Age-Dependent Association of Apolipoprotein E Genotypes With Stroke Subtypes in a Japanese Rural Population

Yoshihiro Kokubo, MD; Anisul Haque Chowdhury, MBBS; Chigusa Date, PhD; Tetsuji Yokoyama, MD; Hatiki Sobue, MD; Heizo Tanaka, MD

Background and Purpose—The association between apolipoprotein E (apoE) polymorphisms and stroke has been controversial. These controversies may be due to inaccurate classification of stroke and differences in age ranges. We investigated the association between apoE genotypes and stroke subtypes (confirmed by CT or MRI findings) by case-control study in a Japanese rural population.

Methods—First-ever-stroke patients (n=322; cerebral infarction, n=201, intracerebral hemorrhage, n=84, and subarachnoid hemorrhage, n=37) aged 40 to 89 years were recruited from Hokueutsu Hospital, Japan. Healthy controls (n=1126) were selected from the general population in the same area. ApoE genotypes were determined by restriction fragment-length polymorphism analysis.

Results—Compared with apoE ε3/ε3 subjects, ε2 carriers had a 2-fold risk of cerebral infarction (OR 1.9, 95% CI 1.1 to 3.2). Among cerebral infarction patients, ε2 carriers had increased risks of cortical infarction (OR 2.4, 95% CI 1.3 to 4.6) (an anatomic subtype) and atherothrombosis (OR 3.9, 95% CI 1.7 to 9.0) and cardioembolism (OR 4.9, 95% CI 1.6 to 14.4) but not lacunar infarction (clinical subtypes). ApoE ε4 carriers had a 2.5-fold risk of subarachnoid hemorrhage (OR 2.5, 95% CI 1.1 to 5.4). ApoE ε2/ε2 subjects had an increased risk of intracerebral hemorrhage (OR 4.4, 95% CI 1.0 to 19.7). ApoE ε3/ε4 subjects showed ≈2-fold increased risk of atherothrombosis (OR 2.1, 95% CI 1.0 to 4.1) and intracerebral hemorrhage (OR 1.8, 95% CI 1.0 to 3.3). The association between ε2 and stroke was accentuated in subjects aged 70 years or older but not in those aged 40 to 69 years.

Conclusions—Our study suggests that apoE ε2 is a risk factor for atherothrombosis, cardioembolism, and intracerebral hemorrhage, whereas ε4 is a risk factor for atherothrombosis, intracerebral hemorrhage, and subarachnoid hemorrhage. The occurrence of stroke may be affected by interaction between age and apoE gene polymorphisms. (Stroke. 2000;31:1299-1306.)

Key Words: apolipoproteins ■ genetics ■ stroke classification ■ Japan

Stroke is a major cause of morbidity and mortality in both developed and developing countries.1,2 Although some classic risk factors for stroke, such as hypertension,3 alcohol consumption,4,5 and cigarette smoking,6 have been established, the relationship between serum cholesterol levels and the risk of stroke seems to differ between Western and Japanese populations. In Western populations, a positive association between higher cholesterol levels and cerebral infarction has been noted,7 whereas in Japan, an inverse relationship between serum cholesterol levels and the occurrence of intracerebral hemorrhage (ICH) or cerebral infarction was observed.3,8,9 These facts led us to study the relationship between cholesterol-related genetic factors and the risk of stroke.

Recent developments in techniques of molecular biology have shed light on previously unknown risk factors for stroke, eg, apolipoprotein genetic polymorphisms. Among apolipoprotein genetic polymorphisms, only apolipoprotein E (apoE) polymorphisms contribute to interindividual variations in total cholesterol (TC) and HDL cholesterol levels in the Japanese population.10 The apoE gene is polymorphic, consisting of 3 common alleles (ε2, ε3, and ε4) and 6 different genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4).11 It explains 7% of the variations in TC levels in Caucasians12 and 2.3% of variations in Japanese.10 The apoE ε2 allele is associated with lower and the ε4 allele with higher levels of TC and LDL cholesterol than the ε3 allele.13 Meanwhile, a recent study has shown that knockout mice lacking apoE develop spontaneous atherosclerosis at an early age.14 Although apoE is an essential part of the lipoprotein metabolism, its association with stroke has been controversial.

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This article encompasses the doctoral dissertation of Dr Kokubo.

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(Table 1). ApoE ε2 has been reported to be associated with ischemic cerebrovascular disease (ICVD),15 whereas apoE ε4 was associated not only with ICVD16–18 but also with large-vessel ICVD.19 Conversely, apoE was shown to be unrelated to cerebral infarction in Western populations20–22 and to both cerebral infarction and ICH in the Japanese population.23 A cohort study reported the protective effect of ε2 in an older population,24 but apoE was not identified as a risk factor for stroke in other populations.25,26 These inconsistencies may be due to inaccurate classifications of stroke, differences in age ranges, and small sample sizes. An accurate classification of stroke subtypes is crucial because strokes are heterogeneous in origin. Most previous studies, however, were unable to perform accurate type-specific analyses owing to their small sample sizes.15,20,22,23 In this study (the Hokuetsu Stroke Study), we examined the association of apoE polymorphisms with stroke subtypes in a Japanese population.

Methods

Stroke Subjects

First-ever-stroke patients aged 40 to 89 years were sequentially recruited from Hokuetsu Hospital in Shibata City, which is located in the northern part of Niigata Prefecture, Japan, from November 1997 to June 1999. Of these patients, 10 subjects were excluded from this study (3 were dead on arrival, 2 were transported to other hospitals, and 5 declined to give consent). ApoE genotypes were undeterminable in an additional 5 subjects. Ultimately, 322 patients were enrolled in the current analysis.

Hokuetsu Hospital is a hospital that specializes in neurosurgery and emergency medicine with CT and MRI facilities. Electrocardiography, cardiac and carotid ultrasonography, and MR angiography are performed routinely for cerebral infarction patients at this hospital. Of the stroke patients taken to this hospital, 87.9% (n = 283) were from Shibata City proper and its nearby vicinities and 12.1% (n = 39) were from other areas. There are only 2 neurosurgical hospitals with CT and MRI facilities in Shibata City. According to the stroke surveillance system of Shibata City in 1998, 47.1% of registered stroke patients were taken to this hospital, 31.4% to the Prefectural Hospital, and 21.5% to other hospitals (data not shown). Thus, we conclude that patients taken to Hokuetsu Hospital come from a broad spectrum of the population in Shibata City.

Definition and Classification of Stroke

We included patients with neurological symptoms lasting ≥24 hours accompanied by corresponding focal density changes detected by CT or MRI, and excluded patients suffering from epidural (subdural) hematoma, brain tumors, and accidental or iatrogenic stroke. Final diagnosis of stroke subtypes was confirmed by serial CT or MRI, and excluded patients suffering from epidural (subdural) hematoma, brain tumors, and accidental or iatrogenic stroke. Final diagnosis of stroke subtypes was confirmed by serial CT or MRI findings.27 Cerebral infarction was identified by gradual or sometimes rapid development of focal neurological symptoms and signs, such as hemiparesis, sensory impairment, and a low-density area in the CT image. On the basis of these investigations and clinical findings, cerebral infarction patients were further subgrouped according to anatomic (cortex, penetrating region, and others) and clinical classifications.27 The clinical classifications used were as follows: (1) atherothrombosis, when low-density areas (>15 mm in major diameter) on CT images did not accompany any cardiac source of embolism; (2) lacunar, when low-density areas (3 to 15 mm in major diameter) on CT images accompanied by high-density material in the proximal portion of a penetrating artery; (3) cardioembolism, when low-density areas (3 to 15 mm in major diameter) on CT images were accompanied by corresponding focal density changes detected by CT or MRI, and were not due to other causes. Transient ischemic attack (TIA) was defined as attacks lasting <24 hours, and the clinical presentations were consistent with the territory of a single cerebral artery.

TABLE 1. Review of Strokes Associated With ApoE Alleles

<table>
<thead>
<tr>
<th>First Author (Published Year)</th>
<th>Study Subjects</th>
<th>Case (Control),* n</th>
<th>Average Age, y</th>
<th>Allele, %†</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Couderc15 (1993)</td>
<td>ICVD</td>
<td>69 (68)</td>
<td>72 (72)</td>
<td>7 (1)</td>
<td>85 (93)</td>
</tr>
<tr>
<td>Coris21 (1995)</td>
<td>ICVD</td>
<td>104 (94)</td>
<td>71 (72)</td>
<td>6 (7)</td>
<td>82 (78)</td>
</tr>
<tr>
<td>Hachinski22 (1996)</td>
<td>ICVD</td>
<td>85 (85)</td>
<td>65 (65)</td>
<td>9 (9)</td>
<td>77 (84)</td>
</tr>
<tr>
<td>Kessler19 (1997)</td>
<td>Case all</td>
<td>227 (225)</td>
<td>62 (63)</td>
<td>9 (7)</td>
<td>76 (81)</td>
</tr>
<tr>
<td>Large-vessel disease</td>
<td></td>
<td>70</td>
<td>...</td>
<td>7</td>
<td>74</td>
</tr>
<tr>
<td>Lacunar</td>
<td></td>
<td>34</td>
<td>...</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>Cardioembolism</td>
<td></td>
<td>53</td>
<td>...</td>
<td>10</td>
<td>78</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>70</td>
<td>...</td>
<td>8</td>
<td>79</td>
</tr>
<tr>
<td>Nakata23 (1997)</td>
<td>Thrombosis</td>
<td>55 (61)</td>
<td>66 (67)</td>
<td>2 (6)</td>
<td>89 (90)</td>
</tr>
<tr>
<td>Martaglione17 (1998)</td>
<td>Survived ICVD</td>
<td>100 (108)</td>
<td>66 (61)</td>
<td>6 (8)</td>
<td>76 (85)</td>
</tr>
<tr>
<td>Peng18 (1999)</td>
<td>ICVD</td>
<td>90 (90)</td>
<td>63 (63)</td>
<td>8 (11)</td>
<td>79 (83)</td>
</tr>
</tbody>
</table>

NA indicates no association; . . . , data not shown in the reference. Values in parentheses indicate those for controls.

*For cohort studies, number of stroke events along with number of stroke subjects or cohort members in parentheses.
†In some cases, percentages may not sum to 100% due to rounding.
‡Only subjects with affected cerebral hemisphere were included.
§Only subjects with ε3/ε4 heterozygosity were included.

Methods

Stroke Subjects

First-ever-stroke patients aged 40 to 89 years were sequentially recruited from Hokuetsu Hospital in Shibata City, which is located in the northern part of Niigata Prefecture, Japan, from November 1997 to June 1999. Of these patients, 10 subjects were excluded from this study (3 were dead on arrival, 2 were transported to other hospitals, and 5 declined to give consent). ApoE genotypes were undeterminable in an additional 5 subjects. Ultimately, 322 patients were enrolled in the current analysis.

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diameter) were present on CT image22; (3) cardioembolism, when the sudden onset of focal neurological symptoms was accompanied by evidence of a source of cardiac embolism, which was sometimes observed as hemorrhagic infarction on CT images; and (4) unclassified, when no cause was detected. The causes of embolism that were considered were atrial fibrillation, recent myocardial infarction, mitral stenosis, sick sinus syndrome, and emboli identified by cardiac ultrasonography. Angiography was not performed in patients who were elderly or who did not give consent.

ICH was diagnosed when rapid evolution of focal neurological signs, quick progression into coma, signs of meningeal irritation, headache, and high-density areas in CT findings were observed. Subarachnoid hemorrhage (SAH) was diagnosed when such clinical observations as the sudden onset of severe headaches with a relatively momentary disturbance in consciousness, signs of meningeal irritation, absence of focal neurological signs, and presence of blood in the cerebrospinal fluid or the subarachnoidal space was indicated by high-density regions on CT images.

Control Subjects
Controls were selected from healthy subjects who underwent the 1998 health examinations of Shibata City, covering the 4 areas of Akadani, Iijimino, Matsura, and Yonekura (see Figure 1 of Reference 10). We invited 2841 subjects aged 40 to 89 years (1819 women and 1022 men) to participate in this study. Of these, 1165 (41%) subjects agreed to participate. Thirty-six subjects with histories of stroke were excluded. ApoE genotypes were undeterminable in 3 subjects and were ultimately determined in 1126 (792 women and 334 men).

Participant Characteristics
Well-trained interviewers recorded histories of smoking, drinking, use of lipid-reducing drugs, and various diseases (hypertension, diabetes mellitus, and ischemic heart disease) from all participants, both cases and controls. Smoking and drinking habits were categorized into current or nonsmoker/drinker, respectively. The presence or absence of disease histories was recorded. The presence of atrial fibrillation was investigated by ECG. Height and weight were measured to calculate the body mass index (weight in kilograms divided by the square of height in meters). If patients were unconscious, drowsy, or demented, their close relatives were interviewed.

Blood Sample Collection
Venous blood samples from controls were obtained without regard to the time of the last meal. In 221 subjects (68.6%) with stroke, blood samples were obtained within 24 hours of stroke onset. Serum TC and HDL levels were measured with an autoanalyzer (Hitachi 7170) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan.29 This study was approved by the ethics review committee of the Medical Center for Cancer and Cardiovascular Diseases, Japan.29 The frequency of smoking, drinking, and personal histories of hypertension, diabetes mellitus, and ischemic heart disease, atrial fibrillation, and the use of antihyperlipidemic drugs were presented with adjustment for sex and age. The frequencies of genotypes in the ORs of all strokes associated with ApoE genotypes were calculated and graphically demonstrated with the following conditional logistic regression model:

$$\logit p = \alpha + \beta_1 \times e2 + \beta_2 \times e2 \times age + \beta_3 \times e4 + \beta_4 \times e4 \times age,$$

where e2 and e4 are dummy variables to express e2 and e4 carriers, respectively; age is in years; $\beta_i$ through $\beta_4$ are regression coefficients; and $\alpha$ is the intercept for the ith stratum. Sex and age in 5-year increments were used for group matching. The CI of the OR at a given age was calculated with the variance and covariance of the regression coefficients.33 All analyses were conducted with the SAS statistical package (release 6.12, SAS Institute Inc.).

Results
Stroke and Control Subjects
As shown in Table 2, the average age for all stroke subjects was slightly higher than that for controls, but this difference was found not to be significant by t test ($P=0.29$). The percentage of males in cases was much higher than that in controls ($\chi^2 = 87.7, df = 1, P<0.001$). Smoking and drinking habits were significantly more prevalent in cases than in controls when adjusted for age and sex. The frequencies of personal histories of hypertension, diabetes mellitus, ischemic heart disease, atrial fibrillation, and the use of antihyperlipidemic drugs were also significantly higher in cases.

| Sex, % male | 58.0 | 29.7 |
| Age, y† | 67.9±11.0 | 64.3±10.5 |
| Body mass index, kg/m²‡ | 23.0±0.2 | 23.0±0.1 |
| Smoking habit, %§ | 31.1 | 14.5 |
| Drinking habit, % | 32.9 | 24.9 |
| Hypertension, % | 46.0 | 31.3 |
| Diabetes mellitus, % | 16.1 | 3.3 |
| Ischemic heart disease, % | 15.8 | 2.6 |
| Atrial fibrillation, % | 11.9 | 1.3 |
| Antihyperlipidemic drugs, % | 7.3 | 4.6 |

*OR (95% CI) adjusted for age and sex.
†Mean±SD.
‡Least square mean±SE adjusted for age and sex by ANCOVA.
§Percentages were adjusted for age and sex by direct methods.

relationships between the apoE genotypes or alleles and stroke risk were expressed in terms of ORs, adjusted for the possible confounding effects of age, sex, smoking, drinking, hypertension, and diabetes mellitus by a conditional logistic regression model in which sex and age in 5-year increments were used for group matching, and other variables were included as covariates. ORs for apoE $\varepsilon2/\varepsilon2$, $\varepsilon2/\varepsilon3$, $\varepsilon2/\varepsilon4$, $\varepsilon3/\varepsilon4$, and $\varepsilon4/\varepsilon4$ genotypes were calculated with $\varepsilon3/\varepsilon3$ as a reference. To examine single-gene effects, ORs for $\varepsilon2$ carriers ($\varepsilon2/\varepsilon2$ and $\varepsilon2/\varepsilon3$) and $\varepsilon4$ carriers ($\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$) were also calculated, with $\varepsilon3/\varepsilon3$ as a reference. ApoE $\varepsilon2/\varepsilon4$ subjects were excluded from this analysis. Because different relationships between apoE genotypes and stroke have been reported in the elderly and middle-aged individuals,34,35 analyses were performed not only for all ages but also according to age groups: the middle-aged (40 to 69 years) and the elderly (70 to 89 years). In addition, age-dependent continuous changes in the ORs of all strokes associated with apoE genotypes were calculated and graphically demonstrated with the following conditional logistic regression model:

$$\logit p = \alpha + \beta_1 \times e2 + \beta_2 \times e2 \times age + \beta_3 \times e4 + \beta_4 \times e4 \times age,$$

where e2 and e4 are dummy variables to express e2 and e4 carriers, respectively; age is in years; $\beta_i$ through $\beta_4$ are regression coefficients; and $\alpha$ is the intercept for the ith stratum. Sex and age in 5-year increments were used for group matching. The CI of the OR at a given age was calculated with the variance and covariance of the regression coefficients.33 All analyses were conducted with the SAS statistical package (release 6.12, SAS Institute Inc.).

Statistical Analyses
Comparisons of sex and age between case and control groups were made by $\chi^2$ test and Student’s t test, respectively. The sex- and age-adjusted least square means of body mass index were compared between the 2 groups by ANCOVA. The percentages of smokers, drinkers, subjects with disease histories, atrial fibrillation, and use of antihyperlipidemic drugs were presented with adjustment for sex and age in 5-year increments by direct method, and the Mantel-Haenszel method31 was used to calculate the adjusted ORs with 95% CIs. The

ApoE Genotyping
DNA was extracted from white blood cells with Puregene (Genta Systems, Inc.). ApoE genotyping was performed by polymerase chain reaction (PCR), digestion of the PCR products with restriction enzyme HhaI, and electrophoresis on polyacrylamide gel.30

Statistical Analyses
Comparisons of sex and age between case and control groups were made by $\chi^2$ test and Student’s t test, respectively. The sex- and age-adjusted least square means of body mass index were compared between the 2 groups by ANCOVA. The percentages of smokers, drinkers, subjects with disease histories, atrial fibrillation, and use of antihyperlipidemic drugs were presented with adjustment for sex and age in 5-year increments by direct method, and the Mantel-Haenszel method31 was used to calculate the adjusted ORs with 95% CIs. The

ApoE Polymorphisms and the Risk of Stroke
Table 3 shows the distribution of apoE genotypes and alleles according to stroke subtypes. The frequencies of genotypes in
controls were 1.0% for $e2/e2$, 6.3% for $e2/e3$, 0.7% for $e2/e4$, 72.8% for $e3/e3$, 18.1% for $e3/e4$, and 11.1% for $e4/e4$. There were no significant differences in allele frequencies between age groups ($\chi^2=0.03$, $df=2$, $P=0.85$) and sexes ($\chi^2=0.22$, $df=2$, $P=0.64$) in controls. The apoE allele frequencies of controls ($e2$, 0.046; $e3$, 0.849; and $e4$, 0.105) were not significantly different from the data of Shibata City in 1990 (1.2 o.r. for $e2$, 0.052; $e3$, 0.855; $e4$, 0.093; $\chi^2=1.36$, $df=2$, $P=0.51$) and data from 5 other previous Japanese studies (pooled estimate $n=1139$; $e2$, 0.047; $e3$, 0.855; $e4$, 0.098; $\chi^2=0.31$, $df=2$, $P=0.86$) (reviewed in Reference 10). There was, however, a significant difference between cases and controls in the frequencies of apoE genotypes ($\chi^2=25.0$, $df=5$, $P<0.001$) and alleles ($\chi^2=21.5$, $df=2$, $P<0.001$). In addition, our controls showed significantly lower frequencies of both $e2$ and $e4$ than found in Western studies (pooled estimate $n=3406$; $e2$, 0.079; $e3$, 0.786; $e4$, 0.135; $\chi^2=23.6$, $df=2$, $P<0.001$). No one with an unclassified stroke had an apoE $e4$ allele.

The results of logistic regression analyses are presented in Table 4. Compared with apoE $e3/e3$ subjects, the risk of all strokes associated with apoE $e2/e2$ increased nearly 5-fold

### Table 3. Distribution of ApoE Genotypes and Allele Frequencies According to Stroke Subtypes*

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>$e2/e2$</td>
<td>$e2$</td>
</tr>
<tr>
<td>$e2/e3$</td>
<td>$e3$</td>
</tr>
<tr>
<td>$e2/e4$</td>
<td>$e4$</td>
</tr>
<tr>
<td>$e3/e3$</td>
<td></td>
</tr>
<tr>
<td>$e3/e4$</td>
<td></td>
</tr>
<tr>
<td>$e4/e4$</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. OR (95% CI) for ApoE Genotypes and Alleles According to Stroke Subtypes*

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Carriers†</th>
</tr>
</thead>
<tbody>
<tr>
<td>$e2/e2$</td>
<td>$e2$</td>
</tr>
<tr>
<td>$e2/e3$</td>
<td>$e3$</td>
</tr>
<tr>
<td>$e2/e4$</td>
<td>$e4$</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are percentages. In some cases, percentages may not sum to 100% due to rounding.
†Numbers in anatomic classification do not sum to those in cerebral infarction due to doubling classification.

... Indicates no cases.

*OR and 95% CIs were estimated by conditional logistic regression models adjusting for confounding variables. ApoE $e3/e3$ was used as the reference in these analyses.
†Genotypes containing $e2$ or $e4$ alleles indicate $e2$ or $e4$ carrier, respectively. ApoE $e2/e4$ was excluded from these analyses.
‡$P=0.07$; §$P=0.05$; $\mid P=0.005$. 
showed a 2-fold increased risk for atherothrombosis and that apoE2 is associated with cerebral infarction (atherothrombosis and cardioembolism) and that apoE3/4 is associated with atherothrombosis and ICH, which is inconsistent with some previous studies.20-26 Such inconsistencies may be due to inaccurate stroke classification, small sample sizes,15,20,23 different age ranges, or the removal of fatal cases in the acute phase.16,17 We believe our study has several advantages over previous studies. First, we diagnosed and classified stroke subjects by serial CT or MRI findings. We did not use hospital-discharge or death records to prevent misclassification. Second, our sample size is one of the largest of all previous studies (Table 1). Third, we used wider age ranges so that we could consider age-dependent associations with stroke. Finally, cases were recruited in their very acute phases, as early as possible within a 24-hour period after stroke onset, thus allowing us to recruit fatal cases into our study.

Before we discuss the association of apoE with stroke, we briefly refer to the association of lipids with stroke in Western and Japanese populations. In Western populations, cerebral infarction is positively associated with TC levels7; however, its association with ICH is conflicting.7,34 Conversely, previous Japanese studies3,8,9 have demonstrated the inverse association of TC levels with ICH, as well as a weak but inverse association with cerebral infarction, which is consistent with our study (data not shown). The mechanism for the occurrence of stroke hypothetically involves smooth muscle degeneration and a weakening of the endothelium in intracerebral arteries due to low cholesterol levels,35 high alcohol intake, and some aspects of the traditional Japanese diet, such as high salt and carbohydrate and low fat and animal protein intakes.1,36 These may be further aggravated by hypertension and lead to ICH in Japanese populations.3,37 We do not refer to the difference in lipids levels between cases and controls in the present study, because the potential cause-effect reversal of biochemical variables limits the accuracy of case-control studies.38,39 Thus, we focused our study on genetic risk factors for stroke.

ApoE is a plasma protein involved in the metabolism of lipids.12 It binds to either of 2 distinct receptors, the LDL and reference in these analyses. *P < 0.05; †P < 0.005.

### TABLE 5. OR (95% CIs) for ApoE Allele According to Stroke Subtypes Categorized by Age*

<table>
<thead>
<tr>
<th></th>
<th>40–69 y</th>
<th></th>
<th>70–89 y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>e2 Carrier (95% CI)</td>
<td>e4 Carrier (95% CI)</td>
</tr>
<tr>
<td>All strokes</td>
<td>169</td>
<td>1.2 (0.6–2.3)</td>
<td>1.4 (0.9–2.2)</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td>91</td>
<td>1.3 (0.6–2.8)</td>
<td>1.1 (0.6–2.0)</td>
</tr>
<tr>
<td>Anatomic classification</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>54</td>
<td>1.5 (0.6–3.8)</td>
<td>1.4 (0.7–3.0)</td>
</tr>
<tr>
<td>Penetrating region</td>
<td>20</td>
<td>0.9 (0.2–4.6)</td>
<td>1.4 (0.5–4.5)</td>
</tr>
<tr>
<td>Others</td>
<td>28</td>
<td>0.6 (0.1–3.1)</td>
<td>0.9 (0.3–2.4)</td>
</tr>
<tr>
<td>Clinical classification</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Atherothrombosis</td>
<td>38</td>
<td>3.0 (1.0–9.3)</td>
<td>2.3 (1.0–5.7)</td>
</tr>
<tr>
<td>Lacunar</td>
<td>20</td>
<td>0.7 (0.1–3.6)</td>
<td>0.7 (0.2–2.8)</td>
</tr>
<tr>
<td>Cardioembolism</td>
<td>12</td>
<td>0.8 (0.1–7.3)</td>
<td>1.6 (0.4–6.0)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>21</td>
<td>1.0 (0.3–3.6)</td>
<td>...</td>
</tr>
<tr>
<td>ICH</td>
<td>50</td>
<td>1.2 (0.4–3.6)</td>
<td>2.0 (1.0–4.1)</td>
</tr>
<tr>
<td>SAH</td>
<td>28</td>
<td>1.0 (0.2–5.2)</td>
<td>2.0 (0.7–5.2)</td>
</tr>
</tbody>
</table>

... indicates no cases.

*ORs and 95% CIs were estimated by conditional logistic regression models adjusting for confounding variables. ApoE2/3 or 3/4 was used as the reference in these analyses.

Kokubo et al Age-Dependent ApoE and Stroke Subtype Associations

### Discussion

This is the first report to have examined the association of apoE genetic polymorphisms with stroke subtypes by use of CT or MRI findings. Our results suggest that apoE2 is associated with cerebral infarction (atherothrombosis and cardioembolism) and that apoE3/4 is associated with atherothrombosis and ICH, which is inconsistent with some previous studies.20-26 Such inconsistencies may be due to inaccurate stroke classification, small sample sizes,15,20,23 different age ranges, or the removal of fatal cases in the acute phase.16,17 We believe our study has several advantages over previous studies. First, we diagnosed and classified stroke subjects by serial CT or MRI findings. We did not use hospital-discharge or death records to prevent misclassification. Second, our sample size is one of the largest of all previous studies (Table 1). Third, we used wider age ranges so that we could consider age-dependent associations with stroke. Finally, cases were recruited in their very acute phases, as early as possible within a 24-hour period after stroke onset, thus allowing us to recruit fatal cases into our study.

Before we discuss the association of apoE with stroke, we briefly refer to the association of lipids with stroke in Western and Japanese populations. In Western populations, cerebral infarction is positively associated with TC levels; however, its association with ICH is conflicting.7,34 Conversely, previous Japanese studies have demonstrated the inverse association of TC levels with ICH, as well as a weak but inverse association with cerebral infarction, which is consistent with our study (data not shown). The mechanism for the occurrence of stroke hypothetically involves smooth muscle degeneration and a weakening of the endothelium in intracerebral arteries due to low cholesterol levels, high alcohol intake, and some aspects of the traditional Japanese diet, such as high salt and carbohydrate and low fat and animal protein intakes.1,36 These may be further aggravated by hypertension and lead to ICH in Japanese populations.3,37 We do not refer to the difference in lipids levels between cases and controls in the present study, because the potential cause-effect reversal of biochemical variables limits the accuracy of case-control studies.38,39 Thus, we focused our study on genetic risk factors for stroke.

ApoE is a plasma protein involved in the metabolism of lipids.12 It binds to either of 2 distinct receptors, the LDL and
lipoprotein remnant receptors. Compared with apoE ε3, ε2 has been associated less with VLDL and LDL and more with HDL. In addition, ε2 is catabolized more slowly than ε3. ε4 has been associated more with VLDL and LDL and less with HDL. ApoE ε4 is also catabolized more rapidly than ε3. In our controls, TC levels were found to be significantly lower in ε2 carriers (age- and sex-adjusted mean 4.75 mmol/L, P<0.001) and higher in ε4 carriers (5.46 mmol/L, P<0.001) than in apoE ε3/ε3 subjects (5.27 mmol/L).

De Andrade et al reported that apoE ε2 might be associated with carotid artery atherosclerosis. In a separate study, the direct atherogenic role of ε2 has been associated with lower-limb atheromatosis in the absence of dyslipidemia. Although the mechanism of the observed association between ε2 and carotid atherosclerosis is unknown, it is likely due to the known effects of ε2 in causing a delayed clearance of triglyceride-rich lipoproteins. It is therefore possible that apoE ε2 may contribute to the occurrence of atherothrombosis in the Japanese population.

We also found apoE ε3/ε4 to be more common in patients with atherothrombosis than in those with other types of cerebral infarction, supporting previous studies reporting that ε4 carriers showed a significantly increased degree of atherosclerosis among younger adults and patients with coronary disease. In addition, apoE ε4 has been associated with dementia. Therefore, stroke with dementia might contribute in part to the association of ε4 with atherothrombosis.

Our results also show that the association of ε2 carriers with stroke was more prominent in the elderly group than in the middle-aged group (Table 5). The Figure shows the continuous changes in the ORs of all strokes associated with ε2 and ε4 carriers according to age. ApoE ε2 carriers tended to have an increased OR for all strokes with aging, whereas ε4 carriers tended to show the opposite effect. Such age-dependent ε2 and ε4 associations suggest that the 2 alleles may play a role in atherosclerosis through different mechanisms and possibly with different time courses over the lifespan. However, differences in some other biological, socioeconomic, or lifestyle factors (diet in particular) may influence such an association; for example, the fat intake of the elderly is lower than that of younger people. Carriers of the ε2 allele may have a greater chance of endothelial weakening in intracerebral arteries because of lower cholesterol levels, whereas the diminished impact of the ε4 allele on LDL levels in elderly groups may also explain our findings of a decreased association between ε4 and stroke in elderly subjects. Such age-dependent changes in the association of the ε2 or ε4 alleles with stroke have also been suggested in previous studies. Positive associations between the ε4 allele and stroke have been detected only in subjects aged <70 years on average. Conversely, a positive association between the ε2 allele and stroke has been found in subjects aged >70 years on average. In cohort studies, Ferrucci et al reported that the protective effect of ε2 decreased progressively with age and after 80 years was no longer statistically significant, whereas Kuusisto et al and Basun et al found no association between apoE and cerebral infarction in elderly subjects.

Atherosclerosis in larger arteries has been related chiefly to lipid levels and hypertension, whereas the arteriosclerotic process in smaller arteries has been related to hypertension. Our results demonstrate the associations of apoE ε2 with atherothrombosis and cortical infarction (larger arteries) and the association of ε4 with atherothrombosis but no association of apoE ε2 or ε4 with penetrating artery region or lacunar infarction (smaller arteries). Therefore, it is thought that apoE may affect only larger arteries, although this remains to be studied further.

Normolipidemic subjects with apoE ε2/ε2 have an elevated and prolonged postprandial hypertriglyceridemic response after a high-fat meal and a delayed clearance of chylomicron remnants. In addition, a single high-fat meal transiently reduces endothelial function in normocholesterolemic healthy subjects, probably owing to the accumulation of triglyceriderich lipoproteins. Therefore, ε2 might cause endothelial dysfunction resulting in the production of free radical superoxide anions, thus causing the deactivation of nitric oxide. Endothelial dysfunction in thrombotic vessels might hypothetically enhance or facilitate dislodgment of thrombi/emboli, thus causing embolic stroke.

In the present study, both apoE ε2/ε2 and ε3/ε4 were observed to be associated with ICH. ApoE ε2 may contribute to ICH, which is in agreement with previous studies demonstrating an inverse association between TC levels and ICH. However, differences in some other biological, socioeconomic, or lifestyle factors (diet in particular) may influence such an association; for example, the fat intake of the elderly is lower than that of younger people. ApoE ε4 has been associated with an increased vascular deposition of the β-amyloid peptide, whereas apoE ε2 appears to promote degenerative changes in the amyloid-
laden vessel wall. Both effects are specific to the vasculopathy of cerebral amyloid angiopathy, whereas cerebral amyloid angiopathy–related hemorrhages have made up only 10% of ICH cases. In the present study, the ε2 (14%, n=3) and ε4 (24%, n=5) alleles occurred more frequently among patients with lobar hemorrhage (n=21) than among patients with hemorrhage in other locations, but the differences were not statistically significant. It is thought that apoE ε2 and ε4 may contribute in part to the increased risk of ICH.

In the present study, the ε4 allele was also associated with SAH. However, the underlying mechanism remains to be resolved.

In conclusion, our study has shown a positive age-dependent effect between apoE ε2 and the risks of atherothrombosis, cardioembolism, and ICH, with this effect being prominent in the elderly group. Meanwhile, a positive age-dependent effect between ε4 and the risk of atherothrombosis has been shown in the middle-aged group. ApoE and hypertension may affect larger arteries. Additional examinations of the effects of and relationship between apoE and lifestyles with regard to stroke risk will be helpful in the prevention of stroke through the promotion of more suitable lifestyles.

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References


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