Genome-Wide Scan for White Matter Hyperintensity
The Framingham Heart Study

Anita L. DeStefano, PhD; Larry D. Atwood, PhD; Joseph M. Massaro, PhD; Nancy Heard-Costa, PhD; Alexa Beiser, PhD; Rhoda Au, PhD; Philip A. Wolf, MD; Charles DeCarli, MD

Background and Purpose—White matter hyperintensity (WMH) volume is associated with aging and cerebrovascular disease and has been demonstrated to have a high heritability in the Framingham Heart Study as well as in other studies. We performed a genome-wide linkage analysis to identify chromosomal regions that may harbor genes influencing WMH in a family-based sample of the Framingham Heart Study.

Methods—Brain magnetic resonance scans were performed, and WMH and total cranial volume (TCV) were quantified as previously described on 2259 cohort and offspring participants. The outcome used for linkage analysis was an age specific (within 10-year age groups) z-score for the natural logarithm of the ratio of WMH to TCV. This z-score was based on 2230 individuals after excluding 26 participants with neurological conditions other than stroke and 3 individuals whose ages were out of range. Variance component linkage analysis included 747 individuals (mean age = 62.16 ± 12.43 years) with both magnetic resonance measure and genotype information in 237 families. Mean percent WMH to TCV was 0.098 ± 0.175 with a range of 0.00025% to 1.37% in the linkage analysis subjects.

Results—A maximum multipoint logarithm of the odds (LOD) score = 3.69, which indicates significant evidence of linkage, was observed at 4 cM on chromosome 4. A suggestive peak with LOD = 1.78 was observed at 95 cM on chromosome 17.

Conclusion—We have significant evidence that a gene influencing WMH volume is located on chromosome 4 of the human genome. (Stroke. 2006;37:77-81.)

Key Words: aging ■ cerebrovascular disorders ■ linkage (genetics)

Increased signal intensity of cerebral white matter is a common MRI finding. Although these white matter hyperintensities (WMH) are a recognized consequence of advancing age, they are also increased in prevalence and severity in association with cerebrovascular risk factors.1–7 Although the pathophysiology of WMH remains unclear, the consistent association between cerebrovascular risk factors and aging suggests that WMH could serve as a phenotypic marker for both the aging process as well as cerebrovascular disease. Recent MRI studies have examined genetic influence on a variety of brain morphologies8–12 including WMH.13–15 A genetic study of WMH among a group of older male twins14 found a heritability of 0.73. Three subsequent studies found genetic associations between WMH, hypertension and stroke suggesting that the high heritability of WMH may reflect shared genetic risk for cerebrovascular disease.15–17 A second analysis that examined associations between differences in WMH volumes18 among the monozygotic twins of the first report,14 however, found significant correlations, independent of familial factors, between monozygotic twin difference in WMH and differences in prevalent cerebrovascular risk factors such as glucose, cholesterol and systolic blood pressure, leaving the etiology for the high heritability of WMH unclear.

The most recent genetic analysis of WMH based on individuals from the offspring of the Framingham Heart Study (FHS)13 included a large group of men and women ranging in age from 34 to 88 years and confirmed the high heritability of WMH. Estimated heritability of WMH was 0.55 with gender-specific heritability estimates of 0.78 for females and 0.52 for males. Analysis in the FHS suggested that heritability of WMH might indicate pleiotropy with complex aging traits, complex cerebrovascular risk traits, or both. Given the confirmed high heritability of WMH, we conducted a genome-wide linkage analysis of members from the FHS to identify chromosomal region(s) harboring genes influencing WMH.

Methods

Subjects and Outcome Variables
The National Heart, Lung, and Blood Institute’s Framingham Cohort Study was established in 1948 with the enrollment of 5209 men and...
women between 28 and 62 years of age residing in Framingham, Massachusetts. In 1971, recruitment began of 5124 offspring of the original participants and their spouses. All participants from the original and offspring cohorts are white. Starting in 1999 MRIs were obtained on surviving study participants. All individuals gave informed consent and the Boston University Institutional Review Board approved all protocols.

Subjects were imaged on a Siemens Magnetom 1 tesla field strength magnetic resonance machine using a double spin-echo coronal imaging sequence of 4-mm contiguous slices from nasion to occiput. After the MR scan was obtained, digital information was transferred to a central location and was processed under the supervision of a single individual (C.D.). All analyses were performed blind to any subject personal identifying information. MRI quantification was performed with a custom-written computer program (Quanta 6.1) operating on a Unix, Solaris platform. Image evaluation was based on a semiautomatic segmentation analysis that involves operator-guided removal of nonbrain elements as previously described. In brief, nonbrain elements were manually removed from the image by operator guided tracing of the dura matter within the cranial vault including the middle cranial fossa, but above the posterior fossa and cerebellum. The resulting measure of the cranial vault was defined as the total cranial volume (TCV) and served as an estimate of head size to correct for recognized gender differences.

For segmentation of brain from CSF, a difference image was created by the subtraction of the second echo image from the first echo image. Image intensity nonuniformities were then removed from the difference image, and the resulting corrected image was modeled as a mixture of 2 gaussian probability functions with the segmentation threshold determined at the minimum probability between these 2 distributions.

For segmentation of WMH from brain matter, the first and second echo images were summed after removal of CSF and correction of image intensity nonuniformities. A repeat gaussian distribution was fitted to the summed image data and a segmentation threshold for WMH was a priori determined as 3.5 SDs in pixel intensity above the mean of the fitted distribution of brain parenchyma. Morphometric erosion of 2 exterior image pixels was applied to the image before modeling to remove the effects of partial volume CSF pixels on WMH determination. WMH measures for the Framingham Heart Study have been published previously.

Volume of the WMH relative to brain size was determined as the ratio of WMH to TCV (WMHV=WMH/TCV). The distribution of WMHV was markedly skewed and hence the natural log transformation was applied (LWMHV). There was a linear relationship between LWMHV and age; therefore, linear regression was used to obtain the residual of LWMHV adjusted for age. These residuals were then used to obtain an age-specific (within 10-year age groups) z-score. This z-score (ZLWMHV), which is an age-adjusted value of the natural log of the WMH to brain volume ratio, was the final variable used for linkage analysis. Gender was not included as a covariate in the regression models because only minor differences between men and women are observed in these data.

MR measures were available on 245 original cohort and 2014 offspring cohort participants. Twenty-six individuals were excluded because of neurological conditions other than stroke, such as multiple sclerosis, that might influence WMH. Three individuals were excluded because their ages fell outside the 10-year groups used to define ZLWMHV. Therefore, a total of 2230 participants were included in computations to define the trait used in linkage analysis.

Genotyping
DNA was extracted from whole blood or buffy coat specimens using a standard protocol. A 10 cM density genome scan was performed (marker set 8A, average heterozygosity 0.77) on individuals with DNA available from the largest Framingham Heart Study families by the NHLBI Mammalian Genotyping Service laboratory at the Marshfield Clinic (Marshfield, WI). DNA samples were sent in multiple batches to the genotyping service, and currently genotype data are available on 356 families. Details regarding markers and primers are available from Research Genetics (http://www.marshmed.org/genetics/default.htm). Genotype data were cleaned as previously described. Genotype data were available on 1886 individuals.

Statistical Analysis
There were 747 individuals with both MR measure and genotype information in 237 families used in linkage analysis. There were an average of 3.2 individuals with both phenotype and genotype data per family; the largest family had 8 individuals with complete information. Families used for linkage analysis contributed 137 sibships of size 2 (sibpairs), 64 sibships of 3, 12 sibships of 4, 5 sibships of 5 and 1 sibship of 6 individuals. Multipoint variance component linkage analysis to the 22 autosomal chromosomes was conducted using Genehunter. Linkage was assessed by fitting a polygenic model that does not incorporate genetic marker information and comparing it to a model that incorporates genotype data across a chromosome. The log (base 10) of the ratio of the likelihoods of the polygenic and marker specific models is the logarithm of the odds (LOD) score, the traditional measure of genetic linkage. A LOD score of 0 indicates that there is no evidence of linkage. It has been suggested that for allele sharing linkage methods a LOD score ≥3.6 is significant evidence of linkage, whereas LOD scores between 2.2 and 3.6 are suggestive evidence of linkage. However, LOD scores <2.2 may warrant further investigation and, hence, all chromosomal regions yielding LOD scores >1.5 will be reported.

Results
Figure 1 shows the distribution of the percent WMH to total cranial volume in individuals with both genotype and phenotype data used in linkage analysis. Descriptive statistics for these individuals are shown in Table. The population is relatively young compared with previous genetic studies of WMH and generally healthy. Linkage analysis in traits that deviate from a normal distribution, specifically traits with large kurtosis, have been shown to yield false-positive results. The skewness and kurtosis of ZLWMHV in this subset of individuals are −0.48 and 1.72, respectively.

Figure 2 shows the LOD score plots for all chromosomes. A maximum multipoint LOD = 3.69, which meets the criterion for significant evidence of linkage, was observed at 4 cM on chromosome 4. This peak is flanked by markers AFM196xb6 (telomeric) and GATA22G05 (centromeric). A suggestive peak with LOD = 1.78 was observed at 95 cM on chromosome 17. There were no other linkage peaks that yielded a LOD score >1.0.

Discussion
Published studies suggest a large genetic component to the individual expression of WMH volume. As previously noted, WMH are common to advancing age and cerebrovascular risk factors. We, therefore, hypothesized that WMH could serve as a phenotypic marker for both the aging process as well as cerebrovascular disease. We note, however, that the previously published high heritability estimates for WMH indicate possible pleiotropy with complex aging traits, complex cerebrovascular risk traits, or both.

The current study examined genetic linkage of WMH volume in a sample of generally healthy individuals ranging in age from 36 to 93. Stroke was observed in only 0.02% of the individuals with both genotype and phenotype data. Therefore, the linkage peaks we observed may harbor genes more likely involved in the genetic regulation of WMH.
volume via the aging process rather than cerebrovascular risk. Genome-wide scans for rare Mendelian forms of stroke have resulted in the identification of causative genes. For example, mutations in Notch3 are responsible for cerebral autosomal-dominant arteriopathy with subcortical infarctions and leukoencephalopathy (CADASIL).\(^{31}\) Genome-wide linkage analyses of common forms of stroke have also led to the identification of phosphodiesterase 4D (\textit{PDE4D}) and arachidonate 5-lipoxygenase-activating protein (\textit{FLAP}) as stroke susceptibility genes.\(^{32,33}\) The linkage peaks identified in this study do not overlap with these known stroke loci (see Gulcher et al 2005 for review).\(^{34}\)

Neuropathological studies of WMH generally separate age-related WMH from the more extensive WMH associated with cerebral ischemia.\(^{35}\) Subependymal gliosis, irregularity of the ependymal lining and adjacent myelin pallor\(^{35-40}\) are generally identified in association with these types of WMH, suggesting oligodendrocyte injury might result from complex age-related phenomena. Oligodendrocytes express a number of heat shock proteins after oxidative stress, suggesting the possibility that oxidative stress may be involved in diseases of myelin.\(^{40}\) The free radical hypothesis of aging\(^{41,42}\) strongly suggests that age-related mitochondrial dysfunction is important in the generation of reactive oxidation species,\(^{42,43}\) which could be involved in oligodendrocyte injury. Given our presumption that the genetics of WMH in this population reflects the aging process, we focused our investigation of genes under the linkage peaks on those related to mitochondrial function.

Results of our genome scan identified one significant linkage peak, on chromosome 4p, in a well-studied region that harbors the gene, huntingtin, responsible for Huntington disease.\(^{44}\) Despite the role of huntingtin in neurodegenerative disease there is no evidence that the wild-type huntingtin gene influences WMH volume. The distribution of the huntingtin protein does not coincide with the locations of WMH seen in this study.\(^{45}\) There were genes in this linkage peak whose products are involved in mitochondria, which in turn may influence the aging process. These include GrpE-like 1, alternatively known as the human mitochondrial GrpE protein (\textit{HMGE}) [OMIM 606173], collapsin response mediator protein-1 (\textit{CRMP1}) also known as dihydropyrimidinase-related protein-1 (\textit{DRP1}), which promotes mammalian cell death [OMIM 602462], and leucine zipper/EF-hand-containing transmembrane protein 1 (\textit{LETM1}), which is an evolutionarily conserved mitochondrial protein [OMIM 604407]. Although our main focus was the aging process, we also looked for vascular related genes; however, aside from these mitochondrial genes there were no obvious candidates in the chromosome 4 region.

This study represents the first published genome wide linkage analysis of WMH. In this regard, this study requires further investigation to confirm and refine our initial observations. Strengths of the study include that WMH volume was measured in multiple generations of a large population-based cohort, which was ascertained without respect to clinical characteristics, and that demonstrates high heritability for the trait. Weaknesses include the mostly healthy, white

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<th>Descriptive Characteristics of the Individuals With Both Genotype and Phenotype Data Used in Linkage Analysis</th>
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<tr>
<td><strong>Mean ± SD</strong></td>
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<tr>
<td>Age</td>
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<td>Percent WMH to TCV</td>
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<td>ZLWMHV</td>
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Figure 1. Distribution of percent WMH relative to TCV in individuals with both MRI measure and genotype data.
population that might limit the variability of WMH and therefore limit our ability to detect genetic linkage. Additionally, the distribution of the measured trait of interest, WMH volume, is quite skewed (Figure 1) and linkage analysis was performed on a log transformed variable. This transformation is conservative and will reduce false-positive linkage findings but may also reduce the power to detect linkage. The mild skewness that remains after transformation is at a level which has been shown by simulation study to have slight effect on type I error rate.30 Future work will entail further investigation of the genes of interest under the linkage peak by genotyping single nucleotide polymorphism markers followed by association analysis.

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