Heritability of Leukoaraiosis in Hypertensive Sibships

Stephen T. Turner, Clifford R. Jack, Myriam Fornage, Thomas H. Mosley, Eric Boerwinkle, Mariza de Andrade

Abstract—Ischemic damage to the subcortical white matter of the brain, referred to as leukoaraiosis, is a frequent complication of hypertension-related microvascular disease and contributes to the risk of stroke and vascular dementia. A large genetic contribution to this late-life form of target organ damage was suggested by a study of elderly male twins. As part of the Genetic Epidemiology Network of Arteriopathy (GENOA), 483 non-Hispanic white subjects were recruited to undergo MRI for determination of the brain volume of leukoaraiosis (291 women and 192 men from 210 sibships providing 434 sibling pairs; mean age±SD=65.2±7.3 years). The GENOA–Rochester sibships contain 2 or more siblings with essential hypertension diagnosed before age 60. The frequency distribution of the volume of leukoaraiosis was positively skewed, with a median value of 6.61 cm³ (interquartile range: 4.77 to 9.08 cm³). Variance component models were used to estimate the heritability (ie, the proportion of phenotypic variation caused by additive genetic factors). After logarithm transformation of the volume of leukoaraiosis, the estimated heritability (±SE) was 0.802±0.102 (P<0.0001). Adjustments for sex, age, systolic blood pressure, and brain volume reduced the heritability estimate to 0.671±0.110 (P<0.0001). This evidence of strong genetic influence on the susceptibility to leukoaraiosis justifies efforts to localize the responsible genes and characterize the predisposing genetic polymorphisms. (Hypertension. 2004;43[part 2]:483-487.)

Key Words: blood pressure ■ hypertension, genetic ■ genetics ■ brain ■ ischemia

With aging, the penetrating arterioles supplying blood to subcortical areas of the brain undergo remodeling that impairs autoregulation and predisposes to ischemic damage. The resulting neuropathologic changes, characterized by loss of myelin, increased water content, and gliosis, appear as areas of hyperintensity on MRI, which are referred to as leukoaraiosis. Large volumes of leukoaraiosis have been implicated in a number of pathologic conditions, including stroke and dementias of the Alzheimer and vascular types. Besides age, the most consistently identified risk factor for leukoaraiosis is hypertension, which accelerates arteriolosclerosis and is associated with cortical and lacunar infarctions. Moreover, hypertension in midlife is a risk factor for cognitive dysfunction later in life, even in persons without clinically diagnosed cerebrovascular events. Ischemic damage to the subcortical white matter, manifest as leukoaraiosis, may mediate some of the contribution of hypertension to risk of stroke and cognitive dysfunction.

Most target organ damage from hypertension is caused by ischemia, which results from obstructive atherosclerosis that can acutely interrupt blood flow or from arteriolosclerosis that chronically impairs perfusion within target organs. Familial studies have established that atherosclerosis, and each of its major biological risk factors, has a genetic basis. In contrast, studies to document a familial or genetic predisposition to arteriolosclerosis are almost nonexistent. Given some overlap in the risk factors for atherosclerosis and arteriolosclerosis (eg, hypertension), a shared genetic predisposition may be assumed; however, the processes are sufficiently distinct anatomically, histologically, and clinically to suggest that some of the causative factors may differ. Genetic influences on measures of brain morphology and function have been reported, including ventricular enlargement in response to aging. To our knowledge, all such studies involving noninvasive imaging of the brain used small samples of twins, which may not be representative of the general population. The heritability of leukoaraiosis estimated in 74 monozygotic and 71 dizygotic male twins who were 68 to 79 years old at MRI was 0.71, which is surprisingly high for a condition that develops so late in life. Therefore, our objective was to estimate the heritability of leukoaraiosis in a larger sample that included women and did not rely on twins. To accomplish this, we sampled an established cohort of siblings participating in a study to...
identify and characterize genetic determinants of hypertension and associated target organ complications.

Materials and Methods

Sample
The 483 non-Hispanic white subjects in the present study (291 women and 192 men) were members of sibships that were initially enrolled in Rochester, Minn between July 1997 and August 1999 in the Genetic Epidemiology Network of Arteriopathy (GENOA) of the Family Blood Pressure Program (FBPP).9 The FBPP, sponsored by the National Heart, Lung, and Blood Institute, is designed to identify and characterize genetic determinants of hypertension and its associated cardio and renal complications. An ancillary study, Genetics of Microangiopathic Brain Injury (GMBI), was designed to extend the assessment of target organ damage to include ischemic damage to the subcortical white matter of the brain determined by MRI, referred to as leukoaraiosis.2 For the GENOA–Rochester cohort, the Mayo Clinic diagnostic index and medical record linkage system of the Rochester Epidemiology Project were used to identify non-Hispanic white residents of Olmsted County with a diagnosis of essential hypertension made before age 60. When an eligible proband had at least one sibling who also reported hypertension, all available members of the sibships were invited to the Mayo Clinic for an initial study visit. Between December 2000 and October 2002, 815 of the original 1583 GENOA–Rochester participants returned for a second study visit. Between August 2001 and August 2003, 494 (of the 815) also underwent MRI of the brain as part of the GMBI ancillary study. The only exclusions to recruitment were history of stroke or neurologic disease, implanted metal devices, or no available sibling. Eleven subjects who underwent MRI were excluded for stroke or neurologic disease, implanted metal devices, or no available sibling. The 483 brain scans used in the analyses were performed on average (+SD) 10.5±3.8 months after the second GENOA study visit (range: 0 to 26.9 months). Study protocols were approved by the human studies review board of the Mayo Clinic, and informed consent was obtained from all participants.

Study Visits
Blood pressure measurements were made with random zero sphygmomanometers and cuffs appropriate for arm size. Three readings were taken from the right arm after the participant rested in the sitting position for at least 5 minutes; the last 2 readings were averaged for the analyses. Blood was drawn after an overnight fast of at least 8 hours. Serum creatinine, glucose, total cholesterol, triglycerides, and HDL cholesterol were measured by standard enzymatic methods. Each prescription medication recorded at the study visit was assigned a code based on mechanism of action. The diagnosis of hypertension was confirmed if a previous diagnosis of hypertension and use of prescription antihypertensive medication were reported, or if the average systolic or diastolic blood pressure was ≥140 mm Hg or ≥90 mm Hg, respectively. Diabetes was diagnosed if the subject reported treatment with insulin or oral hypoglycemic agents, or if the serum glucose concentration (fasting) was ≥126 mg/dL.

Brain MRI and Determination of Leukoaraiosis Volume
All scans were performed on identically equipped Sigma 1.5 Tesla MRI scanners (GE Medical Systems, Waukesha, Wis) under the supervision of Mayo Clinic neuroradiologists. Symmetric head positioning with respect to orthogonal axes was verified by a series of short scout scans. Total intracranial volume (head size) was measured from T1-weighted sagittal images, with each set consisting of 192 contiguous 5-mm-thick slices with no interslice gap that were obtained with the following sequence: repetition time=500 milliseconds, echo time=20 milliseconds, repetitions=2, time=2.5 minutes, field of view=24 cm. Brain and leukoaraiosis volumes were determined from axial fluid-attenuated inversion recovery images, with each set consisting of 192 contiguous 3-mm interleaved slices with no interslice gap obtained with the following sequence: echo time=144.8 ms, inversion time=2600 ms, repetition time=11 000 ms, bandwidth=±32 kHz, echo train length=22, time=8 minutes, field of view=24 cm, matrix=256×192.11 A fluid-attenuated inversion recovery image is a T2-weighted image with the signal of cerebrospinal fluid nulled, such that brain pathology appears as the brightest intracranial tissue. Interactive image processing steps were performed by a research associate who had no knowledge of the subjects’ personal or medical histories or biological relationships. A fully automated algorithm was used to segment each slice of the edited multislice fluid-attenuated inversion recovery sequence into voxels assigned to 1 of 3 categories: brain, cerebrospinal fluid, or leukoaraiosis. The mean absolute error of this method is 1.4% for brain volume and 6.6% for leukoaraiosis volume, and the mean test–retest coefficient of variation is 0.3% for brain volume and 1.4% for leukoaraiosis volume.12

Statistical Analyses
Descriptive statistics included means and SD for quantitative measures and percentages for categorical traits. Before estimating regression relationships between traits or heritabilities, skewed distributions were normalized by logarithm transformation.13 Covariates predictive of interindividual differences in leukoaraiosis volume were identified by use of regression tree-based recursive partitioning methods.14 A generalized estimating equations approach, assuming an identity link and allowing for correlation within sibships, was used to estimate relationships between risk factors and leukoaraiosis volume.13 Variance components methods were used to estimate the heritability of leukoaraiosis volume by fitting a statistical model that incorporates fixed effects for measured covariates and variance components for genetic effects16 By modeling the level of a quantitative trait, y, for the ith individual as 

\[ y_i = \mu + \sum g_j X_{ij} + e_i \]

where \( \mu \) is the trait mean, \( X_{ij} \) is the jth covariate, and \( g_j \) is its regression coefficient, total variation in a trait is partitioned into 

\[ \sigma^2 = g_j^2 \]

 additive genetic factors relative to the total phenotypic variance (before adjustment for covariates) or to the residual phenotypic variance after adjustment for covariates. Heritability was estimated for the logarithm-transformed measure of leukoaraiosis volume before and after adjustments for the identified covariates. Statistical significance was assessed by comparing twice the difference in natural logarithm likelihoods between a model with genetic effects estimated and a model with these effects constrained to zero. Test statistics were considered statistically significant at P=0.05.

Descriptive statistics and regression relationships were calculated using SAS (SAS Institute, Cary, NC). Data transformations and tree-based recursive partitioning were performed using S-PLUS (Insightful Corporation, Seattle, Wash). Heritabilities were estimated using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program.17

Results

Sample Description
The 483 subjects were from 210 sibships with a distribution of sibship sizes that provided 434 sibling pairs (Table 1). Of the 383 subjects, 228 were women (59.5%), and the mean age of subjects was 65.2 years. Consistent with the sampling of sibships with ≥2 hypertensive members, 79.1% of subjects had hypertension and 74% were treated with antihypertensive agents, or if the serum glucose concentration (fasting) was ≥126 mg/dL.
Interindividual Variation in Volume of Leukoaraiosis

The distribution of the volume of leukoaraiosis was positively skewed, with values ranging from 1.2 to 61.9 cm$^3$ (Figure). Before fitting the subsequent statistical models, which assume that the measure of leukoaraiosis volume is normally distributed, the raw measurements were logarithm-transformed, which reduced the skewness measure from 3.55 to 0.78. A multiple linear regression model that included sex, age, systolic blood pressure, and brain volume was in the model; other characteristics in Table 2 made a significant contribution to prediction of leukoaraiosis volume when sex, age, systolic blood pressure, and brain volume were each associated with greater volume of leukoaraiosis. Although women tended to have less leukoaraiosis than men (Table 2), sex did not make a significant additional contribution to the prediction of leukoaraiosis volume when other predictors were in the model. None of the other characteristics in Table 2 made a significant contribution to prediction of leukoaraiosis volume when sex, age, systolic blood pressure, and brain volume were in the model; nor did the effects of age, systolic blood pressure, or brain volume differ significantly between sexes. As expected, brain volume and head size were highly correlated (Spearman $r$=0.87, $P<0.0001$).

Heritability of Leukoaraiosis

The point estimate of the heritability ($\pm$SE) of the logarithm-transformed measure of leukoaraiosis volume was 0.802 (95% CI: 0.565 to 0.966) (Table 4). Together, the covariates were estimated to account for 31.4% of the interindividual variation in this measure of leukoaraiosis (Table 4), which is close to the estimate from the preceding linear regression model that used a generalized estimating equations approach to control for intrasibship correlations (32.3%) (Table 3). Age

TABLE 2. Descriptive Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women N=291</th>
<th>Men N=192</th>
<th>Pooled N=483</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65.0±7.5</td>
<td>65.5±7.0</td>
<td>65.2±7.3</td>
</tr>
<tr>
<td>BMI, kg · m$^{-2}$</td>
<td>29.6±6.1</td>
<td>29.8±4.4</td>
<td>29.7±5.5</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>95.2±15.6</td>
<td>106.4±12.2</td>
<td>99.7±15.4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>136.2±16.5</td>
<td>133.7±15.9</td>
<td>135.2±16.3</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>72.3±8.7</td>
<td>75.4±9.4</td>
<td>73.5±9.1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>79.7</td>
<td>78.1</td>
<td>79.1</td>
</tr>
<tr>
<td>BP medication, %</td>
<td>74.6</td>
<td>73.4</td>
<td>74.1</td>
</tr>
<tr>
<td>Cholesterol, mg · dl$^{-1}$</td>
<td>207.2±32.8</td>
<td>193.5±33.0</td>
<td>201.8±33.5</td>
</tr>
<tr>
<td>HDL cholesterol, mg · dl$^{-1}$</td>
<td>56.3±13.7</td>
<td>43.8±10.8</td>
<td>51.3±14.0</td>
</tr>
<tr>
<td>Triglycerides, mg · dl$^{-1}$</td>
<td>166.9±91.8</td>
<td>149.5±94.5</td>
<td>160.0±93.2</td>
</tr>
<tr>
<td>Lipid medication, %</td>
<td>30.2%</td>
<td>38.5%</td>
<td>33.5%</td>
</tr>
<tr>
<td>Creatinine, mg · dl$^{-1}$</td>
<td>0.8±0.2</td>
<td>1.0±0.2</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Glucose, mg · dl$^{-1}$</td>
<td>102.7±20.4</td>
<td>110.8±27.6</td>
<td>105.9±23.8</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>12.0</td>
<td>16.7</td>
<td>13.9</td>
</tr>
<tr>
<td>Ever smokers, %</td>
<td>38.8</td>
<td>59.4</td>
<td>47.0</td>
</tr>
<tr>
<td>Head size, cm$^3$</td>
<td>1377±110</td>
<td>1572±119</td>
<td>1455±148</td>
</tr>
<tr>
<td>Brain volume, cm$^3$</td>
<td>1089±96</td>
<td>1233±107</td>
<td>1142±120</td>
</tr>
<tr>
<td>Leukoaraiosis volume, cm$^3$</td>
<td>8.48±7.35</td>
<td>9.02±6.65</td>
<td>8.69±7.08</td>
</tr>
</tbody>
</table>

Entries are means±SD for quantitative traits or percentages for categorical traits. BMI indicates body mass index; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein.

TABLE 3. Linear Regression Model Based on Generalized Estimating Equations Predicting Interindividual Variation in the Logarithm Transformed Measure of Leukoaraiosis Volume

<table>
<thead>
<tr>
<th>Predictor</th>
<th>$\beta±$SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−2.601±0.507</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>−0.0273±0.0626</td>
<td>0.666</td>
</tr>
<tr>
<td>Age</td>
<td>0.0435±0.0041</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP</td>
<td>0.00467±0.00197</td>
<td>0.020</td>
</tr>
<tr>
<td>Brain volume</td>
<td>0.000997±0.000283</td>
<td>0.001</td>
</tr>
<tr>
<td>Model</td>
<td>0.323</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Sample of 483 subjects included 291 women and 192 men. $\beta$ indicates regression coefficient; SE, standard error; $P$, statistical significance; SBP, systolic blood pressure; $R^2$, proportion of interindividual variation explained by predictors in the model.
TABLE 4. Heritability Estimates for MRI-Determined Volume of Leukoaraiosis (Logarithm-Transformed)

<table>
<thead>
<tr>
<th>Covariate Adjustments</th>
<th>h²±SE</th>
<th>P</th>
<th>Proportion of Variation Caused by Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.802±0.102</td>
<td>&lt;0.0001</td>
<td>NA</td>
</tr>
<tr>
<td>Sex, age</td>
<td>0.701±0.108</td>
<td>&lt;0.0001</td>
<td>0.278</td>
</tr>
<tr>
<td>Sex, age, head size</td>
<td>0.683±0.110</td>
<td>&lt;0.0001</td>
<td>0.305</td>
</tr>
<tr>
<td>Sex, age, brain volume</td>
<td>0.687±0.109</td>
<td>&lt;0.0001</td>
<td>0.296</td>
</tr>
<tr>
<td>Sex, age, SBP, brain volume</td>
<td>0.671±0.110</td>
<td>&lt;0.0001</td>
<td>0.314</td>
</tr>
</tbody>
</table>

h² indicates heritability estimate; SBP, systolic blood pressure; NA, not applicable.

and sex accounted for most of the variation attributable to covariates; the additional variation explained by brain volume (or by head size) was <2%. After adjustments for all covariates, the estimated heritability of the logarithm-transformed measure of leukoaraiosis volume was reduced to 0.671 (95% CI: 0.436 to 0.864).

**Discussion**

This study, which included men and women from sibships with 2 or more hypertensive members, suggests that genetic variation makes a substantial contribution to interindividual differences in the susceptibility for leukoaraiosis. Although the pathogenesis of the anatomic changes manifesting as leukoaraiosis is debated, most evidence and expert opinion favors ischemia as the predominant mechanism.

With aging and chronic elevation of blood pressure, sclerotic remodeling of penetrating arterioles impairs autoregulation and impedes blood flow to the subcortical white matter. Among the strongest support for such a mechanism has been the consistent associations of leukoaraiosis with hypertension and clinical manifestations of cerebral ischemia, including stroke and vascular dementia.

The estimated heritability of leukoaraiosis exceeds estimates for other heritable risk factors for cerebrovascular disease, including blood pressure levels and hypertension. Consistent with previous clinic and population-based studies, we found that older age and higher systolic blood pressure were associated with greater volume of leukoaraiosis. However, because all subjects had diagnosed hypertension or ≥2 hypertensive siblings, categorically defined hypertension was not a predictor of leukoaraiosis. Even though most hypertensive subjects were treated with medication, measured systolic blood pressure still made a small contribution to the prediction of leukoaraiosis. The relatively greater predictive effects of age may in part be explained by its indexing the duration of exposure to hypertension. Because measures of plasma lipids, smoking, and glucose intolerance were not predictive of leukoaraiosis, we may infer that most additive genetic factors contributing to leukoaraiosis act via novel pathways not reflected in known risk factors for atherosclerotic cerebrovascular disease.

The high estimated heritability of leukoaraiosis among siblings of both sexes is close to a previous estimate in older male twins. This suggests the finding is robust to differences in family sampling design and sex. Unfortunately, the numbers of male–male sibling pairs (n=64) and female–female sibling pairs (n=169) were too small to formally assess the possibility of gender differences in the heritability of leukoaraiosis. Because our subjects had hypertension or were from families with a high prevalence of hypertension, they may have more leukoaraiosis than age- and sex-matched individuals from the general population. Even though we controlled for differences in measured blood pressure, it is still possible that the heritability estimates of leukoaraiosis volume may be inflated. Moreover, the present sample only included non-Hispanic white individuals. Because black individuals have more hypertension and greater susceptibility to microvascular complications, extension of this investigation to samples including black subjects is indicated.

Because our analysis did not estimate shared environments, a further caveat regarding the heritability estimates is that the genetic contribution may be overestimated. Although the siblings were on average 65 years old and all lived in separate households, it is possible that environments shared early in life contribute to familial aggregation of leukoaraiosis volume. Study of second-degree relatives and spouses could help resolve this question. Whereas environmental rather than genetic factors are typically viewed as relatively more important in the pathogenesis of conditions that develop late in life, the reverse appears to be true for measures of cognitive ability, the heritability of which increases from childhood to adulthood and old age. Possible age-dependence of leukoaraiosis heritability remains to be determined. Associations between cognitive measures and brain structure and regional differences in the magnitude of genetic influence on brain morphology has supported the possibility of familial susceptibility to degenerative diseases, particularly those affecting highly genetically determined regions of the brain.

Studies attempting to relate measured genetic variation to interindividual differences in leukoaraiosis are few, but there appear to be many genes that are plausible candidates to contribute to subcortical white matter ischemia and the development of leukoaraiosis. Genetic variants that influence levels of established intermediate risk factors, particularly hypertension, have been the subject of most investigations. Associations have been reported with polymorphisms in the genes encoding angiotensinogen, angiotensin-converting enzyme, paraoxonase PON1, apolipoprotein(a), and methylene tetrahydrofolate reductase. Associations with variants in the gene encoding apolipoprotein E have been inconsistent. The substantial heritability of leukoaraiosis, even after adjustment for the identified covariates, suggests that there are likely to be numerous other genetic pathways besides those acting through known risk factors for cerebrovascular disease.

Genes encoding angiotensinogen, angiotensin-converting enzyme, paraoxonase PON1, apolipoprotein(a), and methylene tetrahydrofolate reductase.
vascular disease. Although there are no reports of genome-wide scans yet, such an undertaking may reveal previously unsuspected mechanisms acting through novel pathways.

Perspectives

Efforts are underway to identify and characterize the genetic determinants of hypertension and its cardiac and renal complications.10 Likewise, the genetic architecture of brain morphology and function and their responses to aging continue to be areas of intense investigation. As mortality caused by atherosclerotic complications decreases, target organ complications of ischemia caused by arteriolosclerosis are bound to increase with aging of the population. The combined application of high-resolution, noninvasive imaging and genomic technologies promises to advance understanding of the pathogenesis of leukoaraiosis and provide for better detection, evaluation, treatment, and prevention of this emerging risk factor for dementia.

Acknowledgments

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