The *LOC387715* Polymorphism and Age-Related Macular Degeneration: Replication in Three Case–Control Samples

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Purpose. Age-related macular degeneration (AMD) is a multifactorial blinding disease in the elderly. *LOC387715* harbors a single-nucleotide polymorphism that has an association with AMD. This study was conducted to confirm the association between *LOC387715* and AMD and to refine estimates of the impact of this gene variation in using samples from three studies: an Australian population-based study and two U.S. clinic-based case–control studies.

Methods. Cases and controls were collected from a National Eye Institute (NEI) clinical protocol ($n = 240$), the Age-Related Eye Disease Study (AREDS; $n = 488$), and the Blue Mountains Eye Study (BMES; $n = 851$). After DNA extraction, subjects were genotyped for the *LOC387715* Ala69Ser polymorphism (rs10490924).

Results. The combined NEI and AREDS samples yielded odds ratios (ORs) of 2.61 (95% CI 1.89–3.61, $P = 1.42 \times 10^{-9}$) and 8.59 (95% CI 4.49–16.5, $P = 3.56 \times 10^{-13}$) for the heterozygous and homozygous risk alleles, respectively. The corresponding odds ratios in the BMES sample were 1.69 (95% CI: 1.25–2.28, $P = 0.0007$) and 2.20 (95% CI: 1.05–4.62, $P = 0.038$) for the heterozygous and homozygous groups. Neither set of samples showed statistically significant interaction with smoking, although there appeared to be a trend of interaction between smoking and *LOC387715* for risk of advanced AMD.

Conclusions. Although these data from three case–control samples support an AMD genetic risk marker harbored within *LOC387715*, the nested case–control data from the population-based BMES samples showed lower estimates than from the clinic-based samples. This may be because the BMES samples consisted of largely early AMD cases while the clinic-based AMD samples consisted exclusively of advanced cases. (*Invest Ophthalmol Vis Sci.* 2007;48:1128–1132) DOI:10.1167/iovs.06-0999

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Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly of the Western World.[1] In the United States, approximately 1.75 million people are estimated to have advanced AMD and 7.3 million people have the early stages of AMD defined by large retinal drusen. The prevalence of AMD may increase with an aging population. By 2020, it is estimated that approximately 2.95 million people will have advanced AMD, and an additional 6.4 million white individuals will have the early stages of AMD in at least one eye.[2]

The etiology of AMD remains elusive. To date, age and cigarette smoking have been identified as consistent AMD risk factors.[3–7] Studies have indicated a significant genetic component to AMD risk.[8] Associations between AMD and various single nucleotide polymorphisms (SNPs) have been widely reported.[9–26]

Chromosome 10 region q26 has been an AMD-implicated locus in several linkage studies.[27–31] One recent study found significant association signals for pleckstrin homology domain–containing family A, member 1 (*PLEKHA1*) and the hypothetical gene *LOC387715*.[32] However, the study was unable to distinguish between the two. Two subsequent retrospective case–control studies have concluded that *LOC387715* is the source of the linkage signal at 10q26.[33,34] The association between *LOC387715* and AMD appears to be enhanced in the subgroup of cigarette smokers, whereas the association between *CFH* and AMD does not.[14,34] Existing *LOC387715* studies have calculated population attributable risks ranging from 36% to 57%.[32,34]

We sought to replicate and refine knowledge of the association between *LOC387715* and AMD in three different samples: a population-based, nested case–control study derived from the Blue Mountains Eye Study (BMES) in Australia and two clinic-based case–control study samples from the United States. We also wanted a better characterization of the odds ratio and population attributable risk of bearing disease susceptibility alleles at rs10490924.

Methods

Study Subjects

Each participant signed the informed consent that was part of the protocol approved by the National Eye Institute (NEI) Institutional Review Board, the AREDS clinical site, or the University of Sydney. All human research adhered to the tenets of the Declaration of Helsinki.

The clinic-based NEI case–control study samples included 103 clinically diagnosed advanced AMD cases and 137 age-matched controls from the greater Washington, DC, area who were evaluated by the AREDS ophthalmologists at the NEI.[19,20,35] In addition, DNA samples of 488 subjects (296 with advanced AMD and 192 control subjects) from the AREDS Genetic Repository were obtained and included as the second clinic-based sample in the study. NEI and AREDS patients and control subjects were self-identified as whites of non-Hispanic descent.

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Because AREDS participants were recruited under criteria identical with the NEI subjects, we combined all subjects from the two sources, to reach an adequate sample size for the study (NEI+AREDS). NEI+AREDS included 172 subjects with geographic atrophy, 227 subjects with neovascular AMD, and 329 control subjects.

The patients with AMD in the NEI and AREDS case–control studies all were diagnosed with the advanced stage of the disease as defined by the guidelines of the AREDS study, based on fundus photographs.[35] Patients with advanced AMD had geographic atrophy of at least 175 μm in diameter, involving the center of the macula and/or choroidal neovascularization defined as nondrusenoid retinal pigment epithelial (RPE) detachment, serous or hemorrhagic retinal detachment, hemorrhage under the retina or the RPE, or subretinal fibrosis, with drusen in at least one eye.[35] The normal control subjects were clinically evaluated showing an absence of drusen or less than five small drusen (<63 μm), no evidence of significant extramacular drusen, and an absence of all other retinal disease affecting the photoreceptors and/or outer retinal layers such as high myopia, retinal dystrophies, central serous retinopathy, vein occlusion, diabetic retinopathy, uveitis, and other retinal diseases. Retinal photographs were obtained in all patients and the AREDS control subjects. Smoking data were collected for AREDS patients by a detailed administered questionnaire.[3] Smoking data for the NEI patients were collected similarly. However, the questionnaire contained only three questions that asked whether the study participants had ever smoked, if they currently smoked, and, if so, how often. Demographic information on all study groups is summarized in Table 1.

Collection and clinical evaluation of subjects in the BMES has been described.[36,37] Briefly, the BMES is a population-based cohort study of common eye diseases and health-related parameters among suburban residents aged 49 years or older in the Blue Mountains region of Australia, near Sydney. The area has a stable and ethnically homogenous population. Retinal photographs were taken of at least one eye in 99%, or of both eyes in 98%, of study participants. During the second survey of the BMES (1997–2000), 2334 of 3654 baseline participants (75% of survivors), together with 1174 (85% of newly eligible) persons who moved to the area and who were within the eligible age group at the time, were examined and photographed. From this second survey population of the BMES ($n = 3508$), AMD was diagnosed by grading the retinal photographs, with the graders masked to the participant's identity. All advanced AMD cases were adjudicated and confirmed by a retinal specialist (PM). Early AMD was defined in either eye from (1) the presence of large (125 μm or larger in diameter), soft indistinct or reticular drusen within the macular area or (2) the presence of both large soft distinct drusen within the macula and retinal pigment abnormalities in the absence of late AMD. This closely followed the definition of early AMD used in the Beaver Dam, Wisconsin, population.[38] Advanced (late) AMD was defined as the presence of neovascular or atrophic AMD. Neovascular AMD was defined as serous or hemorrhagic detachment of the sensory retina or retinal pigment epithelium (RPE), the presence of subretinal or sub-RPE hemorrhages, or subretinal fibrous scar tissue. Geographic atrophy was defined as a discrete area greater than 175 μm in diameter characterized by a sharp border and the presence of visible choroidal vessels.[36] Smoking history was obtained with an interviewer-administered questionnaire. Participants were asked whether they had ever smoked and, if so, at what age they commenced smoking, as well as whether and when they had quit smoking.[39] DNA samples of 851 subjects consisting of 283 cases and 568 control subjects were sent to the NEI for genotyping. After 16 subjects who could not be genotyped were excluded, 835 subjects including 278 cases (54 late and 224 early AMD) and 557 age-, sex-, and smoking status–matched control subjects were included in the analyses.

DNA Extraction

Venous whole blood (10 mL) was collected from the NEI study subjects. Genomic DNA was extracted and isolated (QIAamp DNA Blood Maxi kit; Qiagen, Valencia, CA).

Construction of the rs10490924 DNA Standard

Standard DNA SNP templates were made to serve as genotyping assay references. Genomic DNA of heterozygous *LOC387715* (rs10490924) was PCR amplified with the primers 5′-GTGGAGAAGGAGCCAGTGAC-3′ and 5′-CAGTGTCAGGTGGTGCTGAG-3′. The 158-bp PCR fragment was inserted into a vector (pGEM-T Easy; Promega, Madison, WI). The ligation product was transformed to JM109 high-efficiency competent cells (Promega). Ten colonies were collected to determine the specific SNP types using the restriction fragment length polymorphism (RFLP) assay. The colonies corresponding to each allele were propagated in LB (Luria-Bertani) broth for extraction of plasmid DNA that later served as the genotype specific DNA standard. The mixture of the plasmids containing two different alleles served as the heterozygous control.

SNP Typing

The typing of *LOC387715* (rs10490924) was performed by PCR-RFLP using the primers just described. The PCR annealing temperature was 61°C. RFLP analysis was conducted with enzyme *Fnu*4HI. Samples with G alleles were cut at the 66th bp from the PCR product of 158 bp. Another *Fnu*4HI site at the 136th bp was used as the internal restriction control. DNA fragments were separated on 15% TBE polyacrylamide gels and visualized after ethidium bromide staining. Standard control DNA for both alleles and a heterozygous control were included on each genotyping plate. To confirm the accuracy of our genotyping method, we also genotyped 91% of all samples (Taqman SNP Genotyping Assay; Assay ID C_29934973_20; Applied Biosystems, Foster City, CA). The call rate was 98%. There was a 97% concurrence between results.

Four additional SNPs were analyzed in 190 NEI samples (95 controls, 95 AMD cases) and 190 BM samples (115 controls, 75 AMD cases). rs10490923, rs1045216, and rs2421016 within the 50 kbp next to rs10490924 were analyzed (Taqman SNP Genotyping Assay; ID C_29808405_20, C_2761690_10 and C_2761667_10, respectively; Applied Biosystems). The SNP rs4253004 located in 10q11.23 which is far away from rs10490924 was analyzed by PCR-RFLP, with the primers 5′-AAGTAGAAAGGCGCTGCTGA-3′ and 5′-GCCCACAAGGAAACAGAGAC-3′. The PCR annealing temperature was 60°C. RFLP analysis was conducted with the enzyme *Bse*NI. The SNP site was cut at 171 bp from the PCR product of 231 bp.

Statistical Analysis

Hardy-Weinberg equilibrium for each SNP was tested by the χ^2 procedure. Logistic regression was performed (SAS; ver. 9.1; SAS Institute,

Table 1. Demographic Characteristics of Study Participants

Group	N	Age (mean ± SD)	Ever Smokers n (%)	Current Smokers n (%)	Advanced AMD n (%)	Female n (%)
NEI+AREDS Control	329	72 ± 9	159 (48.3)	12 (3.64)	0 (0)	183 (55.6)
NEI+AREDS AMD	399	79 ± 5	233 (58.4)	30 (7.52)	399 (100)	229 (57.4)
BMES Control	557	74.9 ± 7.9	274 (49.2)	50 (9.0)	0 (0)	338 (60.7)
BMES AMD	278	75.6 ± 8.5	137 (49.3)	24 (8.6)	54 (19.4)	168 (60.1)

Demographic information is for subjects for whom a *LOC387715* genotype was successfully obtained.

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Table 2. Distribution of *LOC387715*-G/T among Cases and Controls from the AREDS and NEI Protocols

Genotypes	Control	Case	Geographic Atrophy		Choroidal Neovascularization		Combined	
			P	OR (95%CI) vs. GG	P	OR (95%CI) vs. GG	P	OR (95%CI) vs. GG
GG	217 (66.0%)	148 (37.1%)	Reference	1	Reference	1	Reference	1
GT	100 (30.4%)	182 (45.6%)	3.40×10^{-7}	2.87 (1.91–4.30)	2.29×10^{-6}	2.48 (1.70–3.61)	1.42×10^{-9}	2.61 (1.89–3.61)
TT	12 (3.65%)	69 (17.3%)	1.73×10^{-6}	6.43 (3.00–13.79)	2.39×10^{-11}	10.12 (5.12–20.01)	3.56×10^{-13}	8.59 (4.49–16.46)
GT+TT	112 (34.0%)	251 (69.2%)	2.07×10^{-8}	3.24 (2.20–4.78)	2.92×10^{-10}	3.0 (2.32–4.70)	9.06×10^{-15}	3.25 (2.39–4.41)
G	534 (81.2%)	478 (59.9%)	Reference	1	Reference	1	Reference	1
T	124 (18.8%)	320 (40.1%)	3.97×10^{-11}	2.64 (1.97–3.54)	5.46×10^{-17}	3.10 (2.37–4.06)	1.80×10^{-18}	2.88 (2.26–3.67)

Cary, NC) to compare genotype and allele frequencies in cases and controls and to estimate ORs. Analyses were simplified by coding smokers into two groups: ever smoked and never smoked. Hypothesis testing was at the two-sided 0.05 level. Linkage disequilibrium (LD) between SNPs was analyzed using the Web-based SNPAnalyzer (http://www.istech21.com/phar/phar_a01.html). The normalized disequilibrium coefficient D' was used as the parameter for determining the strength of association between the SNPs. With the range of possible D' values lying between -1 and 1, a greater association (co-inheritance) was established when the D' value was closer to 1.

Results

The control groups showed no significant deviation from Hardy-Weinberg equilibrium with respect to the *LOC387715* polymorphism rs10490924 ($P > 0.05$). Within the NEI+AREDS samples, a strong association signal was present at rs10490924. An OR of 2.61 was found for individuals heterozygous for the risk allele, and an OR of 8.59 was determined for individuals homozygous for the risk allele (Table 2). The association was adjusted for age and gender. We also stratified the analysis of the NEI+AREDS samples according to AMD subtype to ensure that the *LOC387715* SNP effect was not subtype specific. The 398 NEI+AREDS AMD samples consisted of 171 (43%) patients with diagnosed geographic atrophy and 227 (57%) with neovascular AMD. The strong association between *LOC387715* and AMD was present regardless of AMD subtype (Table 2). The minor allele frequencies for subjects with diagnosed geographic atrophy, and those subjects with choroidal neovascularization were 0.380 and 0.419, respectively.

In agreement with a previous report, rs10490923, rs1045216, and rs2421016 showed high linkage disequilibrium with rs10490924 in control groups (all $D' > 0.85$).[33] However, the strength of the association of those SNPs with AMD was lower than rs10490924 (data not shown). The rs4253004 located in 10q11.23 was not in linkage disequilibrium with rs10490924, and the comparison between control and AMD groups found no association in this SNP. Those results further indicate that rs10490924 is the major risk allele in this locus.

We did not find a statistically significant interaction between smoking status and the *LOC387715* SNP. However, a substantially higher joint effect was present, as shown by ORs from both the gene marker and current smoking (Table 3). To confirm the quality of our smoking data, we tested for an association between smoking and AMD. We found a significant association between smoking and AMD in our case-control samples with an OR of 1.46 (95% CI: 1.10–2.04; $P = 0.0110$).

The nested case–control sample from the BMES cohort also showed a significantly increased prevalence of the *LOC387715* SNP among the AMD patients compared with the control subjects. The ORs were 1.69 and 2.20 for G/T versus G/G and T/T versus G/G, respectively (Table 4). The BMES data did not demonstrate statistically significant interaction between ever smoking and *LOC387715* ($P = 0.9529$). Because ever smoking itself did not have a statistically significant association with AMD within the BMES ($P = 0.5312$), we also tested for an interaction between current smoking status and AMD. No trend of interaction was apparent in the combination of early, intermediate, and late AMD cases (data not shown). However, a trend of joint effect from both factors was evident when only late AMD cases were classified as AMD in the analysis (Table 5).

Table 3. Combined Risk of Smoking and the *LOC387715* SNP in NEI+AREDS Samples

Smoking Status	G/G	G/T	T/T
Never smoked	1	2.95 (1.82, 4.77)	6.34 (2.84, 14.2)
Smoked	1.55 (1.02, 2.36)	3.68 (2.36, 5.75)	23.3 (6.92, 78.6)

Data are ORs (95% CI). Results of three comparisons were significant ($P > 0.05$).

Discussion

We reproduced the strong association signal at *LOC387715* rs10490924 using samples taken from the AREDS and an NEI AMD clinical protocol, which could be useful for future meta-analysis. The OR estimates from our combined case–control analysis support the estimates in existing publications.[32–34] Our data also suggest that the *LOC387715* SNP at rs10490924 is associated with both atrophic AMD and neovascular AMD. Thus, the association appears to be inclusive for all subtypes of advanced AMD. Despite the large number of samples and high prevalence of a history of smoking in our case–control studies, we did not have sufficient evidence of statistical interaction between smoking and *LOC387715*. In the study by Rivera et al.,[33] there was also no evidence of an interaction with smoking. They never explicitly tested for an interaction. However, the minor allele frequencies for AMD patients who were smokers and nonsmokers were not significantly different (0.404 vs. 0.414, smoking and nonsmoking, respectively).[33] Notably, the

Table 4. Distribution of *LOC387715*-G/T among the Nested Cases and Controls from the BMES

Genotypes	Control	Case	P	OR (95% CI) vs. GG
GG	362 (65.0%)	144 (51.8%)	Reference	1
GT	179 (32.1%)	120 (43.2%)	0.0007	1.69 (1.25–2.28)
TT	16 (2.90%)	14 (5.00%)	0.038	2.20 (1.05–4.62)
GT+TT	195 (35.0%)	134 (48.2%)	0.0003	1.73 (1.29–2.32)
G	903 (81.1%)	408 (73.4%)	Reference	1
T	211 (18.9%)	148 (26.6%)	0.0004	1.55 (1.22–1.97)

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TABLE 5. Combined Risk of Smoking and the *LOC387715* SNP for Advanced AMD in the BMES Samples

Smoking Status	G/G	G/T	T/T
Nonsmoker	1	1.47 (0.78, 2.75)	2.12 (0.59, 7.63)
Smoker	1.12 (0.25–5.03)	5.36 (1.62–17.78)	13.25 (1.12–156.61)

Data are the ORs (95% CI). The ORs were adjusted for age and sex because age and sex matching were not performed between advanced AMD cases and all other subjects. Results of three comparisons were not significant ($P > 0.05$).

study that found evidence of this gene–environment interaction had a younger control group (average control age, 66.7 years).[34] The control groups in the present study and the study by Rivera et al.[33] both had average ages of 72 years. Clear geographic differences between the study populations exist as well, with the interaction finding being discovered in the Southeastern United States, whereas the studies that did not find an interaction were conducted in the Northeastern United States and Germany.[33,34] The present study includes populations from two distinct geographic areas: the United States and Australia.

Clinic-based case–control studies have inherent limitations. Information and selection bias may lead to an overestimation of effect size. Large cohort studies are necessary for properly estimating population attributable risk and relative risk.[40,41] A nested case–control sample from population-based studies similar to the BMES has many advantages, such as minimized sample selection bias, but also limitations such as small numbers of advanced AMD cases. For these reasons, the ORs and attributable risk (AR) estimate for *LOC387715* (AR = 9.4%) calculated from the BMES data can be interpreted as the AR for early and intermediate AMD, as most of the AMD cases in the BMES had early and intermediate stages of AMD. This also explains the lower OR observed from the BMES sample, as a previous study has shown that the frequency of the *LOC387715* risk allele is significantly higher in patients with advanced AMD than in those with early and intermediate AMD.[33] Smoking is also most strongly associated with advanced AMD in the BMES population.[39] The small proportion of advanced AMD cases in this sample limits our ability to assess the interaction between this gene marker and smoking appropriately, indicated by the very wide confidence intervals around the estimated risk for advanced AMD found in subjects with combined current smoking and *LOC387715* T/T genotype.

As yet, no biological function is known for *LOC387715*. The gene contains two exons encoding a protein of 107 amino acids. *LOC387715* is expressed in the placenta and appears to be weakly expressed in the retina.[33] Because *PLEKHA1* and *LOC387715* are in strong linkage disequilibrium and *PLEKHA1* encodes TAPP1, which remodels the intracellular actin cytoskeleton and plays a role in lymphocyte activation,[42,43] both may affect RPE function and AMD development. Additional functional studies are needed to clarify the role of this gene in AMD pathogenesis.

In summary, our findings from multiple independent studies in Australia and the United States confirm the association of *LOC387715* and AMD. Our data showed a possible interaction between smoking and advanced AMD, although the study did not confirm any overall interaction between smoking and *LOC387715*.

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