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Risk factors for progression of brain atrophy in aging
Six-year follow-up of normal subjects

C. Enzinger, MD; F. Fazekas, MD; P.M. Matthews, MD, DPhil, FRCP; S. Ropele, PhD; H. Schmidt, MD; S. Smith, DPhil; and R. Schmidt, MD

Abstract—Objectives: To determine the rate of brain atrophy in neurologically asymptomatic elderly and to investigate the impact of baseline variables including conventional cerebrovascular risk factors, APOE ε4, and white matter hyperintensity (WMH) on its progression. Methods: We assessed the brain parenchymal fraction at baseline and subsequent annual brain volume changes over 6 years for 201 participants (F/M = 96/105; 59.8 ± 5.9 years) in the Austrian Stroke Prevention Study from 1.5-T MRI scans using SIENA (structural image evaluation using normalization of atrophy)/SIENAX (an adaptation of SIENA for cross-sectional measurement)/(www.fmrib.ox.ac.uk/fsl). Hypertension, cardiac disease, diabetes mellitus, smoking, and regular alcohol intake were present in 64 (31.8%), 60 (29.9%), 5 (2.5%), 70 (39.3%), and 40 (20.7%) subjects, respectively. Plasma levels of fasting glucose (93.7 ± 18.6 mg/dL), glycated hemoglobin A (HbA1c; 5.6 ± 0.7%), total cholesterol (228.3 ± 40.3 mg/dL), and triglycerides (127.0 ± 75.2 mg/dL) were determined. WMH was rated as absent (n = 56), punctate (n = 120), early confluent (n = 14), and confluent (n = 11). Results: The baseline brain parenchymal fraction of the entire cohort was 0.80 ± 0.02 with a mean annual brain volume change of −0.40 ± 0.29%. Univariate analysis demonstrated a higher rate of brain atrophy in older subjects (p = 0.0001), in those with higher HbA1c (p = 0.0001), higher body mass index (p = 0.02), high alcohol intake (p = 0.04), severe WMH (p = 0.03), and in APOE ε4 carriers (p = 0.07). Multivariate analysis suggested that baseline brain parenchymal fraction, HbA1c, and WMH score explain a major proportion of variance in the rates of brain atrophy in the cohort (corrected $R^2 = 0.27$; $p = 0.0001$). Conclusions: Neurologically asymptomatic elderly experience continuing brain volume loss, which appears to accelerate with age. Glycated hemoglobin A (HbA1c) was identified as a risk factor for a greater rate of brain atrophy. Clustering of factors associated with the so-called metabolic syndrome in subjects with high HbA1c suggests a link between this syndrome and late-life brain tissue loss.

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Our current knowledge on the evolution of brain atrophy during aging predominantly relies on an extrapolation of cross-sectional imaging findings. Risk factors including hypertension, diabetes mellitus, alcohol, hyperlipidemia, cigarette smoking, and elevated plasma homocysteine have been implicated in an acceleration of this process. However, the strength of these conclusions is limited by the application of either visual or semiquantitative techniques to assess brain atrophy in these studies.

The rate of global brain atrophy observed in healthy subjects is much lower than in those with disease. Likewise, a potential modulation of the progression of global brain atrophy by risk factors in otherwise healthy subjects is expected to result in relatively minor differences in the magnitude of longitudinal brain volume changes. Consequently, using a highly sensitive measure of brain volume change is needed to test this assumption. Recent advances in neuroimaging have resulted in fully automated measurement techniques of brain atrophy.

With an aging population, identification of factors that contribute to increased brain atrophy could be important to cognitive impairment. We therefore readdressed this issue and set out to overcome the limitations of previous studies by using a precise, fully automated method to measure brain atrophy over a 6-year period in a large cohort of normal elderly community-dwelling volunteers. This allowed investigation of the impact of a wide range of baseline variables including conventional vascular and...
Table 1 Baseline characteristics of Austrian Stroke Prevention Study Phase II participants with MRI follow-up over 6 years and those without MRI follow-up over 6 years

<table>
<thead>
<tr>
<th>Risk factors, n (%)</th>
<th>Participants with MRI follow-up, n = 201</th>
<th>Participants without MRI follow-up, n = 212</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial hypertension</td>
<td>64 (31.8)</td>
<td>74 (34.9)</td>
<td>0.27†</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (2.5)</td>
<td>13 (6.1)</td>
<td>0.38†</td>
</tr>
<tr>
<td>Current or previous smoking</td>
<td>79 (39.3)</td>
<td>90 (42.4)</td>
<td>0.25†</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>60 (29.9)</td>
<td>89 (42.0)</td>
<td>0.007†</td>
</tr>
<tr>
<td>Continuous variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>138.8 ± 19.9</td>
<td>140.6 ± 20.0</td>
<td>0.03‡</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>85.8 ± 8.4</td>
<td>85.7 ± 9.5</td>
<td>0.52‡</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8 ± 3.6</td>
<td>26.3 ± 3.6</td>
<td>0.17‡</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>228.3 ± 40.3</td>
<td>230.1 ± 40.4</td>
<td>0.71‡</td>
</tr>
</tbody>
</table>

Data are presented as mean ± 1 SD or n (%).

* Student t test; † Chi-square test; ‡ Mann–Whitney U test.

less well-established risk factors, APOE ε4, and the scored severity of white matter changes on the progression of brain atrophy.

Methods. We included 201 participants of the Austrian Stroke Prevention Study (ASPS), for whom MRI scans were available both at baseline and at 6-year follow-up (table 1). The sample exclusively consisted of white subjects of central European origin, and the length of education ranged from 9 to 18 years. At the time of the examination, 47.3% were retired, 10.6% were blue-collar and 64% white-collar workers, 4.6% were self-employed, 9.6% were academics, and 11.2% were stay-at-home wives or husbands. No unemployed individual participated in the study.

The ASPS is a single-center, prospective, follow-up study on the cerebrovascular effects of vascular and genetic risk factors in the normal elderly population of Graz, Austria. At the start of the study, we randomly selected 2,007 individuals aged 50 to 75 years without neuropsychiatric disease from our community register. A total of 509 study participants underwent brain MRI at baseline. Follow-up examinations were done at 3 and 6 years. Absolute changes of brain volume within the 3-year periods were small (i.e., within or close to the measurement error) for a considerable proportion of individuals. We therefore opted to use only the 6-year interval data, which provided a more robust estimation of brain volume changes. The selection and sampling procedures and the design of the study have been described elsewhere. Individuals were excluded from the study if they had a history of neuropsychiatric disease, including cerebrovascular attacks and dementia, or an abnormal neurologic examination determined on the basis of a structured clinical interview and physical and neurologic examinations. Stroke represents an end point in the ASPS. The development of cognitive decline during follow-up does not constitute an exclusion criterion.

A detailed description of the assessment of risk factors within the setting of the ASPS is given elsewhere. In short, both historical information and laboratory findings were considered for risk factor diagnosis. Only baseline findings were considered in the present analysis. Arterial hypertension was considered present if there was a history of arterial hypertension with repeated blood pressure readings higher than 160/95 mm Hg, if an individual was treated for arterial hypertension, or if the three readings of the systolic and diastolic blood pressure exceeded this limit. Diabetes mellitus was coded as present if a subject was treated for diabetes at the time of examination or if the fasting blood glucose level at one examination exceeded 140 mg/dL. Cardiovascular disease was assumed to be present if there was evidence of cardiac abnormalities known to be a source of cerebral embolism, evidence of coronary heart disease according to the Rose questionnaire or appropriate EKG findings (Minnesota codes: I, 1 to 3; IV, 1 to 3; or V, 1 to 2), or if an individual presented signs of left ventricular hypertrophy on echocardiography or EKG (Minnesota codes: III, 1 or IV, 1 to 3). Study participants were asked whether they ever smoked and whether they currently smoked. Information on self-reported alcohol drinking habits was available for 194 subjects and graded as total abstinence, occasional, moderate (one drink per day), or strong (two or more drinks per day) alcohol consumption. A body mass index (BMI) was calculated as kilograms (body weight) per square meters (height).

A lipid status, including triglyceride levels and total cholesterol, was determined for each participant. We also measured the plasma levels of fasting glucose and of the percentage of glycated hemoglobin A (HbA₁c) of study participants, as described previously.

High molecular weight DNA was extracted from peripheral whole blood using Quiagen genomic tips. APOE genotyping was done in 175 participants according to the method of Hixson and Vernier. Thirty-four (19.4%) subjects carried at least one APOE ε4 allele (genotypes ε2/ε4, ε3/ε4, and ε4/ε4). MRI was obtained on 1.5-T scanners from the same manufacturer (Gyroscan S15 and ACS; Philips Medical Systems, Eindhoven, the Netherlands). Identical MRI protocols were used at baseline and at follow-up, generating proton density and T2-weighted sequences in transverse orientation (repetition time (TR)/echo time (TE) = 2,000 to 2,500 msec/30 and 90 msec) and T1-weighted images in a sagittal plane (TR/TE = 600/30 msec). The slice thickness was 5 mm, and the matrix size was 128 × 256 pixels in the sagittal plane and 256 × 256 pixels in the axial plane. A sagittal and coronal pilot ensured consistency in image angulation throughout the study.

The process of rating white matter lesions according to our scheme into absent (grade 0), punctate (grade 1), early confluent (grade 2), and confluent (grade 3) has been described. Early confluent and confluent lesions are correlated with ischemic tissue damage, whereas punctate lesions appear to be of nonischemic origin.

For further analysis, we therefore compared subjects with white matter hyperintensity (WMH) grades 2 and 3 (n = 25, 12.5%) with those with WMH grades 0 and 1 (n = 176, 87.5%). Scans were also analyzed for the presence of clinically silent ischemic infarcts and lacunae (defined as focal lesions involving the basal ganglia, the internal capsule, the thalamus, or the brainstem not exceeding a maximal diameter of 10 mm). None of the
individuals exhibited an MRI pattern suggestive of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.¹⁴

Structural image evaluation using normalization of atrophy (SIENA) was used for the automated assessment of longitudinal annual brain volume change (aBVC) and SIENAX (an adaptation of SIENA for cross-sectional measurement) was used for estimation of the cross-sectional brain parenchymal fraction (BPF) using T2-weighted scans. These methods are described in detail elsewhere.¹⁴,¹⁵ Briefly, SIENA performs segmentation of brain from nonbrain tissue of the head and estimates the outer skull surface (for both time points) and uses these results to register the two images, while correcting (normalizing) for imaging geometry changes. Then the registered segmented brain images are used to find brain volume measures, measured on the basis of the image edges. SIENAX also performs segmentation of brain from nonbrain tissue and estimates the outer skull surface at a single time point. The brain and skull images are then registered to a standard space brain and skull image pair. This step normalizes for skull size. Next, a probabilistic brain mask derived in standard space is applied to make sure that certain structures such as eyes/optic nerves have not been included in brain segmentation. Finally, tissue-type segmentation is carried out and a (normalized) brain volume estimate is produced. Here we used the brain percentage of a central slab of slices after normalization to standard space as an estimate of baseline brain volume. This BPF was calculated as parenchyma volume divided by parenchyma volume plus CSF volume. The SIENA and SIENAX software are freely available as part of the FMRIB Software Library (www.fmrib.ox.ac.uk/fsl).

Statistical analyses. The Statistical Package of Social Sciences (PC+; version 11.5; SPSS Inc., Chicago, IL) was used for data analysis. Categoric variables were tested by Pearson’s χ² test or by a 2 × 2 Fisher exact test in case of contingency tables containing fewer than five cases. Fulfillment of or deviation from normal distribution of continuous variables was tested by Kolmogorov-Smirnov statistics with a significance level after Liliefors and additional inspection of histograms. Normally distributed continuous variables were compared with Student’s t test or one-way analysis of variance (ANOVA). The Mann–Whitney U test and the Kruskal–Wallis test were used as analogue nonparametric tests. Spearman’s rank correlation coefficients were calculated. After log-transformation of nonparametric variables, a linear multiple regression analysis was carried out to assess the relative and independent contribution of different variables on the progression of brain atrophy. Only variables significant at p < 0.2 in univariate analyses were selected. Within the model, an automated selection method was chosen to identify variables with significant contribution (forward stepwise regression, cutoff p = 0.05), as the variables were expected to be correlated. Based on a priori hypotheses, the following variables were entered in the multiple regression analyses with aBVC as a dependent variable: model 1: HbA1c, age, BMI, alcohol consumption, APOE ε4 status; model 2: variables of model 1 plus WMH score at baseline; model 3: variables of model 2 plus BPF at baseline. A multivariate linear model was chosen because it allows one to assess and account for pseudocorrelations between independent variables and to include both continuous and interval-scaled variables. Inter correlation between explaining variables was assessed by collinearity diagnostics yielding eigenvalues, variance inflation factors, and values for tolerance. The level of significance was set at 0.05 in all cases. Data are quoted as mean ± 1 SD or as median ± 1 SD in the absence of normal distribution, unless otherwise stated.

Results. The mean BPF of the entire cohort was 0.80 ± 0.02 (range 0.73 to 0.84; women: 0.80 ± 0.02 vs men: 0.89 ± 0.02; p = 0.14). Over the 6-year observation period, the study participants experienced a mean aBVC of −0.40 ± 0.29% (range −1.47 to 0.32%; women: −0.39 ± 0.26% vs men: −0.41 ± 0.31%; p = 0.76). Increasing age was correlated with a higher rate of brain atrophy (i.e., loss of brain volume, r = −0.34; p = 0.0001) and inversely correlated with BPF at baseline (r = −0.35; p = 0.0001). Brain atrophy rates in subjects aged 65 to 75 years were twice as high as those in subjects aged 50 to 54 years (−0.55 ± 0.29% vs −0.28 ± 0.23%; p = 0.0001; table 2). The rate of brain atrophy varied considerably, even between individuals of identical or comparable age, with a further increase in variability in older age groups (figure 1).

None of the conventional major vascular risk factors such as diabetes, hypertension, cardiac disease, smoking, hypercholesterolemia, and hypertriglyceridemia were significantly associated with either baseline BPF or subsequent aBVC (data not shown). Aside from age, the most significant association between a single factor and both baseline brain volume and subsequent rate of atrophy was found for HbA1c. The rate of atrophy increased with increasing HbA1c (see table 2). Rates of atrophy above the median HbA1c (>5.6%; aBVC: −0.49 ± 0.25%) were twice as high as those within the lowest quartile (HbA1c: 4.4 to 5.2%; aBVC: −0.24 ± 0.17%; p = 0.0001; figure 2). There was only a weak positive correlation between HbA1c and age (r = 0.16; p = 0.02), so age could not explain the magnitude of the brain volume changes observed with increasing HbA1c levels. A weaker relationship was observed between brain atrophy and increasing BMI. Increasing BMI was significantly associated with greater longitudinal brain volume loss (see table 2). HbA1c levels and BMI also demonstrated a modest correlation (r = 0.22; p = 0.002).

Subjects within the highest quartile of HbA1c (5.9 to 9.0%) had a higher BMI than those within the lowest quartile of HbA1c (HbA1c: 4.4 to 5.2%; BMI: 28.0 vs 25.6; p = 0.003), higher fasting glucose levels (105.2 vs 88.4 mg/dL; p = 0.0001), higher total cholesterol (238.6 vs 223.1 mg/dL; p = 0.04), and a trend toward higher mean diastolic blood pressure (86.2 vs 85.4 mm Hg; p = 0.07). No difference was found in the levels of triglycerides (162.2 vs 139.3 mg/dL; p = 0.17).

A J-formed association existed between the extent of alcohol ingestion and progression of brain atrophy (see table 2). Abstainers exhibited a higher atrophy rate than occasional or moderate alcohol consumers. Heavier drinkers experienced the highest annual brain atrophy rate observed (−0.54 ± 0.26%), followed by the five diabetics in the study (−0.54 ± 0.12%) and 24 subjects with early confluent (score 2) to confluent (score 3) WMH at baseline (−0.53 ± 0.34%, see table 2). Individuals with WMH scores of 2 or 3 showed both a lower baseline brain volume (p = 0.05) and a greater rate of atrophy compared with individuals with only punctate or absent white matter changes (p = 0.03). APOE ε4 carriers had a trend toward a higher rate of brain parenchyma loss (p = 0.07).

To better assess the independent contributions of baseline variables to the progression of brain atrophy, we performed a multivariate regression analysis, generating three different models based on a priori hypotheses (table 3). The first model consisted of demographic factors, vascular risk factors, and APOE ε4 status, shown to be significant in the univariate analyses. Model 1 showed that increasing age and HbA1c correlated with brain volume change. In models 2 and 3, imaging findings were included. In the second model, in addition to the variables of the first model, the WMH score was entered. Age, HbA1c, and WMH score were determined to be independent risk factors for greater longitudinal brain volume loss. Finally, in a third model, BPF at baseline was additionally entered. This third model consisted of the following independent predictors of the rate of brain atrophy (listed in descending order of significance): age, HbA1c, and WMH score.
order): BPF at baseline, HbA1c, and WMH score at baseline. These three variables explained almost one-third of the variation in atrophy rate in the entire cohort (corrected $R^2 = 0.27$). The factor age did not contribute significantly to this model anymore and was excluded.

**Discussion.** We used a fully automated method to determine the rate of the progression of brain atrophy over a 6-year period in a large cohort of elderly individuals free of neuropsychiatric disease. We then used these data to identify baseline variables related to increased risk of greater rates of brain atrophy. This approach yielded three major findings. First, we observed an increased rate of brain atrophy with age, earlier than previously suspected. Second, our data suggest a link between increased circulatory glucose concentrations (as evidenced by increased HbA1c) and brain volume loss. Third, considering imaging findings, baseline brain volume independently predicted subsequent brain volume change.

A mean rate of annualized global brain atrophy of $-0.4\%$ was observed in this population of normal volunteers aged 50 to 75 years, consistent with the notion of a "shrinking brain." The observed magnitude of annual brain parenchyma loss lies within the range reported by other groups in similar cohorts\textsuperscript{17,26-29} although a precise comparison is hampered by methodologic differences between studies. We found that the rate of atrophy increased with age in the cohort. Thus, the change in brain volume in subjects in the highest age quartile was twofold higher than in those in the lowest age quartile. This corroborates and extends earlier preliminary evidence\textsuperscript{26,29} suggesting that age-

### Table 2 Univariate associations between baseline characteristics, BPF, and subsequent aBVC over 6 years

<table>
<thead>
<tr>
<th>Continuous variables</th>
<th>Range</th>
<th>BPF at baseline</th>
<th>$p$ Value</th>
<th>% aBVC</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st quartile</td>
<td>50–54</td>
<td>0.810 ± 0.015</td>
<td>0.015</td>
<td>-0.283 ± 0.225</td>
<td></td>
</tr>
<tr>
<td>2nd quartile</td>
<td>55–59</td>
<td>0.810 ± 0.013</td>
<td>0.013</td>
<td>-0.326 ± 0.251</td>
<td></td>
</tr>
<tr>
<td>3rd quartile</td>
<td>60–64</td>
<td>0.802 ± 0.019</td>
<td>0.326</td>
<td>-0.436 ± 0.305</td>
<td></td>
</tr>
<tr>
<td>4th quartile</td>
<td>65–75</td>
<td>0.794 ± 0.019</td>
<td>0.001*</td>
<td>-0.549 ± 0.292</td>
<td></td>
</tr>
<tr>
<td><strong>HbA1c, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st quartile</td>
<td>4.4–5.2</td>
<td>0.813 ± 0.141</td>
<td>0.141</td>
<td>-0.241 ± 0.169</td>
<td></td>
</tr>
<tr>
<td>2nd quartile</td>
<td>5.3–5.5</td>
<td>0.803 ± 0.019</td>
<td>0.374</td>
<td>-0.374 ± 0.331</td>
<td></td>
</tr>
<tr>
<td>3rd quartile</td>
<td>5.6–5.8</td>
<td>0.801 ± 0.023</td>
<td>0.467</td>
<td>-0.467 ± 0.288</td>
<td></td>
</tr>
<tr>
<td>4th quartile</td>
<td>5.9–9.0</td>
<td>0.803 ± 0.016</td>
<td>0.02†</td>
<td>-0.474 ± 0.271</td>
<td></td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st quartile</td>
<td>20.3–23.9</td>
<td>0.805 ± 0.019</td>
<td>0.017</td>
<td>-0.324 ± 0.252</td>
<td></td>
</tr>
<tr>
<td>2nd quartile</td>
<td>24.0–26.5</td>
<td>0.806 ± 0.168</td>
<td>0.018</td>
<td>-0.355 ± 0.261</td>
<td></td>
</tr>
<tr>
<td>3rd quartile</td>
<td>26.6–28.6</td>
<td>0.805 ± 0.021</td>
<td>0.75†</td>
<td>-0.433 ± 0.309</td>
<td></td>
</tr>
<tr>
<td>4th quartile</td>
<td>28.7–41.1</td>
<td>0.801 ± 0.018</td>
<td>0.50‡</td>
<td>-0.486 ± 0.304</td>
<td></td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>115</td>
<td>0.804 ± 0.018</td>
<td>0.018</td>
<td>-0.404 ± 0.292</td>
<td></td>
</tr>
<tr>
<td>Occasionally</td>
<td>39</td>
<td>0.807 ± 0.022</td>
<td>0.341</td>
<td>-0.341 ± 0.300</td>
<td></td>
</tr>
<tr>
<td>1 drink/day</td>
<td>23</td>
<td>0.807 ± 0.016</td>
<td>0.387</td>
<td>-0.387 ± 0.249</td>
<td></td>
</tr>
<tr>
<td>≥2 drinks/day</td>
<td>17</td>
<td>0.795 ± 0.023</td>
<td>0.13‡</td>
<td>-0.546 ± 0.262</td>
<td></td>
</tr>
<tr>
<td><strong>APOEe4 allele</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>34</td>
<td>0.802 ± 0.021</td>
<td>0.45‡</td>
<td>-0.385 ± 0.280</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>141</td>
<td>0.805 ± 0.187</td>
<td>0.07‡</td>
<td>-0.385 ± 0.280</td>
<td></td>
</tr>
<tr>
<td><strong>WMH score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 and 1</td>
<td>177</td>
<td>0.806 ± 0.017</td>
<td>0.05‡</td>
<td>-0.380 ± 0.275</td>
<td></td>
</tr>
<tr>
<td>2 and 3</td>
<td>24</td>
<td>0.794 ± 0.027</td>
<td>0.03‡</td>
<td>-0.529 ± 0.343</td>
<td></td>
</tr>
</tbody>
</table>

Values represent means ± 1 SD.

* Analysis of variance; † Kruskal–Wallis test; ‡ Chi-square test.

BPF = brain parenchymal fraction; aBVC = annual brain volume change; HbA1c = glycated hemoglobin A; APOEe4- carriers = genotypes ε2/ε4, ε3/ε4, and ε4/ε4; WMH = white matter hyperintensity.
related global brain volume loss is not simply a linear process, as was assumed previously.\textsuperscript{2,5,30,31} Although a smaller study has proposed that global brain atrophy rates do not accelerate until after age 70 years,\textsuperscript{28} these data (particularly in conjunction with those from the much younger group studied previously)\textsuperscript{32} suggest that rates of atrophy increase progressively from early age on. This finding has implications for the monitoring of treatment responses\textsuperscript{33} and for future attempts to predict the onset of neurodegenerative disorders from serial brain volume measurements.\textsuperscript{15}

The second major finding was unexpected. In contrast to previous studies,\textsuperscript{7,8} no independent association between the evolution of brain atrophy and most conventional vascular risk factors was found in this cohort of healthy elderly individuals, although different definitions of risk factors might have contributed to this discrepancy. The only highly significant correlate of the rate of brain atrophy other than age was greater HbA$_1c$. This was also evident cross-sectionally: subjects with higher HbA$_1c$ levels had significantly lower brain volumes at baseline. This effect was not explained by the weak association of HbA$_1c$ levels and age, although the possibility of an interaction between HbA$_1c$ and age (similar to that between diabetes and aging)\textsuperscript{36} should be considered.

Several studies reported an association between diabetes and increased atrophy,\textsuperscript{1,6,9-11} but, to our knowledge, there have been no reports so far linking HbA$_1c$ and the progression of atrophy in individuals without diabetes. A high rate of atrophy was noted in the five subjects with diabetes included in this study, but this small number precluded meaningful statistical analysis. It should also be noted that the diagnostic criteria used might have led to an underestimation of the frequency of diabetes within this cohort. Interestingly, a significantly higher rate of brain atrophy was found even in subjects with HbA$_1c$ at levels regarded as normal in clinical practice (third quartile: 5.6 to 5.8%).\textsuperscript{35} Recent research indicates that both obesity and type 2 diabetes, previously considered peripheral metabolic disorders, involve dysfunction of the CNS.\textsuperscript{36}

Evidence suggests a link between this finding and correlates of the metabolic syndrome, also referred to as syndrome X or insulin resistance syndrome.\textsuperscript{37} The National Cholesterol Education Program’s Adult Treatment Panel III report defined the following six components of the metabolic syndrome: abdominal overweight, atherogenic dyslipidemia, increased blood pressure, insulin resistance and glucose intolerance, proinflammatory state, and prothrombotic state.\textsuperscript{36} Adults within the highest quartile of HbA$_1c$...
levels exhibited changes suggestive of the presence of four of these six factors. They had a higher BMI, elevated cholesterol and fasting glucose levels, and a higher diastolic blood pressure. Factors establishing the presence of proinflammatory or prothrombotic states, the remaining two factors required, were not analyzed in this study. Greater age is a risk factor for all these components.37

Extrapolation from diabetic populations suggests that hyperglycemia in these healthy subjects likely is associated with hyperinsulinemia.34,36 Until recently, the brain was described as “an insulin insensitive organ,” but identification of insulin and its receptors in the CNS has challenged that notion.38 Chronic peripheral hyperinsulinemia is a common finding in subjects with chronic hyperglycemia and obese subjects.36 It downregulates blood-brain barrier insulin receptors and reduces insulin transport to the brain.38 Low concentrations of CNS insulin can reduce acetylcholine neurotransmission and cerebral blood flow.38 Proinflammatory effects of hyperinsulinemia may potentiate neurodegeneration.34,38 Even hyperglycemia itself might contribute to neuronal apoptosis, mediated by increased glucose levels in the brain that lead to subsequent oxidative stress, enhanced glycation end products, and increased lipid peroxidation.39

HbA1c expresses the extent of glycation as a percentage of total hemoglobin A. Glycation is the post-translational, nonenzymatic covalent chemical linkage of glucose to proteins occurring in tissues that are exposed to glucose. Advanced glycation end products have been directly linked with the long-term complications associated with poorly controlled glycemia.35 It is widely accepted that HbA1c reflects the prevailing glycemia over the preceding 120 days.35 However, an important issue to consider is that a substantial proportion (as high as 62%) of the population variance in HbA1c levels may also be genetically determined and therefore not essentially glycemia related.35 The observed higher atrophy rate along with increased HbA1c might therefore represent a genetically mediated phenomenon. The clustering of several other factors implicated in the pathogenesis of the metabolic syndrome in individuals with increased HbA1c makes this seem less likely.

In line with a previous cross-sectional study,44 a higher scored severity of cerebral WMH also independently predicted a higher rate of brain atrophy in our study, although to a smaller extent than BPF. Early confluent and confluent WMH have been correlated histopathologically with increasing ischemic tissue damage with loss of fibers, cavitations, arteriolosclerosis, microcystic infarcts, and rarefaction of myelin.23 Microvascular abnormalities including thickening of the capillary basement membrane and endothelial cell degeneration of microvessels have also been found in aging, but these changes tend to be more pronounced in age-matched individuals with diabetes.34 This therefore suggests an additional modest contribution of vascular mechanisms to the progression of brain atrophy during aging. In this context, it is also interesting that a recent subanalysis of the Framingham study on the genetic variation in WMH volume proposed WMH to be an excellent genetic marker of brain aging, based on a high heritability of WMH even among individuals in whom the prevalence of cerebrovascular brain injury was generally low.44 Consistent with existing literature,12 we also observed a correlation between the extent of
alcohol consumption and brain atrophy, which appeared to be J shaped.

Technical weaknesses of our study could arise from variability in scanner performance over time. However, it is unlikely that this significantly affected the validity of the measurement of longitudinal brain volume change, as the method is relatively robust against drifts in image geometry.25 Also, such effects would then have randomly affected the investigated group. In the interpretation of our data, different measurement errors of the methods used have to be considered. For a single time point (cross-sectional) analysis, a brain volume accuracy of 0.5 to 1% (SIENAX) has been reported, whereas for a two time point (longitudinal) estimation of brain volume (SIENA), a substantially lower brain volume change error of approximately 0.15% has been found.25 A higher sensitivity of SIENA compared with semiautomated methods in the detection of subtle differences in atrophy rates has been reported recently.40

It is important to realize that our results may not be generalizable to the full healthy aging population. As shown in table 1, participants attending to MRI follow-up tended to be more educated and less frequently affected by diabetes and had lower systolic blood pressure and less frequent cardiac disease compared with subjects without MRI follow-up. Attrition is a common phenomenon in studies of aging, and (as in our sample) subjects adhering to follow-up more likely demonstrate a more favorable risk factor and sociodemographic profile.41 Recruitment methods that place high demands on older people, such as volunteering to come to a university center for examination and testing, may overrepresent particular types of older adults (although these might be expected to be those in best health, potentially masking results).42

Although a link between HbA1c and the progression of brain atrophy appears biologically plausible, several caveats need to be considered. Apart from causality, associations in observational studies can arise from shared risk factors or a common genetic susceptibility (e.g., consider the high heritability of brain volumes in twins).43,44 Further, many brain changes occur in parallel with aging, and correlational approaches make it challenging to relate particular changes in the brain with particular conditions. Interestingly, in our study, only one-third of the variation in the brain atrophy rate could be explained even by our best regression model. Baseline brain volume, which was entered only in the third regression model, independently predicted subsequent brain volume change and caused age per se to drop out of the model. This suggests that baseline brain volume has to be regarded as an intermediate factor in the association between age and progression of brain atrophy, i.e., it seems to contain information over and above the effect of age on the individual susceptibility to the aging process. The observed differences in brain volume changes were small, and the significance of such differences in subjects without disease is not yet clear.45 Data from individuals followed who later developed neuropsychological deficits suggest that even modest increases in atrophy rate contribute to later cognitive and physical impairments,16,17 which remains to be tested in this cohort. Given a prevalence of 15% of the metabolic syndrome in middle-aged and elderly Europeans without diabetes,46 this may be an important condition to target if aging of the brain is to be limited.

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References
