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

Joab Chapman, MD, PhD; Ningshan Wang, MD; Therese A. Treves, MD; Amos D. Korczyn, MD, MSc; Natan M. Bornstein, MD

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 ε4 (APOE ε4).

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 ε4 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
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.22 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 and PCR conditions were used as previously described by Greengard et al.23 The resulting 206 bp fragments are digested by Mnl I into 123, 47, and 36 bp fragments in wild-type alleles or 159 and 47 bp alleles in FVL alleles.

Detection of APOE ε4 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 ε4 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 ε4 allele is a significant risk factor for AD, we also examined the presence of this allele in the study population. The distribution of APOE ε4 allele genotypes in each group are presented in Table 2. As expected, the AD group had a significantly higher ε4 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 ε4 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 ε4 allele frequency did not differ significantly between the VD and AD groups. Because the APOE ε4 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 ε4. 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 ε4 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-
hensively. As expected, the APOE ε4 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,25,29 APOE ε4 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 a risk factor for both types of 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 ε4 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 ε4 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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References


TABLE 3. MTHFR C677T Allele in Patients With and Without APOE ε4 Alleles

<table>
<thead>
<tr>
<th>MTHFR C677T</th>
<th>APOE ε4</th>
<th>No APOE ε4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−/−</td>
<td>+/+</td>
</tr>
<tr>
<td>VD</td>
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<td>11</td>
</tr>
<tr>
<td>AD</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

Number of patients with each MTHFR C677T genotype presented separately for those with and without APOE ε4 alleles in each of the study groups. Difference between groups was significant for patients without APOE ε4 alleles (P<0.05, χ² test).


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