Human Genome Epidemiology (HuGE) Review

Association between Apolipoprotein E Polymorphisms and Age-related Macular Degeneration: A HuGE Review and Meta-Analysis

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A possible association between apolipoprotein E polymorphisms and age-related macular degeneration has been investigated numerous times, with conflicting results. A previous analysis pooling results from four studies (Schmidt et al., Ophthalmic Genet 2002;23:209–23) suggested an association, but those investigators did not document allele frequencies, the magnitude of the association, or the possible genetic mode of action. Thus, the authors searched MEDLINE from 1966 to December 2005 for any English-language studies reporting genetic associations. Data and study quality were assessed in duplicate. Pooling was performed while checking for heterogeneity and publication bias. Frequencies of the $E_2$ and $E_4$ alleles in Caucasians were approximately 8% and 15%, respectively. Allele- and genotype-based tests of association indicated a risk effect of up to 20% for $E_2$ and a protective effect of up to 40% for $E_4$. $E_2$ appeared to act in a recessive mode and $E_4$ in a dominant mode.

There appears to be a differential effect of the $E_2$ and $E_4$ alleles on the risk of age-related macular degeneration, although the possibility of survivor bias needs to be ruled out more definitively.

ApoE; apolipoproteins E; epidemiology; genetics; macular degeneration; meta-analysis; polymorphism, genetic

Abbreviations: AMD, age-related macular degeneration; ApoE, apolipoprotein E; CI, confidence interval; OR, odds ratio.

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Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world (1–4), accounting for half of all new cases of registered blindness (5). With an aging population, the burden of AMD is set to grow, with almost 30 percent of persons aged 75 years or older showing early signs of disease (6–8). The pathologic hallmark of the disease is drusen, deposits of protein and lipid, in the retinal pigment epithelium or Bruch’s membrane. This maculopathy progresses to degeneration in two forms: 1) geographic atrophy, in which there is loss of retinal pigment epithelium and photoreceptors, and 2) neovascular AMD, in which there is choroidal neovascularization and hemorrhages.

Little is known about the pathogenesis of AMD. Smoking is the only established risk factor, although other cardiovascular disease risk factors (e.g., high cholesterol, hypertension) may also play a role (9). There also appears to be a genetic component, as supported by a number of lines of evidence: familial aggregation (10–13), segregation analysis (14, 15), twin studies (10, 16, 17), and several linkage studies (18–24) culminating in a meta-analysis (25). Although several monogenic forms of macular dystrophy have been described and their genes identified (for reviews, see Yates et al. (15) and Gorin et al. (26)), these have not shed light on sporadic AMD.

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Apolipoprotein E (ApoE) is a lipid transport protein that acts as a ligand for the low density lipoprotein receptor, but it is also involved in the repair and maintenance of neuronal cell membranes in the central and peripheral nervous system. The ApoE gene is located at chromosome 19q13.2, and three major forms, originally identified by isoelectric focusing, have been described. These isoforms are defined by amino acid changes at positions 112 and 158: Alleles E2, E3, and E4 are defined respectively by cysteine/cysteine, cysteine/arginine, and arginine/arginine at these two sites (27, 28). ApoE is involved in clearance of chylomicrons and very low density lipoproteins from the circulation via specific receptors on liver and peripheral cells. The E2 form of ApoE has decreased affinity for the receptor, whereas the E3 and E4 forms have higher affinity. Mutations in ApoE lead to type III hyperlipoproteinemia, in which there is an increase in triglycerides and cholesterol and premature cardiovascular disease (29). ApoE has also been linked to Alzheimer’s disease. Since 1993, when Saunders et al. (30) reported an ApoE polymorphism and AMD to ascertain whether there is a genetic effect on AMD susceptibility and, if so, to estimate the magnitude of that effect and the possible genetic mode of action (37, 38).

MATERIALS AND METHODS

Search strategy

We searched MEDLINE (US National Library of Medicine) for all relevant articles published from January 1966 through November 23, 2005, using the PubMed search engine. The search strategy was “macular degeneration” and “apolipoprotein E*” or “apoE” or “APOE” or “Apo E.” Results were limited to English-language papers.

Inclusion criteria

Any human population-based association study, regardless of sample size, was included if it met the following criteria (we use the term “population-based” to refer to individual sporadic cases rather than familial cases or family-based study designs (e.g., sibling pairs)):

- The investigators determined the association between the ApoE polymorphism and AMD. The alleles and genotypes for this polymorphism were, respectively: E2, E3, and E4; and E2E2, E2E3, E2E4, E3E3, E3E4, and E4E4.
- The outcome was AMD and there were at least two comparison groups (e.g., AMD vs. control (non-AMD) groups). For those studies in which AMD was graded (i.e., drusen, pigment abnormalities in retinal pigment epithelium, geographic atrophy, and choroidal neovascularization), these gradings were collapsed into only one AMD group.

- There were sufficient results for extraction of data (i.e., the number of subjects with each genotype in the AMD and control groups).

We also reviewed the reference lists of the retrieved articles to identify publications on the same topic. Where there were multiple publications from the same study group, the most complete and recent results were used.

Data extraction

Data were extracted independently and in duplicate by two reviewers (T. A. and B. S.) using a standardized data extraction form. Data on covariables such as mean age, gender, and ethnicity were also extracted for each study. Any disagreement was adjudicated by a third author (A. J.).

Quality score assessment

The quality of the studies was independently assessed by two reviewers (B. S. and M. M.) using a quality assessment score developed for genetic association studies (39). This score was based on both traditional epidemiologic considerations and genetic issues (40). Total scores ranged from 0 (worst) to 12 (best). Any disagreement was adjudicated by a third author (A. J.).

Statistical analysis

Hardy–Weinberg equilibrium was assessed for each study using the chi-squared test (41–43). The summary prevalence of all alleles was estimated and characterized using only the data on controls (39). Both per-allele analysis and per-genotype analysis were performed.

Per-allele analysis. The association between ApoE polymorphisms and AMD was first determined using the per-allele approach. Allele frequencies were calculated for studies reporting only genotype data.

The Q test for heterogeneity was performed separately for two odds ratios (ORs), that is, E2 versus E3 (OR1) and E4 versus E3 (OR2). Logistic regression analysis was used to determine the overall gene effect. Bivariate meta-analysis with Bayesian methods. The

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log odds ratios were modeled accounting for both between- 
and within-study variation. Two separate lambda values 
(reflecting the genetic model), which were the ratios of log 
OR4 to log OR1 for λ1 and log OR2 to log OR1 for λ2, 
were estimated. These parameters capture information about 
the genetic mode of action, as follows: The model is a recessive 
model if λ = 0, a dominant model if λ = 1, a codominant 
model if λ = 0.5, and a homozygous or heterosis model if λ > 1 or λ < 0.

For per-allele and per-genotype analyses, we took two 
approaches for handling Hardy-Weinberg disequilibrium. 
Firstly, we performed sensitivity analyses by including 
and excluding studies not in Hardy-Weinberg equilibrium. 
Secondly, we included all studies regardless of Hardy-Weinberg 
equilibrium and instead adjusted for the degree of disequilibrium 
using the inbreeding coefficient (F) as described 
by Trikalinos et al. (48). Briefly, for per-allele analyses, 
the variance of the odds ratio was adjusted by 1 + F (49).

Case and control groups were combined for estimation of F 
using the method described by Ayres and Balding (50). For 
genotype analysis, predicted genotype frequencies were 
estimated in control groups (50), and we used the predicted 
frequencies instead of the observed frequencies in the 
summary analysis.

We also performed subgroup analysis in Caucasians. 
Publication bias was assessed using Egger’s test (51, 52). In 
addition, we conducted a cumulative meta-analysis to assess 
whether the gene effect changed over time (52). All analyses 
were performed using Stata, version 9.0 (53), except for the 
per-genotype analysis, which was performed using 
WinBUGS 1.4.1 (54). For Bayesian modeling, a vague prior 
distribution, representing the lack of prior information about 
parameter values (i.e., log odds ratios and λ), was specified 
using normal-distribution priors for both log odds ratios 
and λ. A “burn-in” of 10,000 iterations was carried out 
for the models, followed by 50,000 iterations for parameter 
estimates. A p value less than 0.05 was considered statistically 
significant, except for tests of heterogeneity, where a 
level of 0.10 was used.

RESULTS

Studies identified

Twenty-eight studies were identified by our search strate-
gies. Eighteen of these studies were not eligible (five were 
not association studies (55–59), five were reviews (3, 8, 15, 
26, 60), three were family-based studies (20, 61, 62), two 
were animal studies (63, 64), one reported only methods 
(65), one enrolled diabetic subjects with AMD (66), and 
one was a duplicate (67)), leaving 10 studies (4, 8, 31–36, 
68, 69) for inclusion in this analysis. The 10 studies are 
described in table 1. Among them, eight studies were carried 
out in Caucasians and two in Asians. The mean age ranged 
from 70.9 years to 81.0 years for cases and from 37.0 years to 
76.6 years for controls. The percentage of males ranged from 
31.6 percent to 63.3 percent.

All studies had case-control designs in which cases and 
controls were selected from hospitals, except for one study 
in which cases and controls had been randomly selected 
from the community (4); in only one of these studies were 
controls age- and gender-matched. In the two studies by 
Schmidt et al. (8, 68), only sporadic cases were used for 
one (68), and only two study groups (from the University 
of California, Los Angeles, and Erasmus University) were 
used for the other (8). The quality of studies ranged from 3 
to 11, out of a possible score of 12 (see appendix tables 1 and 
2). In all studies, investigators used DNA genotyping 
rather than protein isoforms to determine ApoE status.

Summary prevalences of the E2 allele were similar for 
Caucasians and Asians (8.2 percent (95 percent confidence 
interval (CI): 7.3, 9.0) vs. 9.1 percent (95 percent CI: 
6.3, 11.7)) but were more divergent for E4 (14.9 percent 
(95 percent CI: 13.8, 16.0) vs. 8.1 percent (95 percent CI: 
5.5, 10.6)).

ApoE and AMD

Allele-based methods. Among the 10 studies included, 
one did not observe Hardy-Weinberg equilibrium (33) (see 
table 2), leaving nine studies (seven of Caucasians and two 
of Asians) for assessing the association between the ApoE 
gene and AMD. OR1 (E2 vs. E3) and OR2 (E4 vs. E3) 
were estimated for each study (table 2). Neither OR1 (E2 vs. E3) 
nor OR2 (E4 vs. E3) showed any evidence of heterogeneity 
(OR1: χ² = 4.50, df = 8, p = 0.81; OR2: χ² = 9.16, df = 8, 
p = 0.33). Logistic regression indicated that the overall gene 
effect was significant (likelihood ratio test: 26.39, df = 2, 
p < 0.01). The summary OR1 and OR2, obtained using bi-
variate meta-analysis, were 1.17 (95 percent CI: 1.01, 1.35) 
and 0.67 (95 percent CI: 0.57, 0.78), respectively. This 
means that patients who had an E2 allele were approximately 
17 percent more likely to have AMD than patients with the 
E3 allele. Conversely, persons with an E4 allele were approx-
imately 33 percent less likely to have AMD than persons 
with allele E3.

Using Egger’s test, there was no evidence of publication 
bias or a study-size effect for OR1 and OR2 (p = 0.56 and 
p = 0.68, respectively). Sensitivity analysis including the 
one study not in Hardy-Weinberg equilibrium produced 
similar results; OR1 and OR2 were 1.20 (95 percent CI: 
1.01, 1.43) and 0.61 (95 percent CI: 0.49, 0.77), respect-
ively. Taking into account the degree of Hardy-Weinberg 
disequilibrium by adjusting the variance of the odds ratios 
with the inbreeding coefficient F produced similar results; 
the summary OR1 and OR2 were 1.20 (95 percent CI: 
1.01, 1.42) and 0.61 (95 percent CI: 0.48, 0.77), respectively. 
Performing the analysis only among Caucasians in whom 
genotypes were in Hardy-Weinberg equilibrium yielded 
similar results for the E4 allele (OR2 = 0.65, 95 percent 
CI: 0.54, 0.79) but a slightly greater point estimate for the 
E2 allele (OR1 = 1.31, 95 percent CI: 1.08, 1.58).

Genotype-based methods. Table 3 shows the frequen-
cies of the ApoE genotype in case and control groups. We 
estimated the genotype effects for E2E2, E2E3, and E2E4 
in each study by assigning the E2E3 genotype as the reference group (table 4). Cells with a zero count had 0.5 added. Two Asian 
studies (34, 36) did not have E2E2 genotypes in either case 
groups or control groups, and thus results for these studies

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could not be summarized. The summary odds ratios for the \( E_2 E_2 \) and \( E_2 E_3 \) genotypes were 1.05 (95 percent CI: 0.52, 2.20) and 1.22 (95 percent CI: 0.96, 1.61), respectively. These point estimates can be interpreted as meaning that persons with the \( E_2 E_2 \) and \( E_2 E_3 \) genotypes had 5 percent and 22 percent higher risks of developing AMD than persons with the \( E_3 E_3 \) genotype, although these effects did not reach statistical significance. The estimated \( \lambda \) was 0.27 (95 percent CI: ~3.98, 4.93), which suggests a largely recessive mode of action, although the confidence interval was wide.

There was no evidence of publication bias due to the size of the study (Egger’s test: for OR3 and OR4, \( p = 0.78 \) and \( p = 0.85 \), respectively). Cumulative meta-analysis was performed for OR3 and OR4. It showed that the summary OR3 was a bit different in the first two studies (4, 35) and was not much changed, whereas the cumulative OR4 did not change much over time (figure 1). Sensitivity analysis conducted by

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**TABLE 1. General characteristics of studies included in a meta-analysis of apolipoprotein E polymorphisms and age-related macular degeneration**

<table>
<thead>
<tr>
<th>First author and reference no.</th>
<th>Year of publication</th>
<th>Study design</th>
<th>Ethnicity</th>
<th>Mean age (years)</th>
<th>Cases</th>
<th>Controls</th>
<th>Quality score*</th>
<th>Cases Design</th>
<th>Controls Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klaver (4)</td>
<td>1998</td>
<td>Case-control</td>
<td>Caucasian</td>
<td>81.0</td>
<td>69.0</td>
<td>38.5</td>
<td>11</td>
<td>Advanced AMD, combined</td>
<td>Controls without AMD, without ophthalmologic examination</td>
</tr>
<tr>
<td>Souied (35)</td>
<td>1998</td>
<td>Age- and sex-matched case-control</td>
<td>Caucasian</td>
<td>73.8</td>
<td>74.9</td>
<td>35.2</td>
<td>8</td>
<td>AMD with drusen upon ophthalmologic examination, combined</td>
<td>Controls without AMD, without ophthalmologic examination</td>
</tr>
<tr>
<td>Pang (36)</td>
<td>2000</td>
<td>Case-control</td>
<td>Asian</td>
<td>71.8</td>
<td>69.7</td>
<td>3</td>
<td>3</td>
<td>AMD with drusen or changes in retinal pigment epithelium by fundus examination, combined</td>
<td>Controls without eye disease (except cataract), confirmed upon ophthalmologic examination</td>
</tr>
<tr>
<td>Schmidt (68)</td>
<td>2000</td>
<td>Case-control</td>
<td>Caucasian</td>
<td>75.5</td>
<td>68.1</td>
<td>42.7</td>
<td>9</td>
<td>AMD with extensive or intermediate drusen (&gt;63 ( \mu )m, grade 3) with/without retinal pigment epithelium detachment, geographic atrophy (grade 4), or exudative lesion (grade 5), combined</td>
<td>Controls with drusen &lt;63 ( \mu )m (grade 1) or nonextensive intermediate drusen (&gt;63 ( \mu )m), confirmed with ophthalmologic examination</td>
</tr>
<tr>
<td>Simonelli (33)</td>
<td>2001</td>
<td>Case-control</td>
<td>Caucasian</td>
<td>71.8</td>
<td>37.0</td>
<td>58.6</td>
<td>5</td>
<td>AMD with geographic atrophy, choroidal neovascularization, detachment of retinal pigment epithelium, subretinal hemorrhage, or retinal scarring, combined</td>
<td>Controls without AMD, without ophthalmologic examination</td>
</tr>
<tr>
<td>Schmidt (8)</td>
<td>2002</td>
<td>Case-control</td>
<td>Caucasian</td>
<td>73.9</td>
<td>75.3</td>
<td>31.6</td>
<td>10</td>
<td>Same as in Schmidt (68)</td>
<td>Same as in Schmidt (68)</td>
</tr>
<tr>
<td>Schultz (31)</td>
<td>2003</td>
<td>Case-control</td>
<td>Caucasian</td>
<td>78.2</td>
<td>72.5</td>
<td>10</td>
<td>AMD, combined</td>
<td>Controls without AMD, based on fundus photographs</td>
<td></td>
</tr>
<tr>
<td>Baird (32)</td>
<td>2004</td>
<td>Case-control</td>
<td>Caucasian</td>
<td>77.3</td>
<td>76.6</td>
<td>32.9</td>
<td>10</td>
<td>Advanced AMD, combined</td>
<td>Controls with normal fundus or drusen &lt;63 ( \mu )m upon ophthalmologic examination</td>
</tr>
<tr>
<td>Gotoh (34)</td>
<td>2004</td>
<td>Case-control</td>
<td>Asian</td>
<td>70.9</td>
<td>69.4</td>
<td>63.3</td>
<td>7</td>
<td>Advanced AMD, combined</td>
<td>Controls without AMD, confirmed by ophthalmologic examination</td>
</tr>
<tr>
<td>Zareparsi (69)</td>
<td>2004</td>
<td>Case-control</td>
<td>Caucasian</td>
<td>79.2</td>
<td>74.6</td>
<td>37.2</td>
<td>9</td>
<td>Advanced AMD, combined</td>
<td>Controls without AMD, confirmed by ophthalmologic examination</td>
</tr>
</tbody>
</table>

* Total scores ranged from 0 (worst) to 12 (best) (see Materials and Methods). 
† AMD, age-related macular degeneration.
including one study not in Hardy-Weinberg equilibrium showed similar results: OR3, OR4, and \( \lambda \) were 1.06 (95 percent CI: 0.51, 2.05), 1.31 (95 percent CI: 1.01, 1.69), and 0.42 (95 percent CI: –4.21, 5.43), respectively.

Results of analysis taking Hardy-Weinberg disequilibrium into account were slightly different; OR3 and OR4 were 1.18 (95 percent CI: 0.61, 2.71) and 1.32 (95 percent CI: 1.02, 1.70), with the latter reaching statistical significance. \( \lambda \) increased to 0.65 (95 percent CI: –3.82, 5.32), suggesting more clearly a codominant mode of action.

We also estimated the genotype effects for \( E_4E_4 \) and \( E_3E_4 \) as compared with \( E_3E_3 \) (table 5). Again, we could not summarize data for the two Asian studies (34, 36), since there was no one with the \( E_4E_4 \) genotype in either case groups or control groups in those studies. The pooled OR5 (\( E_4E_4 \) vs. \( E_3E_3 \)) and OR6 (\( E_3E_4 \) vs. \( E_3E_3 \)) were 0.85 (95 percent CI: 0.44, 1.75) and 0.62 (95 percent CI: 0.46, 0.90), respectively; that is, persons with the \( E_4E_4 \) and \( E_3E_4 \) genotypes were approximately 15 percent and 38 percent less likely to have AMD than persons with the \( E_3E_3 \) genotype. The estimated \( \lambda \) was 1.17 (95 percent CI: –4.51, 5.71), which suggests a dominant mode of action.

There was no publication bias due to study size (for OR5 and OR6, \( p = 0.10 \) and \( p = 0.92 \), respectively). The cumulative meta-analysis for OR5 and OR6 (figure 2) showed that the summary OR4 did not change over time, whereas the

### TABLE 2. Allele frequencies in studies of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls

<table>
<thead>
<tr>
<th>First author and reference no.</th>
<th>Cases</th>
<th>Controls</th>
<th>( E_2/E_3 )</th>
<th>( E_2/E_4 )</th>
<th>( E_3/E_3 )</th>
<th>( E_3/E_4 )</th>
<th>( E_4/E_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klaver (4)</td>
<td>176</td>
<td>22</td>
<td>142</td>
<td>12</td>
<td>1,802</td>
<td>163</td>
<td>3,357</td>
</tr>
<tr>
<td>Souied (35)</td>
<td>232</td>
<td>23</td>
<td>192</td>
<td>17</td>
<td>336</td>
<td>21</td>
<td>266</td>
</tr>
<tr>
<td>Schmidt (68)</td>
<td>202</td>
<td>21</td>
<td>150</td>
<td>31</td>
<td>744</td>
<td>60</td>
<td>575</td>
</tr>
<tr>
<td>Simonelli (33)†</td>
<td>174</td>
<td>17</td>
<td>152</td>
<td>5</td>
<td>2568</td>
<td>153</td>
<td>2,149</td>
</tr>
<tr>
<td>Schmidt (8)</td>
<td>196</td>
<td>24</td>
<td>156</td>
<td>16</td>
<td>146</td>
<td>12</td>
<td>118</td>
</tr>
<tr>
<td>Schultz (31)</td>
<td>208</td>
<td>19</td>
<td>170</td>
<td>19</td>
<td>226</td>
<td>18</td>
<td>180</td>
</tr>
<tr>
<td>Baird (32)</td>
<td>398</td>
<td>39</td>
<td>310</td>
<td>49</td>
<td>246</td>
<td>17</td>
<td>185</td>
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<tr>
<td>Zareparsi (69)</td>
<td>1,258</td>
<td>116</td>
<td>1,022</td>
<td>120</td>
<td>410</td>
<td>33</td>
<td>320</td>
</tr>
<tr>
<td>Gotoh (34)</td>
<td>170</td>
<td>9</td>
<td>149</td>
<td>12</td>
<td>164</td>
<td>14</td>
<td>135</td>
</tr>
<tr>
<td>Pang (36)</td>
<td>274</td>
<td>27</td>
<td>231</td>
<td>16</td>
<td>266</td>
<td>25</td>
<td>221</td>
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</tbody>
</table>

* HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.
† Not included in calculation of summary OR.

### TABLE 3. Genotype frequencies in studies of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls

<table>
<thead>
<tr>
<th>First author and reference no.</th>
<th>Cases</th>
<th>Controls</th>
<th>( E_2E_2 )</th>
<th>( E_2E_3 )</th>
<th>( E_2E_4 )</th>
<th>( E_3E_3 )</th>
<th>( E_3E_4 )</th>
<th>( E_4E_4 )</th>
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<tr>
<td>Klaver (4)</td>
<td>80</td>
<td>0</td>
<td>20</td>
<td>5</td>
<td>56</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Souied (35)</td>
<td>116</td>
<td>0</td>
<td>20</td>
<td>3</td>
<td>82</td>
<td>8</td>
<td>3</td>
<td>8</td>
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<tr>
<td>Schmidt (68)</td>
<td>101</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>58</td>
<td>21</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Simonelli (33)†</td>
<td>87</td>
<td>0</td>
<td>16</td>
<td>1</td>
<td>66</td>
<td>4</td>
<td>0</td>
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<td>98</td>
<td>1</td>
<td>21</td>
<td>1</td>
<td>60</td>
<td>15</td>
<td>0</td>
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<td>104</td>
<td>2</td>
<td>15</td>
<td>0</td>
<td>69</td>
<td>17</td>
<td>1</td>
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<tr>
<td>Baird (32)</td>
<td>199</td>
<td>2</td>
<td>28</td>
<td>7</td>
<td>122</td>
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<td>1</td>
<td>104</td>
<td>10</td>
<td>406</td>
<td>106</td>
<td>2</td>
<td>2</td>
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<tr>
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<td>0</td>
<td>8</td>
<td>1</td>
<td>65</td>
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<td>0</td>
</tr>
<tr>
<td>Pang (36)</td>
<td>137</td>
<td>0</td>
<td>24</td>
<td>3</td>
<td>97</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\( E_2 \), \( E_3 \), and \( E_4 \), respectively.

Am J Epidemiol 2006;164:813–822
summary OR derived from the first two studies (4, 35) was a bit different from that derived after inclusion of the third study. Including the one study not in Hardy-Weinberg equilibrium yielded an OR of 0.83 (95 percent CI: 0.47, 1.71), 0.59 (95 percent CI: 0.43, 0.83), and 1.43 (95 percent CI: –4.53, 5.98), respectively. Adjusting for Hardy-Weinberg disequilibrium yielded similar results; OR, OR6, and k were 0.81 (95 percent CI: 0.45, 1.64), 0.60 (95 percent CI: 0.45, 0.82), and 1.35 (95 percent CI: –4.39, 5.81), respectively.

**DISCUSSION**

Our study found that the prevalences of ApoE alleles E2 and E4 were largely similar between Caucasians and Asians. Assuming a per-allele model, which allowed us to conduct one overall test of association and avoid multiple comparisons, we found that ApoE gene polymorphisms were indeed associated with AMD, with the E4 allele appearing to be protective and E2 appearing to be a risk allele. Over half of the studies included (5/9) were of good quality, with a quality score of 10 or above out of 12. Exploring this association in more detail allowed us to estimate the magnitude of the association and the possible genetic mode of action. Results for the E2 allele did not reach statistical significance, but point estimates appeared to indicate up to a 20 percent increase in risk of AMD and suggested a recessive model. Results for the E4 allele did reach statistical significance and indicated that E4 might act dominantly, with the presence of at least one E4 allele providing up to a 38 percent reduction in the risk of AMD. These results are strengthened by the facts that there was no evidence of heterogeneity and that including the one study not in Hardy-Weinberg equilibrium gave us similar results. Egger’s test evaluates whether small studies produce different results than larger studies. If so, publication bias is a possibility. In this meta-analysis, the result of Egger’s test was not significant, but with only 10 studies we had limited power to detect such an effect. The indications of a risk

<table>
<thead>
<tr>
<th>First author and reference no.</th>
<th>Cases</th>
<th>Controls</th>
<th>OR3 (E2E2 vs. E3E3)</th>
<th>OR4 (E2E3 vs. E3E3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klaver (4)</td>
<td>0</td>
<td>20</td>
<td>56</td>
<td>9</td>
</tr>
<tr>
<td>Souied (35)</td>
<td>0</td>
<td>20</td>
<td>82</td>
<td>1</td>
</tr>
<tr>
<td>Schmidt (68)</td>
<td>2</td>
<td>13</td>
<td>58</td>
<td>4</td>
</tr>
<tr>
<td>Schmidt (8)</td>
<td>1</td>
<td>21</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Simonelli (33)†</td>
<td>0</td>
<td>16</td>
<td>66</td>
<td>12</td>
</tr>
<tr>
<td>Schmidt (8)</td>
<td>1</td>
<td>21</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Schultz (31)</td>
<td>2</td>
<td>15</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Baird (32)</td>
<td>2</td>
<td>28</td>
<td>122</td>
<td>2</td>
</tr>
<tr>
<td>Zareparsi (69)</td>
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<td>104</td>
<td>406</td>
<td>0</td>
</tr>
<tr>
<td>Summary OR</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* CI, confidence interval.
† Not included in calculation of summary OR.

**TABLE 4. Estimation of the summary odds ratios (ORs) OR3 (E2E2 vs. E3E3) and OR4 (E2E3 vs. E3E3) in an analysis of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls**

**FIGURE 1.** Odds ratios (ORs) OR3 (E2E2 vs. E3E3) and OR4 (E2E3 vs. E3E3) in a cumulative meta-analysis of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls. Horizontal bars, 95% confidence interval.

*Am J Epidemiol* 2006;164:813–822
effect of $E_2$ and a protective effect of $E_4$ are consistent with results from an earlier, smaller pooling study (8). In addition, the two main types of AMD may have different etiologies (9) and hence may have different genetic susceptibilities. However, there was insufficient information provided in the papers to meta-analyze these two types of AMD separately. Combining both as a single AMD outcome would introduce measurement error in the outcome factor and could lead to a bias towards the null. This bias makes our significant results more robust.

Nevertheless, there are two major concerns. Firstly, this $E_4$ effect is the opposite of that found for cardiovascular disease; that is, $E_4$ is associated with increased mortality and decreased longevity. Hence, one might expect that any survivor bias would “deplete” the $E_4$ allele among persons old enough to develop AMD, and this decreased odds ratio might therefore be spurious. This remains a possibility because, although most studies had similar age distributions in cases and controls, cases had older mean ages than controls. In defense of our results is the finding that all studies but one were in Hardy-Weinberg equilibrium, and the one not in Hardy-Weinberg equilibrium was excluded from the summary analysis. However, it is puzzling that the risk allele for cardiovascular disease should have a beneficial effect for AMD if the mechanism is still related to cholesterol metabolism. This could indicate a type I (i.e., false-positive) error, although there are many examples of pleiotropy in biology (i.e., multiple functions for the same protein or gene), and it is difficult to predict the direction of a genetic effect given the biology. Secondly, the studies included in this meta-analysis were all small or medium-sized case-control studies. There is some evidence that smaller studies tend to overstate genetic effects in comparison with larger studies (70).

Despite these potential problems, our results are statistically robust and point to some interesting directions for future research. In particular, this review indicates the need for confirmation of these results in a large-scale, long-term longitudinal study in which survivor bias might be detected.

### TABLE 5. Estimation of the summary odds ratios (ORs) OR$_5$ ($E_4E_4$ vs. $E_3E_3$) and OR$_6$ ($E_3E_4$ vs. $E_3E_3$) in an analysis of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls

<table>
<thead>
<tr>
<th>First author and reference no.</th>
<th>Cases</th>
<th>Controls</th>
<th>$E_4E_4$ vs. $E_3E_3$</th>
<th>$E_3E_4$ vs. $E_3E_3$</th>
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</thead>
<tbody>
<tr>
<td>Klaver (4)</td>
<td>0</td>
<td>10</td>
<td>56</td>
<td>20</td>
</tr>
<tr>
<td>Souied (35)</td>
<td>3</td>
<td>8</td>
<td>82</td>
<td>2</td>
</tr>
<tr>
<td>Schmidt (68)</td>
<td>3</td>
<td>21</td>
<td>58</td>
<td>10</td>
</tr>
<tr>
<td>Simonelli (33)†</td>
<td>0</td>
<td>4</td>
<td>66</td>
<td>9</td>
</tr>
<tr>
<td>Schmidt (8)</td>
<td>0</td>
<td>15</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>Schultz (31)</td>
<td>1</td>
<td>17</td>
<td>69</td>
<td>1</td>
</tr>
<tr>
<td>Baird (32)</td>
<td>2</td>
<td>38</td>
<td>122</td>
<td>2</td>
</tr>
<tr>
<td>Zareparsi (69)</td>
<td>2</td>
<td>106</td>
<td>406</td>
<td>0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>OR$_5$</th>
<th>95% CI*</th>
<th>OR$_6$</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klaver (4)</td>
<td>0.216</td>
<td>0.013, 3.621</td>
<td>0.409</td>
<td>0.208, 0.805</td>
</tr>
<tr>
<td>Souied (35)</td>
<td>1.773</td>
<td>0.341, 9.219</td>
<td>0.253</td>
<td>0.114, 0.559</td>
</tr>
<tr>
<td>Schmidt (68)</td>
<td>1.164</td>
<td>0.310, 4.366</td>
<td>1.005</td>
<td>0.574, 1.761</td>
</tr>
<tr>
<td>Simonelli (33)†</td>
<td>0.715</td>
<td>0.041, 12.421</td>
<td>0.263</td>
<td>0.100, 0.691</td>
</tr>
<tr>
<td>Schmidt (8)</td>
<td>0.262</td>
<td>0.010, 6.571</td>
<td>0.901</td>
<td>0.396, 2.052</td>
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<tr>
<td>Schultz (31)</td>
<td>1.029</td>
<td>0.104, 10.130</td>
<td>0.766</td>
<td>0.380, 1.545</td>
</tr>
<tr>
<td>Baird (32)</td>
<td>0.566</td>
<td>0.078, 4.105</td>
<td>0.581</td>
<td>0.338, 0.997</td>
</tr>
<tr>
<td>Zareparsi (69)</td>
<td>0.585</td>
<td>0.398, 0.861</td>
<td>0.624</td>
<td>0.459, 0.904</td>
</tr>
</tbody>
</table>

* CI, confidence interval.  
† Not included in calculation of summary OR.
ACKNOWLEDGMENTS

Conflict of interest: none declared.

REFERENCES


(Appendix tables follow)
APPENDIX TABLE 1. Criteria for methodological quality assessment of molecular association studies included in an analysis of apolipoprotein E polymorphisms and age-related macular degeneration

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Quality score*</th>
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<tbody>
<tr>
<td>Representativeness of cases</td>
<td></td>
</tr>
<tr>
<td>A. Consecutive/randomly selected from case population with clearly defined random frame</td>
<td>2</td>
</tr>
<tr>
<td>B. Consecutive/randomly selected from case population without clearly defined random frame or with extensive inclusion criteria</td>
<td>1</td>
</tr>
<tr>
<td>C. Method of selection not described</td>
<td>0</td>
</tr>
<tr>
<td>Representativeness of controls</td>
<td></td>
</tr>
<tr>
<td>D. Controls were consecutive/randomly drawn from the same area (ward/community) as cases with the same criteria</td>
<td>2</td>
</tr>
<tr>
<td>E. Controls were consecutive/randomly drawn from a different area than cases</td>
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</tr>
<tr>
<td>F. Not described</td>
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</tr>
<tr>
<td>Ascertainment of AMD† cases</td>
<td></td>
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<tr>
<td>G. Clearly described objective criteria for diagnosis of AMD</td>
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</tr>
<tr>
<td>H. Not described</td>
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</tr>
<tr>
<td>Ascertainment of controls</td>
<td></td>
</tr>
<tr>
<td>I. Ocular examinations were performed on controls by ophthalmologists to prove that controls did not have AMD</td>
<td>2</td>
</tr>
<tr>
<td>J. Article merely stated that controls were subjects who did not have AMD; no proof provided</td>
<td>1</td>
</tr>
<tr>
<td>K. Not described</td>
<td>0</td>
</tr>
<tr>
<td>Ascertainment of genotyping examination</td>
<td></td>
</tr>
<tr>
<td>L. Genotyping done under “blind” conditions</td>
<td>1</td>
</tr>
<tr>
<td>M. Unblinded or not mentioned</td>
<td>0</td>
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<tr>
<td>Test for Hardy-Weinberg equilibrium</td>
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<tr>
<td>N. Hardy-Weinberg equilibrium in control group</td>
<td>2</td>
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<td>O. Hardy-Weinberg disequilibrium in control group</td>
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<td>P. Hardy-Weinberg equilibrium not checked</td>
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<tr>
<td>Association assessment</td>
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</tr>
<tr>
<td>Q. Assessed association between genotypes and AMD with appropriate statistic and adjusting confounders</td>
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</tr>
<tr>
<td>R. Assessed association between genotypes and AMD with appropriate statistic without adjusting confounders</td>
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</tr>
<tr>
<td>S. Inappropriate statistic used</td>
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</tr>
</tbody>
</table>

* Total scores ranged from 0 (worst) to 12 (best) (see Materials and Methods).
† AMD, age-related macular degeneration.

APPENDIX TABLE 2. Details of quality assessment (quality scores*) for the studies included in an analysis of apolipoprotein E polymorphisms and age-related macular degeneration

<table>
<thead>
<tr>
<th>First author and reference no.</th>
<th>Representativeness of cases</th>
<th>Representativeness of controls</th>
<th>Ascertainment of age-related macular degeneration</th>
<th>Ascertainment of controls</th>
<th>Ascertainment of genotyping examination</th>
<th>Test for Hardy-Weinberg equilibrium</th>
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<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td>8</td>
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<tr>
<td>Pang (36)</td>
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<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
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<td>9</td>
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<tr>
<td>Simonelli (33)</td>
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<td>2</td>
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<td>2</td>
<td>0</td>
<td>2</td>
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<td>10</td>
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<tr>
<td>Baird (32)</td>
<td>1</td>
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</tbody>
</table>

* Total scores ranged from 0 (worst) to 12 (best) (see Materials and Methods).