ACE, MTHFR, Factor V Leiden, and APOE Polymorphisms in Patients With Vascular and Alzheimer’s Dementia

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Background and Purpose—There is a growing interest in the use of genetic markers in the differential diagnosis of dementia. In the current study we examined the usefulness of genetic risk factors for vascular disease as markers for vascular dementia (VD).

Methods—The groups included 41 patients with VD, 49 patients with dementia of the Alzheimer’s type, and 40 age-matched control subjects without dementia. These patients were genotyped for vascular disease–associated polymorphisms in the genes coding for methylenetetrahydrofolate reductase (MTHFR), angiotensin-converting enzyme (ACE), factor V Leiden (FVL), and a common genetic risk factor for AD, apolipoprotein E e4 (APOE e4).

Results—There was no significant association between ACE, MTHFR, and FVL genotypes with VD whether compared with subjects with AD or with control subjects. There was a higher frequency of APOE e4 alleles in patients with AD (30%, P=0.016) and VD (26%, P=0.07) compared with control subjects (15%).

Conclusions—VD is not associated with the genetic risk factors for vascular disease examined in this study, indicating that the pathogenesis of VD may differ from other vascular diseases. (Stroke. 1998;29:1401-1404.)

Key Words: angiotensin-converting enzymes ■ apolipoprotein E ■ dementia ■ factor V Leiden ■ methylenetetrahydrofolate reductase

Vascular dementia is arguably the second most common cause of dementia, following AD. Moreover, it has been suggested that above the age of 85 years it is more common than AD1 and may possibly be the single most common risk factor for dementia worldwide.2 As summarized in a recent review,1 VD is associated with multiple large infarcts, white matter ischemia, silent infarcts, or even a single strategically placed infarct and is linked with risk factors that include age, race, sex, education, hypertension, cigarette smoking, myocardial infarction, diabetes, hypercholesterolemia, and genetic diseases such as autosomal dominant hereditary cerebral amyloidosis and cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy. In contrast to AD, which is in the most part untreatable, there is a definite potential for the prevention of vascular brain disease. However, differentiation between AD and VD is problematic4 because of the overlap of clinical syndromes and current clinical diagnostic standards. This problem is also highly pertinent to dementia research, and different pathological criteria have been used in different studies.5,6

The use of biological markers offers the potential of more accurate diagnosis of dementia.74 Among relevant markers are a number of common mutations, which are genetic risk factors for peripheral and cerebral vascular diseases. These include polymorphisms and mutations in the genes coding for ACE,7-9 MTHFR,10 and FVL.11-13 The ACE gene D allele linked to high levels of circulating ACE is a risk factor for myocardial infarcts, cardiovascular disease, coronary heart disease, hypertension, diabetes mellitus, and strokes, all of which are risk factors for VD.7-9,14-16 MTHFR is an important factor in the metabolism of homocysteine, an established risk factor for stroke.17 Severe deficiency of MTHFR (<20% of normal activity) leads to severe mental and vascular disorders, whereas a common T677C polymorphism codes for a thermostable type of the enzyme associated with vascular disease.18-19 An arginine to glutamine mutation at codon 506 in the gene coding for FVL is associated with protein C resistance and a hypercoagulable state.11 This mutation has been associated with thrombosis, mainly venous.20

In the present study the ACE, MTHFR, and FVL polymorphisms were evaluated as markers for VD. Because the study included patients with AD, we also examined the most common genetic risk factor for AD, the APOE e4 allele, as an important covariate.

Subjects and Methods

Subjects

The study population included 130 subjects screened at the memory clinic of the Tel Aviv Medical Center. Of these, 41 patients, whose average age of onset was 74.3±7.2 years (±SD), were diagnosed clinically as probable VD on the basis of NINDS-AIREN criteria.2

All patients had a Hachinski Ischemic Score of 7 or above.1 Brain CT revealed that 41% of the VD patients had small-vessel lacunar...
infarcts, 38% had large-artery cortical infarcts, 12% had white matter changes without discreet infarcts, and 9% had only diffuse brain atrophy.

Another group of 49 patients were diagnosed clinically on the basis of the DSM-III-R and NINCDS-ADRDA criteria as probable AD. Their average age of onset was 74.8 ± 9.7 years. A normal control group included 40 healthy subjects without dementia accompanying patients with dementia, whose average age was 72.7 ± 7.8 years. Genomic DNA was isolated from peripheral blood cells by standard procedures.

Detection of the ACE Gene Polymorphism
The primers and PCR conditions were based on those described by Rigat et al.12 The PCR fragments were separated by electrophoresis in a 2% agarose gel and the D and I polymorphisms were identified as 190 and 490 bp bands, respectively.

Detection of the T677C Polymorphism Coding for Thermolabile MTHFR
The primers for analysis and PCR conditions were based on those described by Greengard et al.12 The resulting 206 bp fragments are digested by Hinf I recognition sequence with resulting 175 and 23 bp fragments.

Detection of FVL Alleles
The primers and PCR condition were used as previously described by Greengard et al.12 The resulting 206 bp fragments are digested by Mnl I into 123, 47, and 36 bp fragments in wild-type alleles or 159 and 43 bp alleles in FVL alleles.

Detection of APOE e4 Alleles
Primers and PCR conditions were used as described by us.23

Statistical Analysis
A χ2 analysis was used to compare allele frequencies between the 3 groups. We hypothesized before the study that ACE D, MTHFR T677C, and FVL alleles would be most common in the VD group, whereas APOE e4 alleles would be most common in the AD group.

Results
The respective genotypes in VD, AD, and control subjects are presented in Table 1. The ACE D allele frequency in these groups was 68%, 66%, and 66%, respectively. The MTHFR C677T allele frequencies in these groups were 41%, 44%, and 43%, respectively (nonsignificant differences). One FVL allele was found in a single control patient (1% allele frequency), 2 VD patients (2% allele frequency), and none of the AD patients. These differences were not significant statistically.

Because the APOE e4 allele is a significant risk factor for AD, we also examined the presence of this allele in the study population. The distribution of APOE e4 allele genotypes in each group are presented in Table 2. As expected, the AD group had a significantly higher e4 allele frequency (30%) than the control group (15%, P = 0.016 by Fisher’s exact test). A χ2 test on the data from all 3 groups revealed a trend to statistical significance (P < 0.07), and the APOE e4 allele frequency in VD patients (26%) was higher than in control subjects; this difference was of borderline statistical significance (P = 0.07 by Fisher’s exact test). The APOE e4 allele frequency did not differ significantly between the VD and AD groups. Because the APOE e4 allele is a significant risk factor for dementia, we analyzed the results obtained with the other genetic markers examined while controlling for the presence of e4. None of these subgroup analyses was significant except for the distribution of MTHFR alleles, the results of which are presented in Table 3. The differences between the groups are due in the main to a lower MTHFR C677T allele frequency in VD patients with no APOE e4 alleles, which was unexpected.

Discussion
The genetic vascular risk factors, ACE D, MTHFR C677T, and FVL, were not associated in the present study with VD. It is interesting to note that the ACE D allele frequency in the population examined in this study is high relative to other reports and similar to some European reports.24 The effect of this allele on the prevalence of hypertension or vascular disease in population studies has not been examined compre-

### Table 1. ACE and MTHFR Genotypes in Dementia Groups and Control Subjects

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>ACE</th>
<th>ACE</th>
<th>ACE</th>
<th>ACE</th>
<th>MTHFR</th>
<th>MTHFR</th>
<th>MTHFR</th>
<th>MTHFR</th>
<th>MTHFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>VD</td>
<td>1/1</td>
<td>20</td>
<td>18</td>
<td>68%</td>
<td>14</td>
<td>20</td>
<td>7</td>
<td>41%</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>4/4</td>
<td>17</td>
<td>18</td>
<td>66%</td>
<td>15</td>
<td>16</td>
<td>9</td>
<td>43%</td>
<td></td>
</tr>
</tbody>
</table>

In each group of patients the number with each genotype of ACE and MTHFR are indicated together with the allele frequencies of ACE D and MTHFR C677T. There were no significant differences in the distribution of the alleles or genotypes between the groups.
hensively. As expected, the APOE e4 allele was a significant risk factor for AD, and in addition was a risk factor for VD though this was of borderline statistical significance. These results are compatible with data previously reported by our group,25 Japanese groups,26–28 and to a variable degree in other studies.25,28–38 The reasons for differences between the studies probably include the age of the populations, the diagnostic criteria used, and ethnic and environmental factors.

VD is probably complex, clinically, radiologically, and in origin. The potential overlap with AD is indicated by the fact that in our study, as in others,25 APOE e4 alleles appear to be a risk factor for both types of dementia. One explanation for the lack of association between known genetic vascular risk factors and VD is the variability in the lesions associated with dementia. For example, hypertension is associated with small-vessel disease,8 whereas a tendency to thrombosis may be associated with major vessel occlusion. However, if these factors were significantly associated with a subgroup of patients, one would expect at least a trend in the VD group compared with the control group, which was not found in the present study. Interestingly, among patients with dementia who did not carry the APOE e4 allele, those with a diagnosis of VD had significantly fewer MTHFR C677T alleles when compared with control subjects or those diagnosed with AD. This difference was not hypothesized at the onset of the study, and we consider it to be a chance occurrence.

VD and AD are probably not mutually exclusive diagnoses. This may be due to parallel processes or to an overlap in the pathogenesis of these syndromes. It is interesting to note that in pathological studies, most patients with VD also have changes typical of AD.39 This may explain the difficulty of using APOE e4 as a specific marker for AD.

In conclusion, VD is not associated with the genetic risk factors for vascular disease examined in this study.

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