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Genetic associations with brain microbleeds

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Genetic associations with brain microbleeds

Systematic review and meta-analyses

ABSTRACT

Objective: We performed a systematic review and meta-analyses to assess the evidence for genetic associations with brain microbleeds (BMBs).

Methods: We sought all published studies of the association between any genetic polymorphism and BMBs studied in a total of >100 people. We critically appraised studies, and calculated pooled odds ratios (ORs) using the generic inverse variance fixed effects method. We used I^2 and χ^2 statistics to assess heterogeneity, and fail-safe N estimates to assess the robustness of our results.

Results: Only the *APOE* ε2/3/4 polymorphism had been studied in >100 people (10 studies, 7,351 participants). Compared with people with the ε3/ε3 genotype, carriers of the ε4 allele (ε4+) were statistically significantly more likely to have BMBs in any location (ε4+ vs ε3/ε3: pooled OR 1.22, 95% confidence interval [CI] 1.05–1.41, *p* = 0.01). For strictly lobar BMBs, this association appeared slightly stronger (ε4+ vs ε3/ε3: pooled OR 1.35, 95% CI 1.10–1.66, *p* = 0.005). The association of ε4+ genotypes with strictly lobar BMBs was reasonably robust to potential publication and reporting biases.

Conclusions: Given the known associations of *APOE* alleles with lobar intracerebral hemorrhage and cerebral amyloid angiopathy, these findings support the concept that strictly lobar BMBs may be an imaging biomarker of cerebral amyloid angiopathy. *Neurology*® **2011;77:158–167**

GLOSSARY

BMB = brain microbleed; **CAA** = cerebral amyloid angiopathy; **CI** = confidence interval; **GRE** = gradient-recalled echo; **HWE** = Hardy-Weinberg equilibrium; **ICH** = intracerebral hemorrhage; **OR** = odds ratio.

Brain microbleeds (BMBs) are minute deposits of blood products, seen as focal areas of signal loss typically <10 mm in diameter on haem-sensitive T2*-weighted gradient-recalled echo (GRE) MRI sequences. BMBs occur in 5% to 38% of apparently healthy people, increasing in frequency with age, hypertension, and smoking.[1-3] They are particularly prevalent (>50%) in people with a history of intracerebral hemorrhage (ICH).[1] Lobar BMBs are considered a diagnostic marker of cerebral amyloid angiopathy (CAA),[4] which appears to be the major cause of lobar, sometimes recurrent ICH in the elderly.[5]

Understanding the vascular pathology underlying BMBs should be enhanced by identifying genetic risk factors. There is particular interest in whether *APOE* genotype might confer susceptibility to BMBs. The *APOE* gene on chromosome 19 has 3 common alleles—ε2, ε3, and ε4—giving rise to 6 different genotypes: ε2/2, ε2/3, ε2/4, ε3/3 (the wild type, occurring in just under two-thirds of people in most populations[6]), ε3/4, and ε4/4. Associations of the *APOE* ε2 or ε4 alleles with both ICH (particularly lobar ICH) and CAA have been described,[7-11] and strictly lobar BMBs have now been included in the modified Boston diagnostic criteria for CAA.[4] We would therefore expect an association between these alleles and BMBs, especially those in a strictly lobar location.

We therefore carried out a systematic review of the literature on genetic variants associated with BMBs and, where appropriate, meta-analyses of the relationship between polymorphisms in any gene (including *APOE*) and BMBs.

METHODS **Search strategy and selection criteria.** We sought articles published in full in any language, obtaining translations where necessary, which studied an association between any genetic polymorphism and BMBs detected on MRI in humans. We used comprehensive electronic search strategies (most recent search date July 2010) including a list of 13 different medical subject heading and text word terms for BMBs and 13 and 17 different medical subject heading and text word terms for genes or polymorphisms in Medline (from 1966) and Embase (from 1980) respectively to identify relevant articles (appendix). Two authors (S.S.M. and L.P.) read the titles, abstracts, and, where necessary, full text of all identified studies to identify potentially relevant studies, resolving any uncertainties with a third author (C.L.M.S.). At least 2 authors (S.S.M. and one or both of C.A.J. and C.L.M.S.) read in full all articles selected as potentially relevant and, for each genetic polymorphism, determined the number of independent studies and the total number of participants included. Two authors (S.S.M. and L.P.) hand-searched the reference lists of all included studies and any related review articles to identify relevant studies missed in the initial search. Where a polymorphism had been studied in a total of >100 participants, we selected the relevant studies for detailed methodologic assessment and meta-analyses. We decided not to review data for polymorphisms studied in ≤100 participants since conclusions drawn on the basis of such small numbers would be likely to be unreliable.

Data extraction. For each selected study, we extracted information on year of publication; country in which the study was conducted; types of participants studied; number, ethnicity, and mean age of participants; genotyping method; whether genotypes were stated to be in Hardy-Weinberg equilibrium (HWE); and blinding of scanning staff to genotypes and of genotyping staff to scan results. We also extracted information on the quality and features of the brain imaging methods used. These included the criteria specified to identify a BMB (both appearance and size), the awareness of potential BMB mimics, the MRI sequence used, the number of MRI scan readers, and any available data on interobserver and intraobserver agreement of BMB rating. We assessed each study against a checklist of key quality indicators (clearly defined BMB criteria, awareness of ≥2 BMB mimics, ≥2 BMBs raters, raters independent, interobserver and intraobserver agreement recorded, genotypes stated to be in HWE, blinding of BMB raters to genotype and genotyping staff to BMB status, adjustment of results for other risk factors) that we developed from the above methodologic, imaging, and laboratory criteria and with reference to both the Strengthening the Reporting of Genetic Association Studies statement[12] and the ideal characteristics for a study of BMBs.[1] However, we did not include or exclude studies on the basis of these quality indicators.

Two authors (S.S.M. and C.A.J.) independently extracted published data from each study on the number of participants with at least one BMB for each genotype or group of genotypes, or published crude or adjusted odds ratios (ORs) derived from these data. S.S.M. and C.A.J. discussed and resolved any disagreements with a third author (C.L.M.S.). For *APOE* studies,

where possible, we subdivided the participants into those with an ε2-containing (ε2+), ε4-containing (ε4+), or ε3/3 genotype, and excluded the small proportion with ε2/4 genotypes, allowing the most consistent treatment of data across studies. Where available, we extracted data on the relationship between *APOE* and BMBs in either strictly lobar or not strictly lobar distributions, assigning participants with BMBs restricted to a lobar location to the strictly lobar group and all others with BMBs to the not strictly lobar group. If studies had not published these data, we approached the corresponding author for additional unpublished data on location-specific BMB-genotype associations in an effort to include all available data in our meta-analyses. If the same participants had been included in more than one publication, we only included those from the publication with the larger number of participants.

Statistical analysis. We performed meta-analyses using Cochrane RevMan (version 4.2) software. We describe here methods used for analyses of the *APOE* ε2/3/4 polymorphism since this turned out to be the only polymorphism studied in >100 participants. Where studies provided the necessary data, we calculated directly study-specific ORs for the presence vs absence of BMBs, comparing participants with an ε2+ or ε4+ genotype vs the ε3/3 genotype, and otherwise used the relevant published crude or adjusted ORs. We used the generic inverse variance fixed effect meta-analysis method to obtain pooled ORs, weighting each study by the inverse of the square of the standard error of its study-specific OR. Where both crude and adjusted ORs were available for a particular study, we used the crude OR for meta-analysis, and compared the crude and adjusted ORs to assess for the possibility of confounding. Unless stated otherwise, we considered a *p* value of <0.05 to be statistically significant.
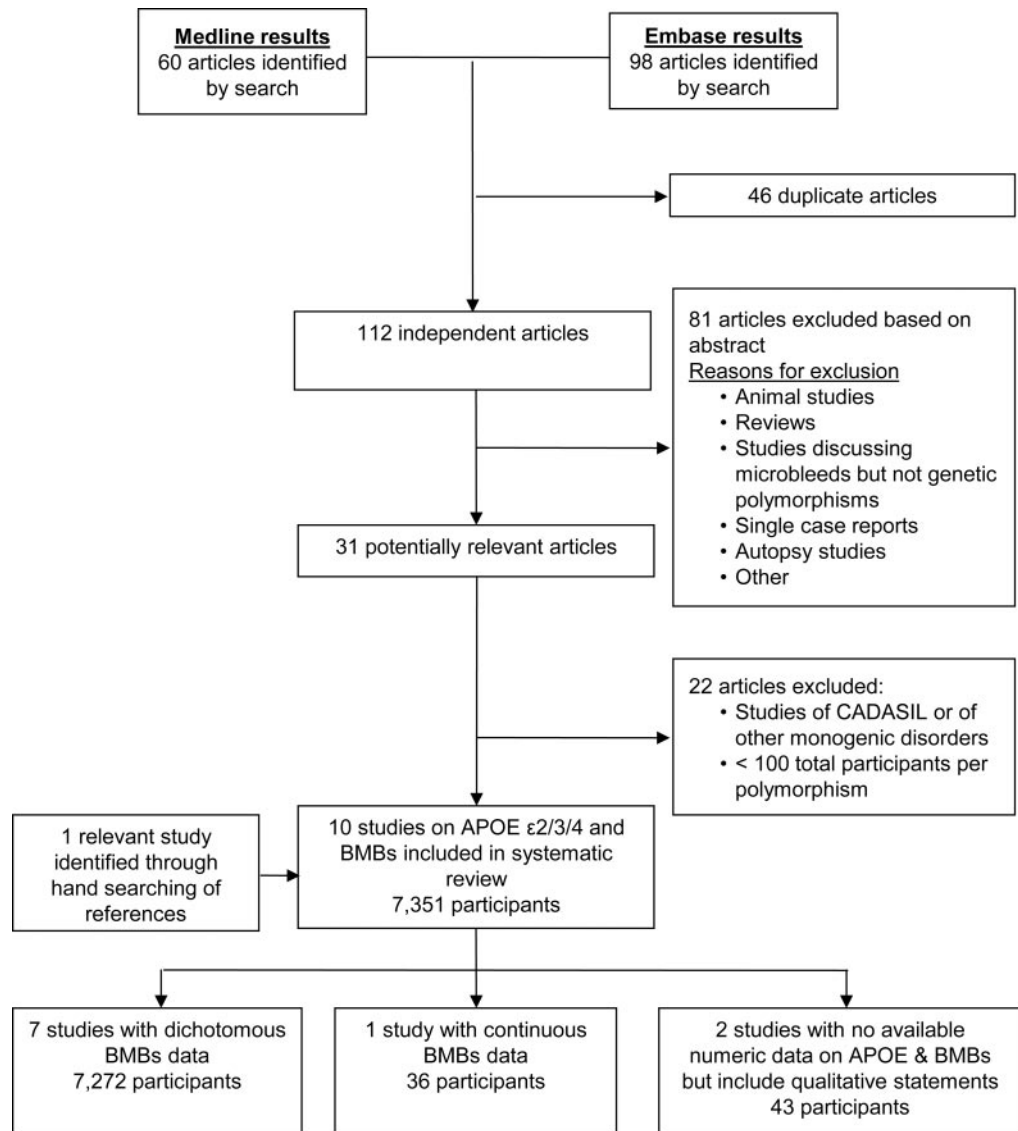
We assessed statistical heterogeneity between studies using both the χ^2 test and the I^2 statistic.[13] We performed prespecified subgroup analyses based on study size (above or below the mean study size), ethnicity, and type of study population (healthy individuals compared with those recruited on the basis of existing neurologic conditions), to explore potential modification of effect by these factors, assessing differences between subgroups with χ^2 statistics. We also assessed the effects of genotype on strictly lobar and not strictly lobar BMBs separately.

To assess the potential effect on our results of unpublished negative studies or studies not reporting or able to provide the data required for our meta-analyses (i.e., the potential for publication and reporting biases), we calculated the "fail-safe N" for any meta-analysis which produced a significant result (*p* < 0.05). We used a modification of Rosenthal's method,[14] in which we determined the size of a notional study with a null result (OR = 1) required to bring any significant result to a just nonsignificant level (*p* = 0.05), assuming the overall prevalence of BMBs and the distribution of genotypes in this notional study to be the average of these for the studies included. Since we carried out several tests of significance, we repeated our fail-safe N calculations based on a statistical significance cutoff of *p* = 0.01.

We prepared this report with reference to the Meta-analysis of Observational Studies in Epidemiology reporting guidelines.[15]

RESULTS We identified 112 articles in our initial search of Medline and Embase, of which 31 were potentially relevant to this systematic review. Only the *APOE* ε2/3/4 polymorphism had been studied in a total of >100 participants. Ten studies assessed the association between *APOE* ε2/3/4 genotypes and

Figure 1   Study selection and exclusion


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┌─────────────────────────┐        ┌─────────────────────────┐
│  Medline results        │        │  Embase results         │
│  60 articles identified │        │  98 articles identified │
│  by search              │        │  by search              │
└─────────────────────────┘        └─────────────────────────┘
```

46 duplicate articles

112 independent articles

81 articles excluded based on abstract
Reasons for exclusion
• Animal studies
• Reviews
• Studies discussing microbleeds but not genetic polymorphisms
• Single case reports
• Autopsy studies
• Other

31 potentially relevant articles

22 articles excluded:
• Studies of CADASIL or of other monogenic disorders
• < 100 total participants per polymorphism

1 relevant study identified through hand searching of references

10 studies on APOE ε2/3/4 and BMBs included in systematic review
7,351 participants

7 studies with dichotomous BMBs data
7,272 participants

1 study with continuous BMBs data
36 participants

2 studies with no available numeric data on APOE & BMBs but include qualitative statements
43 participants

BMB = brain microbleed; CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

BMBs in a total of 7,351 participants (figure 1). Seven studies which included 7,272 participants (99% of the total) presented dichotomous data for BMBs (i.e., presence vs absence per genotype or group of genotypes), enabling their results to be pooled in meta-analyses.[2,3,16-21] The remaining 3 presented continuous data (i.e., mean number of BMBs per genotype) or purely qualitative statements about the association between *APOE* and BMBs.[22-24]

Study characteristics. Study populations comprised either healthy people from the general population (3 studies, 5,977 participants) or those with neurologic (mainly cerebrovascular) conditions (7 studies, 1,374 participants). Most participants were of European origin, but one study was conducted in Asians.[17] Participants were middle-aged to elderly (mean age per study

ranged from 45 to 76 years). Studies had a mean of 735 (range 20 to 3,689) participants. Prevalence of BMBs in the study populations varied from 5% in the Framingham and Framingham offspring population-based cohort to 65% in a population of people hospitalized with intracerebral hemorrhage (table 1).

Imaging characteristics varied between the studies. BMBs were typically defined as rounded focal areas of signal loss, but the size criteria varied. All studies but one[17] considered BMB mimics, most commonly calcification in the basal ganglia (although none of the studies appeared to use CT to investigate this) (table e-1 on the *Neurology*® Web site at www.neurology.org). The larger studies generally fulfilled more of our quality indicators for a study on BMB genetics than smaller studies, but none fulfilled all 10 (table 2). The studies with

Table 1 Characteristics of included studies

Study references	Types of participants	Total study population[a]/no. scanned and genotyped/no. of these with BMBs[b]	Country	Ethnicity[c]	% Male[d]	Mean age, y[d]
Studies included in meta-analysis						
16	Population-based; participants from the Framingham original and offspring cohorts	472/368/17[b]	USA	Mostly white	45[e]	61[e]
17	Patients with "neurologic abnormalities" (70% stroke or TIA, others vertigo, headache, parkinsonism, dementia) requiring MRI	414/414/117	Korea	Korean	52	66
18	Memory clinic patients attending a Dutch university Alzheimer center 2002–2005	772/438/62	Netherlands	Assumed white/ Dutch	53[e]	66[e]
19	Patients admitted to a German teaching hospital with nontraumatic primary ICH, 1997–2000	193/101/65[b]	Austria	Central European	NR	68[e]
20	Patients ≥50 years with TIA or ischemic stroke admitted to hospital, 2003–2005	342/342/89	Belgium	Assumed white/ Belgian	62	71
2	Population-based; participants born in Reykjavik 1907–1935 who participated in the Reykjavik study in 1967 and survived to be reexamined in 2002	1,962/1,920/214	Iceland	Assumed white/ Icelandic	42[e]	76[e]
3, 21	Population-based; participants living in Rotterdam	3,979/3,689/565	Netherlands	Assumed white/ Dutch	46[e]	60[e]
Studies with missing or incompatible data not included in meta-analysis						
22	DNA-proven HCHWA-D mutation carriers	27/20/13[b]	Netherlands	Assumed white/ Dutch	52[e]	49[e]
24	DNA-confirmed CADASIL *NOTCH 3* mutation carriers	36/36/?	Netherlands	Assumed white/ Dutch	47	45
23	Patients with primary ICH who fulfilled their criteria for CAA (1: age ≥55 y; 2: lobar primary ICH with exclusion of other causes)	50/23/19	Portugal	Assumed white/ Portuguese	50[e]	72[e]

Abbreviations: BMB = brain microbleed; CAA = cerebral amyloid angiopathy; CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; HCHWA-D = Hereditary Cerebral Haemorrhage with Amyloidosis–Dutch type; ICH = intracerebral hemorrhage; NR = not reported.

[a] As described under "types of participants."
[b] Estimated from published % where necessary, as marked.
[c] Assumed indicates ethnicity not directly stated.
[d] Of number of participants scanned and genotyped, unless stated otherwise.
[e] Mean age and % male of total study population.

data unavailable or unsuitable for our meta-analyses were much smaller in size than the included studies and performed less well against our methodologic quality indicators (table 2).[22-24] The largest study was of high quality and contributed over half of the participants included in the meta-analyses.[3,21] Where both crude and adjusted ORs were available, they were similar.[17,20]

Association between *APOE* ε2/3/4 polymorphism and BMBs. In the 7 studies with dichotomous data on BMBs, pooled results showed that, compared with people with an ε3/3 genotype, those with an ε4+ genotype had an increased odds of having one or more BMBs (OR 1.22, 95% confidence interval [CI] 1.05–1.41, $p = 0.01$; figure 2A). There was no difference between the odds of having BMBs among those with an ε2+ vs those with an e3/3 genotype (OR 1.14, 95% CI 0.94–1.40, $p = 0.2$; figure 3A). There was no evidence from χ^2 or I^2 statistics of any

heterogeneity between the studies' results for either of these genotype group comparisons (figures 2A and 3A), and there were no differences between the pooled results of studies divided into subgroups according to study size, ethnicity, or type of study population (data not shown).

Two studies had published sufficient data for inclusion in our meta-analyses of the association between *APOE* genotypes and strictly lobar vs not strictly lobar BMBs.[3,17,21] We sought additional unpublished data from 3 other studies that had not included them in their publications, of which 2 provided them,[18,20] and one did not.[2] The 4 studies with location-specific data available included 4,883 (66%) of all 7,351 participants. The association between ε4+ genotypes and BMBs was slightly stronger and more significant for participants with BMBs in a strictly lobar location vs those with no BMBs

Table 2 Summary of key quality indicators

| Study references | Study size | BMB criteria clearly defined | Awareness of ≥2 mimics[a] | ≥2 Raters of BMBs | Raters independent | Observer agreement | | HWE stated | Blinding of | | Adjusted results for other risk factors | No. of quality indicators fulfilled (out of 10) |
						Inter-rater	Intrarater		Scanners to genotype	Genotypers to scan result		
Studies included in meta-analyses												
16	368	✓	✓	✓	✓	✓[b]	✗	✗	✓	✗	✓	7
17	414	✓	✗	✗	✗	✗	✗	✗	✗	✗	✓	2
18	438	✓	✓	✗	✗	✗	✗	✗	✓	✗	✗	4
19	101	✓	✗	✗	✗	✗	✗	✗	✓	✗	✗	2
20	342	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	9
2	1920	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	6
3, 21	3689	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	9
Studies with incompatible or missing data												
22	20	✓	✓	✓	✗	✓	✗	✗	✓	✗	✗	5
24	36	✓	✓	✗	✗	✗	✗	✗	✓	✗	✗	3
23	23	✗	✗	✗	✗	✗	✗	✓	✗	✗	✗	1

Abbreviations: BMB = brain microbleed; HWE = Hardy-Weinberg equilibrium.

[a] There were 4 mimics considered: calcification/nonhemorrhagic iron deposits; sulcal flow voids; cavernous malformations; hemorrhagic transformations of cerebral infarcts.
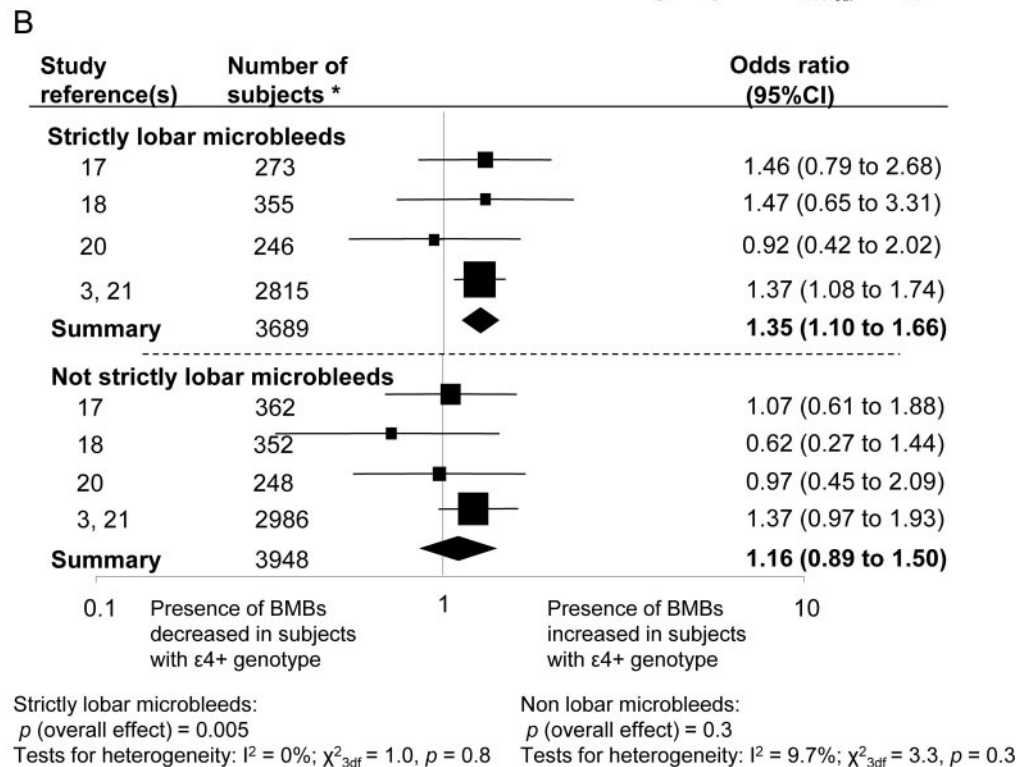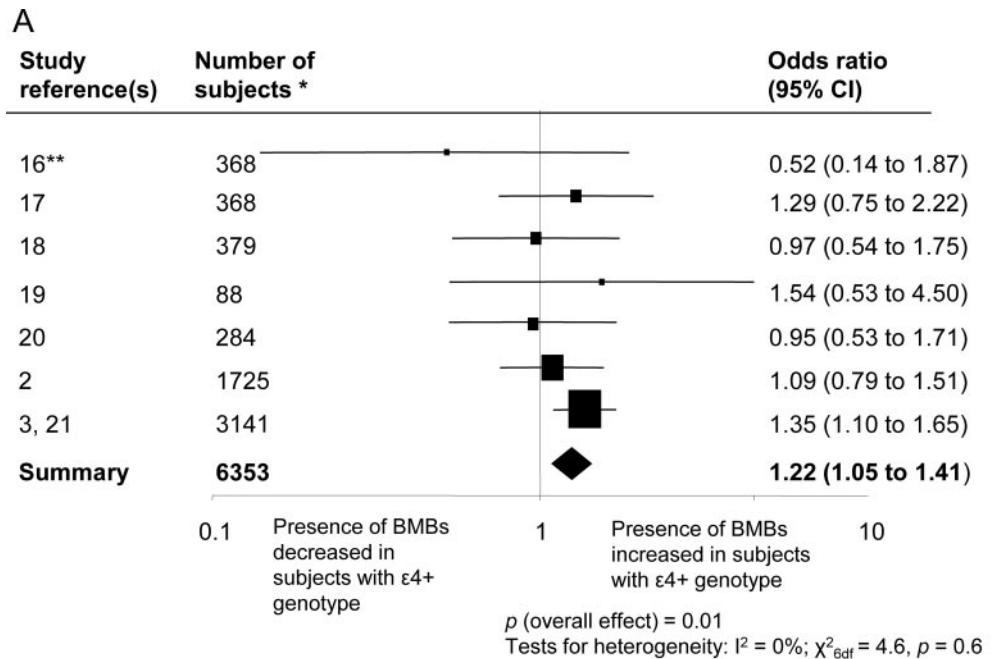
[b] Only had fair to moderate interobserver agreement (others reporting on this found substantial or almost perfect agreement; see table e-1).

(OR 1.35, 95% CI 1.10–1.66, $p = 0.005$), while it was slightly weaker and nonsignificant for those with not strictly lobar BMBs vs those with no BMBs (OR 1.16, 95% CI 0.89–1.50) (figure 2B). We could not carry out a formal significance test for the difference between these results for strictly lobar and not strictly lobar BMBs since both used the same no BMBs comparison group. However, we noted the 95% CIs for these results to be overlapping. There was no association of ε2+ genotypes with either strictly lobar or not strictly lobar BMBs (OR for strictly lobar BMBs 1.20, 95% CI 0.92–1.57, $p = 0.2$; OR for not strictly lobar BMBs 1.03, 95 CI 0.73–1.46, $p = 0.9$) (figure 3B).

Of the studies not contributing data to the meta-analyses of *APOE* associations with BMBs in strictly lobar vs not strictly lobar locations, 2 studies (between them including 469 participants) mentioned that they did not find an association of *APOE* genotype with lobar BMBs.[15,18] However, one study including 1,920 participants with data on *APOE* genotype and BMB location did not comment on the associations of *APOE* genotypes with BMBs by location and was unable to provide these unpublished data for inclusion in our location-specific meta-analyses.[2] Fail-safe N calculations showed that it would require null studies of around 7,700 and 4,800 participants respectively to bring the associations of ε4+ genotypes with any BMBs and with strictly lobar BMBs to a just statistically nonsignificant level (i.e., $p = 0.05$). When we set the cutoff for statistical significance at $p = 0.01$, the association of ε4+ genotypes with any BMBs became of borderline significance only while the fail-safe N study size for the association with strictly lobar BMBs was around 940 participants.

DISCUSSION Our systematic review and meta-analyses of genetic associations with BMBs suggest that ε4 *APOE* allele carriers are at higher risk of having BMBs, particularly in strictly lobar brain locations. Our data would also be compatible with an association between ε2+ genotypes and strictly lobar BMBs but this could only be considered speculative, since this association was not significant in our analyses. Given the reported associations of *APOE* genotype with CAA and lobar intracerebral hemorrhage, our data support strictly lobar BMBs as a possible imaging biomarker of CAA.[7,8,10,25] *APOE* ε4 and ε2 alleles may increase the risk of developing cerebral small cortical vessel fragility and consequent leakage of tiny amounts of blood particularly within the lobar regions, because patients who had lobar ICH and carried either of these alleles developed new BMBs at increased frequency compared to ε3/3 allele carriers.[26] Several animal studies have shown that *APOE*

**Figure 2** Meta-analysis of the association between ε4+ vs ε3/3 genotypes and brain microbleeds (BMBs)

A

Study reference(s)	Number of subjects *	Odds ratio (95% CI)
16**	368	0.52 (0.14 to 1.87)
17	368	1.29 (0.75 to 2.22)
18	379	0.97 (0.54 to 1.75)
19	88	1.54 (0.53 to 4.50)
20	284	0.95 (0.53 to 1.71)
2	1725	1.09 (0.79 to 1.51)
3, 21	3141	1.35 (1.10 to 1.65)
Summary	**6353**	**1.22 (1.05 to 1.41)**

0.1 — Presence of BMBs decreased in subjects with ε4+ genotype — 1 — Presence of BMBs increased in subjects with ε4+ genotype — 10

p (overall effect) = 0.01
Tests for heterogeneity: $I^2 = 0\%$; $\chi^2_{6df} = 4.6$, $p = 0.6$

B

Study reference(s)	Number of subjects *	Odds ratio (95%CI)
Strictly lobar microbleeds		
17	273	1.46 (0.79 to 2.68)
18	355	1.47 (0.65 to 3.31)
20	246	0.92 (0.42 to 2.02)
3, 21	2815	1.37 (1.08 to 1.74)
Summary	**3689**	**1.35 (1.10 to 1.66)**
Not strictly lobar microbleeds		
17	362	1.07 (0.61 to 1.88)
18	352	0.62 (0.27 to 1.44)
20	248	0.97 (0.45 to 2.09)
3, 21	2986	1.37 (0.97 to 1.93)
Summary	**3948**	**1.16 (0.89 to 1.50)**

0.1 — Presence of BMBs decreased in subjects with ε4+ genotype — 1 — Presence of BMBs increased in subjects with ε4+ genotype — 10

Strictly lobar microbleeds:
p (overall effect) = 0.005
Tests for heterogeneity: $I^2 = 0\%$; $\chi^2_{3df} = 1.0$, $p = 0.8$

Non lobar microbleeds:
p (overall effect) = 0.3
Tests for heterogeneity: $I^2 = 9.7\%$; $\chi^2_{3df} = 3.3$, $p = 0.3$

(A) BMBs in any location. (B) BMBs in strictly lobar or not strictly lobar locations. Studies are displayed in order of publication date. The squares represent study-specific odds ratios (ORs), with their size proportional to their statistical weight by the generic inverse variance method. Horizontal lines represent 95% confidence intervals (CIs). Diamonds represent pooled ORs, and their width represents the 95% CI for the relevant pooled OR. *Number of participants included in analysis. For some studies, where numbers of participants per genotype were not provided, we estimated the total number of participants with ε4+ or ε3/3 genotypes, assuming the distribution of the genotypes to be the average of the included studies with available data on each genotype. This provides a rough estimate of numbers of participants included in the comparison, but has no effect on the study-specific OR, standard error, or meta-analysis calculations. **Study used slightly different comparison groups (ε4+ vs ε4−).

ε4 glycoprotein product enhances deposition of β-amyloid peptide within the vessels causing smooth muscle loss and vessel wall thickening with an in-creased risk of vessel leakage.[27] The ε2 allele has been demonstrated to increase fibrinoid necrosis of amy-loid laden vessels, and to be associated with an in-

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Figure 3 Meta-analysis of the association between ε2+ vs ε3/3 genotypes and brain microbleeds (BMBs)
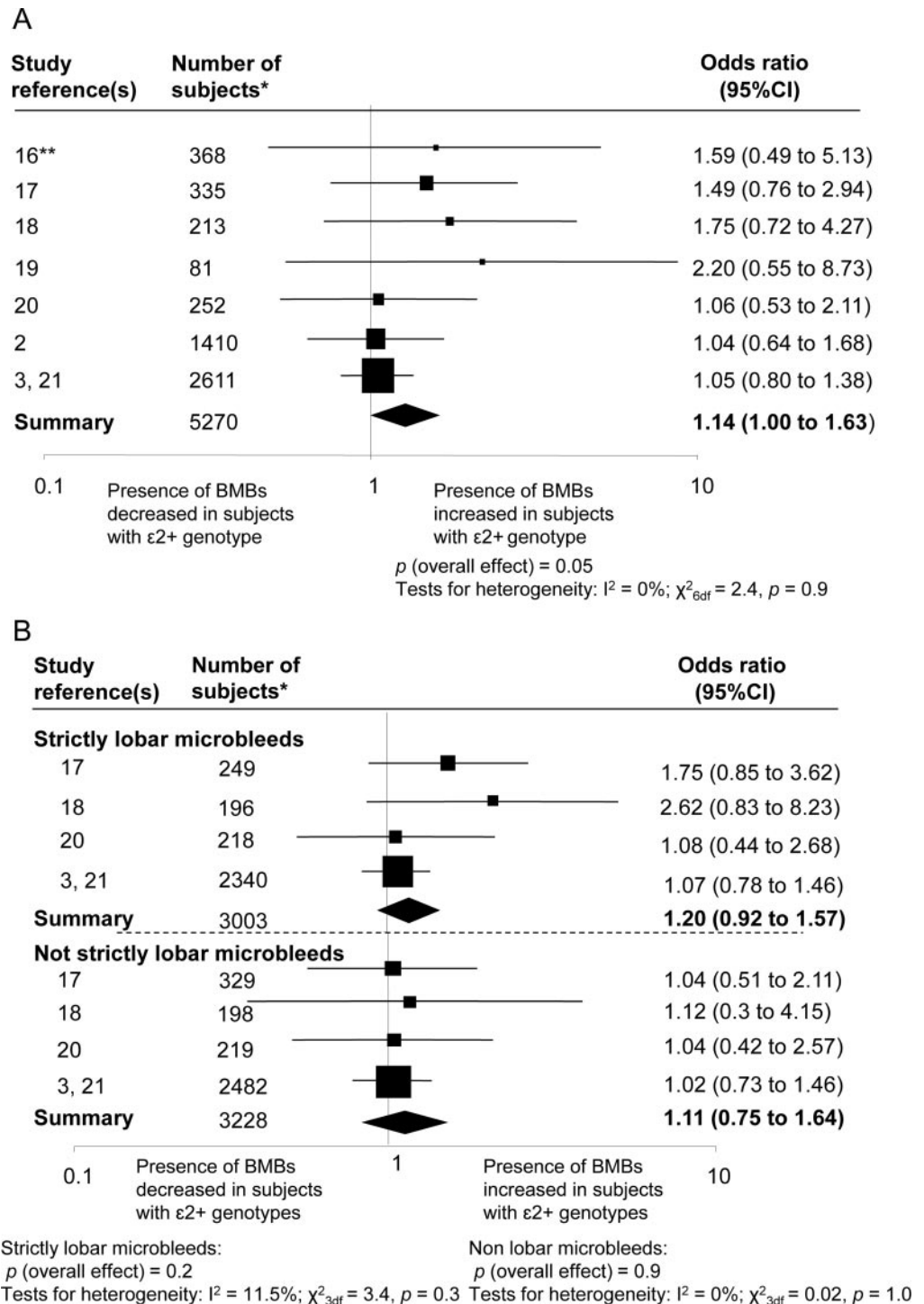
(A) BMBs in any location. (B) BMBs in strictly lobar or not strictly lobar locations. Notation as for figure 2. *Number of participants included in analysis. For some studies, where numbers of participants per genotype were not provided, we estimated the total number of participants with ε2+ or ε3/3 genotypes, assuming the distribution of the genotypes to be the average of the included studies with available data on each genotype. This provides a rough estimate of numbers of participants included in the comparison, but has no effect on the study-specific OR, standard error, or meta-analysis calculations. **Study used slightly different comparison groups (ε2+ vs ε2−). CI = confidence interval.

creased incidence of newly developed BMBs on follow-up scans.[28,29]

By its restriction to fully published studies, our systematic review is potentially subject to publication bias.[30] Furthermore, although we sought some additional unpublished data, only 4 studies in around two-thirds of the total number of participants from relevant studies contributed data on the association

between *APOE* polymorphisms and BMB location. The results may therefore be subject to reporting bias (i.e., those studies which found an association between lobar BMBs and *APOE* genotype may have been more likely to report their location-specific findings). Our fail-safe N calculations would suggest that a substantial amount of data from a null study or studies would be required to render nonsignificant ($p \geq 0.05$) the demonstrated associations of the $\epsilon 4$ allele with BMBs and with strictly lobar BMBs. However, with a more stringent significance level of $p < 0.01$ (accounting for multiple testing), the association of $\epsilon 4+$ genotypes with any BMBs was only borderline, while the association with strictly lobar BMBs would become nonsignificant with the addition to our meta-analysis of a further 940 participants with a null result. Since this is not implausible, we conclude that the demonstrated $\epsilon 4+$ associations are reasonably robust to the potential effects of publication and reporting bias, but not completely secure.

Although variation in imaging characteristics between studies may have led to a variation in the size of associations detected between genotype and BMBs, we did not detect any such heterogeneity. None of the studies included in our review fulfilled all our methodologic quality indicators for a study on the association between genotype and BMBs. Encouragingly, however, the larger studies which provided the most weight in the meta-analyses performed better against our quality indicators, increasing confidence in the overall pooled OR. Furthermore, a low-quality score does not necessarily indicate a poor quality study but one for which important methodologic quality indicators are incompletely reported. The results of our meta-analyses depend critically on the inclusion of the largest study which contributed over half of the participants. Reassuringly, this study was of high quality.[3,21]

A further limitation of our study is that analyses for *APOE* used a dominant genetic model, while it seems likely that an additive model would be both biologically more accurate and statistically somewhat more powerful, and may have improved our ability to detect a significant association with the *APOE* $\epsilon 2$ allele.[11,21] However, since our analyses relied mainly on the availability of published data, we had to use the dominant model used in the publications. While an individual participant data meta-analysis with inclusion of unpublished data from all studies would have allowed us to analyze the data more flexibly with an additive genetic model, it would have required considerably more time and resources, and may well have been possible only for a more limited dataset.

We have therefore demonstrated an association between $\epsilon 4$-containing *APOE* genotypes and BMBs, and have shown that the association with strictly lobar BMBs is reasonably robust. Future studies in this area would be improved by the use of a consistent definition of BMBs[5] and a robust method of rating BMBs, such as the Brain Observer Microbleed Scale[31] or Microbleed Anatomic Rating Scale,[32] to improve the comparability of different studies and facilitate future meta-analyses. The statistical power of future studies may be improved by treating BMBs as a continuous or at least ordered categorical variable, rather than as a dichotomous variable, as the majority of studies (and so our meta-analyses) have up to now. While only the *APOE* genotype has been studied in large numbers of people so far within the published literature, genome-wide association studies may soon be able to identify other, similarly important genes.

No.	Search history	Results
Appendix	**Electronic database search strategies**	
Medline search strategy[a]		
1	(microbleed$ or microh?emorrhag$ or micro-h?emorrhag$ or micro-bleed$ or microscopic bleeds$ or microscopic h?emorrhag$ or dot like h?emosiderin spot or dot-like h?emosiderin spot or multifocal signal loss or hypointense lesion or hypointense foci or lacunar h?emorrhag$ or minute h?emorrhag$).tw.	840
2	Limit 1 to (humans and yr = "1966-July 2010")	724
3	Exp genetics/ or exp genotype/ or exp inheritance patterns/ or exp "linkage (genetics)"/ or exp genes/ or exp genome/	897,875
4	Limit 3 to (humans and yr = "1966-July 2010")	716,242
5	(Polymorphi$ or genotype$ or gene or genes or genetic$ or allele$ or mutat$).tw.	1,518,187
6	Limit 5 to (humans and yr = "1966-July 2010")	836,523
7	4 or 6	1,076,430
8	2 and 7	60
Embase search strategy[a]		
1	(microbleed$ or microh?emorrhag$ or micro-h?emorrhag$ or micro-bleed$ or microscopic bleed$ or microscopic h$emorrhag$ or dot like h?emosiderin spot or dot-like h?emosiderin spot or multifocal signal loss or hypointense lesion or hypointense foci or lacunar h?emorrhag$ or minute h?emorrhag$).tw.	858
2	Limit 1 to (human and yr = "1980-July 2010")	698
3	Exp genetics/ or exp hereditary/ or exp genetic disorder/ or genetic epidemiology/ or exp genetic analysis/ or exp population genetic parameters/ or quantitative trait/ or exp molecular genetics/ or exp genetic parameters/ or exp gene mapping/	2,026,335
4	Limit 3 to (human and yr = "1980-July 2010")	1,256,529
5	(polymorphi$ or genotype$ or gene or genes or genetic$ or allel$ or mutat$).tw.	1,301,707
6	Limit 5 to (human and yr = "1980- July 2010")	641,489
7	4 or 6	1,326,351
8	2 and 7	98

[a] Search software used: OvidSP_UI03.02.02.104, SourceID 52336.

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