American Journal of Epidemiology Advance Access published August 17, 2006

American Journal of Epidemiology

DOI: 10.1093/aje/kwj279

Human Genome Epidemiology (HuGE) Review

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

Ammarin Thakkinstian[1,2], Steve Bowe[2], Mark McEvoy[2], Wayne Smith[2], and John Attia[2]

[1] Clinical Epidemiology Unit, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.
[2] Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Newcastle, New South Wales, Australia.

Received for publication February 9, 2006; accepted for publication April 5, 2006.

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.

Editor's note: *This article is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/default.htm).*

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.

Correspondence to Dr. John Attia, Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Newcastle, New South Wales, 2300 Australia (e-mail: John.Attia@newcastle.edu.au).

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 E_2, E_3, and E_4 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 E_2 form of *ApoE* has decreased affinity for the receptor, whereas the E_3 and E_4 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 increased frequency of the E_4 allele in a small prospective series of Alzheimer's disease patients visiting a memory disorders clinic, multiple studies have confirmed an increased risk of Alzheimer's disease among persons with the E_4 allele.

The genetic association between *ApoE* and AMD has been investigated multiple times, although the results have been inconsistent (4, 8, 31–36). A previous study summarizing results from four groups (8) suggested a protective effect of the E_4 allele and a risk effect of the E_2 allele in comparison with the most common allele, E_3. We pooled the results of all available population-based studies of the association between *ApoE* 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: E_2, E_3, and E_4; and E_2E_2, E_2E_3, E_2E_4, E_3E_3, E_3E_4, and E_4E_4.
- 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 (T. A.).

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, E_2 versus E_3 (OR_1) and E_4 versus E_3 (OR_2). Logistic regression analysis was used to determine the overall gene effect. Bivariate meta-analysis with the maximum likelihood method was used to estimate the summary odds ratios (44–46). OR_1 and OR_2 and their 95 percent confidence intervals are reported.

Per-genotype analysis. Once a gene effect was confirmed, per-genotype analysis was used to ascertain the genetic model. Two separate per-genotype analyses—E_2E_2, E_2E_3, and E_3E_3 and E_4E_4, E_3E_4, and E_3E_3—were separately explored by assigning E_3E_3 as the reference group. The genotype effects were estimated using the model-free approach (47), in which no assumptions about genetic models are required. OR_3 (E_2E_2 vs. E_3E_3), OR_4 (E_2E_3 vs. E_3E_3), OR_5 (E_4E_4 vs. E_3E_3), and OR_6 (E_3E_4 vs. E_3E_3) were estimated using multivariate meta-analysis with Bayesian methods. The

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 OR_4 to log OR_3 for λ_1 and log OR_6 to log OR_5 for λ_2, were estimated. These parameters capture information about the genetic mode of action, as follows: The model is a recessive model if $\lambda = 0$, a dominant model if $\lambda = 1$, a codominant model if $\lambda = 0.5$, and a homozygous or heterosis model if $\lambda > 1$ or $\lambda < 0$.

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 strategies. 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 E_2 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 E_4 (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. OR_1 (E_2 vs. E_3) and OR_2 (E_4 vs. E_3) were estimated for each study (table 2). Neither OR_1 (E_2 vs. E_3) nor OR_2 (E_4 vs. E_3) showed any evidence of heterogeneity (OR_1: $\chi^2 = 4.50$, df $= 8$, $p = 0.81$; OR_2: $\chi^2 = 9.16$, 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 OR_1 and OR_2, obtained using bivariate 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 E_2 allele were approximately 17 percent more likely to have AMD than patients with the E_3 allele. Conversely, persons with an E_4 allele were approximately 33 percent less likely to have AMD than persons with allele E_3.

Using Egger's test, there was no evidence of publication bias or a study-size effect for OR_1 and OR_2 ($p = 0.56$ and $p = 0.68$, respectively). Sensitivity analysis including the one study not in Hardy-Weinberg equilibrium produced similar results; OR_1 and OR_2 were 1.20 (95 percent CI: 1.01, 1.43) and 0.61 (95 percent CI: 0.49, 0.77), respectively. 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 OR_1 and OR_2 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 E_4 allele ($OR_2 = 0.65$, 95 percent CI: 0.54, 0.79) but a slightly greater point estimate for the E_2 allele ($OR_1 = 1.31$, 95 percent CI: 1.08, 1.58).

Genotype-based methods. Table 3 shows the frequencies of the *ApoE* genotype in case and control groups. We estimated the genotype effects for E_2E_2 and E_2E_3 in each study by assigning the E_3E_3 genotype as the reference group (table 4). Cells with a zero count had 0.5 added. Two Asian studies (34, 36) did not have E_2E_2 genotypes in either case groups or control groups, and thus results for these studies

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

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

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

could not be summarized. The summary odds ratios for the E_2E_2 and E_2E_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_2E_2 and E_2E_3 genotypes had 5 percent and 22 percent higher risks of developing AMD than persons with the E_3E_3 genotype, although these effects did not reach statistical significance. The estimated λ 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 OR_3 and OR_4, $p = 0.78$ and $p = 0.85$, respectively). Cumulative meta-analysis was performed for OR_3 and OR_4. It showed that the summary OR_3 was a bit different in the first two studies (4, 35) and was not much changed, whereas the cumulative OR_4 did not change much over time (figure 1). Sensitivity analysis conducted by

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

First author and reference no.	Cases				Controls				HWE* *p* value	E_2/E_3		E_4/E_3	
	No.	Allele			No.	Allele				OR_1*	95% CI*	OR_2	95% CI
		E_2	E_3	E_4		E_2	E_3	E_4					
Klaver (4)	176	22	142	12	1,802	163	1,357	282	0.087	1.289	0.800, 2.079	0.407	0.223, 0.743
Souied (35)	232	23	192	17	336	21	265	50	0.673	1.512	0.813, 2.810	0.469	0.263, 0.839
Schmidt (68)	202	21	150	31	744	60	575	109	0.594	1.342	0.791, 2.276	1.090	0.704, 1.689
Simonelli (33)†	174	17	152	5	2568	153	2,149	266	0.001	1.571	0.927, 2.662	0.266	0.108, 0.654
Schmidt (8)	196	24	156	16	146	12	118	16	0.856	1.513	0.727, 3.149	0.756	0.363, 1.574
Schultz (31)	208	19	170	19	226	18	180	28	0.716	1.118	0.567, 2.201	0.718	0.387, 1.334
Baird (32)	398	39	310	49	246	17	185	44	0.143	1.369	0.753, 2.490	0.665	0.425, 1.038
Zareparsi (69)	1,258	116	1,022	120	410	33	320	57	0.053	1.101	0.733, 1.653	0.659	0.469, 0.926
Gotoh (34)	170	9	149	12	164	14	135	15	0.352	0.582	0.244, 1.389	0.725	0.328, 1.604
Pang (36)	274	27	231	16	266	25	221	20	0.799	1.033	0.582, 1.835	0.765	0.387, 1.514
Summary OR										1.167	1.006, 1.354	0.671	0.573, 0.784

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

including one study not in Hardy-Weinberg equilibrium showed similar results: OR_3, OR_4, and λ 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; OR_3 and OR_4 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. λ 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 OR_5 (E_4E_4 vs. E_3E_3) and OR_6 (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 λ 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 OR_5 and OR_6, $p = 0.10$ and $p = 0.92$, respectively). The cumulative meta-analysis for OR_5 and OR_6 (figure 2) showed that the summary OR_5 did not change over time, whereas the

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

First author and reference no.	Cases							Controls						
	No.	Genotype						No.	Genotype					
		E_2E_2	E_2E_3	E_2E_4	E_3E_3	E_3E_4	E_4E_4		E_2E_2	E_2E_3	E_2E_4	E_3E_3	E_3E_4	E_4E_4
Klaver (4)	88	0	20	2	56	10	0	901	9	130	15	500	227	20
Souied (35)	116	0	20	3	82	8	3	168	1	15	4	104	42	2
Schmidt (68)	101	2	13	4	58	21	3	372	4	44	8	225	81	10
Simonelli (33)	87	0	16	1	66	4	0	1,284	12	111	17	903	232	9
Schmidt (8)	98	1	21	1	60	15	0	73	0	11	1	47	13	1
Schultz (31)	104	2	15	0	69	17	1	113	0	15	3	71	23	1
Baird (32)	199	2	28	7	122	38	2	123	2	10	3	69	37	2
Zareparsi (69)	629	1	104	10	406	106	2	205	0	29	4	119	53	0
Gotoh (34)	85	0	8	1	65	11	0	82	0	13	1	55	12	1
Pang (36)	137	0	24	3	97	13	0	133	0	24	1	89	19	0

TABLE 4. Estimation of the summary odds ratios (ORs) OR_3 (E_2E_2 vs. E_3E_3) and OR_4 (E_2E_3 vs. E_3E_3) in an analysis of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls

First author and reference no.	Cases			Controls			E_2E_2 vs. E_3E_3		E_2E_3 vs. E_3E_3	
	E_2E_2	E_2E_3	E_3E_3	E_2E_2	E_2E_3	E_3E_3	OR_3	95% CI*	OR_4	95% CI
Klaver (4)	0	20	56	9	130	500	0.466	0.026, 8.117	1.374	0.796, 2.371
Souied (35)	0	20	82	1	15	104	0.422	0.018, 10.500	1.691	0.815, 3.507
Schmidt (68)	2	13	58	4	44	225	1.939	0.347, 10.851	1.146	0.579, 2.268
Simonelli (33)†	0	16	66	12	111	903	0.543	0.032, 9.279	1.972	1.103, 3.524
Schmidt (8)	1	21	60	0	11	47	2.355	0.094, 59.132	1.495	0.656, 3.407
Schultz (31)	2	15	69	0	15	71	5.144	0.243, 109.079	1.029	0.468, 2.264
Baird (32)	2	28	122	2	10	69	.566	0.078, 4.105	1.583	0.726, 3.454
Zareparsi (69)	1	104	406	0	29	119	0.882	0.036, 21.790	1.051	0.664, 1.664
Summary OR							1.046	0.519, 2.201	1.221	0.956, 1.612

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

summary OR_6 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_5, OR_6, and λ 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_5, OR_6, and λ 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 E_2 and E_4 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 E_4 allele appearing to be protective and E_2 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 E_2 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 E_4 allele did reach statistical significance and indicated that E_4 might act dominantly, with the presence of at least one E_4 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

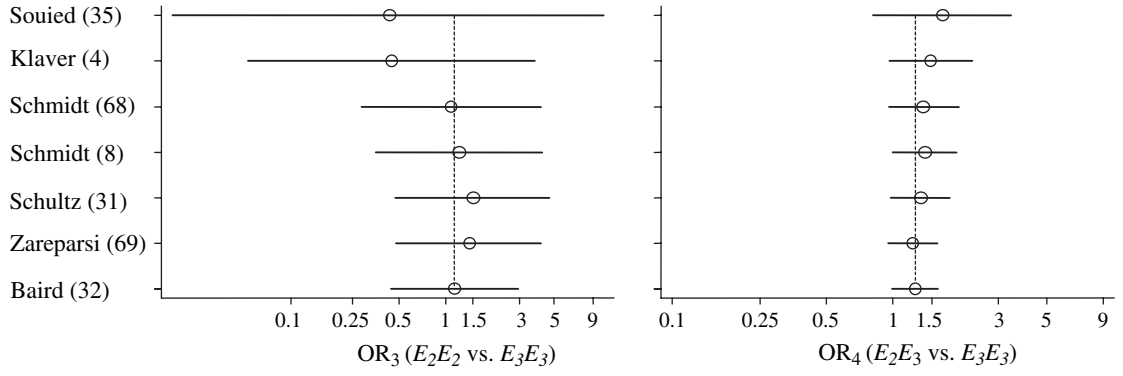

FIGURE 1. Odds ratios (ORs) OR_3 (E_2E_2 vs. E_3E_3) and OR_4 (E_2E_3 vs. E_3E_3) in a cumulative meta-analysis of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls. Horizontal bars, 95% confidence interval.

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

First author and reference no.	Cases			Controls			E_4E_4 vs. E_3E_3		E_3E_4 vs. E_3E_3	
	E_4E_4	E_3E_4	E_3E_3	E_4E_4	E_3E_4	E_3E_3	OR_5	95% CI*	OR_6	95% CI
Klaver (4)	0	10	56	20	227	500	0.216	0.013, 3.621	0.409	0.208, 0.805
Souied (35)	3	8	82	2	42	104	1.773	0.341, 9.219	0.253	0.114, 0.559
Schmidt (68)	3	21	58	10	81	225	1.164	0.310, 4.366	1.005	0.574, 1.761
Simonelli (33)†	0	4	66	9	232	903	0.715	0.041, 12.421	0.263	0.100, 0.691
Schmidt (8)	0	15	60	1	13	47	0.262	0.010, 6.571	0.901	0.396, 2.052
Schultz (31)	1	17	69	1	23	71	1.029	0.104, 10.130	0.766	0.380, 1.545
Baird (32)	2	38	122	2	37	69	0.566	0.078, 4.105	0.581	0.338, 0.997
Zareparsi (69)	2	106	406	0	53	119	1.47	0.070, 30.828	0.585	0.398, 0.861
Summary OR							0.847	0.444, 1.751	0.624	0.459, 0.904

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

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.

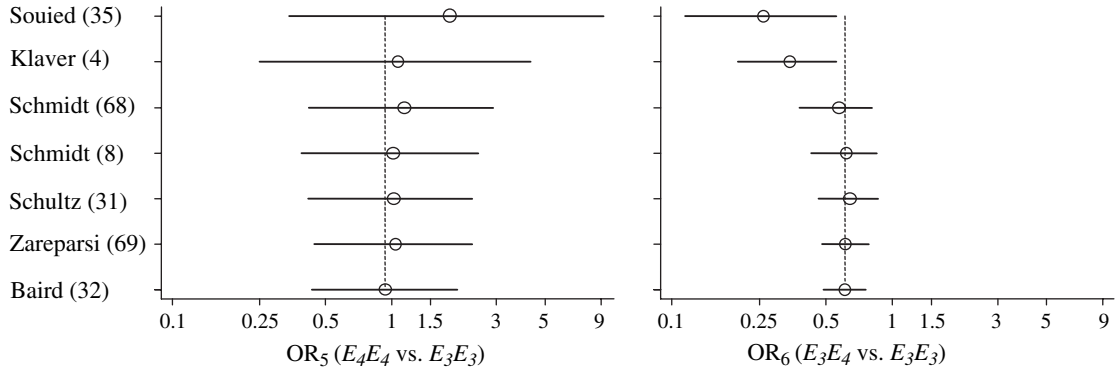

FIGURE 2. Odds ratios (ORs) OR_5 (E_4E_4 vs. E_3E_3) and OR_6 (E_3E_4 vs. E_3E_3) in a cumulative meta-analysis of apolipoprotein E polymorphisms among patients with age-related macular degeneration and controls. Horizontal bars, 95% confidence interval.

ACKNOWLEDGMENTS

Conflict of interest: none declared.

REFERENCES

1. Mitchell P, Smith W, Attebo K, et al. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. Ophthalmology 1995;102:1450–60.
2. VanNewkirk MR, Nanjan MB, Wang JJ, et al. The prevalence of age-related maculopathy: The Visual Impairment Project. Ophthalmology 2000;107:1593–600.
3. Pang CP, Lam DS. Differential occurrence of mutations causative of eye diseases in the Chinese population. Hum Mutat 2002;19:189–208.
4. Klaver CC, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet 1998;63:200–6.
5. Evans J, Wormald R. Is the incidence of registrable age-related macular degeneration increasing? Br J Ophthalmol 1996;80: 9–14.
6. Klein R, Klein B, Linton K. Prevalence of age-related maculopathy: The Beaver Dam Eye Study. Ophthalmology 1992; 99:933–43.
7. Vingerling JR, Dielemans I, Hofman A, et al. The prevalence of age-related maculopathy in the Rotterdam Study. Ophthalmology 1995;102:205–10.
8. Schmidt S, Klaver C, Saunders A, et al. A pooled case-control study of the apolipoprotein E (*APOE*) gene in age-related maculopathy. Ophthalmic Genet 2002;23:209–23.
9. Tomany SC, Wang JJ, Van Leeuwen R, et al. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. Ophthalmology 2004;111:1280–7.
10. Klein ML, Mauldin WM, Stoumbos VD. Heredity and age-related macular degeneration. Observations in monozygotic twins. Arch Ophthalmol 1994;112:932–7.
11. Seddon J, Ajani U, Mitchell B. Familial aggregation of age-related maculopathy. Am J Ophthalmol 1997;123: 199–206.
12. Klaver CC, Wolfs RC, Assink JJ, et al. Genetic risk of age-related maculopathy. Population-based familial aggregation study. Arch Ophthalmol 1998;116:1646–51.
13. Silvestri G, Johnston PB, Hughes AE. Is genetic predisposition an important risk factor in age-related macular degeneration? Eye 1994;8:564–8.
14. Heiba IM, Elston RC, Klein BE, et al. Sibling correlations and segregation analysis of age-related maculopathy: The Beaver Dam Eye Study. Genet Epidemiol 1994;11:51–67.
15. Yates JR, Moore AT. Genetic susceptibility to age related macular degeneration. J Med Genet 2000;37:83–7.
16. Meyers SM, Greene T, Gutman FA. A twin study of age-related macular degeneration. Am J Ophthalmol 1995;120:757–66.
17. Hammond CJ, Webster AR, Snieder H, et al. Genetic influence on early age-related maculopathy: a twin study. Ophthalmology 2002;109:730–6.
18. Klein ML, Schultz DW, Edwards A, et al. Age-related macular degeneration. Clinical features in a large family and linkage to chromosome 1q. Arch Ophthalmol 1998;116:1082–8.
19. Weeks DE, Conley YP, Tsai HJ, et al. Age-related maculopathy: an expanded genome-wide scan with evidence of susceptibility loci within the 1q31 and 17q25 regions. Am J Ophthalmol 2001;132:682–92.
20. Weeks DE, Conley YP, Tsai HJ, et al. Age-related maculopathy: a genomewide scan with continued evidence of susceptibility loci within the 1q31, 10q26, and 17q25 regions. Am J Hum Genet 2004;75:174–89.
21. Majewski J, Schultz DW, Weleber RG, et al. Age-related macular degeneration—a genome scan in extended families. Am J Hum Genet 2003;73:540–50.
22. Schick JH, Iyengar SK, Klein BE, et al. A whole-genome screen of a quantitative trait of age-related maculopathy in sibships from the Beaver Dam Eye Study. Am J Hum Genet 2003;72:1412–24.
23. Seddon JM, Santangelo SL, Book K, et al. A genomewide scan for age-related macular degeneration provides evidence for linkage to several chromosomal regions. Am J Hum Genet 2003;73:780–90.
24. Abecasis GR, Yashar BM, Zhao Y, et al. Age-related macular degeneration: a high-resolution genome scan for susceptibility loci in a population enriched for late-stage disease. Am J Hum Genet 2004;74:482–94.
25. Fisher SA, Abecasis GR, Yashar BM, et al. Meta-analysis of genome scans of age-related macular degeneration. Hum Mol Genet 2005;14:2257–64.
26. Gorin MB, Breitner JC, De Jong PT, et al. The genetics of age-related macular degeneration. Mol Vis 1999;5:29.
27. Weisgraber KH, Rall SC Jr, Mahley RW. Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. J Biol Chem 1981;256: 9077–83.
28. Rall SC Jr, Weisgraber KH, Innerarity TL, et al. Structural basis for receptor binding heterogeneity of apolipoprotein E from type III hyperlipoproteinemic subjects. Proc Natl Acad Sci U S A 1982;79:4696–700.
29. National Center for Biotechnology Information, National Library of Medicine. +107741. Apolipoprotein E; APOE. In: OMIM: Online Mendelian Inheritance in Man. (Database). Bethesda, MD: National Library of Medicine, 2006. (http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=107741).
30. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43: 1467–72.
31. Schultz DW, Klein ML, Humpert A, et al. Lack of an association of apolipoprotein E gene polymorphisms with familial age-related macular degeneration. Arch Ophthalmol 2003; 121:679–83.
32. Baird PN, Guida E, Chu DT, et al. The epsilon2 and epsilon4 alleles of the apolipoprotein gene are associated with age-related macular degeneration. Invest Ophthalmol Vis Sci 2004;45:1311–15.
33. Simonelli F, Margaglione M, Testa F, et al. Apolipoprotein E polymorphisms in age-related macular degeneration in an Italian population. Ophthalmic Res 2001;33:325–8.
34. Gotoh N, Kuroiwa S, Kikuchi T, et al. Apolipoprotein E polymorphisms in Japanese patients with polypoidal choroidal vasculopathy and exudative age-related macular degeneration. Am J Ophthalmol 2004;138:567–73.
35. 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;125:353–9.
36. Pang CP, Baum L, Chan WM, et al. The apolipoprotein E epsilon4 allele is unlikely to be a major risk factor of age-related macular degeneration in Chinese. Ophthalmologica 2000;214:289–91.
37. Thakkinstian A, McElduff P, D'Este C, et al. A method for meta-analysis of molecular association studies. Stat Med 2005;24:1291–306.

38. Thakkinstian A, D'Este C, Eisman J, et al. Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. J Bone Miner Res 2004;19:419–28.
39. Thakkinstian A, McEvoy M, Minelli C, et al. Systematic review and meta-analysis of the association between β2-adrenoceptor polymorphisms and asthma: a HuGE review. Am J Epidemiol 2005;162:201–11.
40. Attia J, Thakkinstian A, D'Este C. Systematic review of meta-analyses of molecular association studies: methodologic lesson for genetic epidemiology. J Clin Epidemiol 2003;56: 297–303.
41. Nam J. Simple test for the Hardy-Weinberg law for HLA data with no observed double blanks. Biometrics 1995;51:354–7.
42. Hernandez JL, Weir BS. A disequilibrium coefficient approach to Hardy-Weinberg testing. Biometrics 1989;45:53–70.
43. Chen JJ, Thomson G. The variance for the disequilibrium coefficient in the individual Hardy-Weinberg test. Biometrics 1999;55:1269–72.
44. van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. Stat Med 2002;21:589–624.
45. Van Houwelingen HC, Zwinderman KH, Stijnen T. A bivariate approach to meta-analysis. Stat Med 1993;12:2273–84.
46. Thompson JR, Minelli C, Abrams KR, et al. Meta-analysis of genetic studies using Mendelian randomization—a multivariate approach. Stat Med 2005;24:2241–54.
47. Minelli C, Thompson JR, Abrams KR, et al. The choice of a genetic model in the meta-analysis of molecular association studies. Int J Epidemiol 2005;34:1319–28.
48. Trikalinos TA, Salanti G, Khoury MJ, et al. Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. Am J Epidemiol 2006; 163:300–9.
49. Schaid DJ, Jacobsen SJ. Biased tests of association: comparisons of allele frequencies when departing from Hardy-Weinberg proportions. Am J Epidemiol 1999;149:706–11.
50. Ayres KL, Balding DJ. Measuring departures from Hardy-Weinberg: a Markov chain Monte Carlo method for estimating the inbreeding coefficient. Heredity 1998;80:769–77.
51. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315: 629–34.
52. Egger M, Smith GD, Altman DG. Systematic reviews in health care: meta-analysis in context. London, United Kingdom: BMJ Books, 2001.
53. Stata Corporation. Stata statistical software, release 9.0. College Station, TX: Stata Corporation, 2005.
54. Spiegelhalter D, Thomas A, Best N, et al. WinBUGS user manual. Version 1.4, January 2003. Cambridge, United Kingdom: MRC Biostatistics Unit, Institute of Public Health, 2003. (http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/manual14.pdf).
55. Anderson DH, Ozaki S, Nealon M, et al. Local cellular sources of apolipoprotein E in the human retina and retinal pigmented epithelium: implications for the process of drusen formation. Am J Ophthalmol 2001;131:767–81.
56. Anderson DH, Talaga KC, Rivest AJ, et al. Characterization of beta amyloid assemblies in drusen: the deposits associated with aging and age-related macular degeneration. Exp Eye Res 2004;78:243–56.
57. Ishida BY, Bailey KR, Duncan KG, et al. Regulated expression of apolipoprotein E by human retinal pigment epithelial cells. J Lipid Res 2004;45:263–71.
58. Malek G, Li CM, Guidry C, et al. Apolipoprotein B in cholesterol-containing drusen and basal deposits of human eyes with age-related maculopathy. Am J Pathol 2003;162: 413–25.
59. Mullins RF, Russell SR, Anderson DH, et al. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 2000;14:835–46.
60. Kuhrt H, Hartig W, Grimm D, et al. Changes in CD44 and ApoE immunoreactivities due to retinal pathology of man and rat. J Hirnforsch 1997;38:223–9.
61. Kenealy SJ, Schmidt S, Agarwal A, et al. Linkage analysis for age-related macular degeneration supports a gene on chromosome 10q26. Mol Vis 2004;10:57–61.
62. Jun G, Klein BE, Klein R, et al. Genome-wide analyses demonstrate novel loci that predispose to drusen formation. Invest Ophthalmol Vis Sci 2005;46:3081–8.
63. Kliffen M, Lutgens E, Daemen MJ, et al. The APO*E3-Leiden mouse as an animal model for basal laminar deposit. Br J Ophthalmol 2000;84:1415–19.
64. Malek G, Johnson LV, Mace BE, et al. Apolipoprotein E allele-dependent pathogenesis: a model for age-related retinal degeneration. Proc Natl Acad Sci U S A 2005;102: 11900–5.
65. Robman L, McNeil J, Dimitrov P, et al. Methodology of the Cardiovascular Health and Age-Related Maculopathy (CHARM) Study. Ophthalmic Epidemiol 2004;11:161–79.
66. Santos A, Salguero ML, Gurrola C, et al. The epsilon4 allele of apolipoprotein E gene is a potential risk factor for the severity of macular edema in type 2 diabetic Mexican patients. Ophthalmic Genet 2002;23:13–19.
67. van Leeuwen R, Klaver CC, Vingerling JR, et al. Cholesterol and age-related macular degeneration: is there a link? Am J Ophthalmol 2004;137:750–2.
68. Schmidt S, Saunders AM, De La Paz MA, et al. Association of the apolipoprotein E gene with age-related macular degeneration: possible effect modification by family history, age, and gender. Mol Vis 2000;6:287–93.
69. Zareparsi S, Reddick AC, Branham KE, et al. Association of apolipoprotein E alleles with susceptibility to age-related macular degeneration in a large cohort from a single center. Invest Ophthalmol Vis Sci 2004;45:1306–10.
70. Ioannidis JP, Trikalinos TA, Ntzani EE, et al. Genetic associations in large versus small studies: an empirical assessment. Lancet 2003;361:567–71.

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

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

* 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

First author and reference no.	Representativeness of cases	Representativeness of controls	Ascertainment of age-related macular degeneration	Ascertainment of controls	Ascertainment of genotyping examination	Test for Hardy-Weinberg equilibrium	Association assessment	Total score
Klaver (4)	2	2	1	2	0	2	2	11
Souied (35)	1	1	1	1	1	2	1	8
Pang (36)	0	0	1	2	0	0	0	3
Schmidt (68)	1	2	1	1	0	2	2	9
Simonelli (33)	1	1	1	2	0	0	0	5
Schmidt (8)	1	2	1	2	0	2	2	10
Schultz (31)	1	2	1	2	0	2	2	10
Baird (32)	1	2	1	2	0	2	2	10
Gotoh (34)	0	0	1	2	0	2	2	7
Zareparsi (69)	1	2	1	2	0	2	2	10

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