Genetics

Genetic Determinants of Age-Related Macular Degeneration in Diverse Populations From the PAGE Study

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Purpose. Substantial progress has been made in identifying susceptibility variants for AMD in European populations; however, few studies have been conducted to understand the role these variants play in AMD risk in diverse populations. The present study aims to examine AMD risk across diverse populations in known and suspected AMD complement factor and lipid-related loci.

Methods. Targeted genotyping was performed across study sites for AMD and lipid trait-associated single nucleotide polymorphism (SNPs). Genetic association tests were performed at individual sites and then meta-analyzed using logistic regression assuming an additive genetic model stratified by self-described race/ethnicity. Participants included cases with early or late AMD and controls with no signs of AMD as determined by fundus photography. Populations included in this study were European Americans, African Americans, Mexican Americans, and Singaporeans from the Population Architecture using Genomics and Epidemiology (PAGE) study.

Results. Index variants of AMD, rs1061170 (*CFH*) and rs10490924 (*ARMS2*), were associated with AMD at $P = 3.05 \times 10^{-8}$ and $P = 6.36 \times 10^{-6}$, respectively, in European Americans. In general, none of the major AMD index variants generalized to our non-European populations with the exception of rs10490924 in Mexican Americans at an uncorrected P value < 0.05. Four lipid-associated SNPS (*LPL* rs328, *TRIB1* rs6987702, *CETP* rs1800775, and *KCTD10/MVK* rs2338104) were associated with AMD in African Americans and Mexican Americans ($P < 0.05$), but these associations did not survive strict corrections for multiple testing.

Conclusions. While most associations did not generalize in the non-European populations, variants within lipid-related genes were found to be associated with AMD. This study highlights the need for larger well-powered studies in non-European populations.

Keywords: age-related macular degeneration, *CFH* Y402H, *ARMS2* A69S, PAGE Study, genetic epidemiology

Age-related macular degeneration is the third leading cause of visual impairment worldwide,[1] commonly affecting seniors more than any other form of blindness. Unlike many other common, complex disorders, substantial progress has been made in identifying susceptibility variants for AMD. The most widely replicated loci are complement factor H (*CFH*) and the age-related maculopathy susceptibility-2 (*ARMS2*)/HtrA serine peptidase 1 (*HTRA1*) complex.[2–6] Multiple polymorphisms within the chromosome 10q26 region have been proposed as the functional variation including *ARMS2* A69S, the *HTRA1* promoter variant rs11200638 adjacent to *ARMS2,*[7,8] and a complex insertion/deletion variant in the untranslated region (UTR) of *ARMS2*.[9,10]

Before the successful identification of genetic factors, studies of epidemiological risk variables for AMD have consistently found that particular demographic groups experience a greater burden of disease. Those at an increased risk are women, older individuals, smokers, and individuals of European descent.[11–13] Prevalence rates of any AMD vary by race and ethnicity with non-Hispanic whites (7.3%) experiencing a higher burden than non-Hispanic blacks (2.4%), and Mexican Americans (5.1%) in a study of the U.S. population over the age of 40.[14] The prevalence of AMD in Asian populations is similar to that observed in European-descent populations at approximately 6.8%.[15] Some studies have identified blood pressure, body mass index, physical activity, and lipids as environmental modifiers of AMD risk, but these findings are not universal.[12,16,17] Cigarette smoking is a significant environmental risk factor and has been shown to modify the effect of *ARMS2* A69S in some,[18,19] but not all,[20,21] studies. Individuals with bilateral AMD were found to be more likely to have been heavy smokers (odds ratio [OR] = 5.1) than with those who presented with unilateral AMD.[22]

A substantial body of research has implicated lipid levels as a major risk factor in AMD, particularly high-density lipoprotein (HDL) cholesterol, though the relationship between serum HDL levels and AMD risk is inconsistent.[23,24] Reynolds and colleagues[23] found a protective effect of higher HDL levels against AMD. Conversely, the Rotterdam Study found that higher levels of HDL increased the risk (OR = 1.20 per standard deviation increase) of incident AMD.[24] The exact role that lipids play in AMD pathology is still unknown, but new insights may be garnered from genetic association studies that have found evidence of a correlation between lipid-trait genes and AMD risk. Recently, a meta-analysis of over 17,000 advanced AMD cases confirmed the findings of variants previously associated with HDL cholesterol on susceptibility to AMD that had been observed in two previous genome-wide association studies (GWASs).[25–27]

Many of these associations were first discovered and described in European-descent case-control populations. Thus, more data are needed to clarify the role of these variants individually on disease susceptibility, in combination with each other and other risk factors for AMD, in population-based samples, and in other racial/ethnic groups. A major goal of the Population Architecture Using Genomics and Epidemiology (PAGE) study is to describe the underlying genetic architecture of common, complex diseases such as AMD across diverse populations.

Materials and Methods

Study Populations

Participants from three PAGE study sites are included in this study: the Atherosclerosis Risk in Communities (ARIC) study, the Cardiovascular Health Study (CHS), and Epidemiologic Architecture for Genes Linked to Environment (EAGLE) study accessing the Third National Health and Nutrition Examination Survey (NHANES III). In addition to data from the PAGE study sites, additional data are presented from the Singapore Prospective Study Programme (SP2) and the Singapore Malay Eye Study (SiMES). Brief descriptions of each study are given in Table 1. The original phenotypic focus of each study varies from cardiovascular traits (ARIC and CHS), type 2 diabetes and cardiovascular diseases (SP2), ocular traits (SiMES), and a representative sample of the United States regardless of health status (EAGLE).

The ARIC study is a population-based cohort study that included 15,792 women and men aged between 45 and 64 years at recruitment in 1987 through 1989.[28] The participants were selected by probability sampling from four US communities: suburbs of Minneapolis, Minnesota; Washington County, Maryland; Jackson, Mississippi; and Forsyth County, North Carolina. Retinal photographs were taken at the third visit, allowing inclusion of a subset of participants in this study of AMD.[29] A digitized, 45° color fundus photograph was taken of one eye from participants aged between 48 and 72 years. We graded AMD according to a modified version of the Wisconsin Age-Related Maculopathy Grading System.[30] Current smoking

Table 1. Description of Study Participants, by Study Site

	ARIC				CHS				EAGLE	
	European American		African American		European American		African American		European American	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
n	289	3128	34	582	351	1918	31	381	190	664
Age	65.1 (3.1)	64.3 (3.0)	63.9 (3.1)	64.0 (3.1)	72.4 (4.6)	70.7 (4.2)	72.4 (4.4)	71.5 (4.7)	76.4 (8.1)	71.2 (7.5)
Body mass index	27.8 (4.6)	27.7 (4.8)	29.1 (3.9)	29.8 (5.9)	26.1 (4.2)	26.5 (4.2)	28.9 (6.0)	28.7 (4.8)	26.8 (5.7)	27.1 (4.7)
Total fasting cholesterol	209.6 (35.0); $n = 249$	208.8 (36.7); $n = 2647$	210.9 (31.6); $n = 30$	209.3 (38.4); $n = 501$	210.0 (36.7)	213 (39.0)	198.9 (29.2)	210.1 (35.9)	230.0 (79.5)	228.6 (76.0)
Fasting HDL cholesterol	50.0 (16.5)	51.5 (18.4)	53.1 (20.0)	55.4 (18.1)	56.0 (16.6)	54.3 (15.4)	59.4 (14.4)	58.8 (15.0)	52.7 (15.5)	66.7 (113.9)
Female, %	49.1	51.3	44.1	63.6	57.0	60.0	74.2	65.1	66.8	54.5
Current smokers, %	61.6	60.1	58.8	50.5	9.6	8.7	12.9	13.3	44.2	52.0

Means and SDs are given unless otherwise noted.

* Study site did not collect fasting data; statistics are shown for nonfasting data.

Investigative Ophthalmology & Visual Science

was defined by "Do you now smoke cigarettes"? After the institutional review board at every participating university approved the ARIC Study protocol, written informed consent was obtained from each participant.

The CHS study is a population-based longitudinal study of risk factors for cardiovascular disease in adults aged 65 years or older, recruited at four field centers (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; Pittsburgh, Pennsylvania).[31] Overall, 5201 predominantly white individuals were recruited from 1989 to 1990 from random samples of Medicare eligibility lists, followed by an additional 687 African Americans recruited from 1992 to 1993. Starting in 1989, participants underwent standardized clinical exams at enrollment, which included blood pressure, lipid profiles, echocardiography of the heart, carotid ultrasound, cranial magnetic-resonance imaging (MRI), and fundus photography. Retinal photographs were taken with a 45° nonmydriatic camera of one randomly selected eye from participants.[29,32] The status of AMD was graded according to the modified Wisconsin Age-Related Maculopathy classification scheme.[30] The main outcomes are coronary heart disease, angina, heart failure, stroke, transient ischemic attack, claudication, and mortality. Current smoking status was self-reported at baseline.

The National Health and Nutrition Examination Surveys (NHANES), conducted by the National Center for Health Statistics at the Centers for Disease Control and Prevention (CDC), are US population–based, cross-sectional surveys collected without regard to health status and include detailed demographic, health, lifestyle, laboratory, clinical, and physical examination data for study participants. The National Health and Nutrition Examination Surveys was conducted in two phases between 1988 and 1994, and DNA samples were collected in the second phase (1991–1994).[33,34] Genetic NHANES III consists of 7159 DNA samples, and the method of collection has been previously described.[35,36] We used study participant data from NHANES III, of which 3131 had available fundus photographs and laboratory measurements of serum cotinine (ng/mL). Participants aged older than 40 years were selected to have a nonstereoscopic, 45° color fundus photograph taken of one randomly selected eye. Age-related macular degeneration was graded according to the Wisconsin Age-Related Maculopathy Grading System.[30] Current smokers were defined as those who answered "yes" to the question "Do you smoke cigarettes now?" or those with cotinine levels $>$ 15 ng/mL. All procedures were approved by the CDC Ethics Review Board and written informed consent was obtained from all participants. Because no identifying information is available to the investigators, Vanderbilt University's Institutional Review Board determined that this study met the criteria of "nonhuman subjects."

The Singapore Prospective Study Programme is a population-based cohort consisting of Singaporean Chinese participants aged between 40 and 80 years.[37] Initially, subjects (n = 10,747) were invited from four population-based cross-sectional surveys conducted in Singapore (1982–1998) to participate in a repeat examination from 2004 to 2007.[38–41] Participants who were successfully re-contacted and completed a questionnaire (n = 7744; 76.8% response rate) were then invited to attend a clinic health examination that included physicals and ocular assessment, retinal photography, and collection of biologic specimens, of which 5163 (66.7% of those who completed the questionnaire or 51.2% of all eligible subjects) attended. Retinal photographs were taken of both eyes with a 45° digital retinal camera and are available for 4110 participants.[42] We graded AMD according to the Wisconsin Age-Related Maculopathy Grading System.[43] A structured interviewer-administered questionnaire was used to collect information about smoking status. Current smoking status was self-reported.

The Singapore Malay Eye Study is a population-based cross-sectional study of urban Singaporean Malay adults, conducted to assess prevalence, risk factors, and the public health impact of common age related eye diseases.[44] An age-stratified (by 10-year age group) random sample of the Malay population residing in 15 residential districts in Southwestern Singapore age 40 to 80 years was drawn from the computer-generated random list of 16,069 Malay names provided by the Ministry of Home Affairs. Of 4168 eligible participants, 3280 (overall response rate 78.7%) participated in the study, conducted from August 2004 through June 2006. Retinal photographs were taken of both eyes in participants with a digital retinal camera and AMD was graded according to the Wisconsin Age-Related Maculopathy Grading System.[45] A questionnaire was used to collect information about smoking status, with participants self-reporting current smoking status.

This research adhered to the tenets of the Declaration of Helsinki. Approval for the study was obtained from the appropriate institutional review boards at all participating institutions, and all study participants gave informed consent

Table 1. Extended

EAGLE				SP2		SiMES	
African American		Mexican American		Asian		Asian	
Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
30	209	47	270	21	206	107	863
70.2 (7.5)	68.5 (6.9)	69.3 (7.0)	67.2 (6.3)	69.3 (5.3)	67.1 (4.9)	71.3 (5.4)	69.0 (5.6)
31.0 (6.3)	28.2 (6.2)	28.6 (5.3)	28.4 (5.3)	21.3(3.3)	23.2 (3.1)	25.5 (5.8)	26.2 (5.0)
235.7 (56.2)	229.9 (104.5)	256.6 (145.9)	222.1 (70.9)	197.2 (30.9)	208.8 (34.8)	220.4 (42.5)*	224.2 (46.4)*
63.2 (19.5)	71.2 (116.8)	84.6 (180.0)	53.9 (71.6)	54.1 (11.6)	54.1 (11.6)	54.1 (11.6)*	50.2 (11.6)*
60.0	47.4	44.7	44.1	19.05	46.60	29.91	51.68
50.0	58.9	53.2	54.8	38.10	22.82	48.57	36.74

Investigative Ophthalmology & Visual Science

where appropriate. All work for studies conducted in the United States were HIPAA-compliant.

AMD Phenotype Definition

At all study sites, fundus photographs were graded according to a modified version of the Wisconsin Age-related Maculopathy Scale.[30] Early AMD cases were aged at least 60 years and included participants with: soft drusen, depigmentation of the retinal pigment epithelium in the presence of soft and/or hard drusen, or hyperpigmentation in the presence of soft and/or hard drusen. Late AMD included individuals aged 60 years and older with geographic atrophy, subretinal hemorrhage, subretinal fibrous scarring, or sensory serous subretinal detachments. Controls were aged at least 60 years with gradable retinal photographs showing an absence of hallmark AMD features. Demographic characteristics of the study participants are presented in Table 1.

Genotyping

As part of the larger PAGE study, SNPs were selected for genotyping based on previous GWAS and candidate gene studies for a variety of common human diseases and traits including AMD; lipid-traits (HDL cholesterol, low density lipoprotein cholesterol, and triglycerides); body mass index/obesity; type 2 diabetes; hypertension; and inflammation (C-reactive protein), to name a few. For this study, we included both AMD and lipid-trait associated SNPs reported in genome-wide association studies or the National Human Genome Research Institute's GWAS catalog as of 2009.[46] A total of 57 SNPs were selected for analysis and included the two primary AMD variants (*CFH* rs1061170 and *ARMS2* rs10490924); other variants involved in the complement system pathway (*CFH* rs800292, rs1065489, rs3753394, rs3766404, rs6677604, rs800292; *CFI* rs10033900, rs11726949; *C2* rs547154), and variants associated with lipid-related traits (Supplementary Table S1). As described in Matise et al.,[47] genotyping in the PAGE Study was performed at each PAGE study site independently using a variety of genotyping assays and platforms. Not all 57 SNPs were genotyped in each study site (Supplementary Table S1). For quality control, all PAGE study sites genotyped 360 DNA samples (CEU, YRI, CHB, JPN, and MEX) from the International HapMap Project, including 77 parent-child trios.[47]

In ARIC, the *CFH* Y402H variant (rs1061170) was previously genotyped in a candidate gene study using the TaqMan assay and polymerase chain reaction amplification (Applied Biosystems, Foster City, CA, USA).[48] Genotypes were called using the ABI 7900HT and the sequence detection system software (ABI PRISM 7700; Applied Biosystems). Genotyping of DNA for the *ARMS2* A69S variant (rs10490924) was part of a GWAS using a commercial array (GeneChip SNP Array 6.0; Affymetrix, Inc., Santa Clara, CA, USA).[49] The high density lipoprotein–associated SNPs used in this study were genotyped as previously described.[50]

In the Cardiovascular Health Study, *CFH* Y402H and *ARMS2* were not genotyped. Genotyping data for other variants were obtained from two sources. First, SNP genotyping was conducted in the Houston central lab using TaqMan (see details above for ARIC). The second source of genotyping data from CHS was performed at the General Clinical Research Center's phenotyping/genotyping Laboratory at Cedars-Sinai using a beadchip system (Illumina 370CNV BeadChip; Illumina, Inc., San Diego, CA, USA). Genotypes were called using commercial software (Illumina BeadStudio; Illumina, Inc.).[49]

In Epidemiologic Architecture for Genes Linked to Environment, *CFH* Y402H (rs1061170) and *ARMS2* A69S (rs10490924) were genotyped by the Center for Human Genetics Research DNA Resources Core in NHANES III using a commercial assay system (Sequenom iPLEX Gold; Illumina, Inc.) according to the manufacturer's instructions. Blinded duplicates were genotyped as required by CDC, and both SNPs passed quality control metrics required by CDC. The following lipid trait-associated SNP data were accessed from existing genotype data in Genetic NHANES: rs3890182 (*ABCA1*), rs3135506 (*APOA5*), rs1800775 (*CETP*), rs1323432 (*GRIN3A*), rs1800588 (*LIPC*), and rs328 (*LPL*).[51] The remaining lipid trait-associated SNPs were genotyped using commercial assay system (Sequenom or Illumina BeadXpress; Illumina, Inc.). All genotype data reported here were deposited into the NHANES III Genetic database and are available for secondary analysis through CDC.

In Singapore Prospective Study Programme, Chinese samples were previously genotyped as controls for a type 2 diabetes case control study which used beadchip kits (Illumina HumanHap 610 Quad or 1MDuo-v3 BeadChips; Illumina, Inc.) as part of a psoriasis case control study which used a commercial beadchip kit (Illumina HumanHap 550 BeadChip; Illumina, Inc.). Genotyping details have been published elsewhere.[52]

Genotyping methods with SiMES followed those of the SP2 cohort as previously described.[53]

Genotyping for any given SNP, in a particular population, was not always available across all study sites. Therefore, we provide the following maximum and minimum ranges that include cases and controls for genotyping data by population: European Americans ($n = 4546$–6540), African Americans ($n = 796$–1267), Mexican Americans ($n = 275$–317), and Asians ($n = 972$–1197).

Statistical Analysis

Each study site performed tests of association locally using a common analysis protocol prior to meta-analysis. Early and late cases of AMD were combined for analyses to increase power. Each genetic variant was tested for association with AMD using logistic regression assuming an additive genetic model stratified by self-described race/ethnicity (e.g., European American, African American, Mexican American, and Asian). Single nucleotide polymorphisms available for analysis by study site are given in Supplementary Table S1. All models were adjusted for site of ascertainment (model 1, minimally adjusted). Models 2 and 3 were adjusted for age; sex; body mass index (BMI); smoking status (current versus ever/never); and HDL cholesterol, fasting ($\geq$8 hours; model 2) or regardless of fasting status (model 3). Participants on lipid-lowering medications were excluded in both models 2 and 3. Asians were represented by the SP2 cohort only in model 2 given the lack of fasting HDL-C in the SiMES cohort.

Local analyses were conducted using data analysis and statistical software: Stata 9.0 (StataCorp, LP, College Station, TX, USA; ARIC); SAS v9.3 (SAS Institute, Inc., Cary, NC, USA; CHS), SAS v9.2 (by EAGLE using the Analytic Data Research by Email [ANDRE] portal of the CDC Research Data Center in Hyattsville, MD, USA), and genome data analysis software (PLINK v1.06; Harvard University, Cambridge, MA, USA; SP2/SiMES).

After analyses were conducted locally at each site, meta-analyses using summary statistics were carried out using a fixed-effects, inverse-variance weighted approach implemented in a metal analysis helper (METAL; University of Michigan, Ann Arbor, MI, USA).[53] Between-study heterogeneity was tested for in METAL with the Cochran's *Q*-test. Genomic control was applied on the combined meta-analysis and not each specific cohort analysis to avoid overcorrection of

Investigative Ophthalmology & Visual Science

population stratification. Meta-analysis results were plotted using Synthesis-View.[54]

All P values listed in the manuscript and tables are uncorrected for multiple hypothesis testing. The following Bonferroni corrected thresholds were calculated by population based on the number of available SNPs in that population across all study sites regardless of whether the SNP was available at each study site: European Americans (0.05/49 SNPs) = 0.0010; African Americans (0.05/47 SNPs) = 0.0010; Mexican Americans (0.05/31 SNPs) = 0.0016; and Asians (0.05/43 SNPs) = 0.0012.

Results

Study Population Characteristics

The meta-analysis is composed of multiple studies within the PAGE study (ARIC, CHS, and EAGLE) and Asian cohorts comprised of SP2 and SiMES (Table 1). Combined, this meta-analysis includes various racial/ethnic groups: European Americans (830 cases and 5710 controls); African Americans (95 cases and 1172 controls); Mexican Americans (47 cases and 270 controls); Singaporean Chinese (21 cases and 206 controls); and Singaporean Malays (107 cases and 863 controls). All study sites ascertained both men and women. Smoking status was defined as current versus ever/never smoker and varied across studies (Table 1). The average BMI was greater than 25 kg/m^2 across all studies regardless of case status, except in the ethnically Chinese population (SP2).

Replication of Previously Associated AMD Variants in European Americans

We meta-analyzed tests of associations for up to 19 SNPs previously associated with AMD. Among European Americans, 13 AMD SNPs were tested, and 7/13 (54%) were significant at an uncorrected $P < 0.05$ in model 1 (Table 1 and Supplementary Table S2; Fig. 1). As expected, both *CFH* rs1061170 (OR = 1.55; $P = 3.05 \times 10^{-8}$) and *ARMS2* rs10490924 (OR = 1.55; $P = 6.36 \times 10^{-6}$) were strongly associated with AMD risk (Table 2) at a Bonferroni corrected P value ($P < 0.001$). The genetic effect sizes estimated here were smaller for these variants compared with previous reports with other studies estimating risk between 2.7 and 4.6 for heterozygotes (Fig. 2).[2,3,55,56] Three additional *CFH* SNPs were associated with AMD risk at $P < 0.05$ in European Americans: missense rs800292 (OR = 0.58; $P = 3.80 \times 10^{-5}$), intergenic rs3753394 (OR = 1.25; $P = 0.03$), and intronic rs6677604 (OR = 0.77; $P = 0.04$; Supplementary Table S2). The remainder of the complement factor SNPs that were tested failed to replicate at this liberal significance threshold. Two lipid-related variants previously associated with AMD[25,26,56] were nominally associated with AMD in European Americans: *CETP* rs3764261 (OR = 1.14; $P = 0.04$) and *ABCA1* rs1883025 (OR = 0.82; $P = 0.03$). After adjustment for age, sex, BMI, smoking status, and HDL-C (model 3), only *CFH* rs1061170, *ARMS2* rs10490924, and *CFH* rs800292 remained significant at a Bonferroni corrected P value (Supplementary Table S3; Supplementary Fig. S2).

Generalization of Previously Associated AMD Variants to Diverse Populations

We consider a previously associated AMD variant to have generalized if the same variant was associated in a different population with the same direction of effect as observed in the original population (in this case, European-descent populations). For Mexican Americans, only one study contributed data toward generalization: EAGLE accessing NHANES III, which included 47 cases and 270 controls. Among tests of association for variants previously associated with AMD, 1/8 (12%) in model 1 was significant at an uncorrected $P < 0.05$ in Mexican Americans. The association between *ARMS2* rs10490924 (OR = 1.63; $P = 0.04$) and AMD in Mexican Americans has been previously reported for EAGLE accessing NHANES III.[57] The association between *ARMS2* rs10490924 and AMD in Mexican Americans is still significant ($P = 0.05$) in this study after adjustment for model 3 covariates (Supplementary Table S3). However, after strict correction for multiple testing, none of the SNPs tested in Mexican Americans was associated with AMD.

Among African Americans, none of the 13 previously associated SNPs were associated with AMD in model 1 in this population at the liberal significance threshold of $P < 0.05$. Of note is the test of association between AMD and *ARMS2* rs10490924. A previous report accessing only NHANES III data consisting of 30 cases and 209 controls suggested this variant was marginally associated with AMD in the opposite direction compared with European Americans.[57] In this meta-analysis, the test of association was expanded to include an additional 34 cases and 582 controls from ARIC. The resulting point estimate of the genetic effect size (OR = 0.83; 95% confidence interval [CI]: 0.51–1.33) was consistent with the original report by Spencer et al.,[57] but the test of association was no longer significant ($P = 0.43$).

Similar to African Americans, in Asians none of the previously associated AMD variants were associated with AMD in model 1 in this population at a liberal significance of $P < 0.05$. While *CFH* rs1061170 failed to generalize in terms of statistical significance ($P = 0.24$), the point estimate of the genetic effect (OR = 4.43; 95% CI: 0.35–55.29) was in the same direction as the effect sizes observed for European-descent populations (Table 2; Figs. 1, 2).

Lipid-Associated SNPs and AMD

Given that recent GWAS have highlighted the association of genes traditionally involved in lipid pathways as mediators of AMD risk, we tested an additional 44 lipid-trait variants in these diverse populations for association with AMD.[26,56,58] Among the variants associated with lipid traits that were not previously associated with AMD in European-descent populations, only *LIPC* rs261332 (model 1) was marginally associated with AMD in European Americans at $P = 0.052$ (Table and Supplementary Table S2; Fig. 1); *LIPC* rs261332 (OR = 0.77) was only tested in ARIC with a total of 289 cases.

In non–European-descent populations, several lipid-associated SNPs were associated with AMD. Among 95 and 1172 African American cases and controls, respectively, 3/41 (7%) SNPs that were previously associated with a lipid trait were associated with AMD at the uncorrected threshold of $P < 0.05$ in model 1. These single nucleotide polymorphisms include *LPL* rs328 (OR = 1.75; $P = 0.03$); *TRIB1* rs6987702 (OR = 1.61; $P = 0.04$); and *CETP* rs1800775 (OR = 1.57; $P = 0.04$; Table 2 and Supplementary Table S2; Fig. 1). In Mexican Americans, 1/29 (3%) lipid-related SNPs tested reached significance at an uncorrected $P < 0.05$ (model 1). This variant was rs2338104 (*KCTD10/MVK*), previously associated with HDL cholesterol, associated here with AMD (OR = 1.69; $P = 0.02$).[59,60] The associations observed for *LPL* rs328 and *TRIB1* rs6987702 in African Americans and *KCTD10/MVK* rs2338104 in Mexican Americans remained significant after adjusting for covariates including fasting HDL-C (Supplementary Table S3). Of the 35 lipid-associated SNPs tested in the SP2 and SiMES meta-analysis of Asians, none of the association tests reached significance at an uncorrected $P < 0.05$. After strict correction for multiple

Investigative Ophthalmology & Visual Science

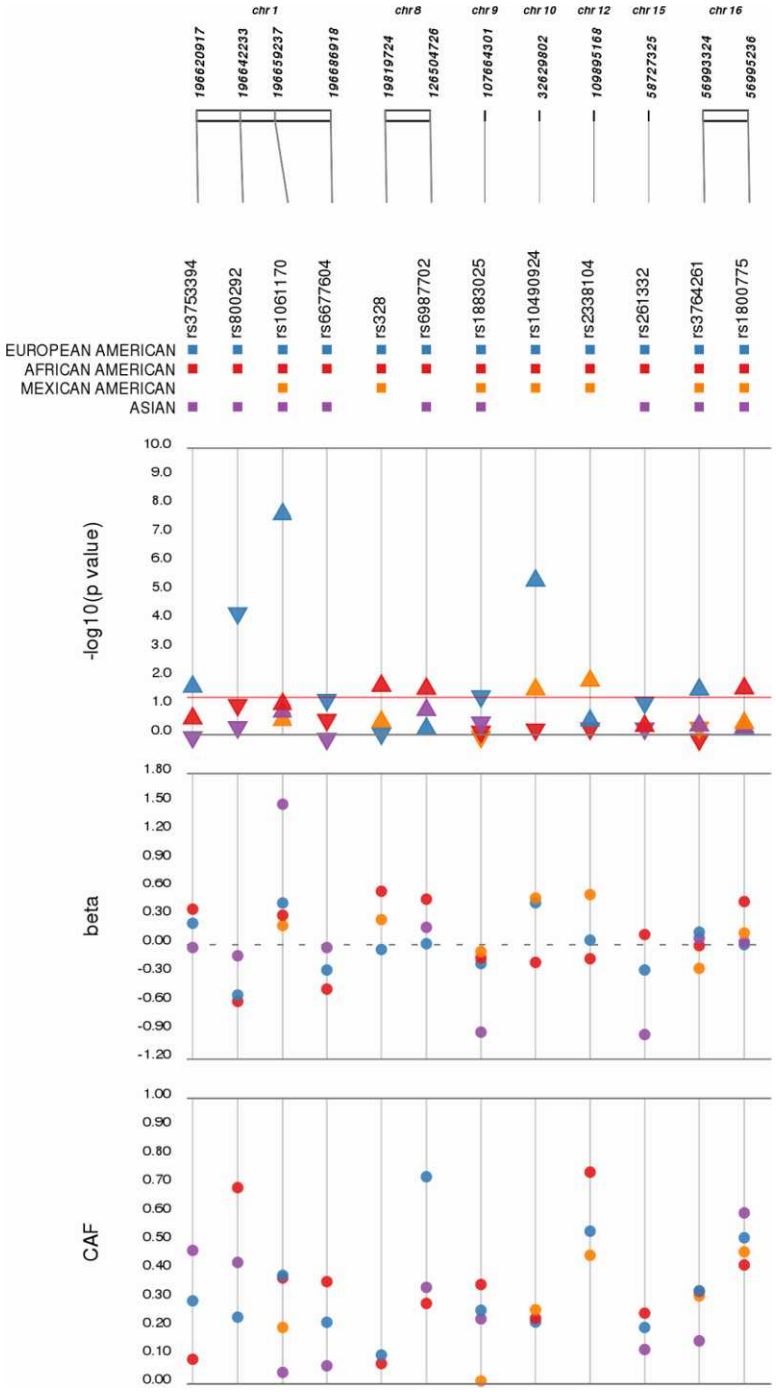

Figure 1. Synthesis view plot of nominally significant ($P < 0.05$) meta-analysis association results for model 1 which is minimally adjusted only for site of ascertainment, for all race/ethnicities. Statistical (*P*) values are represented by the *colored arrows* and are transformed by the –log10, with the threshold of $P = 0.05$ marked by the *red line. Colored arrows* also show the direction of effect (beta). Betas, *P* values, and coded allele frequencies (CAF) are plotted by race/ethnicity.

testing none of the lipid-associated SNPs were associated with AMD in any population.

Discussion

We tested up to 57 SNPs representing 19 previously associated AMD variants and 38 previously associated lipid trait variants for association with AMD in European Americans, African Americans, Mexican Americans, and Asians. At an uncorrected *P* value for multiple testing ($P < 0.05$), we replicated up to 54% (7/13) of the previously reported associations for AMD tested here in European Americans. Only one previously reported AMD association (*ARMS2* rs10490924) generalized to Mexican Americans (model 1; Table 2), whereas none generalized to African Americans and Asians. In contrast, several associations were observed between lipid trait-associated variants and AMD in African Americans and Mexican Americans.

Table 2. Significant AMD Meta-Analysis Association Results

Rsid	Gene	Chr	OR (95% CI)	Direction of Effect*	*P* Value	Coded Allele	CAF	Race/Ethnicity
rs1061170	*CFH*	1	1.55 (1.34–1.78)	+,.,+	**3.05×10^{-8}**	C	0.37	European American
			1.36 (0.91–2.04)	+,.,+	0.13		0.38	African American
			1.22 (0.70–2.13)	†	0.47		0.20	Mexican American
			4.43 (0.35–55.29)	†	0.24		0.04	Asian
rs800292	*CFH*	1	0.59 (0.47–0.74)	†	**3.80×10^{-5}**	A	0.24	European American
			0.55 (0.29–1.03)	†	0.06		0.69	African American
			0.89 (0.68–1.15)	-,-,-,+	0.36		0.42	Asian
rs3753394	*CFH*	1	1.25 (1.03–1.51)	†	0.03	T	0.29	European American
			1.45 (0.60–3.53)	†	0.41		0.08	African American
			0.97 (0.75–1.25)	+,-,+,-	0.80		0.54	Asian
rs6677604	*CFH*	1	0.77 (0.61–0.97)	†	0.04	A	0.22	European American
			0.63 (0.31–1.28)	†	0.20		0.37	African American
			0.97 (0.54–1.75)	-,-,+,+	0.92		0.05	Asian
rs328	*LPL*	8	0.95 (0.79–1.14)	-,-,+	0.60	G	0.10	European American
			1.75 (1.06–2.91)	+,+,+	0.03		0.07	African American
			1.30 (0.55–3.08)	†	0.53		<0.01	Mexican American
rs6987702	*TRIB1*	8	1.01 (0.87–1.17)	-,+,.	0.93	T	0.73	European American
			1.61 (1.03–2.52)	+,+,.	0.04		0.29	African American
			1.20 (0.90–1.58	+,+,+,+	0.21		0.43	Asian
rs1883025	*ABCA1*	9	0.82 (0.69–0.96)	-,.,-	0.03	A	0.26	European American
			0.87 (0.57–1.33)	-,.,-	0.52		0.35	African American
			0.93 (0.57–1.54)	†	0.78		<0.01	Mexican American
			0.40 (0.08–1.91)	.,-,.,.	0.25		0.23	Asian
rs10490924	*ARMS2*	10	1.55 (1.29–1.81)	+,.,+	**6.36×10^{-6}**	T	0.22	European American
			0.83 (0.51–1.33)	+,.,-	0.43		0.24	African American
			1.63 (1.02–2.60)	†	0.04		0.26	Mexican American
rs2338104	*KCTD10*	12	1.05 (0.92–1.20)	.,-,+	0.50	G	0.53	European American
			0.86 (0.61–1.22)	-,-,+	0.40		0.74	African American
			1.69 (1.08–2.64)	†	0.02		0.45	Mexican American
rs261332	*LIPC*	15	0.77 (0.60–0.98)	†	0.05	A	0.20	European American
			1.11 (0.57–2.18)	†	0.75		0.25	African American
			0.39 (0.05–3.45)	.,-,.,.	0.40		0.12	Asian
rs1800775	*CETP*	16	1.00 (0.87–1.16)	-,.,+	0.98	C	0.52	European American
			1.57 (1.03–2.38)	+,.,+	0.04		0.42	African American
			1.13 (0.70–1.83)	†	0.59		0.46	Mexican American
			1.03 (0.79–1.34)	+,+,+,-	0.84		0.51	Asian
rs3764261	*CETP*	16	1.14 (1.01–1.28)	+,+,-	0.04	T	0.33	European American
			0.99 (0.71–1.40)	-,+,-	0.96		0.33	African American
			0.78 (0.46–1.32)	†	0.37		0.31	Mexican American
			1.07 (0.74–1.53)	+,-,+,+	0.73		0.17	Asian

Each study site performed tests of association using logistic regression assuming an additive genetic model. Data were meta-analyzed using a fixed-effects inverse-variance weighted approach. Results are shown for nominally significant tests at an uncorrected threshold ($P < 0.05$) adjusted for site of ascertainment (model 1). Bold *P* values are those that met strict Bonferroni correction.

* Direction of effect is given for ARIC, CHS, and EAGLE for European Americans, African Americans, and Mexican Americans, if data are available. Direction of effect is given for Asians for SiMES and SP2 1M, 550, and 610 platforms. Otherwise, the study site is set to missing (".").

† Only a single study site is represented.

Factors That Impact Generalization of AMD-Associated Variants

The lack of replication and/or generalization could be due to power. Indeed, sample sizes for non–European-descent populations were limited. For the two most strongly associated variants observed in European Americans (rs1061170 *CFH* and rs10490924 *ARMS2*), we had greater than 90% power to detect published effect sizes of 2.41 and 2.94 in African Americans and Mexican Americans, assuming an additive genetic model at a *P* value of 0.05.[56] Therefore, compared with the effect sizes described in the literature for European Americans (Fig. 2), we were generally well powered to detect previously reported associations with these two SNPs in African Americans and Mexican Americans. In the present study, the direction of effect for *CFH* rs1061170 (OR = 1.36) in African Americans was the same as that previously reported in European Americans (OR = 1.80–4.60; Fig. 2); however, the direction of effect for *ARMS2* rs10490924 (OR = 0.83) was opposite that of published studies of European Americans. In Mexican Americans, the direction of effect was the same for both variants as that observed in European Americans. The consistent direction of effect observed in Mexican Americans may represent European admixture at or surrounding this genomic region. However, due to the limited genetic data available in the present study, we could not explicitly test this hypothesis. In the Asian population, we were underpowered to detect an association for *CFH* rs1061170 due to the limited number of cases ($n = 8$) genotyped for this particular variant; *ARMS2* rs10490924 was not genotyped in this Asian population.

Investigative Ophthalmology & Visual Science

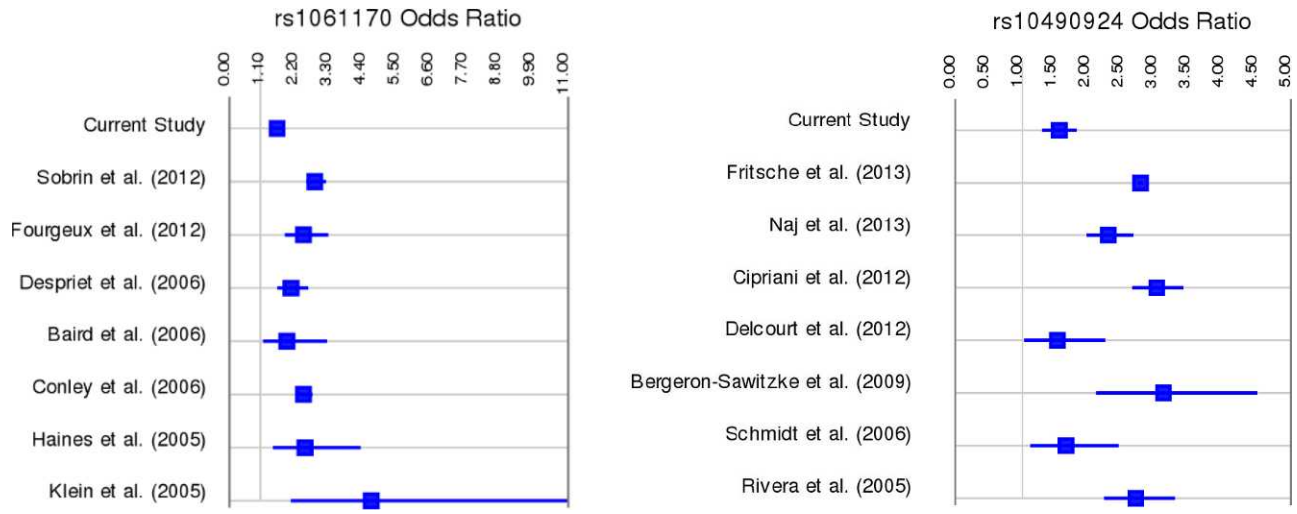

Figure 2. Forest plots with odds ratios and confidence intervals for previously reported associations of *CFH* rs1061170 and *ARMS2* rs10490924 compared with results for current study for European-descent populations.

Although our study was powered to detect published effect sizes in African Americans and Mexican Americans, these previous estimates were based on studies of European-descent individuals and may not be representative of the risk of AMD in diverse populations explored here.

Differences in linkage disequilibrium could also have adversely affected our ability to generalize associations originally identified in European-descent populations. In this study, we only tested the index variants reported in the literature. The index variants are often not the causal or functional variant; rather, the index variant is in linkage disequilibrium (LD) or "tags" the unknown functional variant. Failure to genotype or target the functional or causal variant can potentially reduce power to detect the association if the genotyped variant is not in perfect LD with the true functional or causal variant.

Fine-mapping or GWAS data are not available for this study; therefore, LD patterns cannot be directly compared across the study populations described here. However, examination of LD patterns in HapMap III for Europeans (CEU), African Americans in Southwest US (ASW), Mexican Americans (MEX), and Han Chinese in Beijing (CHB) suggests that LD differs in AMD-associated genomic regions across populations as expected.[61,62] For example, in the region of *ARMS2* (rs10490924), Haploview[63] plots of CEU (Supplementary Fig. S2a) show rs10490924 in perfect LD ($r^2 = 1$) or near perfect LD ($r^2 > 0.90$) with four intronic SNPs (rs3750848, rs3750847, rs2284665, rs932275) in an approximately 17-kb region. These single nucleotide polymorphisms are also in perfect/near perfect LD with rs10490924 in HapMap MEX (Supplementary Fig. S2c). However in HapMap ASW (Supplementary Fig. S2b), neither rs2284665 nor rs932275 are in perfect LD although they are still correlated to rs10490924 ($r^2 = 0.47$ and $r^2 = 0.70$, respectively). In HapMap CHB, rs10490924 is again in perfect LD with rs3750848 and rs3750847 and high LD with rs2284665 and rs932275 ($r^2 = 0.86$ and $r^2 = 0.63$, respectively). Many other variants upstream of rs10490924 are in moderate LD ($r^2 > 0.25$) in CEU and MEX. There is less evidence of LD present in ASW and CHB. Generalization of rs10490924 in Mexican Americans (Table 2) but not in African Americans in our study coupled with LD patterns suggest that rs10490924 may not be a major factor behind AMD susceptibility in African Americans.

Power in this study could have also been affected by differences in allele frequencies between populations. In a comprehensive review of estimates of *CFH* Y402H frequency, four studies of *CFH* Y402H in African-descent populations were identified.[49,64,65] The allele frequency estimates from these four studies range from 0.34 to 0.43, in line with our estimate of 0.38. It is intriguing that despite a high frequency of the risk allele similar to European-descent populations, the prevalence of AMD in African and African-descent populations is much lower.[66] This suggests that other genetic and/or environmental factors are at play. Of the four race/ethnicities represented, Mexican Americans and Asians had the lowest frequency of Y402H (0.20 and 0.04, respectively). The lower frequency of this variant may account for at least some of the difference in prevalence of AMD in Hispanics compared with European-descent populations.[66]

Cholesteryl ester transfer protein (*CETP*) variant rs3764261, which has been associated with HDL levels and AMD in European-descent populations, was replicated in our European American analyses (uncorrected $P < 0.05$).[25,57,67,68] The allele frequency for this SNP was 0.33 in our European Americans, similar to the 0.32 and 0.31 in the African Americans and Mexican Americans, respectively. A study by Chen et al.[25] found that rs3764261 trended toward significance in a Japanese population with a frequency of 0.22. In the present study, the Singaporean population had a similar frequency of 0.17, but was not associated with AMD. As with *CFH* Y402H, the frequency of the rs3764261 allele is similar across diverse populations, but does not appear to contribute to AMD risk in all populations suggesting that this variant may only be "tagging" the casual variant.

AMD Risk and Lipid Trait-Associated Variants

Lipid levels and lipid metabolism have been associated with susceptibility and progression of AMD in various populations.[24,69,70] The cholesteryl ester transfer protein is a plasma glycoprotein involved in the transport and removal of lipoproteins from blood circulation and as a component of the reverse cholesterol transport pathway. Variants in *CETP* have been found to cause an increase or decrease in blood lipid levels with corresponding high or low blood levels of CETP.[71,72] This gene has been identified in various studies as a modifier of AMD susceptibility.[25,56] Our study found nominally significant associations with *CETP* variants, rs1800775 and rs3764261, in European Americans and African Americans. These variants of *CETP*, along with other lipid-

related genes in our study, were found to be nominally significant (rs328 [*LPL*] and rs6987702 [*TRIB1*]) in African Americans; and rs2338104 (*KCTD10/MVK*) in Mexican Americans. We observed more of these lipid-related associations in diverse populations than the European American population supporting the role of lipids in disease susceptibility across diverse populations. Still, more studies are needed to elucidate the role they play in risk of AMD due to limited sample size.

Overall Limitations and Strengths

The major limitation for the PAGE study of age-related macular degeneration was small sample size. As expected, the European-descent samples (~6500) constitute the largest population. Sample sizes for African Americans, Mexican Americans, and Asians were smaller, which adversely impacted power to detect associations. Sample size was further impacted by the fact that not all SNPs were genotyped or available in all study populations. Also, as already emphasized, only the index variant was genotyped in PAGE. The lack of GWAS-level or fine-mapping data abolished our ability to examine differences in LD in these study populations.

Despite the small sample sizes, a major strength of the present study is the diversity of populations included. Several large-scale studies have examined the genetic risk variants associated with AMD in European-descent,[8,57,59,73–75] Japanese,[25,76–78] and Chinese populations.[79–82] Indeed, some of these previous GWAS for AMD have included many of the same European American and Singaporeans examined here. In contrast, only a handful of limited genotyping studies have examined various Hispanic and African American groups in association with AMD and complement factor genes, *APOE*, and *ARMS2/HTRA1*.[58,67,83–85] Compared with previous publications, this study contributes new data beyond *CFH* and *ARMS2/HTRA1*in African Americans and Mexican Americans. These new data, coupled with the cumulative data collected in European American and Asian populations highlight shared as well as unique associations for AMD across diverse populations.

Conclusions

In summary, we have characterized the genetic architecture of known AMD-related variants and variants in cholesterol pathways in the three major race/ethnicities in the United States, and Chinese and Malay individuals from Singapore. Although limited in scope, this study has identified potential genetic factors influencing the risk of AMD in diverse populations. The potential exists for elucidating population-specific causative pathways as highlighted by the presence or lack of an association of *CFH* Y402H and *ARMS2* A69S across populations.

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Investigative Ophthalmology & Visual Science

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