

resistance is associated with the plaque-type pathology of AD,<sup>3</sup> even though there are some controversial findings.<sup>4,5</sup>

Along with insulin resistance and diabetes, dyslipidemia is an important metabolic disorder. In humans, however, there are few studies regarding the association between dyslipidemia and AD-related pathology.<sup>6,7</sup> In this study, to clarify the relationship between abnormal lipid metabolism and AD, we searched for evidence of AD-related pathologic risk by examining the associations between lipid profiles and the typical AD-related pathologic outcomes, neuritic plaques (NPs) and neurofibrillary tangles (NFTs).

**METHODS Subjects.** The design of the Hisayama Study has been described in detail elsewhere.<sup>3,8-10</sup> In the present study, we examined a series of autopsy samples of Hisayama residents from October 1, 1998, to March 31, 2003. During this period, 290 residents in Hisayama died and 214 were autopsied (autopsy rate 73.8%). The clinical data for the present study were collected from a clinical examination performed in 1988, as described previously.<sup>9</sup> Briefly, of a total of 3,227 residents aged 40–79 years included in the study registry, 2,587 (participation rate, 80.2%) took part in a clinical examination in 1988. Of the 214 autopsy cases, we excluded 3 subjects whose brain specimens were inadequate for evaluation, and 64 subjects who did not complete the fasting blood protocol in 1988. Finally, 147 subjects who underwent both the fasting blood protocol and brain autopsy were included in the present study. None of the 147 subjects showed signs of dementia at the clinical examination in 1988. The study subjects mostly overlapped with those in our previous study, in which we reported the association of insulin resistance with the plaque-type pathology of AD.<sup>3</sup>

**Standard protocol approvals, registrations, and patient consents.** The study was approved by the Ethics Committee of the Faculty of Medicine, Kyushu University, and was performed in accordance with the ethical standards described in the 5th revision of the Declaration of Helsinki, 2000. Written informed consent was obtained from all study subjects.

**Risk factors.** In the clinical examination performed in 1988, blood samples were collected on the morning after an overnight fast. We used values of total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), triglycerides (TG), TC/HDLC, LDLC/HDLC, and non-HDLC as lipid profiles. Levels of TC, HDLC, and TG were determined enzymatically. LDLC was calculated using the Friedewald formula ( $LDLC = TC - HDLC - TG/5$ ).<sup>11</sup> Non-HDLC was defined as  $non-HDLC = TC - HDLC$ . Other risk factors were also measured as described previously.<sup>3</sup> *APOE* genotyping was determined by direct sequencing. The homozygous  $\epsilon 4$  genotype was not found among these participants, and those who carried one copy of the  $\epsilon 4$  allele were categorized as *APOE*  $\epsilon 4$  carriers.

**Assessment of neuropathologic changes.** Brain specimens in each case included the middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, anterior cingulate gyrus, amygdala, hippocampus with entorhinal and transento-

rhinal cortex, calcarine cortex, basal ganglia including the nucleus basalis of Meynert, thalamus, substantia nigra, locus ceruleus, and dorsal vagal nucleus. Sections were routinely stained using hematoxylin-eosin, Klüver-Barrera stain, and a modified Bielschowsky method. Specimens from each subject were immunostained with antibodies against phosphorylated tau (AT8, mouse monoclonal, 1:500; Innogenetics, Belgium). The assessment of AD pathology was conducted according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) guidelines<sup>12</sup> and to Braak stage.<sup>13,14</sup> For the pathologic assessment of cerebrovascular diseases, any type of cerebral infarction or hemorrhage was recorded according to gross examination and microscopic assessment, regardless of clinical features.

**Statistical analyses.** Mean or geometric mean values of continuous data among the NP or NFT groups were adjusted for age and sex and compared by analysis of covariance. Proportions of categorical data were adjusted for age and sex by direct method and compared by logistic regression analysis. We also used logistic regression analysis to determine relationships between risk factors and pathologic outcome, which are expressed as odds ratios (OR) and 95% confidence intervals (CI). Model 1 was adjusted for age and sex. Model 2 was adjusted for model 1 plus systolic blood pressure, fasting blood glucose levels, fasting insulin levels, body mass index, smoking habit, regular exercise, and cerebrovascular disease. Model 3 was adjusted for model 2 plus *APOE*  $\epsilon 4$  carrier.

Each lipid profile was divided into 4 groups to compare the risk of NPs among quartiles. Missing values (2 for LDL cholesterol, 1 for fasting insulin levels, 7 for *APOE*  $\epsilon 4$  carrier, and 1 for the grading of Braak stage) were excluded from the analysis. In addition, subjects were divided into high or low groups at the boundary of the most unfavorable quartile to compare the risk of NPs. Significance was defined as  $p < 0.05$ .

**RESULTS** The demographic characteristics of the study subjects at clinical examination are described in table 1. The mean age at death was 76 years in subjects without NPs (CERAD = 0) and 83 years in those with NPs (CERAD = 1 to 3). There was no clear selection bias regardless of autopsy, according to a comparison of demographic characteristics between our study subjects and those who did not undergo autopsy (data not shown). After the clinical examination in 1988, 34.0% ( $n = 50$ ) of subjects developed dementia; specifically, 17.7% ( $n = 26$ ) were Alzheimer-type dementia, 13.6% ( $n = 20$ ) were vascular dementia, and 2.0% ( $n = 3$ ) were mixed-type dementia.

The frequencies of NPs were categorized into the following 4 groups by CERAD criteria: 32.0% ( $n = 47$ ) for none (score 0), 15.7% ( $n = 23$ ) for sparse (score 1), 15.0% ( $n = 22$ ) for moderate (score 2), and 37.4% ( $n = 55$ ) for frequent (score 3). The extent of NFTs was classified into the following 4 groups by Braak stage: 13.0% ( $n = 19$ ) for stage 0, 17.8% ( $n = 26$ ) for stage I to II, 43.8% ( $n = 64$ ) for stage III to IV, and 25.3% ( $n = 37$ ) for stage V to VI. Prevalence of cerebrovascular disease at autopsy

**Table 1 Demographic characteristics of 147 subjects according to the presence of NPs or NFTs<sup>a</sup>**

Variables	Without NPs (CERAD = 0) (n = 47)	With NPs (CERAD = 1 to 3) (n = 100)	Without NFTs (Braak stage = 0) (n = 19)	With NFTs (Braak stage = I to VI) (n = 127)
Male sex, %	41.9	46.5	51.3	43.9
Age at medical examination, y	63 ± 1	71 ± 1 <sup>b</sup>	62 ± 2	69 ± 1 <sup>b</sup>
Fasting plasma glucose, mmol/L	5.7 ± 0.2	6.0 ± 0.1	5.6 ± 0.3	5.9 ± 0.1
Fasting insulin, μU/mL	4.5 (4.0, 5.2)	5.5 (5.0, 6.0) <sup>b</sup>	5.1 (4.1, 6.2)	5.2 (4.8, 5.6)
Systolic blood pressure, mm Hg	143.3 ± 3.6	137.5 ± 2.4	135.4 ± 5.7	139.8 ± 2.1
Diastolic blood pressure, mm Hg	78.1 ± 1.9	76.0 ± 1.3	76.4 ± 2.9	76.5 ± 1.1
TC, mmol/L	4.9 ± 0.2	5.4 ± 0.1 <sup>b</sup>	5.5 ± 0.3	5.2 ± 0.1
LDLC, mmol/L	3.0 ± 0.2	3.6 ± 0.1 <sup>b</sup>	3.8 ± 0.2	3.4 ± 0.1
HDLC, mmol/L	1.4 ± 0.1	1.3 ± 0.03	1.3 ± 0.1	1.3 ± 0.0
TG, mmol/L	1.0 (0.9, 1.2)	1.2 (1.1, 1.3)	1.1 (0.9, 1.4)	1.1 (1.0, 1.2)
TC/HDLC	3.7 ± 0.2	4.6 ± 0.1 <sup>b</sup>	4.5 ± 0.3	4.3 ± 0.1
LDLC/HDLC	2.4 ± 0.2	3.0 ± 0.1 <sup>b</sup>	3.0 ± 0.3	2.8 ± 0.1
Non-HDLC, mmol/L	3.5 ± 0.2	4.2 ± 0.1 <sup>b</sup>	4.2 ± 0.3	3.9 ± 0.1
Body mass index, kg/m <sup>2</sup>	21.8 ± 0.5	21.9 ± 0.3	21.6 ± 0.7	21.9 ± 0.3
Current smoking, %	49.5	43.6	59.4	40.3
Regular exercise, %	6.6	5.2	0.2	8.6
APOE ε4 carrier, %	0.03	21.8 <sup>b</sup>	17.6	14.5

Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; HDLC = high-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol; NFT = neurofibrillary tangle; NP = neuritic plaque; TC = total cholesterol; TG = triglycerides.

<sup>a</sup> Values are %, mean ± SE, or geometric mean (95% prediction interval). Geometric means of fasting insulin and triglycerides are shown due to the skewed distribution. Values are adjusted for age and sex except for sex and age at medical examination.

<sup>b</sup>  $p < 0.05$ . Male sex is adjusted for age. Age at medical examination is adjusted for sex.

was 59.2% (n = 87), which included any type of infarction (n = 73), hemorrhage (n = 10), or Binswanger type change (n = 6).

As shown in tables 2 and 3, we compared adjusted mean or geometric mean values of each lipid profile among groups according to CERAD score for NPs or Braak stage for NFTs. In the age- and sex-adjusted analyses (model 1), the subjects with NPs (CERAD score 1 to 3) showed significantly higher TC, LDLC, TC/HDLC, LDLC/HDLC, and non-HDLC levels compared to subjects without NPs (CERAD score 0). These associations remained significant even after multivariate model analysis (model 2 and 3). Test for trend among 4 CERAD stages revealed a limited dose-response relationship after multivariate model analysis. Unfavorable lipid metabolism was significantly associated with plaque-type AD pathology even in sparse to moderate stages (CERAD = 1 or 2). In contrast, we found no significant association between any lipid profile and NFT pathology (Braak stage I to VI vs stage 0).

To confirm these associations, we compared the risk of NPs among quartiles of each lipid profile in

table 4. Compared with the lowest quartile (Q1) of TC, age- and sex-adjusted risks of NPs (model 1) were constant in the second (Q2) and the third (Q3) quartiles, but were significantly increased in the highest quartile (Q4). This relationship remained significant even after multivariate adjustment (model 2). Further adjustment for *APOE* genotype resulted in a higher increased risk of NPs (model 3). In a similar way, the highest quartiles of LDLC, TC/HDLC, LDLC/HDLC, and non-HDLC showed increased risk for NPs compared with the lowest respective quartiles. These findings suggested that the relationship between lipid profiles and the presence of NPs may fit with threshold models but not with linear models.

Additionally, table 4 shows ORs for the presence of NPs relative to lipid profile levels, namely low or high. We set the threshold level between Q3 and Q4 (lipid profiles excluding HDLC) or between Q1 and Q2 (HDLC). NPs were found in 86.1% of subjects with high TC (>5.80 mmol/L) and in 62.2% of people with low TC (≤5.80 mmol/L). Compared with low TC, the age- and sex-adjusted risk of NPs was significantly increased for high TC (model 1). After multivariate adjustments (models 2 and 3), this relationship remained significant. In a similar way, high levels of LDLC, TC/HDLC, LDLC/HDLC, and non-HDLC showed significantly increased risk for NPs compared with low levels, even after multivariate adjustments. When we performed similar analyses in which we narrow down the subjects with NPs to the group of CERAD = 2 to 3 (table e-1 on the *Neurology*<sup>®</sup> Web site at www.neurology.org) or CERAD = 3 (table e-2), similar associations between the lipid profiles and NPs were observed. The similar findings were observed even in the sensitivity analyses that excluded 26 cases with Alzheimer-type dementia (table e-3), or those that excluded 28 *APOE* ε4 carriers (data not shown). Because of the limited sample size, we could not perform sex-specific analyses.

**DISCUSSION** Using a series of autopsy cases from a general Japanese population, we found that high levels of TC, LDLC, TC/HDLC, LDLC/HDLC, and non-HDLC were significantly associated with plaque-type AD pathology. Our findings also suggest that the relationship between these lipid profiles and NPs may have certain threshold levels.

Because lipid metabolism is closely related to *APOE* genotype,<sup>15</sup> which is a strong risk factor for AD pathogenesis,<sup>16</sup> we compared the results of 2 multivariate models (model 2 and 3). The relationship between HDLC levels and the risk of NPs was diminished after adjustment for *APOE* genotype,

Table 2 Adjusted mean or geometric mean values of each lipid profile according to CERAD score<sup>a</sup>

	Model 1					Model 2					Model 3				
	CERAD score					CERAD score					CERAD score				
	0 (n = 47)	1 (n = 23)	2 (n = 22)	3 (n = 55)	p for trend (p [1-3 vs 0])	0 (n = 47)	1 (n = 23)	2 (n = 22)	3 (n = 55)	p for trend (p [1-3 vs 0])	0 (n = 47)	1 (n = 23)	2 (n = 22)	3 (n = 55)	p for trend (p [1-3 vs 0])
TC, mmol/L	4.87	5.47 <sup>b</sup>	5.66 <sup>b</sup>	5.32	0.07	4.85	5.47 <sup>b</sup>	5.70 <sup>b</sup>	5.34 <sup>b</sup>	0.05	4.82	5.42 <sup>b</sup>	5.69 <sup>b</sup>	5.36	0.049
LDLC, mmol/L	3.05	3.54	3.83 <sup>b</sup>	3.46	0.07	3.02	3.55	3.86 <sup>b</sup>	3.47 <sup>b</sup>	0.05	3.01	3.53	3.85 <sup>b</sup>	3.50	0.007
HDLC, mmol/L	1.36	1.25	1.33	1.23	0.08	1.35	1.29	1.33	1.22	0.11	1.31	1.26	1.33	1.26	0.62
TG, mmol/L	1.00	1.30	0.99	1.22	0.20	1.02	1.25	1.00	1.22	0.25	1.06	1.25	1.01	1.15	0.77
TC/HDLC	3.73	4.64 <sup>b</sup>	4.47 <sup>b</sup>	4.56 <sup>b</sup>	0.006	3.76	4.51 <sup>b</sup>	4.50 <sup>b</sup>	4.59 <sup>b</sup>	0.004	3.87	4.58 <sup>b</sup>	4.51	4.50 <sup>b</sup>	0.009
LDLC/HDLC	2.39	2.93 <sup>b</sup>	3.06 <sup>b</sup>	2.96 <sup>b</sup>	0.01	2.38	2.87	3.07 <sup>b</sup>	2.99 <sup>b</sup>	0.008	2.45	2.92	3.07 <sup>b</sup>	2.94	0.06
Non-HDLc, mmol/L	3.51	4.21 <sup>b</sup>	4.33 <sup>b</sup>	4.10 <sup>b</sup>	0.02	3.50	4.18 <sup>b</sup>	4.37 <sup>b</sup>	4.12 <sup>b</sup>	0.01	3.51	4.16 <sup>b</sup>	4.36 <sup>b</sup>	4.10 <sup>b</sup>	0.002

Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; HDLC = high-density lipoprotein cholesterol; LDLc = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglycerides.

<sup>a</sup> Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, and cerebrovascular disease. Model 3 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, cerebrovascular disease, and APOE ε4 carrier. Geometric mean of triglyceride is shown due to the skewed distribution.

<sup>b</sup> p < 0.05 vs CERAD score = 0.

which suggested that *APOE* genotype was a confounding factor that had distorted the relationship between HDLC and NPs. Meanwhile, adjustment for *APOE* genotype resulted in a greater increased risk of NPs in association with high levels of TC, LDLc, and non-HDLc. These findings indicated that lipid profiles, such as TC, LDLc, and non-HDLc, may be significant risk factors for NPs and that these relationships were independent from *APOE* genotype.

There was a limited dose-response relationship between the lipid profiles and CERAD score after multivariate model analysis, which might be diminished by an epidemiologic competing effect, indicating that subjects with very high lipid profiles at the clinical examination probably died earlier as a result of cardiovascular disease, for example. Moreover, there might be a threshold effect, indicating that serum cholesterol in excess of a certain threshold level would trigger the plaque formation even though the further development of AD pathology might be modified by different factors. To control the serum cholesterol below a threshold level would decrease the risk of plaque formation, which might contribute to the prevention of AD.

Our analyses using quartiles suggested possible threshold levels to be approximately 6 mmol/L for TC and 4 mmol/L for LDLc. TC/HDLC, LDLc/HDLC, and non-HDLc are primarily the indexes for prediction of coronary heart disease based on a linear relationship<sup>17</sup>; nevertheless, our results also showed certain threshold levels for these indexes. This suggests that the increased risk of NP formation is less associated with atherosclerotic vascular factors. Lipid profiles were measured in blood samples; however, peripheral lipid profiles could be quite different from cholesterol metabolism in the brain. There may be a homeostatic regulation of cholesterol across the blood-brain barrier, which might adopt a threshold in the periphery. It is difficult to further estimate exact threshold levels due to the limited sample size of this study. Further studies with a larger sample size are needed to determine this issue.

The absence of a consistent association between the lipid profiles and NFT pathology in the present study might be due to the relatively small sample size; nevertheless, NFT pathology was less associated with disturbed lipid metabolism than was the formation of NPs, and NFT pathology is considered to be a consequence of Aβ deposition in the amyloid cascade hypothesis.<sup>18</sup> Lipid profiles may act upstream of the cascade, and might trigger AD pathogenesis. This is similar to the relationship between diabetes-related factors and NP pathology that we have previously reported.<sup>3</sup> The dissociation with the NFT could be

Table 3 Adjusted mean or geometric mean values of each lipid profile according to Braak and Braak staging<sup>a</sup>

	Model 1						Model 2						Model 3					
	Braak stage		Braak stage		Braak stage		Braak stage		Braak stage		Braak stage		Braak stage		Braak stage		Braak stage	
	0 (n = 19)	I, II (n = 26)	III, IV (n = 64)	V, VI (n = 37)	p for trend	p (I-VI vs 0)	0 (n = 19)	I, II (n = 26)	III, IV (n = 64)	V, VI (n = 37)	p for trend	p (I-VI vs 0)	0 (n = 19)	I, II (n = 26)	III, IV (n = 64)	V, VI (n = 37)	p for trend	p (I-VI vs 0)
TC, mmol/L	5.44	5.08	5.17	5.43	0.78	0.38	5.46	5.03	5.19	5.43	0.82	0.33	5.49	5.07	5.17	5.42	0.92	0.32
LDLC, mmol/L	3.75	3.25	3.34	3.45	0.59	0.12	3.77	3.21	3.36	3.45	0.58	0.11	3.83	3.24	3.36	3.46	0.50	0.09
HDLC, mmol/L	1.28	1.29	1.28	1.31	0.80	0.87	1.28	1.29	1.27	1.33	0.64	0.88	1.25	1.30	1.26	1.34	0.47	0.62
TG, mmol/L	1.09	1.06	1.03	1.34	0.17	0.97	1.11	1.07	1.05	1.28	0.33	0.94	1.14	1.08	1.04	1.24	0.56	0.73
TC/HDL	4.44	4.15	4.24	4.42	0.88	0.55	4.46	4.11	4.30	4.34	0.96	0.49	4.58	4.11	4.33	4.32	0.73	0.32
LDLC/HDL	2.96	2.72	2.75	2.81	0.78	0.46	2.98	2.69	2.78	2.78	0.69	0.41	3.07	2.70	2.81	2.78	0.55	0.28
Non-HDL, mmol/L	4.16	3.79	3.88	4.13	0.84	0.35	4.19	3.74	3.92	4.10	0.92	0.29	4.24	3.77	3.91	4.08	0.91	0.23

Abbreviations: HDLC = high-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglycerides.

<sup>a</sup> Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, and cerebrovascular disease. Model 3 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, cerebrovascular disease, and APOE ε4 carrier. Geometric mean of triglycerides is shown due to the skewed distribution.

another example that plaques and NFT are driven by very different factors.

Cholesterol may be associated with levels of the amyloid-precursor-protein metabolite Aβ, although the effects of cholesterol on Aβ metabolism, amyloid fibrillogenesis, and toxicity are not well understood and the results reported so far are controversial.<sup>19,20</sup> Aβ, apoE, cholesterol, and cholesterol oxidase have been shown to colocalize in the core of fibrillary plaques in transgenic mice models of AD,<sup>21,22</sup> which suggests that cholesterol and apoE are involved in fibrillar plaque formation. Previous studies have also found that levels of serum cholesterol, especially in the form of LDLC in patients with AD, were significantly higher when compared to age-matched controls.<sup>23</sup> A change in membrane properties, including stiffness and fluidity, has been suggested to influence activities of membrane-bound proteins and enzymes, including secretases. The high cholesterol content in lipid rafts, membrane regions where these enzymes are located, facilitates the clustering of the β and γ secretases with their substrates into an optimum configuration, thereby promoting the undesirable pathogenic cleavage of amyloid precursor protein.<sup>24</sup>

There are few previous studies that have investigated the association between hypercholesterolemia and AD-related pathology.<sup>6,7</sup> Of these, the Honolulu-Asia Aging Study was a population-based study which reported that the constituents of HDLC may play a role in the formation of AD pathology. The discrepancy between these and our results may reflect differences in study design. One difference is in the observation period between the evaluation of cholesterolemia and autopsy. Because the observation period in our study was relatively long (10–15 years) compared with the Honolulu-Asia Aging Study (<8 years), our study design might reduce the possibility of reverse causality; the presence of AD might affect the lifestyle of the subjects and their lipid profiles. Another retrospective study shows that serum hypercholesterolemia may be a risk factor for the development of AD amyloid pathology.<sup>6</sup> This study was not population-based and the increased risk is observed only among subjects younger than 55 years of age; however, significant association between serum cholesterol and the development of amyloid pathology is consistent with our findings.

Meanwhile, the relationship between cholesterol levels and clinical manifestation of dementia is less clear.<sup>25</sup> Epidemiology studies show controversial findings; high cholesterol levels in midlife may increase risk for subsequent dementia and AD<sup>26–29</sup> or low cholesterol levels in late life have been predictive of subsequent dementia.<sup>30</sup> Differences in study designs, length of observational periods, analytical

Table 4 Multivariate-adjusted ORs and 95% CIs for presence of NPs (CERAD score 1-3 vs 0) according to lipid profile levels<sup>a</sup>

Quantiles of lipid profiles	Range	No. of subjects with NPs/total (%)	Model 1		Model 2		Model 3	
			OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
<b>TC, mmol/L</b>								
Q1	≤4.48	23/37 (62.2)						
Q2 (vs Q1)	>4.48 and ≤5.20	23/37 (62.2)	1.1 (0.4-3.1)	0.93	0.9 (0.3-2.9)	0.8302	1.1 (0.3-4.4)	0.93
Q3 (vs Q1)	>5.20 and ≤5.80	23/37 (62.2)	1.0 (0.3-3.1)	0.96	0.7 (0.2-2.6)	0.62	0.7 (0.2-3.1)	0.65
Q4 (vs Q1)	>5.80	31/36 (86.1)	6.8 (1.8-25.4)	0.005	8.2 (1.9-35.2)	0.004	23.1 (3.8-141.6)	0.0007
Q4 (vs Q1-3)			6.6 (2.1-20.5)	0.001	9.6 (2.7-34.1)	0.0005	24.8 (4.7-130.5)	0.0002
<b>LDLC, mmol/L</b>								
Q1	≤2.75	22/37 (59.5)						
Q2 (vs Q1)	>2.75 and ≤3.35	24/36 (66.7)	1.6 (0.5-5.2)	0.39	1.1 (0.3-3.9)	0.87	1.0 (0.2-4.0)	0.97
Q3 (vs Q1)	>3.35 and ≤4.02	23/37 (62.2)	1.2 (0.4-3.6)	0.75	1.1 (0.3-3.8)	0.86	1.5 (0.4-6.0)	0.61
Q4 (vs Q1)	>4.02	30/35 (85.7)	7.5 (1.9-29.0)	0.004	8.1 (1.9-34.0)	0.005	13.5 (2.5-73.1)	0.003
Q4 (vs Q1-3)			5.8 (1.8-18.4)	0.003	7.5 (2.2-25.3)	0.001	11.6 (2.7-49.4)	0.0009
<b>HDLC, mmol/L</b>								
Q4	>1.50	20/31 (64.5)						
Q3 (vs Q4)	>1.27 and ≤1.50	24/38 (63.2)	0.7 (0.2-2.1)	0.49	0.7 (0.2-2.5)	0.63	0.9 (0.2-3.8)	0.94
Q2 (vs Q4)	>1.04 and ≤1.27	25/41 (61.0)	0.9 (0.3-2.7)	0.86	1.0 (0.3-3.3)	0.94	1.0 (0.2-3.8)	0.95
Q1 (vs Q4)	≤1.04	31/37 (83.8)	3.2 (0.9-11.5)	0.07	2.8 (0.7-11.0)	0.15	1.7 (0.4-7.8)	0.49
Q1 (vs Q2-4)			3.8 (1.3-10.9)	0.01	3.1 (1.1-9.2)	0.04	1.8 (0.6-5.6)	0.34
<b>TG, mmol/L</b>								
Q1	≤0.81	26/38 (68.4)						
Q2 (vs Q1)	>0.81 and ≤1.11	25/36 (69.4)	0.9 (0.3-2.8)	0.88	1.0 (0.3-3.4)	>0.99	0.9 (0.2-3.3)	0.87
Q3 (vs Q1)	>1.11 and ≤1.56	22/38 (57.9)	0.5 (0.2-1.5)	0.21	0.5 (0.2-1.7)	0.28	0.6 (0.2-2.1)	0.40
Q4 (vs Q1)	>1.56	27/35 (77.1)	2.7 (0.8-8.9)	0.11	3.1 (0.8-12.4)	0.10	2.7 (0.6-12.2)	0.19
Q4 (vs Q1-3)			3.5 (1.2-9.6)	0.02	4.0 (1.3-12.8)	0.02	3.5 (1.0-12.3)	0.05
<b>TC/HDLC</b>								
Q1	≤3.32	21/37 (56.8)						
Q2 (vs Q1)	>3.32 and ≤4.09	23/37 (62.2)	1.1 (0.4-3.2)	0.86	1.4 (0.4-4.4)	0.62	1.2 (0.3-4.4)	0.77
Q3 (vs Q1)	>4.09 and ≤5.10	24/38 (63.2)	1.8 (0.6-5.5)	0.27	2.6 (0.7-9.2)	0.14	1.8 (0.4-7.7)	0.41
Q4 (vs Q1)	>5.10	32/35 (91.4)	13.0 (2.8-59.9)	0.001	18.1 (3.1-105.5)	0.001	19.7 (2.6-149.4)	0.004
Q4 (vs Q1-3)			9.7 (2.5-37.1)	0.0009	9.7 (2.3-40.1)	0.002	13.1 (2.5-68.6)	0.002
<b>LDLC/HDLC</b>								
Q1	≤2.00	24/38 (63.2)						
Q2 (vs Q1)	>2.00 and ≤2.64	23/35 (65.7)	1.1 (0.4-3.1)	0.90	1.0 (0.3-3.3)	>0.99	1.0 (0.3-3.5)	0.96
Q3 (vs Q1)	>2.64 and ≤3.48	21/37 (56.8)	1.1 (0.4-3.2)	0.92	1.3 (0.4-4.3)	0.68	1.2 (0.3-4.9)	0.75
Q4 (vs Q1)	>3.48	31/35 (88.6)	5.7 (1.4-23.0)	0.01	6.9 (1.4-32.7)	0.02	7.9 (1.2-50.5)	0.03
Q4 (vs Q1-3)			5.5 (1.7-18.1)	0.005	6.0 (1.7-21.8)	0.007	7.0 (1.5-32.0)	0.01
<b>Non-HDLC, mmol/L</b>								
Q1	≤3.29	23/38 (60.5)						
Q2 (vs Q1)	>3.29 and ≤3.86	24/37 (64.9)	1.0 (0.4-3.1)	0.94	0.9 (0.3-3.0)	0.82	0.7 (0.2-2.9)	0.65
Q3 (vs Q1)	>3.86 and ≤4.61	22/37 (59.5)	1.0 (0.4-3.1)	0.95	1.0 (0.3-3.3)	0.95	0.7 (0.2-2.9)	0.64
Q4 (vs Q1)	>4.61	31/35 (88.6)	8.5 (2.1-34.6)	0.003	10.1 (2.1-48.2)	0.004	13.1 (2.3-75.9)	0.004
Q4 (vs Q1-3)			8.2 (2.4-28.2)	0.0008	10.7 (2.8-40.5)	0.0005	16.5 (3.5-77.6)	0.0004

Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; CI = confidence interval; HDLC = high-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol; NP = neuritic plaque; OR = odds ratio; TC = total cholesterol; TG = triglycerides.

<sup>a</sup> Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, cerebrovascular disease, and regular exercise. Model 3 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, cerebrovascular disease, and APOE ε4 carrier.

strategies, and the age at the occurrence of high cholesterol may influence observations.<sup>31</sup> Our study evaluated how cholesterol affects the neuropathologic process of AD; however, dyslipidemia might also affect mechanisms other than NP formation in the onset of dementia or AD, such as cell-membrane maintenance or synaptic function.

There are some limitations to our present study. First, the crude, semiquantitative evaluation of NPs (CERAD) and NFTs (Braak stage) could affect the results of the present study. Second, the medical history of dyslipidemia, such as disease duration, use of medication, and complications, were not considered in this study. Medication or change of lifestyle between the clinical examination and death might affect the lipid profiles during a follow-up period; therefore, the association between lipid profiles and AD pathology could be underestimated in this study.

Despite these limitations, our study has several strengths. The main advantage over other studies is the direct measurement of lipid profiles, such as TC, TG, and HDLC, more than a decade before subjects died. We included community-based subjects, who had detailed metabolic characterization at midlife based on comprehensive blood testing, and we systematically assessed AD pathology. Accordingly, the data included in this study are valuable for the examination of metabolic risk factors for AD pathology. In the Hisayama Study, both participation rate of clinical examinations and autopsy rate have remained at high levels. Therefore, our results could apply to other Japanese populations.

As part of the Hisayama Study, we have shown that dyslipidemia, in addition to insulin resistance, may be an independent risk factor for NP formation. Due to the long follow-up period, a number of other factors may have come into play. Nonetheless, our study clearly makes the point that lipid profiles may contribute directly or indirectly to plaque burden in the brain. Because a direct measurement of LDLC may be unreliable, and for the purpose of additional consideration of very low-density lipoprotein and intermediate density lipoprotein cholesterol, the values of non-HDL cholesterol might help to predict the development of NPs. Further studies are required to determine if there is a causal link between dyslipidemia and the development of NPs or other AD-related pathologies. In the future, adequate control of cholesterol, in addition to the control of diabetes, might contribute to a strategy for the prevention of AD.

#### AUTHOR CONTRIBUTIONS

Dr. Matsuzaki: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, statistical analysis. Dr. Sasaki: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, obtaining funding.

Dr. Hata: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data, statistical analysis. Dr. Hirakawa: analysis or interpretation of data, acquisition of data. Dr. Fujimi: analysis or interpretation of data, acquisition of data. Dr. Ninomiya: drafting/revising the manuscript, acquisition of data. Dr. Suzuki: drafting/revising the manuscript, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data. Dr. Kanba: analysis or interpretation of data, study supervision. Dr. Kiyohara: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, study supervision, obtaining funding. Dr. Iwaki: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision, obtaining funding.

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# Apolipoprotein Genotype for Prediction of Alzheimer's Disease in Older Japanese: The Hisayama Study

Tomoyuki Ohara, MD,\*<sup>†</sup> Toshiharu Ninomiya, MD, PhD,<sup>‡</sup> Michiaki Kubo, MD, PhD,<sup>§</sup>  
Yoichiro Hirakawa, MD,\*<sup>‡</sup> Yasufumi Doi, MD, PhD,<sup>‡</sup> Jun Hata, MD, PhD,\* Toru Iwaki, MD, PhD,<sup>†</sup>  
Shigenobu Kanba, MD, PhD,<sup>†</sup> and Yutaka Kiyohara, MD, PhD\*

**OBJECTIVES:** To estimate the effects of the apolipoprotein E (APOE)- $\epsilon$ 4 allele on the development of dementia and to elucidate its usefulness in the risk prediction of dementia in Japanese.

**DESIGN:** Prospective cohort study.

**SETTING:** The Hisayama Study, in Japan.

**PARTICIPANTS:** Five hundred twenty-three participants with deoxyribonucleic acid samples from a population of 1,073 community-dwelling participants without dementia aged 60 to 79.

**MEASUREMENTS:** The risk estimates of the APOE- $\epsilon$ 4 allele on the development of all-cause dementia, Alzheimer's disease (AD), and vascular dementia (VaD).

**RESULTS:** During 17 years of follow-up, 136 participants developed dementia, 81 of whom had AD and 39 VaD. After adjusting for age, sex, education, smoking, alcohol intake, systolic blood pressure, use of antihypertensive agents, glycosylated hemoglobin, serum total cholesterol, body mass index, and regular exercise, the risks of all-cause dementia and AD were significantly higher in APOE- $\epsilon$ 4 carriers than in noncarriers, but no such association was observed for VaD (all-cause dementia: hazard ratio (HR) = 1.81,  $P = .004$ ; AD: HR = 3.42,  $P < .001$ ; VaD: HR = 1.08,  $P = .86$ ). The area under the receiver operating characteristic curve was significantly greater when the APOE genotype was incorporated into a model with potential risk factors for AD (0.74 vs 0.68,  $P = .02$ ). Other measures of model discrimination (net reclassification improvement: 0.18,  $P = .01$ ; integrated discrimination improvement: 6.25,  $P < .001$ ) also confirmed this improvement in AD risk assessment.

**CONCLUSION:** The APOE- $\epsilon$ 4 allele is a risk factor for AD in the Japanese population. Information on APOE genotype improves AD risk assessment substantially beyond a model based on potential risk factors. *J Am Geriatr Soc* 59:1074–1079, 2011.

**Key words:** dementia; Alzheimer's disease; vascular dementia; cohort study; APOE

Dementia is a major cause of disability and premature death in older adults.<sup>1</sup> Alzheimer's disease (AD) has been found to be the most common form of dementia in population-based prospective studies conducted in Western countries. Conversely, vascular dementia (VaD) has been found to be more prevalent in Japan than in Western countries,<sup>2–7</sup> although in recent years the incidence of AD in Japanese has risen to nearly the same level as in Western studies,<sup>4–7</sup> with the result that the burden of AD has been increasing gradually in Japan.<sup>3</sup>

The apolipoprotein (APOE)- $\epsilon$ 4 allele has been identified as a susceptibility genotype for AD,<sup>8</sup> but few cohort studies have examined this possibility in Asians.<sup>9,10</sup> Moreover, only a few studies have assessed whether the APOE- $\epsilon$ 4 genotype can improve the accuracy with which AD can be predicted.<sup>11,12</sup> An enhanced risk assessment would be of great clinical value if it could more accurately identify people who are at high risk of AD.

Toward this end, a community-based prospective cohort study for evaluating risk factors for dementia in Japanese was established. One achievement of this study was that it verified the subtypes of dementia using a detailed neurological and morphological examination, including neuroimaging and autopsy.<sup>4,13</sup> The purposes of this study were to elucidate the association between the APOE- $\epsilon$ 4 allele and the development of dementia and its subtypes, and to investigate the effect of the APOE genotype on the accuracy of AD prediction in Japanese.

From the Departments of \*Environmental Medicine, <sup>†</sup>Neuropsychiatry, and <sup>‡</sup>Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; and <sup>§</sup>Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN Yokohama Institute, Kanagawa, Japan.

Address correspondence to Toshiharu Ninomiya, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka City 812-8582, Japan. E-mail: nino@intmed2.med.kyushu-u.ac.jp

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## METHODS

### Study Population

A population-based prospective study of cerebrocardiovascular diseases was established in 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area of Kyushu Island, Japan. Full community surveys of the health status and neurological condition of residents aged 40 and older have been repeated since 1961.<sup>14</sup> Additionally, comprehensive surveys of cognitive impairment in older adults have been conducted every 6 or 7 years since 1985, and collection of genomic deoxyribonucleic acid (DNA) was started in 2000.

In 1988, 1,073 residents aged 60 to 79 (89.6% of the total population in this age group) participated in a health examination; 534 individuals for whom DNA samples were not available (416 died before the collection of DNA samples was begun, and 118 did not consent to the genomic study) were excluded from the study. The characteristics of the excluded participants are shown in Table 1. Additionally, after excluding 13 individuals with dementia at baseline, two of whom died before starting the follow-up, and one for whom we failed to identify the APOE genotype, the remaining 523 participants (205 men and 318 women) were enrolled in this study.

### Follow-Up Survey

The participants were followed prospectively for 17 years, from December 1988 to November 2005. Detailed infor-

mation about the follow-up survey on dementia has been described elsewhere.<sup>3,4,13</sup> Briefly, a daily monitoring system among the study team and local physicians or members of the town's Health and Welfare Office was established. Regular health examinations were given annually to obtain information on any stroke or dementia that the monitoring network missed. Health status was also checked yearly by mail or telephone for any participant who did not undergo regular examinations or who had moved out of town. Follow-up screening surveys of cognitive function were conducted in 1992, 1998, and 2005.<sup>3,4</sup> When new neurological symptoms, including cognitive impairment, were suspected, the study physician and psychiatrist carefully evaluated the participant, conducting comprehensive investigations including interviews of the family or attending physician, physical and neurological examinations, and a review of the clinical records.

### Diagnosis of Dementia

The diagnosis of dementia was made based on the guidelines of the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised*.<sup>15</sup> Participants diagnosed with AD met the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria,<sup>16</sup> and participants diagnosed with VaD met the National Institute of Neurological Disorders and Stroke—Association Internationale pour la Recherche et l'Enseignement en

**Table 1. Baseline Characteristics of Study Subjects**

Characteristic	Subjects Included in the Study			Subjects for Whom DNA Samples Were Unavailable (n = 534)
	Overall (n = 523)	Noncarrier (n = 415)	Carrier (n = 108)	
Age, mean ± SD	66.8 ± 4.9	67.1 ± 5.0	66.1 ± 4.3	70.4 ± 5.6 <sup>##</sup>
Male, n (%)	205 (39.2)	149 (35.9)	56 (51.9) <sup>**</sup>	289 (45.9) <sup>#</sup>
Education ≤6 years, n (%)	48 (9.2)	42 (10.1) <sup>*</sup>	6 (5.6)	72 (13.5) <sup>#</sup>
Systolic blood pressure, mmHg, mean ± SD	135.3 ± 20.2	135.3 ± 20.0	135.4 ± 20.9	142.6 ± 24.4 <sup>##</sup>
Use of antihypertensive agents, n (%)	116 (22.2)	93 (22.4)	23(21.3)	149 (27.9) <sup>#</sup>
Glycosylated hemoglobin, %, mean ± SD	5.6 ± 0.7	5.7 ± 0.7	5.6 ± 0.8	5.7 ± 0.8
Serum total cholesterol, mg/dL, mean ± SD	212.7 ± 40.9	212.1 ± 39.5	215.1 ± 46.2	204.6 ± 46.2 <sup>##</sup>
Body mass index, kg/m <sup>2</sup> , mean ± SD	22.8 ± 3.0	22.7 ± 3.0	22.9 ± 3.2	22.0 ± 3.2 <sup>##</sup>
Smoker, n (%)	101 (19.3)	72 (17.3)	29 (26.9) <sup>*</sup>	150 (28.1) <sup>##</sup>
Alcohol drinker, n (%)	131 (25.0)	94 (22.7)	37 (34.3) <sup>*</sup>	142 (26.6)
Regular exercise (≥3 times/wk), n (%)	81 (15.5)	63 (15.2)	18 (16.7)	71 (13.3)
Duration of follow-up, mean ± SD	16.0 ± 2.4	16.1 ± 2.2	15.6 ± 3.0	9.7 ± 4.8 <sup>##</sup>
Died during follow-up, n (%)	68 (13.0)	57 (13.7)	11 (10.2)	416 (77.9) <sup>##</sup>
Developed all-cause dementia, n (%)	136 (26.0)	102 (24.6)	34 (31.5)	154 (28.8)
Age at onset of all-cause dementia, mean ± SD	82.9 ± 5.9	83.5 ± 6.0	81.2 ± 5.4	80.9 ± 5.9 <sup>##</sup>
Developed Alzheimer's disease, n (%)	81 (15.5)	52 (12.5)	29 (26.9) <sup>**</sup>	67 (12.6)
Age at onset of Alzheimer's disease, mean ± SD	83.0 ± 5.7	83.8 ± 5.7	81.6 ± 5.5	82.0 ± 5.5
Developed vascular dementia, n (%)	39 (7.5)	31 (7.5)	8 (7.4)	61 (11.4) <sup>#</sup>
Age at onset of vascular dementia, mean ± SD	82.4 ± 6.1	83.2 ± 6.3	79.3 ± 4.1	80.2 ± 5.8

Differences were estimated using the Student *t*-test or the chi-square test as appropriate.

*P* < <sup>\*</sup>.05, <sup>\*\*</sup>.01: ε4 allele (+) vs ε4 allele (-).

*P* < <sup>#</sup>.05, <sup>##</sup>.01: subjects for whom deoxyribonucleic acid (DNA) samples were not available vs subjects included in the study.

APO = apolipoprotein; SD = standard deviation.

Neurosciences criteria.<sup>17</sup> Possible or probable dementia subtypes were diagnosed according to clinical information, including neuroimaging. Definite dementia subtypes were also determined on the basis of clinical and neuropathological information in participants with dementia who underwent autopsy. The diagnostic procedure for autopsy cases was reported previously.<sup>18</sup> Expert neurologists and psychiatrists adjudicated every dementia case.

During the 17-year follow-up period, 136 participants (45 men and 91 women) developed dementia, and 68 of these died. The mean age of onset of dementia was  $83 \pm 6$  years. Of those who developed dementia, 116 (85.3%) underwent evaluation with neuroimaging, and 61 (44.9%) were subjected to brain autopsy examination. Fifty-seven of the 136 participants were evaluated with both examinations, resulting in 120 participants (88.2%) having a morphological examination. Of the participants with dementia, eight had mixed AD and VaD and were counted as cases in both dementia subtypes. Finally, 81 participants had AD and 39 VaD.

### Single Nucleotide Polymorphism Selection and APOE Genotyping

Genomic DNA was extracted from peripheral blood leukocytes or autopsy tissues using a standard method. Two single nucleotide polymorphisms (SNPs; rs429358 and rs7412) were genotyped using the multiplex polymerase chain reaction–based Invader assay (Third Wave Technologies, Madison, WI)<sup>19</sup> in a blinded fashion to the clinical information of study samples. The accuracy of genotype identification was tested in 94 samples by direct sequencing using the ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, CA), and the concordance rate was 100%. The APOE genotypes were classified into  $\epsilon 2/\epsilon 2$ ,  $\epsilon 2/\epsilon 3$ ,  $\epsilon 3/\epsilon 3$ ,  $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 4$ , and  $\epsilon 4/\epsilon 4$  based on the haplotypes of rs429358 and rs7412. The frequencies of the APOE genotypes in the whole sample were as follows:  $\epsilon 2/\epsilon 2$ , three (0.6%);  $\epsilon 2/\epsilon 3$ , 45 (8.6%);  $\epsilon 3/\epsilon 3$ , 367 (70.2%);  $\epsilon 2/\epsilon 4$ , four (0.8%);  $\epsilon 3/\epsilon 4$ , 94 (18.0%); and  $\epsilon 4/\epsilon 4$ , 10 (1.9%). These genotyped data were strictly controlled under condition of anonymity so that individuals could not be identified.

### Risk Factors

At the baseline examination, each participant completed a self-administered questionnaire covering medical history, antihypertensive treatment, educational status, alcohol consumption, smoking habit, and physical activity. Alcohol consumption and smoking habit were classified as current use or not. Participants engaging in sports at least three times per week during their leisure time were classified as physically active. Sitting blood pressure was measured using a sphygmomanometer three times at the right upper arm after at least 5 minutes of rest, and the mean of the three measurements was used in the analysis. Body height and weight were measured in light clothing without shoes, and body mass index (BMI;  $\text{kg}/\text{m}^2$ ) was calculated. Glycosylated hemoglobin (HbA1c) was measured using high-performance liquid chromatography (HLC-723Hb, TOSOH, Tokyo, Japan). Plasma total cholesterol levels were measured enzymatically.

### Statistical Analysis

The Cox proportional hazards model was used to estimate the multivariable-adjusted probabilities of event-free survival and the multivariable-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) of dementia in relation to the APOE genotype. The assumption of the proportional hazards was checked graphically using the log cumulative hazard plots for each dementia subtype according to APOE genotype. The heterogeneity in the relationship between subgroups was tested by adding multiplicative interaction terms to the relevant Cox model.

To compare the accuracy of risk assessment for AD development between the models adjusted for potential risk factors with and without the APOE genotype, receiver operating characteristic (ROC) curves for the model were plotted. The consistency in the area under the ROC curve (AUC) between models was estimated using DeLong's method.<sup>20</sup> The greater discriminative value of the APOE genotype was further examined using two measures previously described:<sup>21</sup> net reclassification improvement and integrated discrimination improvement. In this analysis, the probability of the risk of AD over 17 years was classified into clinically meaningful categories of less than 10%, 10% to 20%, and more than 20%. The individual probabilities were estimated using the Cox proportional hazards model. SAS (version 9.2, SAS Institute, Inc., Cary, NC) and STATA (version 9.2, Stata Corp., College Station, TX) were used to perform statistical analysis. Two-sided  $P < .05$  was considered statistically significant in all analyses.

### Ethical Considerations

This study was conducted with the approval of the ethics committees of the Kyushu University Faculty of Medicine and of the RIKEN Yokohama Institute. Written informed consent was obtained from all participants.

### RESULTS

The baseline characteristics of participants are summarized in Table 1 according to the presence or absence of the APOE- $\epsilon 4$  allele. Mean age did not differ between APOE- $\epsilon 4$  carriers and noncarriers, but the proportion of men was higher among APOE- $\epsilon 4$  carriers. The frequencies of smoking and alcohol intake were higher and the frequency of low educational status ( $\leq 6$  years) was lower in APOE- $\epsilon 4$  carriers than in noncarriers.

As shown in Table 2, APOE- $\epsilon 4$  carriers had significantly higher incidence rates of all-cause dementia (33.0% vs 20.8%,  $P = .008$ ) and AD (28.8% vs 10.2%,  $P < .001$ ) over the 17-year follow-up than noncarriers after adjusting for age, sex, education, smoking, and alcohol intake; no significant differences were observed for VaD. Participants who were APOE- $\epsilon 4$  carriers had significantly greater risk of all-cause dementia (HR = 1.72, 95% CI = 1.15–2.56) than those who were APOE- $\epsilon 4$  noncarriers, after adjusting for the aforementioned risk factors. This association remained significant even in the fully adjusted model including age, sex, education, smoking, alcohol intake, systolic blood pressure, use of antihypertensive agents, HbA1c, serum total cholesterol, BMI, and regular exercise (HR = 1.81, 95% CI = 1.21–2.72). With regard to subtypes of dementia, APOE- $\epsilon 4$  carriers had a 3.4 times (95% CI = 2.12–5.51)

**Table 2. Association Between the Apolipoprotein (APOE)-ε4 Allele and Development of Dementia**

APOE-ε4 Allele Genotype	Events, n	Participants, n	Adjusted Incidence for 17 Years (%) <sup>*</sup>	Hazard Ratio (95% Confidence Interval) P-Value		
				Crude	Model 1 <sup>*</sup>	Model 2 <sup>†</sup>
<b>All-cause dementia</b>						
Negative	102	415	20.8	1.00 (reference)	1.00 (reference)	1.00 (reference)
Positive	34	108	33.0	1.35 (0.92–1.99) .13	1.72 (1.15–2.56) <.001	1.81 (1.21–2.72) .004
<b>AD</b>						
Negative	52	415	10.2	1.00 (reference)	1.00 (reference)	1.00 (reference)
Positive	29	108	28.8	2.28 (1.45–3.59) <.001	3.15 (1.97–5.02) <.001	3.42 (2.12–5.51) <.001
<b>VaD</b>						
Negative	31	415	6.5	1.00 (reference)	1.00 (reference)	1.00 (reference)
Positive	8	108	7.7	1.04 (0.48–2.27) .91	1.19 (0.54–2.64) .66	1.08 (0.48–2.43) .85

For the analysis of incidence of Alzheimer's disease (AD), vascular disease (VaD) cases were censored and vice versa.

<sup>\*</sup>Risk estimates were adjusted for age, sex, education, smoking, and alcohol intake.

<sup>†</sup>Risk estimates were adjusted for confounding factors included in Model 1 plus systolic blood pressure, use of antihypertensive medication, glycosylated hemoglobin, serum total cholesterol, body mass index, and regular exercise.

greater risk of AD than APOE-ε4 noncarriers in the fully adjusted model, but no such association was observed for VaD. Competing risk models were also run using VaD as a competing risk in the AD model and vice versa. The results remained; in the fully adjusted competing risk model, APOE-ε4 carriers had a greater risk of AD (HR = 2.76, 95% CI = 1.98–3.73) and VaD (HR = 1.09, 95% CI = 0.62–1.91) than noncarriers.

Additionally, the relationship between the number of APOE-ε4 alleles and the risk of AD was elucidated. The fully adjusted HR of AD increased linearly with increasing number of APOE-ε4 alleles (HR = 3.04, 95% CI = 1.84–5.04 for one APOE-ε4 allele; HR = 9.76, 95% CI = 3.62–26.29 for two APOE-ε4 alleles; *P* for trend <.001).

To evaluate the influence of APOE genotype on the accuracy of AD risk assessment, the AUCs of models with and without the APOE genotype were compared. The AUC was significantly greater after adding information on the APOE genotype to the model, including other potential risk factors, namely, age, sex, education, smoking, alcohol intake, systolic blood pressure, use of antihypertensive agents, HbA1c, serum total cholesterol, BMI, and regular exercise (from 0.68, 95% CI = 0.62–0.75 to 0.74, 95% CI = 0.69–0.80; *P* for difference in the area = .02). Reclassifications for participants who did or did not develop AD are summarized in Table 3. When the model with the APOE genotype was used, 15 participants were correctly reclassified into a higher risk category, and 14 were inappropriately reclassified into a lower risk category of participants who developed AD. Alternatively, 137 participants were correctly reclassified into a lower risk category, and 62 were inappropriately reclassified into a higher risk category of participants who did not develop AD. After the addition of the APOE genotype, the net reclassification improvement was estimated as 0.18 ( $Z_{NRI} = 2.47, P = .01$ ), and the integrated discrimination improvement was estimated as 6.25 ( $Z_{IDI} = 3.75, P < .001$ ).

**DISCUSSION**

This long-term prospective study of a general Japanese population demonstrated that APOE-ε4 carriers had a

greater risk of developing AD, but not VaD, than noncarriers. This association remained unchanged even after controlling for confounding factors including age, sex, education, smoking, alcohol intake, systolic blood pressure, use of antihypertensive agents, HbA1c, serum total cholesterol, BMI, and regular exercise. To the knowledge of the authors, this is the first prospective study showing that the incorporation of the APOE genotype into a model with

**Table 3. Reclassification of the 17-Year Predicted Absolute Risk of the Development of Alzheimer's Disease (AD)**

Model 1	Model 1 + APOE Genotype			Total
	Participants, n			
	<10% risk	10–20% risk	>20% risk	
<b>Participants who developed AD</b>				
<10% risk	5	3 <sup>*</sup>	2 <sup>*</sup>	10
10–20% risk	12 <sup>†</sup>	9	10 <sup>*</sup>	31
>20% risk	0 <sup>†</sup>	2 <sup>†</sup>	38	40
Total	17	14	50	81
<b>Participants who did not develop AD</b>				
<10% risk	103	28 <sup>*</sup>	1 <sup>*</sup>	132
10–20% risk	128 <sup>†</sup>	67	33 <sup>*</sup>	228
>20% risk	0 <sup>†</sup>	9 <sup>†</sup>	73	82
Total	231	104	107	442

Model 1 includes age, sex, education, smoking, alcohol intake, systolic blood pressure, use of antihypertensive medication, glycosylated hemoglobin, serum total cholesterol, body mass index, and regular exercise.

Participants were categorized according to the 17-year predicted absolute risks of the development of AD, which were estimated by using the relevant Cox model.

Participants reclassified into

<sup>\*</sup>higher- and

<sup>†</sup>lower-risk categories after including the apolipoprotein E (APOE) genotype in the model.

Net reclassification improvement was estimated as 0.18 ( $Z_{NRI} = 2.47, P = .01$ ).

potential risk factors improved the ability to predict AD in a general population. These findings may provide a useful guide to estimate the risk of AD for the general population.

The APOE- $\epsilon$ 4 allele has been found to be an important genetic risk factor for AD in a large majority of epidemiological studies,<sup>8</sup> but few population-based prospective studies have provided evidence for the association between the APOE- $\epsilon$ 4 allele and the incidence of AD for Asians.<sup>9,10</sup> The Honolulu-Asia Aging Study<sup>9</sup> of Japanese-American men has evaluated the association between the APOE- $\epsilon$ 4 allele and the risk of developing AD and VaD. The results showed that APOE- $\epsilon$ 4 carriers had a significant 2.4 times greater risk of AD than noncarriers, but no such association was observed for VaD. The Kame Project,<sup>10</sup> a prospective study of Japanese Americans living in King County, Washington, in the United States, also reported that the APOE- $\epsilon$ 4 allele was a strong risk factor for AD. These findings are in accordance with those of the current study.

In the present study, adding the APOE genotype to potential risk factors significantly increased the AUC, although the influence of APOE genotype on the validity of AD risk assessment is inconsistent in previous studies.<sup>11,12</sup> In a hospital-based cross-sectional study, adding the APOE genotype to the clinical information significantly increased the AUC for discriminating true cases of AD.<sup>11</sup> Conversely, the Honolulu-Asia Aging Study<sup>12</sup> showed no significant differences in the AUC for detecting AD between models with and without the APOE genotype. It has been acknowledged that the AUC analysis is insensitive to change in the prediction ability even when a new marker is statistically significant and independently associated with risk.<sup>22</sup> Thus, the current study also evaluated new measures of model discrimination, namely net reclassification improvement and integrated discrimination improvement statistics, which appeared to be more sensitive to change in the prediction ability of the outcomes between risk assessment models than the AUC analysis.<sup>21</sup> The estimates of these statistics showed that the addition of the APOE genotype to the model with potential risk factors improved the discriminatory property of the model for AD prediction in participants without dementia at baseline. These findings suggest that APOE genotype is an important risk factor for predicting accurately the occurrence of AD over time.

APOE is the most common susceptibility gene for AD, but the mechanism underlying its action in the development of AD is not completely understood. A widely accepted hypothesis is that  $\beta$ -amyloid accumulates in the brain, aggregating to form oligomers, plaques, and cerebrovascular deposits.<sup>23</sup> The APOE- $\epsilon$ 4 allele is implicated in disordered trafficking of  $\beta$ -amyloid peptide<sup>24</sup> and stimulates its deposition.<sup>25</sup> A postmortem neuropathological study demonstrated that people with AD with the APOE- $\epsilon$ 4 allele had stronger amyloid deposition than those without it.<sup>26</sup> These findings indicate that the APOE- $\epsilon$ 4 allele may lead to poorer clearance of  $\beta$ -amyloid, causing AD.

The strengths of the current study include its longitudinal population-based study design, long duration of follow-up, and accuracy in the diagnosis of dementia, including its subtypes. Some limitations should be noted. First, there was selection bias in the study population. Individuals excluded from the study had more cardiovascular risk factors, greater mortality, and higher cumulative inci-

dence of VaD than those included in the study at baseline (Table 1), but there was no significant difference in the cumulative incidence of AD between participants excluded and included. Thus, the generalizability of the findings in regard to VaD may be limited. Nevertheless, the present findings provide useful information for assessing the risk of AD. Second, despite the use of detailed findings from autopsy, brain imaging, and clinical information, a certain degree of subtype misclassification cannot be excluded because the boundary between VaD and AD is not always discernible, and participants with dementia sometimes have mixed neurodegenerative and vascular pathology.<sup>27</sup> However, a sensitivity analysis using only events determined to be pure cases of AD and VaD did not make any material difference in the findings (data not shown).

In conclusion, the APOE- $\epsilon$ 4 allele is an independent risk factor for the development of AD in a general Japanese population. Moreover, the fact that adding the APOE genotype to the model significantly improved its ability to predict the risk of AD suggests that the APOE- $\epsilon$ 4 allele should be considered an essential risk factor for predicting AD. Further investigations are required to establish a more-reliable risk assessment for AD.

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**Conflict of Interest:** The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this paper.

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**Author Contributions:** Tomoyuki Ohara and Toshiharu Ninomiya: study coordinators. Tomoyuki Ohara: study concept, design, data collection, endpoint adjudication, interpretation of data, and writing the manuscript. Toshiharu Ninomiya: study concept, design, data collection, statistical analysis, interpretation of data, and writing the manuscript. Michiaki Kubo: study concept, genotyping, interpretation of data, and writing the manuscript. Yoichiro Hirakawa: data collection, statistical analysis, and interpretation of data. Yasufumi Doi and Jun Hata: data collection and interpretation of data. Toru Iwaki and Shigenobu Kanba: endpoint adjudication and interpretation of data. Yutaka Kiyohara: study performance, obtaining supporting sources, and writing of manuscript. All authors critically reviewed the manuscript and approved the final version.

**Sponsor's Role:** The supporting sources had no role in the study design or conduct of the study; collection, management, analysis, or interpretation of the data; writing the report; or in the decision to submit the manuscript for publication.

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# Midlife and Late-Life Blood Pressure and Dementia in Japanese Elderly

## The Hisayama Study

Toshiharu Ninomiya, Tomoyuki Ohara, Yoichiro Hirakawa, Daigo Yoshida, Yasufumi Doi, Jun Hata, Shigenobu Kanba, Toru Iwaki, Yutaka Kiyohara

**Abstract**—The associations between blood pressure and dementia have been inconclusive. We followed up a total of 668 community-dwelling Japanese individuals without dementia, aged 65 to 79 years, for 17 years and examined the associations of late-life and midlife hypertension with the risk of vascular dementia and Alzheimer disease using the Cox proportional hazards model. During the follow-up, 76 subjects experienced vascular dementia and 123 developed Alzheimer disease. The age- and sex-adjusted incidence of vascular dementia significantly increased with elevated late-life blood pressure levels (normal: 2.3, prehypertension: 8.4, stage 1 hypertension: 12.6, and stage 2 hypertension: 18.9 per 1000 person-years;  $P_{\text{trend}} < 0.001$ ), whereas no such association was observed for Alzheimer disease ( $P_{\text{trend}} = 0.88$ ). After adjusting for potential confounding factors, subjects with prehypertension and stage 1 or stage 2 hypertension had 3.0-fold, 4.5-fold, and 5.6-fold greater risk of vascular dementia, respectively, compared with subjects with normal blood pressure. Likewise, there was a positive association of midlife blood pressure levels with the risk of vascular dementia but not with the risk of Alzheimer disease. Compared with those without hypertension in both midlife and late life, subjects with midlife hypertension had an  $\approx 5$ -fold greater risk of vascular dementia, regardless of late-life blood pressure levels. Our findings suggest that midlife hypertension and late-life hypertension are significant risk factors for the late-life onset of vascular dementia but not for that of Alzheimer disease in a general Japanese population. Midlife hypertension is especially strongly associated with a greater risk of vascular dementia, regardless of late-life blood pressure levels. (*Hypertension*. 2011;58:00-00.) • Online Data Supplement

**Key Words:** prospective studies ■ aged ■ hypertension ■ vascular dementia ■ Alzheimer disease

Vascular dementia (VaD) has been acknowledged to be more prevalent in Japan as compared with Western countries.<sup>1-5</sup> We reported previously that the prevalence of VaD did not apparently decrease over the past 2 decades,<sup>2</sup> despite the fact that the incidence of stroke significantly decreased because of the improvement of blood pressure (BP) lowering therapy since the 1970s.<sup>6</sup> In addition, the incidence of Alzheimer disease (AD) in Japanese studies is greater to almost same degree as that of Western studies in recent years,<sup>1,4,5</sup> resulting in a drastic increase in the burden of dementia in Japan.<sup>2</sup>

Hypertension and dementia are common disorders in the elderly.<sup>7,8</sup> Because hypertension has been shown to be a major risk factor for cerebrovascular disease, higher BP was likely to be strongly associated with a greater risk of VaD.<sup>9</sup> In addition, recent evidence has emerged to imply that vascular factors may be involved in AD, which has traditionally been considered a primarily neurodegenerative disorder.<sup>10,11</sup> However, the results of observational longitudinal studies showing

the effects of BP on the risks of dementia and its subtypes are inconsistent.<sup>12,13</sup> In particular, some studies have suggested that midlife hypertension is a risk factor for late-life dementia, whereas lower diastolic BP in late life may be related to increased risks of dementia and AD.<sup>12</sup> These facts raise the possibilities that the effects of hypertension on the development of dementia may be different between midlife and late life, possibly because the longitudinal changes related to hypertension in the brain may begin earlier in the adult life span.<sup>12,13</sup> However, few studies have compared the effects of midlife and late-life hypertension on the development of dementia in an identical population.

The purposes of this study were to investigate the association of BP levels in midlife and late life with the development of dementia and its subtypes in a general Japanese population and to elucidate whether the effects of hypertension on the risk of dementia are different between midlife and late life in an identical population. The findings from this study are expected to provide clear evidence of the diverse

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From the Departments of Medicine and Clinical Science (T.N., Y.H., Y.D., J.H.) and Environmental Medicine (T.N., T.O., Y.H., D.Y., Y.D., J.H., Y.K.), Neuropsychiatry (T.O., S.K.), and Neuropathology (T.I.), Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Correspondence to Toshiharu Ninomiya, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka City 812-8582, Japan. E-mail nino@intmed2.med.kyushu-u.ac.jp

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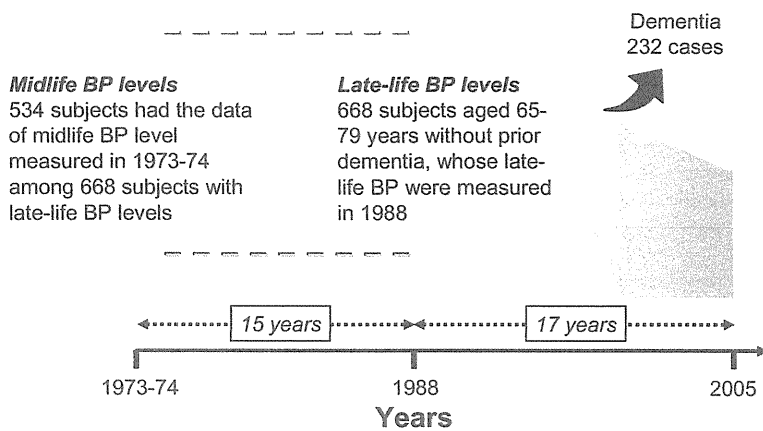


Figure. Diagram of the study design. BP indicates blood pressure.

associations of BP levels with the risk of dementia in midlife and late life.

## Methods

### Study Population

A population-based prospective study of cerebro-cardiovascular diseases was established in 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. Full community surveys of health status and neurological conditions of the residents aged  $\geq 40$  years have been repeated since 1961.<sup>6</sup> In addition, comprehensive surveys of cognitive function in the elderly, including neuropsychological tests, have been conducted every 6 or 7 years since 1985.<sup>2</sup>

In 1988, a total of 682 residents aged 65 to 79 years (90.5% of the total population in this age group) participated in a health checkup. After excluding 12 subjects with dementia at baseline and 2 subjects who died before starting the follow-up, the remaining 668 subjects (266 men and 402 women) were enrolled in this study to investigate the association between late-life BP and the risk of dementia. Among them, 534 subjects (210 men and 324 women) had also participated in a health checkup conducted in 1973–1974 and were included in the analysis of the effects of midlife BP on the risk of late-life dementia (Figure). Written, informed consent was obtained from the participants at baseline in 1988. This study was conducted with the approval of the ethics committee of the Kyushu University Faculty of Medicine.

### BP Categories

Sitting BP was measured with a sphygmomanometer 3 times at the right upper arm after  $\geq 5$  minutes of rest, and the mean of the 3 measurements was used in the analysis. BP levels measured in 1973–1974 and 1988 were classified into 4 categories according to the criteria of the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7).<sup>14</sup>

The extended Materials and Methods section provides detailed information on the follow-up survey, diagnosis of dementia, other risk factors, and statistical analysis. Please see the online Data Supplement at <http://hyper.ahajournals.org>.

## Results

The clinical characteristics of the study population according to BP levels defined by the criteria of JNC-7 in late life (1988) are summarized in the top part of Table 1. The mean age of the overall population was  $72 \pm 4$  years old. Subjects with higher BP levels were likely to be older. The proportion of subjects using antihypertensive agents and the proportion with diabetes mellitus increased gradually with higher BP levels. There were no associations of BP levels with the mean

serum total cholesterol levels and the proportion of women, educational status, or smoking habit. Likewise, the clinical characteristics according to BP levels at midlife (1973–1974) are shown in the bottom part of Table 1. The mean age at midlife was  $57 \pm 4$  years old. The mean values of age, serum total cholesterol, and body mass index and the proportion of the use of antihypertensive agents increased with higher midlife BP levels.

During the 17-year follow-up period, 232 subjects developed dementia of some kind. Of these, 199 (85.8%) underwent evaluation with neuroimaging, and 115 (49.6%) received a general autopsy examination; in 106 cases, both were performed. Thus, 208 subjects in all (89.7%) had some kind of morphological examination. Among dementia cases, 15 AD cases and 13 VaD cases had other coexisting subtypes of dementia, of which 8 cases were a mixed type of AD and VaD. These cases were counted as events in the analysis for each subtype. In all, 123 subjects developed AD, and 76 developed VaD.

First, we estimated the associations between late-life BP levels defined by the criteria of JNC-7 and the risk of dementia (Table 2 and Figure S1, available in the online Data Supplement at <http://hyper.ahajournals.org>). The age- and sex-adjusted incidence of all-cause dementia showed an increasing linear trend with the rise of late-life BP levels ( $P_{\text{trend}}=0.07$ ). In regard to subtypes of dementia, the age- and sex-adjusted incidence of VaD significantly increased with elevated late-life BP levels ( $P_{\text{trend}} < 0.001$ ). Meanwhile, there were no significant associations between late-life BP levels and the age- and sex-adjusted incidence of AD ( $P_{\text{trend}}=0.88$ ). Table 2 also shows the multivariate-adjusted hazard ratios of all-cause dementia and its subtypes across late-life BP levels defined by the criteria of JNC-7. After adjusting for potential confounding factors (age, sex, education level, use of antihypertensive agents, diabetes mellitus, chronic kidney disease, serum total cholesterol, body mass index, history of stroke, smoking habits, and alcohol intake), the risk of VaD increased progressively with elevated BP levels. This relationship was not altered substantially after adjusting for the above-mentioned confounding factors and serum homocysteine. No clear associations were observed between BP levels and the risks of all-cause dementia and AD. There was no evidence of heterogeneity in these associations between the

Table 1. Clinical Characteristics of Study Population

Characteristics	Blood Pressure Levels Defined by JNC-7				P for Trend
	Normal	Prehypertension	Stage 1 Hypertension	Stage 2 Hypertension	
Risk factors according to blood pressure levels in 1988					
No. of participants	106	227	200	135	
Age, mean (SD), y	71 (4)	71 (4)	73 (4)	73 (4)	<0.001
Women, %	54.7	63.9	57.0	63.0	0.58
Educational status, %					
≤6 y	11.3	12.1	18.6	13.6	0.06*
7 to 9 y	50.9	64.1	54.3	57.6	
≥10 y	37.8	23.8	27.1	28.8	
Systolic blood pressure, mean (SD), mm Hg	109 (8)	130 (6)	149 (6)	177 (17)	<0.001
Diastolic blood pressure, mean (SD), mm Hg	65 (7)	72 (8)	79 (9)	84 (11)	<0.001
Antihypertensive agents, %	3.8	22.0	34.0	51.1	<0.001
Diabetes mellitus, %	4.7	13.7	12.0	25.9	<0.001
Chronic kidney disease, %	8.5	15.9	16.5	25.4	0.001
Serum total cholesterol, mean (SD), mmol/L	5.1 (1.1)	5.5 (1.1)	5.3 (1.1)	5.4 (1.2)	0.24
Body mass index, mean (SD), kg/m <sup>2</sup>	20.4 (2.5)	22.2 (2.9)	22.3 (3.1)	23.2 (3.7)	<0.001
Serum homocysteine, mean (SD), μmol/L	9.4 (4.0)	9.4 (3.4)	10.5 (7.4)	10.8 (5.9)	0.01
History of stroke, %	1.9	4.4	7.5	9.6	0.005
Smoking habits, %	25.5	17.2	20.0	20.7	0.69
Alcohol intake, %	16.0	21.1	24.6	30.4	0.006
Risk factors according to blood pressure levels in 1973–1974					
No. of participants	122	185	153	74	
Age, mean (SD), y	56 (4)	57 (4)	58 (4)	58 (4)	<0.001
Women, %	57.4	60.0	58.2	73.0	0.10
Educational status, %					
≤6 y	8.2	13.0	17.0	18.9	0.24*
7 to 9 y	69.7	60.0	60.1	58.1	
≥10 y	22.1	27.0	22.9	23.0	
Systolic blood pressure, mean (SD), mm Hg	109 (7)	129 (7)	147 (7)	178 (16)	<0.001
Diastolic blood pressure, mean (SD), mm Hg	67 (6)	76 (7)	85 (7)	94 (11)	<0.001
Antihypertensive agents, %	0.0	2.7	12.4	20.3	<0.001
Diabetes mellitus, %	0.8	0.5	3.9	1.4	0.18
Chronic kidney disease, %	3.3	1.7	3.9	4.1	0.49
Serum total cholesterol, mmol/L, mean (SD)	4.9 (0.8)	4.9 (0.8)	5.0 (0.8)	5.2 (0.8)	0.03
Body mass index, mean (SD), kg/m <sup>2</sup>	21.4 (2.6)	22.2 (2.7)	22.8 (3.0)	23.2 (4)	<0.001
History of stroke, %	0.0	0.0	1.3	1.4	0.11
Smoking habits, %	36.1	35.1	30.1	29.7	0.21
Alcohol intake, %	20.5	28.1	34.0	25.7	0.13

JNC-7 indicates the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

\*Educational status was tested by using  $\chi^2$  test.

sexes and between subjects with or without antihypertensive agents (all  $P_{\text{heterogeneity}} > 0.10$ ).

Likewise, we investigated the associations between midlife BP levels defined by the criteria of JNC-7 and the risk of dementia developing in late life. Greater midlife BP levels were associated linearly with the increased risk of VaD but not AD (Table 3). Reflecting the rising risk of VaD, there was an increasing linear trend in the risk of all-cause dementia

with higher BP levels in midlife. Sensitivity analyses, in which the all of the event cases were definite cases of dementia as determined by brain autopsy, did not make any material differences in these findings (Table S1).

In addition, we examined the effects of systolic BP and diastolic BP levels measured in late life and in midlife on the risk of incident VaD (Figure S2). In multivariate analysis, the risk of VaD significantly increased with elevated systolic BP



**Table 2. Association Between Late-Life Blood Pressure and the Risk of Dementia During 17-Y Follow-Up**

Late-Life BP Levels Defined by JNC-7	No. of Events	No. of Participants	Age- and Sex-Adjusted Incidence, per 10 <sup>3</sup> PYs (95% CI)	Age-, Sex-, and Education-Adjusted		Multivariable-Adjusted (Model A)*		Multivariable-Adjusted (Model B)†	
				HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
<b>All-cause dementia</b>									
Normal	33	106	28.0 (17.9 to 38.1)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Prehypertension	71	227	27.9 (21.2 to 34.5)	0.86 (0.57 to 1.31)	0.49	0.84 (0.54 to 1.29)	0.42	0.89 (0.57 to 1.39)	0.60
Stage 1 hypertension	75	200	32.0 (24.8 to 39.3)	1.07 (0.71 to 1.63)	0.73	1.08 (0.69 to 1.68)	0.74	1.12 (0.71 to 1.79)	0.62
Stage 2 hypertension	53	135	37.4 (27.4 to 47.5)	1.28 (0.82 to 1.98)	0.28	1.12 (0.68 to 1.87)	0.65	1.16 (0.68 to 1.98)	0.59
<i>P</i> for trend			0.07	0.09		0.25		0.29	
<b>Vascular dementia</b>									
Normal	2	106	2.3 (−0.9 to 5.4)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Prehypertension	19	227	8.4 (4.6 to 12.3)	3.91 (0.91 to 16.85)	0.07	3.01 (0.68 to 13.31)	0.15	3.20 (0.71 to 14.36)	0.13
Stage 1 hypertension	29	200	12.6 (8.0 to 17.2)	6.46 (1.53 to 27.21)	0.01	4.46 (1.02 to 19.42)	0.046	4.72 (1.05 to 21.28)	0.04
Stage 2 hypertension	26	135	18.9 (11.6 to 26.3)	9.98 (2.35 to 42.35)	0.002	5.57 (1.22 to 25.49)	0.03	7.26 (1.54 to 34.17)	0.01
<i>P</i> for trend			<0.001	<0.001		0.009		0.003	
<b>Alzheimer disease</b>									
Normal	22	106	17.9 (9.8 to 26.0)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Prehypertension	39	227	14.0 (9.4 to 18.6)	0.71 (0.42 to 1.20)	0.20	0.73 (0.42 to 1.27)	0.27	0.74 (0.42 to 1.27)	0.27
Stage 1 hypertension	39	200	16.8 (11.5 to 22.0)	0.86 (0.51 to 1.47)	0.58	0.95 (0.54 to 1.68)	0.87	0.93 (0.52 to 1.65)	0.80
Stage 2 hypertension	23	135	14.7 (8.7 to 20.7)	0.84 (0.46 to 1.52)	0.56	0.84 (0.42 to 1.66)	0.61	0.67 (0.33 to 1.37)	0.27
<i>P</i> for trend			0.88	0.92		0.97		0.55	

BP indicates blood pressure; HR, hazard ratio; PYs, person-years; JNC-7, the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

\*Model A was adjusted for potential confounding covariates in late life, namely, age, sex, education level, use of antihypertensive agents, diabetes mellitus, chronic kidney disease, serum total cholesterol, body mass index, history of stroke, smoking habits, and alcohol intake.

†Model B was adjusted for potential confounding covariates included in model 1 plus serum homocysteine.

levels in both late life ( $P_{\text{trend}}=0.0100$ ) and midlife ( $P_{\text{trend}}<0.0001$ ). Every 10-mm Hg increment in systolic BP in late life and midlife was associated with an 18% (95% CI: 7% to 31%) and a 24% (95% CI: 12% to 37%) higher risk of incident VaD, respectively. Such an association was also seen for diastolic BP levels in midlife ( $P_{\text{trend}}=0.001$ ) but not for diastolic late-life BP levels ( $P_{\text{trend}}=0.60$ ). The risk of VaD increased by 37% (95% CI: 9% to 72%) per 10-mm Hg increment in midlife diastolic BP. When we divided the systolic BP category of <120 mm Hg or the diastolic BP category of <80 mm Hg into 2 categories of <110 and 110 to 119 mm Hg or <70 and 70 to 79 mm Hg, respectively, the subjects with the lowest systolic or diastolic BP level did not have a greater risk of VaD than those with second-lowest systolic or diastolic BP level (Table S2). There was no evidence of linear or J-curve associations of systolic BP and diastolic BP levels in late life or in midlife with the risks of AD (Table S2).

Finally, we estimated the effect of the change in BP levels from midlife to late life on the risk of the development of dementia (Table 4). Compared with those having BP levels of <140/90 mm Hg in both midlife and late life, subjects with BP levels of <140/90 mm Hg in midlife and  $\geq 140/90$  mm Hg in late life had a 3.32-fold greater risk of VaD after adjusting for potential confounding factors, whereas subjects with midlife BP levels of  $\geq 140/90$  mm Hg had an  $\approx 5$ -fold greater risk of VaD, regardless of late-life BP levels. Reflecting the increasing risk of VaD, the risk of all-cause dementia

tended to be greater in subjects with midlife BP levels of  $\geq 140/90$  mm Hg. There was no clear association of any elevation in BP levels with the risk of AD.

## Discussion

In the present study, we demonstrated a clear association of higher BP levels in both midlife and late life with a greater risk of the development of VaD, whereas such associations were not observed for AD. Intriguingly, subjects with higher BP levels in midlife were at increasing risks of late-life onset of all-cause dementia and VaD, regardless of late-life BP levels. These findings lend support to the hypothesis that the vascular damages related to hypertension in the brain begin earlier in the life span and are gradually becoming less reversible.<sup>12,13</sup> Therefore, it would be reasonable to suppose that the optimal control of midlife BP levels is clinically important to reduce the risk of late-life dementia in the general Japanese population.

Several prospective studies have examined the association between late-life BP and incident dementia, but the findings have been inconsistent.<sup>12,15–22</sup> Several cohort studies failed to reveal a significant association between higher late-life BP and the risk of all-cause dementia or AD,<sup>16–19</sup> whereas other studies reported a positive association with VaD.<sup>15,16</sup> Our findings were comparable with the latter. In contrast, a few studies have reported that lower late-life BP predisposed elderly subjects, especially those aged  $\geq 80$  years, to all-cause dementia or AD.<sup>20–22</sup> In a randomized control trial conducted

**Table 3. Association Between Midlife Blood Pressure and the Risk of Dementia in Late Life**

Midlife BP Levels Defined by JNC-7	No. of Events	No. of Participants	Age-, Sex-, and Education-Adjusted		Multivariable-Adjusted*	
			HR (95% CI)	P	HR (95% CI)	P
<b>All-cause dementia</b>						
Normal	38	122	1.00 (reference)		1.00 (reference)	
Prehypertension	56	185	0.92 (0.60 to 1.40)	0.68	0.92 (0.60 to 1.41)	0.71
Stage 1 hypertension	66	153	1.51 (1.00 to 2.29)	0.05	1.73 (1.12 to 2.65)	0.01
Stage 2 hypertension	33	74	1.79 (1.11 to 2.90)	0.02	1.95 (1.18 to 3.24)	0.01
P for trend			0.001		<0.001	
<b>Vascular dementia</b>						
Normal	4	122	1.00 (reference)		1.00 (reference)	
Prehypertension	15	185	2.29 (0.75 to 6.99)	0.15	2.38 (0.77 to 7.30)	0.13
Stage 1 hypertension	26	153	5.12 (1.76 to 14.93)	0.003	5.96 (2.00 to 17.77)	0.001
Stage 2 hypertension	18	74	8.92 (2.95 to 26.93)	<0.001	10.07 (3.25 to 31.25)	<0.001
P for trend			<0.001		<0.001	
<b>Alzheimer disease</b>						
Normal	26	122	1.00 (reference)		1.00 (reference)	
Prehypertension	33	185	0.80 (0.47 to 1.35)	0.4	0.77 (0.45 to 1.31)	0.34
Stage 1 hypertension	31	153	1.09 (0.63 to 1.87)	0.76	1.26 (0.72 to 2.21)	0.42
Stage 2 hypertension	12	74	0.97 (0.48 to 1.96)	0.94	1.05 (0.50 to 2.22)	0.89
P for trend			0.72		0.45	

BP indicates blood pressure; HR, hazard ratio; JNC-7, the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

\*Data were adjusted for potential confounding covariates in late life, namely, age, sex, education level, use of antihypertensive agents, diabetes mellitus, chronic kidney disease, serum total cholesterol, body mass index, history of stroke, smoking habits, and alcohol intake.

in the very elderly, BP lowering did not increase the risk of dementia, but the BP levels achieved in the intervention group were still >140/90 mm Hg.<sup>23</sup> Therefore, BP lowering for very elderly people may be implemented with caution. To

date, it might be said that there is no strong evidence to indicate that elevated late-life BP is a risk factor for dementia.

Several longitudinal studies have examined the associations between midlife BP and the risk of dementia.<sup>24–28</sup> The

**Table 4. Effects of Change in Blood Pressure Levels From Midlife to Late Life on the Risk of Dementia**

BP Levels, mm Hg		No. of Events	No. of Participants	Percentage of Use of Antihypertensive Agents		Age-, Sex-, and Education-Adjusted		Multivariable-Adjusted*	
Midlife	Late-Life			Midlife	Late-Life	HR (95% CI)	P	HR (95% CI)	P
<b>All-cause dementia</b>									
<140/90	→ <140/90	59	197	0.5	9.2	1.00 (reference)		1.00 (reference)	
<140/90	→ ≥140/90	35	110	3.6	21.8	1.05 (0.69 to 1.61)	0.81	1.13 (0.73 to 1.75)	0.58
≥140/90	→ <140/90	35	78	10.3	42.3	1.74 (1.14 to 2.66)	0.01	1.88 (1.19 to 2.96)	0.007
≥140/90	→ ≥140/90	64	149	17.5	55.0	1.68 (1.17 to 2.42)	0.005	1.64 (1.09 to 2.46)	0.02
<b>Vascular dementia</b>									
<140/90	→ <140/90	6	197	0.5	9.2	1.00 (reference)		1.00 (reference)	
<140/90	→ ≥140/90	13	110	3.6	21.8	3.71 (1.40 to 9.83)	0.008	3.29 (1.21 to 8.94)	0.02
≥140/90	→ <140/90	14	78	10.3	42.3	6.68 (2.55 to 17.52)	<0.001	5.32 (1.9 to 14.89)	0.001
≥140/90	→ ≥140/90	30	149	17.5	55.0	6.94 (2.86 to 16.88)	<0.001	4.72 (1.83 to 12.17)	0.001
<b>Alzheimer disease</b>									
<140/90	→ <140/90	41	197	0.5	9.2	1.00 (reference)		1.00 (reference)	
<140/90	→ ≥140/90	18	110	3.6	21.8	0.79 (0.45 to 1.39)	0.41	0.91 (0.51 to 1.62)	0.74
≥140/90	→ <140/90	14	78	10.3	42.3	1.00 (0.54 to 1.85)	1.00	1.23 (0.64 to 2.34)	0.53
≥140/90	→ ≥140/90	29	149	17.5	55.0	1.16 (0.71 to 1.90)	0.55	1.29 (0.74 to 2.26)	0.37

BP indicates blood pressure; HR, hazard ratio.

\*Data were adjusted for potential confounding covariates in late life, namely, age, sex, education level, use of antihypertensive agents, diabetes mellitus, chronic kidney disease, serum total cholesterol, body mass index, history of stroke, smoking habits, and alcohol intake.

Honolulu-Asia Aging Study revealed that the risks for both AD and VaD increased in Japanese-American men with untreated hypertension in midlife.<sup>24,25</sup> The results of community-based studies conducted in Finland<sup>26</sup> and in China<sup>27</sup> also showed that elevated systolic BP in midlife increased the risk of AD in late life. Conversely, the Hiroshima Study<sup>28</sup> in Japan demonstrated that higher midlife systolic BP was linked to late-life onset of VaD but not to AD. This finding is in accord with ours. The discrepancies in the findings among these studies may be attributable to the difficulty of distinguishing between dementia subtypes. Patients with dementia sometimes have mixed neurodegenerative and vascular pathology.<sup>29</sup> Recently, cognitive impairment in association with vascular factors has received much attention as a treatable condition and has been termed “vascular cognitive impairment,” which can occur either alone or in association with AD.<sup>11</sup> Careful ascertainment of the dementia type, using clinical information, neuroimaging, and brain autopsy, may be necessary to assess the true effects of vascular risk factors on the development of dementia. Therefore, we have ascertained the relationship between BP and each dementia subtype in the sensitivity analysis using only definite cases determined by autopsy. Another possible explanation is that the diverse findings may reflect that controlling for confounding factors such as diabetes mellitus and metabolic disorders was lacking or insufficient in the previous studies.

Most notably, the present study demonstrated that subjects with midlife BP of  $\geq 140/90$  mm Hg still had a greater risk of VaD, even if their late-life BP was reduced to  $<140/90$  mm Hg. Elevated BP has been found likely to cause small-vessel disease and white-matter lesions.<sup>30,31</sup> Long exposure to poorly controlled midlife hypertension presumably worsens arteriosclerotic changes and lipohyalinosis in the deep subcortical white matter circuit, which may be less reversible by BP reduction once these changes are established.<sup>13,32</sup> The present findings, therefore, strongly support that hypertension and relevant cardiovascular morbidity in midlife have a great impact on the etiology of VaD.

The strengths of our study include its longitudinal population-based design, long follow-up, evaluation of neuropathology and neuroimaging data where needed for the ascertainment of dementia types. On the other hand, several limitations of the present study should be noted. First, the fact that there were only 3 measurements of BP on only one occasion in midlife and on another in late life may have led to some degree of misclassification of BP levels. Such a limitation would weaken the association found in the present study, biasing the results toward the null hypothesis. To obtain a precise estimate of the association, a study in which multiple measurements of BP are taken on separate occasions is needed. Second, we were unable to obtain potential confounding factors, such as depressed mood and apolipoprotein E genotype. The lack of this information would result in a bias toward overdiagnosis of dementia and reduce the accuracy of our findings.

## Perspectives

The present study clearly demonstrated that elevated midlife and late-life BP levels are significant risk factors for the late-life onset of VaD but not for AD in a general Japanese population. Higher midlife BP is especially considered to be strongly associated with greater risks of all-cause dementia and VaD, regardless of BP levels in late life. These findings highlight certain important facts, that BP-related pathophysiological processes of dementia begin many years before any symptoms appear and that a clinical history of hypertension and related comorbid disease at that point is likely to have a great impact on the establishment of the disease. To the extent that the adverse effects of long-standing hypertension on small brain vessels and the subsequent development of dementia are less reversible, optimal management of hypertension as early as possible in the life cycle may be an effective approach to preventing late-life dementia in the general population.

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## Disclosures

None.

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