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Genetic Factors In Non-smokers with Age-related Macular Degeneration Revealed Through Genome-wide Gene-Environment Interaction Analysis

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SUMMARY

Relatively little is known about the interaction between genes and environment in the complex etiology of age-related macular degeneration (AMD). This study aimed to identify novel factors associated with AMD by analyzing gene-smoking interactions in a genome-wide association study of 1207 AMD cases and 686 controls of Caucasian background with genotype data on 668,238 single nucleotide polymorphisms (SNPs) after quality control. Participants' history of smoking at least 100 cigarettes lifetime was determined by a self-administered questionnaire. SNP associations modeled the effect of the minor allele additively on AMD using logistic regression, with adjustment for age, sex, and ever/never smoking. Joint effects of SNPs and smoking were examined comparing a null model containing only age, sex, and smoking against an extended model including genotypic and interaction terms. Genome-wide significant main effects were detected at three known AMD loci: *CFH* (P=7.51×10^{-30}), *ARMS2* (P=1.94×10^{-23}), and *RDBP*/*CFB*/*C2* (P=4.37×10^{-10}), while joint effects analysis revealed three genomic regions with P<10^{-5}. Analyses stratified by smoking found genetic associations largely restricted to non-smokers, with one notable exception: the chromosome 18q22.1 intergenic SNP rs17073641 (between *SERPINB8* and *CDH7*), more strongly associated in non-smokers (OR=0.57, P=2.73×10^{-5}), with an inverse association among smokers (OR=1.42, P=0.00228), suggesting that smoking modifies the effect of some genetic polymorphisms on AMD risk.

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Keywords

Age-related macular degeneration; age-related maculopathy; genome wide association studies (GWAS); gene-environment interaction; genome wide gene-environment interaction studies; smoking; smoking-gene interactions

Introduction

Age-related macular degeneration (AMD) is the leading cause of severe visual impairment in older adults in the developed world (Congdon *et al.*, 2004). AMD is responsible for more than half of all blindness in the US, with approximately 7.3 million persons aged 40 years and older diagnosed with intermediate AMD and an additional 1.75 million people affected with advanced AMD (Friedman *et al.*, 2004). With an aging population, the worldwide prevalence of this debilitating condition is expected to increase more than 50% by 2020 (Friedman *et al.*, 2004). Multiple inherited and environmental exposures contribute to risk and severity of this common disease. The strongest risk factor associated with AMD development is age (AREDS Research Group, 2000, McCarty *et al.*, 2001, Smith *et al.*, 2001). The incidences of various features of AMD also increase with age, including early changes such as multiple small drusen, intermediate features like large drusen and pigmentary changes, and advanced findings of choroidal neovascularization and geographic atrophy (Friedman *et al.*, 2004, Klein *et al.*, 2007). Other risk factors for the condition include female gender and Northern European Causasian background, as well as modifiable factors including antioxidant intake, hypertension, and obesity (Klein *et al.*, 2004, Tomany *et al.*, 2004). Cigarette smoking is the strongest modifiable risk factor for AMD, contributing to a two-fold increase in risk (Seddon *et al.*, 2005, McCarty *et al.*, 2001, Tomany *et al.*, 2004, Vingerling *et al.*, 1996).

Among non-modifiable risk factors for AMD, several genes have been identified through linkage and association studies, amongst which the most replicated are *CFH* (encoding complement factor H) (Haines *et al.*, 2005, Klein *et al.*, 2005, Hageman *et al.*, 2005, Edwards *et al.*, 2005), *CFB*/*C2* (encoding complement factor B and complement component 2, respectively) (Maller *et al.*, 2006, Gold *et al.*, 2006), *ARMS2* (age-related maculopathy susceptibility 2)/*HTRA1* (encoding HtrA serine peptidase 1) (Rivera *et al.*, 2005, Yang *et al.*, 2006), *C3* (encoding complement component 3) (Maller *et al.*, 2007, Yates *et al.*, 2007), and *APOE* (encoding apolipoprotein E) (Souied *et al.*, 1998, Klaver *et al.*, 1998). Two recent genome-wide association studies (GWAS) of AMD (Chen *et al.,* 2010, Neale *et al.,* 2011) implicated variation in several new candidate genes for AMD including *LIPC* (hepatic lipase), *ABCA1* (ATP-binding cassette, subfamily A member 1) and *TIMP3* (encoding metalloproteinase inhibitor 3), among others.

The role of smoking as a modifier of genetic risk for AMD has been evaluated in candidate gene studies of several of these major AMD loci. Our group previously identified suggestive evidence for interaction of smoking history with the *APOE* ε2 allele on AMD risk (Schmidt *et al.*, 2005), and we also reported synergistic interaction between cigarette smoking and genotypic variants in *ARMS2* (Schmidt *et al.*, 2006). Other groups observed contradictory evidence for some of these interactions (Conley *et al.*, 2006, DeAngelis *et al.*, 2007, Hughes *et al.*, 2007). More recently, a significant interaction with smoking was reported for AMD-associated variants in the *NOS2A* gene (Ayala-Haedo *et al.*, 2010).

While genetic studies like those mentioned here have clearly implicated roles for genes in the complement pathway and chronic inflammation, much of AMD's genetic contribution, with heritability (h^2) estimated to be between 45% and 71% (Hammond *et al.*, 2002, Seddon

et al., 2005), remains unknown and recent work has indicated that *ARMS2*/*HTRA1* and complement pathway genes explain at most 50% of the disease heritability (Maller *et al.*, 2006). Furthermore, while some pathological effects of smoking on AMD progression are known, it is unclear how this powerful risk factor may alter the role of genes outside of the known AMD risk genes in the complement pathway and *ARMS2*. With the powerful effect of smoking on AMD risk, it is also unclear if the association of genetic variants with disease may be masked due to undetected gene-smoking interactions in genome-wide association studies. For these reasons, we performed a genome-wide association study, analyzing data on 668,238 SNPs from 1207 persons with AMD and 686 persons free of disease. With the availability of detailed smoking history data, we also examined whether smoking history modified the effects of genomic variants on AMD risk, and performed secondary analyses to examine whether any genetic variants newly demonstrate association when accounting for smoking history.

Subjects and Methods

Ascertainment

Study participants were recruited from the Duke University Eye Center (DUEC), the Vanderbilt Eye Institute (VEI), and the Bascom Palmer Eye Institute (BPEI) at the University of Miami Miller School of Medicine. All recruitment was performed under research protocols approved by the Institutional Review Boards at each institution, and written informed consent was obtained from all participants.

All participants were examined by a retinal specialist by slit-lamp biomicroscopy and dilated fundus examination, including indirect ophthalmoscopy. Fundus imaging was also obtained from all subjects. Images were scored using a modified grading system based on the Age-Related Eye Disease Study (AREDS; AREDS Research Group, 2000) described in detail elsewhere (Schmidt *et al.*, 2000). Briefly, the grading system was scored from 1 to 5. The 1 and 2 categories corresponded to controls. The rest corresponded to early (grade 3) and advanced (grades 4 and 5) stages of AMD (For the complete grading list please see Table 1 footnotes).

DNA Cleaning and Genotyping

Whole blood obtained from participants underwent DNA extraction using a standardized protocol (Puregene; Gentra Systems, Minneapolis, MN). We performed high-density genotyping using the Affymetrix 6.0 chips on 1276 cases and 715 controls following the recommended protocol, with genotype calling using the Birdseed program in APT BirdSuite 1.8.6 (Korn *et al.*, 2008), with genotyping efficiency greater than 95%. Quality assurance was performed through inclusion of one CEPH control genotyped several times per 96-well plate. Technicians were blinded to affection status and quality-control samples.

Sample Quality Control

After genotyping, multiple quality control procedures were performed per sample, including assessing sample efficiency (the proportion of valid genotype calls to attempted calls within a sample). Samples with efficiency less than 0.95 were dropped from the analysis (N=58). Differences between reported gender and gender estimated from reporting of heterozygous genotypes of X-linked SNPs resulted in 13 inconsistent samples being dropped from the analysis. We used the program Graphical Representation of Relatedness (GRR) (Abecasis *et al.*, 2001) to test relatedness between samples, and 22 samples with proportion of alleles shared IBD ($\widehat{\pi}$) > 0.40 were dropped from the analysis.

We examined the case-control sample for evidence of population substructure by identifying a set of 10,000 SNPs with minor allele frequency (MAF)>0.25, selected for minimal between-SNP linkage disequilibrium (r^2<0.20) and spread evenly across the autosomal chromosomes. We analyzed this subset of SNPs via principal components analysis in the program EIGENSTRAT (Price *et al.*, 2006), generating principal component loadings for each genotyped individual, removing outliers (N=11) identified by using the top ten principal components over five iterations with a threshold of six standard deviations. The top three principal components were examined and found to have small loadings, suggesting the general absence of major population substructure, and thus population substructure was not adjusted for in association analyses.

After exclusions, we analyzed data on 1893 Northern European Caucasian participants, including 1207 cases (individuals graded 3, 4, or 5) and 686 controls (individuals graded 1 or 2).

SNP Quality Control

Quality control was performed to remove any low quality SNPs. Efficiency of individual SNPs was estimated as the proportion of samples with genotype calls for a given SNP, and SNPs with efficiency less than 0.95 were dropped from analysis. SNPs with MAF<0.05 were dropped from analysis because of low statistical power to detect association. Hardy-Weinberg Disequilibrium (HWD) statistics were calculated among controls with the Fisher's exact test in the PLINK software package (Purcell *et al.*, 2007) and SNPs with $P<10^{-6}$ for HWD were dropped from analysis. After these quality control measures, 668,238 SNPs remained for association analysis.

Covariate Data

A detailed smoking history was obtained by self-administered questionnaire. Participants were asked if they had smoked more than 100 cigarettes in their lifetimes; an affirmative answer lead to the description of the number of cigarettes per day, the age at which they had started smoking, whether they had quit, and if so, when. From these measures, a binary measure of "ever" or "never" smoking was coded.

The total number of individuals with smoking history and other environmental risk information (1,419 Northern European Caucasian participants) was included in the analysis of SNP/smoking interaction (894 AMD cases [grades 3, 4 and 5] and 525 unrelated controls [grades 1 and 2]). A full description of the participants by clinical findings and smoking status is presented in Table 1.

Statistical Analysis

Genotype Imputation

To provide more comprehensive coverage of the genome, genome-wide imputation was performed using the MaCH 1.0.16 software package (Li & Abecasis, 2006) with HapMap phase 2 (release 22) CEPH Utah pedigree (CEU) reference haplotypes and genotype data passing quality control. Imputation quality was assessed using R^2. Only results for SNPs imputed with R^2≥0.50 were examined. In analyses of imputation data, 2,543,887 SNPs in total were examined.

Association Analyses

Association analysis was performed using logistic regression to test association of genotypes with AMD under an additive model, with covariate adjustment for age and sex. To identify variants with associations potentially masked by association at *ARMS2* and *CFH*, we also

performed association analyses conditioning on minor allele dosage of the most significantly associated SNPs in *ARMS2* (rs10490924) and *CFH* (rs1831282) both separately and together in models also adjusting for age and sex. All analyses were performed using the PLINK software package (Purcell *et al.*, 2007). Quantile-quantile plots (Fig. 4) of the associations suggest the absence of systematic bias in the tests of association, with the primary analysis demonstrating a genomic inflation factor of λ=1.07.

Stratification by Smoking Status and Analysis of SNP-Smoking Interactions

Using covariate data on smoking history, we performed a genome-wide investigation of SNP-smoking interaction in AMD risk using logistic regression models including terms for SNP-specific effects modeled additively, ever/never smoking, and a SNP-smoking interaction term. Statistical interactions were identified for follow-up if a two degree-of-freedom (2df) test of association, comparing a null model without SNP main effect and SNP-smoking interaction to an extended model including both terms, generated $P<10^{-5}$. This approach has more statistical power to identify gene-environment interactions than marginal tests of independence of gene and environment in cases only or examining the statistical significance of interaction terms directly (Kraft *et al.*, 2007). Furthermore, examining genome-wide association results from the 2df test of interaction could identify potential novel genetic associations that may not have been previously observed due to confounding or mediating effects of smoking.

We also conducted stratified analyses by dividing our dataset into ever smokers (548 cases and 278 controls) and never smokers (346 cases and 247 controls). Within each stratum, we examined SNP associations in genomic regions with $P<10^{-5}$ from the 2df test to assess patterns of association with AMD using a logistic regression model with adjustment for age and sex.

Replication Analysis

To provide replication for the associations observed in primary data analysis of the discovery dataset, data from genome-wide genotyping and, for a subset of SNPs, individual SNPs genotyped via Taqman assay were examined in an independent set of 122 cases and 86 controls on the 79 SNPs with associations of $P<10^{-5}$ from discovery analyses. Replication analyses were performed in a manner analogous to the approach described for discovery analyses. Combined association analyses pooled data from both discovery and replication case-control datasets to strengthen statistical power to detect associations through increased sample size, and as with the replication data, were performed in a manner analogous to discovery analyses

Results

Dataset Characteristics

Table 1 depicts the demographic characteristics of the cases and controls examined in discovery analyses. We examined 1207 AMD cases (those with Grades 3, 4, or 5), with an average age of 77.6 years at examination (standard deviation (SD): ± 7.9 years), and 686 controls (those with Grades 1 or 2) with a significantly lower average age at examination of 70.9 years (SD: ± 7.8 years) ($P=2.5\times10^{-67}$ from $t_{df=2}$). Cases were more frequently female (63.5%) than controls (55.4%) (P=0.002). The frequency of smokers was higher among cases (N=548; 61.3%) than controls (N=278; 53.0%) (P=0.002). The significant differences in age, sex, and smoking history between the two groups suggested that these might be important confounders in the genetic associations, and therefore both age and sex were included as covariates in primary analysis with subsequent analyses also incorporating ever/never smoking.

Results from Genome-wide Association

Examining associations of genomic variants with AMD, 67 SNPs distributed over eight genomic regions demonstrated associations with $P<10^{-5}$ (Table 2; Figure 1; Supplementary Table 2). Similar to the prior published GWAS of AMD, the strongest associations fell in the chromosome 1q31.3 gene *CFH* (encoding complement factor H) at rs1831282 (OR (95% CI): 0.40 (0.35, 0.47), $P=7.51\times10^{-30}$) and in the *ARMS2* (age-related maculopathy susceptibility 2) locus at rs10490924 (OR (95% CI): 2.25 (1.92, 2.63), $P=1.94\times10^{-23}$) on chromosome 10q26.13. While the most commonly associated variant in *CFH*, Y402H (rs1061170) (Li *et al.*, 2006, Baird *et al.*, 2008, Zareparsi *et al.*, 2005) was not genotyped as part of the GWAS, the strongest signal in *CFH* at rs1831282 is at a SNP in high LD ($D'=0.98$; $r^2=0.90$) with the Y402H SNP in HapMap Caucasian (CEU) samples of Northern European Caucasian background. Multiple variants in both genes demonstrated associations with genome-wide statistical significance ($P<5.00\times10^{-8}$), including several potentially functional SNPs, such as the nonsynonymous coding SNPs rs800292 (V62I) in *CFH* ($P=7.37\times10^{-14}$) and rs10490924 (A69S) in *ARMS2* ($P=1.94\times10^{-23}$). Variation on chromosome 6p21.32 near loci *RDBP* and *CFB*/*C2* was also strongly associated with AMD, with the strongest association at rs522162 (OR (95% CI): 0.41 (0.31, 0.54), $P=4.37\times10^{-10}$). This SNP was in high LD ($D'=1.00$; $r^2=1.00$) with the *CFB* coding variant R32Q (rs641153), associated strongly with AMD in previous studies (Gold *et al.*, 2006, Spencer *et al.*, 2007, McKay *et al.*, 2009) but not genotyped in this study. In addition to these genome-wide significant associations, several regions demonstrated modestly significant evidence of association ($P<10^{-5}$), including chromosomes 2p23.3, 2q37.3, 4q32.2, 15q26.2, and 17q22,

Examining replication data on 122 cases and 86 controls passing quality control confirmed associations at the *ARMS2*, *CFH*, and *RDBP*/*CFB*/*C2* loci, as well as further strengthening the association observed for the associated variant at 17q22 ($P=1.73\times10^{-6}$), however neither the pattern nor strength of associations with borderline statistical significance at other loci (2p23.3, 4q32.2, 15q26.2, and 2q37.3) appeared to replicate in this smaller dataset. Several SNPs in genes proximal to strongly associated genes, like rs2253755 in *HTRA1* near *ARMS2* and rs406936 in *SKIV2L* near *RDBP*/*CFB*/ *C2* (Supplementary Table 2), demonstrated much stronger associations with the inclusion of replication data (rs2253755, discovery $P=1.45\times10^{-6}$ → combined $P=3.13\times10^{-9}$; rs406936, discovery $P=8.01\times10^{-6}$ → combined $P=6.80\times10^{-7}$). This is potentially the result of the clustering of multiple AMD-related genes in close proximity, in particular for *SKIV2L*, for which evidence exists that it is associated with AMD independently of *RDBP*/*CFB*/*C2* (Kopplin *et al.,* 2010).

To minimize potential effects of misclassification by affection status, we performed tests of association comparing a subset of cases with neovascular AMD (Grade 5) to controls with little or no evidence of AMD or drusen development (Grade 1) (Table 3; Fig. 2; Supplementary Table 2). While several regions demonstrated associations of $P<10^{-5}$, only those of variants in *CFH* and *ARMS2* attained genome-wide statistical significance (rs424535 in *CFH*, $P=6.54\times10^{-11}$; rs10490924 in *ARMS2*, $P=9.15\times10^{-26}$).

We also examined three models of associations conditioning on the genotypic effects of the most significantly associated variants in *ARMS2* and *CFH*, rs10490924 and rs1831282 respectively, with two models conditioning on each variant individually and one model conditioning on both (Table 4). Among associations conditioning on rs10490924 in *ARMS2* with $P<10^{-5}$, we observed several new associations with $P<10^{-5}$ at variants on chromosomes Xq27.1, 11p13, and 12q15, with associations of *CFH* variants also remaining highly significant (rs403846, OR (95% CI): 0.40 (0.34, 0.47); $P=1.09\times10^{-28}$). Conditioning on rs1831282 in *CFH*, *ARMS2* variation maintained highly significant association, with novel associations in chromosomes 11q22.1 and 14q21.3 without previous association at $P<10^{-5}$. Conditioning on both the *ARMS2* and *CFH* variants, only associations in the

RDBP/*CFB*/*C2* region maintained genome-wide statistical significance, with variants in few other regions demonstrating associations with $P<10^{-5}$. Associations near other AMD candidate loci including *C3*, *APOE*, *LIPC*, *ABCA1*, and *TIMP3* were largely unchanged after conditioning on either the *ARMS2* or *CFH* variants investigated (data not shown).

Genome-wide Interaction Analysis of Ever/Never Cigarette Smoking

We investigated differences in exposure to the single strongest modifiable environmental risk factor for AMD, cigarette smoking, to determine if smoking may modify genetic susceptibility to AMD. Overall, six genomic regions demonstrated evidence of both strong main effects and interaction with $P<10^{-5}$ from the 2df joint tests (Table 5; Fig. 3; Supplementary Table 3), including the regions containing genes *CFH* ($P=2.00\times10^{-23}$ from 2df test), *RDBP*/*CFB*/*C2* ($P=3.61\times10^{-8}$ from 2df test), and *ARMS2* ($P=1.55\times10^{-17}$ from 2df test). Parsing the terms contributing to associations in these three genes, the SNP main effects were of strong genome-wide statistical significance ($P<5.00\times10^{-8}$), however no nominally significant interactive effects were observed ($P<0.05$).

Several other genomic regions report $P<10^{-5}$ for the 2df joint tests, including variants on chromosomes 5p15.2, 6q16.2, and 18q22.1 (Table 5). The chromosome 18q22.1 variant, rs17073641, with $P=7.00\times10^{-7}$ from the 2-df joint test, is notable for both a strong main effect ($P=1.52\times10^{-5}$) and strong interactive effect ($P=1.19\times10^{-7}$). Stratified analyses of this SNP reveal an increased risk of AMD among ever smokers (OR (95% CI) = 1.42 (1.13, 1.77), $P=0.00228$) for each copy of the C allele at this locus (MAF = 0.45), but a strong protective effect of this allele among never smokers (OR (95% CI) = 0.57 (0.44, 0.74), $P=2.73\times10^{-5}$).

As several loci including *HTRA1* (Tam *et al.*, 2008) and *NOS2* (Ayala-Haedo *et al.*, 2010) have demonstrated evidence for gene-smoking interactions in AMD in previous studies, we examined effect modification of variants in and around (10kb upstream or downstream) these genes. Of the three SNPs in/near *NOS2* observed, rs11657662, rs9898084, and rs9895785, none of these demonstrated nominally statistically significant main effects ($P<0.05$) or interactive effects (2df $P<0.05$ or P for interaction <0.05). In *HTRA1*, which is located 5kb downstream of *ARMS2*, several variants with strong overall genetic effect on AMD (2df $P<0.05$) were observed at the 5′ end of the gene near *ARMS2*, however only one variant (rs2672587; 2df $P=3.06\times10^{-13}$) demonstrated statistical significance for the joint (2df) test of interaction.

In analyses stratified by ever/never smoking status, association findings for SNPs with $P<10^{-5}$ from the 2-df test differed greatly between strata (Table 5; Supplementary Table 4). Results for ever smokers resembled the associations reported for unstratified analyses, with the most statistically significant associations being observed in known AMD genes (*CFH*: OR (95% CI) = 0.38 (0.30, 0.48), $P=7.7\times10^{-16}$; *ARMS2*: 2.51 (1.97, 3.21), $P=1.06\times10^{-13}$; *RDBP*/*CFB*/*C2*: 0.34 (0.22, 0.52), $P=7.91\times10^{-7}$). One gene with suggestive association that was specific to ever smokers, *GLIS3* (OR (95% CI) = 0.60 (0.48, 0.76), $P=2.31\times10^{-5}$) is also known to be involved in eye development. In addition, several novel loci were implicated in the analysis of individuals who had never smoked, suggesting different underlying genetic associations for nonsmokers. The only genome-wide significant association observed among nonsmokers was at *CFH* SNP rs572515 (OR (95% CI) = 0.37 (0.28, 0.50), $P=1.74\times10^{-11}$), with similar size and directionality of effect as observed in unstratified analyses of that SNP (OR (95% CI) = 0.42 (0.36, 0.49), $P=7.77\times10^{-28}$). *ARMS2* was only suggestively associated in this stratum (OR (95% CI) = 1.87 (1.42, 2.47), $P=7.64\times10^{-6}$) (unstratified association: (OR (95% CI) = 2.25 (1.92, 2.63), $P=1.94\times10^{-23}$).

Examining a subset of loci that demonstrated modest evidence for interaction (*P* for interaction < 10^{-4}) but did not have significant main and interactive effects ($P<10^{-5}$) in the 2-df test, three strong association signals among non-smokers were in or near genes known to be involved in retinal cell function (intergenic SNP rs4619440, between *RGS9* and *AXIN2*: OR (95% CI) = 0.28 (0.16, 0.49), $P=7.59\times10^{-6}$; *EYS1*: 0.51 (0.38, 0.68), $P=6.33\times10^{-6}$; *DGKI*: 0.55 (0.41, 0.72), $P=2.41\times10^{-5}$).

Discussion

Our data confirms the association with AMD of variation at three loci, namely *ARMS2*, *CFH*, and *RDBP*/*CFB*/*C2*, and observed strong associations in several novel genomic regions, including chromosomes 15q26.2 and 17q22. Furthermore, incorporating a set of replication cases and controls strengthened associations at *ARMS2*, *CFH*, *RDBP*/*CFB*/*C2*, and the novel signal at 17q22. The chromosome 17q22 region is notable as several previous studies have implicated this region in disorders of vision; it is near a strong linkage signal on 17q21-17q22 to high myopia (hypothetical locus named myopia 5 [high grade, autosomal dominant] (*MYP5*)) (Paluru *et al.*, 2003). A chromosome 17q21.33 gene encodes collagen type 1 alpha 1 (*COL1A1*), an extracellular matrix gene which is expressed in the scleral wall and also implicated in myopia (Inamori *et al.*, 2007), though this gene is at a considerable distance from the association signal (approximately 4Mb downstream). While historically myopia has not shown association with AMD (Goldberg *et al.*, 1988, Wang *et al.*, 1998, Wong *et al.*, 2002), myopic degeneration has some similarities to AMD including loss of central vision due to photoreceptor cell degeneration; variation in this region may contribute to common early pathologic features of both myopia and AMD and thus subsequently increase risk of either outcome.

Notably, this study is the first to present results from a genome-wide scan for joint effects of genomic variation and history of ever/never smoking. Though none of the associations attained statistical significance, our analysis identified several novel regions with potential gene-smoking interaction and support gene-smoking interactions at the major risk loci *ARMS2* and *CFH*, each of which were initially described in prior candidate gene studies. A study of 103 discordant sibling pairs demonstrated that a smoking history of 10 pack-years or more increased risk of neovascular AMD 144-fold among those carrying the CC genotype at the Y402H (rs1061170) polymorphism of *CFH* relative to those with the CT or TT genotype (DeAngelis *et al.*, 2007). A study looking at ever/never smoking and Y402H similarly observed a positive interaction between the two in AMD risk (Baird *et al.*, 2008). In the present study, we observed a modest interaction of ever/never smoking with rs572515 which is in high LD ($D'=0.98$, $r^2=0.81$) with the common Y402H-proxy SNP, rs1831282. Likewise, we observed modest evidence for a gene-smoking interaction at *ARMS2*, specifically the functional SNP rs10490924 (A69S), providing additional confirmation for this interaction which we previously described in a subset of this sample (Schmidt *et al.*, 2006).

Among novel gene-smoking interactions detected is the interaction of rs522162 in the *RDBP*/*CFB*/*C2* region with ever/never smoking, for which the minor allele demonstrated a stronger protective association among smokers (OR (95% CI) = 0.34 (0.22, 0.52), $P=7.91\times10^{-7}$) than among non-smokers (OR (95% CI) =0.46 (0.28, 0.75), $P=0.00199$). We also identified variants in two regions, 6q16.2 and 18q22.1, with potential AMD-smoking interactions, the latter of which demonstrated a notable risk-increasing effect among ever smokers and protective effect among never smokers. We also observed interesting new evidence of a strong association on chromosome 5p15.2 among only individuals who smoked.

Although the strongest and most consistent association on chromosome 6 lies in the 6p21.32 region near the *RDBP* and *CFB*/*C2* loci, several lines of evidence suggest that loci near 6q16.2 approximately 66 Mb away may also contribute to macular disease. A linkage study of North Carolina macular dystrophy (Small *et al.*, 1992) identified a locus on chromosome 6q16 that contributed to an autosomal-dominant form of macular degeneration. Furthermore, studies of different forms of rare macular dystrophy have also identified linkage of Stargardt-like dominant progressive macular dystrophy to the 6q14-6q16 region, (Stone *et al.*, 1994, Kniazeva *et al.*, 1999) as have several studies on rare familial forms of macular degeneration and drusen development (Kniazeva *et al.*, 2000, Holz *et al.*, 1995). Furthermore, the associated variants on 6q16.2 including rs4840097 are near the *MCHR2* gene (melanin-concentrating hormone receptor 2), which is expressed in brain and is part of the rhodopsin family of genes involved in photoreceptor development.

Similarly, variation near chromosome 18q22.1 contributes to several disorders of vision that may share some common pathological features with AMD. One study identified variation in 18q22 that may contribute to pigmentary glaucoma (Andersen *et al.*, 1999); a more recent case study found that a deletion in 18q22.1 may have contributed to the occurrence of pigment dispersion syndrome (Mikelsaar *et al.*, 2007), which can lead to pigmentary glaucoma if untreated. *CDH7* also lies in 18q22 and encodes cadherin 7, which is most highly expressed in the retina (Wohrn *et al.*, 1998, Etzrodt *et al.*, 2009), particularly in the ganglion cell and amacrine layers (Liu *et al.*, 2007).

Potentially most interesting among our novel findings is the observed association of a chromosome 5p15.2 variant rs553169 with AMD among smokers (P=1.88×10^{-7}) but not among nonsmokers (P=0.175). This SNP is proximal to *SEMA5A*, encoding Semaphorin 5A, which inhibits axonal growth by retinal ganglion cells (Goldberg *et al.*, 2004). This region was also mapped as the strongest linkage signal (*MCDR3*, LOD=3.61) in a study examining a well-documented four-generation English family with a phenotype with features similar to North Carolina Macular Dystrophy, including retinal pigment epithelium abnormalities and the formation of drusen-like deposits (Michaelides *et al.*, 2003). Further examination of associations in this region among smokers may identify a susceptiblity gene for AMD for which effects may be limited to those with a history of smoking.

While these separate pieces of evidence suggest that chromosomes 6q16.2 and 18q22.1 may both hold vision-related loci, and 5p15.2 may hold an AMD locus with effects only among smokers, replication of these findings in datasets enriched with subjects with well-characterized smoking history data is necessary to confirm these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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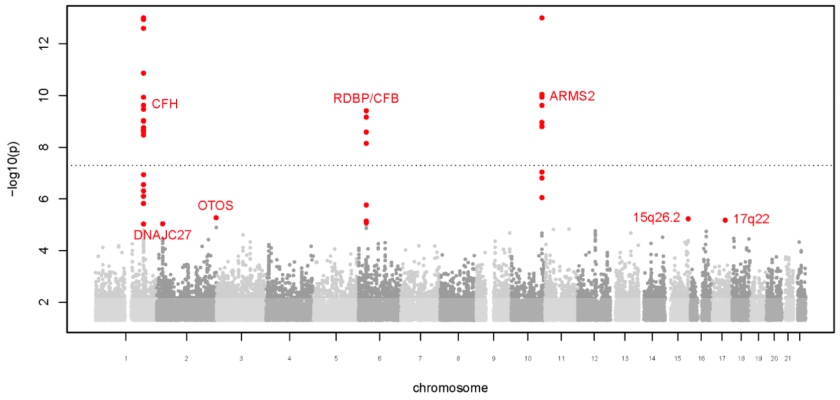

Figure 1.

Manhattan plots of observed –log *P*-values for AMD (y-axis) by chromosome (x-axis) from analysis of the discovery dataset of 1,207 AMD cases and 686 controls in the basic model adjusting for sex and age-at-exam. All SNPs with associations below $P < 10^{-5}$ are highlighted in red. SNPs with associations $P < 10^{-13}$ that reside in the *CFH* and *ARMS2* regions are not depicted here. The dotted line demarcates the threshold for genome-wide statistical significance, $P < 5.00 \times 10^{-8}$.

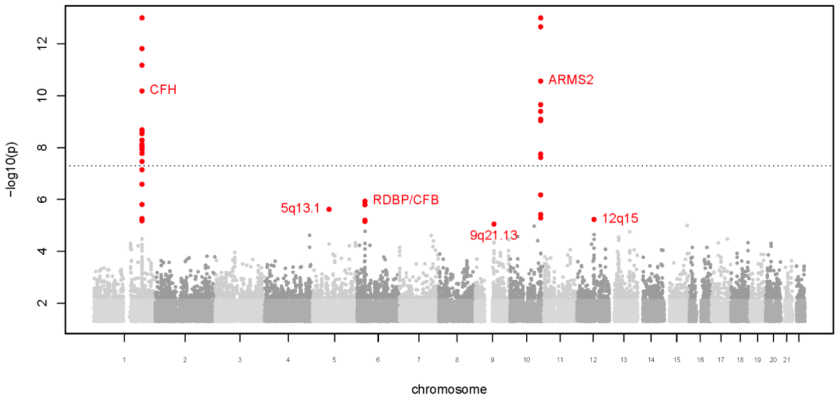

Figure 2.

Manhattan plots of observed –log *P*-values for AMD (y-axis) by chromosome (x-axis) from analysis of the discovery dataset of 720 grade 5 AMD cases and 513 grade 1 controls in the basic model adjusting for sex and age-at-exam. All SNPs with associations below $P < 10^{-5}$ are highlighted in red. SNPs with associations $P < 10^{-13}$ that reside in the *CFH* and *ARMS2* regions are not depicted here. The dotted line demarcates the threshold for genome-wide statistical significance, $P < 5.00 \times 10^{-8}$.

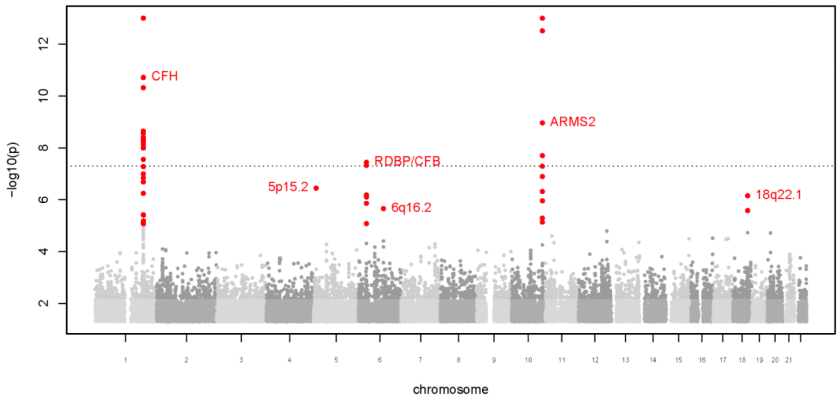

Figure 3.

Manhattan plots of observed –log *P*-values for the 2-df test of association with AMD from gene-smoking interaction analysis (y-axis) by chromosome (x-axis). Analyses were performed on the discovery dataset of 894 AMD cases and 525 controls for whom data on smoking history were available, and modeling adjusted for sex and age-at-exam, as well as ever/never smoking. The two degree-of-freedom test evaluates statistical significance of the inclusion of both genetic main effect and interaction terms with the potential to identify associated variants masked by the effect of smoking. All SNPs with associations below $P < 10^{-5}$ are highlighted in red. SNPs with associations $P < 10^{-13}$ that reside in the *CFH* and *ARMS2* regions are not depicted here. The dotted line demarcates the threshold for genome-wide statistical significance, $P < 5.00 \times 10^{-8}$.

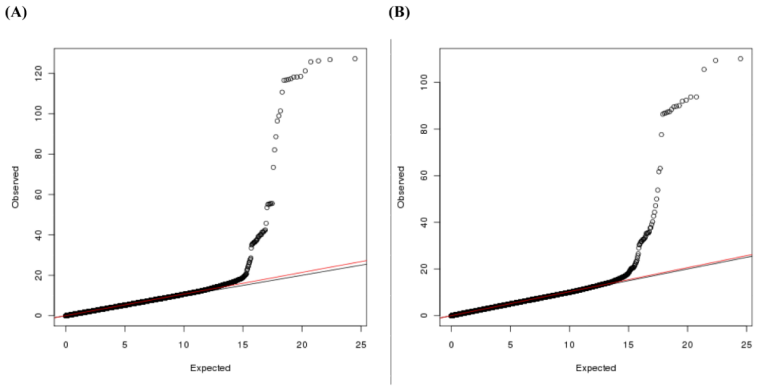

Figure 4.

Quantile-quantile plots of observed (y-axis) vs. expected (x-axis) *P*-values for LOAD from the AMD discovery sample under the basic model adjusting for age-at-exam and sex. Plot **(A)** examines the results from all cases vs. all controls in the discovery sample, whereas **(B)** examines the results from association among grade 5 cases vs. grade 1 controls. The genomic inflation factor for analysis of all cases and controls is estimated to be $\lambda = 1.07$, whereas for the analysis of grade 5 cases vs. grade 1 controls, $\lambda = 1.03$.

Table 1

Descriptive statistics for the AMD discovery and replication datasets.

AMD Grade	N	Female (%)	Mean Age (SD)	Ever Smoked (%)	Never Smoked (%)
Discovery					
Cases	*1207*	*767 (63.5%)*	*77.62 (7.89)*	*548 (45.4%)*	*346 (28.7%)*
5	720	456 (63.3%)	78.48 (7.6)	349 (48.5%)	185 (25.7%)
4	148	93 (62.8%)	79.89 (7.8)	57 (38.5%)	43 (29.1%)
3	339	218 (64.3%)	74.8 (7.82)	142 (41.9%)	118 (34.8%)
Controls	*686*	*380 (55.4%)*	*70.86 (7.81)*	*278 (40.5%)*	*247 (36%)*
2	173	90 (52.0%)	71.75 (8.25)	83 (48%)	62 (35.8%)
1	513	290 (56.5%)	70.56 (7.64)	195 (38%)	185 (36.1%)
TOTAL	**1893**	**1147 (60.6%)**	**75.17 (8.47)**	**826 (43.6%)**	**593 (31.3%)**
Replication					
Cases	*137*	*86 (62.8%)*	*77.07 (8.44)*	*27 (19.7)*	*16 (11.7)*
5	83	56 (67.4%)	77.59 (9.00)	11 (13.3)	4 (4.8%)
4	20	12 (60.0%)	80.25 (5.57)	3 (15.0%)	2 (10.0%)
3	34	18 (52.9%)	73.91 (7.56)	13 (38.2%)	10 (29.4%)
Controls	*91*	*59 (64.8%)*	*72.70 (6.05)*	*11 (12.1%)*	*4 (4.4%)*
2	13	10 (76.9%)	74.92 (8.27)	4 (30.8%)	1 (7.7%)
1	78	49 (62.8%)	72.33 (5.58)	7 (9.0%)	3 (3.8%)
TOTAL	**228**	**145 (63.6%)**	**75.32 (7.86)**	**38 (16.7%)**	**20 (8.8%)**
Combined	**2121**	**1292 (60.9%)**	**75.18 (8.41)**	**864 (40.7%)**	**613 (28.9%)**

Grade 1: No drusen or small (<63 μm) non-extensive drusen without retinal pigment epithelium (RPE) abnormalities

Grade 2: Extensive small drusen or non-extensive intermediate drusen and/or RPE hyperpigmentation or hypopigmentation

Grade 3: Extensive intermediate drusen or any large soft drusen (> 125 μm), including drusenoid RPE detachment

Grade 4: Geographic atrophy (area of RPE atrophy with sharp margins, usually visable choroidal vessels, at least 175 μm diameter)

Grade 5: Extensive AMD, including nondrusenoid RPE detachment, choroidal neovascularization, subretinal hemorrhage or fibrosis, or photocoagulation scar consistent with treatment of AMD

Table 2

Genome-wide Association Results for AMD in the discovery (1,207 cases / 686 controls), replication (122 cases/ 86 controls), and combined (1,329 cases/ 772 controls) analyses. Association signals represent SNPs demonstrating $P \leq 10^{-5}$ in the discovery dataset with either **(A)** the strongest associations within each locus or **(B)** SNPs with potential functional effects. In tests of association, the major allele was used as reference. Genes with previously published case-control association signals at $P \leq 5.00\times10^{-8}$ are denoted with *.

SNP	Band	CH:MB	Nearest Gene	Role	MA	MAF	Discovery Analysis		Replication Analysis		Combined Analysis	
							OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
(A) Most Statistically Significant Associations by Genomic Region												
rs1831282	1q31.3	1:194.9	*CFH**	Intron	C	0.45	0.40 (0.35, 0.47)	7.51×10^-30	0.53 (0.34, 0.81)	0.00331	0.42 (0.36, 0.48)	1.03×10^-31
rs713586	2p23.3	2:25.0	*DNAJC27*	Downstream	G	0.49	1.41 (1.22, 1.64)	6.58×10^-6	0.95 (0.63, 1.43)	0.804	1.35 (1.18, 1.56)	2.56×10^-5
rs4854022	2q37.3	2:240.7	*OTOS*	Promoter	G	0.23	1.52 (1.27, 1.82)	6.81×10^-6	1.08 (0.65, 1.80)	0.758	1.46 (1.23, 1.74)	1.34×10^-5
rs10857341	4q32.2	4:162.8	*FSTL5*	Intron	T	0.05	0.47 (0.34, 0.64)	2.70×10^-6	1.11 (0.44, 2.79)	0.829	0.52 (0.38, 0.69)	1.31×10^-5
rs522162	6p21.32	6:32.0	*RDBP/CFB**	3′-UTR	G	0.07	0.41 (0.31, 0.54)	4.37×10^-10	0.61 (0.28, 1.33)	0.217	0.43 (0.33, 0.55)	1.90×10^-10
rs10490924	10q26.13	10:124.2	*ARMS2**	Missense (A69S)	A	0.35	2.25 (1.92, 2.63)	1.94×10^-23	2.56 (1.59, 4.12)	1.03×10^-4	2.28 (1.96, 2.65)	7.61×10^-27
rs10438448	15q26.2	15:92.3	--	--	C	0.29	1.48 (1.25, 1.74)	4.71×10^-6	0.89 (0.56, 1.42)	0.627	1.40 (1.20, 1.63)	2.71×10^-5
rs6503782	17q22	17:52.5	--	--	T	0.49	0.71 (0.62, 0.83)	6.74×10^-6	0.70 (0.45, 1.08)	0.103	0.71 (0.62, 0.82)	1.73×10^-6
(B) Most Statistically Significant Associations Among Potentially Functional SNPs												
rs800292	1q31.3	1:194.9	*CFH**	Missense (V62I)	T	0.17	0.47 (0.39, 0.58)	7.37×10^-14	0.53 (0.31, 0.91)	0.0203	0.48 (0.40, 0.58)	4.41×10^-15
rs4854022	2q37.3	2:240.7	*OTOS*	Promoter	G	0.23	1.52 (1.27, 1.82)	6.81×10^-6	1.08 (0.65, 1.80)	0.758	1.46 (1.23, 1.74)	1.34×10^-5
rs10490924	10q26.13	10:124.2	*ARMS2**	Missense (A69S)	A	0.35	2.25 (1.92, 2.63)	1.94×10^-23	2.56 (1.59, 4.12)	1.03×10^-4	2.28 (1.96, 2.65)	7.61×10^-27

CH:MB, chromosome:position (in mega base pairs, build 19); MA, minor allele; MAF, minor allele frequency; OR, odds ratio; P, *P*-value.

Nearest Gene is determined as most proximal gene within 50kb 5′ or 3′ of the variant.

Genes with previous case-control genome-wide statistically significant associations: *ARMS2, CFB/C2/RDBP, CFH.*

Table 3

Genome-wide Association Results for AMD using extreme sampling of Grade 5 AMD cases and Grade 1 controls in the discovery (720 Grade 5 cases / 513 Grade 1 controls), replication (70 Grade 5 cases /73 Grade 1 controls), and combined (790 Grade 5 cases /586 Grade 1 controls) analyses. Association signals represent the strongest associations within each locus where SNPs demonstrate $P \leq 10^{-5}$ in the discovery dataset. In tests of association, the major allele was used as reference. Genes with previously published case-control association signals at $P \leq 5.00\times10^{-8}$ are denoted with *.

SNP	Band	CH:MB	Nearest Gene	Role	MA	MAF	Discovery Analysis		Replication Analysis		Combined Analysis	
							OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs424535	1q31.3	1:195.0	*CFH**	Intron	T	0.31	0.51 (0.41, 0.62)	6.54×10^-11	0.65 (0.38, 1.12)	0.118	0.52 (0.43, 0.63)	2.38×10^-11
rs12516844	5q13.1	5:68.1	--	--	G	0.11	0.49 (0.37, 0.66)	2.40×10^-6	1.43 (0.58, 3.51)	0.432	0.55 (0.42, 0.73)	2.66×10^-5
rs522162	6p21.32	6:32.0	*RDBP/CFB**	3′-UTR	G	0.07	0.42 (0.29, 0.59)	1.17×10^-6	0.48 (0.18, 1.29)	0.144	0.42 (0.30, 0.59)	3.34×10^-7
rs10781185	9q21.13	9:75.9	--	--	T	0.28	1.63 (1.32, 2.01)	6.56×10^-6	1.03 (0.58, 1.80)	0.930	1.51 (1.24, 1.83)	4.15×10^-5
rs10490924	10q26.13	10:124.2	*ARMS2*	Missense (A69S)	A	0.37	3.07 (2.49, 3.78)	9.15×10^-26	3.29 (1.76, 6.14)	1.86×10^-4	3.09 (2.54, 3.77)	4.55×10^-29
rs11178531	12q15	12:69.7	--	--	A	0.45	1.54 (1.28, 1.86)	5.94×10^-6	0.55 (0.32, 0.95)	0.0318	1.36 (1.14, 1.62)	5.10×10^-4
rs6614655	Xp11.22	23:50.9	--	--	C	0.19	2.02 (1.50, 2.70)	2.65×10^-6	0.65 (0.31, 1.37)	0.254	1.72 (1.32, 2.25)	6.87×10^-5

CH:MB, chromosome:position (in mega base pairs, build 19); MA, minor allele; MAF, minor allele frequency; OR, odds ratio; *P, P*-value.

Nearest Gene is determined as most proximal gene within 50kb 5′ or 3′ of the variant.

Genes with previous case-control genome-wide statistically significant associations: *ARMS2, CFB/C2/RDBP, CFH.*

Table 4

Genome-wide Association Results for AMD from analyses conditioning on the most strongly associated SNPs in main analysis from the *ARMS2* and *CFH* genes: (a) conditioning on the *ARMS2* SNP rs10490924; (b) conditioning on the *CFH* SNP rs1831282; and (c) conditioning on both rs10490924 from *ARMS2* and rs1831282 from *CFH*. Association signals represent the strongest associations within each locus where SNPs demonstrate $P \leq 10^{-5}$ in the discovery dataset. Genes with previously published case-control association signals at $P \leq 5.00\times10^{-8}$ are denoted with *.

SNP	Band	CH:MB	Nearest Gene	Role	MA	MAF	Conditional Analysis		Discovery Analysis	
							OR (95% CI)	*P*	OR (95% CI)	*P*
(A) Associations by Genomic Region Conditioning on ARMS2 SNP										
rs403846	1q31.3	1:195	*CFH**	Intron	G	0.4512	0.40 (0.34, 0.47)	1.09×10^-28	0.40 (0.35, 0.47)	2.07×10^-29
rs541862	6p21.32	6:32	*CFB**	Intron	C	0.06914	0.42 (0.31, 0.56)	5.38×10^-9	0.41 (0.31, 0.54)	6.94×10^-10
rs10438448	15q26.2	15:92.3	--	--	C	0.2894	1.51 (1.27, 1.79)	2.77×10^-6	1.47 (1.24, 1.74)	5.88×10^-6
rs6503782	17q22	17:52.5	Near *FGF13*	--	T	0.4855	0.70 (0.60, 0.81)	2.83×10^-6	0.71 (0.62, 0.83)	6.66×10^-6
rs12556103	Xq27.1	X:138.1	--	--	G	0.4081	0.67 (0.56, 0.79)	4.27×10^-6	0.70 (0.59, 0.83)	2.49×10^-5
rs728749	11p13	11:35.9	--	--	G	0.3229	0.69 (0.58, 0.81)	4.54×10^-6	0.71 (0.61, 0.83)	1.54×10^-5
rs11178531	12q15	12:69.7	--	--	A	0.448	1.43 (1.22, 1.67)	7.36×10^-6	1.39 (1.20, 1.62)	1.71×10^-5
(B) Associations by Genomic Region Conditioning on CFH SNP rs1831282										
rs10490924	10q26.13	10:124.2	*ARMS2**	Missense (A69S)	A	0.3482	2.27 (1.92, 2.68)	3.99×10^-22	2.27 (1.93, 2.66)	7.61×10^-24
rs522162	6p21.32	6:32	*RDBP**	3′-UTR	G	0.06917	0.43 (0.32, 0.57)	1.00×10^-8	0.41 (0.31, 0.54)	3.89×10^-10
rs1384200	11q22.1	11:97.9	--	--	A	0.2611	1.55 (1.30, 1.86)	1.94×10^-6	1.46 (1.23, 1.74)	1.48×10^-5
rs10857341	4q32.2	4:162.8	*FSTL5*	Intron	T	0.05482	0.46 (0.33, 0.64)	3.51×10^-6	0.47 (0.34, 0.64)	2.46×10^-6
rs4854022	2q37.3	2:240.7	*OTOS*	Downstream	G	0.2269	1.57 (1.30, 1.90)	3.55×10^-6	1.53 (1.27, 1.83)	5.39×10^-6
rs2261151	14q21.3	14:48.3	--	--	T	0.147	1.71 (1.36, 2.14)	3.75×10^-6	1.56 (1.26, 1.94)	5.26×10^-5
(C) Associations by Genomic Region Conditioning on ARMS2 SNP rs10490924 and CFH SNP rs1831282										

SNP	Band	CH:MB	Nearest Gene	Role	MA	MAF	Conditional Analysis		Discovery Analysis	
							OR (95% CI)	*P*	OR (95% CI)	*P*
rs541862	6p21.32	6:32	*CFB**	Intron	C	0.06914	0.43 (0.32, 0.59)	8.32×10^-8	0.41 (0.31, 0.54)	6.94×10^-10
rs728749	11p13	11:35.9	--	--	G	0.3229	0.68 (0.57, 0.80)	6.88×10^-6	0.71 (0.61, 0.83)	1.54×10^-5
rs6503782	17q22	17:52.5	--	--	T	0.4855	0.70 (0.60, 0.82)	8.48×10^-6	0.71 (0.62, 0.83)	6.66×10^-6
rs2261151	14q21.3	14:48.3	--	--	T	0.147	1.69 (1.34, 2.14)	8.70×10^-6	1.56 (1.26, 1.94)	5.26×10^-5

CH:MB, chromosome:position (in mega base pairs, build 19); MA, minor allele; MAF, minor allele frequency; OR, odds ratio; P, *P*-value.

Nearest Gene is determined as most proximal gene within 50kb 5′ or 3′ of the variant.

Genes with previous case-control genome-wide statistically significant associations: *ARMS2, CFB/C2/RDBP, CFH.*

Table 5

Strongest regional associations results from genome-wide association analyses of AMD stratified by ever/never smoking status in the discovery dataset. Association signals in section **(A)** represent the strongest associations within each locus where two the degree-of-freedom (2df) test demonstrates $P \leq 10^{-5}$ in the discovery dataset. Otherwise, association signals depicted are the strongest SNPs associations ($P \leq 10^{-3}$) in the discovery dataset within stratum, among either **(A)** smokers or **(B)** non-smokers. Genes with previously published case-control association signals at $P \leq 5.00\times10^{-8}$ are denoted with *.

SNP	Band	CH:MB	Nearest Gene	Role	MA	MAF	2-df Joint Test	Test of Interaction		Ever Smokers (548 cases/ 278 controls)		Never Smokers (346 cases/ 247 controls)	
							P	Main Effect *P*	Interaction *P*	OR (95% CI)	*P*	OR (95% CI)	*P*
(A) Most statistically significant 2-df joint test ($P<10^{-5}$)													
rs403846	1q31.3	1:195.0	*CFH**	Intron	G	0.45	2.00×10^-23	2.35×10^-11	0.969	0.40 (0.32, 0.50)	7.85×10^-15	0.37 (0.28, 0.50)	1.74×10^-11
rs553169	5p15.2	5:10.0	--	--	A	0.29	3.58×10^-7	0.187	9.10×10^-6	0.53 (0.42, 0.67)	1.88×10^-7	1.22 (0.92, 1.62)	0.175
rs522162	6p21.32	6:32.0	*RDBP**	3′-UTR	G	0.07	3.61×10^-8	0.00201	0.311	0.34 (0.22, 0.52)	7.91×10^-7	0.46 (0.28, 0.75)	0.00198
rs4840097	6q16.2	6:100.4	--	--	G	0.31	2.22×10^-6	8.31×10^-4	5.09×10^-7	0.64 (0.51, 0.80)	1.29×10^-4	1.64 (1.22, 2.20)	0.000968
rs10490924	10q26.13	10:124.2	*ARMS2**	Missense (A69S)	A	0.35	1.55×10^-17	4.06×10^-6	0.103	2.51 (1.97, 3.21)	1.06×10^-13	1.87 (1.42, 2.47)	7.64×10^-6
rs17073641	18q22.1	18:60.6	--	--	C	0.45	7.00×10^-7	1.52×10^-5	1.19×10^-7	1.42 (1.13, 1.77)	0.00228	0.57 (0.44, 0.74)	2.73×10^-5
(B) Most statistically significant associations among smokers ($P<10^{-5}$)													
rs572515	1q31.3	1:194.9	*CFH**	Intron	G	0.49	2.49×10^-22	2.97×10^-9	0.466	0.38 (0.30, 0.48)	7.77×10^-16	0.42 (1.79, 3.16)	2.40×10^-9
rs10490924	10q26.13	10:124.2	*ARMS2**	Missense (A69S)	A	0.34	1.55×10^-17	4.06×10^-6	0.103	2.51 (1.97, 3.21)	1.06×10^-13	1.87 (1.42, 2.47)	7.64×10^-6
rs553169	5p15.2	5:10.0	--	--	A	0.29	3.58×10^-7	0.187	9.10×10^-6	0.53 (0.42, 0.68)	1.88×10^-7	1.22 (0.92, 1.62)	0.175
rs522162	6p21.32	6:32.0	*RDBP**	3′-UTR	G	0.07	3.61×10^-8	0.00201	0.311	0.34 (0.22, 0.52)	7.91×10^-7	0.46 (0.28, 0.75)	0.00199
rs37935	7q21.22	7:79.9	--	--	G	0.40	6.30×10^-5	0.777	0.00169	0.60 (0.48, 0.76)	1.57×10^-5	1.03 (0.79, 1.34)	0.824
rs2224492	9p24.2	9:4.2	*GLIS3*	Intron	C	0.32	8.90×10^-5	0.880	0.00335	0.60 (0.48, 0.76)	2.31×10^-5	1.01 (0.77, 1.34)	0.934
(C) Most statistically significant associations among non-smokers ($P<10^{-5}$)													

SNP	Band	CH:MB	Nearest Gene	Role	MA	MAF	2-df Joint Test	Test of Interaction		Ever Smokers (548 cases/ 278 controls)		Never Smokers (346 cases/ 247 controls)	
							P	Main Effect *P*	Interaction *P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs403846	1q31.3	1:195.0	*CFH**	Intron	G	0.45	2.00×10^-23	2.35×10^-11	0.969	0.40 (0.32, 0.50)	7.85×10^-15	0.37 (0.28, 0.50)	1.74×10^-11
rs624851	6q12	6:65.3	*EYS*	Missense (L1419S)	A	0.32	6.72×10^-5	1.57×10^-5	0.00526	0.92 (0.72, 1.16)	0.465	0.51 (0.38, 0.68)	6.33×10^-6
rs4619440	17q24.1	17:60.9	--	--	T	0.07	3.21×10^-5	9.78×10^-6	0.00413	0.80 (0.54, 1.20)	0.283	0.28 (0.16, 0.49)	7.59×10^-6
rs10490924	10q26.13	10:124.2	*ARMS2**	Missense (A69S)	A	0.35	1.55×10^-17	4.06×10^-6	0.103	2.51 (1.97, 3.21)	1.06×10^-13	1.87 (1.42, 2.47)	7.64×10^-6
rs7240748	18q23	18:72.6	--	--	T	0.46	1.12×10^-4	2.01×10^-5	0.00133	0.99 (0.79, 1.23)	0.894	0.54 (0.41, 0.71)	1.40×10^-5
rs4557782	9q13.1	9:38.7	--	--	G	0.14	1.09×10^-4	2.18×10^-5	3.45×10^-4	1.08 (0.79, 1.48)	0.621	0.41 (0.28, 0.62)	1.66×10^-5
rs12535157	7q33	7:136.7	*DGKI*	Intron	A	0.34	5.10×10^-5	1.38×10^-5	8.97×10^-5	1.12 (0.89, 1.41)	0.322	0.55 (0.41, 0.72)	2.41×10^-5

CH:MB, chromosome:position (in mega base pairs, build 19); MA, minor allele; MAF, minor allele frequency; OR, odds ratio; P, *P*-value.

Nearest Gene is determined as most proximal gene within 50kb 5′ or 3′ of the variant.

Genes with previous case-control genome-wide statistically significant associations: *ARMS2, CFB/C2/RDBP, CFH.*