indel locus than at A69S. There were 10 individuals with discordant genotypes at ARMS2 A69S and the HTRA1 promoter. Eight of these 10 individuals were cases, and half had more risk alleles at the ARMS2 A69S. Also, there were 10 individuals who were discordant at the ARMS2 indel and the HTRA1 promoter. Seven of these 10 individuals were cases, and 4 of them had more risk alleles at the indel locus. Thus, analyses of the A69S SNP, the ARMS2 indel, and the HTRA1 promoter SNP did not favor any one genetic variant as a likely functional risk factor for AMD, because their respective risk alleles were about equally distributed among the cases in each pairwise comparison of the loci. Furthermore, a power analysis based on the empiric data (Supplementary Table S2, http://www iovs org/cgi/content/full/51/4/2191/DC1) suggests that the sample was underpowered to distinguish the causative disease locus (power = 0.12 at α = 0.05), and that a sample size of 6247 subjects would be necessary (power ≥ 80% at α = 0.05) to distinguish these alleles by case-control test for genetic association.

Finally, we analyzed the effect of smoking, family history, and statin use as risk factors for AMD (Table 3). We found family history to be a significantly associated risk factor. Of the patients, 31% reported a family history of AMD versus 17% of the control subjects (P = 7.36 × 10⁻⁴, OR = 3.12). The case patients were also less likely to be on a statin than were the

Table 1. Single-Marker Genotypic Association of AMD

<table>
<thead>
<tr>
<th>Marker</th>
<th>Gene</th>
<th>Relative Position</th>
<th>Risk Allele</th>
<th>WT Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10490924</td>
<td>ARMS2</td>
<td>A69S</td>
<td>T</td>
<td>G</td>
<td>OR</td>
</tr>
<tr>
<td>rs3750848</td>
<td>ARMS2</td>
<td>Intron</td>
<td>G</td>
<td>T</td>
<td>0.36, 0.23</td>
</tr>
<tr>
<td>rs11200638</td>
<td>HTRA1</td>
<td>Promoter</td>
<td>A</td>
<td>G</td>
<td>0.36, 0.24</td>
</tr>
<tr>
<td>rs932275</td>
<td>HTRA1</td>
<td>Intron</td>
<td>A</td>
<td>G</td>
<td>0.36, 0.24</td>
</tr>
</tbody>
</table>

1, no indel; 2, indel.
allele frequency in our controls (21% vs. 23% respectively, allele frequency in the HapMap CEU was similar to the risk
istically constrained German population. Of interest, the risk
sent a more pan-European population than the more geograph-

2.9, respectively), may stem from the reduced power of our
straints of the two populations and may illustrate the effect of
between these two different European-derived Caucasian pop-

DISCUSSION

We have characterized the relationship between six genetic
variants highly associated with AMD in the 17-kb region sur-
rounding ARMS2 and HTRA1 on 10q26. The coverage of SNPs
genotyped in the most recent phase (phase 3) of the HapMap
project (www.HapMap.org) do not sufficiently cover the 17-kb
region spanning the six risk loci, and this deficit motivated our
analysis to define the LD structure of the six genetic loci with
roles in AMD. Furthermore, and to the best of our knowledge,
our significant findings represent the first replication of the
indel with AMD in a Caucasian population. All six risk alleles
were found in 35% to 36% of the AMD population versus 23%
to 24% of the control population, and the most strongly asso-
ciated locus was A69S (P = 3.31 × 10⁻³, OR = 1.86). Recent
larger studies in a German population of Caucasian individuals
(760 cases, 549 controls) report a larger effect for the A69S risk
allele with AMD (42% in cases vs. 19% in controls; OR = 2.9;
P = 2.8 × 10⁻²⁰). The reduced effect of the risk allele in our
study, when compared to the German study (OR = 1.86 vs.
2.9, respectively), may stem from the reduced power of our
smaller sample size. Furthermore, due to different population
migration histories, our European-derived subjects may repres-
sent a more pan-European population than the more geograph-
ically constrained German population. Of interest, the risk
allele frequency in the HapMap CEU was similar to the risk
allele frequency in our controls (21% vs. 23% respectively, P ≤
0.5, data not shown). Therefore, it may be that geographic
constraints alter the susceptibility to AMD induced by the A69S
risk allele. A phylogenetic analysis of genome-wide variation
between these two different European-derived Caucasian pop-
ulations may be used to visualize the relative geographic con-
straints of the two populations and may illustrate the effect of
such constraints on the risk allele frequency.

Our results replicate the previously reported association be-
tween the ARMS2 A69S variant and AMD and independently
confirm the association of AMD with the indel polymorphism.
We found exceptions, however, to the previously reported concordance between the various SNPs at the locus spanning
ARMS2 and HTRA1, and our results suggest an incomplete
linkage in the region. There were 36 individuals who demon-
strated incomplete LD across the region. Of these, 8 had
discordant genotypes at A69S and the indel, 10 had discordant
genotypes at A69S and the HTRA1 promoter, and 10 were
discordant at the indel and the HTRA1 promoter. These dis-
cordant patients represent novel haplotypes, which could
prove extremely useful in determining which of the genes
(ARMS2 vs. HTRA1) has the biggest influence on the develop-
ment of AMD—an area of controversy and uncertainty. How-
ever, given that AMD is a complex disease relying on multiple
genetic and environmental risk factors, our sample consisted of
too few discordant individuals to draw conclusions as to which
SNP and gene is most causative of AMD. Nonetheless, finding
additional minor novel haplotypes in more diverse populations
will generate important hypotheses to be tested toward deter-
mining the causality of risk alleles in AMD.

Although the relatively small sample size in this study pre-
ccludes the possibility of determining which of the variants
associated with the ARMS2 and HTRA1 at the 10q26 locus is
more likely to be responsible for the increased risk of AMD, our
data suggest that >6000 samples would have to be analyzed to
be able to discriminate (power ≥ 80% at α = 0.05) among the
genetic variants for potential causation. Although it may be
possible to achieve such a large number of subjects via further
recruitment and/or collaboration with other investigators who
are studying the ARMS2 gene in patient populations, careful
characterization and functional testing of these loci, both in
vitro and in vivo, may also be indicated to try to resolve the
controversy of which genetic variant in which gene underlies
the increased risk of AMD. Another option is to perform the
association in another ethnic group (i.e., African Americans),
which may distinguish HTRA1 from ARMS2, based on a differ-
ent LD block structure.

Susceptibility to AMD also depends on the complex inter-
action of many other susceptibility factors, including a positive
family history, multiple environmental factors such as age and
smoking,13–15 and, less definitively, use of statins.43 Of these
risk factors, we found family history to be the most significant
risk factor for AMD with 31% of the cases reporting a positive
family history versus 17% of the controls (P ≤ 7.36 × 10⁻⁴,
OR = 3.12). We did not find an association between smoking
history and AMD, with 46% of the case patients reporting a
positive smoking history versus 53% of the control group (P ≤
0.474, OR = 0.85).

Table 2. Haplotype Genotypic Association with AMD

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG2GAA</td>
<td>0.35</td>
<td>0.22</td>
<td>2.20 × 10⁻⁴</td>
</tr>
<tr>
<td>GT1GGG</td>
<td>0.63</td>
<td>0.76</td>
<td>6.81 × 10⁻⁴</td>
</tr>
<tr>
<td>GT1CGA</td>
<td>0.01</td>
<td>0.02</td>
<td>4.30 × 10⁻¹</td>
</tr>
</tbody>
</table>

1, no indel; 2, indel.

Table 3. Effect of Family History, Smoking History, and Statin Use as Risk Factors for AMD

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Case n (Freq)</th>
<th>Control n (Freq)</th>
<th>P</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history vs. no family history</td>
<td>68 (0.31)</td>
<td>18 (0.17)</td>
<td>7.36 × 10⁻⁴</td>
<td>3.12</td>
</tr>
<tr>
<td>Ever-smoker vs. never-smoker</td>
<td>117 (0.46)</td>
<td>77 (0.53)</td>
<td>0.474</td>
<td>0.83</td>
</tr>
<tr>
<td>Ever-statin vs. never-statin</td>
<td>121 (0.51)</td>
<td>90 (0.65)</td>
<td>2.09 × 10⁻²</td>
<td>0.55</td>
</tr>
</tbody>
</table>
We found that the lack of statin use was a statistically significant risk factor for AMD, with a smaller portion having the case group having ever consumed a statin compared with those in the control group (51% vs. 65%, P ≤ 2.09 × 10^{-2}, OR = 0.55). The pathogenesis of AMD is thought to involve an inflammatory process, and it has been hypothesized that statin use has a protective role in AMD due to its anti-inflammatory property. However, this potential protective role has not been consistently demonstrated.55 Although more of our control patients did take statins, larger and longer population-based studies are necessary to further evaluate the role of statins in the development of AMD.

To conclude, our results replicate the previously reported association between the high-risk alleles (rs10490924, rs5795939, rs57939917, rs11200638, and rs932275) and AMD and independently confirm for the first time an association of AMD and the indel (del443ins54) polymorphism in a Caucasian population. The novel haplotypes we identified from 36 individuals with discordant alleles spanning the ARMS2-HTRA1 region provide unique opportunities to gauge the relative phenotypic contributions of each of these genetic risk factors. Particular focus on those individuals discordant at del443ins54 in the 3’ UTR of ARMS2, rs10490924 in the exon of ARMS2, and rs11200638 in the promoter region of HTRA1 may be useful, as these markers are more likely to be functional, given their location. With additional discordant patients, it may be possible to further determine which of the risk alleles and genes (ARMS2 vs. HTRA1) have the biggest impact on disease susceptibility, which has been an ongoing area of controversy. Future work should also focus on the function of the normal ARMS2 protein and analyze larger populations to further define the linkage structure of the region with a focus on phenotypic effects of the various haplotypes involving 10q26 on AMD.

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