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CFH, *ELOVL4*, *PLEKHA1* and *LOC387715* genes and susceptibility to age-related maculopathy: AREDS and CHS cohorts and meta-analyses

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Age-related maculopathy (ARM) is an important cause of visual impairment in the elderly population. It is of crucial importance to identify genetic factors and their interactions with environmental exposures for this disorder. This study was aimed at investigating the *CFH*, *ELOVL4*, *PLEKHA1* and *LOC387715* genes in independent cohorts collected using different ascertainment schemes. The study used a case–control design with subjects originally recruited through the Cardiovascular Health Study (CHS) and the Age-Related Eye Disease Study (AREDS). *CFH* was significantly associated with ARM in both cohorts ($P \leq 0.00001$). A meta-analysis confirmed that the risk allele in the heterozygous or homozygous state (OR, 2.4 and 6.2; 95% CI, 2.2–2.7 and 5.4–7.2, respectively) confers susceptibility. *LOC387715* was also significantly associated with ARM in both cohorts ($P \leq 0.00001$) and a meta-analysis confirmed that the risk allele in the heterozygous and homozygous state (OR, 2.5 and 7.3; 95% CI, 2.2–2.9 and 5.7–9.4, respectively) confers susceptibility. Both *CFH* and *LOC387715* showed an allele-dose effect on the ARM risk, individuals homozygous at either locus were at more than two-fold risk compared to those heterozygous. *PLEKHA1*, which is closely linked to *LOC387715*, was significantly associated with ARM status in the AREDS cohort, but not the CHS cohort and *ELOVL4* was not significantly associated with ARM in either cohort. Joint action of *CFH* and *LOC387715* was best described by independent multiplicative effect without significant interaction in both cohorts. Interaction of both genes with cigarette smoking was insignificant in both cohorts. This study provides additional support for the *CFH* and *LOC387715* genes in ARM susceptibility via the evaluation of cohorts that had different ascertainment schemes regarding ARM status and through the meta-analyses.

INTRODUCTION

Age-related maculopathy (ARM) is a leading cause of central blindness in the elderly of industrialized nations. The prevalence of ARM is expected to increase because of the aging of these populations (1). The etiology of ARM is complex, with environmental as well as genetic susceptibility playing a role. Association-based analyses are generally more sensitive to small genetic effects than linkage-based analyses and are extremely valuable for fine mapping of disease-related genes (2). Case–control association studies with the use of unrelated individuals may have advantages over family-based studies, especially when a multilocus genetic model is anticipated (3,4), however, such studies are potentially sensitive to the ascertainment scheme for the case and control cohorts. For this reason, there is value in assessing candidate genes

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in populations from projects with different study designs. This current study investigates the complement factor H (*CFH*) gene, the elongation of very long chain fatty acid-like 4 (*ELOVL4*) gene, the pleckstrin homology domain-containing protein (*PLEKHA1*) gene, and the hypothetical *LOC387715* gene in two such distinct cohorts.

The association of the *CFH* gene with ARM susceptibility has been established in samples of European American descent (5–10) as well as in samples from the United Kingdom (11), Germany (12), France (13), Iceland (14) and Japan (15).

Three studies support the *PLEKHA1*/*LOC387715* locus on chromosome 10q26 (12,16,17). The study by Jakobsdottir *et al*. (16) reported that the *PLEKHA1*/*LOC387715* locus was significantly associated with ARM status, however, strong linkage disequilibrium between *PLEKHA1* and *LOC387715* in the independent family-based and case–control populations utilized for the study meant that a role for one gene over the other could not be determined (16). Evidence that the hypothetical *LOC387715* gene was more likely to be the gene accounting for susceptibility to ARM came from a study by Rivera *et al*. (12) that utilized two independent case–control samples (12) and a study by Schmidt *et al*. that utilized both family-based and case–control studies (17). All three studies indicated that the association of this region on chromosome 10q26 with ARM status was independent of the association with *CFH* that had been previously reported in all three populations (6,9,12). In addition, based on the Schmidt *et al*. study, the effect of the *LOC387715* locus appears to be modified by smoking history (17).

Two studies have evaluated a potential role for *ELOVL4* in ARM in humans. Ayyagari *et al*. (18) evaluated the gene and found no significant association with ARM status in their sporadic case–control analysis. However, Conley *et al*. found a significant association of *ELOVL4* and ARM status in familial and sporadic case–control analyses (9). The difference in findings between these studies may be related to the proportion of cases with exudative ARM in each population, since Conley *et al*. found that *ELOVL4* was especially associated with the exudative sub-phenotype (9). These results indicate that additional studies are needed to establish or refute a relationship between *ELOVL4* and ARM.

The two cohorts utilized for this study were the Cardiovascular Health Study (CHS), a population-based cohort of individuals 65 years and older at baseline for which ARM status was not a factor for ascertainment (19), and the Age-Related Eye Disease Study (AREDS), a cohort of individuals aged 55–80 years participating in a randomized controlled clinical trial of anti-oxidant and zinc intervention for which ARM status was a factor for ascertainment (20). These cohorts have been previously described (21,22).

This study was designed to evaluate the *CFH*, *ELOVL4*, *PLEKHA1* and *LOC387715* genes in two independent cohorts with very different ascertainment schemes in relation to ARM status and then to incorporate the findings into meta-analyses. Association of a gene with susceptibility to ARM regardless of ascertainment scheme would further increase the evidence that the association is real and would enhance the likelihood that evaluation of the gene(s) would accurately identify at risk individuals.

RESULTS

To further evaluate *CFH*, *ELOVL4*, *PLEKHA1* and *LOC387715* in ARM, we genotyped previously reported SNPs within all four genes in samples from the AREDS and CHS studies. Separate analyses were performed on each data set, using a total of 701 non-Hispanic white ARM patients and 175 controls from the AREDS study, and a total of 126 non-Hispanic white ARM patients and 1051 controls from the CHS study (see Table 1 for sample sizes and other characteristics of the data, and Table 2 for genotype frequencies). The disease status of subjects at their last follow-up visit was the primary endpoint evaluated for AREDS subjects. The AREDS subjects include controls of grade 1 and cases (grades 3–5) with moderate ARM and advanced ARM in one or both eyes. The ARM disease status of CHS subjects was evaluated by Dr Gorin, using monocular, non-mydriatic fundus photographs taken at the 8-year follow-up visit. The majority of CHS cases had moderate ARM including multiple drusen with and without pigment epithelial changes (equivalent to AREDS grade 3) with a small number of cases having geographic atrophy (GA) or choroidal neovascular membranes (CNV) and the CHS controls are of AREDS grade 1 with the exclusion of those cases with significant extramacular drusen.

Association analyses

For each gene, *CFH*, *ELOVL4*, *PLEKHA1* and *LOC387715*, association of one non-synonymous SNP with ARM was assessed by a χ^2 statistic. The magnitude of the effect of each variant was estimated by odds ratios (ORs) and population attributable risks (PARs). To evaluate whether the variants confer risk similarly to mild/moderate and advanced ARM, ORs were calculated for each grade and subtype (GA and CNV) separately using the AREDS data.

CFH. The association of the Y402H variant in *CFH* with ARM is extremely significant ($P \leq 0.00001$) in both the AREDS and CHS cohorts (Table 3), confirming earlier findings by ourselves (9,16) and others (5–7,12). The estimated ORs for Y402H in *CFH* suggest that the variant confers similar risk to all stages of ARM and both forms of advanced ARM, GA and CNV (Fig. 1 and Supplementary Material, Table S2). An allele-dose effect appears to be present, with carriers of two C alleles at higher risk of ARM than carriers of one C allele (Table 4 and Supplementary Material, Fig. S1). Despite the increased risk in carriers of two C alleles, the PAR is similar for the two risk genotypes, owing to relatively high frequency of the CT genotype compared to the CC genotype in the general population. PAR estimates derived from the CHS data set suggest that the CT and CC genotypes explain 27% and 25% of ARM in the non-Hispanic white population, respectively.

ELOVL4. The M299V variant in *ELOVL4* is significantly associated ($P = 0.034$) with exudative ARM in the AREDS sample (Table 3), in agreement with our previous findings (9). However, no ORs are statistically significant at 95% significance level (Fig. 1 and Supplementary Material, Table S2 and Supplementary Material, Fig. S2). These

Table 1. Characteristics of the study populations

	Mean (SD) age	Clinical subtypes				Total	No. males (%)
		Neither	GA only	CNV only	Both		
AREDS data							
Controls (1)	76.53 (4.44)	175	—	—	—	175	86 (49)
Cases (345)	79.46 (5.23)	123	147	278	153	701	293 (42)
Cases (45)	79.54 (5.23)	27	147	278	153	605	253 (42)
Cases (3)	78.93 (5.22)	96	0	0	0	96	40 (42)
Cases (4)	78.83 (5.23)	24	59	149	34	266	124 (47)
Cases (5)	80.10 (5.17)	3	88	129	119	339	129 (38)
CHS data							
Controls	70.27 (3.92)	1051	—	—	—	1051	455 (43)
Cases	73.22 (4.84)	100	15	9	2	126	55 (44)

In the AREDS cohort, mean age and phenotypic classification is based on age at last fundus photography. The number in the parentheses denotes the disease severity according the AREDS grading method. In the CHS cohort mean age is based on age at baseline visit, but retinal evaluation was done at 8-year follow-up visit.

results do not exclude the potential role of *ELOVL4* in ARM, but do not strongly support it. The small number of individuals with exudative ARM did not allow for subphenotype analysis in the CHS cohort.

PLEKHA1 and LOC387715. The association of the S69A variant in *LOC387715* with all presentations of ARM is extremely significant ($P \leq 0.00001$) in both the AREDS and CHS data sets (Table 3), confirming earlier findings by ourselves (16) and others (12,17). The A320T variant in *PLEKHA1*, which is located on the same haplotype block as *LOC387715*, is highly significant ($P = 0.00004$) in the AREDS sample but only borderline significant ($P = 0.08$) in the CHS sample. The degree of linkage disequilibrium between A320T and S69A is statistically significant in both AREDS ($D' = 0.66$) and CHS ($D' = 0.65$) controls. In order to identify which gene, *PLEKHA1* or *LOC387715*, more likely harbors the true ARM-predisposing variant, we applied the haplotype method (23). According to the haplotype method, the relative frequency of alleles at neutral variants is expected to be the same in cases and controls for a haplotype containing all the predisposing variants. The results based on applying the method suggest that S69A in *LOC387715*, and not A320T in *PLEKHA1*, is an ARM-predisposing variant (Supplementary Material, 'Distinguishing between *PLEKHA1* and *LOC387715*—Results'). Further, by permutation testing, the null hypothesis: H_0: the S69A variant in *LOC387715* fully accounts for the ARM predisposition to the *PLEKHA1–LOC387715* haplotype block, is not rejected ($P = 0.92$ in the AREDS data, $P = 0.45$ in the CHS data), while a similar hypothesis for A320T is rejected ($P \leq 0.0001$ in the AREDS data, $P = 0.0002$ in the CHS data).

The S69A variant in *LOC387715* shows different risk patterns than Y402H in *CFH*. The variant appears to increase the risk of severe ARM substantially more than the risk of mild ARM (Fig. 1, Supplementary Material, Table S2 and Supplementary Material, Fig. S4) in the AREDS data where severity of disease is differentiated. For example, the OR for AREDS cases of grade 3, who carry one or two T alleles, is 3.07 (95% CI 1.82–5.17), while the OR for AREDS cases, with CNV in both eyes, who carry one or two T alleles, is 7.21 (95% CI 4.24-12.27). Similar to *CFH*, S69A shows an allele-dose effect without dramatic differences in the PAR of the GT and TT genotypes (Table 4 and Supplementary Material, Fig. S4). Since only four AREDS controls are TT homozygous at S69A, point estimates and CIs, for recessive and homozygote contrasts, derived from regular logistic regression were compared with estimates from exact regression [models fitted in SAS software release 8.2 (SAS Institute Inc., Cary, NC, USA)]. These quality checks revealed no major differences in point estimates (which is the basis of the PAR estimates) and lower confidence limits (which is the basis of comparison with the ORs), but the upper confidence limits were higher (results not shown).

Interaction analyses

We used logistic regression modeling to build a model of the joint contribution of *CFH* and *LOC387715*, *CFH* and cigarette smoking and *LOC387715* and cigarette smoking. A series of models were fitted in order to draw inferences about the most likely and most parsimonious model(s). As described by North *et al*. (24), models were compared using the Akaike information criterion (AIC). When the most parsimonious model had been identified we estimated joint ORs of the risk factors. Separate estimates were calculated from each cohort. In order to maximize the AREDS sample size, no subphenotype or subgrade analyses were performed; AREDS cases of grade 3–5 were compared with AREDS controls of grade 1.

In a previous article (16), we found no evidence of interacting effects of the *CFH* and *PLEKHA1*/*LOC387715* loci; the joint action of the two loci was best described by independent multiplicative effects (additive on a log-scale). Rivera *et al*. (12) reported that S69A in *LOC387715* acted independently of Y402H in *CFH*. Schmidt *et al*. (17) also arrived at the same most parsimonious model. The AREDS and CHS data also suggest that the two genes contribute independently to disease risk. The best fitting model (the model with the smallest AIC) derived from the AREDS data is an additive model with an interaction term. This model, with AIC of 721.4, does however not provide a significantly better fit (AIC difference <2) than a simpler additive model with AIC of 723.0. The additive model is the most parsimonious model (AIC = 635.1) derived from the CHS data and is also the best fitting model (Table 5). Joint ORs for combinations of risk genotypes at Y402H and S69A were computed to further understand the joint action of the two loci (Supplementary Material, Table S4). Using all cases regardless of severity, the AREDS data suggest that individuals heterozygous for the risk allele at one of the loci and homozygous for the non-risk allele at the other are more susceptible to ARM than individuals with no-risk allele at both loci (for the CT–GG joint genotype, OR 2.8, 95% CI 1.6–5.0; for the TT–GT joint genotype, OR 3.2, 95% CI 1.7–6.0). The ARM risk more than doubles if a person is heterozygous at both loci (for the CT–GT joint genotype, OR 7.2, 95% CI 3.8–13.5) and being homozygous for the risk allele for at least one of the

Table 2. Genotype distributions by ARM status

Gene (Variant) and genotypes	Genotype frequencies in				
	AREDS cases ($n = 701$)	CHS cases ($n = 126$)	AREDS controls ($n = 175$)	CHS controls ($n = 1051$)	HapMap (CEU)
CFH (Y402H)					
TT	0.170	0.264	0.434	0.448	—
CT	0.435	0.482	0.416	0.450	—
CC	0.395	0.255	0.150	0.103	—
ELOVL4 (M299V)					
AA	0.781	0.742	0.711	0.802	0.717
AG	0.195	0.250	0.259	0.174	0.233
GG	0.024	0.008	0.030	0.024	0.050
PLEKHA1 (A320T)					
GG	0.474	0.411	0.339	0.346	0.317
AG	0.443	0.460	0.464	0.476	0.467
AA	0.084	0.129	0.196	0.178	0.217
LOC387715 (S69A)					
GG	0.313	0.442	0.645	0.604	0.583
GT	0.492	0.408	0.331	0.353	0.400
TT	0.195	0.150	0.023	0.043	0.017

AREDS cases are of grades 3–5 and AREDS controls of grade 1. Genotype counts are available by each grade and subphenotype in Table S1 of the Supplementary Material. Description of the HapMap CEU populations is given in the Supplementary Material.

Table 3. Results of allele- and genotype-association tests

Evaluated contrast in AREDS or CHS	*CFH*		*ELOVL4*		*PLEKHA1*		*LOC387715*	
	P-value for test		*P*-value for test		*P*-value for test		*P*-value for test	
	Allele	Genotype	Allele	Genotype[a]	Allele	Genotype	Allele	Genotype
AREDS								
1 versus 345	**≤0.00001**	**≤0.00001**	0.06775	0.13963	**0.00004**	**0.00004**	**≤0.00001**	**≤0.00001**
1 versus 5	**≤0.00001**	**≤0.00001**	0.20518	0.32438	**≤0.00001**	**≤0.00001**	**≤0.00001**	**≤0.00001**
1 versus 5 (GA)[b]	**≤0.00001**	**≤0.00001**	0.10465	0.21869	**0.04131**	**0.03862**	**≤0.00001**	**≤0.00001**
1 versus 5 (CNV)[c]	**≤0.00001**	**≤0.00001**	**0.03445**	**0.04851**	**≤0.00001**	**≤0.00001**	**≤0.00001**	**≤0.00001**
CHS	**≤0.00001**	**≤0.00001**	0.33832	0.07819	0.07626	0.22544	**≤0.00001**	**≤0.00001**

P-values < 0.05 are bolded.
[a]Two-sided *P*-values from Fisher's exact test.
[b]ARM cases have GA in both eyes.
[c]ARM cases have CNV in both eyes.

loci further increases the risk. The joint ORs estimated from the CHS data show a similar pattern, but having only one risk allele is not sufficient to increase the risk (for the CT–GG joint genotype, OR 1.3, 95% CI 0.6–2.7; for the TT–GT joint genotype, OR 1.2, 95% CI 0.5–2.8).

A recent study (17) reported a strong statistical interaction between genotypes at S69A and smoking, both on binary (ever versus never smoked) and continuous scale (pack-years of smoking). We fail to replicate this finding in both the AREDS and CHS data sets (Table 5). Results from the AREDS sample suggests that the joint effects of Y402H and smoking are best described by independent multiplicative effects, without significant dominance or interacting effects. On the other hand, the model that best describes the CHS data includes only additive effects of Y402H. Results from the AREDS data suggest that the joint effects of S69A and smoking are best described by independent multiplicative effects, without significant dominance or interacting effects. The CHS data implicate a model with only S69A. When smoking exposure is a continuous variable (pack-years of smoking) and the S69A genotypes are coded in additive fashion, the interaction term is not significant ($P = 0.40$) in the CHS data. Pack-years of cigarette smoking were not available for participants in the AREDS study. To further understand the combined effect of the genes and cigarette smoking, joint ORs of risk genotypes at each gene and smoking were estimated from the AREDS data (Supplementary Material, Table S7). The results suggest that, while the risk of ARM due to any of the risk genotypes (at Y402H and S69A) is elevated in smokers, both genes have substantially more influence on ARM risk than cigarette smoking. Both the model fitting approach and a simple χ^2 test ($P = 0.71$) show that the main effects of cigarette smoking are insignificant (on binary scale) in the CHS data.

APOE results

Main effects of the *APOE* gene in ARM were tested using the CHS data. Neither the distribution of *APOE*-ε4 carriers

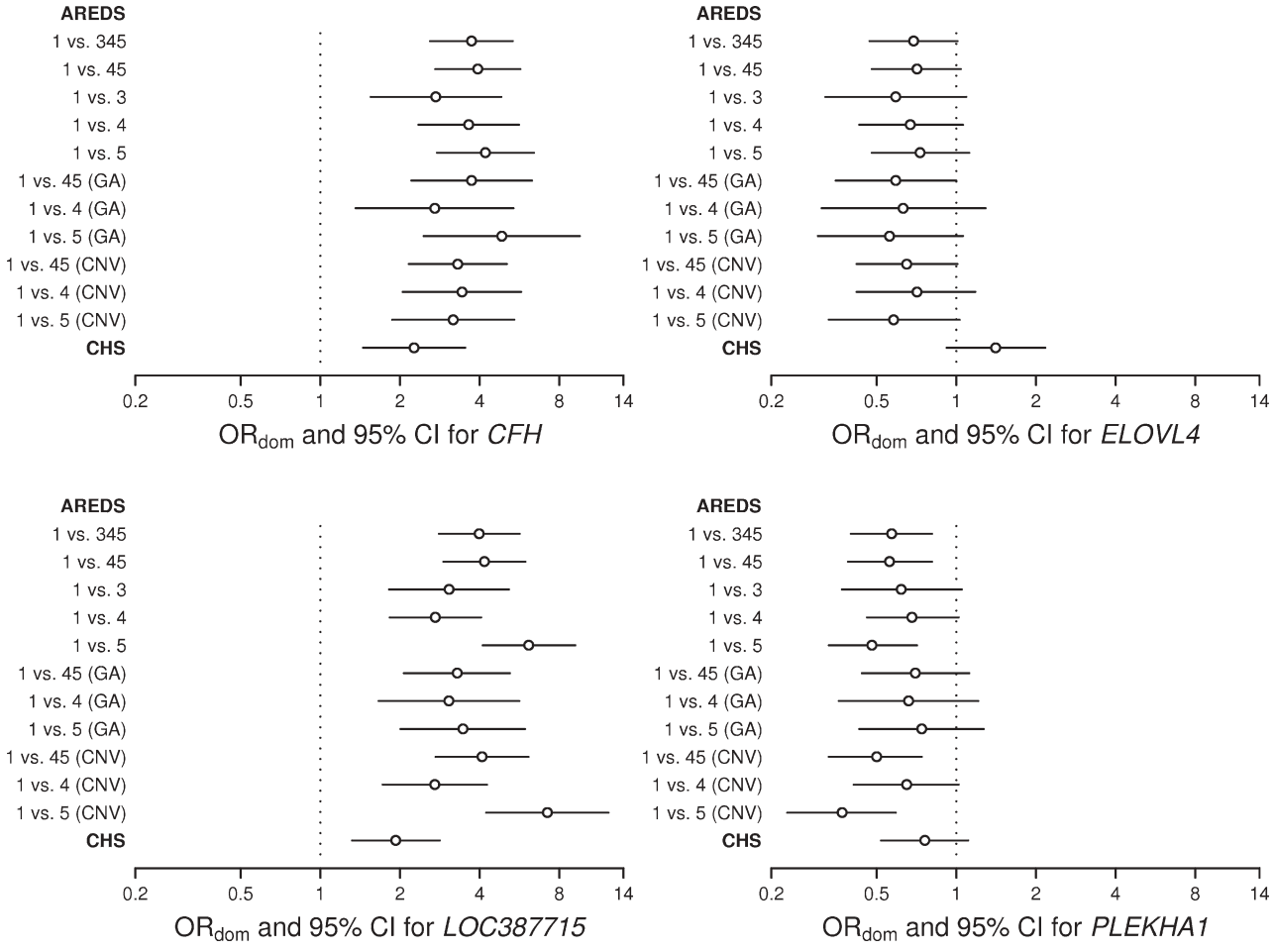

Figure 1. Estimated crude ORs and 95% CIs for *CFH*, *ELOVL4*, *PLEKHA1* and *LOC387715* genes. Carriers of one or two risk alleles (RR+RN) are compared with those subjects homozygous for the non-risk allele (NN). The solid lines denote the 95% CI corresponding to an OR (open circle). The dotted vertical line marks the null value of an OR of 1. The contrasts that were evaluated in AREDS and CHS cohorts are given on the vertical axis.

($P = 0.41$) nor *APOE*-ε2 ($P = 0.42$) carriers was significantly different between cases and controls, when compared to *APOE*-ε3/ε3.

Meta-analyses

Meta-analysis of CFH. We used a meta-analysis approach to pool estimated ORs for Y402H from 11 independent data sets [including the CHS and AREDS cohorts reported here (Supplementary Material, Table S10)]. This resulted in the analysis of 5451 cases and 3540 controls all of European or European American descent. The results confirm the increased ARM risk due to the C allele in the non-Hispanic white population (Fig. 2 and Supplementary Material, Table S11). The pooled estimates have narrower CI than any individual study, and non-overlapping CI for hetero- and homozygote ORs: $OR_{het} = 2.43$ (95% CI 2.17–2.72) and $OR_{hom} = 6.22$ (95% CI 5.38–7.19), when assuming homogeneity across studies. When the analysis is performed under heterogeneity, the point estimates are essentially the same and the CIs are slightly wider. Leave-one-out sensitivity analyses, under a fixed effect model, show that no study has dramatic influence on the pooled estimates (Supplementary Material, Table S11). The study by Rivera *et al*. (12) changes the estimates more than any other study; when the study is excluded, the OR_{dom} and OR_{het} are approximately 0.2 higher, while the OR_{rec} and OR_{hom} are lowered by approximately 0.2. The Rivera *et al*. study is the only study where the genotype distribution, in the control group, deviates from Hardy–Weinberg equilibrium [HWE ($P = 0.03$)]. The allele and genotype distributions, in cases and controls, are strikingly similar across studies. However, the genotype distribution in CHS cases differs from the other studies and the frequency of the CC risk genotype is lower compared to other cohorts (Supplementary Material, Fig. S5).

Meta-analysis of LOC387715. Meta-analysis of the risk associated with S69A in ARM included five independent data sets [including the CHS and AREDS cohorts reported here (Supplementary Material, Table S12)]. This resulted in the analysis of 3147 cases and 2381 controls all of European or European American descent. The studies of *LOC387715* are more heterogeneous than the studies of *CFH*; OR_{dom} and OR_{het} differ significantly across studies ($P < 0.01$ and 0.02,

Table 4. ORs and PAR% for subjects who are hetero- and homozygous for Y402H in *CFH* and S69A in *LOC387715*

Evaluated contrast in AREDS or CHS	Y402H in *CFH*				S69A in *LOC387715*			
	Heterozygotes (CT versus TT)		Homozygotes (CC versus TT)		Heterozygotes (GT versus GG)		Homozygotes (TT versus GG)	
	OR_{het}	PAR%	OR_{hom}	PAR%	OR_{het}	PAR%	OR_{hom}	PAR%
AREDS								
1 versus 345	2.66 (1.81,3.92)	43 (29,54)	6.69 (4.08,10.98)	37 (24,48)	3.06 (2.13,4.39)	42 (33,50)	17.26 (6.22,47.89)	41 (36,46)
1 versus 45	2.82 (1.89,4.19)	45 (31,56)	7.06 (4.27,11.70)	38 (24,50)	3.18 (2.20,4.60)	43 (34,52)	18.30 (6.57,50.93)	43 (37,48)
1 versus 3	1.93 (1.04,3.60)	30 (−3,52)	4.95 (2.46,9.95)	29 (−2,50)	2.45 (1.42,4.23)	34 (13,49)	11.89 (3.70,38.19)	32 (18,43)
1 versus 4	2.67 (1.67,4.27)	43 (24,57)	6.33 (3.60,11.16)	35 (16,50)	2.34 (1.55,3.53)	32 (19,43)	8.19 (2.80,24.00)	24 (16,31)
1 versus 5	2.94 (1.87,4.63)	47 (29,60)	7.71 (4.46,13.34)	41 (23,54)	4.32 (2.85,6.57)	54 (43,63)	32.07 (11.30,91.01)	57 (50,64)
1 versus 45 (GA)	2.54 (1.44,4.48)	41 (15,59)	7.04 (3.69,13.41)	38 (12,56)	2.81 (1.74,4.52)	39 (23,52)	10.14 (3.28,31.31)	28 (17,38)
1 versus 4 (GA)	1.68 (0.78,3.61)	23 (−21,51)	5.55 (2.48,12.41)	32 (−8,57)	2.74 (1.46,5.17)	38 (13,56)	7.57 (1.97,29.06)	22 (5,36)
1 versus 5 (GA)	3.47 (1.69,7.14)	53 (20,72)	8.65 (3.92,19.09)	44 (7,66)	2.86 (1.63,5.02)	40 (19,55)	12.02 (3.65,39.57)	32 (17,45)
1 versus 45 (CNV)	2.48 (1.57,3.93)	40 (21,55)	5.60 (3.21,9.78)	32 (13,47)	3.30 (2.17,5.01)	45 (33,55)	15.34 (5.32,44.25)	38 (30,46)
1 versus 4 (CNV)	2.78 (1.61,4.80)	44 (20,61)	5.24 (2.74,10.01)	30 (4,50)	2.44 (1.53,3.90)	34 (17,47)	6.58 (2.07,20.90)	19 (9,28)
1 versus 5 (CNV)	2.17 (1.22,3.86)	34 (6,54)	6.00 (3.12,11.53)	34 (7,53)	5.24 (3.02,9.10)	60 (43,72)	35.22 (11.47,108.17)	60 (46,70)
CHS	1.82 (1.13,2.92)	27 (1,46)	4.22 (2.39,7.42)	25 (3,42)	1.58 (1.05,2.39)	17 (−1,32)	4.75 (2.56,8.80)	14 (1,25)

95% CIs are given in the parentheses. Results for the *ELOVL4* and *PLEKHA1* genes are given in Table S2 of the Supplementary Material. Results for evaluations of dominance and recessive effects are given in Supplementary Material, Table S2.

respectively). The results support earlier findings of the association of the T allele with increased ARM risk (Supplementary Material, Table S13). Carriers of two T alleles are at substantially higher risk than are carriers of one T allele; when accounting for between-study variation, the OR_{het} and OR_{hom} are 2.48 (95% CI 1.67–3.70) and 7.33 (95% CI 4.33–12.42), respectively. The genotype distribution is similar across all control populations and across all ARM populations, except the CHS ARM population (Supplementary Material, Fig. S6).

DISCUSSION

During the past year, major discoveries of associations of the *CFH* and *PLEKHA1*/*LOC387715* genes with ARM were published. A number of reports established a strong association of the Y402H coding change in *CFH* with ARM and three reports found an association, of similar magnitude as the association of Y402H, of the S69A coding change in *LOC387715* with ARM. Both of those genes lie within chromosomal regions, *CFH* on 1q31 and *LOC387715* on 10q26, consistently identified by family-based linkage studies (25–31).

Because the majority of the studies of Y402H and all three studies of S69A were specially designed to search for (and find) genes involved in ARM complex etiology, it is possible that they overestimate the effect size of the risk alleles at Y402H and S69A. Therefore, we analyzed two independent case–control cohorts with varying inclusion and exclusion criteria based on ARM status, the AREDS and CHS cohorts. The AREDS cohort did have inclusion and exclusion criteria relevant to severity of ARM and both affected and non-affected individuals were enrolled (20). In contrast, the CHS cohort is a population-based cohort that utilized community-based recruitment of individuals 65 years and older with no inclusion and exclusion criteria relevant to ARM status (19). Retinal assessments in the CHS cohort were not conducted until the 8-year follow-up visit. Given the difference in ascertainment of subjects into the two studies, replication of association of a candidate gene in both cohorts greatly strengthens the support for its causal involvement in ARM pathogenesis.

We evaluated previously reported associations of four genes, *CFH* (1q31), *ELOVL4* (6q14), *PLEKAH1* (10q26) and *LOC387715* (10q26). Variants in both *CFH* and *LOC387715* are extremely significantly ($P \leq 0.00001$) associated with ARM in both AREDS and CHS cohorts. Both variants show an allele-dose effect on the ARM risk and a model of independent multiplicative contribution of the two genes is most parsimonious in both AREDS and CHS cohorts. The A320T coding change in the *PLEKHA1* gene, adjacent to and in linkage disequilibrium with *LOC387715* on 10q26, is significantly associated with ARM in the AREDS cohort ($P = 0.00004$), but not in the CHS cohort ($P = 0.08$). Because of extensive linkage disequilibrium between *PLEKHA1* and *LOC387715* in our initial study population we could not, with reasonable certainty, distinguish between their association signals. Our results based on applying the haplotype method to both the AREDS and CHS cohorts, combined with the findings of Rivera *et al*. (12), who used conditional haplotype analysis and detected, for the first time, a weak expression of LOC387715 in the retina, and Schmidt *et al*. (17), who detected only a weak association signal at *PLEKHA1*, indicate that S69A in *LOC387715* is most likely the major ARM-predisposing variant on 10q26. The results of the haplotype method show that *PLEKHA1* is not sufficient to account for the ARM-predisposition at 10q26; however, we cannot exclude the possibility that A320T in *PLEKHA1* may be on a causative haplotype with S69A and other unknown variants.

The replication of associations of *CFH* and *LOC387715* genes with ARM in AREDS and CHS cohorts, two cohorts with different study designs, continues to provide strong support for their involvement in ARM. Variable findings for *PLEKHA1* in AREDS and CHS cohorts do however need to be considered in the light of differences between the two cohorts. In addition to differences in ascertainment of the

Table 5. Results of fitting two-factor models by logistic regression

Two-Factor Model	AREDS data		CHS data	
	AIC	AIC difference	AIC	AIC difference
Y402H (Factor 1) and S69A (Factor 2)				
ADD1	799.3	77.9	652.7	17.6
ADD2	786.1	64.7	656.0	21.0
ADD-BOTH	**723.0**	**1.7**	**635.1**	**0.0**
DOM1	801.2	79.8	654.4	19.3
DOM2	786.9	65.5	656.0	21.0
DOM-BOTH	726.5	5.1	636.3	1.3
ADD-INT	721.4	0.0	635.8	0.8
ADD-DOM	724.3	3.0	638.8	3.8
DOM-INT	—	—	637.8	2.8
Y402H (Factor 1) and Smoking (ever versus never)				
ADD1	787.3	6.0	**677.3**	**0.0**
SMOKE	848.3	67.0	700.6	23.3
ADD1-SMOKE	**781.3**	**0.0**	679.1	1.8
DOM1	789.3	8.0	679.0	1.7
ADD1-SMOKE-INT	783.2	1.8	678.3	1.0
DOM1-SMOKE-INT	786.6	5.3	681.9	4.6
S69A (Factor 2) and Smoking (ever versus never)				
ADD2	774.0	6.1	**745.6**	**0.1**
SMOKE	842.9	75.0	765.2	19.8
ADD2-SMOKE	**767.9**	**0.0**	747.3	1.8
DOM2	774.7	6.7	745.5	0.0
ADD2-SMOKE-INT	769.7	1.8	749.1	3.7
DOM2-SMOKE-INT	772.4	4.4	748.9	3.4

Detailed model definitions are given in the 'Materials and Methods–Interaction Analyses' section. AIC difference is the difference from the AIC of the best fitting model. Most parsimonious model is in bold. Model with best fit (lowest AIC) has AIC difference = 0.

case and control populations, the evaluation of retinal changes, documentation of retinal findings and prevalence of advanced ARM differed between the two cohorts. In the CHS study, fundus photography was only available for one randomly selected eye and the photography was performed with non-dilated pupils and these limitations could certainly influence the sensitivity to detect disease pathology, although this is more likely to influence the detection of early retinal changes. The proportion of advanced ARM in the entire CHS cohort that was evaluated at the 8-year follow-up evaluation was ~1.3% (21) compared to ~17% in the AREDS (22) and the variation in the proportion of advanced ARM disease pathology between the two cohorts could lead to variation in findings, especially if a gene is more likely to influence progression of the disease. In addition, one important difference between these two cohorts is the timing of the retinal evaluations. AREDS participants had retinal evaluations conducted at baseline as well as during follow-up evaluations, whereas CHS participants had retinal evaluations done eight or more years after enrollment, when they would have been at least 73 years old. It is possible that survival to the retinal evaluation for the CHS participants could bias the population available for this particular type of study. Potential confounding issues related to the use of the AREDS cohort are that subjects in categories other than the unaffected group were randomized into a clinical trial using vitamin and mineral supplements to evaluate the impact of these on ARM progression and there is some evidence indicating that unaffected subjects in category 1 have different demographic characteristics than affected subjects in the other categories (22). It is not clear whether these could impact the results of our study, but it should be considered when findings are interpreted.

As mentioned previously, most studies that have investigated the genetic etiology of ARM were designed to optimize identification of regions of the genome housing susceptibility genes for ARM and for ARM candidate gene testing. Published attributable risks range from 43 to 68% (5,6,16,17) for the Y402H variant in *CFH* and from 36 to 57% (16,17) for the S69A variant in *LOC387715*. Interestingly, the PARs for the CHS population are lower than those previously published: 41% for the Y402H variant in *CFH* and 27% for the S69A variant in *LOC387715* (Supplementary Material, Table S2). Because the majority of the CHS cases have moderate ARM the PAR estimates derived from the CHS data are not completely comparable with estimates from previous studies in which the proportion of patients with advanced ARM was considerably higher. However, they are comparable to estimates derived from using AREDS cases of grade 3. Those estimates are within the previously published range of PARs: 49% for Y402H in *CFH* and 45% for S69A in *LOC387715*. These findings may indicate that the ARM attributed to these two susceptibility variants may be lower than previously thought, given that the CHS cohort was not ascertained based on ARM status. A prospective design is needed to more precisely estimate the relative risks, which are approximated by ORs estimated from retrospective case–control designs, and corresponding PARs.

We were not able to replicate the association of *ELOVL4* with overall ARM (9). The number of individuals with exudative ARM allowed us to perform subphenotype analysis in the AREDS, but not the CHS cohort. Subphenotype analysis was especially important with regard to *ELOVL4*, where our previous findings indicated a role for *ELOVL4* in exudative ARM; this is trending towards significance in the AREDS cohort. Given the lack of strong association and significant ORs for *ELOVL4* in ARM susceptibility in both cohorts and the lack of association reported by Ayyagari *et al*., it is very unlikely that *ELOVL4* plays a substantial role in ARM susceptibility. The power to detect an OR of 0.6 for overall ARM is reasonable, with type I error rate 5%, minor allele frequency 0.15 and population prevalence 6% the power is ~81% in AREDS and ~69% in CHS. The power to detect the same effect in exudative ARM is only ~53% in AREDS data, under the same conditions. Therefore, the possibility that *ELOVL4* plays a role in overall ARM is unlikely but mild effect in exudative ARM cannot be refuted. These power estimates were performed using Quanto (32).

We also used the CHS cohort to test whether the ε4 or ε2 alleles of the *APOE* gene are associated with ARM. In several studies, the ε2 allele is suggested to contribute to disease risk and the ε4 allele has been found to protect from ARM. Our results do not reach statistical significance and do not support the hypothesized role of the gene in ARM pathogenesis.

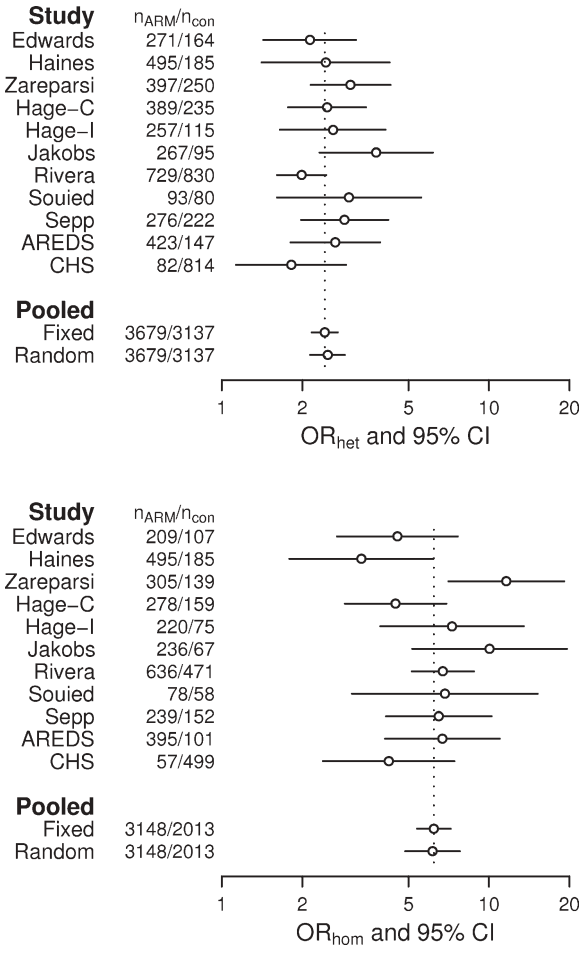

Figure 2. Estimated ORs and 95% CIs, derived from data sets included in meta-analysis of Y402H in *CFH*, and pooled estimates from fixed and random effect models. The top figure shows OR_{het} (OR for CT heterozygotes compared to TT) and the bottom figure shows OR_{hom} (OR for CC homozygotes compared to TT). 'Hage-C' and 'Hage-I' denote estimates derived from the Columbia and Iowa cohorts of Hageman *et al*., respectively, and 'Jakobs' denotes estimates from the Jakobsdottir *et al*. paper. 'Fixed' denotes pooled estimates derived from all the studies assuming the between-study variability is due to chance. 'Random' denotes pooled estimates derived from all the studies allowing for heterogeneity across studies. 'n_{ARM}' is the total number of ARM cases included in the estimates and 'n_{con}' is the total number of controls without ARM included in the estimates. For the Haines *et al*. study 'n_{ARM}' and 'n_{con}' refer to the whole sample (individuals of all genotypes). The dotted vertical line marks the point estimate of the pooled OR under homogeneity ('Fixed').

The AREDS and CHS data support the independent contribution of Y402H in *CFH* and S69A in *LOC387715* to ARM susceptibility. A multiplicative risk model for these two variants is the most parsimonious based on evaluation of the AREDS and CHS cohorts; this model was also supported by our previous paper (16) as well as data presented by Rivera *et al*. and Schmidt *et al*. (12,17). The ARM risk appears to increase as the total number of risk alleles at Y402H and S69A increases (Supplementary Material, Table S4).

Prior to the discovery of *CFH* and *LOC387715* cigarette smoking was one of the more important known ARM-related risk factors. Cigarette smoking is generally accepted as a modifiable risk factor for ARM; van Leeuwen *et al*. provide a review of the epidemiology of ARM and discuss the support of smoking as ARM risk factor (33). Schmidt *et al*. (17) recently reported statistically significant interaction between *LOC387715* and cigarette smoking in ARM. Their data suggested that the association of *LOC387715* with ARM was primarily driven by the gene effect in heavy smokers. Our own analyses of interaction do not support this finding and the AREDS data suggest that the joint action of S69A and smoking is multiplicative.

A role for *CFH* and *LOC387715* in ARM susceptibility is further supported via the results of our meta-analysis. The meta-analysis, which include the CHS and AREDS cohorts reported in this article, indicates that having one or two copies of the risk allele at *CFH* or *LOC387715* increases the risk of ARM, and those who have two copies are at higher risk. The combined results from all studies as well as the results from each independent study were remarkably tight (Figs 2 and 3). One known limitation of meta-analysis is the susceptibility to publication bias. Generally, such bias is a result of non-publication of negative findings (34). In the case of *CFH* and *LOC387715*, all published studies have reported strong association with ARM in the same direction, with the risk allele for *CFH* being the allele that codes for histidine and the risk allele for *LOC387715* being the allele that codes for serine. We expect the preferential publication of statistically significant associations to show random directionality if the significant association is a false-positive result (35). It is therefore unlikely that the consistency of the association of *CFH* and *LOC387715* with ARM is a result of publication bias.

While the results of our statistical analyses are in agreement with *LOC387715* being the major ARM-related gene on 10q26, they do not prove causality. The possible causal role of *CFH* in ARM pathogenesis has been further supported by the localization of its protein within drusen deposits of ARM patients and involvement in activation of the complement pathway. Regarding *LOC387715*, little is currently known about the biology of the gene and nothing about how its protein may affect ARM susceptibility. Until recently the expression of LOC387715 appeared limited to the placenta, but recently weak expression was reported in the retina (12), which opens up the possibility of a tissue-specific role of the gene.

In summary, our results continue to support a role of both *CFH* and *LOC387715* in etiology of ARM, given that both genes harbor variants highly associated with ARM, regardless of how the subjects were ascertained. Evaluation of *PLEKHA1* and *ELOVL4* in the AREDS and CHS cohorts demonstrates that these genes are much less likely to play role in ARM susceptibility. The *CFH* and *LOC387715* genes appear to act independently in a multiplicative way in ARM pathogenesis and individuals homozygous for the risk alleles at either locus are at highest risk. The continued support for these genes in ARM susceptibility will hopefully bring us closer to being able to utilize the information in these genes to identify at risk individuals and provide a rational basis for future clinical trials to test preventive therapies in high-risk cohorts as well as to provide insights into the basic pathogenesis of this condition.

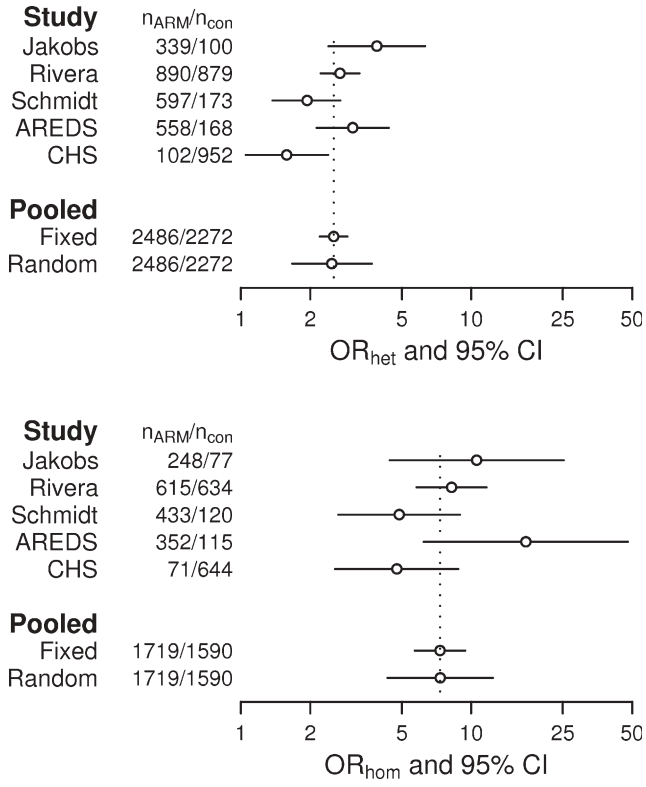

Figure 3. Estimated ORs and 95% CIs, derived from data sets included in meta-analysis of S69A in *LOC387715*, and pooled estimates from fixed and random effect models. The top figure shows OR_{het} (OR for GT heterozygotes compared to GG) and the bottom figure shows OR_{hom} (OR for TT homozygotes compared to GG). 'Jakobs' denote estimates from the Jakobsdottir *et al*. paper. 'Fixed' denotes pooled estimates derived from all the studies assuming the between-study variability is due to chance. 'Random' denotes pooled estimates derived from all the studies allowing for heterogeneity across studies. 'n_{ARM}' is the total number of ARM cases included in the estimates and 'n_{con}' is the total number of controls without ARM included in the estimates. For the Haines *et al*. study, 'n_{ARM}' and 'n_{con}' refer to the whole sample (individuals of all genotypes). The dotted vertical line marks the point estimate of the pooled OR under homogeneity ('Fixed').

MATERIALS AND METHODS

Cardiovascular health study (CHS) participants—sampling and phenotyping

CHS is a population-based, longitudinal study primarily designed to identify factors related to cardiovascular disease in those aged 65 and older. Retinal assessments were performed at the 8-year follow-up visit. Community-based recruitment took place in Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh, PA. Medicare eligibility lists of the Health Care Financing Administration were utilized to identify individuals who were aged 65 and older. Individuals aged 65 years and older living in the households of list members were also eligible. Inclusion criteria were minimal and included being non-institutionalized, expected to remain in the area for at least 3 years, able to give informed consent, not wheelchair-bound, not receiving hospice care and not receiving radiation or chemotherapy for cancer (19). DNA samples from the CHS from participants who consented for genetic studies were used for this research. Only DNA samples from subjects who had a retinal examination where the findings fit our criteria of a case or control were included in this study.

CHS subjects usually had the retina of one randomly selected eye photographed and the photographs were graded by Dr Gorin using the same classification model that was described in prior publications (29). Only Caucasian individuals are included in the analysis, as the sample size of other groups with ARM is too small for reasonable results: there were 180 black controls but only three cases, and five controls of other races. All CHS cases ($n = 126$) used for analyses are 'Type A', which falls into our most stringent model for clinical classification (29). Individuals in this category are clearly affected with ARM based on extensive and/or coalescent drusen, pigmentary changes (including pigment epithelial detachments) and/or the presence of end-stage disease (GA and/or CNV membranes). Very few CHS cases had end-stage ARM, GA or CNV (Table 1); therefore, analyses of specific subtypes of ARM were not conducted. All CHS controls ($n = 1051$) were of AREDS grade 1. A few potential controls ($n = 22$) had unclear signs of GA or CNV and were excluded from analyses.

Age-related eye disease study (AREDS) participants—sampling and phenotyping

AREDS is a prospective, multicenter study of the natural history of ARM and age-related cataract with a clinical trial of high-dose vitamin and mineral supplementation embedded within the study. Individuals recruited into the AREDS study were men and women aged 55–80 years at enrollment; these individuals were required to be free of any condition or illness that would hinder long-term follow-up. Inclusion criteria were minimal and included having ocular media clear enough to allow for fundus photography and either no evidence of ARM in either eye or having ARM in one eye while the other maintained good vision (20/30 or better) (20). DNA samples from subjects who consented for genetic studies from the NEI-AREDS Genetic Repository were used for this research.

ARM status was assigned using the AREDS ARM grading system and based on phenotypes assigned at the most recent follow-up visit. Again, only Caucasian individuals are included in the analysis, as the sample size of other groups is too small for reasonable results: there are only 15 African American, two Hispanic and three individuals of other races. AREDS cases ($n = 701$) consisted of grade 3, 4 and 5. AREDS subjects of grade 3 ($n = 96$) have ARM but do not suffer from end-stage ARM, subjects of grade 4 ($n = 266$) have end-stage ARM in one eye and subjects of grade 5 ($n = 339$) have end-stage ARM in both eyes. AREDS controls ($n = 175$) have AREDS grade 1 (grade 2 individuals were excluded prior to analyses).

Genotyping

The M299V variant in *ELOVL4* (rs3812153), the Y402H variant in *CFH* (rs1061170) and the S69A variant in *LOC387715* (rs10490924) were genotyped using RFLP techniques. The primers, annealing temperatures and restriction

endonuclease for each assay were: 5′-AGATGCCGATGTTG TTAAAAG-3′ (F), 5′-CATCTGGGTATGGTATTAAC-3′ (R), 50°C and *BspH*I for *ELOVL4*; 5′-TCTTTTTGTG CAAACCTTTGTTAG-3′ (F), 5′-CCATTGGTAAAACAA GGTGACA-3′ (R), 52°C and *Nla*III for *CFH*; 5′-GCA CCTTTGTCACCACATTA-3′ (F), 5′-GCCTGATCATCTGC ATTTCT-3′ (R), 54°C and *Pvu*II for *LOC387715*.

The A320T variant in PLEKHA1 (rs1045216) was genotyped using 5′ exonuclease Assay-on-Demand TaqMan assays (Applied Biosystems Incorporated). Amplification and genotype assignments were conducted using the ABI7000 and SDS 2.0 software (Applied Biosystems Incorporated). For all genotyping conducted for this research, double-masked genotyping assignments were made for each variant, compared and each discrepancy addressed using raw data or by re-genotyping.

Association analyses

SNP-disease association was measured with allele- and genotype χ^2 tests, and *P*-values were simulated using 100 000 replicates; in cases with one or more expected cell numbers less than five, the Fisher's exact test was used. The strength of the association was estimated by crude OR and PAR. A general formula was used to calculate the PAR: $PAR = P_r(OR - 1)/(1+P_r(OR - 1))$, where P_r is the prevalence of the risk factor in the general population. Estimates of P_r were derived from the CHS controls; this is reasonable, because the CHS subjects were not selected on the basis of ARM disease status, and the number of CHS controls is large ($n = 1{,}051$). Confidence intervals for the PARs were derived using asymptotic normal distribution of $\log(1 - PAR)$ and transforming to an interval for the PAR. The CIs derived in this way are likely to be too narrow when the risk factor is rare ($P_r < 0.1$) and sample sizes are small (36). For comparison purposes, ORs adjusted (OR_{adj}) for age and gender were estimated. Logistic regression models were used to calculate both crude and adjusted ORs, using R (37). The less frequent allele in the control group was considered the risk allele, and the OR and OR_{adj} were calculated by comparing those homozygous for the risk allele (RR) to the baseline group [those homozygous for the normal allele (NN)] and comparing those heterozygous for the risk allele (RN) to the baseline group. The contrasts for dominance (RR and RN versus NN) and recessive (RR versus RN and NN) effects were also evaluated.

Distinguishing between *PLEKHA1* and *LOC387715*

We employed the haplotype method (23) to identify which one of the two loci, A320T in *PLEKHA1* or S69A in *LOC387715*, is more likely the actual disease predisposing variant in the 10q26 region. The basis of the haplotype method is simple and elegant [for a mathematical proof, see Valdes and Thomson (23)]. If all predisposing variants are included on a haplotype, then the neutral variants are expected to be in the same ratio in cases and controls on a particular disease-predisposing haplotype, although the actual frequencies may differ. On the other hand, if not all predisposing variants have been identified, equality in the ratios of haplotype frequencies of non-predisposing variants is not expected. The expected ratios for the A320T–S69A haplotype are formulated in the Supplementary Material. Two null hypotheses were tested: one that A320T fully accounts for the ARM predisposition to the *PLEKHA1–LOC387715* haplotype block, and the other that S69A fully accounts for the ARM predisposition to the *PLEKHA1–LOC387715* haplotype block (for details on the hypotheses and permutation procedure to generate *P*-values, see the Supplementary Material). The program SNPHAP (38) was used to estimate haplotype frequencies and individual haplotypes. SNPHAP uses the EM algorithm to calculate a maximum likelihood estimate of haplotype frequencies given the unphased genotype data. The posterior probabilities of individual haplotype assignments exceed 87% for every individual typed at both A320T and S69A. For 80% of the haplotype assignments the underlying genotype at one or both loci is homozygous and hence the posterior probability is 100%.

Interaction analyses

The analyses of interaction were three-fold: first, we tested for interacting genetic effects of Y402H in *CFH* and S69A in *LOC387715* in both CHS and AREDS samples, then we tested for interaction of both Y402H and S69A with smoking history in both CHS and AREDS samples and finally we calculated joint ORs of the three risk factors.

We followed a modeling strategy proposed by North *et al*. (24). Series of logistic regression models are fitted to the AREDS and CHS data sets in order to find the model that best describes the joint effects of *CFH* and *LOC387715*. For each genotype, models allowing for additive effects (ADD1, ADD2 and ADD-BOTH), and models which incorporate dominance effects (DOM1, DOM2 and DOM-BOTH) are fitted. The ADD1 model includes only the term x_1 for additive effects of *CFH*, coded as -1 for genotype TT at Y402H, as 0 for genotype CT and as 1 for genotype CC. The ADD2 includes only model term x_2 for additive effects of *LOC387715*, coded as -1 for genotype GG at S69A, as 0 for genotype GT and as 1 for genotype TT. The ADD-BOTH models the joint additive effects of *CFH* and *LOC387715*. The DOM1 incorporates dominance effects to ADD1, and includes x_1 and z_1, coded as 0.5 for genotype CT and -0.5 for genotypes TT and CC at Y402H. The DOM2 model similarly incorporates dominance effects to ADD2, and includes x_2 and z_2, coded as 0.5 for genotype GT and -0.5 for genotypes GG and TT at S69A. DOM-BOTH models the joint dominance effects of *CFH* and *LOC387715*. Three further models, that model the interaction between *CFH* and *LOC387715* are fitted: ADD-INT includes the product term $x_1{}^*x_2$, ADD-DOM includes $x_1{}^*x_2$, $x_1{}^*z_2$ and $z_1{}^*x_2$ and DOM-INT includes $x_1{}^*x_2$, $x_1{}^*z_2$, $z_1{}^*x_2$ and $z_1{}^*z_2$.

The above modeling strategy was modified to investigate the joint effects of *CFH* and smoking, and the joint effects of *LOC387715* and smoking. The modified approach is the same as used by Schmidt *et al*. (17) to test for interaction between *LOC387715* and smoking. The coding scheme is the same, as above, except that smoking is coded as 0 for never smokers and 1 for ever smokers. The models fitted for the effects of *CFH* and smoking are: ADD1, SMOKE,

ADD1-SMOKE, DOM1, ADD1-SMOKE-INT and DOM1-SMOKE-INT, and the models fitted for the effects of *LOC387715* and smoking are: ADD2, SMOKE, ADD2-SMOKE, DOM2, ADD2-SMOKE-INT and DOM2-SMOKE-INT.

All models were compared by the AIC. Models for which the AIC differed by <2 are considered indistinguishable (24), and the model with fewer parameters was chosen as the most parsimonious model. Since adjusting for age and gender did not affect the estimates of ORs for Y402H nor S69A (Supplementary Material, Table S3), and to keep number of parameters as small as possible, no adjustment was made for these covariates when modeling interaction. Based on the results of the above interaction analyses, joint ORs were calculated.

APOE analyses

Previous studies have reported possible protective and harmful effects of the apolipoprotein E (*APOE*) gene in ARM. The ε4 allele may have protective effects (39–43), whereas the least frequent allele, ε2, may increase the risk of ARM (39,43). The *APOE* variant was genotyped by CHS and its association with ARM was assessed in this study. Individuals were classified by *APOE* genotype into individuals with *APOE*-ε3/ε3 genotype, and *APOE*-ε2 and *APOE*-ε4 carriers (denoted *APOE*-ε2/* and *APOE*-ε4/*, respectively); individuals with *APOE*-ε2/ε4 genotype were included in both the *APOE*-ε2/* and *APOE*-ε4/* groups. χ^2 tests were used to test for differences in distributions of *APOE*-ε3/ε3 and *APOE*-2ε/*, and *APOE*-3ε/3ε and *APOE*-4ε/*, genotypes in controls and cases.

Meta-analyses

We undertook a meta-analysis approach to pool estimated OR from previously published reports on *CFH* and *LOC387715* and the two reports presented here. Initially data were analyzed, assuming the between-study variation is due to chance, and fixed-effects model was employed. Under the fixed-effect model, the maximum likelihood estimator of the pooled OR is an average of individual estimates, weighted by the inverse of their variances, and the variance of the pooled OR is estimated by the inverse of the sum of individual weights. Meta-analyses under homogeneity were performed in R (37). The assumption of homogeneity was checked using a χ^2 test. However, tests of homogeneity tend to have low power, and therefore, for comparison, we also pooled the OR in a random effects setting. Meta-analyses under heterogeneity were performed using the method of restricted maximum likelihood (REML), as implemented in SAS Proc Mixed [SAS software release 8.2 (SAS Institute Inc.)]. The pooled REML estimator is identical to the DerSimonian-Laird estimator (44,45). The SAS codes by van Houwelingen *et al*. (45) were modified to perform the analyses under heterogeneity. A literature search was performed in PubMed in May 2006 and was limited to the English language. *CFH* studies were found by entering the search phrase: (CFH or 'Complement Factor H') and ('Age-related macular degeneration' or 'Age-related maculopathy' or AMD or ARM). Similarly, *LOC387715* studies were found using the search phrase: *LOC387715* and 'Age-related macular degeneration' or 'Age-related maculopathy' or AMD or ARM. The only inclusion criterion was that the research participants were Caucasian.

The Y402H variant within *CFH* has been found strongly associated with ARM in 11 studies (5–14,16); two of these 11 studies are ours, so only the results from our Jakobsdottir *et al*. (16) paper, that evaluated all contrasts, were used in meta-analysis. The Klein *et al*. (7) study used a small subset of the AREDS sample, and the Magnusson *et al*. (14) paper only reported allele-based ORs and no genotype counts. Therefore, these two studies were not included. Results from the Haines *et al*. (6) study were included in pooled estimates of ORs for hetero- and homozygotes; genotype counts were not available to evaluate contrasts for dominance and recessive effects. Three studies have reported highly associated variant, S69A, within the hypothetical *LOC387715* (12,16,17). All three reports on *LOC387715* were included in the meta-analysis. Research participants in all studies of *CFH* and *LOC387715* are non-Hispanic whites of European and European American descent. Supplementary Material, Tables S10 and S12 summarize the studies included in the meta-analyses of *CFH* and *LOC387715*, respectively.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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Conflict of Interest statement. The authors of this manuscript have no conflicts of interest, financial or otherwise, to disclose, though the University of Pittsburgh has filed a patent application regarding the use of variants in *PLEKHA1* and *LOC387715* for the determination of genetic risk associated with the development of ARM.

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