Genomic Susceptibility Loci for Brain Atrophy, Ventricular Volume, and Leukoaraiosis in Hypertensive Sibships

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Objective: To localize susceptibility genes for alterations in brain structure associated with risk of stroke and dementia. We conducted genomewide linkage analyses for magnetic resonance imaging (MRI) measures of brain atrophy, ventricular, and subcortical white matter hyperintensity (leukoaraiosis) in 689 non-Hispanic white (673 sibling pairs; median age, 61 years) and 544 non-Hispanic black participants (503 sibling pairs; median age, 64 years) from sibships with at least 2 members with essential hypertension.

Design, Setting, and Patients: We determined brain, ventricular, and leukoaraiosis volumes from axial fluid-attenuated inversion recovery MRI; we calculated brain atrophy, ventricular, and subcortical white matter atrophy volumes. Microsatellite markers (n=451) distributed across the 22 autosomes were genotyped, and we used variance components methods to estimate heritability and assess evidence of genetic linkage for each MRI measure.

Main Outcome Measures: Brain atrophy ventricular volume, and leukoaraiosis determined from fluid-attenuated inversion recovery MRI.

Results: In both races, the heritability of each MRI measure was statistically greater than 0 (P<.001), ranging in magnitude from 0.42 (for ventricular volume in blacks) to 0.69 (for brain atrophy in blacks). Based on multipoint logarithm of odds scores (MLS), the strongest evidence of genetic linkage was observed for brain atrophy on chromosomes 1 (MLS, 3.49 at 161 cM; P<.001) and 17 (MLS, 3.08 at 188 cM; P<.001) in whites; for ventricular volume on chromosome 12 (MLS, 3.67 at 49 cM; P<.001) in blacks and chromosome 10 (MLS, 2.47 at 100 cM; P<.001) in whites; and for leukoaraiosis on chromosome 11 (MLS, 2.21 at 118 cM; P<.001) in whites and chromosome 22 (MLS, 2.02 at 36 cM; P= .001) in blacks.

Conclusions: The MRI measures of structural brain injury are heritable in non-Hispanic black and white sibships ascertained through hypertensive sibling pairs. The susceptibility loci for brain atrophy, ventricular volume, and leukoaraiosis identified by linkage analyses differ among MRI measures and between races.

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MULTICIPICITY OF VASCULAR and neurodegenerative processes contributes to changes in brain structure with age. Despite success in identifying rare single gene mutations that cause stroke or dementia, most genes making smaller contributions to risk remain unknown.1,2 Difficulties in identifying genetic polymorphisms with small effects on clinical endpoints are among the motivations for studying the underlying contributory disease processes, which progress asymptotically for decades before a clinical event. Magnetic resonance imaging (MRI) of the brain has been used as a noninvasive method to obtain accurate and reproducible quantitative measures of alterations in brain structure, including cerebral atrophy, ventricular enlargement, and the volume of subcortical white matter hyperintensity (leukoaraiosis).3 These MRI measures of structural brain injury are heritable, associated with hypertension and other risk factors for arteriosclerosis, and predictive of stroke and dementia.3,6 The goal of the present study was to localize regions of the genome that harbor DNA sequence variations influencing MRI measures of structural brain injury, that is, total cerebral brain atrophy, ventricular volume, and leukoaraiosis. We studied non-Hispanic white and black sibships at increased risk of structural brain injury by virtue of previously diagnosed hypertension in 2 or more members of each sibship. Our primary approach was univariate linkage analysis for each MRI measure of the brain. We also conducted bivariate linkage analyses for pairwise combinations of brain MRI measures and of each brain MRI measure with measures of blood pressure level and pulsation, attempting to leverage greater statistical power to identify loci with pleiotropic ef-
METHODS

SUBJECTS

The 1233 study participants consisted of 689 non-Hispanic white adults (410 women and 279 men) and 544 non-Hispanic black adults (379 women and 165 men) from sibships enrolled in the Genetic Epidemiology Network of Arteriopathy (GENOA) of the Family Blood Pressure Program, designed to identify genetic determinants of hypertension in multiple ethnic groups.8 The Mayo Clinic diagnostic index and medical record linkage system were used to identify non-Hispanic white residents of Olmsted County, Minnesota, with a diagnosis of essential hypertension made before 60 years of age. Non-Hispanic blacks were recruited from hypertensive probands in a probability sample of black residents of that community aged 45 to 64 years.9 Sibships in which either index hypertensive sibling was known to have impaired kidney function (eg, serum creatinine level, ≥2.0 mg/dL [to convert to micromoles per liter, multiply by 88.4]) were not recruited; otherwise, all available members of the index sibships were invited to an initial examination (1996-2000). A second phase of the Family Blood Pressure Program was designed to identify target organ complications, wherein participants whose brain MRIs were not analyzable, the most common reason was unsuspected prior cortical/hemispheric brain infarction (n=36 [53%]), followed by other anatomic abnormalities (n=17 [23%]), artifacts related to subject or technical factors (n=8 [12%]), and failure to complete the MRI (n=7 [10%]). The analyses were conducted on a subset of 1233 participants with analyzable brain MRIs, measurements of linkage markers that were consistent with reported pedigree structure, and at least 1 sibling with these same data available.

STUDY PROTOCOL

Study protocols were approved by the Human Studies Review Board of each institution, and written informed consent was obtained from participants. Height was measured by means of a wall stadiometer and weight by means of an electronic balance, with body mass index calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured with a random zero sphygmomanometer (Hawksley & Sons Ltd, West Sussex, England) and a cuff appropriate for arm size. The second and third of 3 readings, taken from the right arm after the participant sat for at least 5 minutes, were averaged for the analyses. Mean arterial pressure was calculated as:

\[
\text{Mean arterial pressure} = \frac{\text{systolic blood pressure} + (2 \times \text{diastolic blood pressure})}{3},
\]

and pulse pressure was calculated as systolic blood pressure - diastolic blood pressure. The diagnosis of hypertension was confirmed if a prior diagnosis of hypertension and use of prescription antihypertensive medication were reported, or if the systolic or diastolic blood pressures averaged at least 140 mm Hg or at least 90 mm Hg, respectively.

Magnetic resonance imaging of the brain was performed at both institutions on identically equipped 1.5-T MRI scanners (Signa; GE Medical Systems, Waukesha, Wisconsin) under the supervision of neuroradiologists. The methods for semiautomated MRI measurements of brain anatomy have been described previously.7 Total intracranial volume was measured from T1-weighted spin-echo sagittal images, each set consisting of 32 contiguous 3-mm-thick interleaved sections with no interstice gap, a field of view of 24 cm, and a matrix of 256 × 192, obtained with the following sequence: scan time, 2.5 minutes; echo time, 14 milliseconds; 2 repetitions; and repetition time, 500 milliseconds. Brain, ventricular, and leukoaraiosis volumes were determined from axial fluid-attenuated inversion recovery images, each set consisting of 48 contiguous 3-mm-thick interleaved sections with no interstice gap, a field of view of 22 cm, and a matrix of 256 × 160, obtained with the following sequence: scan time, 9 minutes; echo time, 144.8 milliseconds; inversion time, 2600 milliseconds; repetition time, 11 seconds; bandwidth, ±15.6 kHz; and an average of 1 signal. A fluid-attenuated inversion recovery image is a T2-weighted image with the signal of cerebrospinal fluid nullled, such that brain pathology appears as the brightest intracranial tissue. Interactive image-processing steps were performed by a research associate at the Mayo Clinic who had no knowledge of the subjects’ personal or medical histories or biological relationships. A fully automated algorithm was used to segment each section of the edited multislice fluid-attenuated inversion recovery sequence into voxels assigned to 1 of the following 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.8 The difference between total intracranial volume and brain volume provided a measure of brain atrophy. Brain images with cortical/hemispheric infarctions were excluded from the analyses because of the distortion of the leukoaraiosis volume estimates that would be introduced in the automated segmentation algorithm. Lacunar infarctions and periventricular white matter hyperintensities were included in the leukoaraiosis intensity category and therefore in the leukoaraiosis volume estimates.

A set of 451 microsatellite markers distributed across the 22 autosomes (Cooperative Human Linkage Center/Weber screening set 9.0) was genotyped using standard polymerase chain reaction methods by the Mammalian Genotyping Center of the Marshfield Medical Research Foundation, Marshfield, Wisconsin, which provided the ordering of markers and their genetic map distances. Inconsistencies of the genotypes with pedigree structure were identified by the Lange and Go-radia algorithm as implemented in the PedCheck software.9 Instances that could not be resolved as genotyping errors were considered missing data.

STATISTICAL ANALYSES

Because distributions of ventricular and leukoaraiosis volumes were positively skewed, the raw MRI trait values were transformed for the genetic analyses using the empirical normal quantile transformation,10 which was effective in normalizing the distributions.11 Adjustments for sex, age, and total intracranial volume (for brain atrophy and ventricular volume), or brain volume (for leukoaraiosis) were incorporated as covariates in the genetic models. Genetic and environmental correlations between the adjusted (transformed) trait values were estimated by means of variance decomposition using maximum likelihood methods,13 and phenotypic correlations between traits were calculated on the basis of genetic and environmental correlations.14 Sibship structure was verified using the MERLIN software program.15 Univariate and bivariate genome-wide linkage analyses were conducted with an R/S-plus Library MULTIC routine16 that uses the variance components approach.17 We also conducted univariate linkage analyses for
pairwise combinations of brain MRI measures and of each brain MRI measure with a measure of steady-state blood pressure level and a measure of blood pressure pulsation. Both blood pressure level and pulsation are influenced by genetic factors, and each may make an additive, independent contribution to risk of structural brain injury. Multivariate linkage analyses provided greater statistical power to identify loci with pleiotropic effects on genetically correlated traits and permitted assessment of overlap of blood pressure loci with those predisposing to structural brain injury. A rationale for the bivariate analyses of pulse pressure, in addition to mean arterial pressure, was the high percentage of GENOA participants treated with antihypertensive medications (Table 1), which may lower both systolic and diastolic blood pressure levels but have less impact on the calculated difference between them.

The multipoint identity-by-descent sharing among pairs of relatives was calculated using SimWalk2 software and was based on the pedigree relationships for 1239 white and 1482 black GENOA participants in whom linkage markers were measured. A likelihood ratio test (LRT) was used to test for genetic linkage, in which the LRT is defined as \(-2 \times (\log \text{likelihood under the null hypothesis} - \log \text{likelihood under the alternative hypothesis})\). Under the null hypothesis, the linked genetic factors are restricted to equal 0, and the asymptotic distribution of the LRT is a mixture of \(\chi^2\) statistics. All logarithm of odds (LOD) scores for the linkage analyses were calculated from the LRT values as \(\frac{1}{2} \times \log_{10}(\text{LRT})\). Conventionally, univariate multipoint LOD scores (MLs) of at least 3.00 are considered statistically significant evidence of linkage (\(P \leq 1 \times 10^{-8}\)). MLs of at least 2.00 as suggestive evidence (\(P \leq .001\)), and MLs of at least 1.30 as tentative evidence (\(P \leq .007\)). Because the bivariate linkage analyses have greater degrees of freedom, higher LOD score thresholds are required for the bivariate MLs to achieve comparable levels of statistical significance (ie, \(\geq 4.00\), \(\geq 2.87\), and \(\geq 2.06\), respectively). However, because we performed multiple univariate and bi-
The intermediate level of evidence (MLS, 2.00-2.99) was met in whites on chromosome 11 (at 130 cM from pter) and in blacks on chromosome 22 (Table 3). Three other MLS values achieved the lowest level of evidence (1.30-1.99), all in blacks (on chromosomes 4, 13, and 20).

For the MRI measure of leukoaraiosis, 2 MLS values achieved the intermediate level of evidence of genetic linkage (MLS, 2.00-2.99), one in whites on chromosome 11 and the other in blacks on chromosome 22 (Table 3 and Figure 2).
Figure 3. Two additional MLS values achieved the lowest level of evidence (1.30-1.99), on chromosome 21 at 13 cM from pter in white and at 58 cM from pter in blacks. Genetic correlations were significantly greater than 0 between MRI measures of brain atrophy and ventricular volume in both whites (0.38; \(P < .001\)) and blacks (0.45;
Figure 2. Ventricular volume multipoint logarithm of odds (LOD) score plots calculated from magnetic resonance images for univariate genetic linkage analysis. Subjects included 689 non-Hispanic whites and 544 non-Hispanic blacks from sibships with at least 2 members with essential hypertension. Within the chromosome 12p12.2-12q12 linkage region identified in blacks (38.5-55.7 cM from pter) (see also Table 3), 4 single-nucleotide polymorphisms (SNPs) in the phosphodiesterase 3A gene (rs1444644, rs1444645, rs10505865, and rs1444629) were associated with total and regional cerebral volumes; 1 SNP in the contactin 1 gene (rs10506176) was associated with temporal brain volume; and 1 SNP in the leucine-rich repeat kinase 2 gene (rs10506151) was associated with frontal and temporal brain volumes.6
In whites, leukoaraiosis was also genetically correlated with brain atrophy \((0.22; P < .001)\) and ventricular \((0.40; P < .001)\), but neither correlation differed significantly from 0 in blacks \((P = .59)\). The bivariate linkage analyses provided evidence of 9 loci with pleiotropic effects on pairwise combinations of brain MRI measures, based on bivariate MLS values that satisfied the intermediate or lower level of evidence of bivariate linkage (ie, bivariate MLS, 2.06-3.99) (chromosomes 2, 4, 5, 8, 10, 13, and 16 in whites and 13 and 22 in blacks).
Results of this study provide evidence of genetic influences on MRI measures of structural brain injury in non-Hispanic black and white sibships in which at least 2 siblings had essential hypertension. The estimated heritabilities for MRI measures of brain atrophy, ventricular volume, and leukoaraiosis were similar in magnitude to those reported initially for a cohort of elderly male twins \(^24\), \(^25\) and subsequently for the community-based Framingham Heart Study cohort. \(^26\) The present study provides estimates of heritability in hypertensive sibships and in non-Hispanic black sibships, which were not included in previous samples. Consistency of the heritability estimates with samples that were not ascertained through hypertension suggests independence of the larger genetic effects detectable by linkage analyses from those with major effects on blood pressure. This latter inference is also consistent with previous genetic analyses that adjusted brain MRI measures for blood pressure and other risk factors for arteriosclerosis \(^27\) and with results of our bivariate linkage analyses that detected only 2 loci (and at only the lowest level of statistical support) with pleiotropic effects on brain MRI and blood pressure measures.

The chromosomal regions defined by 1-LOD score-down intervals around the 2 highest MLS peaks for each MRI measure contain numerous plausible candidate genes for structural brain entry; potentially relevant metabolic, vascular, and neurodegenerative disorders contributing to structural brain injury have been mapped to these regions (Table 3) \(^28\)-\(^68\). For example, within the intervals identified by the highest level of evidence of genetic linkage (ie, MLSs \(>3.00\)), the chromosome 1p12-1q23.3 region for brain atrophy and the chromosome 12p12.2-12q12 region for ventricular volume include genes implicated in Parkinson disease (\(PARK10\) and \(LRRK2\), respectively), and the chromosome 17p13.3-17p12 region for brain atrophy includes genes implicated in type 2 diabetes mellitus (\(SLC2A4\)) and the metabolic insulin-resistance syndrome (\(AOMS2\)). The chromosome 1 and 12 regions also harbor genes implicated in hypertension (eg, \(SELE\), \(HHTY4\), and \(HTNB\)) and atherosclerosis (eg, \(SELP\), \(APOA2\), and \(CRP\)), which, like diabetes and insulin resistance, are risk factors for structural brain injury. \(^69\) Moreover, diabetes, hypertension, and other risk factors for arteriosclerosis have been implicated in Alzheimer disease, \(^70\) in which brain atrophy and ventricular enlargement are prominent features. Because biologically plausible candidate genes may be located under linkage peaks by chance alone, comparisons with results from other genome scans is indicated.

The Framingham Heart Study has reported results of genome scans for brain MRI measures. \(^5\) An initial scan for white matter hyperintensity volume used 387 highly polymorphic linkage markers in 2259 family members. \(^5\) The significant linkage criterion was satisfied by a single locus on chromosome 4p16.2 (LOD, 3.69) that was noted to harbor genes contributing to brain injury, including the gene for Huntington disease (\(HTT\)). This region corresponds to one of the linkage regions for brain atrophy in our white sample (0-24 cM from 4 pter) (Table 3 and Figure 1). Subsequently, results of genomewide association analyses of 100 000 single-nucleotide polymorphisms (SNPs) have been reported for 705 members of the largest Framingham pedigrees. \(^6\) Comparison with the 2 highest MLS peaks that we observed for each brain MRI measure validates association of candidate genes within the 6 regions. Within the chromosome 12p12.2-12q12 linkage region identified for ventricular volume in blacks (38.5-55.7 cM from pter) (Table 3), the Framingham investigators noted that 4 SNPs in the phosphodiesterase 3A gene (\(PDE3A\); \(rs1444644\), \(rs1444645\), \(rs10505865\), and \(rs1444629\)) were associated with total and regional cerebral volumes, 1 SNP in the contactin 1 gene (\(CNTN1\); \(rs10506176\) was associated with temporal brain volume, and 1 SNP in the leucine-rich repeat kinase 2 gene (\(LRRK2\); \(rs10506151\)) was associated with frontal and temporal brain volumes. The \(PDE3A\) ex-
pressed in human platelets plays a role in activation.\textsuperscript{71} CNTNI has been associated with cerebellar ataxia in an animal model.\textsuperscript{72} and LRRK2 has been associated with Parkinson disease\textsuperscript{73} and tau pathology.\textsuperscript{74} Within the chromosome 11q23.1-1q25 region for leukoaraiosis in whites (108-139 cm from pter) (Table 3), the Framingham investigators noted that 1 SNP in the beta-amyloid B precursor protein-cleaving enzyme 1 gene (BACE1; rs1261791) was associated with parietal brain volume. Variation in BACE1 has been previously associated with Alzheimer disease.\textsuperscript{75}

We attempted to leverage the genetic correlations among traits by performing bivariate linkage analyses with greater statistical power\textsuperscript{76} to localize additional regions with effects on brain MRI measures too small to be detected in univariate linkage analyses and to assess overlap with regions influencing blood pressure. Although results of the genetic correlation analyses supported the concept of pleiotropy, those regions implicated by the bivariate linkage analyses to harbor genes influencing more than 1 MRI measure were also detected in the univariate linkage analyses for the separate MRI measures (as illustrated in Figure 4). Moreover, evidence of overlap between linkage regions influencing blood pressure and brain MRI measures was relatively sparse and statistically weak but consistent with findings in an animal model in which stroke genes detected by linkage analysis were separate from genes determining blood pressure.\textsuperscript{57,70} Hence, the theoretical advantages of multivariate linkage analyses were not realized in the present study.

Results of this and previous studies have several implications for the genetic architecture of MRI measures of structural brain injury. First, high heritabilities of the MRI measures are likely, for the most part, to be the consequence of many genes with small effects (ie, polygenes), inasmuch as relatively few regions had large enough effects to satisfy conventional criteria for significant evidence of genetic linkage or association.\textsuperscript{6} Second, because the regions identified by genetic linkage or association analyses differ among MRI measures,\textsuperscript{3} the genetic correlations among MRI measures are also likely to reflect, for the most part, polygenes. Third, most of the genetic variation in MRI measures of structural brain injury appears to be separate from genetic variation in measures of blood pressure or other established risk factors for arteriosclerosis.\textsuperscript{27} Fourth, because most regions reported to harbor variants influencing a given MRI measure also differ between racial groups and between different samples from the same race,\textsuperscript{4} the larger genetic effects detected by this and previous linkage or association analyses may be context dependent, possibly reflecting gene-gene or gene-environment interactions that are sample specific or study specific.

Our study has the strength of relatively large, well-characterized biracial cohorts; however, it also has limitations. Use of genetic markers to scan the genome is a hypothesis-generating first step toward localizing particular DNA sequence variants that have functional effects contributing to structural brain injury. Replication of linkage or association findings in independent samples will be necessary before undertaking fine mapping of the regions to prioritize which positional candidate genes may merit further investigation for functional variants. In addition, the traditional linkage approach we applied may have insufficient power to detect many additional loci hypothesized to have smaller effects on brain MRI measures.\textsuperscript{79} Despite apparent independence of the genes influencing brain MRI measures from those influencing blood pressure, because our results are based on sibships with at least 2 members diagnosed as having essential hypertension before 60 years of age in whom the full range of untreated blood pressure levels was not observed, extension of inferences to groups of different risk factor profiles must be cautious.

The present study confirms a large genetic component of interindividual variation in MRI measures of structural brain injury and makes a first step toward localizing regions of the genome that may harbor DNA sequence variants meriting further investigation as potential risk factors for stroke and dementia.

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Author Contributions: Dr Turner had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Turner, Jack, Mosley, Boerwinkle, and de Andrade. Acquisition of data: Turner, Fornage, Jack, Mosley, Kardia, and Boerwinkle. Analysis and interpretation of data: Turner, Mosley, Knopman, Kardia, Boerwinkle, and de Andrade. Drafting of the manuscript: Turner. Critical revision of the manuscript for important intellectual content: Turner, Fornage, Jack, Mosley, Knopman, Kardia, Boerwinkle, and de Andrade. Administrative, technical, and material support: Turner, Jack, and Mosley. Study supervision: Turner and de Andrade.

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