The association between complement component 2/complement factor B polymorphisms and age-related macular degeneration: A HuGE review and meta-analysis

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Human Genome Epidemiology (HuGE) Review

Systematic Review and Meta-Analysis of the Association Between Complement Component 3 and Age-related Macular Degeneration: A HuGE Review and Meta-Analysis

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The authors performed a meta-analysis to estimate the magnitude of polymorphism effects for the complement component C3 gene (*C3*) and their possible mode of action on age-related macular degeneration (AMD). The meta-analysis included 16 and 7 studies for rs2230199 and rs1047286, respectively. Data extraction and risk of bias assessments were performed in duplicate, and heterogeneity and publication bias were explored. There was moderate evidence for association between both polymorphisms and AMD in Caucasians. For rs2230199, patients with CG and GG genotypes were 1.44 (95% confidence interval (CI): 1.33, 1.56) and 1.88 (95% CI: 1.59, 2.23) times more likely to have AMD than patients with the CC genotype. For rs1047286, GA and AA genotypes had 1.27 (95% CI: 1.15, 1.41) and 1.70 (95% CI: 1.27, 2.11) times higher risk of AMD than did GG genotypes. These gene effects suggested an additive model. The population attributable risks for the GG/GC and AA/GA genotypes are approximately 5%–10%. Subgroup analysis by ethnicity indicates that these variants are very infrequent in Asians and that the observed gene effects are based largely on the high frequency within Caucasian populations. This meta-analysis supports the association between *C3* and AMD and provides a robust estimate of the genetic risk.

complement component factor 3; epidemiology; genetic association studies; genetics; macular degeneration; meta-analysis

Abbreviations: AMD, age-related macular degeneration; C3, complement component 3; CFB, complement factor B; CFH, complement factor H; CI, confidence interval; HWD, Hardy-Weinberg disequilibrium; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SNP, single nucleotide polymorphism.

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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% of those older than 75 years showing early signs of the disease (1, 6, 7). The pathologic hallmark of AMD is drusen, deposits of protein and lipid located between the retina and the retinal pigment epithelium, at which stage the condition is termed early AMD. In a considerable proportion of people with these changes, progression of these early features can result in geographic atrophy, in which there is loss of retinal pigment epithelium and photoreceptors, and/or an acute exudative phenomenon due to neovascularization (''neovascular AMD'') in the macular tissues. Late AMD is the term used to describe the condition when either geographic atrophy or neovascular changes are detected.

Since 2005, research into genetic influences on early and late AMD pathophysiology has implicated the *ARMS2* locus including *LOC387715*/serine protease *HTRA1* at 10q26 (8–12)

in addition to several genes involved in the complement pathway, including variants in the alternative complement pathway genes complement factor H (*CFH*) and complement factor B (*CFB*). Additional independent variants in genes encoding classical complement pathway components, such as complement component 2 (*C2*) (13–19) and complement component 3 (*C3*) (20–35), have also been identified.

The gene *C3*, located on 19p13.3-p13.2 (Online Mendelian Inheritance in Man (OMIM) + 120700), has 2 single nucleotide polymorphisms (SNPs), rs2230199 C>G and rs1047286 G>A, reported to be highly associated with AMD and which are in high linkage disequilibrium ($r^2 = 0.85$) (27). *C3* is an acute-phase reactant, meaning that there is increased synthesis of *C3* during any inflammatory process. The minor allele frequencies for rs2230199 range from 3.2% to 6.8% in African Americans, 16.9% to 20.6% in Europeans, and 0.8% in sub-Saharan Africans (36); rs1047286 is virtually nonexistent in African Americans and is present in 23.9%–50% of Europeans (37). The *C3* polymorphisms may contribute to early or late AMD via *CFH*, which acts as a cofactor with the *C3b* inactivator to regulate the activity of *C3* convertases (38), or may act independently and directly on disease pathophysiology (39).

The gene effects reported for *C3* on AMD have varied across studies. We therefore conducted a systematic review to pool the results of all available population-based association studies between *C3* (rs2230199 and rs1047286) and AMD with the following aims: first, to estimate the prevalence of the minor alleles of rs2230199 and rs1047286 by ethnicity; and second, to ascertain if there are genetic effects on AMD susceptibility and, if present, to estimate the magnitude of that genetic effect and the underlying genetic model.

MATERIALS AND METHODS

Search strategy

Studies were identified through Medline and Embase databases by using PubMed and Ovid search engines. One author (A. T.) identified relevant studies up to July 2010 using the search strategy: (gene *or* allele *or* polymorphism) *and* (macular degeneration) *and* (''complement component 3'' *or* C3 *or* ''complement factor 3'') *or* (''complement component 2'' *or* C2 *or* ''complement factor 2''). The reference lists of the retrieved articles were also reviewed 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.

Inclusion criteria

One author (A. T.) reviewed all titles or abstracts of the identified studies in order to select those for inclusion. Any human population-based association study, regardless of ethnicity or sample size, was included if it met the following criteria:

- Reported *C3* SNPs at rs2230199 (R102E C>G) and/or rs1047286 (L314P G>A).
- The outcome of interest was AMD, and there were at least 2 comparison groups (i.e., AMD vs. control group).
- There were sufficient results for extraction of data (i.e., number of participants for each genotype in AMD and control groups). Where eligible papers provided insufficient information, we contacted authors by e-mail for additional information.

Outcome measurement

The definition of AMD varied between studies and used various grading systems (i.e., international classification age-related maculopathy (22, 28, 31, 35, 40), age-related eye disease study grading (20, 23, 24, 26, 34), clinical age-related maculopathy system (29), Wisconsin age-related maculopathy grading (16), and others (21, 30, 33)) (refer to Web Table 1, which is posted on the *Journal*'s Web site (http://aje.oxfordjournals.org/)). Briefly, early AMD (grades 2 and 3) was defined as the presence of soft indistinct drusen (≥125 μm) and/or pigmentary abnormalities. Late AMD (grade 4) was defined as either geographic atrophy and/or features indicating the presence of neovascularization or mixed when geographic atrophy was present in 1 eye and neovascularization in the fellow eye. Control participants were defined as those without early or late AMD with no evidence of drusen or pigmentary abnormalities in both eyes. Unless specifically stated, the term AMD represents both early and late AMD combined. For studies that provided subcategories of AMD disease status, gradings were collapsed into a single AMD group. Cases were classified according to AMD diagnosis in the worse eye.

Data extraction

Summary data for *C3* were extracted independently and in duplicate by 2 authors (A. T., M. M.) using a standardized data extraction form. Covariates, such as mean age, percent male, percent smoker, and ethnicity, were also extracted. Any disagreements between the 2 authors were resolved by consensus.

Risk of bias assessment

The quality of studies was independently assessed by 2 authors (A. T., M. M.) by using a risk-of-bias score for genetic association studies. This was modified on the basis of both traditional epidemiologic considerations and genetic issues (41–44) (Appendix Table 1). The score consists of 4 domains:

- Information bias: Ascertainment of diagnosis of AMD and controls and genotyping methods were assessed.
- Confounding bias: Population stratification and other confounder effects were considered.
- Selective reporting of outcomes.
- Hardy-Weinberg equilibrium (HWE) was assessed in the control groups of each included study.

Each domain was rated by the following question: Is there a low risk of bias? The answer was categorized as yes, no,

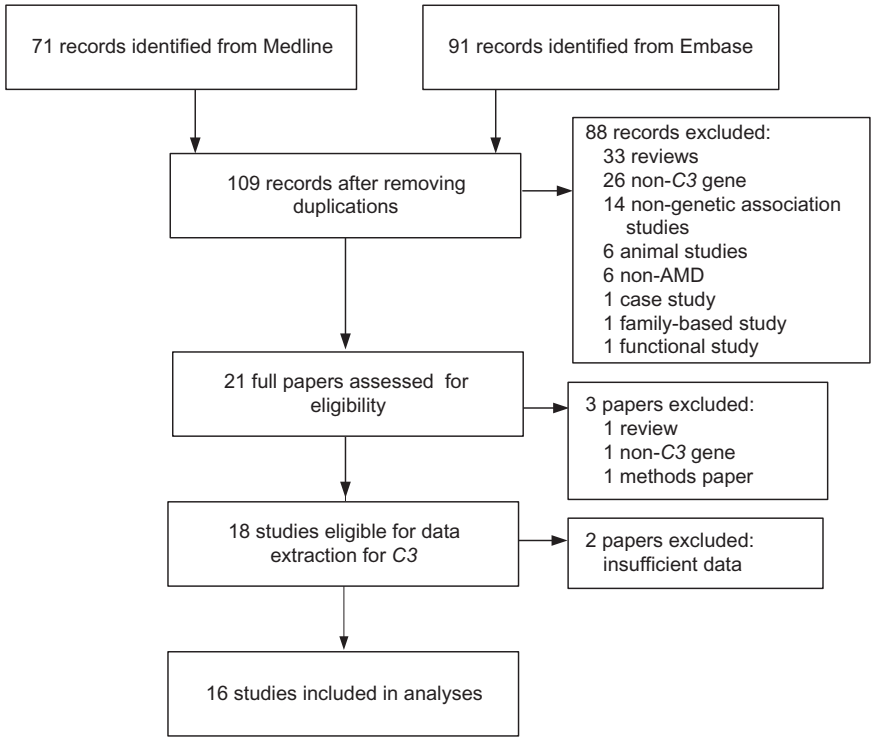

Figure 1. Flow for identifying and selecting studies in this meta-analysis. AMD, age-related macular degeneration; *C3*, complement component 3 gene.

and unclear, which refer to low risk, high risk, and unclear if insufficient information was available for assessment.

Statistical analysis

HWE was assessed in the control group of each study by using an exact test. The analyses were performed as follows:

- Pooled allele prevalence: Only data from control groups of included studies were used for pooling allele prevalence. Overall prevalence of the minor allele was pooled for each SNP separately by ethnicity by use of a random effect model if heterogeneity was present.
- Overall test of genetic association: The Q test for heterogeneity was assessed, and I^2 was used to quantify the degree of heterogeneity for each polymorphism separately for 2 odds ratios (ORs) (i.e., AA vs. aa (OR_1) and Aa vs. aa (OR_2)), where AA, Aa, and aa are common homozygous, heterozygous, and minor homozygous genotypes, respectively. If heterogeneity was present in at least 1 odds ratio (Q test: $P < 0.1$ or $I^2 > 25\%$), the cause of heterogeneity was explored by fitting covariates (e.g., age, percent male, percent smoker, or AMD phenotype) in a meta-regression model if the data for these covariables were available (45–48). A mixed-effects hierarchical model with logit link function (42) was applied to determine the overall gene effect by using the xtmelogit command in STATA software (StataCorp LP, College Station, Texas). Summary genotyping data between cases and controls were expanded by using the expand command before running the mixed model. The genotypes were considered as fixed effects in the model, whereas the study was considered as a random effect. A likelihood ratio test was then applied to assess whether the gene effect was significant.
- Magnitude and genetic model: Once a gene effect was confirmed, the per-genotype analysis was used to ascertain the genetic model. The genotype effects were estimated by using the model-free approach (49). OR_1 and OR_2 were estimated by using multivariate meta-analysis with Bayesian methods in which both between- and within-study variation was considered. A parameter lambda (λ) (i.e., the ratio of $\log(OR_2)$ vs. $\log(OR_1)$) was calculated to reflect the genetic model. λ close to 0, 1, and 0.5 suggests recessive, dominant, and additive models, respectively; a λ greater than 1 or less than 0 suggests a homozygous or heterosis model.

Two approaches were applied for handling Hardy-Weinberg disequilibrium (HWD). First, sensitivity analysis was performed by including and excluding studies not in HWE. Second, all studies were included regardless of HWD and instead adjusted for the degree of disequilibrium by using the inbreeding coefficient (F) as described by Trikalinos et al. (50). Briefly, data in the control group were used to estimate the inbreeding coefficient (F). The predicted genotype frequencies were then estimated (51) and used instead of the observed frequencies in the summary analysis of magnitude and genetic model.

Publication bias was assessed by using the Egger test and funnel plot (52, 53). A contour-enhanced funnel plot was used to detect publication bias due to the small-study

Table 1. General Characteristics of Studies Included in the Meta-Analysis, 2007–2010

First Author, Year (Reference No.)	Age, years	Male, %	Smoke, %	Design	Type of Case	Type of Control
Yates, 2007 (35)	77.9	42.2	57	Case-control	Advanced AMD, 74.6%	Non-AMD
Edwards, 2008 (23)				Case-control	Large or advanced AMD	AMD grade 1
Scholl, 2008 (31)	73.5	43.6	47.5	Case-control	CNV, 69.6%	Non-AMD
Seitsonen, 2008 (33)	76			Case-control	Acute exudative or disciform lesion, 87%	Pigment abnormality diameter, <250 μm; hard drusen, ≤5 μm
Spencer, 2008 (34)	73.7	38.7	57.4	Case-control	AMD grades 3–5	AMD grades 1 and 2
Bergeron-Sawitzke, 2009 (20)	65.4	45.7	46.5	Age-sex-race–matched case-control	AMD grades 3–5	Non-AMD
Cui, 2010 (21)	66.1	55.3		Case-control	Exudative AMD	Non-AMD
Despriet, 2009 (22)	68.6	41.5	66.1	Rotterdam cohort	Early and late, 22.2%	Non-AMD
Despriet, 2009 (22)	76.8	42.6	66	Case-control	Early and late, 75.1%	Non-AMD
Francis, 2009 (24)				AREDS cohort	GA/CNV	AMD grade 1
Francis, 2009 (24)	76.7	33.2		CEIMDC case-control	GA/CNV	Drusen, <63 μm in diameter
Gu, 2009 (26)	74.2	53.3	48.4	Case-control	Early and late, 66.5%	Blood donors
Park, 2009 (27)				Cohort	Early and late, 54.6%	AMD grade 1
Pei, 2009 (28)	69.9	53	45.8	Age-sex–matched case-control	CNV	Non-AMD
Reynolds, 2009 (29)		50	54.4	Case-control	AMD grade 4 (GA)/grade 5 (CNV) in one or both eyes	AMD grade 1 in both eyes
Scholl, 2009 (30)	75.6	38.1	49.5	Case-control	GA	
Liu, 2010 (40)	64.2	45.4		Age-matched case-control	CNV, 66.4%, and drusen, 33.6%	Non-AMD
McKay, 2010 (16)	74.9	38.5		Age-matched case-control	GA/CNV	Non-AMD

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-related Eye Disease Study; CEIMDC, Casey Eye Institute Macular Degeneration Center; CNV, choroidal neovascularization; GA, geographic atrophy.

effect (54–57). Trim and fill meta-analysis was applied to impute missing studies (58). The population attributable risk for risk genotypes was determined (59, 60). Analyses were performed by using STATA, version 11.0 (61), and WinBugs 1.4.2 (62) software with beta vague prior distributions for estimation of parameters (i.e., λ and odds ratio). The models were run for a burn-in of 10,000 iterations, followed by 50,000 iterations for parameter estimates. $P < 0.05$ was considered statistically significant, except for tests of heterogeneity where a level of 0.10 was used.

RESULTS

Identifying studies

Seventy-one and 91 studies were located from Medline and Embase (Figure 1), respectively. After duplicates were removed, 109 titles or abstracts were screened, with 88 determined to be ineligible. The reasons for ineligibility are shown in Figure 1. After retrieval and review of the publications for the 21 remaining studies, we excluded a further 3, leaving 18 for data extraction for rs2230199 and rs1047286 SNPs for consideration in this review. Two studies did not report gene frequencies according to AMD groups, and corresponding authors were contacted for additional data but did not respond. This left 16 studies with sufficient data for analysis. Agreement between the 2 author/reviewers on data extraction was 93.8%. Disagreements were resolved by discussion and consensus. Characteristics of these 16 studies are described in Table 1.

Risk of bias assessment

Agreement between both authors (A.T., M. M.) on risk of bias assessments was 94.8%. Disagreement was solved by consensus. As described in Table 2, the criteria for diagnosis of AMD and controls were clearly described for all included studies, and risk of ascertainment bias was therefore less likely. Among 16 studies, quality control for genotyping was unclear or not mentioned in 7 (43.7%). Risk of bias from population stratification was present in 2 (12.5%) studies as the result of imbalance of ethnicity between cases and controls in 1 study and use of related family members in the other. Risk of confounding bias was high in 4 studies (25.0%) because adjustment for

Table 2. Determination of Risk Assessment Bias by Included Studies of Meta-Analysis, 2007–2010[a]

First Author, Year (Reference No.)	Ascertainment of AMD	Ascertainment of Control	Quality Control for Genotyping	Population Stratification	Confounding Bias	Selective Outcome Report	HWE
Yates, 2007 (35)	Yes	Yes	Unclear	No	Yes	Yes	No
Edwards, 2008 (23)	Yes	Yes	Yes	Yes	No	Yes	Yes
Scholl, 2008 (31)	Yes	Yes	No	Yes	Yes	Yes	Yes
Seitsonen, 2008 (33)	Yes	Yes	No	Yes	Yes	Yes	Yes
Spencer, 2008 (34)	Yes	Yes	Yes	Yes	Yes	No	Yes
Bergeron-Sawitzke, 2009 (20)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cui, 2010 (21)	Yes	Yes	Yes	Yes	No	Yes	Yes
Despriet, 2009 (22)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Francis, 2009 (24)	Yes	Yes	No	Yes	Yes	Yes	No
Gu, 2009 (26)	Yes	Yes	No	No	No	Yes	No
Park, 2009 (27)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pei, 2009 (28)	Yes	Yes	No	Yes	Yes	Yes	Yes
Reynolds, 2009 (29)	Yes	Yes	Yes	Yes	Yes	Yes	Unclear
Scholl, 2009 (30)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Liu, 2010 (40)	Yes	Yes	Yes	Yes	No	Yes	Yes
McKay, 2010 (16)	Yes	Yes	Unclear	Yes	Yes	No	Yes

Abbreviations: AMD, age-related macular degeneration; HWE, Hardy-Weinberg equilibrium.

[a] For each domain, the following question was asked: Was there low risk of bias? Answers were "yes," "no," and "unclear" for low risk, high risk, and unclear information, respectively.

confounders was not mentioned in the reports. Two studies reported significant association between some SNPs and AMD while nonsignificant SNPs were not reported, indicating that selective outcome reporting might be present. Assessing HWE was unclear or not described in 4 studies (25.0%).

rs2230199

Sixteen studies (16, 20–24, 26–31, 33–35, 40) assessed the association between rs2230199 and AMD. Among these, 13 studies were in Caucasian (16, 20, 22–24, 26, 27, 29–31, 33–35) and 3 were in Asian populations (21, 28, 40). The publications by Despriet et al. (22) and Francis et al. (24) included 2 independent substudies, and data from these were treated separately in the remaining analyses. Minor G-allele frequencies in the control group ranged from 0.003 to 0.221 (Table 3). HWE was not observed in 2 studies (22, 26). Pooling the G-allele frequency for Caucasians (without these 2 studies) and Asians by using a random-effects model yielded estimates of 0.193 (95% confidence interval (CI): 0.181, 0.205) and 0.007 (95% CI: 0.001, 0.013), respectively. Sensitivity analysis including the 2 studies did not substantially change this estimate of 0.194 (95% CI: 0.181, 0.208) for Caucasians.

Genotype frequencies for AMD and control groups from the included studies are shown in Table 4. The pooled sample sizes were 6,803 versus 5,967 in cases and controls, respectively, in individuals of European descent and 511 versus 511 in Asians. Gene effects for GG versus CC (OR_1) and CG versus CC (OR_2) were estimated for each included study. Among 13 Caucasian studies, moderate heterogeneity was present for both OR_1 (chi-square = 23.0, df = 12; $P = 0.028$; $I^2 = 47.8\%$) and OR_2 (chi-square = 21.07, df = 12; $P = 0.041$; $I^2 = 44.7\%$) (Figure 2). An overall genetic effect was therefore tested by using hierarchical logit regression, in which between-study variation was fitted as a random effect. The overall gene effect was significant (likelihood ratio = 112.37; $P < 0.001$) with pooled OR_1 and OR_2 of 1.88 (95% CI: 1.59, 2.23) and 1.44 (95% CI: 1.33, 1.56), respectively, indicating that GG and GC genotypes had 88% and 44% significantly higher risk of AMD than did CC genotypes. The estimated λ was 0.71 (95% CI: 0.37, 0.98) (i.e., the genetic mode of effect might be either additive or dominant). Sensitivity analysis including the 2 studies not in HWE yielded similar results (i.e., the OR_1 and OR_2 were 2.09 (95% CI: 1.80, 2.44) and 1.45 (95% CI: 1.35, 1.57) with a λ of 0.69 (95% CI: 0.39, 0.98)). Results were also consistent with adjustment for genotype frequencies in the control group (i.e., the OR_1 and OR_2 were 2.06 (95% CI: 1.77, 2.40) and 1.46 (95% CI: 1.35, 1.57), and the λ was 0.58 (95% CI: 0.38, 0.85)). Among 3 studies in Asians, there was no one with the GG genotype; thus, data were insufficient for assessing genetic effects.

Exploring sources of heterogeneity was performed by fitting the AMD subphenotype, age, percent male, and percent smoking in a meta-regression model. Among 13 Caucasian studies, 8 studies (16, 20, 23, 24, 29, 30, 34) had patients with advanced AMD (i.e., geographic atrophy, choroidal neovascularization, or both), while the other 5 studies (22, 27, 31, 33, 35) had a mixture of patients with early or advanced disease. Although the AMD case subphenotype was nonsignificantly associated with the gene effect, it did reduce the I^2 from 44.7% to 17.1% for OR_2 but not for OR_1.

Table 3. Allele Frequencies for rs2230199 and Estimated Pooled Prevalence of Major and Minor Alleles, by Ethnicity, of Studies Included in the Meta-Analysis, 2007–2010[a]

First Author, Year (Reference No.)	Ethnicity	No. of Alleles	G Allele		C Allele		HWE *P* Value
			Frequency, no.	Allele Prevalence	Frequency, no.	Allele Prevalence	
Cui, 2010 (21)	Asian	322	1	0.003	321	0.997	1.000
Liu, 2010 (40)	Asian	440	4	0.009	436	0.991	1.000
Pei, 2009 (28)	Asian	260	4	0.015	256	0.985	1.000
Pooled prevalence				0.007 (0.001, 0.013)[b]		0.993 (0.987, 0.999)	
Bergeron-Sawitzke, 2009 (20)	Caucasian	430	76	0.177	354	0.823	0.345
Despriet, 2009 (22)	98% Caucasian, Rotterdam cohort	4,874	1,018	0.209	3,856	0.791	0.668
Despriet, 2009 (22)[a]	Caucasian, case-control	336	53	0.158	283	0.842	0.037
Edwards, 2008 (23)	Caucasian	598	108	0.181	490	0.819	0.115
Francis, 2009 (24)	Caucasian, AREDS	644	126	0.196	518	0.804	0.726
Francis, 2009 (24)	Caucasian, CEIMDC	368	78	0.212	290	0.788	0.267
Gu, 2009 (26)[a]	91.5% Caucasian	686	176	0.257	510	0.743	0.023
McKay, 2010 (16)	Caucasian	872	193	0.221	679	0.779	0.126
Park, 2009 (27)	Caucasian	592	108	0.182	484	0.818	0.562
Reynolds, 2009 (29)	Caucasian	116	25	0.216	91	0.784	0.712
Scholl, 2009 (30)	Caucasian	1,168	204	0.175	964	0.825	0.317
Scholl, 2008 (31)	Caucasian	134	18	0.134	116	0.866	1.000
Seitsonen, 2008 (33)	Caucasian	210	32	0.152	178	0.848	0.703
Spencer, 2008 (34)	Caucasian	572	121	0.212	451	0.788	1.000
Yates, 2007 (35)	Caucasian	1,356	268	0.198	1,088	0.802	0.717
Pooled prevalence				0.193 (0.181, 0.205)		0.807 (0.795, 0.819)	
Overall pooled prevalence				0.155 (0.104, 0.206)		0.845 (0.794, 0.896)	

Abbreviations: AREDS, Age-related Eye Disease Study; CEIMDC, Casey Eye Institute Macular Degeneration Center; HWE, Hardy-Weinberg equilibrium.

[a] Not included in pooling because of departure from HWE.

[b] Numbers in parentheses, 95% confidence interval.

A subgroup analysis for advanced cases alone gave more homogeneous OR_1 and OR_2 ($I^2 = 28.8\%$ and 0%, respectively). The pooled corresponding odds ratios were 2.27 (95% CI: 1.76, 2.92) and 1.65 (95% CI: 1.46, 1.87) (i.e., those having GG and CG genotypes were about 2.27 and 1.65 times, respectively, more likely to have advanced AMD than those with the CC reference genotype). The estimated λ was 0.67 (95% CI: 0.37, 0.97) (i.e., the additive model was most likely). Pooling OR_1 and OR_2 for studies with mixed early and late AMD patients increased the degree of heterogeneity I^2 to 57.0% and 64.0%, respectively, and diluted the corresponding odds ratios (i.e., OR = 1.61, 95% CI: 1.28, 2.03; OR = 1.30, 95% CI: 1.17, 1.44), with a λ of 0.55 (95% CI: 0.14, 0.96). Again, the additive model was more likely.

For other covariables, the analysis could be performed with some Caucasian studies (9 studies for age and gender, 7 studies for smoking) where summary data for these variables were available. Results of meta-regression suggested that percent smoking was associated with OR_1 and OR_2 and could reduce the degree of heterogeneity of OR_1 and OR_2 from 57.7% and 60.0% to zero for both odds ratios. Percent smoking ranged from 46.5% to 66.1% with a median of 54.4% among 7 studies. Subgroup analysis was therefore performed in 3 and 4 studies, in which smoking prevalence was $\leq$54.4% and >54.4%, respectively. The heterogeneity was reduced in the former group ($I^2 = 0$ for both ORs) but not for the latter group ($I^2 = 63.7\%$ and 73.8% for OR_1 and OR_2). The genetic effects OR_1 and OR_2 were 3.15 (95% CI: 1.74, 5.62) and 1.67 (95% CI: 1.28, 2.16) for the former groups and 1.60 (95% CI: 1.27, 2.01) and 1.30 (95% CI: 1.16, 1.45) for the latter groups.

Fitting age did not explain heterogeneity for OR_1 but did decrease I^2 from 48.5% to 33.2% for OR_2. Among 9 studies where the data were available, mean age ranged from 65.4 to 77.9 years with a median of 74.3 years. The heterogeneity

Table 4. Genotype Frequencies for rs2230199 Between AMD and Control Groups and Genotype Effects of Studies Included in the Meta-Analysis, 2007–2010

First Author, Year (Reference No.)	AMD				Controls				GG/CC		CG/CC	
	No. of Subjects	Genotype			No. of Subjects	Genotype			OR_1	95% CI	OR_2	95% CI
		CC	CG	GG		CC	CG	GG				
Bergeron-Sawitzke, 2009 (20)	421	227	164	30	215	148	58	9	2.17	1.00, 4.71	1.84	1.28, 2.65
Despriet, 2009 (22)[a]	1,175	711	405	59	2,437	1,529	798	110	1.15	0.83, 1.6	1.09	0.94, 1.27
Despriet, 2009 (22)[b,c]	331	197	110	24	168	123	37	8	1.87	0.82, 4.3	1.86	1.20, 2.87
Edwards, 2008 (23)	443	236	167	40	299	205	80	14	2.48	1.31, 4.69	1.81	1.31, 2.51
Francis, 2009 (24)[d]	672	324	293	55	322	207	104	11	3.19	1.63, 6.25	1.8	1.36, 2.39
Francis, 2009 (24)[e]	202	115	80	7	184	117	56	11	0.65	0.24, 1.73	1.45	0.95, 2.23
Gu, 2009 (26)[c]	769	317	270	182	343	198	114	31	3.67	2.41, 5.58	1.48	1.12, 1.96
McKay, 2010 (16)	437	220	175	42	436	270	139	27	1.91	1.14, 3.2	1.55	1.16, 2.05
Park, 2009 (27)	1,216	668	477	71	296	196	92	8	2.6	1.23, 5.5	1.52	1.16, 2
Reynolds, 2009 (29)	97	44	41	12	58	36	19	3	3.27	0.86, 12.49	1.77	0.88, 3.56
Scholl, 2009 (30)	97	54	35	8	584	394	176	14	4.17	1.67, 10.4	1.45	0.92, 2.3
Scholl, 2008 (31)	112	68	35	9	67	50	16	1	6.62	0.81, 53.93	1.61	0.80, 3.22
Seitsonen, 2008 (33)	151	101	44	6	105	76	26	3	1.5	0.36, 6.21	1.27	0.72, 2.25
Spencer, 2008 (34)	701	353	289	59	286	178	95	13	2.29	1.22, 4.28	1.53	1.14, 2.06
Yates, 2007 (35)	1,079	561	438	80	678	438	212	28	2.23	1.43, 3.49	1.61	1.31, 1.98
Pooled odds ratio[f]	6,803				5,967				1.88	1.59, 2.23	1.44	1.33, 1.56
Cui, 2010 (21)[g]	150	147	3	0	161	160	1	0	1.09	0.02, 55.19	3.27	0.34, 31.74
Pei, 2009 (28)[g]	123	120	3	0	130	126	4	0	1.05	0.02, 53.33	0.79	0.17, 3.59
Liu, 2010 (40)[g]	238	230	8	0	220	216	4	0	0.94	0.02, 47.55	1.95	0.58, 6.56
Pooled odds ratio	511				511						1.57	0.67, 3.66

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR_1 and OR_2, degree of heterogeneity for each polymorphism separately for 2 odds ratios; HWE, Hardy-Weinberg equilibrium.

[a] Rotterdam cohort.

[b] Case-control design.

[c] Not included in pooling because of departure from HWE.

[d] Age-related Eye Disease Study.

[e] Casey Eye Institute Macular Degeneration Center.

[f] $\lambda = 0.71$ (95% CI: 0.37, 0.98).

[g] Continuing correction by adding 0.5 in all cells for OR_1.

was worse in a subgroup of 4 studies where the mean age was less than 74.3 years ($I^2 = 79.3\%$) but decreased to $I^2 = 0$ where the mean age was greater than 74.3 years. The OR_1 and OR_2 were 1.48 (95% CI: 1.15, 1.92) and 1.25 (95% CI: 1.10, 1.4) for the younger group and 1.97 (95% CI: 1.46, 2.64) and 1.54 (95% CI: 1.33, 1.77) for the older age group.

Publication bias was assessed in both OR_1 and OR_2 by using Egger's test and suggested no evidence of publication bias for either odds ratio (coefficient = 1.50, $P = 0.098$ for OR_1; coefficient = 1.68, $P = 0.060$ for OR_2). Contour-enhanced funnel plots (Figure 3, A and B) indicated that some studies were in significant areas (i.e., from $P < 0.01$ to $P < 0.05$), and some studies were within the nonsignificant area for both odds ratios. ''Metatrim'' analysis suggested that 4 and 6 nonsignificant studies were missing, and analysis adjusting for the presumed missing studies resulted in OR_1 and OR_2 of 1.54 (95% CI: 0.83, 2.84) and 1.16 (95% CI: 0.86, 1.57), respectively.

rs1047286

Seven studies, 5 in Caucasians (22, 23, 27, 34, 35) and 2 in Asians (21, 40), reported association between rs1047286 and AMD. Minor A-allele frequencies ranged from 0.172 to 0.220 for Caucasians and from 0.003 to 0.005 for Asians (Table 5). One case-control study by Despriet et al. (22) did not observe HWE and thus was not included in pooling. Among 5 Caucasian studies, the pooled prevalence of the A allele was 0.196 (95% CI: 0.181, 0.211) and was similar when including the study not observing HWE. The pooled prevalence of 0.004 (95% CI: 0.002, 0.005) was very rare in Asians.

Genotype odds ratios for studies in Caucasians are described in Table 6. There was moderate-to-high heterogeneity observed for OR_1 (AA vs. GG) (chi-square = 5.85, df = 4; $P = 0.211$; $I^2 = 31.6\%$) and OR_2 (GA vs. GG) (chi-square = 22.78, df = 4; $P < 0.001$; $I^2 = 82.4\%$) (Figure 4, A and B). A mixed-random logit model suggested that overall

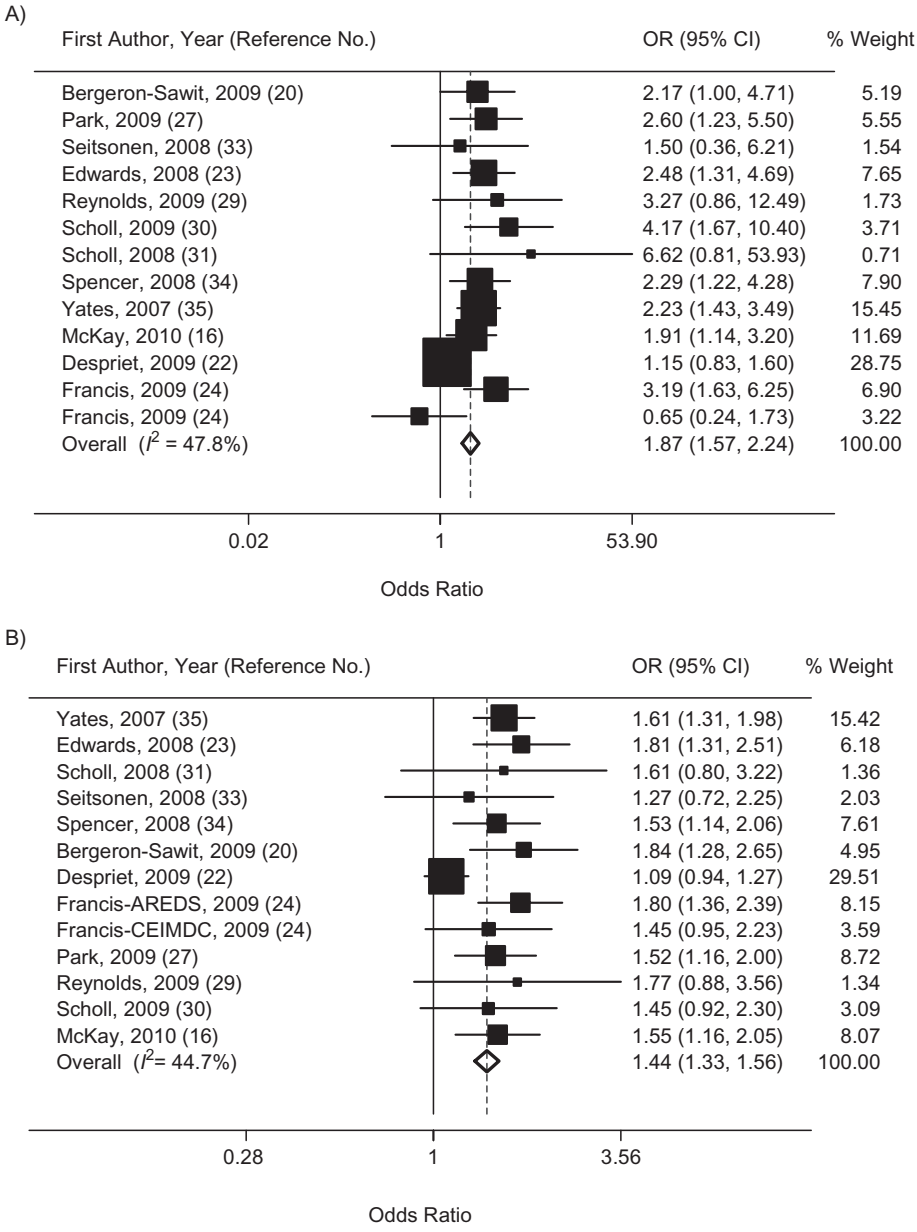

Figure 2. Forest plots for rs2230199. Individual and pooled odds ratio estimates for GG versus CC (part A) and GC versus CC (part B). The size of each square is proportional to the percent weight that each study contributed in the pooled odds ratio. The pooled odds ratio is indicated by the diamond. AREDS, Age-related Eye Disease Study; CEIMDC, Casey Eye Institute Macular Degeneration Center; CI, confidence interval (horizontal lines); OR, odds ratio.

genotype effects were present (likelihood ratio = 37.60; $P < 0.001$). The pooled OR_1 and OR_2 were 1.70 (95% CI: 1.37, 2.11) and 1.27 (95% CI: 1.15, 1.41), respectively (i.e., having AA and GA genotypes was associated with 70% and 27% significantly higher odds of having AMD). The estimated λ was 0.63 (95% CI: 0.24, 0.97), indicating that an additive mode of effect was more likely.

Sensitivity analysis, which included the study not observing HWE (22), resulted in little change of the observed genetic effect. The OR_1 and OR_2 were 1.69 (95% CI: 1.37, 2.08) and 1.30 (95% CI: 1.18, 1.44) with a λ of 0.69 (95% CI: 0.34, 0.98). Adjusting genotype frequencies for the inbreeding coefficient also resulted in similar genetic effects (i.e., corresponding odds ratios were 1.65 (95% CI: 1.35, 2.02) and 1.29 (95% CI: 1.17, 1.43) with a λ of 0.68 (95% CI: 0.32, 0.98)).

Because heterogeneity was reduced for OR_1 (AA vs. GG), exploring the cause of heterogeneity was performed only for OR_2 (GA vs. GG). Pooling within 2 studies where cases were classified as advanced AMD yielded less heterogeneous gene

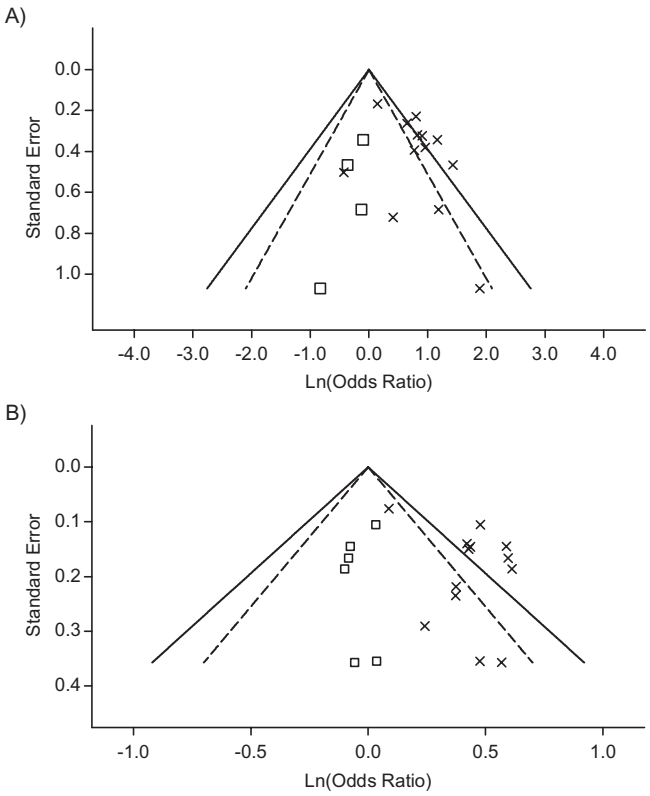

Figure 3. Contour-enhanced funnel plots for rs2230199 showing AA versus GG (part A) and GA versus GG (part B). These plots indicate that some studies were in significant areas where $P < 0.01$ (solid lines) and where $P = 0.01$–<0.05 (dashed lines); other studies were in the nonsignificant area (the area between the 2 dashed lines). Hollow squares refer to filled studies, and X's refer to included studies.

effects ($I^2 = 49.7\%$) but not for pooling the 3 studies with mixed early and advanced AMD ($I^2 = 88.7\%$). The OR_1 and OR_2 in the advanced cases were 1.98 (95% CI: 1.27, 3.09) and 1.51 (95% CI: 1.22, 1.88), respectively. The pooled gene effects were diluted where cases were mixed early and advanced AMD, with corresponding odds ratios of 1.62 (95% CI: 1.26, 2.07) and 1.21 (95% CI: 1.07, 1.36). Only 2 studies reported mean age, smoking, and percent male, and this was insufficient to explore sources of heterogeneity.

There was no evidence of publication bias in the pooled estimate of the 5 Caucasian studies for either odds ratio (coefficient = 2.71, $P = 0.173$ for OR_1; coefficient = 6.91, $P = 0.075$ for OR_2). Figure 5, A and B, displays the contour-enhanced funnel plots for OR_1 and OR_2. Some studies lie in the significant areas (i.e., from $P < 0.01$ to $P < 0.05$) for OR_1 and OR_2, while some of the studies fall in nonsignificant areas (white area, $P > 0.05$). Two studies were nonsignificant for both odds ratios and showed moderate-to-high standard errors with moderate gene effects. Results of "metatrim" analysis suggested 3 and 2 missing studies for the OR_1 and OR_2, respectively. Adjustment for missing studies resulted in nonsignificance for both odds ratios ($OR_1 = 1.36$, 95% CI: 0.99, 1.88; $OR_2 = 1.19$, 95% CI: 0.93, 1.53).

DISCUSSION

We have performed a systematic review and meta-analysis of associations between *C3* rs2230199 and rs1047286 SNPs and AMD, respectively, including 13,792 (12,770 Caucasians, 1,022 Asians) and 8,887 (8,118 Caucasians, 769 Asians) subjects for each SNP, respectively. The results suggest robust associations in Caucasians (i.e., carrying GG and GC genotypes for rs2231099 would increase risks of AMD by approximately 88% and 44% compared with the CC genotype). The risk of AMD was similar for rs1047286 (i.e., those with AA and GA genotypes were 70% and 27% more likely to have AMD compared with the GG genotype). The genetic mode of action could be additive or dominant for both SNPs. Sensitivity analyses including and excluding studies not observing HWE or adjusted for departure from HWE yielded consistent results.

The genetic effects for rs2230199 were moderately heterogeneous for both GG and CG groups compared with the CC genotype group. Candidate sources of heterogeneity (AMD case subphenotype, age, and percent smoking) were explored. Performing subgroup analyses based on advanced cases yielded only more homogeneous genetic effects than with mixed early and advanced cases together. In addition, the genetic effects were clearer (i.e., stronger, in the advanced cases than in the mixed cases; GG and CG genotypes carried 2.3 and 1.7 times higher risks of advanced AMD than did CC genotypes with corresponding risks of 1.6 and 1.3 in the mixed cases). This is perhaps understandable given that not all cases with intermediate levels of pathology will progress to the visually disabling advanced forms of AMD. It is also evident that the mixing of advanced and intermediate disease cases leads to a dilution of the genetic effect observed.

The observed genetic effects were reasonably consistent in both magnitude and direction between the pooled group and within the subgroups of age and smoking, although heterogeneity was still present in some subgroups (i.e., age, <74.4 years; percent smoke, >54.4%). In addition, we also found a paradoxical age effect (i.e., gene effects were higher in those aged >74.4 years than in those aged <74.4 years). It might be expected that those with a genetic predisposition to AMD might manifest earlier, although this might be as a result of better phenotyping in the older group or a different pathophysiologic mechanism in the younger group. Indeed, inclusion of younger cases may encompass macular dystrophies with pathologic presentation similar to that of AMD, rather than a purely age-related disorder with different but overlapping underlying genetics. The observed genetic effects also differ on the basis of ethnicity as a result of much lower minor G-allele frequencies in Asians with respect to those originating from a European background (i.e., 0.007 (95% CI: 0.001, 0.013) vs. 0.193 (95% CI: 0.181, 0.205), respectively). Unfortunately, there were only 3 Asian studies (21, 28, 40) and, as a consequence, estimation of genetic effects yielded very large confidence intervals (i.e., $OR_1 = 1.03$, 95% CI: 0.00, 150.11; $OR_2 = 1.55$, 95% CI: 0.22, 10.62).

The commonality of the risk alleles for both SNPs in Caucasians (19.3%–19.6%) is indicative of an important effect at a population level. The proportions of GG/CG

Table 5. Allele Frequencies of rs1047285 Polymorphism in Control Groups of Studies Included in the Meta-Analysis, 2007–2010

First Author, Year (Reference No.)	No. of Alleles	A Allele		G Allele		HWE *P* Value
		Frequency, no.	Allele Prevalence	Frequency, no.	Allele Prevalence	
Liu, 2010 (40)	440	2	0.005	438	0.995	0.950
Cui, 2010 (21)	322	1	0.003	321	0.997	1.000
Pooled prevalence			0.004 (0.002, 0.005)[a]		0.996 (0.995, 0.998)	
Despriet, 2009 (22)[b]	4,838	989	0.204	3,849	0.796	0.532
Despriet, 2009 (22)[c,d]	332	62	0.187	270	0.813	0.039
Park, 2009 (27)	582	100	0.172	482	0.828	1.000
Edwards, 2008 (23)	598	104	0.174	494	0.826	0.067
Spencer, 2008 (34)	572	126	0.220	446	0.780	1.000
Yates, 2007 (35)	1,352	269	0.199	1,083	0.801	0.468
Pooled prevalence			0.196 (0.181, 0.211)		0.804 (0.789, 0.819)	
Overall pooled prevalence			0.139 (0.067, 0.210)		0.861 (0.790, 0.933)	

Abbreviation: HWE, Hardy-Weinberg equilibrium.
[a] Numbers in parentheses, 95% confidence interval.
[b] Rotterdam cohort.
[c] Case-control design.
[d] Not included in pooling because of departure from HWE.

and AA/GA genotypes for the former and the latter SNPs in the control population are about 34%, and the pooled odds ratios for these genotypes are 1.49 and 1.32, respectively. The population attributable risks for the combined genotypes GG/CG and AA/GA are 8.6% and 5.9% (i.e., the *C3* polymorphisms at rs2230199 and rs1047286 contribute about 5%–9% of all AMD in Caucasians, although the effect is not as high as that of the *CFH* Y402H polymorphism, in which the population attributable risk was 58.9% for carrying the C risk allele (12).

These results are robust given our meticulous methodology. We pooled only genetic association studies making no assumptions about genetic models (49). We avoided multiple comparisons by doing 1 overall test of the genetic association (63) and checked for HWE in the control groups. Sensitivity analysis was performed by pooling studies with and without observance of HWE and adjustment for HWD before pooling. Genetic effects were consistent for all Caucasian studies. Egger's test was used to assess publication bias resultant from small study effects because such studies tend to overestimate effect sizes compared with large studies, or they tend to be less publishable because of unfavorable gene effects (54, 55). Although the test did not suggest small-study effects for any odds ratios, contour-enhanced

Table 6. Genotype Frequencies of rs1047286 Polymorphism in AMD and Control Groups of Studies Included in the Meta-Analysis, 2007–2010

First Author, Year (Reference No.)	AMD				Non-AMD				AA Versus GG		GA Versus GG	
	No. of Subjects	GG	GA	AA	No. of Subjects	GG	GA	AA	OR_1	95% CI	OR_2	95% CI
Despriet, 2009 (22)[a]	1,926	615	295	53	4,838	1,536	777	106	1.25	0.89, 1.76	0.95	0.81, 1.12
Despriet, 2009 (22)[b,c]	662	179	127	25	332	114	42	10	1.59	0.74, 3.44	1.93	1.26, 2.93
Park, 2008 (27)	880	243	159	38	598	209	76	14	2.33	1.23, 4.43	1.8	1.29, 2.5
Edwards, 2009 (23)	2,426	682	471	60	582	199	84	8	2.19	1.03, 4.65	1.64	1.24, 2.17
Spencer, 2008 (34)	1,402	374	276	51	572	174	98	14	1.69	0.91, 3.14	1.31	0.98, 1.76
Yates, 2007 (35)	1,660	439	324	67	1,352	437	209	30	2.22	1.42, 3.49	1.54	1.24, 1.92
Pooled odds ratio[d]									1.70	1.37, 2.11	1.27	1.15, 1.41
Liu, 2010 (40)[e]	238	236	2	0	220	218	2	0	0.92	0, 2,387.72	0.92	0.13, 6.61
Cui, 2010 (21)[e]	300	148	2	0	322	160	1	0	1.08	0, 2,816.13	2.16	0.19, 24.09

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR_1 and OR_2, degree of heterogeneity for each polymorphism separately for 2 odds ratios.
[a] Rotterdam cohort.
[b] Case-control design.
[c] Not included in pooling because of departure from HWE.
[d] $\lambda = 0.63$ (95% CI: 0.24, 0.97).
[e] Continuing correction by adding 0.5 in all cells for OR_1.

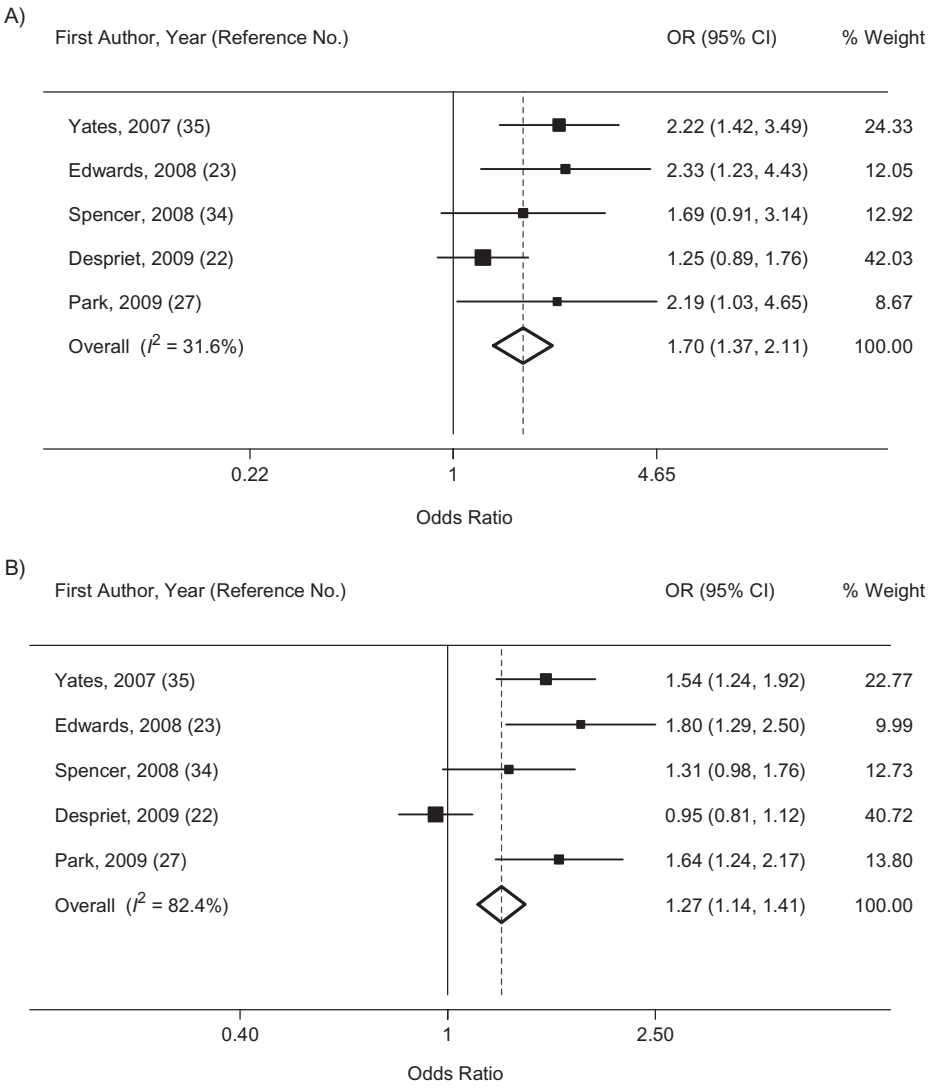

Figure 4. Forest plots for rs1047286. Individual and pooled odds ratio estimates for AA versus GG (part A) and GA versus GG (part B). The size of each square is proportional to the percent weight that each study contributed in the pooled odds ratio. The pooled odds ratio is indicated by the diamond. CI, confidence interval (horizontal lines); OR, odds ratio.

funnel plots indicated 2–6 missing studies for both SNPs. Regression-based adjustment was applied to estimate the gene effects adjusting for presumed missing or unidentified studies, and the gene effects were still present.

The level of evidence for our meta-analysis was graded following recommendations published by Ioannidis et al. (44). Three components were assessed: amount of evidence, replication, and protection of bias. The amount of evidence was categorized as level A, B, or C if the total number of minor genotypes was >1,000, 100–1,000, or <100, respectively. Our meta-analysis had minor GG and AA genotype numbers of 730 and 441 for rs2230199 and for rs1047286, respectively. The evidence should thus be categorized as moderate B level. The degree of heterogeneity and P value were used to assess replication. The I^2 of our pooled values was 44.7%–47.8% for rs2230199 and 31.6%–82.4% for rs1047286. P values for the overall genetic effect tests of these corresponding SNPs were 3.98×10^{-25} and 6.84×10^{-9}, which were far less than the recommended threshold for replication of both genome-wide ($P < 10^{-8}$) and genetic association ($P < 0.05$) studies. Replication for our meta-analysis was therefore categorized as strong. For protection of bias, the process of study selection and flow of results were clearly described and, thus, selection bias should be minimized. Only studies observing HWE were included in the main pooling, limiting potential bias due to genotyping error or population stratification. All included studies clearly described the differential diagnosis of AMD and also control subjects, resulting in less biased outcome measures. In addition, there was no evidence of publication bias due to

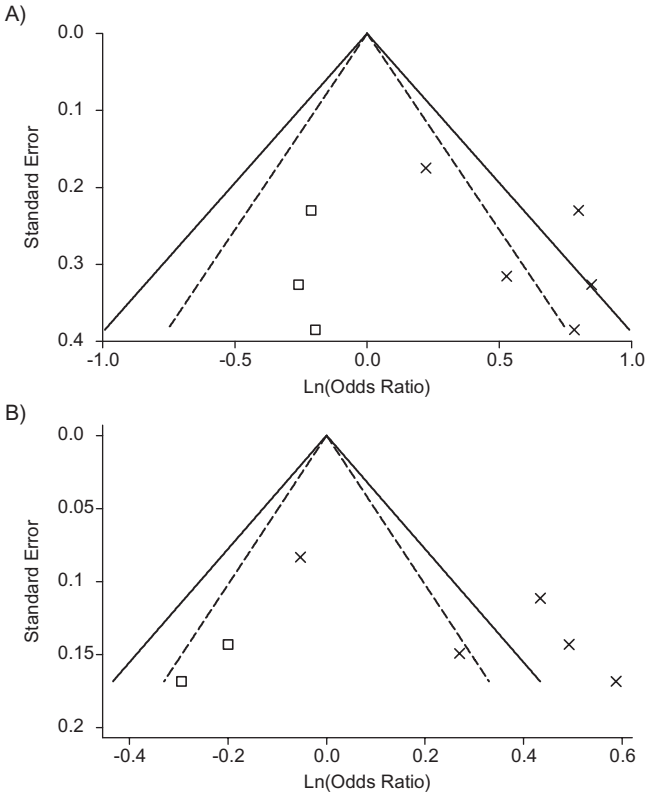

Figure 5. Contour-enhanced funnel plots for rs1047286 showing GG versus CC (part A) and GC versus CC (part B). These plots indicate that some studies were in significant areas where $P < 0.01$ (solid lines) and where $P = 0.01$–<0.05 (dashed lines); other studies were in the nonsignificant area (the area between the 2 dashed lines). Hollow squares refer to filled studies, and X's refer to included studies.

small study effects. Risk of bias for our meta-analysis should be minimized and, thus, categorized as level A. Combining the 3 components yields a level of evidence of BAA.

Given the high linkage disequilibrium between both loci (r^2 ranged from 0.80 to 0.85) (22, 27, 34), it is likely that the 2 SNPs are capturing the same prognostic information. However, because of lack of individual-level data, we were unable to assess the haplotype effects of these 2 loci together.

In summary, this meta-analysis provides robust evidence for an association between *C3* and AMD, and it provides estimates for carriage of risk alleles for rs2230199 and rs1047286 resulting in increased odds of AMD by ~1.8-fold in Caucasians. The low minor allele frequency of these variants within Asian populations suggests that the effect of these SNPs at a population-based level is greatly diminished, although our results suggest a nonsignificant overlap in the effect size and direction with that observed within Caucasians. That said, variation in haplotype structure according to ethnicity across the *C3* gene is not assessed by this study, and the consequence of perturbation of the C3 protein and its contribution to AMD on the Asian continent cannot be dismissed. We contend that the polymorphic variability in this gene contributes approximately 5%–10% of all AMD in Caucasian populations.

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(Appendix follows)

APPENDIX

The search strategy for Embase (Elsevier) comprised the following:

1. Gene
2. Allele
3. Polymorphism
4. Macular degeneration
5. "Complement component 3"
6. "Complement factor 3"
7. C3
8. "Complement component 2"
9. "Complement factor 2"
10. C2
11. (1 *or* 2 *or* 3)
12. (5 *or* 6 *or* 7)
13. (8 *or* 9 *or* 10)
14. 11 *and* 4 *and* (12 *or* 13)

Appendix Table 1. Risk of Bias Assessment for Genetic Association Studies of AMD of Studies Included in the Meta-Analysis, 2007–2010

Domain and Item	Low Risk of Bias
Information bias	
Ascertainment of AMD	
Clearly described objective criteria of diagnosis of AMD	Yes
Not clearly described	No
Did not mention	Unclear
Ascertainment of controls	
Controls were non-AMD proved by ocular examination	Yes
Just mentioned that controls were subjects who did not have AMD without ocular examination	No
Not described	Unclear
Ascertainment of genotyping examination	
Genotyping done under "blind" conditions of case specimens and control specimens	Yes
Genotyping of cases and controls was performed together	Yes
Genotyping error rate $< 5\%$	Yes
Quality control procedure (e.g., reanalysis of random specimens, by using different genotyping methods for analysis, analysis if replicate sample)	Yes
Unblind	No
Genotyping error rate $> 5\%$	No
Did not mention what was done	Unclear
Confounding bias	
Population stratification	
No difference in ethnic origin between cases and controls	Yes
Use of controls who were not related to cases/use of genomic controls	Yes
Use of some controls who came from the same family	No
No report of what was done	Unclear
Other confounding bias	
Controls for confounding variables (e.g., age, gender, smoking) in analysis	Yes
Not controlled for confounding variables	No
Not mentioned	Unclear
Selective reporting (for replication studies)	
Reported results of all polymorphisms mentioned in the objectives, nonsignificant or not	Yes
Reported results of only significant polymorphisms	No
HWE	
HWE in the control group	Yes
HWD in the control group	No
HWE not checked	No

Abbreviations: AMD, age-related macular degeneration; HWD, Hardy-Weinberg disequilibrium; HWE, Hardy-Weinberg equilibrium.