Associations Among Plasma Lipoprotein Subfractions as Characterized by Analytical Capillary Isotachophoresis, Apolipoprotein E Phenotype, Alzheimer Disease, and Mild Cognitive Impairment

To the Editor:

Alzheimer disease (AD) is the most common form of dementia, and the central pathogenic event is the abnormal accumulation of amyloid β-protein (Aβ) in extracellular amyloid deposits and cerebral blood vessels. CAD is a complex and genetically heterogeneous disease. Mild cognitive impairment (MCI), a cognitive disorder in the transition between normal cognition and AD, is a known risk factor for AD, with a conversion rate of ~10% per year. Apolipoprotein E (apoE), a lipid transporter, has been found to be associated with the development of AD and an increased risk of MCI. Cholesterol has also been identified as a risk factor for AD. Mild cognitive impairment (MCI) and AD are thought to be part of a continuous spectrum of cognitive decline. The apoE4 isoform or APOE-ε4 allele is associated with the development of AD and an increased risk for AD, with a conversion rate of ~10% per year.

Although CAD is a prevalent finding in AD, whether or not plasma lipoprotein subfractions are associated with MCI and AD has not yet been investigated. The separation and determination of lipoprotein subfractions are generally labor-intensive and time-consuming. Recently, however, the research group of Schmitz and coworkers developed a new automated technique to separate and quantify lipoprotein subfractions in minutes using capillary isotachophoresis (cITP). Therefore, in the present study, we investigated the associations among lipoprotein subfractions as determined by cITP, apoE phenotype, MCI, and AD.

Twenty-eight patients with MCI, 47 patients with AD, and 26 nondemented control subjects were evaluated at the Neurology Department of Fukuoka University Hospital between 2002 and 2003. Global cognitive impairment was assessed by the Mini-Mental State Examination (MMSE) and Clinical Dementia Rating (CDR). Patients with MCI (CDR=0.5) met the diagnostic criteria for MCI formulated by the Mayo Clinic Alzheimer Disease Research Center (MCADRC) in Rochester, MN. Patients with AD met the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD and had a CDR score >1. Two patients with AD and one patient with MCI received pravastatin. Nondemented control subjects were volunteers who underwent a standard medical check up and were judged to be in good health. They were required to have a CDR score of 0. This study was approved by the Ethics Committee of Fukuoka University, and samples were collected only after the participants had given their informed consent.

ApoE phenotypes were determined by using isoelectric focusing, as described previously, and were confirmed by apoE genotyping. ApoE phenotype–genotype nonconcordance was found in one control subject (E2/E3 and E3/E3), and the results of apoE genotyping were used. Capillary isotachophoresis of plasma lipoproteins was performed on a Beckman P/ACE MDQ system (Beckman-Coulter Inc.) according to the method of Bottcher et al with some modifications, as described previously. Plasma lipoprotein was stained with NBD C6-ceramide (Molecular Probes, Inc.), a lipophilic dye. The peak area for each cITP lipoprotein subfraction relative to that of the internal marker was presented as the level of the cITP lipoprotein. Agarose gel electrophoresis and differential staining

### Frequency Distribution of Apolipoprotein E (ApoE) Phenotype, Serum Levels of Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), and ApoB, and Levels of Plasma Lipoprotein Subfractions as Characterized by Capillary Isotachophoresis (cITP) in Nondemented Subjects and Patients With Mild Cognitive Impairment (MCI) and Alzheimer Diseases (AD)

<table>
<thead>
<tr>
<th>ApoE phenotype</th>
<th>Non-demented Subjects (n=26)</th>
<th>MCI Patients (n=28)</th>
<th>AD Patients (n=47)</th>
<th>P* (ANCOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/E3 + E3/E3</td>
<td>20 (76.9%)</td>
<td>21 (75.0%)</td>
<td>19 (40.4%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E3/E4 + E4/E4</td>
<td>6 (23.1%)</td>
<td>7 (25.0%)</td>
<td>28 (59.6%)*†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>186±6</td>
<td>211±5*</td>
<td>206±5*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>99±6</td>
<td>123±4*</td>
<td>117±4*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>87±5</td>
<td>102±4*</td>
<td>99±3*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>cITP lipoprotein subfractions [peak area relative to internal markers (RPA)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>1.74±0.12</td>
<td>2.13±0.11</td>
<td>2.25±0.10*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>iHDL</td>
<td>2.86±0.07</td>
<td>3.02±0.08</td>
<td>3.02±0.07</td>
<td>n.s.</td>
</tr>
<tr>
<td>sHDL</td>
<td>0.67±0.05</td>
<td>0.77±0.03</td>
<td>0.77±0.03</td>
<td>n.s.</td>
</tr>
<tr>
<td>Chylomicron/remnants</td>
<td>0.33±0.05</td>
<td>0.30±0.03</td>
<td>0.28±0.03</td>
<td>n.s.</td>
</tr>
<tr>
<td>VLDL/IDL</td>
<td>0.60±0.06</td>
<td>0.50±0.05</td>
<td>0.52±0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>fLDL</td>
<td>0.96±0.07</td>
<td>1.18±0.09</td>
<td>1.16±0.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>sLDL</td>
<td>2.24±0.19</td>
<td>3.32±0.15*</td>
<td>3.39±0.13*</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM.

*P<0.05, vs control subjects; †P<0.05, AD vs MCI, as assessed by a χ² analysis or Scheffe’s multiple comparison test.

hHDL indicates high density lipoprotein; iHDL, fast-migrating HDL; iHDL, intermediate-migrating HDL; sHDL, slow-migrating HDL; VLDL, very low density lipoprotein; fLDL, intermediate density lipoprotein; sLDL, slow-migrating LDL.
were performed using a Rapid Electrophoresis system (REP, Helena Laboratories) according to the method of Kido et al. REP Lipo-30 plate and CHOL/TRIG COMBO (K.K. Helena Kenkyujo) were used as the agarose gel and staining reagents, respectively.

Patients with AD included significantly more females (87.2% versus 61.5%, \(P < 0.05\)), were older (76.9 ± 0.6 years versus 74.2 ± 0.9 years), and had lower MMSE scores (18.8 ± 0.8 versus 29.2 ± 0.2) than nondemented control subjects. Patients with AD included a significantly higher percentage of subjects carrying the apoE4 isoform than controls and patients with MCI (Table). Serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and apoB in patients with both MCI and AD were significantly higher than those in controls as assessed by Scheffe’s multiple comparison test (Table). The differences among groups were significant after adjusting for age and sex by an analysis of covariance (Table).

The Figure shows typical electropherograms of plasma lipoprotein subfractions as characterized by cITP (left) and serum lipid profiles analyzed by agarose gel electrophoresis (right) for a young normal healthy subject (A), a patient with MCI (B), and a patient with AD (C). As shown, plasma lipoproteins were separated into 7 fractions by capillary isotochophoresis: three high-density lipoprotein (HDL) fractions (peaks 1 to 3: fast [f]-, intermediate [i]-, and slow [s]-migrating HDL), a chylomicron/remnants fraction (peak 4), a very low density lipoprotein (VLDL)/intermediate density lipoprotein (IDL) fraction (peak 5), and two LDL fractions (peaks 6 to 7: fast- and slow-migrating LDL). The three cITP HDL subfractions (HDL, iHDL, and sHDL) corresponded to \(\alpha\) lipoprotein in agarose gel electrophoresis (Figure). The fLDL and sLDL corresponded to \(\beta\) lipoprotein in agarose gel electrophoresis (Figure). The peak area relative to internal marker (RPA) for each lipoprotein fraction in patients with MCI and AD and control subjects is shown in the Table. Patients with AD had a significantly higher RPA for fHDL than control subjects, and patients with both AD and MCI had a significantly higher RPA for sLDL than control subjects. A multiple logistic regression analysis showed that the associations between cITP sLDL and MCI (odds ratio [95% CI]: 10 [2.4 to 40]) and between cITP sLDL and AD (9.6 [2.5 to 48]) were independent of apoE phenotype (\(P < 0.01\)) after adjusting for age and sex.
This is the first examination of the association between charged-based LDL subfractions and MCI or AD. Because cITP separates plasma lipoproteins based on electric mobility, the fLDL fraction carries a more-negative electric charge than the sLDL fraction. The electronegative fLDL could be an atherogenic LDL because we found that this LDL fraction inversely correlates with the size of LDL and increases with the oxidation of LDL (unpublished data). We did not detect a significant association between the cITP fLDL subfraction and either MCI or AD, possibly because of the relatively small number of control subjects (Table). However, we found that the major LDL (sLDL) fraction was associated with both MCI and AD. Our findings support the notion that the increased synthesis of cholesterol is related to the progression of cognitive impairment. Our finding that the association between the cITP sLDL subfraction and AD was independent of the apoE4 isoform is consistent with other reports that the association between the serum TC level and AD is independent of the apoE genotype.18

Our finding that the cITP sLDL subfraction was increased in both MCI and AD patients suggests that early lipid-lowering therapy in MCI patients could be beneficial, considering that MCI is a known risk factor for AD. In fact, simvastatin has been shown to significantly decrease Aβ42 in the cerebrospinal fluid of patients with mild Alzheimer disease, but not in severely affected patients.19

In conclusion, the charge-based major LDL subfraction as characterized by cITP was associated with both MCI and AD, independent of apoE isoforms, suggesting that the combination of apoE4 and cITP sLDL may be a better indicator of AD than the apoE4 isoform alone. Further large-scale studies are needed to confirm these findings.

Acknowledgments

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