Association of Variants in the \textit{LIPC} and \textit{ABCA1} Genes with Intermediate and Large Drusen and Advanced Age-Related Macular Degeneration

Yi Yu,\textsuperscript{1} Robyn Reynolds,\textsuperscript{1} Jesen Fagerness,\textsuperscript{2,3} Bernard Rosner,\textsuperscript{4} Mark J. Daly,\textsuperscript{2,3} and Johanna M. Seddon\textsuperscript{1,5}

\textbf{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.

\textbf{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 \textit{CFH}, \textit{C2}, \textit{C3}, \textit{CFB}, \textit{CFI}, \textit{APOE}, and \textit{ARMS2/HTRA1} genes/regions and the novel genes \textit{LIPC}, \textit{CETP}, and \textit{ABCA1} in the high-density lipoprotein (HDL) cholesterol pathway were genotyped. Associations between stage of AMD and SNPs were assessed using logistic regression.

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

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

**Ge**-related macular degeneration (AMD) is a common, complex, chronic eye disease.\textsuperscript{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.\textsuperscript{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).\textsuperscript{3} GA features well-demarcated borders of atrophy that develop as the macular neurosensorial 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,\textsuperscript{4} they have one common hallmark, which is drusen between the RPE and Bruch’s membrane.\textsuperscript{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\textsuperscript{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.\textsuperscript{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.\textsuperscript{1,2}

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.\textsuperscript{13} Cigarette smoking and higher body mass index (BMI) increase the risk of advanced AMD and its progression\textsuperscript{14–16} and also modify genetic susceptibility.\textsuperscript{16,20}

At least two independent variants in the complement factor H (\textit{CFH}) gene are associated with advanced AMD.\textsuperscript{21–26} Variants of several other genes in the alternative complement pathway, including \textit{CFB}/\textit{C2}\textsuperscript{24,27} \textit{C3}\textsuperscript{28,29} and \textit{CFI}\textsuperscript{30} also contribute to the risk of developing advanced AMD. The \textit{ARMS2/HTRA1} region on chromosome 10 confers a high risk of advanced AMD and is not in the complement pathway.\textsuperscript{31,32} Both \textit{CFH} and the \textit{ARMS2/HTRA1} region have been related to large, soft,
or cuticular drusen.\textsuperscript{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 \textit{LIPC}, which regulates triglyceride hydrolysis and plasma high density lipoprotein cholesterol (HDL-C) levels,\textsuperscript{37,38} decreases the risk of advanced AMD.\textsuperscript{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 \textit{CETP} and \textit{ABCA1}, may also be involved in the etiology of AMD.\textsuperscript{39,40} The \textit{APOE} gene, a widely expressed cholesterol transporter, has been shown to be associated with AMD in some studies, but results are inconsistent.\textsuperscript{41–44} Although it is well known that lipids are major components of drusen,\textsuperscript{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, \textit{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.

\section*{Materials and Methods}

\subsection*{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 (CARSMS) grades\textsuperscript{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 grade 4. For eyes without any of the above signs of advanced AMD, grades were determined by the highest drusen category score\textsuperscript{45,46} among the longitudinal records. Eyes with large, soft drusen (≥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.

\subsection*{SNP Selection}

We assessed variants in lipoprotein metabolism genes, including a functional variant (rs1061170 in the hepatic lipase (\textit{LIPC}) gene on 15q22, which was associated with advanced AMD with genome-wide significance ($P = 1.34 \times 10^{-5}$) in our previous GWAS\textsuperscript{46}; SNP rs1880925 in the ATP-binding cassette subfamily A member 1 (\textit{ABCA1}) gene on 9q31; and SNP rs5764261 in the cholesterol ester transfer protein (\textit{CETP}) gene on 16q21, which had suggestive associations with advanced AMD in previous GWAS\textsuperscript{39,40} and haplotypes in the apolipoprotein E (\textit{APOE}) gene which have been reported to be associated with AMD.\textsuperscript{41–42} We also genotyped a nonsynonymous SNP rs10499024 in the \textit{ARMS2/HTRA1} q26 region of chromosome 10 which is associated with advanced AMD but not known to be related to the complement pathway.\textsuperscript{31,52–54} In addition, five known risk variants associated with advanced AMD in complement genes were genotyped: SNP rs1061170 in exon 9 of the \textit{CFH} gene in 1q31, which results in a substitution of histidine for tyrosine (Y402H) in the protein sequence; SNP rs9352739 in the complement component 2 (\textit{C2}) gene, which results in an amino acid change E318D in exon 7; SNP rs641153 in the complement factor B (\textit{CFB}) gene, which results in an amino acid change R32Q; SNP rs2230199 in the complement component 3 (\textit{C3}) gene, which results in an amino acid change D110H in exon 2, and SNP rs10033900 in the complement factor 1 (\textit{C1}) gene on chromosome 4.\textsuperscript{51–53}

\subsection*{Genotyping}

DNA was extracted from blood samples of the participants. SNPs in the \textit{CFH}, \textit{ARMS2/HTRA1}, \textit{C2}, \textit{C3}, \textit{CFB}, and \textit{C1} genes were genotyped at the Broad Institute Center for Genotyping and Analysis (iPLEX assay; Sequenom, San Diego, CA; http://www.sequenom.com/Genetic-Analyses/Applications/iPLEX-Genotyping/iPLEX-Overview.aspx). For SNPs not compatible with the assay, such as \textit{APOE}, and other SNPs newly reported to be associated with advanced AMD, including \textit{LIPC}, \textit{CETP}, and \textit{ABCA1} genes regions, DNA samples were genotyped (TaqMan 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).

\begin{table}
\centering
\begin{tabular}{lcccc}
\hline
 & \textbf{Control} & \textbf{Intermediate} & \textbf{Large} & \textbf{GA} & \textbf{NV} \\
\hline
\textbf{Subjects, n} & 221 & 814 & 949 & 259 & 823 \\
\textbf{Mean age ± SD, y} & 77.0 ± 4.6 & 78.1 ± 4.2 & 78.9 ± 4.9 & 79.5 ± 5.5 & 80.7 ± 5.1 \\
\textbf{Sex} & & & & & \\
\textbf{Female} & 116 (52) & 463 (57) & 548 (58) & 129 (50) & 477 (58) \\
\textbf{Male} & 105 (48) & 351 (43) & 401 (42) & 130 (50) & 346 (42) \\
\textbf{Education} & & & & & \\
\textbf{≥High school} & 155 (70) & 587 (72) & 656 (69) & 163 (65) & 475 (57) \\
\textbf{≥High school} & 66 (30) & 226 (28) & 292 (31) & 96 (37) & 350 (43) \\
\textbf{Smoking} & & & & & \\
\textbf{Never} & 105 (47) & 438 (54) & 473 (50) & 108 (42) & 321 (39) \\
\textbf{Past} & 101 (46) & 342 (42) & 442 (46) & 134 (52) & 417 (51) \\
\textbf{Current} & 15 (7) & 34 (4) & 34 (4) & 17 (6) & 85 (10) \\
\textbf{BMI} & & & & & \\
\textbf{≤25} & 65 (31) & 280 (35) & 339 (37) & 78 (31) & 220 (27) \\
\textbf{25–29.9} & 88 (41) & 337 (43) & 403 (44) & 105 (41) & 345 (43) \\
\textbf{≥30} & 60 (28) & 174 (22) & 179 (19) & 70 (28) & 240 (30) \\
\textbf{Antioxidants} & & & & & \\
\textbf{No} & 128 (58) & 418 (51) & 442 (47) & 136 (53) & 411 (50) \\
\textbf{Yes} & 93 (42) & 396 (49) & 507 (53) & 123 (47) & 412 (50) \\
\hline
\end{tabular}
\caption{Characteristics of Subjects in Each Phenotype Group}
\end{table}

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

This research complied with the Declaration of Helsinki and was approved by the Institutional Review Board.
| SNP (Gene) | Subjects, n | rs1046801 LIPC | 63–124 μm n (%) | ≥125 μm n (%) | rs188305 ABCA1 | 63–124 μm n (%) | ≥125 μm n (%) | rs3764261 CETP | 63–124 μm n (%) | ≥125 μm n (%) | rs1061170 CHH | 63–124 μm n (%) | ≥125 μm n (%) | rs10190924 ARMS2/HTRA1 | 63–124 μm n (%) | ≥125 μm n (%) | rs9332739 C2 | 63–124 μm n (%) | ≥125 μm n (%) | rs2230199 C3 | 63–124 μm n (%) | ≥125 μm n (%) | rs641153 CFB | 63–124 μm n (%) | ≥125 μm n (%) | rs10033900 CFI | 63–124 μm n (%) | ≥125 μm n (%) |
|-----------|-------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 63/65 μm  | 221         | 98 (45)        | 91 (35)         | 148 (58)       | 99 (46)        | 95 (44)        | 25 (10)        | 102 (47)       | 98 (45)        | 18 (8)         | 160 (73)       | 58 (27)        | 106 (49)       | 27 (13)        | 147 (68)       | 65 (30)        | 5 (2)          | 193 (89)       | 23 (11)        | 140 (65)       | 68 (31)        | 8 (4)          | 175 (80)       | 43 (20)        | 65 (30)        |
| n (%)     |             | 414 (51)       | 326 (41)        | 473 (50)       | 440 (55)       | 318 (39)       | 48 (6)          | 358 (44)       | 366 (45)       | 85 (11)        | 620 (77)       | 185 (25)       | 366 (45)       | 108 (15)       | 523 (65)       | 254 (31)       | 33 (4)         | 737 (91)       | 73 (9)         | 506 (65)       | 273 (34)       | 28 (3)         | 876 (82)       | 72 (8)         | 506 (65)       |
|           |             | 473 (50)       | 396 (42)        | 452 (53)       | 548 (58)       | 357 (36)       | 58 (6)          | 413 (44)       | 428 (45)       | 102 (11)       | 732 (78)       | 208 (22)       | 442 (47)       | 119 (21)       | 505 (53)       | 362 (39)       | 81 (9)         | 786 (92)       | 72 (8)         | 539 (57)       | 363 (38)       | 45 (5)         | 876 (82)       | 72 (8)         | 539 (57)       |
| GA (n (%))| 259         | 148 (58)       | 91 (35)         | 177 (7)        | 153 (60)       | 87 (34)        | 16 (6)          | 113 (44)       | 115 (45)       | 28 (11)        | 210 (82)       | 45 (18)        | 107 (42)       | 94 (36)        | 104 (40)       | 114 (44)       | 41 (16)        | 247 (96)       | 11 (4)         | 129 (50)       | 106 (41)       | 24 (0)         | 230 (89)       | 28 (11)        | 129 (50)       |
| NV (n (%))| 825         | 452 (53)       | 353 (41)        | 64 (6)         | 472 (58)       | 315 (38)       | 34 (4)          | 336 (41)       | 359 (44)       | 125 (15)       | 660 (80)       | 160 (20)       | 368 (45)       | 322 (40)       | 251 (31)       | 395 (48)       | 171 (21)       | 784 (96)       | 30 (4)         | 397 (49)       | 351 (43)       | 67 (8)         | 750 (92)       | 64 (8)         | 397 (49)       |
| EA        |             | T             | T               | T              | T              | A              | A               | A              | A              | A              | A              | A              | A              | T              | A              | A              | A              | T              | C              | G              | G              | T              | T              | T              | T              |
| OR (95% CI)|             | 0.79 (0.62–0.99) | P = 0.045 | P = 0.045 | 0.78 (0.62–0.99) | P = 0.041 | 0.68 (0.53–0.87) | 0.66 (0.52–0.84) | 0.63 (0.5–0.81) | 0.7 (0.55–0.9) | P = 4.4 × 10⁻³ | 7.7 × 10⁻⁴ | P = 3.0 × 10⁻⁴ | 2.7 × 10⁻⁴ | 9.8 × 10⁻⁴ | 8.1 × 10⁻³ | 1.0 × 10⁻¹⁴ | 3.4 × 10⁻² | 6.3 × 10⁻⁵ | 1.2 × 10⁻¹⁴ | 1.1 × 10⁻⁲ | 1.72 (1.31–2.25) | 1.72 (1.31–2.25) | 1.35 (1.08–1.68) | 1.35 (1.08–1.68) | 1.35 (1.08–1.68) | 1.35 (1.08–1.68) |

EA, effective allele of which the genetic effect was 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 TaqMan) were similar. None of the SNPs was significant (P < 10^{-7}) 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.59

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 and subjects into case and control groups by their worse eye grades. To analyze the genetic effects on different stages of AMD, we classified 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 × 10^{-5}), ARMS2/HTRA1 (P = 6.5 × 10^{-5}), ABCA1 (P = 7.7 × 10^{-5}) and LIPC (P = 0.041) were significant. However, only ABCA1 (P = 4.4 × 10^{-5}) and LIPC (P = 0.045) were significantly related to intermediate drusen. Associations between advanced AMD and CFH (rs1061170, P = 1.0 × 10^{-14}), C2 (rs93532739, P = 1.2 × 10^{-4}), C3 (rs2230199, P = 1.1 × 10^{-4}), CFH (rs641153, P = 1.2 × 10^{-5}), CFI (rs10033900, P = 8.0 × 10^{-5}), and ARMS2/HTRA1 (rs10490924 P = 2.7 × 10^{-16}) were confirmed. The genes LIPC (rs10468017 P = 1.8 × 10^{-5}) and ABCA1 (rs1883025 P = 3.0 × 10^{-5}) in the cholesterol/lipoprotein pathways were also significantly associated with advanced AMD. Associations between advanced AMD and CETP (rs5764261, 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 × 10^{-5}) were significantly related to intermediate drusen. LIPC (P = 0.01), ABCA1 (P = 7.8 × 10^{-5}), CFH (P = 1.7 × 10^{-5}), ARMS2/HTRA1 (P = 4.6 × 10^{-5}), C2 (P = 1.6 × 10^{-5}), C3 (P = 7.6 × 10^{-5}), 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

<table>
<thead>
<tr>
<th>EA†</th>
<th>Intermediate Drusen OR (95% CI)</th>
<th>Large Drusen OR (95% CI)</th>
<th>GA OR (95% CI)</th>
<th>NV OR (95% CI)</th>
<th>Advanced AMD OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10468017</td>
<td>T 0.77 (0.61–0.99)</td>
<td>0.79 (0.62–1.01)</td>
<td>0.8 (0.57–1.12)</td>
<td>0.73 (0.54–1)</td>
<td>0.7 (0.53–0.93)</td>
</tr>
<tr>
<td></td>
<td>LIPC P = 0.04</td>
<td>P = 0.06</td>
<td>P = 0.19</td>
<td>P = 0.05</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>rs1883025</td>
<td>T 0.71 (0.56–0.91)</td>
<td>0.64 (0.5–0.82)</td>
<td>0.64 (0.45–0.91)</td>
<td>0.55 (0.4–0.76)</td>
<td>0.61 (0.46–0.81)</td>
</tr>
<tr>
<td></td>
<td>ABCA1 P = 6.9 × 10^{-5}</td>
<td>P = 3.9 × 10^{-4}</td>
<td>P = 0.01</td>
<td>P = 3.0 × 10^{-4}</td>
<td>P = 7.8 × 10^{-4}</td>
</tr>
<tr>
<td>rs3764261</td>
<td>A 1.1 (0.86–1.4)</td>
<td>1.12 (0.87–1.44)</td>
<td>1.08 (0.76–1.52)</td>
<td>1.29 (0.96–1.74)</td>
<td>1.21 (0.92–1.59)</td>
</tr>
<tr>
<td></td>
<td>CETP P = 0.46</td>
<td>P = 0.37</td>
<td>P = 0.68</td>
<td>P = 0.09</td>
<td>P = 0.17</td>
</tr>
<tr>
<td></td>
<td>APOE EA† 0.89 (0.62–1.28)</td>
<td>0.85 (0.58–1.24)</td>
<td>0.67 (0.39–1.16)</td>
<td>0.61 (0.38–0.97)</td>
<td>0.68 (0.44–1.04)</td>
</tr>
<tr>
<td></td>
<td>CFI P = 0.52</td>
<td>P = 0.39</td>
<td>P = 0.15</td>
<td>P = 0.04</td>
<td>P = 0.07</td>
</tr>
<tr>
<td>rs1061170</td>
<td>C 0.98 (0.78–1.23)</td>
<td>1.37 (1.09–1.73)</td>
<td>2.32 (1.67–3.21)</td>
<td>3.38 (2.52–4.54)</td>
<td>2.77 (2.14–3.59)</td>
</tr>
<tr>
<td></td>
<td>CFH P = 0.86</td>
<td>P = 7.1 × 10^{-5}</td>
<td>P = 4.9 × 10^{-7}</td>
<td>P = 6.3 × 10^{-16}</td>
<td>P = 1.7 × 10^{-14}</td>
</tr>
<tr>
<td>rs10490924</td>
<td>T 1.2 (0.9–1.61)</td>
<td>1.72 (1.29–2.29)</td>
<td>2.98 (2.05–4.35)</td>
<td>3.82 (2.76–5.31)</td>
<td>3.35 (2.48–4.55)</td>
</tr>
<tr>
<td></td>
<td>ARMS2/HTRA1 P = 0.22</td>
<td>P = 2.1 × 10^{-4}</td>
<td>P = 1.3 × 10^{-8}</td>
<td>P = 1.0 × 10^{-15}</td>
<td>P = 4.6 × 10^{-14}</td>
</tr>
<tr>
<td>rs93532759</td>
<td>C 0.68 (0.41–1.15)</td>
<td>0.58 (0.34–0.99)</td>
<td>0.36 (0.15–0.88)</td>
<td>0.23 (0.11–0.48)</td>
<td>0.27 (0.14–0.54)</td>
</tr>
<tr>
<td></td>
<td>C2 P = 0.15</td>
<td>P = 0.05</td>
<td>P = 0.02</td>
<td>P = 9.2 × 10^{-5}</td>
<td>P = 1.6 × 10^{-4}</td>
</tr>
<tr>
<td>rs2230199</td>
<td>G 1.1 (0.82–1.47)</td>
<td>1.4 (1.04–1.87)</td>
<td>1.71 (1.17–2.51)</td>
<td>1.97 (1.39–2.8)</td>
<td>1.72 (1.26–2.36)</td>
</tr>
<tr>
<td></td>
<td>C3 P = 0.52</td>
<td>P = 0.03</td>
<td>P = 5.5 × 10^{-3}</td>
<td>P = 1.5 × 10^{-4}</td>
<td>P = 7.6 × 10^{-4}</td>
</tr>
<tr>
<td>rs641153</td>
<td>T 0.91 (0.65–1.37)</td>
<td>0.69 (0.45–1.05)</td>
<td>0.7 (0.37–1.35)</td>
<td>0.41 (0.23–0.74)</td>
<td>0.53 (0.31–0.88)</td>
</tr>
<tr>
<td></td>
<td>CFH P = 0.64</td>
<td>P = 0.08</td>
<td>P = 0.28</td>
<td>P = 2.8 × 10^{-3}</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>rs10033900</td>
<td>T 1.11 (0.89–1.38)</td>
<td>1.16 (0.92–1.47)</td>
<td>1.35 (0.99–1.85)</td>
<td>1.3 (0.98–1.72)</td>
<td>1.28 (0.99–1.65)</td>
</tr>
<tr>
<td></td>
<td>CHI P = 0.57</td>
<td>P = 0.20</td>
<td>P = 0.06</td>
<td>P = 0.07</td>
<td>P = 0.06</td>
</tr>
</tbody>
</table>

*Significant results (P < 0.05) are shown in bold.
† 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 LPC and based on multivariate logistic models controlling for all genetic, demographic, and behavioral risk factors. The TT genotype of LPC (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 × 10⁻³). The CT genotype of LPC (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 LPC 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 × 10⁻³, OR [95% CI] = 0.41 [0.23–0.74]; CT: P = 5.8 × 10⁻⁵, OR [95% CI] = 0.62 [0.44–0.87]), and advanced AMD (TT: P = 3.5 × 10⁻³, OR [95% CI] = 0.35 [0.17–0.71]; CT: P = 6.8 × 10⁻⁵, 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 LPC 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.orglookup/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 LPC 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.⁵⁹,⁶⁰,⁶¹ 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, smoking, education, and antioxidant treatment and other genotypes related to AMD.

<table>
<thead>
<tr>
<th>Table 4. Associations between Drusen and Advanced AMD and LPC and ABCA1 Genotypes in Multivariate Models</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LIPC and ABCA1 Genes, Drusen, and Advanced AMD</strong></td>
</tr>
<tr>
<td><strong>Intermediate Drusen</strong></td>
</tr>
<tr>
<td>rs10468017</td>
</tr>
<tr>
<td>CC</td>
</tr>
<tr>
<td>CT</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>Y402H (rs1883025)</td>
</tr>
<tr>
<td>CC</td>
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<tr>
<td>CT</td>
</tr>
<tr>
<td>TT</td>
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<tr>
<td><strong>Cases/controls, n (%)</strong></td>
</tr>
<tr>
<td>LPC (rs10468017)</td>
</tr>
<tr>
<td>ABCA1 (rs1883025)</td>
</tr>
</tbody>
</table>

*Case/controls are shown in bold.

In our recent study, higher

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**Note:** The image contains a table with details on the relationship between LPC and ABCA1 genotypes and drusen or advanced AMD. The table includes odds ratios and confidence intervals for different genotypes and drusen stages. The study found that the TT homozygous genotype of LPC (rs10468017) was 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 × 10⁻³, OR [95% CI] = 0.41 [0.23–0.74]; CT: P = 5.8 × 10⁻⁵, OR [95% CI] = 0.62 [0.44–0.87]), and advanced AMD (TT: P = 3.5 × 10⁻³, OR [95% CI] = 0.35 [0.17–0.71]; CT: P = 6.8 × 10⁻⁵, 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 LPC 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.
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, suggesting that another mechanism is involved.

LIPC and ABCA1 have been shown to be expressed in the retina and RPE. LiPc and ABCA1 have also been synthesized and expressed in macrophages, which are the predominant cells involved in creating the progressive plaque lesions of atherosclerosis. ABCA1 is believed to suppress atherosclerotic lesions as an anti-inflammatory receptor that exports cholesterol from arterial macrophages. Drusen may develop by a similar mechanism as atherosclerotic plaque, except that lipoproteins accumulated in Bruch’s membrane are likely to be intracellular. Macrophages have also been found in Bruch’s membrane and are related to early and advanced AMD. 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. CFH has also been related to small, hard macular drusen in a sample with typically young average age (30–66 years). 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, CFH and ARMS2/HTRA1 may also have contributed to early-stage drusen accumulation when the patients were younger.

In our previous GWAS, 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 ($P = 1.41 \times 10^{-5}$). 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

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**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.

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References


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