Genetic Determinants of White Matter Hyperintensities on Brain Scans
A Systematic Assessment of 19 Candidate Gene Polymorphisms in 46 Studies in 19 000 Subjects

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Background and Purpose—White matter hyperintensities (WMH) are highly heritable and associated with small artery ischemic stroke, so they may be a useful trait for studying the genetics of small vessel disease. Many studies have attempted to find associations between polymorphisms in various candidate genes and WMH. We aimed to evaluate the evidence for these associations by performing a systematic review and series of meta-analyses.

Methods—We used a comprehensive search strategy to identify studies of the association between any genetic polymorphism and WMH. For all polymorphisms in genes studied in >2000 subjects we performed meta-analyses, calculating pooled odds ratios or standardized mean differences.

Results—We identified 46 studies of polymorphisms in 19 genes in ~19 000 subjects. Most genes were involved in lipid metabolism, control of vascular tone, or blood pressure regulation. Polymorphisms in the apolipoprotein E, angiotensin-converting enzyme, methylenetetrahydrofolate reductase, and angiotensinogen genes had been studied in >2000 subjects and were evaluated by meta-analysis. There was no evidence for an association between apolipoprotein E (ε4+/−), methylenetetrahydrofolate reductase (677 cytosine/thymine polymorphism [C/T]), or angiotensinogen (Met235Thr) and WMH. For the angiotensin-converting enzyme insertion/deletion polymorphism (I/D) there appeared to be a significant association (OR, 1.95; 95% CI, 1.09–3.48), but this may be partly attributable to the small study (mainly publication) and other biases.

Conclusion—No genetic polymorphism has yet shown convincing evidence for an association with WMH. Much larger studies will be needed to detect and confirm genetic associations with this promising trait in the era of genome-wide association studies.

Key Words: genetics ■ meta-analysis ■ white matter hyperintensities

Family history and twin studies suggest a genetic contribution to risk of ischemic stroke. However, studies attempting to identify the individual genes involved have not yet produced convincingly positive results. Reasons include inadequate study size, poor choice of controls, and failure to present data for different ischemic stroke subtypes. Genetic influences may be more important for large artery (atherothrombotic) and small artery (lacunar) stroke than for other ischemic subtypes (mainly cardioembolic stroke), and it is likely that some genetic risk factors differ between them. Identifying these may help us to better understand the pathophysiology of large and small artery cerebrovascular disease.

White matter hyperintensities (WMH) on MR brain scans (or hypointensities on CT brain scans) are associated with a history of, and later progression to, radiologically defined lacunar infarcts and clinically apparent small artery ischemic stroke. WMH are more prevalent in patients with lacunar ischemic stroke than in those with other subtypes, and so they are considered a useful quantitative trait for studying small artery (lacunar) ischemic stroke. It has been suggested that lacunar infarction associated with WMH may reflect 1 subtype of small vessel disease pathology, with isolated lacunar infarction being the other subtype and having a different underlying vascular pathology.

Several terms are used to describe WMH, including leukoariosis, white matter changes, white matter lesions, and age-related white matter changes. For consistency, we use the term WMH throughout this article. WMH can be measured as a volume or using a grading scale. Volume measures produce a quantitative variable that may increase statistical power in genotype–phenotype association studies but require sophisticated analysis tools and MRI protocols. In addition, the volume of WMH may not fully represent their clinical
impact, because distribution and location are also of importance. There are many grading scales in use, some for CT, some for MRI, and some for both. All rate WMH according to extent and severity, some rate periventricular hyperintensities and deep WMH separately, and some rate various parts of the brain separately.

Heritability of WMH volume has been estimated to be between 55% and 80%. Many studies have assessed the association between polymorphisms in a range of candidate genes and WMH, but most have been small with apparently conflicting results and are difficult to interpret in isolation.

In this study we aimed to bring together all studies of the association between any polymorphism and WMH and to perform detailed methodological assessments and meta-analyses of studies of polymorphisms in genes assessed in large numbers of subjects. Our rationale for this approach was that it would enable us to give an up-to-date summary of what is known so far about genetics of WMH and which genetic polymorphisms have been studied; to increase power to detect associations through pooling data; and to explore potential reasons for heterogeneity of study results.

Materials and Methods
We sought articles in any language describing studies of the association between polymorphisms in any gene and WMH using a comprehensive search strategy in Medline (1966 to end of 2007) and Embase (1980 to end 2007), including a range of MeSH and text word terms for genes or genetics and for WMH. For each gene so identified, we calculated the number of studies and subjects and selected for meta-analyses polymorphisms in those genes studied in >2000 subjects.

For each selected gene, we performed supplementary gene-specific searches (replacing the general genetics terms with gene name terms) and checked the reference lists of relevant articles for further studies. We included all studies that had measured the volume or grade of WMH on brain imaging in human subjects. When studies had recruited overlapping subject samples, we included only the largest (with data available) in our analyses.

For each study we extracted information on year of publication; country in which the study was conducted; ethnicity of subjects; nature of the study population (eg, patients with hypertension); total number of subjects; mean age and gender distribution of subjects; reported numbers of subjects. Our rationale for this approach was that it would enable us to give an up-to-date summary of what is known so far about genetics of WMH and which genetic polymorphisms have been studied; to increase power to detect associations through pooling data; and to explore potential reasons for heterogeneity of study results.

Results
We identified 995 articles in the initial search, yielding 45 studies of relevance for this review. (see also supplemental references 1 to 11) and gene-specific searches identified 1 further study. Supplemental Table I, available online at http://stroke.ahajournals.org, shows the numbers of studies (and subjects) for each of 19 genes studied in a total of ~19 000 subjects (ranging between 40 and 8546 for any particular gene). Protein products from these genes are mainly involved in lipid metabolism, vascular tone, or blood pressure regulation.

Four genes (APOE, ACE, MTHFR, and AGT) had been studied in >2000 individuals and we included the relevant polymorphisms in meta-analyses. Characteristics of the relevant studies are presented in supplemental Table II. They were conducted in Europe, Japan, Hong Kong, and the USA, and generally recruited middle-aged to elderly patients. Many recruited hospital patients but some recruited subjects from the general population. Most reported that those performing and reporting brain scans were blind to genotype and that genotypes were in Hardy-Weinberg equilibrium. The method of WMH quantification varied, but most studies used a grading scale and studied only the deep white matter.

Twenty-four studies/substudies (8546 subjects) had assessed the association between WMH and APOE ε2/ε3/ε4 genotypes. Eleven compared the numbers of individuals in lower and upper WMH grades between genotype groups (Figure 1A), 14,16,17,22,23,25,29,30,33 3 compared mean grade (Figure 1B), 15,21,24 4 compared mean volume (Figure 1C), 20,28,34 Random-effects meta-analyses comparing ε4+ with ε4− genotypes found no evidence of association between APOE and WMH (graded WMH dichotomous pooled OR, 0.97; 95% CI, 0.78–1.21; graded WMH continuous pooled standardized mean difference: 0.30, 95% CI, −0.02–0.62; WMH volume pooled standardized mean difference: 0.15, 95% CI −0.04 to 0.33). There was substantial heterogeneity between studies measuring WMH volume (I² = 51%) but otherwise no detectable heterogeneity. Data for meta-analyses were unavailable from 9 relevant studies (4191 subjects, ie, 59% of the total number of subjects from relevant studies). Most of these, including the largest conducted among 3480 subjects and accounting for >80% of
the unavailable data,\textsuperscript{48} reported no significant association between APOE (ε4+ vs ε4−) and WMH (Figure 1).

Nine studies/substudies (2316 subjects) had assessed the association between the ACE I/D polymorphism and WMH.\textsuperscript{16,24,29,37–42} Six compared numbers of subjects with upper and lower WMH grades between genotype groups (Figure 2A).\textsuperscript{16,29,37,38,42} Random-effects meta-analysis comparing deletion-deletion (DD) with deletion-insertion/insertion-insertion (DI/II) suggested a significant association between ACE and WMH (pooled OR, 1.95; 95% CI, 1.09–3.48), but there was substantial heterogeneity between study results (I\textsuperscript{2} = 71%). One small study analyzed WMH grade as a continuous variable.\textsuperscript{41} The result was in the same direction as for the dichotomous analysis, but nonsignificant (Figure 2B). One study measured WMH volume and found no association (Figure 2C).\textsuperscript{39} Data for meta-analyses were unavailable from 2 relevant studies (151 subjects, ie, 7% of the total number of subjects). Neither found an association between ACE I/D and WMH (Figure 2).\textsuperscript{24,40}

Three studies (2796 subjects) had assessed the association between MTHFR 677C/T and WMH.\textsuperscript{29,43,44} All had measured WMH grade and had data available for meta-analysis. Overall, comparing TT with TC/CC, there was no significant association between MTHFR and WMH (random effects OR, 1.10; 95% CI, 0.85–1.43) and no excess heterogeneity (I\textsuperscript{2} = 0%) (Figure 3).

Six studies (2702 subjects) had assessed the association between AGT Met235Thr and WMH.\textsuperscript{38,40,42,45–47} Three measured WMH grade and compared numbers of subjects with upper and lower WMH grades between genotype groups (Figure 4A).\textsuperscript{38,42,45} Random effects meta-analysis comparing Thr Thr with Met Thr/Met Met found no significant association between AGT and WMH (pooled OR, 1.29; 95% CI, 0.62–2.68). One study measured WMH volume and found a
small but significant association (Figure 4B). Data for meta-analyses were unavailable in 2 of relevant studies (922 subjects, ie, 34% of the total number of subjects), but neither found a significant association (Figure 4). Fixed-effect meta-analyses for all these genetic polymorphisms produced similar results. Polymorphisms in many of the other genes that had been studied in much fewer numbers showed preliminary evidence for an association with WMH (eg, CYP11B2, protein kinase on chromosome 19, and intercellular adhesion molecule 1), but far larger samples will have to be studied to confirm or refute these preliminary findings.

**Discussion**

Despite the large number of studies of many genetic polymorphisms (representing mainly lipid metabolism, vascular tone, and blood pressure regulation pathways) and WMH, individual studies and numbers of subjects studied per polymorphism were generally small. Reliable conclusions cannot be drawn when the number of subjects studied is small because of imprecise results. Thus, we only conducted meta-analyses when the total number of subjects was >2000.

Only 4 polymorphisms ($APOE$ $\varepsilon2/\varepsilon3/\varepsilon4$), $MTHFR$ (677C/T), $ACE$ (I/D), and $AGT$ (Met235Thr) reached this threshold. Even with this approach, small study (mainly publication) and other sources of bias must be considered.

We found no convincing association between $APOE$, $MTHFR$, or $AGT$ polymorphisms. Although there was a large proportion of missing data in the $APOE$ and $AGT$ analyses, it is unlikely that the inclusion of any of these missing studies would have led to the identification of an association between $APOE$ or $AGT$ polymorphisms and WMH; indeed, their inclusion would almost certainly have strengthened the conclusion of no association.

For $APOE$, 95% CI of the meta-analyses include the possibility of a small or moderate association with $\varepsilon4$ vs $\varepsilon3\varepsilon2$ genotypes, but studies with unavailable data mainly showed no association and anything other than an extremely modest association seems unlikely. This is consistent with our previous meta-analysis of the association between $APOE$ ($\varepsilon4+/-$) and stroke, in which we found an apparent association with large artery but not small artery ischemic stroke, and suggests that $APOE$ is less important in the etiology of small artery stroke. By contrast, we have reported that $APOE$...
does appear to be associated with carotid intima-media thickness (an intermediate trait associated with large artery stroke), as it is with myocardial infarction, further strengthening the conclusion that APOE may specifically affect large artery (atherothrombotic) disease, perhaps through its effects on cholesterol metabolism.

For MTHFR, the wide 95% CI includes the possibility of an association, but much larger studies will be needed to detect a small to moderate association. ACE (I/D) was the only polymorphism showing an overall association with WMH, but this result may well be prone to reporting bias (when positive results tend to be reported in more detail and highlighted more than negative ones) because none of the 4 studies without available data for meta-analysis found an association.

For all genes included in our meta-analyses, the only studies with individually positive results were those performed in high-risk patients with a clinically evident stroke, infarcts on brain imaging, or hypertension. Whereas this could be the result of an interaction in which genotype has a greater influence on WMH in these high-risk subjects, reporting, publication, and other small study biases are also potential explanations, and further work with very large numbers of high- and low-risk subjects will be required to confirm or refute any real interaction.

Any meta-analysis depends on the data available from the individual contributing studies, and we encountered several limitations. First, because several methods for measuring WMH were used, several separate analyses were generally required for each polymorphism. Within separate analyses, we were able to achieve reasonable consistency. For example, despite several grading scales being used across studies, we were able consistently to pick a cut-off that represented approximately the same amount of WMH. However, many studies combined periventricular and deep WMH, and these may have different etiologies. Second, our analysis was limited by the genotype models used in individual studies. These models were chosen on the grounds of biological plausibility, but we were unable independently to check the appropriateness of these choices. Finally, although the relatively large proportion of unavailable data are a further limitation, we regard it as a strength that we did not make availability of data for meta-analysis a study inclusion criterion, as many meta-analyses do. Our findings clearly show that this approach may be prone to reporting bias. The inclusion of studies with unavailable data would almost certainly have strengthened our conclusions of no evidence of association for APOE (ε4+/−) and AGT (Met235Thr), and weakened the possibility of an association with ACE (I/D). Because of the limitation already imposed by variability in WMH measurement method, and because we could qualitatively incorporate the results of studies without available data, we decided not to seek additional unpublished data, because this would not have materially altered our conclusions.

Two linkage studies of WMH volume have identified candidate regions for further analyses. A 10-cM density microsatellite genome-wide scan among 747 subjects from 237 families in the Framingham study identified a significant logarithm of odds (LOD) score of 3.69 at 4 cM on chromosome 4, a region in which no candidate gene has so far been studied in WMH. A suggestive LOD score of 1.78 was also observed at 95 cM on chromosome 17, within 10 cM of the ACE gene. A 366-microsatellite genome-wide scan in the GENOA (Genetic Epidemiology Network of Arteriopathy) study of 488 subjects from 223 sibships found tentative evidence of linkage (maximum LOD scores of 1.30–1.99) in several novel regions.

There remains a discrepancy between the high heritability estimates for WMH and the lack of identified associated genetic polymorphisms. This could be because most studies so far have been too small to detect moderate association reliably (as evidenced by the CIs in Figures 1 to 4, which show the range of results statistically compatible with the observed data before any bias is considered); that the heritability of WMH has been overestimated; that the polymorphisms studied so far interact with each other, with heterogeneity of effects making associations harder to detect; or, perhaps most likely, that the most strongly associated polymorphisms have yet to be studied. Hence, the large genome-wide association studies that are now being planned...
for WMH will hopefully lead to the identification of more promising candidates.

Conclusion
No polymorphism has been convincingly associated with WMH. The genetics of WMH is a promising area of study, but like many other areas of complex disease, genetics requires much larger studies and internationally agreed measurement methods to allow comparability of study results and to improve opportunities for data pooling and meta-analyses. This is increasingly important in the era of genome-wide association studies in which WMH will be an important and potentially highly informative trait.

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References


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