Apolipoprotein E4 Phenotype in Alzheimer’s Disease

Yasushi Takagi1, Mon Son Choi1, Satoshi Kimura1, Kunihiko Fukuchi1, Tetsumasa Miya1, Kansaku Baba2, Koujiro Sugita3 and Kunihide Gomi1

Abstract: Phenotypes of apolipoprotein E (apoE) were determined with an isoelectric focusing method in 39 patients with sporadic Alzheimer’s disease (AD), 38 patients with dementia from cerebrovascular disease (CVD), and 100 healthy subjects without hyperlipidemia. There was a striking difference in the distribution of apoE phenotypes between patients with AD and healthy subjects (p<0.001). Such a difference was attributable to different frequencies of phenotype E4/3 and E3/3. The apoE4/3 phenotype was detected in 38.5% (15 of 39) patients with AD, more than 3 times higher than in healthy subjects (12.0%, 12 of 100). In contrast, apoE3/3 phenotype, a wild type apoE phenotype, was detected in 81.0% (81 of 100) of healthy subjects but only in 38.5% (15 of 39) of patients with AD. The frequency of apoE-e4 allele assessed on the basis of obtained apoE phenotypes in patients with AD (0.269) was significantly higher than that in healthy subjects (0.08, p<0.001) and in patients with dementia from CVD (0.118, p<0.01). Furthermore, the frequencies of apoE-e4 in AD were 0.190 in patients older than 70 years and 0.333 in patients younger than 70 years. These results indicate a strong association between apoE-e4 and sporadic AD and suggest that apoE-e4 assessed from apoE phenotypes may be a possible risk factor for AD.

Key words: apolipoprotein E (apoE), apoE phenotype, Alzheimer’s disease, apoE-e4

Introduction

Apolipoprotein E (apoE) is a polymorphic protein associated with plasma lipoproteins. It plays an important role in lipid and lipoprotein metabolism through an interaction with the “remnant receptor” (apoE receptor) and the low density lipoprotein (LDL) receptor (apoE/B receptor) of the liver and other organs1. ApoE is unique among apolipoproteins in that it has a special relevance to nervous tissue2. Unlike other lipoproteins, which are made exclusively in the liver, apoE is also produced by Schwann cells in the peripheral nervous system3 and by astrocytes4 and oligodendrocytes in the central nervous system. It is the major apolipoprotein in the cerebrospinal fluid5. ApoE is implicated in the mobilization and redistribution of lipids for growth, maintenance, and repair of myelin and axonal membranes during development and experimental injury in animals6-7.

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Alzheimer’s disease (AD) is by far the most common cause of dementia. Accumulation of large senile plaques and neurofibrillary tangles is a characteristic neuropathologic manifestation of AD. Recent investigations have revealed that apolipoprotein E (apoE) is present in these structures⁸ and that one particular allele, apoE-ε4, is frequently associated with late-onset familial AD and sporadic AD⁹⁻¹¹.

In the present study, we describe the association of the most common apoE phenotypes and alleles with AD.

Materials and Methods

Subjects

Sera were obtained from 39 patients with nonfamilial AD (mean age±SD, 77.0±6.14 years), 38 patients with dementia from cerebrovascular disease (76.0±7.70 years), and 100 healthy volunteers (70.9±7.32 years; Table 1). AD was diagnosed as “probable AD” according to the standard of NINCDS-ADRDA¹². Patients with dementia underwent complete neurologic evaluations, psychiatric assessments, clinical laboratory tests, and a computed tomographic scan of the brain.

ApoE phenotype analysis

Phenotyping of three apoE isoproteins (E2, E3, and E4) was performed with one-dimensional, flat-gel, isoelectric focusing using a commercial kit (Phenotyping ApoE IEF System, Joko, Tokyo, Japan). In brief, 10-µl serum samples were incubated with dithiothreitol and Tween-20 for 15 minutes and applied to 5% polyacrylamide gels containing ampholyte (pH 4.5–8) and urea (3 mol/l). After 2 hours of isoelectric focusing, apoE bands were made visible by immunoblotting. After electrophoresis, the proteins was transferred by simple diffusion. The gel was rinsed briefly in Tris-buffered saline (TBS; 0.25 mmol/l NaCl, 0.03 mmol/l Tris-HCl, pH 8.0), followed by protein transfer onto nitrocellulose membrane (pore size, 0.45 µm) by simple diffusion; the gel-nitrocellulose contact was for 1 hour at room temperature. After protein transfer, the filter was carefully removed from the gel and rinsed in TBS buffer, followed by 60 minutes of incubation with 5% (w/v) nonfat dry milk dissolved in deionized water to saturate any remaining protein binding sites. The filter was then exposed overnight in a 4°C cold chamber to goat-anti-human apoE polyclonal antiserum in TBS buffer followed by three 10-minutes washes in TBS buffer. The washed filter was then incubated in a second antibody, rabbit-anti-goat IgG conjugated with the enzyme alkaline phosphatase, for 30 minutes in TBS buffer. Subsequently, the filter was washed three times in TBS buffer and eventually stained histochemically using 3-indoxyl phosphate.

### Table 1. Characteristics of patients.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sex</th>
<th>No.</th>
<th>Age (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>Female</td>
<td>29</td>
<td>77.7±4.81</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>10</td>
<td>73.6±9.52</td>
</tr>
<tr>
<td>Dementia from CVD</td>
<td>Female</td>
<td>24</td>
<td>76.6±7.60</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>14</td>
<td>73.0±7.83</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>Female</td>
<td>63</td>
<td>69.8±6.53</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>37</td>
<td>72.8±6.53</td>
</tr>
</tbody>
</table>

CVD: cerebrovascular disease.
Model of Multiple Banding Patterns of ApoE Phenotype

<table>
<thead>
<tr>
<th>phenotype</th>
<th>4/4</th>
<th>3/3</th>
<th>2/2</th>
<th>4/2</th>
<th>4/3</th>
<th>3/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E4</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>E3</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>E2</td>
<td></td>
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<td>(+)</td>
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</tr>
</tbody>
</table>

Fig. 1. Model and demonstration of apoE phenotypes. Upper: Model of multiple banding patterns of apoE phenotypes. Lower: Typical patterns of apoE phenotypes.

di-p-toluidine and nitroblue tetrazolium in 50 ml of stock buffer.

Statistical analysis

We estimated allele frequencies in healthy subjects and patients with dementia by counting alleles and calculating sample proportions. Differences in the frequencies of apoE phenotypes and alleles between groups were evaluated with the \( \chi^2 \) test.

Results

ApoE phenotyping by IEF

The illustration of model patterns of apoE phenotypes and the demonstration of samples are shown in Fig. 1. Each of the six most common phenotypes could be easily distinguished. On a gel with control samples, the three bands of apoE 3/3 homozygotes were most obvious because of the high frequency of this phenotype. The three bands of the E2/2 homozygotes were focused at distinct pI (towards the cathode), unlike the E3 bands. There were three E4 bands, but the two E4 bands on the anode side were not recognizable as separate from those of E3; thus, the E4/3 heterozygote had three bands of E3 and one band of E4. The E3/2 heterozygote had six bands, three from E3 and three from E2. The E4/4 homozygote could be distinguished from E4/3 easily, because it had only three bands and the two E4 bands were focused slightly closer to the anode side than was the E3 band. The E4/2 heterozygote had the three bands of E2 as well as the major band of E4.

ApoE phenotypes in AD

There were no appreciable differences in the frequency of apoE phenotypes between healthy
Table 2. ApoE phenotype in patients with Alzheimer’s disease (AD), patients with dementia from CVD, and healthy volunteers (HV).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>AD (n=39)</th>
<th>CVD (n=38)</th>
<th>HV (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoE4/4</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>4/3</td>
<td>15 (38.5)</td>
<td>8 (21.1)</td>
<td>12 (12.0)</td>
</tr>
<tr>
<td>4/2</td>
<td>4 (10.3)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3/3</td>
<td>15 (38.5)</td>
<td>25 (65.8)</td>
<td>81 (81.0)</td>
</tr>
<tr>
<td>3/2</td>
<td>2 (5.1)</td>
<td>4 (10.5)</td>
<td>5 (5.0)</td>
</tr>
<tr>
<td>2/2</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Fig. 2. Frequency distributions of apoE phenotypes in Alzheimer’s disease (AD), dementia from cerebrovascular disease (CVD), and healthy volunteers (HV).

subjects and patients with cerebrovascular dementia (Table 2 and Fig. 2). On the other hand, there was a striking difference in the distribution of apoE phenotypes between patients with AD and healthy subjects (p<0.001). Such differences were mostly attributable to differing frequencies of phenotypes E4/3 and E3/3. In patients with AD, the prevalence of E4/3 phenotype (0.385) was almost three times higher than in healthy control subjects (0.120). In contrast, the prevalence of E3/3 in patients with AD (0.385) was less than half of that in healthy subjects (0.810). Phenotype E4/4 was detected in two healthy subjects and one AD patient.

ApoE alleles assessed from apoE phenotypes

Frequencies of apoE alleles can be assessed on the basis of obtained phenotyping data, assuming there is little discrepancy between the phenotype and genotype of apoE. The frequency of apoE-ε4 allele in AD was estimated to be 0.269, which was markedly higher than those in healthy subjects (0.080; p<0.001) and patients with cerebrovascular dementia (0.118; p<0.01; Figs. 3 and 4). The frequency of apoE-ε4 in AD patients younger than 70 years was 0.333, which was significantly higher than that of patients older than 70
Fig. 3. Frequency of apoE alleles in Alzheimer's disease (AD), dementia from cerebrovascular disease (CVD), and healthy volunteers (HV). ApoE alleles were calculated from the phenotypes of apoE detected on electrophoresis.

Fig. 4. ApoE-ε4 allele in Alzheimer's disease (AD), dementia from cerebrovascular disease (CVD), and healthy volunteers (HV).

Discussion

ApoE has a key role in the clearance of cholesterol from plasma and in reverse cholesterol transport. ApoE has six phenotypes that are controlled by three alleles located on chromosome 19. Recent studies have revealed that one allele, apoE-ε4 (ε4), might be a risk factor for AD. Patients with late-onset familial or sporadic AD patients have an increased frequency of apoE allele, ε4, a finding that suggests apoE4 is associated with increased sus-
ceptibility to disease. ApoE4 has a higher affinity than other apoE isoforms, such as apoE2 and E3, for binding synthetic amyloid β (β/A4) peptide, which is a primary constituent of the senile plaque and congophilic angiopathy in AD.

We have two methods for detecting ε4 allele: genotyping with polymerase chain reaction (PCR) and phenotyping with electrophoresis. Because the polymerase chain reaction method is complicated and time consuming, we used the electrophoresis method for detecting ε4 allele. Electrophoresis can be used to analyze many samples at once; the commercial kit can analyze 25 samples simultaneously. We could determine the phenotype of apoE in almost all samples by the pattern of electrophoresis; however, the phenotype of apoE was difficult to determine in several samples because the pattern of bands differed from the regular patterns. The frequencies of apoE phenotypes in both AD patients and healthy subjects were similar to those reported by Ueki et al. The frequencies of apoE phenotypes in patients with cerebrovascular dementia were a little different from those in healthy subjects. In Japan, some case of cerebrovascular dementia have characteristics of Alzheimer's disease, mixed type. We have to follow up patients carefully if they have ε4 allele.

The frequency of the ε4 allele in AD patients (0.269) was significantly higher than healthy subjects (0.08). Our results indicate that the ε4 allele is increased in frequency in sporadic AD. The ε4 frequency was 0.190 in our sporadic AD patients older than 70 years at onset and was 0.333 in patients younger than 70 years, indicating that the risk of AD associated with ε4 is age-dependent and that AD develops of AD in persons with the ε4 allele at an earlier age.

Of our healthy subjects, two, aged 64 and 70 years, had apoE4/4. They have no findings of dementia at present. If we can follow up these two cases and are able to confirm the onset of AD in the future, the significance of ε4 as a risk factor for AD will be more clear.

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References


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