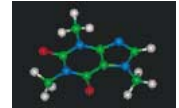


# Plasma Homocysteine Levels, Cerebrovascular Risk Factors, and Cerebral White Matter Changes (Leukoaraiosis) in Patients With Alzheimer Disease



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**Context:** The pathogenesis of leukoaraiosis on computed tomographic (CT) scanning is unknown, but cerebrovascular risk factors for leukoaraiosis show overlap with those for Alzheimer disease (AD).

**Objective:** To investigate the contribution of cerebrovascular risk factors, in particular plasma total homocysteine (tHcy), to leukoaraiosis in patients with AD and controls.

**Design:** Cross-sectional case-control study.

**Setting:** Referral population to a hospital clinic and community volunteers from the Oxfordshire region in England seen between July 1, 1988, and July 1, 2000.

**Participants:** One hundred thirty-seven AD cases (104 confirmed post mortem) and 277 controls matched for age (mean  $\pm$  SD, 73  $\pm$  8 years) and sex.

**Main Outcome Measures:** Cerebrovascular risk factors and leukoaraiosis on CT scans of cases and con-

trols; the odds ratio (OR) of having moderate to severe leukoaraiosis with higher levels of plasma tHcy and cerebrovascular risk factors such as age, sex, systolic blood pressure, smoking, diabetes mellitus, and apolipoprotein E  $\epsilon$ 4 genotype.

**Results:** Leukoaraiosis was more prevalent in AD cases. For a 5- $\mu$ mol/L increase in tHcy levels, the OR for leukoaraiosis was 1.40 (95% confidence interval, 1.02-1.91) independent of other risk factors. The distribution pattern of leukoaraiosis was more marked in the deep white matter than in the periventricular area in individuals with elevated tHcy levels, particularly in patients with AD.

**Conclusions:** Higher tHcy levels are an independent risk factor for moderate to severe leukoaraiosis in individuals with AD and of leukoaraiosis of the deep white matter in particular. The nature of the relationship between tHcy levels and leukoaraiosis in AD requires further longitudinal and intervention studies.

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**L**EUKOARAIOSIS,<sup>1</sup> or white matter low attenuation, on computed tomographic (CT) scanning is frequently found in patients with cerebrovascular disease<sup>2</sup> but has also been associated with dementia severity in Alzheimer disease (AD).<sup>3-6</sup> The pathogenesis of leukoaraiosis is probably multifactorial and, at present, is poorly understood. Cerebrovascular risk factors associated with leukoaraiosis, such as increased blood pressure (BP), diabetes mellitus, the presence of the apolipoprotein E (APOE)  $\epsilon$ 4 genotype, and smoking are also considered to be risk factors for AD.<sup>2,7-11</sup> An increased plasma total homocysteine (tHcy) concentration is a known risk factor for cerebrovascular disease,<sup>12</sup> but moderately elevated levels of tHcy were also associated with confirmed AD in the Oxford Project to Investigate Memory and Ageing (OPTIMA).<sup>13</sup> Other re-

searchers<sup>14-17</sup> have also found that tHcy levels were elevated in individuals with AD compared with controls.

In the present study, we investigated the association between a variety of cerebrovascular disease risk factors and leukoaraiosis in patients with probable or definite AD and in control subjects from the OPTIMA cohort. In particular, we wanted to test whether elevated levels of tHcy were associated with leukoaraiosis and whether such an association was independent of other cerebrovascular risk factors, such as older age, sex, systolic BP (SBP), the presence of the APOE  $\epsilon$ 4 allele, diabetes mellitus, and smoking. Older age and hypertension are considered to be the most important risk factors for leukoaraiosis.<sup>11</sup> Although most studies<sup>18,19</sup> have found that hypertension is a risk factor for leukoaraiosis in patients with AD, one study<sup>20</sup> has found the opposite, that is, that lower SBP is a risk fac-

## PARTICIPANTS, MATERIALS, AND METHODS

### PARTICIPANTS AND CLINICAL INVESTIGATIONS

The OPTIMA is a longitudinal study that started in 1988 and has since recruited more than 600 elderly participants.<sup>13</sup> Patients were usually referred to the OPTIMA by their family physician from the Oxfordshire region because a dementia syndrome was suspected. Controls were community-dwelling, self-caring volunteers who (1) were recruited through lectures or general practices in the Oxfordshire region, (2) were without objective cognitive impairment (Mini-Mental State Examination score  $>24$ ) at first assessment, and (3) were followed for 2 to 7 years to exclude the development of cognitive dysfunction. Informed consent for all participants and ethical approval was obtained before the study. All subjects had undergone extensive medical examination at enrollment, including blood samples, brain scans (CT or magnetic resonance imaging and single-photon emission CT) and measurements of cognitive function (with the *Cambridge Examination for Mental Disorders of the Elderly*,<sup>23</sup> cognitive section [CAMCOG], and Mini-Mental Status Examination). For the present study, the first visit by each subject at which a CT scan had been obtained was selected. We excluded 4 subjects who were younger than 50 years and 8 who had normal-pressure hydrocephalus. We included 277 controls (35 confirmed post mortem) and 137 patients who had been diagnosed as having "probable" or "definite" AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria.<sup>24</sup> Of these, 104 were confirmed post mortem as probable or definite AD according to the Consortium to Establish a Registry for Alzheimer's Disease criteria.<sup>25</sup> The interrater reliability of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association clinical criteria was found to be "substantial," and the accuracy and sensitivity of these criteria (compared with histopathologic criteria) were high.<sup>26</sup>

### LEUKOARAIOSIS

The CT scans were examined independently for leukoariosis by 2 radiologists (H.M.R. and A.M.) who were masked to the clinical diagnosis. The CT scans were rated for leukoariosis in the anterior frontal, posterior frontal, parietal, and occipital brain areas. Within each area, leukoariosis was graded for severity (ranging from 0 [none] to 3 [severe]) and extent (0 indicates none; 1, periventricular leukoariosis; 2, periventricular and deep white matter leukoariosis; and 3, all the white matter involved). For CT examples demonstrating these gradations, refer to our previous study.<sup>27</sup> There

was also a separate score for subjects in whom leukoariosis in the deep white matter was more severe than periventricular leukoariosis (deep white matter pattern of 1). These were compared with the other cases in whom the extent of leukoariosis in any area was greater than 0 (none) but deep white matter leukoariosis was not more severe than periventricular leukoariosis (deep white matter pattern of 0). The interrater reliability (weighted  $\kappa$ ) for these ratings was substantial on average (varying from 0.63–0.79 over the different regions). The codes for severity and extent were multiplied for the scores per area to give leukoariosis scores per area, which were summed to obtain a total leukoariosis score.<sup>27</sup>

### CEREBROVASCULAR RISK FACTORS

Plasma tHcy levels were measured in nonfasting samples obtained between 10 AM and noon that had been stored at  $-70^{\circ}\text{C}$  by high-performance liquid chromatography with fluorescence detection<sup>28</sup> or by immunoassay with fluorescent polarization.<sup>29</sup> Blood pressure was measured with the individual in the supine position. Diabetes mellitus was coded as 0 (not present) or 1 (present but only if medication [eg, insulin or other hypoglycemic agent] was prescribed). Smoking was coded as 0 (never and past smokers) and 1 (current smokers). If the caregiver's response deviated from the patient's response (in 14 instances), the answer of the caregiver was used. Apolipoprotein E allele genotyping was performed using standard methods<sup>30</sup> and coded as 0 (no APOE  $\epsilon 4$  alleles present), 1 (1 APOE  $\epsilon 4$  allele present), and 2 (2 APOE  $\epsilon 4$  alleles present).

### STATISTICAL ANALYSES

To describe potential differences in demographic and clinical variables between groups, we performed  $\chi^2$  analyses for categorical variables and  $t$  tests (2-tailed, unpaired) for continuous variables (**Table 1**).

For the purpose of our logistic analyses (to assess which risk factors predicted the combined severity and extent of leukoariosis), we performed a mean split (mean total leukoariosis score was 2.98) and grouped subjects as 0 (mean score or less, which was overall comparable to none to minimal leukoariosis) vs 1 (which was comparable to moderate to severe leukoariosis).

Last, we looked at the association between the risk factors and the pattern of the leukoariosis by comparing risk factors for subjects in whom leukoariosis of the deep white matter was more severe than that of the periventricular white matter (pattern of 1) to those who had leukoariosis but whose deep white matter was not more affected than the periventricular area (deep white matter pattern of 0). For these analyses,  $\chi^2$  analyses and logistic regression were used. All analyses were performed using statistical software (SPSS version 9.0 for Windows; SPSS Inc, Chicago, Ill) and significance was set at  $P < .05$ .

tor. We therefore used SBP as a continuous variable. In a preliminary study of 195 OPTIMA subjects, the presence of leukoariosis was positively correlated with age, tHcy level, and SBP but not with smoking, vitamin B<sub>12</sub> level, total cholesterol level, or the presence of the APOE  $\epsilon 4$  allele.<sup>21</sup> Because cerebrovascular risk factors are likely to be inter-

related, the relative importance of the individual cerebrovascular risk factors for AD or for leukoariosis remains unclear.<sup>2,8,22</sup> In the present study, we used logistic regression to assess the relative contribution of the individual risk factors to leukoariosis in a larger cohort of cases with AD and controls.

**Table 1. Differences in Demographic and Clinical Variables Between Cases and Controls\***

Variable	Controls (n = 277)	AD Cases (n = 137)	P Value
Demographic variables			
Age, mean (SD), y	73.3 (7.7)	73.9 (9.0)	.50
Sex, % female	50	60	.19
Mini-Mental State Examination score, mean (SD)	28.5 (1.7)	15.9 (8.1)	<.001
CAMCOG <sup>23</sup> score (maximum, 107), mean (SD)	97.6 (6.1)	54.4 (27.5)	<.001
Cerebrovascular risk factors and control variables			
No APOE ε4 allele present, %	77	33	<.001
1 APOE ε4 allele present, %	22	51	
2 APOE ε4 alleles present, %	1	16	
Diabetes mellitus, %	8	5	>.27
Current smokers, %	12	26	<.001
SBP, mean (SD), mm Hg	154.4 (21.8)	148.2 (24.6)	.01
DBP, mean (SD), mm Hg	84.1 (1.09)	85.8 (13.1)	>.16
tHcy, mean (SD), μmol/L [mg/L]	12.8 (3.9) [1.73 {0.41}]	14.7 (4.9) [1.99 {0.66}]	
CT scans			
Extent of leukoaraiosis, %			
PV leukoaraiosis only (vs other)	11	19	.02
Deep white matter leukoaraiosis > PV (vs other)	5	18	<.001
Total leukoaraiosis score (severity × extent over all areas), mean (SD)	1.45 (3.40)	6.08 (9.02)	<.001

\*AD indicates Alzheimer disease; CAMCOG, Cambridge Examination for Mental Disorders of the Elderly;<sup>23</sup> cognitive section; APOE, apolipoprotein E; SBP, systolic blood pressure; DBP, diastolic blood pressure; tHcy, total plasma homocysteine; CT, computed tomographic; and PV, periventricular.

## RESULTS

### CHARACTERISTICS OF THE SAMPLE

The demographic and clinical characteristics for the sample are displayed in Table 1. Cases and controls were similar in terms of age and sex. The CAMCOG and Mini-Mental State Examination scores reflect the cognitive impairment and dementia severity of the cases.

### RISK FACTORS FOR AD

There were approximately twice as many smokers and 3 times as many APOE ε4 allele carriers in the AD group vs the control group. Similar to our earlier findings, tHcy levels were higher in AD cases compared with controls. Systolic BP was significantly lower in cases ( $P<.01$ ), but there was no significant difference in DBP ( $P>.16$ ). There was also no significant difference between groups in the percentage of participants with diabetes mellitus ( $P>.27$ ).

### CT SCAN DATA

As expected, overall extent (**Table 2**) and severity (**Table 3**) of leukoaraiosis were greater in cases. The overall total leukoaraiosis score (severity × extent) was also significantly higher in cases than in controls (Table 1).

**Table 2. Extent of Leukoaraiosis in Different Brain Areas in Cases and Controls\***

Variables	Controls, % (n = 277)	AD Cases, % (n = 137)
Anterior frontal extent		
None	76	47
PV only	16	28
PV + DWM	8	21
All	1	4
Posterior frontal extent		
None	85	66
PV only	7	8
PV + DWM	7	18
All	1	7
Parietal extent		
None	78	47
PV only	6	18
PV + DWM	13	26
All	3	10
Occipital extent		
None	96	72
PV only	3	12
PV + DWM	1	16
All	0	1

\*AD indicates Alzheimer disease; PV, periventricular; and DWM, deep white matter leukoaraiosis. For all variables,  $P<.001$  by  $\chi^2$  analysis.

**Table 3. Severity of Leukoaraiosis in Different Brain Areas in Cases and Controls\***

Variable	Controls, % (n = 277)	AD Cases, % (n = 137)
Anterior frontal severity		
None	76	47
Mild	19	27
Moderate	5	16
Severe	0	10
Posterior frontal severity		
None	85	66
Mild	11	12
Moderate	4	13
Severe	0	9
Parietal severity		
None	78	47
Mild	14	25
Moderate	7	11
Severe	1	18
Occipital severity		
None	96	74
Mild	3	10
Moderate	0	12
Severe	0	7

\*AD indicates Alzheimer disease. For all variables,  $P<.001$  by  $\chi^2$  analysis.

Of cases, 78% had a score that was higher than the overall mean total leukoaraiosis score (of 2.98) compared with 20% of the controls. There was only a small difference in the number of cases who had periventricular leukoaraiosis only compared with controls. However, cases were 3.5 times more likely than controls to have a pattern in which leukoaraiosis of the deep white matter was more severe than that of the periventricular area (Table 1).

## CORRELATIONS OF THE INDIVIDUAL CEREBROVASCULAR RISK FACTORS

Spearman rank correlations showed that, overall, total leukoaraiosis was associated with the *APOE*  $\epsilon 4$  genotype ( $\rho=0.12$ ;  $P<.01$ ), higher levels of tHcy ( $\rho=0.24$ ;  $P<.001$ ), higher SBP ( $\rho=0.13$ ;  $P<.01$ ), and being older

( $\rho=0.43$ ;  $P<.001$ ). Having higher levels of tHcy, in turn, was associated with a higher SBP ( $\rho=0.17$ ;  $P<.001$ ), and both were associated with older age (tHcy level:  $\rho=0.31$ ;  $P<.001$ , SBP:  $\rho=0.15$ ;  $P<.002$ ). There was also a trend for women to be more likely to have worse leukoaraiosis ( $\rho=0.09$ ;  $P=.06$ ). Men, on the other hand, were more likely to have higher levels of tHcy ( $\rho=-0.11$ ;  $P<.02$ ), possibly in part because smoking was associated with having higher levels of tHcy ( $\rho=0.14$ ;  $P<.005$ ), and men were more likely to be smokers ( $\rho=-0.20$ ;  $P<.02$ ). Having diabetes mellitus was only associated with younger age in this cohort ( $\rho=-0.11$ ;  $P<.03$ ).

**Table 4. Vascular Risk Factors in Controls and Cases With or Without Leukoaraiosis\***

Group	Leukoaraiosis		P Value
	None-Minimal	Moderate-Severe	
Controls	(n = 231)	(n = 46 [20%])†	
No <i>APOE</i> $\epsilon 4$ allele present, %	80	68	.003
1 <i>APOE</i> $\epsilon 4$ allele present, %	20	28	
2 <i>APOE</i> $\epsilon 4$ alleles present, %	0	4	
Diabetes mellitus, %	7	8	.76
Sex, % female	52	48	.57
Current smokers, %	12	11	.87
Age, mean (SD), y	72 (8)	78 (7)	<.001
SBP, mean (SD), mm Hg	154 (22)	155 (21)	.77
DBP, mean (SD), mm Hg	84 (11)	84 (10)	.98
tHcy, mean (SD), $\mu\text{mol/L}$ [mg/L]	13 (4) [1.76 {0.54}]	14 (4) [1.89 {0.54}]	.11
MMSE score mean (SD)	29 (2)	28 (2)	.74
AD cases	(n = 78)	(n = 59 [78%])†	
No <i>APOE</i> $\epsilon 4$ allele present, %	31	36	.84
1 <i>APOE</i> $\epsilon 4$ allele present, %	53	49	
2 <i>APOE</i> $\epsilon 4$ alleles present, %	16	15	
Diabetes mellitus, %	6	4	.61
Sex, % female	53	66	.11
Current smokers, %	28	24	.56
Age, mean (SD), y	70 (8)	79 (8)	<.001
SBP, mean (SD), mm Hg	145 (23)	153 (26)	.05
DBP, mean (SD), mm Hg	85 (13)	87 (14)	.28
tHcy, mean (SD), $\mu\text{mol/L}$ [mg/L]	13 (4) [1.76 {0.54}]	17 (6) [2.30 {0.81}]	.001
MMSE score, mean (SD)	17 (8)	14 (8)	.03

\**APOE* indicates apolipoprotein E; SBP, systolic blood pressure; DBP, diastolic blood pressure; tHcy, total plasma homocysteine; MMSE, Mini-Mental State Examination; and AD, Alzheimer disease.

†Percentage of participants with moderate to severe leukoaraiosis per group.

## RISK FACTORS FOR LEUKOARAIOSIS IN CASES AND CONTROLS

**Table 4** shows that controls with moderate to severe leukoaraiosis were on average 6 years older than those who had no or minimal leukoaraiosis. Controls who had moderate to severe leukoaraiosis were also more likely to have 2 *APOE*  $\epsilon 4$  alleles.

Cases with moderate to severe leukoaraiosis were on average 9 years older and had higher levels of tHcy than cases with no or with minimal leukoaraiosis. In cases with no or minimal leukoaraiosis, the SBP was 8 mm Hg lower than that of cases with moderate to severe leukoaraiosis, whereas there was no difference between the SBP for the corresponding groups of controls. None of the other factors were different (sex, presence of diabetes mellitus, smoking, and DBP) for cases and controls having moderate to severe leukoaraiosis vs those with no or minimal leukoaraiosis.

## LOGISTIC REGRESSION ANALYSIS OF RISK FACTORS FOR LEUKOARAIOSIS

A summary of the logistic regression analyses is given in **Table 5**. Overall, the risk for moderate to severe leukoaraiosis increased by a factor of almost 2 for a 5- $\mu\text{mol/L}$  increase in tHcy levels. This was independent of age (model 1) and diagnosis (model 2). The risk for AD cases to have moderate to severe leukoaraiosis was more than 3 times

**Table 5. Summary of the Logistic Regression Analyses\***

Predictor Variable	Model 1		Model 2		Model 3	
	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
tHcy (per 5 $\mu\text{mol/L}$ )	1.78 (1.36-2.29)	<.001	1.42 (1.05-1.91)	.02	1.40 (1.02-1.91)	.04
Age (per year)	1.10 (1.07-1.14)	.001	1.12 (1.08-1.17)	<.001	1.13 (1.09-1.18)	<.001
Sex (being female)	1.51 (0.96-2.38)	.08	1.25 (0.75-2.09)	.40	1.19 (0.69-2.04)	.53
Diagnosis (having AD)			3.74 (2.22-6.20)	<.001	3.02 (1.62-5.62)	.001
Diabetes mellitus					1.43 (0.48-4.31)	.52
Smoking (current)					1.00 (0.49-2.07)	.99
SBP (per 1 mm Hg)					1.00 (0.99-1.02)	.64
Not having an <i>APOE</i> $\epsilon 4$ allele						.22
Having 1 <i>APOE</i> $\epsilon 4$ allele					1.61 (0.88-2.97)	.13
Having 2 <i>APOE</i> $\epsilon 4$ alleles					1.96 (0.74-5.45)	.20

\*Model 1 is adjusted for age and sex; model 2 is adjusted for age, sex, and diagnosis; and model 3 is adjusted for age, sex, diagnosis, and the cerebrovascular risk factors smoking (yes/no), diabetes mellitus (yes/no), systolic blood pressure (SBP), and apolipoprotein E (*APOE*)  $\epsilon 4$  allele genotype (with simple contrast). OR indicates odds ratio; CI, confidence interval; tHcy, total plasma homocysteine; and AD, Alzheimer disease.

†Odds ratios depict the risk for moderate to severe leukoaraiosis.



higher than for controls. When analyses were adjusted for the other cerebrovascular risk factors (model 3), a 5- $\mu$ mol/L increase in tHcy levels was associated with a 40% increase in the risk for moderate to severe leukoaraiosis. None of the other cerebrovascular risk factors had a significant independent association with leukoaraiosis.

We then stratified the analyses for diagnosis. For AD cases, the risk of moderate to severe leukoaraiosis was a factor of 2 higher for every 5- $\mu$ mol/L increase in tHcy levels (odds ratio [OR], 2.01; 95% confidence interval [CI], 1.19-3.39;  $P < .01$ ). Older age was an independent risk factor in AD cases (OR, 1.16, 95% CI, 1.08-1.24;  $P < .001$ ) and controls (OR, 1.12, 95% CI, 1.06-1.18;  $P < .001$ ) for moderate to severe leukoaraiosis. None of the other risk factors had a significant contribution to leukoaraiosis in these analyses.

Last, we investigated factors affecting the distribution pattern of leukoaraiosis by entering all the risk factors simultaneously. Logistic regression showed that for every 5- $\mu$ mol/L increase in tHcy levels, the risk of having a greater degree of deep white matter leukoaraiosis than of periventricular leukoaraiosis was increased by 70% (OR, 1.70; 95% CI, 1.14-2.52;  $P = .009$ ). This effect was independent of age (OR, 1.12; 95% CI, 1.05-1.18) and diagnosis (OR, 3.35; 95% CI, 1.39-8.07). There was also a trend for smoking to be a risk factor for deep white matter leukoaraiosis ( $P = .06$ ). None of the other cerebrovascular risk factors had a significant independent association. We stratified the analyses by diagnosis. For AD cases, the risk of having deep white matter leukoaraiosis was higher with increased tHcy levels: for every 5- $\mu$ mol/L increase in tHcy the OR was 2.18 (95% CI, 1.26-3.78;  $P < .01$ ). In AD cases, smokers (OR, 3.87; 95% CI, 1.01-14.88;  $P < .05$ ) and those who were older (OR, 1.15; 95% CI, 1.06-1.26;  $P < .001$ ) also had increased risk. Older age showed a trend in controls ( $P = .08$ ), but none of the other risk factors had a significant contribution.

#### COMMENT

We found that the risk for moderate to severe leukoaraiosis increased 40% for every 5- $\mu$ mol/L increase in tHcy levels when controlling for diagnosis and other cerebrovascular risk factors. In addition, moderate to severe leukoaraiosis was more prevalent in older subjects and was 3 times more common in AD cases than in age-matched controls. When analyses were stratified by diagnosis, the risk for moderate to severe leukoaraiosis increased by a factor of 2 for every 5- $\mu$ mol/L increase in tHcy levels in cases. In cases and controls, older age was independently associated with moderate to severe leukoaraiosis. Leukoaraiosis in the deep white matter was more common in cases than in controls. In cases, the risk for leukoaraiosis, which was more profound in the deep white matter than in the periventricular areas, was also increased by a factor of 2 with every 5- $\mu$ mol/L increase in tHcy levels, and, independently, was higher in smokers and in elderly individuals. Other cerebrovascular disease risk factors, such as APOE  $\epsilon 4$  genotype, sex, SBP, and diabetes mellitus, did not have an independent effect on leukoaraiosis in these analyses.

Several aspects of this study need to be discussed in more detail. First, these data do not necessarily indicate that

the association between tHcy and leukoaraiosis is limited to patients with AD. The reason for not finding a fitting model for quantified leukoaraiosis in controls may be related to a floor effect in that there was too little leukoaraiosis, as assessed by CT, in this group. Magnetic resonance imaging has been found to be more sensitive for the radiologic detection of white matter changes in healthy elderly individuals.<sup>31</sup> Thus, it is possible that with magnetic resonance imaging, associations of leukoaraiosis with tHcy levels or other variables would be found in controls.

A second, related point is that the tHcy level, leukoaraiosis, and all other data were all taken from the subject's first visit, whereas the postmortem confirmation of AD was from a later date. However, the interval from the first visit to death was only 2 years on average. In addition, all the cases had been diagnosed clinically at the first episode as probable AD. None of the controls converted to the demented group over time, and there were no diagnoses of dementia in our postmortem confirmed controls. An earlier study<sup>26</sup> also showed a high specificity and positive predictive value (both 92%) for the clinical probable AD diagnoses compared with Consortium to Establish a Registry for Alzheimer's Disease histopathologic diagnoses of AD.

A third point for discussion is that the total leukoaraiosis score was used to assess the quantitative association with tHcy concentration and other variables. This score was calculated from the extent and severity scores from each region. It is conceivable that this score was not a good overall measure (eg, if one patient scored "mild" severity in 3 areas, this would be similar to "severe" in only one area). We checked post hoc whether this total score was a valid reflection and found that the correlations between this score and all the separate scores were high ( $\rho > 0.65$ ).

There are several mechanisms through which tHcy could affect leukoaraiosis. First, tHcy is thought to be associated with microvascular cerebral disease, such as vascular endothelial dysfunction.<sup>32</sup> Indeed, one study<sup>33</sup> assumed that white matter changes associated with elevated tHcy levels are due entirely to vascular pathologic mechanisms. Second, tHcy could be a marker for hypomethylation, which might affect the integrity of myelin.<sup>34</sup> Alternatively, tHcy could have direct neurotoxic effects by acting as an *N*-methyl-D-aspartate agonist.<sup>35</sup> In a recent study,<sup>36</sup> an *N*-methyl-D-aspartate antagonist reduced leukoaraiosis in rats. We found in cases that higher tHcy levels and smoking were independently associated with having leukoaraiosis of the deep white matter that was more severe than that of the periventricular area. Leukoaraiosis of the deep white matter may have a different pathogenesis from that of periventricular white matter. Although hyperintensity of the periventricular white matter on magnetic resonance imaging was correlated with age and increasing ventricular dilation, hyperintensity of the deep white matter was presumed to have ischemic origins in patients with AD and Lewy body dementia.<sup>37</sup> A perfusion study<sup>38</sup> of patients with AD also found that deep white matter lesions were associated with reduced cerebral blood flow in the hippocampal region. However, postmortem examination<sup>38</sup> of one of their cases showed no ischemic changes in the deep white matter lesions but

instead showed severe loss of myelin and astrocytic gliosis. In our cohort, a comparison of 10 cases with the lowest and 10 with the highest total leukoaraiosis scores showed that leukoaraiosis was associated with white matter pallor histopathologically but was not typical of severe small vessel disease (Catherine Joachim, MD, unpublished observation, 2001). Which of these mechanisms might be primarily responsible for the association among tHcy level, smoking, and leukoaraiosis of the deep white matter remains to be investigated.

We found no association between the  $\epsilon 4$  allele of *APOE* and leukoaraiosis, although controls with moderate to severe leukoaraiosis were more likely to be homozygous for  $\epsilon 4$  (2 subjects). Other studies<sup>39-42</sup> also did not find an association between *APOE* genotype and the presence of leukoaraiosis in demented patients. The latter study<sup>42</sup> suggested that having the *APOE*  $\epsilon 4$  allele could possibly modify the risk for acquiring dementia but would not affect pathologic processes thereafter. Similarly, it has been reported that having an *APOE*  $\epsilon 4$  allele combined with leukoaraiosis rendered a greater risk for dementia (both AD and vascular dementia), whereas each factor individually was not sufficient.<sup>41</sup>

In the present study, SBP was on average lower in AD cases than in controls, but there was no difference for DBP. In addition, SBP was lower in AD cases without leukoaraiosis (see also Blennow et al<sup>18</sup> and Rezek et al<sup>19</sup>), but AD cases with leukoaraiosis had SBP values similar to those of controls. The nature of the association among SBP, AD, and leukoaraiosis is debated (see the beginning of this article) and probably complex.<sup>43</sup> A longitudinal study<sup>44</sup> showed that 10 to 15 years before the onset of AD, BP values were high in cases but then showed a decrease 1 to 2 years before onset. Dysfunction in central BP autoregulatory mechanisms due to chronic hypertension could lead to reduction of cerebral blood flow at BP levels considered normal for normotensive subjects.<sup>2</sup> Possibly, cases with leukoaraiosis actually had a much higher SBP before a drop before the onset of AD<sup>44</sup> and the concomitant dysfunction in BP autoregulatory mechanisms. Last, in our analyses, SBP was associated with tHcy level and age but did not have an independent effect on leukoaraiosis by itself. The possible interactive effects of the individual risk factors should be investigated in more detail in a future study.

There was a trend for leukoaraiosis to be more severe in women, which is similar to the findings of other investigators.<sup>45</sup> However, men overall had higher levels of tHcy, the main risk factor studied herein. When investigating the interaction between tHcy level and sex on leukoaraiosis post hoc, we found that in men with AD, tHcy levels were not associated with worse leukoaraiosis until levels were greater than 15  $\mu\text{mol/L}$  (tHcy upper tertile). For women, tHcy was associated with worse leukoaraiosis at lower levels as well, possibly because older women have lower levels of sex hormones, such as estradiol, which could counteract some of the detrimental effects of tHcy on the brain.<sup>46</sup>

In summary, tHcy level is strongly and independently associated with moderate to severe leukoaraiosis in patients with AD. In particular, leukoaraiosis of the deep white matter was associated with high tHcy levels.

Level of tHcy is a potentially modifiable risk factor, and the presence of significant leukoaraiosis is increasingly recognized as an important correlate of cerebrovascular events, depression, cognitive decline, and dementia.<sup>47-49</sup> Hence, more research is required to further elucidate this association.

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