Association of Variants in the *LIPC* and *ABCA1* Genes with Intermediate and Large Drusen and Advanced Age-Related Macular Degeneration

Yi Yu,[1] *Robyn Reynolds,*[1] *Jesen Fagerness,*[2,3] *Bernard Rosner,*[4] *Mark J. Daly,*[2,3] *and Johanna M. Seddon*[1,5]

Purpose. Intermediate and large drusen usually precede advanced age-related macular degeneration (AMD). There is little information about which genes influence drusen accumulation. Discovery of genetic variants associated with drusen may lead to prevention and treatments of AMD in its early stages.

Methods. A total of 3066 subjects were evaluated on the basis of ocular examinations and fundus photography and categorized as control (n = 221), intermediate drusen (n = 814), large drusen (n = 949), or advanced AMD (n = 1082). SNPs in the previously identified *CFH*, *C2*, *C3*, *CFB*, *CFI*, *APOE*, and *ARMS2/HTRA1* genes/regions and the novel genes *LIPC*, *CETP*, and *ABCA1* in the high-density lipoprotein (HDL) cholesterol pathway were genotyped. Associations between stage of AMD and SNPs were assessed using logistic regression.

Results. Controlling for age, sex, education, smoking, body mass index, and antioxidant treatment, the number of minor (T) alleles of the genes *LIPC* and *ABCA1* were significantly associated with a reduced risk of intermediate drusen (*LIPC* [P trend = 0.045], *ABCA1* [$P = 4.4 \times 10^{-3}$]), large drusen (*LIPC* [P = 0.041], *ABCA1* [$P = 7.7 \times 10^{-4}$]), and advanced AMD (*LIPC* [$P = 1.8 \times 10^{-3}$], *ABCA1* [$P = 3 \times 10^{-4}$]). After further adjustment for known genetic factors, the protective effect of the TT genotype was significant for intermediate drusen (*LIPC* [odds ratio (OR), 0.56; 95% confidence interval (CI), 0.33–0.94], *ABCA1* [OR, 0.48; 95% CI, 0.27–0.85]), large drusen (*LIPC* [OR, 0.58; 95% CI, 0.34–0.98)], *ABCA1* [OR, 0.41; 95% CI, 0.23–0.74)]), and advanced AMD (*LIPC* [OR, 0.39; 95% CI, 0.21–0.74)], *ABCA1* [OR, 0.35; 95% CI, 0.17–0.71)]). *CFH*, *C3*, *C2*, and *ARMS2/HTRA1* were associated with large drusen and advanced AMD.

Conclusions. *LIPC* and *ABCA1* are related to intermediate and large drusen, as well as advanced AMD. *CFH*, *C3*, *C2*, and *ARMS2/HTRA1* are associated with large drusen and advanced AMD. Genes may have varying effects on different stages of AMD. (*Invest Ophthalmol Vis Sci.* 2011;52:4663–4670) DOI: 10.1167/iovs.10-7070

Age-related macular degeneration (AMD) is a common, complex, chronic eye disease.[1] As a leading cause of vision loss in people older than 60 years, AMD currently affects more than 1.75 million individuals in the United States. This number is expected to increase by more than 50% to 3 million in 2020 due to aging of the population.[2] The most severe visual loss due to AMD occurs when the disease progresses to one of the two advanced forms: geographic atrophy (GA) or choroidal neovascularization (NV).[3] GA features well-demarcated borders of atrophy that develop as the macular neurosensory cells slowly degenerate. NV is characterized by the creation of new blood vessels beneath the retinal pigment epithelial (RPE) layer of the retina, which cause bleeding, subretinal fluid, and scarring of the macula. Although GA and NV are pathophysiologically and clinically distinct,[4] they have one common hallmark, which is drusen between the RPE and Bruch's membrane.[5] Drusen are extracellular deposits composed mainly of lipids and proteins, including esterified and unesterified cholesterol, apolipoproteins, vitronectin, amyloid, complement factor H, and complement component C3.[6–11] Although the mechanism of drusen initiation and development is unclear, it has been hypothesized that age-related lipid accumulation in Bruch's membrane may induce early physiologic changes and lesions in the RPE.[12] These age-related lipid accumulations may then interact with other lipids, local ligands, or additional secreted self-aggregating proteins, such as the complement complex, to manifest as clinically detectable drusen.[5,12]

Genetic and environmental factors both contribute to the risk of developing AMD by various proportions during the early, intermediate, and advanced stages of this disease.[13] Cigarette smoking and higher body mass index (BMI) increase the risk of advanced AMD and its progression[14–19] and also modify genetic susceptibility.[18,20]

At least two independent variants in the complement factor H (*CFH*) gene are associated with advanced AMD.[21–26] Variants of several other genes in the alternative complement pathway, including *CFB/C2*,[24,27] *C3*,[28,29] and *CFI*,[30] also contribute to the risk of developing advanced AMD. The *ARMS2/HTRA1* region on chromosome 10 confers a high risk of advanced AMD and is not in the complement pathway.[31,32] Both *CFH* and the *ARMS2/HTRA1* region have been related to large, soft,

From the [1]Ophthalmic Epidemiology and Genetics Service, Department of Ophthalmology, Tufts Medical Center, Boston, Massachusetts; the [2]Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts; the [3]Broad Institute of Harvard and the Massachusetts Institute of Technology, Program in Medical and Population Genetics, Cambridge, Massachusetts; the [4]Channing Laboratory, Boston, Massachusetts; and the [5]Tufts University School of Medicine, Boston, Massachusetts.

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, May 2010.

Supported by an anonymous donor (JMS); in part by grant R01-EY11309 from the National Institutes of Health, Bethesda, MD; the Massachusetts Lions Eye Research Fund, Inc.; an unrestricted grant from Research to Prevent Blindness, Inc., New York, NY; the American Macular Degeneration Foundation, Northampton, MA; the Elizabeth O'Brien Trust; the Virginia B. Smith Trust; and the Macular Degeneration Research Fund of the Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts Medical Center, Tufts University School of Medicine, Boston, MA.

Submitted for publication December 16, 2010; revised January 28, 2011; accepted February 25, 2011.

Disclosure: **Y. Yu**, None; **R. Reynolds**, None; **J. Fagerness**, None; **B. Rosner**, None; **M.J. Daly**, P; **J.M. Seddon**, P

Corresponding author: Johanna M. Seddon, Tufts Medical Center, 800 Washington Street, 450, Boston, MA, 02111; jseddon@tuftsmedicalcenter.org.

or cuticular drusen.[33–36] Recently, in a well-powered, genome-wide association study (GWAS), we reported that another noncomplement gene, a variant in the hepatic lipase gene *LIPC*, which regulates triglyceride hydrolysis and plasma high density lipoprotein cholesterol (HDL-c) levels,[37,38] decreases the risk of advanced AMD.[39,40] This finding unveils the involvement of proteins described in the plasma HDL pathway in the pathogenesis of AMD. Other genes in the HDL pathway, including *CETP* and *ABCA1*, may also be involved in the etiology of AMD.[39,40] The *APOE* gene, a widely expressed cholesterol transporter, has been shown to be associated with AMD in some studies, but results are inconsistent.[41–44] Although it is well known that lipids are major components of drusen,[11,12] the effects of genetic variants in the recently identified HDL pathway genes on drusen development have not been previously reported. In this study, we analyzed the effects of HDL and LDL genes, *ARMS2/HTRA1* and known genes in the complement pathway on intermediate and large drusen, as well as GA and NV, to investigate and compare their roles in these different stages of AMD.

Materials and Methods

Study Population

Phenotypes for 3066 Caucasian subjects with DNA specimens in the Age-Related Eye Disease Study (AREDS) were evaluated. Each eye of a subject was categorized by using the Clinical Age-Related Maculopathy Staging System (CARMS) grades[45] based on the longitudinal records of ocular examination and fundus photography. Eyes were assigned to neovascular AMD (grade 5) if there was a history of any of the following: hemorrhagic retinal detachment, hemorrhage under the retina or retinal pigment epithelium, or subretinal fibrosis. Eyes with a record of GA, either in the center grid or anywhere within the grid and without any record of hemorrhage, were assigned to a grade 4. For eyes without any of the above signs of advanced AMD, grades were determined by the highest drusen category score[43,44] among the longitudinal records. Eyes with large, soft drusen ($\geq$125μm) and eyes with intermediate drusen (63–124μm) were assigned to grades 3 and 2, respectively. The control group (grade 1) included eyes with either no drusen or only a few small drusen ($<$63μm). The final grade for each subject was based on the eye with the most advanced stage of AMD. This research complied with the Declaration of Helsinki and was approved by the Institutional Review Board.

SNP Selection

We assessed variants in lipoprotein metabolism genes, including a functional variant (rs10468017) in the hepatic lipase (*LIPC*) gene on 15q22, which was associated with advanced AMD with genome-wide significance ($P = 1.34 \times 10^{-8}$) in our previous GWAS[45]; SNP rs1883025 in the ATP-binding cassette subfamily A member 1 (*ABCA1*) gene on 9q31; and SNP rs3764261 in the cholesterol ester transfer protein (*CETP*) gene on 16q21, which had suggestive associations with advanced AMD in previous GWAS[39,40] and haplotypes in the apolipoprotein E (*APOE*) gene which have been reported to be associated with AMD.[41,42] We also genotyped a nonsynonymous SNP rs10490924 in the *ARMS2/HTRA1* q26 region of chromosome 10 which is associated with advanced AMD but not known to be related to the complement pathway.[31,32,47,48] In addition, five known risk variants associated with advanced AMD in complement genes were genotyped: SNP rs1061170 in exon 9 of the *CFH* gene on 1q31, which results in a substitution of histidine for tyrosine (Y402H) in the protein sequence of *CFH*; SNP rs9332739 in the complement component 2 (*C2*) gene, which results in the amino acid change E318D in exon 7; SNP rs641153 in the complement factor B (*CFB*) gene, which results in an amino acid change at R32Q; SNP rs2230199 in the complement component 3 (*C3*) gene, which results in an amino acid change at R102G in exon 3; and SNP rs10033900 in complement factor I (*CFI*) on chromosome 4.[21–32,47,48]

Genotyping

DNA was extracted from blood samples of the participants. SNPs in the *CFH*, *ARMS2/HTRA1*, *C2*, *C3*, *CFB*, and *CFI* genes were genotyped at the Broad Institute Center for Genotyping and Analysis (iPLEX assay; Sequenom, San Diego, CA; http://www.sequenom.com/Genetic-Analysis/Applications/iPLEX-Genotyping/iPLEX-Overview.aspx). For SNPs not compatible with the assay, such as *APOE*, and other SNPs newly reported to be associated with advanced AMD, including *LIPC*, *CETP*, and *ABCA1* genes/regions, DNA samples were genotyped (*Taq*Man assay, with sequence detection on the Prism 7900; ABI, Foster City, CA; https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=601283).

Table 1. Characteristics of Subjects in Each Phenotype Group

		Drusen		Advanced AMD	
	Control	Intermediate	Large	GA	NV
Subjects, *n*	221	814	949	259	823
Mean age ± SD, y	77.0 ± 4.6	78.1 ± 4.2	78.9 ± 4.9	79.5 ± 5.5	80.7 ± 5.1
Sex					
Female	116 (52)	463 (57)	548 (58)	129 (50)	477 (58)
Male	105 (48)	351 (43)	401 (42)	130 (50)	346 (42)
Education					
>High school	155 (70)	587 (72)	656 (69)	163 (63)	473 (57)
≤High school	66 (30)	226 (28)	292 (31)	96 (37)	350 (43)
Smoking					
Never	105 (47)	438 (54)	473 (50)	108 (42)	321 (39)
Past	101 (46)	342 (42)	442 (46)	134 (52)	417 (51)
Current	15 (7)	34 (4)	34 (4)	17 (6)	85 (10)
BMI					
<25	65 (31)	280 (35)	339 (37)	78 (31)	220 (27)
25–29.9	88 (41)	337 (43)	403 (44)	105 (41)	345 (43)
≥30	60 (28)	174 (22)	179 (19)	70 (28)	240 (30)
Antioxidants					
No	128 (58)	418 (51)	442 (47)	136 (53)	411 (50)
Yes	93 (42)	396 (49)	507 (53)	123 (47)	412 (50)

Data are expressed as the number (percentage of total group), unless otherwise noted.

TABLE 2. Associations between Drusen and Advanced AMD and Genetic Factors in Single SNP Models

SNP (Gene)	Controls None, ≤63 μm *n* (%)	Drusen 63–124 μm *n* (%)	Drusen ≥125 μm *n* (%)	Advanced AMD GA *n* (%)	Advanced AMD NV *n* (%)	EA	Intermediate Drusen OR (95% CI) *P**	Large Drusen OR (95% CI) *P**	Advanced AMD OR (95% CI) *P**
Subjects, *n*	221	814	949	259	823				
rs10468017 *LIPC*									
C C	98 (45)	414 (51)	473 (50)	148 (58)	432 (53)	T	0.79 (0.62–0.99)	0.78 (0.62–0.99)	0.68 (0.53–0.87)
T C	93 (43)	326 (41)	396 (42)	91 (35)	333 (41)		**$P = 0.045$**	**$P = 0.041$**	**$P = 1.8 \times 10^{-3}$**
T T	27 (12)	61 (8)	73 (8)	17 (7)	46 (6)				
rs1883025 *ABCA1*									
C C	99 (46)	440 (55)	548 (58)	153 (60)	472 (58)	T	0.7 (0.55–0.9)	0.66 (0.52–0.84)	0.63 (0.5–0.81)
C T	95 (44)	318 (39)	337 (36)	87 (34)	315 (38)		**$P = 4.4 \times 10^{-3}$**	**$P = 7.7 \times 10^{-4}$**	**$P = 3.0 \times 10^{-4}$**
T T	23 (10)	48 (6)	58 (6)	16 (6)	34 (4)				
rs3764261 *CETP*									
C C	102 (47)	358 (44)	413 (44)	113 (44)	336 (41)	A	1.12 (0.88–1.42)	1.1 (0.87–1.4)	1.22 (0.97–1.55)
A C	98 (45)	366 (45)	428 (45)	115 (45)	359 (44)		$P = 0.37$	$P = 0.42$	$P = 0.09$
A A	18 (8)	85 (11)	102 (11)	28 (11)	125 (15)				
APOE									
E2/E3	160 (73)	620 (77)	732 (78)	210 (82)	660 (80)	E4	0.88 (0.62–1.25)	0.89 (0.62–1.27)	0.71 (0.49–1.02)
E4	58 (27)	185 (23)	208 (22)	45 (18)	160 (20)		$P = 0.47$	$P = 0.51$	$P = 0.06$
rs1061170 *CFH*									
T T	83 (38)	336 (42)	307 (32)	57 (22)	125 (15)	C	0.98 (0.78–1.22)	1.34 (1.07–1.67)	2.45 (1.96–3.08)
C T	106 (49)	366 (45)	442 (47)	107 (42)	368 (45)		$P = 0.84$	**$P = 9.8 \times 10^{-3}$**	**$P = 1.0 \times 10^{-14}$**
C C	27 (13)	108 (13)	119 (21)	94 (36)	322 (40)				
rs10490924 *ARMS2/HTRA1*									
G G	147 (68)	523 (65)	505 (53)	104 (40)	251 (31)	T	1.2 (0.9–1.6)	1.76 (1.33–2.32)	3.44 (2.61–4.55)
T G	65 (30)	254 (31)	362 (39)	114 (44)	395 (48)		$P = 0.21$	**$P = 6.3 \times 10^{-5}$**	**$P = 2.7 \times 10^{-18}$**
T T	5 (2)	33 (4)	81 (9)	41 (16)	171 (21)				
rs9332739 C2									
GG	193 (89)	737 (91)	876 (92)	247 (96)	784 (96)	C	0.75 (0.45–1.25)	0.63 (0.38–1.05)	0.32 (0.18–0.57)
CG/CC	23 (11)	73 (9)	72 (8)	11 (4)	30 (4)		$P = 0.27$	$P = 0.074$	**$P = 1.2 \times 10^{-4}$**
rs2230199 *C3*									
C C	140 (65)	506 (63)	539 (57)	129 (50)	397 (49)	G	1.04 (0.79–1.38)	1.31 (0.99–1.74)	1.72 (1.31–2.25)
G C	68 (31)	273 (34)	363 (38)	106 (41)	351 (43)		$P = 0.77$	$P = 0.056$	**$P = 1.1 \times 10^{-4}$**
G G	8 (4)	28 (3)	45 (5)	24 (9)	67 (8)	T			
rs641153 *CFB*									
C C	173 (80)	673 (83)	806 (85)	230 (89)	750 (92)		0.83 (0.55–1.23)	0.69 (0.46–1.03)	0.38 (0.24–0.58)
CT/TT	43 (20)	137 (17)	142 (15)	28 (11)	64 (8)		$P = 0.35$	$P = 0.069$	**$P = 1.2 \times 10^{-5}$**
rs10033900 *CFI*									
C C	65 (30)	225 (28)	235 (25)	56 (22)	187 (23)	T	1.14 (0.92–1.42)	1.15 (0.92–1.44)	1.35 (1.08–1.68)
C T	107 (50)	378 (47)	488 (51)	121 (47)	400 (49)		$P = 0.22$	$P = 0.21$	**$P = 8.0 \times 10^{-3}$**
T T	44 (20)	207 (25)	225 (24)	81 (31)	227 (28)				

EA, effective allele of which the genetic effect were shown for each SNP.

* Test of linear trend for single SNP additive model adjusted for age, sex, education (≤high school vs. >high school), smoking (never, past, current), BMI (<25, 25–29.9, 30+), and antioxidant treatment. Significant results ($P < 0.05$) are shown in bold.

We implemented stringent quality control criteria for each SNP in our dataset. All the SNPs had a greater than 99% genotype call rate. The rates for missing SNPs genotyped on the two platforms (iPLEX and *Taq*Man) were similar. None of the SNPs was significant ($P < 10^{-3}$) in the Hardy-Weinberg equilibrium test or ($P < 10^{-3}$) in the differential missing tests between cases and controls. All quality control steps were performed using PLINK.[49]

Statistical Analysis

To analyze the genetic effects on different stages of AMD, we classified subjects into case and control groups by their worse eye grades. Subjects with a worse eye grade of 1 were used as the control group in all models. The allele frequencies of each SNP in the two drusen groups (intermediate drusen or large drusen; grade 2 or 3) and the allele frequencies in the advanced AMD group (GA or NV; grade 4 or 5) were compared with the allele frequencies in the control group. We tested associations between SNPs and each disease stage by logistic regression (SAS ver. 9.2; SAS, Cary, NC). SNPs were coded as 0, 1, and 2 by the number of risk alleles in the tests for linear trend. Demographic and behavioral covariates, including age (continuous), sex, education (less than or equal to high school or more than high school), smoking (never, past, or current), BMI (<25, 25–29.9, and ≥30), and randomized antioxidant treatment in AREDS were included in the models. Multivariate logistic regression models were used to further adjust for the known genetic factors, in addition to the demographic and behavioral factors. To estimate the effects of specific genotypes of *LIPC* and *ABCA1* variants, we applied polytomous logistic regression in multivariate models.

Results

Among the 3066 Caucasian participants, there were 221 controls with no drusen or only a few small drusen and 814 subjects with intermediate drusen, 949 with large soft drusen, 259 with GA, and 823 with NV. Subjects with GA or NV were classified as having advanced AMD. For each SNP evaluated, the number of participants included in the analyses varied slightly, depending on the genotype call rates, which were all greater than 99% in this study. The distributions of demographic and behavioral characteristics of the participants are listed in Table 1. Numbers in tables may not equal totals due to missing information. Older subjects, those who smoked or had less than a high school education were more likely to have advanced AMD.

Table 2 shows the genotype frequencies of variants in each gene separately for the controls and each case group, and also *P* values for tests for linear trend for the number of effective alleles for each SNP. With adjustment for demographic and behavioral factors, the associations between each SNP and large drusen for *CFH* ($P = 9.8 \times 10^{-3}$), *ARMS2/HTRA1* ($P = 6.3 \times 10^{-5}$), *ABCA1* ($P = 7.7 \times 10^{-4}$), and *LIPC* ($P = 0.041$) were significant. However, only *ABCA1* ($P = 4.4 \times 10^{-3}$) and *LIPC* ($P = 0.045$) were significantly related to intermediate drusen. Associations between advanced AMD and *CFH* (rs1061170, $P = 1.0 \times 10^{-14}$), *C2* (rs9332739, $P = 1.2 \times 10^{-4}$), *C3* (rs2230199, $P = 1.1 \times 10^{-4}$), *CFB* (rs641153, $P = 1.2 \times 10^{-5}$), *CFI* (rs10033900, $P = 8.0 \times 10^{-3}$), and *ARMS2/HTRA1* (rs10490924 $P = 2.7 \times 10^{-18}$) were confirmed. The genes *LIPC* (rs10468017 $P = 1.8 \times 10^{-3}$) and *ABCA1* (rs1883025 $P = 3.0 \times 10^{-4}$) in the cholesterol/lipoprotein pathways were also significantly associated with advanced AMD. Associations between advanced AMD and *CETP* (rs3764261, $P = 0.09$) and *APOE* ($P = 0.06$) were not significant, but were in the same direction as previously reported.

To evaluate whether the associations between drusen and advanced AMD are independent of other genetic factors, we performed multivariate logistic analysis using the demographic and behavioral risk factors for AMD shown in Table 1, plus all the genetic factors (Table 3). The relationship between the case groups and the number of effective alleles was assessed. Only the two HDL genes *LIPC* ($P = 0.04$) and *ABCA1* ($P = 6.9 \times 10^{-3}$) were significantly related to intermediate drusen. *LIPC* ($P = 0.01$), *ABCA1* ($P = 7.8 \times 10^{-4}$), *CFH* ($P = 1.7 \times 10^{-14}$), *ARMS2/HTRA1* ($P = 4.6 \times 10^{-15}$), *C2* ($P = 1.6 \times 10^{-4}$), *C3* ($P = 7.6 \times 10^{-4}$), and *CFB* ($P = 0.01$) were independently associated with advanced AMD. Similar or slightly weaker association signals were observed for these

Table 3. Associations between Drusen and Advanced AMD and Effective Alleles for Genetic Factors in Multivariate Models*

	EA†	Intermediate Drusen OR (95% CI)	Large Drusen OR (95% CI)	GA OR (95% CI)	NV OR (95% CI)	Advanced AMD OR (95% CI)
rs10468017	T	**0.77 (0.61–0.99)**	0.79 (0.62–1.01)	0.8 (0.57–1.12)	**0.73 (0.54–1)**	**0.7 (0.53–0.93)**
LIPC		**$P = 0.04$**	$P = 0.06$	$P = 0.19$	**$P = 0.05$**	**$P = 0.01$**
rs1883025	T	**0.71 (0.56–0.91)**	**0.64 (0.5–0.82)**	**0.64 (0.45–0.91)**	**0.55 (0.4–0.76)**	**0.61 (0.46–0.81)**
ABCA1		**$P = 6.9 \times 10^{-3}$**	**$P = 3.9 \times 10^{-4}$**	**$P = 0.01$**	**$P = 3.0 \times 10^{-4}$**	**$P = 7.8 \times 10^{-4}$**
rs3764261	A	1.1 (0.86–1.4)	1.12 (0.87–1.44)	1.08 (0.76–1.52)	1.29 (0.96–1.74)	1.21 (0.92–1.59)
CETP		$P = 0.46$	$P = 0.37$	$P = 0.68$	$P = 0.09$	$P = 0.17$
APOE	E4	0.89 (0.62–1.28)	0.85 (0.58–1.24)	0.67 (0.39–1.16)	**0.61 (0.38–0.97)**	0.68 (0.44–1.04)
		$P = 0.52$	$P = 0.39$	$P = 0.15$	**$P = 0.04$**	$P = 0.07$
rs1061170	C	0.98 (0.78–1.23)	**1.37 (1.09–1.73)**	**2.32 (1.67–3.21)**	**3.38 (2.52–4.54)**	**2.77 (2.14–3.59)**
CFH		$P = 0.86$	**$P = 7.1 \times 10^{-3}$**	**$P = 4.9 \times 10^{-7}$**	**$P = 6.3 \times 10^{-16}$**	**$P = 1.7 \times 10^{-14}$**
rs10490924	T	1.2 (0.9–1.61)	**1.72 (1.29–2.29)**	**2.98 (2.05–4.35)**	**3.82 (2.76–5.31)**	**3.35 (2.48–4.53)**
ARMS2/HTRA1		$P = 0.22$	**$P = 2.1 \times 10^{-4}$**	**$P = 1.3 \times 10^{-8}$**	**$P = 1.0 \times 10^{-15}$**	**$P = 4.6 \times 10^{-15}$**
rs9332739	C	0.68 (0.41–1.15)	**0.58 (0.34–0.99)**	**0.36 (0.15–0.88)**	**0.23 (0.11–0.48)**	**0.27 (0.14–0.54)**
C2		$P = 0.15$	**$P = 0.05$**	**$P = 0.02$**	**$P = 9.2 \times 10^{-5}$**	**$P = 1.6 \times 10^{-4}$**
rs2230199	G	1.1 (0.82–1.47)	**1.4 (1.04–1.87)**	**1.71 (1.17–2.51)**	**1.97 (1.39–2.8)**	**1.72 (1.26–2.36)**
C3		$P = 0.52$	**$P = 0.03$**	**$P = 5.5 \times 10^{-3}$**	**$P = 1.5 \times 10^{-4}$**	**$P = 7.6 \times 10^{-4}$**
rs641153	T	0.91 (0.6–1.37)	0.69 (0.45–1.05)	0.7 (0.37–1.33)	**0.41 (0.23–0.74)**	**0.53 (0.31–0.88)**
CFB		$P = 0.64$	$P = 0.08$	$P = 0.28$	**$P = 2.8 \times 10^{-3}$**	**$P = 0.01$**
rs10033900	T	1.11 (0.89–1.38)	1.16 (0.92–1.47)	1.35 (0.99–1.85)	1.3 (0.98–1.72)	1.28 (0.99–1.65)
CFI		$P = 0.37$	$P = 0.20$	$P = 0.06$	$P = 0.07$	$P = 0.06$

Significant results ($P < 0.05$) are shown in bold.

* Tests for linear trend in multivariate models which include all the SNPs in the table and also age, sex, education (≤ high school vs. >high school), smoking (never, past, current), BMI (<25, 25–29.9, 30+), antioxidant treatment.

† EA, effective allele of which the genetic effect were shown for each SNP.

variants for NV, GA, and large drusen. The E4 allele in *APOE* was associated with NV ($P = 0.04$).

Table 4 shows the relationship between AMD phenotypes and genotypes for *ABCA1* and *LIPC* based on multivariate logistic models controlling for all genetic, demographic, and behavioral risk factors. The TT genotype of *LIPC* (rs10468017) was protective against intermediate drusen (OR [95% CI] = 0.52 [0.30–0.89]; $P = 0.018$), large drusen (OR [95% CI] = 0.55 [0.31–0.95]; $P = 0.033$), and advanced AMD (OR [95% CI] = 0.37 [0.19–0.71], $P = 2.8 \times 10^{-3}$). The CT genotype of *LIPC* (rs10468017) was not significant, suggesting that the genetic effect of this locus best fits a recessive genetic model. Using the collapsed genotypes of CC and CT as the reference genotype, we found that the TT homozygous genotype of *LIPC* was consistently associated with a reduced risk of intermediate, large drusen, and advanced AMD. Both homozygous and heterozygous genotypes with the T allele of *ABCA1* (rs1883025) were protective against intermediate drusen (TT: $P = 0.011$, OR [95% CI] = 0.48 [0.27–0.85]; CT: $P = 0.037$, OR [95% CI] = 0.70 [0.50–0.98]), large drusen (TT: $P = 3.1 \times 10^{-3}$, OR [95% CI] = 0.41 [0.23–0.74]; CT: $P = 5.8 \times 10^{-3}$, OR [95% CI] = 0.62 [0.44–0.87]), and advanced AMD (TT: $P = 3.5 \times 10^{-3}$, OR [95% CI] = 0.35 [0.17–0.71]; CT, $P = 6.8 \times 10^{-3}$, OR [95% CI] = 0.59 [0.40–0.87]). The genetic effect of the T allele of *ABCA1* (rs1883025) seems to fit an additive genetic model. Although the *P* values for both the *LIPC* and the *ABCA1* genes were higher in the GA group than in the NV group, possibly because of the smaller sample size in the GA group, the estimates of the effects of these genes on GA and NV were similar.

We also tested the effects of interactions between the genetic factors in the complement pathway and genes in other pathways, on risk of drusen phenotypes and advanced AMD (Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-7070/-/DCSupplemental). None of the gene interactions was significantly related to risk of drusen or advanced AMD. The present study may not have sufficient power to evaluate the small effects of interactions between the genetic variants.

Discussion

In this study, we present novel findings that variants in the *LIPC* and *ABCA1* genes in the HDL cholesterol/lipoprotein pathway are likely to be associated with drusen accumulation in the early stages of AMD. This study adds new information beyond the initial discovery, replication and evaluation of these two genes which assessed associations between advanced AMD or a combined phenotype and controls.[39,40,50,51] We evaluated the effects of these genes on separate groups of early, intermediate, and advanced forms of AMD in a large cohort, and controlled for demographic and behavioral factors including age, sex, BMI, smoking, education, and antioxidant treatment and other genes related to AMD.

The SNP rs10468017, located in the promoter region of *LIPC* on chromosome 15, is a functional variant that regulates serum HDL levels by controlling expression level of *LIPC.*[52,53] Serum HDL level was estimated to be 100 μmol/L higher with each copy of the T allele at rs10468017 in a large GWAS study for dyslipidemia.[38] The T allele of rs1883025 in *ABCA1*, which is associated with decreased HDL levels,[38] is also inversely associated with the risk of developing intermediate drusen, large drusen, and advanced AMD in this study. Since the allele in *ABCA1*, which decreases HDL levels, and the allele in *LIPC*, which increases HDL levels, are both associated with decreasing risk of advanced AMD and drusen, it is not likely that these genes influence the risk of AMD through the same pathway(s) that regulate serum HDL levels. In our recent study, higher

Table 4. Associations between Drusen and Advanced AMD and *LIPC* and *ABCA1* Genotypes in Multivariate Models

	Intermediate Drusen (765/203*)		Large Drusen (901/203*)		GA (245/203*)		NV (778/203*)		Advanced AMD (1023/203*)	
	OR (95% CI)	P†	OR (95% CI)	P†	OR (95% CI)	P†	OR (95% CI)	P†	OR (95% CI)	P†
LIPC:rs10468017										
CC	1		1		1		1		1	
TC	0.85 (0.60–1.19)	0.34	0.88 (0.63–1.25)	0.48	0.98 (0.60–1.60)	0.94	0.96 (0.63–1.46)	0.83	0.88 (0.60–1.30)	0.51
TT	0.52 (0.30–0.89)	**0.018**	0.55 (0.31–0.95)	**0.033**	0.48 (0.21–1.10)	0.083	0.39 (0.18–0.81)	**0.012**	0.37 (0.19–0.71)	**2.8×10^{-3}**
TT vs CC/TC	0.56 (0.33–0.94)	**0.029**	0.58 (0.34–0.98)	**0.043**	0.48 (0.21–1.08)	0.076	0.39 (0.19–0.81)	**0.01**	0.39 (0.21–0.74)	**3.4×10^{-3}**
ABCA1:rs1883025										
CC	1		1		1		1		1	
CT	0.70 (0.50–0.98)	**0.037**	0.62 (0.44–0.87)	**5.8×10^{-3}**	0.56 (0.34–0.90)	**0.017**	0.58 (0.39–0.88)	**0.011**	0.59 (0.40–0.87)	**6.8×10^{-3}**
TT	0.48 (0.27–0.85)	**0.011**	0.41 (0.23–0.74)	**3.1×10^{-3}**	0.44 (0.19–1.05)	0.065	0.26 (0.11–0.58)	**1.1×10^{-3}**	0.35 (0.17–0.71)	**3.5×10^{-3}**

* Cases/controls, *n*.

† Multivariate polytomous model including the genotypes of *LIPC* and *ABCA1* and adjusted for age, sex, education (≤high school vs. >high school), smoking (never, past, current), BMI (<25, 25–29.9, 30+), antioxidant treatment, *CFH* Y402H (TT, CT, CC), *ARMS2/HTRA1* (GG, GT, TT), *C2* (GG, CG/CC), *C3* (CC, CG, GG), *CFB* (CC, CT/TT), *CFI* (TT, CT, CC), *CETP* (AA, AC, CC), and APOE (E4, E2/E3). Significant results ($P < 0.05$) are shown in bold.

serum HDL was associated with lower risk of AMD, and *LIPC* was associated with reduced risk of advanced AMD, independent of serum levels of HDL,[51] suggesting that another mechanism is involved.

LIPC[39] and *ABCA1*[54] have been shown to be expressed in the retina and RPE.[55] *LIPC*[56,57] and *ABCA1*[58,59] have also been synthesized and expressed in macrophages, which are the predominant cells involved in creating the progressive plaque lesions of atherosclerosis.[60] *ABCA1* is believed to suppress atherosclerotic lesions as an anti-inflammatory receptor that exports cholesterol from arterial macrophages.[61,62] Drusen may develop by a similar mechanism as atherosclerotic plaque, except that lipoproteins accumulated in Bruch's membrane are likely to be of intraocular origin.[63,64] Macrophages have also been found in Bruch's membrane and are related to early and advanced AMD.[65,66] Combining this evidence with our findings of an inverse association between *LIPC* and *ABCA1* with drusen, it is possible that functional variants regulating expression levels of *LIPC* and *ABCA1* promote cholesterol efflux, reduce the activation of the inflammatory pathway in subretinal macrophages, and result in less drusen accumulation.

Figure 1 shows the absolute difference in the specific risk/protective genotype frequencies of variants in *CFH* (Y402H), *ARMS2/HTRA1*, *ABCA1*, and *LIPC* relative to the genotype frequencies in the control group. The largest differences in TT genotype frequencies in the *ABCA1* and *LIPC* genes are between the control stage and the intermediate drusen stage. For intermediate and large drusen GA and NV, there is little change in frequencies of the TT genotype in the *ABCA1* and *LIPC* genes. This pattern of genotype frequencies in *ABCA1* and *LIPC* is distinctive from the pattern in *ARMS2/HTRA1* and the complement genes, in which the greatest frequency changes are seen between the stage of large drusen and advanced AMD (Fig. 1). Our results suggest that the variants in the HDL genes are involved in drusen initiation and accumulation and that the complement pathway is activated later as a result of inflammatory responses due to drusen accumulation. However, we cannot exclude the possibility that genes in the complement pathway and *ARMS2/HTRA1* are also involved in early stages of AMD, especially considering that so many protein products of complement-related genes constitute the makeup of drusen. It has been reported that variants in the *CFH* gene may cause basal laminar drusen in young adults,[67] and *CFH* has also been related to small, hard macular drusen in a sample with relatively young average age (30–66 years).[68] Most of the individuals with advanced AMD in our dataset with the risk alleles in *CFH* and *ARMS2/HTRA1* are older subjects who had drusen before progression to advanced stages. As strong risk factors for the progression to advanced AMD,[20,69] *CFH* and *ARMS2/HTRA1* may also have contributed to early-stage drusen accumulation when the patients were younger.

In our previous GWAS,[39,40] there was some evidence that the A allele of rs3764261 in *CETP* increased the risk of advanced AMD (OR [95% CI] = 1.12 [1.04–1.20]), although this was not genome-wide significant ($P = 1.41 \times 10^{-3}$). In the present study, the AA genotype at this locus is overrepresented in NV cases compared with the controls, indicating the same direction of genetic effect on this locus, controlling for demographic, behavioral, and other known genetic factors that were not included in our original GWAS. The E4 allele of *APOE* tended to reduce risk of NV; however, the magnitude of the effect in our sample was much smaller than previously reported. Subjects with the E4 allele (mean age, 78.54 ± 4.97 years) were significantly younger ($P = 0.0014$) than subjects without the E4 allele (mean age, 79.24 ± 4.95, years) in this sample. Thus, it is possible that the protective effect of the E4 allele on NV is confounded by the selection pressure of *APOE* in older subjects.

Strengths of the study include the large, well characterized population of subjects in carefully defined AMD stages, recruited from various geographic regions around the United States; the standardized collection of risk factor information; direct measurements of height and weight; and classification of maculopathy by well-documented ophthalmic examinations and fundus photography. The phenotype grades were unlikely to be misclassified because they were assigned without knowledge of risk factors or genotype and were assigned based on phenotypic information from multiple diagnostic records. We evaluated early, intermediate, and advanced AMD stages and expanded the evaluation of the complicated genetic effects on AMD. Results suggest that a functional variant plays an important role during an early stage of AMD. Therefore, it would be helpful to collect samples across all spectra of the disease and analyses could be done separately for different stages. Prospective studies and larger independent samples are needed to confirm and expand upon these findings.

In conclusion, T alleles in two genes in the HDL pathway, *LIPC* and *ABCA1*, are protective for intermediate and large

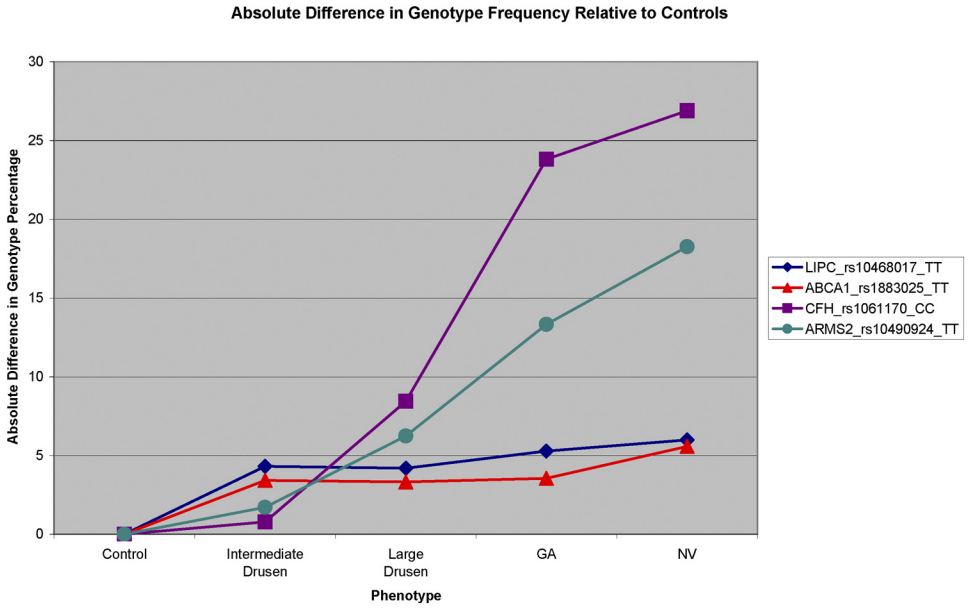

Figure 1. Absolute difference in genotype frequency relative to controls of the TT genotype of rs10468017 in *LIPC*, the TT genotype of rs1883025 in *ABCA1*, the CC genotype of rs1061170 in *CFH*, and the TT genotype of rs10490924 in *ARMS2/HTRA1* of intermediate drusen, large drusen, GA, and NV disease.

drusen phenotypes, as well as advanced AMD, independent of other genetic and environmental factors. These genes in the HDL pathway may play important roles in drusen accumulation in early and intermediate stages of AMD.

Acknowledgments

The authors thank Gordon Huggins, PhD (Tufts Medical Center), for assistance with the genotyping.

References

1. Seddon JM, Sobrin L. Epidemiology of age-related macular degeneration. In: Albert D, Miller J, Azar D, Blodi B, eds. *Albert & Jakobiec's Principles and Practice of Ophthalmology.* Philadelphia: WB Saunders; 2007:413–422.
2. Friedman DS, O'Colmain BJ, Munoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol.* 2004;122:564–572.
3. de Jong PT. Age-related macular degeneration. *N Engl J Med.* 2006;355:1474–1485.
4. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. *Arch Ophthalmol.* 2004;122:598–614.
5. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res.* 2001;20:705–732.
6. Fariss RN, Apte SS, Olsen BR, Iwata K, Milam AH. Tissue inhibitor of metalloproteinases-3 is a component of Bruch's membrane of the eye. *Am J Pathol.* 1997;150:323–328.
7. Hageman GS, Mullins RF, Russell SR, Johnson LV, Anderson DH. Vitronectin is a constituent of ocular drusen and the vitronectin gene is expressed in human retinal pigmented epithelial cells. *FASEB J.* 1999;13:477–484.
8. Luibl V, Isas JM, Kayed R, Glabe CG, Langen R, Chen J. Drusen deposits associated with aging and age-related macular degeneration contain nonfibrillar amyloid oligomers. *J Clin Invest.* 2006;116:378–385.
9. Johnson LV, Leitner WP, Staples MK, Anderson DH. Complement activation and inflammatory processes in drusen formation and age related macular degeneration. *Exp Eye Res.* 2001;73:887–896.
10. Li CM, Clark ME, Rudolf M, Curcio CA. Distribution and composition of esterified and unesterified cholesterol in extra-macular drusen. *Exp Eye Res.* 2007;85:192–201.
11. Wang L, Clark ME, Crossman DK, et al. Abundant lipid and protein components of drusen. *PLoS One.* 2010;5:e10329.
12. Curcio CA, Johnson M, Huang JD, Rudolf M. Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration. *J Lipid Res.* 2010;51:451–467.
13. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. *Arch Ophthalmol.* 2005;123:321–327.
14. Seddon JM, Willett WC, Speizer FE, Hankinson SE. A prospective study of cigarette smoking and age-related macular degeneration in women. *JAMA.* 1996;276:1141–1146.
15. Seddon JM, Cote J, Davis N, Rosner B. Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist-hip ratio. *Arch Ophthalmol.* 2003;121:785–792.
16. Tomany SC, Wang JJ, Van Leeuwen R, et al. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. *Ophthalmology.* 2004;111:1280–1287.
17. Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL 3rd. Risk factors for the incidence of advanced age-related macular degeneration in the Age-Related Eye Disease Study (AREDS). AREDS report no. 19. *Ophthalmology* 2005;112:533–539.
18. Seddon JM, George S, Rosner B, Klein ML. CFH gene variant, Y402H, and smoking, body mass index, environmental associations with advanced age-related macular degeneration. *Hum Hered.* 2006;61:157–165.
19. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration. *Arch Ophthalmol.* 2006;124:995–1001.
20. Seddon JM, Francis PJ, George S, Schultz DW, Rosner B, Klein ML. Association of CFH Y402H and LOC387715 A69S with progression of age-related macular degeneration. *JAMA.* 2007;297:1793–1800.
21. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science.* 2005;308:419–421.
22. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005;308:385–389.
23. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2005;102:7227–7232.
24. Maller J, George S, Purcell S, et al. Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. *Nat Genet.* 2006;38:1055–1059.
25. Li M, Atmaca-Sonmez P, Othman M, et al. CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration. *Nat Genet.* 2006;38:1049–1054.
26. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science.* 2005;308:421–424.
27. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet.* 2006;38:458–462.
28. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. *Nat Genet.* 2007;39:1200–1201.
29. Yates JR, Sepp T, Matharu BK, et al. Complement C3 variant and the risk of age-related macular degeneration. *N Engl J Med.* 2007;357:553–561.
30. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. *Eur J Hum Genet.* 2009;17:100–104.
31. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet.* 2005;14:3227–3236.
32. Yang Z, Camp NJ, Sun H, et al. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. *Science.* 2006;314:992–993.
33. Seddon JM, Reynolds R, Rosner B. Peripheral retinal drusen and reticular pigment: association with CFHY402H and CFHrs1410996 genotypes in family and twin studies. *Invest Ophthalmol Vis Sci.* 2009;50:586–591.
34. Andreoli MT, Morrison MA, Kim BJ, et al. Comprehensive analysis of complement factor H and LOC387715/ARMS2/HTRA1 variants with respect to phenotype in advanced age-related macular degeneration. *Am J Ophthalmol.* 2009;148:869–874.
35. Magnusson KP, Duan S, Sigurdsson H, et al. CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PLoS Med.* 2006;3:e5.
36. Grassi MA, Folk JC, Scheetz TE, Taylor CM, Sheffield VC, Stone EM. Complement factor H polymorphism p.Tyr402His and cuticular drusen. *Arch Ophthalmol.* 2007;125:93–97.
37. Hasham SN, Pillarisetti S. Vascular lipases, inflammation and atherosclerosis. *Clin Chim Acta.* 2006;372:179–183.
38. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet.* 2009;41:56–65.
39. Neale BM, Fagerness J, Reynolds R, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). *Proc Natl Acad Sci U S A.* 2010;107:7395–7400.
40. Chen W, Stambolian D, Edwards AO, et al. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence sus-

ceptibility to age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2010;107:7401–7406.
41. Souied EH, Benlian P, Amouyel P, et al. The epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration. *Am J Ophthalmol.* 1998; 125:353–359.
42. Baird PN, Richardson AJ, Robman LD, et al. Apolipoprotein (APOE) gene is associated with progression of age-related macular degeneration (AMD). *Hum Mutat.* 2006;27:337–342.
43. Losonczy G, Fekete A, Voko Z, et al. Analysis of complement factor H Y402H, LOC387715, HTRA1 polymorphisms and ApoE alleles with susceptibility to age-related macular degeneration in Hungarian patients. *Acta Ophthalmol.* 2011;89:255–262.
44. Fritsche LG, Freitag-Wolf S, Bettecken T, et al. Age-related macular degeneration and functional promoter and coding variants of the apolipoprotein E gene. *Hum Mutat.* 2009;30:1048–1053.
45. Seddon JM, Sharma S, Adelman RA. Evaluation of the clinical age-related maculopathy staging system. *Ophthalmology.* 2006; 113:260–266.
46. The Age-Related Eye Disease Study (AREDS): design implications. AREDS report no. 1. *Control Clin Trials* 1999;20:573–600.
47. Dewan A, Liu M, Hartman S, et al. HTRA1 promoter polymorphism in wet age-related macular degeneration. *Science.* 2006;314:989–992.
48. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am J Hum Genet.* 2005;77:389–407.
49. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559–575.
50. Seddon JM, Reynolds R, Rosner B. Associations of smoking, body mass index, dietary lutein, and the LIPC gene variant rs10468017 with advanced age-related macular degeneration. *Mol Vis.* 2010; 16:2412–2424.
51. Reynolds R, Rosner B, Seddon JM. Serum lipid biomarkers and hepatic lipase gene associations with age-related macular degeneration. *Ophthalmology.* 2010;117:1989–1995.
52. Rufibach LE, Duncan SA, Battle M, Deeb SS. Transcriptional regulation of the human hepatic lipase (LIPC) gene promoter. *J Lipid Res.* 2006;47:1463–1477.
53. Grarup N, Andreasen CH, Andersen MK, et al. The -250G>A promoter variant in hepatic lipase associates with elevated fasting serum high-density lipoprotein cholesterol modulated by interaction with physical activity in a study of 16,156 Danish subjects. *J Clin Endocrinol Metab.* 2008;93:2294–2299.
54. Tserentsoodol N, Gordiyenko NV, Pascual I, Lee JW, Fliesler SJ, Rodriguez IR. Intraretinal lipid transport is dependent on high density lipoprotein-like particles and class B scavenger receptors. *Mol Vis.* 2006;12:1319–1333.
55. Duncan KG, Hosseini K, Bailey KR, et al. Expression of reverse cholesterol transport proteins ATP-binding cassette A1 (ABCA1) and scavenger receptor BI (SR-BI) in the retina and retinal pigment epithelium. *Br J Ophthalmol.* 2009;93:1116–1120.
56. Nong Z, Gonzalez-Navarro H, Amar M, et al. Hepatic lipase expression in macrophages contributes to atherosclerosis in apoE-deficient and LCAT-transgenic mice. *J Clin Invest.* 2003;112:367–378.
57. Gonzalez-Navarro H, Nong Z, Freeman L, Bensadoun A, Peterson K, Santamarina-Fojo S. Identification of mouse and human macrophages as a site of synthesis of hepatic lipase. *J Lipid Res.* 2002; 43:671–675.
58. Schmitz G, Kaminski WE, Porsch-Ozcurumez M, et al. ATP-binding cassette transporter A1 (ABCA1) in macrophages: a dual function in inflammation and lipid metabolism? *Pathobiology.* 1999;67: 236–240.
59. Oram JF, Lawn RM, Garvin MR, Wade DP. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. *J Biol Chem.* 2000;275:34508–34511.
60. Lucas AD, Greaves DR. Atherosclerosis: role of chemokines and macrophages. *Expert Rev Mol Med.* 2001;3:1–18.
61. Tang C, Liu Y, Kessler PS, Vaughan AM, Oram JF. The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor. *J Biol Chem.* 2009;284:32336–32343.
62. Yvan-Charvet L, Wang N, Tall AR. Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses. *Arterioscler Thromb Vasc Biol.* 2010;30:139–143.
63. Curcio CA, Rudolf M, Wang L. Histochemistry and lipid profiling combine for insights into aging and age-related maculopathy. *Methods Mol Biol.* 2009;580:267–281.
64. Curcio CA, Johnson M, Huang JD, Rudolf M. Aging, age-related macular degeneration, and the response-to-retention of apolipoprotein B-containing lipoproteins. *Prog Retin Eye Res.* 2009;28: 393–422.
65. Cherepanoff S, McMenamin PG, Gillies MC, Kettle E, Sarks SH. Bruch's membrane and choroidal macrophages in early and advanced age-related macular degeneration. *Br J Ophthalmol.* 2010; 94:918–925.
66. Killingsworth MC, Sarks JP, Sarks SH. Macrophages related to Bruch's membrane in age-related macular degeneration. *Eye (Lond).* 1990;4(Pt 4):613–621.
67. Boon CJ, Klevering BJ, Hoyng CB, et al. Basal laminar drusen caused by compound heterozygous variants in the CFH gene. *Am J Hum Genet.* 2008;82:516–523.
68. Munch IC, Ek J, Kessel L, et al. Small, hard macular drusen and peripheral drusen: associations with AMD genotypes in the Inter99 Eye Study. *Invest Ophthalmol Vis Sci.* 2010;51:2317–2321.
69. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest Ophthalmol Vis Sci.* 2009;50:2044–2053.