Heritability of MRI Lesion Volume in CADASIL
Evidence for Genetic Modifiers

Christian Opherk, MD; Nils Peters, MD; Markus Holtmannspötter, MD; Andreas Gschwendtner, MD; Bertram Müller-Myhsok; Martin Dichgans, MD

Background and Purpose—The phenotypic expressivity shows striking variability among individuals with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), a small vessel disease caused by mutations in NOTCH3. However, little is known about the factors that underlie this variability. We sought to quantify the contribution of modifying genetic effects to individual differences in the volume of cerebral ischemic lesions.

Methods—One hundred and fifty-one affected individuals (mean age ± SD = 45.7 ± 10.4) from 95 unrelated families with CADASIL underwent MRI. The volume of lesions visible on T2-weighted images and the intracranial volume (ICV) were quantified and vascular risk factors were assessed. Because of a skewed distribution, lesion volume measures were square-root transformed. Variance component methods were used to estimate the heritability of lesion volumes (ie, the proportion of variation caused by additive genetic factors) after adjusting for covariates.

Results—In multivariate analyses, higher age, a larger ICV, and a higher diastolic blood pressure were independently associated with a larger volume of T2-visible lesions (all \(P < 0.05\)). After adjustment for age the point estimate for the heritability of the square-root-transformed measure of T2 lesion volume was 0.634 (SE = 0.286). Adjustment for age, sex, ICV, and diastolic blood pressure increased the estimated heritability to 0.738 (SE = 0.255).

Conclusions—Heritability estimates in CADASIL suggest a strong modifying influence of genetic factors distinct from the causative NOTCH3 mutation on the amount of ischemic brain lesions. These findings justify a systematic search for genetic variants that modify disease progression. (Stroke. 2006;37:2684-2689.)

Key Words: CADASIL ■ genetics ■ white matter hyperintensities

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary small vessel disease caused by mutations in the NOTCH3 gene. Most mutation carriers eventually develop strokes and cognitive impairment. However, there are great discrepancies between individuals regarding the severity of the phenotype. For instance, age of onset for stroke ranges from about 25 to 70 years. Similarly, some individuals develop early disability and dementia whereas others remain asymptomatic until advanced age.

Conventional MR images have been used to quantify the extent of subcortical ischemic lesions in symptomatic and presymptomatic cases. In parallel with the variations in clinical expressivity, there are considerable discrepancies in the volume of T2-hyperintense lesions between individuals of similar age (Figure 1). Such discrepancies are not explained by different NOTCH3 mutations because there are similar variations within families and between unrelated individuals who carry the same mutation. In fact, previous studies found no influence of mutation characteristics on MRI lesion volumes.

The influence of conventional cardiovascular risk factors on disease progression was examined by a recent study which found an association between smoking and an earlier age of onset for stroke/transient ischemic attack. In another study higher systolic blood pressure was a risk factor for the progression of cerebral lesions as determined by serial MRI. However, these findings await confirmation. Until now, higher age is the only firmly established risk factor for disease progression in CADASIL leaving a large proportion of the phenotypic variance unexplained.

The impact of genetic modifiers, ie, cis- or trans-acting genetic factors and nonallelic genetic modifiers on disease progression in CADASIL is unknown. The objective of this study therefore was to quantify the contribution of modifying genetic influences to individual differences in disease severity.
biopsy material (n = 8).12–14 Individuals were excluded if they had /H11005 by the presence of characteristic ultrastructural vascular alterations in confirmed either by a typical mutation in the presence and severity of symptoms.

Symmetric head positioning with respect to orthogonal axes was intracranial volume. All imaging studies were performed on the same 1.5 Tesla system containing a 235 CADASIL families.4 The study was nested within an ongoing natural history study.9,11 Between September 1999 and May 2004, 153 affected subjects underwent MRI at the authors institution. All affected individuals from multiple affected families were invited to partici- subjects were asked to join the study irrespective of the presence and severity of symptoms.

Subjects were included if they had definite CADASIL as con- firmed either by a typical mutation in the NOTCH3 gene (n = 145) or by the presence of characteristic ultrastructural vascular alterations in biopsy material (n = 8).12–14 Individuals were excluded if they had other apparent neurological illness, such as multiple sclerosis or brain tumor that might influence the extent of lesions unrelated to CADASIL or if an MRI could not be performed. Two subjects (one with a typical NOTCH3 mutation) were excluded from the analysis because of insufficient quality of the MR images attributable to motion artifacts.

Cardiovascular risk factors were assessed by a standard question- naire and examination. Blood pressure (BP) recordings were ob- tained after at least 5 minutes of rest in a sitting position using a mercury sphygmomanometer. BP was defined as the mean of 2 successive measurements. Hypertension was defined by a systolic BP > 140 mm Hg, a diastolic BP > 90 mm Hg or use of hypertensive medication together with a past diagnosis of hypertension. Smoking habits were studied in 3 ways: pack-years; ever smokers; and current smokers. Hypercholesterolemia was defined as a cholesterol > 5.7 mmol/L or a previous diagnosis of hypercholesterolemia.

Data were organized into discrete family structures: 90 individuals were in genetically useful relationships including 34 sibling pairs, 19 parent-offspring pairs, 21 first-cousin pairs, 21 avuncular pairs, 6 first-cousin once-removed pairs, 6 grand-avuncular pairs and 1 second-cousin pair. The study protocol was approved by the local ethics committee and informed consent was obtained from all participants.

MRI

All imaging studies were performed on the same 1.5 Tesla system (Magnetom Vision, Siemens) using a standard birdcage headcoil. Symmetric head positioning with respect to orthogonal axes was verified by T1-weighted axial, coronal, and sagittal scouts. Intracranial volume and T2 lesion volumes were determined from dual-echo turbo spin-echo (repetition time = 3300 ms, echo time = 16/98 ms) and T1-weighted spin-echo (repetition time = 768 ms, echo time = 14 ms) images, with each set consisting of 24 contiguous axial slices (slices thickness 5 mm, no gap, field of view 188 × 250 mm², matrix 192 × 256, image resolution of 0.97 × 0.97 × 5 mm³). Slices were positioned to run parallel to a line that joins the most inferoanterior and the inferoposterior parts of the corpus callosum.15

Image Analysis and Postprocessing

Interactive image postprocessing was performed offline and without knowledge of the subjects histories or biological relationships. For the calculation of T2 lesion volumes we applied a previously established 2-step procedure.9,16 First, regions of abnormal hyperin- tense signal were identified and marked by 2 experienced readers (M.H., M.D.) on hardcopies of proton density–weighted scans of the brain. All lesions were cross-checked on T2-weighted images to increase confidence in lesion identification. Using the marked lesions on the hardcopies as a working template, trained radiological technicians applied a local threshold based segmentation technique on the corresponding digital proton density–weighted images. This provided an absolute volume of lesion load. The mean intraobserver coefficient of variation of this method is 2.6%.16 Intracranial volume was calculated from the T2-weighted datasets with sienax, a tool for cross-sectional volumetric measurements, developed as part of the FSL analysis software (University of Oxford FMRI Analysis Group, http://www.fmrib.ox.ac.uk/fsl/). Volumetric measurements using this software have been validated independently for T2-weighted images as well as other image types.17

Statistical Methods

Descriptive statistics and regression analyses were calculated using SPSS version 12.0.1 for Windows (SPSS Inc). Before estimating heritabilities or analyzing the relationships between traits, skewed distributions of dependent variables were normalized by square-root transformation of the raw measurements. Linear multiple regression analysis was carried out to identify covariates predictive of interind- ividual differences in T2-lesion volumes.

Heritabilities including standard errors were estimated using MERLIN and QTDT.18,19 These programs partition the total variance of the trait into the proportion of variance attributable to (1) additive genetic factors, (2) covariates, and (3) other unmeasured factors (eg, shared and nonshared environment), ie, a variance component framework is used. The proportion of variance attributable to additive genetic factors is referred to as the heritability. Estimation of these quantities was performed via maximum likelihood.

Results

Sample Description

The 151 CADASIL subjects were from 95 families and included 64 men and 87 women. Subject demographics and average MRI variables are summarized by sex in Table 1. Intracranial volume was significantly smaller in women (P < 0.001). In contrast, age, T2 lesion volume, and cardiovascular risk factors did not differ significantly between sexes.

Interindividual Variation in T2 Lesion Volume

The distribution of T2 lesion volume was positively skewed with values ranging from 2.4 to 298.5 cm³ (Figure 2, insert). The statistical models assume that the dependent variable is normally distributed. Therefore, the raw measurements (vol- umes) were square-root-transformed. This step reduced the skewness measure from 1.06 to −0.08 (Figure 2).

A multiple linear regression model that included age, sex, intracranial volume, systolic BP, diastolic BP, pack-years and smoking habits, diabetes, and hypercholesterolemia ac-

Figure 1. Variability of MRI lesion volume: T2-weighted images of a 60-year-old male mutation carrier with few ischemic lesions (T2-lesion volume = 45.1 mL; A) and a 49-year-old female mutation carrier with multiple confluent subcortical ischemic lesions (T2-lesion volume = 164.7 mL; B). Both patients were shown to carry the R168C mutation.
Heritability of T2 Lesion Volumes

After adjustment for age the point estimate for the heritability of the square-root–transformed measure of T2 lesion volume was 0.634 (SE=±0.286; Table 3). Adjustment for all identified covariates increased the estimated heritability to 0.738 (SE±0.055). Age was estimated to account for 29% of the interindividual variation in the square-root–transformed measure of T2 lesion volume. All covariates together were estimated to account for 31% of the variation in lesion volume which is close to the estimate from the linear regression model (34%).

To estimate the influence from different disease causing NOTCH3 alleles, we modeled them as a factor that is shared within families. The point estimate for the proportion of variance attributable to this factor was 0% which agrees with previous studies that found no differential effect of NOTCH3 genotypes on T2 lesion volumes. As can be seen in Figure 3, variability of normalized T2 lesion volumes over age is similar across NOTCH3 mutations.

Discussion

CADASIL is a devastating, progressive disorder leading to disability and vascular dementia. However, there is significant variability in the rate of disease progression.2–4,11 Potential factors accounting for this variability include environmental influences, cis- and trans-acting genetic factors, nonallelic genetic modifiers, and stochastic factors. To obtain an estimate on the proportion of variance explained by genetic factors, we estimated the heritability of the volume of MRI lesions as a quantitative marker for disease severity. The results suggest a strong contribution of genetic modifiers to interindividual differences in the volume of ischemic brain lesions in CADASIL.

The heritability estimate is remarkably close to previous estimates of the heritability of incidental white matter hyper-intensity (WMH) volume in elderly subjects. An investigation in elderly male veteran twins found a heritability of 71%.20 Studies in hypertensive sibs and unselected individuals from the Framingham cohort revealed heritabilities of 67% and 55%, respectively.21,22 Incidental WMH have been shown to most often reflect ischemic lesions secondary to small vessel disease.23–28 They therefore share a common pathological basis with MR lesions in CADASIL. Taken together the available data from population-based samples, hypertensive individuals and CADASIL patients suggest a strong genetic influence on subcortical ischemic lesions.

The neuropathological correlates of T2 hypersignals in CADASIL include neuronal and axonal loss, demyelination, mild edema, and reactive gliosis with occasional inflammatory reactions.29,30 Genetic modifiers could act on various levels (eg, vascular endothelial and smooth muscle cells, neurons, oligodendrocytes, inflammatory cells) and through multiple mechanisms including vascular endothelial function cerebral autoregulation, the response of neurons to ischemia, and repair mechanisms.31,32

The specific genetic variants underlying lesion volume variability in CADASIL are still unknown; the only 2 candidate-gene studies published so far found no modifying influence of apolipoprotein E genotype on quantitative MRI metrics.8,33 Recent data from the Framingham cohort suggest that a locus on chromosome 4 influences WMH volume in normal elderly subjects.34 Candidate gene studies in other

### Table 1. Descriptive Characteristics of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>Women (n=87)</th>
<th>Men (n=64)</th>
<th>Total (n=151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (SD)</td>
<td>46.6 (11.3)</td>
<td>44.6 (9.1)</td>
<td>45.7 (10.4)</td>
</tr>
<tr>
<td>Intracranial vol, cm³ (SD)</td>
<td>1274 (109)*</td>
<td>1418 (99)</td>
<td>1334 (127)</td>
</tr>
<tr>
<td>T2 lesion vol, cm³ (SD)</td>
<td>90.5 (54.5)</td>
<td>92.6 (56.3)</td>
<td>90.8 (55.1)</td>
</tr>
<tr>
<td>Square-root–transformed T2 Lesion vol, cm³ (SD)</td>
<td>9.02 (2.88)</td>
<td>9.1 (3.16)</td>
<td>9.05 (2.99)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg (SD)</td>
<td>129.5 (17.6)</td>
<td>130.4 (14.0)</td>
<td>129.9 (16.1)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg (SD)</td>
<td>82.0 (11.5)</td>
<td>83.3 (11.9)</td>
<td>82.5 (11.6)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>23.8</td>
<td>17.7</td>
<td>21.2</td>
</tr>
<tr>
<td>Pack-years (SD)</td>
<td>44.0</td>
<td>43.6</td>
<td>43.8</td>
</tr>
<tr>
<td>Ever smoking, %</td>
<td>34.1</td>
<td>33.9</td>
<td>34.0</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>8.2</td>
<td>1.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>37.9</td>
<td>28.1</td>
<td>33.3</td>
</tr>
</tbody>
</table>

*P<0.001 compared to women.

Figure 2. Frequency distribution of the square-root–transformed measures of T2 lesion volumes determined by MRI in 87 women and 64 men. Insert: Frequency distribution of the non-transformed raw measurements of MRI lesion volumes.
cohort found associations between variants in the renin-angiotensin system or endothelial nitric oxide synthase gene and WMH volume.35–38 These loci/variants represent potential candidate sites for similar studies in CADASIL.

In the multivariate analysis a higher diastolic BP was independently associated with greater lesion volumes. These findings agree with longitudinal data in CADASIL, which showed that higher BP levels are an independent risk factor for MRI progression during follow-up.9,10 It further agrees with epidemiological data suggesting that hypertension is the most important risk factor for WMH, following age.39,40 In the current study, there was no influence of other conventional risk factors on MRI lesion volume. We may therefore infer that most additive genetic factors contributing to cerebral ischemic lesions in CADASIL act through novel pathways not reflected in conventional vascular risk factors. However, this will need to be investigated by targeted studies.

The methodological strengths of this study include a consistent scan protocol, a validated protocol for fully quantitative lesion volume measurements and the inclusion of data from multiple generations. The main limitations are those inherent to a limited sample size, a broad age range, and the assumptions of the genetic model. Thus, for example, our study was not sufficiently powered to calculate heritabilities separately for men and women or for different age groups.22 Also, the estimate is only of additive genetic variation, although this may be relatively robust. Also, the calculation gives no insight into the number of genes or their relative effect. A further caveat regarding the estimates is that the genetic contribution might be overestimated because of shared environmental influences. However, we consider this possibility unlikely because our analyses included distantly related subjects, because most subjects lived in separate households, and because a separate analysis including shared environment had no substantial influence on the results (data not shown). Finally, we cannot exclude an ascertainment bias toward symptomatic subjects or subjects who were mobile and might therefore be less severely affected.

Our analysis was limited to the volume of MRI-visible lesions and cannot be generalized to other disease aspects including the clinical phenotype. Yet, a clinical relevance of T2 lesion volumes has been demonstrated by previous cross-sectional studies which found correlations between MR lesion volumes and multiple clinical scales including scales for disability and cognitive performance.6,9,41 We therefore hypothesize that our heritability estimate reflects a clinically meaningful influence from modifying genetic factors.

Variations in phenotypic expressivity are common in monogenic disorders and a modifying influence from genetic factors has been demonstrated for several neurological conditions including Huntington’s disease,42 spinoocerebellar ataxia type 2,2,31 and sickle cell anemia.44 Identification of the responsible genetic variants has provided insights into disease mechanisms and allowed predicting individual risks44,45 thus improving the counseling of patients and their families.

In conclusion, the heritability estimates provided here justify a systematic search for genetic modifiers in CADASIL. Such studies will demand large samples, probably in the order of several hundred subjects, thus requiring the combined efforts of multiple centers. The combined application of MRI and high-throughput genotyping promises to advance understanding of the pathogenesis of small vessel disease and subcortical ischemic lesions and may eventually result in better prevention and treatment.

TABLE 3. Heritability Estimated for MRI Lesion Volume (square-root–transformed)

<table>
<thead>
<tr>
<th>Covariate Adjustments</th>
<th>h^2 ± SE</th>
<th>P Value</th>
<th>Proportion of Variation Caused by Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.634 ± 0.286</td>
<td>0.0263</td>
<td>0.292</td>
</tr>
<tr>
<td>Age, sex</td>
<td>0.586 ± 0.277</td>
<td>0.0345</td>
<td>0.301</td>
</tr>
<tr>
<td>Age, sex, intracranial volume</td>
<td>0.620 ± 0.263</td>
<td>0.0184</td>
<td>0.302</td>
</tr>
<tr>
<td>Age, sex, diastolic BP</td>
<td>0.684 ± 0.269</td>
<td>0.0110</td>
<td>0.305</td>
</tr>
<tr>
<td>Age, sex, intracranial volume, diastolic BP</td>
<td>0.738 ± 0.255</td>
<td>0.0039</td>
<td>0.312</td>
</tr>
</tbody>
</table>

h^2 indicates heritability estimate.
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Disclosures
None.

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