glucose-oxidase method. Diabetes was defined by the American Diabetes Association criteria in 2003 as follows [18]: fasting plasma glucose of ≥ 7.0 mmol/l and/or 2-h post-load glucose of ≥ 11.1 mmol/l and/or use of anti-diabetic medications.

At baseline, serum insulin levels were measured by a commercial double-antibody solid-phase radioimmunoassay (Phadeseph Insulin; Pharmacia Diagnostics AB, Uppsala, Sweden). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with the formula [fasting plasma glucose (mmol/l) × fasting serum insulin (μU/ml)]/22.5 [19], and the subjects in the top quartile of HOMA-IR distribution were defined as having insulin resistance [20]. Serum specimens collected at the time of high-sensitivity C-reactive protein (hs-CRP) measurement were stored at –20 °C until used in 2002. High-sensitivity CRP concentrations were determined using a modification of the Behring latex-enhanced CRP assay. The categorical variable of hs-CRP was stratified by the median value, as there is no established guideline for the threshold value of hs-CRP in Japanese. HDL cholesterol and triglycerides were all determined enzymatically.

Sitting blood pressure was obtained three times and the average values were used in the analyses. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or current treatment with anti-hypertensive agents. Body height and weight were measured in light clothing without shoes and the BMI was calculated. Obesity was defined as a BMI level of ≥ 25 kg/m^2.

Each subject completed a self-administered questionnaire covering medical history, anti-diabetic and anti-hypertensive treatments, alcohol intake, smoking habits and physical activity at the screening. Diabetes in first- or second-degree relatives was taken to indicate a family history of diabetes. Alcohol intake and smoking habits were classified as either current use or not. Subjects engaging in sports at least three times per week during their leisure time were defined as the regular-exercise group.

Statistical analysis

Magnesium intake was divided into four categories based on the quartile distribution: ≤ 148.5, 148.6–171.5, 171.6–195.5 and ≥ 195.6 mg/day. Because the distributions of HOMA-IR, hs-CRP, triglycerides and dietary variables were skewed, these values were natural log transformed for statistical analyses. Age- and sex-adjusted mean values for possible risk factors were calculated by analysis of covariance and their trends across the quartiles of magnesium intake were tested by multiple regression analysis. Frequencies of risk factors were adjusted for age and sex by the direct method and the trends were examined by a logistic regression model. The incidence of Type 2 diabetes was calculated by a person-year method and was adjusted for the distribution of age and sex in the overall study population using a direct method. The age- and sex-adjusted or multivariable-adjusted hazard ratios with their 95% confidence intervals of magnesium intake for the development of Type 2 diabetes were estimated by Cox proportional hazards model. The median value of continuous variables for magnesium intake in each quartile was used for trend tests. Comparisons of the effects of magnesium intake between participants with and without other Type 2 diabetes risk factors were made by adding an interaction term to the statistical model. $P < 0.05$ was considered statistically significant in all analyses. The software package SAS (version 9.2; SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses.

Ethical considerations

This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research and written informed consent was obtained from the participants.

Results

The baseline characteristics of the study population according to quartiles of magnesium intake are summarized in Table 1. The mean values of age, BMI, total energy, crude fibre, saturated and polyunsaturated fatty acids and vitamin C, and the frequency of regular exercise, increased significantly with increasing magnesium intake quartiles, whereas the mean value of carbohydrates and the frequencies of men and smoking habits declined significantly with increasing quartiles.

The age-and sex-adjusted incidence of Type 2 diabetes according to quartiles of magnesium intake is shown in Table 2. Compared with that in the lowest quartile, the incidence of Type 2 diabetes significantly decreased in the third ($P = 0.01$) and fourth quartiles of magnesium intake ($P = 0.03$) after adjustment for age and sex.

The age- and sex-adjusted and multivariable-adjusted hazard ratios and their 95% confidence intervals for the development of Type 2 diabetes according to quartiles of magnesium intake are also shown in Table 2. The age- and sex-adjusted hazard ratios of Type 2 diabetes increased significantly with elevating quartiles of magnesium intake (P for trend = 0.01). In the multivariate analysis, this association remained substantially unchanged even after adjustment for family history of diabetes, BMI, HDL cholesterol, triglycerides, hypertension, smoking habits, alcohol intake and regular exercise, and intakes of total energy, carbohydrate, crude fibre, saturated and polyunsaturated fatty acids and vitamin C. Compared with the first quartile, the multivariate-adjusted hazard ratio of Type 2 diabetes was 0.67 (95% CI 0.49–0.92, $P = 0.01$) in the third quartile and 0.63 (95% CI 0.44–0.90, $P = 0.01$)

Table 1 Age- and sex-adjusted mean values or frequencies of risk factors for Type 2 diabetes according to quartiles of magnesium intake, 1988

	Quartiles of magnesium intake (mg/day)*				
	≤ 148.5 (n = 499)	148.6–171.5 (n = 500)	171.6–195.5 (n = 500)	≥ 195.6 (n = 500)	P for trend
Age, years	56 (10)	57 (10)	57 (10)	58 (10)	0.001
Men, %	54.3	44.7	39.6	24.6	< 0.001
Fasting plasma glucose, mmol/l	5.5 (0.5)	5.5 (0.5)	5.5 (0.5)	5.5 (0.5)	0.95
Two-hour post-load glucose, mmol/l	6.5 (1.6)	6.6 (1.6)	6.5 (1.6)	6.6 (1.6)	0.41
Family history of diabetes, %	7.4	5.4	9.1	8.3	0.22
BMI, kg/m^2	22.9 (3.0)	22.7 (3.0)	22.8 (3.0)	23.3 (3.0)	0.01
HOMA-IR	1.32 (1.27–1.38)	1.33 (1.28–1.39)	1.40 (1.34–1.46)	1.37 (1.31–1.43)	0.12
hs-CRP, mg/l	0.49 (0.44–0.54)	0.42 (0.38–0.47)	0.41 (0.37–0.46)	0.47 (0.42–0.53)	0.66
HDL cholesterol, mmol/l	1.32 (0.30)	1.29 (0.30)	1.32 (0.30)	1.32 (0.30)	0.61
Triglycerides, mmol/l	1.14 (1.09–1.20)	1.10 (1.05–1.15)	1.12 (1.07–1.18)	1.14 (1.09–1.20)	0.82
Systolic blood pressure, mmHg	133 (19)	130 (19)	129 (19)	131 (19)	0.14
Diastolic blood pressure, mmHg	78 (11)	77 (11)	77 (11)	79 (11)	0.16
Hypertension, %	40.4	34.9	32.9	38.5	0.30
Smoking habits, %	30.3	25.0	20.1	16.9	< 0.001
Alcohol intake, %	32.6	29.3	28.8	30.0	0.18
Regular exercise, %	7.1	10.9	9.9	14.6	0.003
Dietary variables					
Total energy, kcal/day	1674 (354)	1700 (350)	1717 (350)	1779 (355)	< 0.001
Carbohydrate, g/day*	240 (238–243)	234 (231–236)	227 (225–230)	215 (213–218)	< 0.001
Crude fibre, g/day*	3.0 (3.0–3.1)	3.7 (3.6–3.8)	4.2 (4.1–4.3)	4.8 (4.7–4.9)	< 0.001
Saturated fatty acid, g/day*	10.9 (10.6–11.2)	12.0 (11.7–12.3)	12.8 (12.4–13.1)	13.7 (13.3–14.0)	< 0.001
Polyunsaturated fatty acid, g/day*	13.4 (13.0–13.7)	14.8 (14.5–15.2)	16.0 (15.7–16.4)	17.6 (17.2–18.0)	< 0.001
Vitamin C, mg/day*	49.8 (48.3–51.4)	67.2 (65.2–69.3)	81.4 (79.0–83.9)	95.9 (93.0–98.9)	< 0.001

All values are given as means (SD), geometric means (95% CIs) or percentages. Geometric means (95% CIs) are given for HOMA-IR, hs-CRP and triglycerides, and intakes of carbohydrate, crude fibre, saturated fatty acid, polyunsaturated fatty acid and vitamin C.
Age is sex-adjusted and frequency of men is age-adjusted.
Hypertension: blood pressures of ≥ 140/90 mmHg and/or current use of anti-hypertensive medicine.
Because of missing values, analysis of HOMA-IR and hs-CRP was limited to 1998 and 1948, respectively.
*The amount of each dietary variable was adjusted for energy using the regression residual method.
HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

Table 2 Hazard ratios and 95% confidence intervals for the development of Type 2 diabetes according to quartiles of magnesium intake, 1988

	Quartiles of magnesium intake (mg/day)						
	≤ 148.5 (n = 499)	148.6–171.5 (n = 500)	171.6–195.5 (n = 500)	≥ 195.6 (n = 500)	P for trend (across categories)	Continuous log scale*	P-value (continuous)
Median of magnesium intake (mg)	132.9	160.3	182.9	214.7			
No. of events/person-years	129/7452	105/7840	90/7879	93/8080			
Age- and sex-adjusted incidence rate, per 1000 person-years	16.1	13.3	11.1	12.2			
Age- and sex-adjusted hazard ratio (95% CI)	1 (referent)	0.79 (0.61–1.03)	0.68 (0.52–0.90)	0.73 (0.56–0.96)	0.01	0.89 (0.80–0.98)	0.02
Multivariable-adjusted hazard ratio (95% CI)	1 (referent)	0.84 (0.64–1.12)	0.67 (0.49–0.92)	0.63 (0.44–0.90)	0.01	0.86 (0.75–0.99)	0.04

*Hazard ratio per 1-SD increase of log magnesium intake.
Multivariable adjustment was made for age, sex, family history of diabetes, BMI, HDL cholesterol, triglycerides, hypertension, smoking habits, alcohol intake and regular exercise, and intakes of total energy, carbohydrate, crude fibre, saturated fatty acid, polyunsaturated fatty acid and vitamin C.

in the fourth quartile. When estimating the hazard ratio for a 1-SD increment in log-transformed magnesium intake levels, a significant downward trend for the development of Type 2 diabetes was also found, even after multivariate adjustment (hazard ratio 0.86; 95% CI 0.75–0.99; P = 0.04).

We next estimated the multivariate-adjusted hazard ratios and 95% confidence intervals for the development of Type 2 diabetes by a 1-SD increment in log-transformed magnesium intake according to risk factor levels—namely, age, sex, obesity, HOMA-IR, hs-CRP, smoking habits, alcohol intake and regular exercise (Fig. 1; see also Supporting Information, Table S1). The influence of a 1-SD increment in log-transformed magnesium intake on the incidence of Type 2 diabetes was significantly different between subjects with and without insulin resistance, low-grade inflammation or alcohol intake (all $P < 0.05$); the association was stronger, particularly in subjects with these factors, although the interactions were no more significant after a Bonferroni adjustment.

Discussion

In a population-based prospective study, we demonstrated that higher intake of magnesium reduced the risk of Type 2 diabetes, and this association remained robust even after adjustment for confounding factors. Moreover, there were statistically significant interactions of magnesium intake with levels of HOMA-IR, hs-CRP and alcohol intake on the risk of Type 2 diabetes, and the association was stronger in individuals with insulin resistance, low-grade inflammation and a drinking habit. These findings provide valuable insight into the important role of magnesium in the pathogenesis of incident Type 2 diabetes.

In our study, the mean values of age, BMI and all dietary intakes, and the frequencies of men, smoking habits and regular exercise, were significantly different among the quartiles of magnesium intake. Nevertheless, the inclusion of these factors in the model did not attenuate the overall association between magnesium intake and incident Type 2 diabetes. Similar findings have been reported mainly in Western populations that have been shown to have higher insulin resistance than Asians [4,6,7]. However, in Asians,

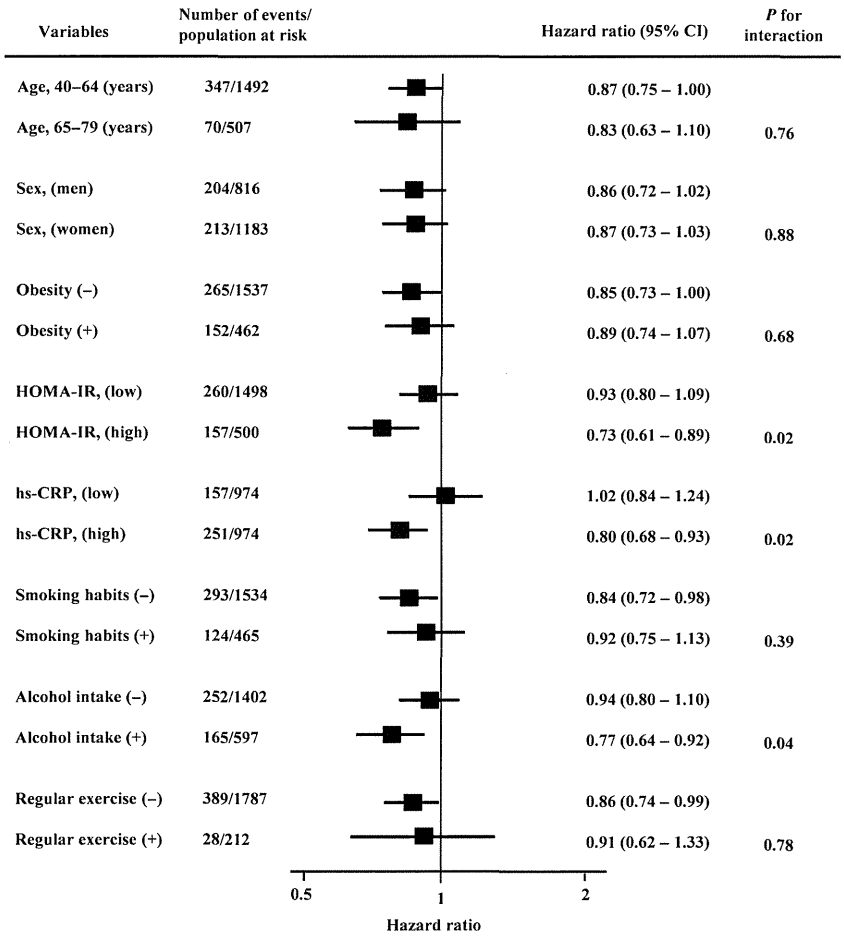

FIGURE 1 Multivariate-adjusted hazard ratios and 95% confidence intervals (CIs) for the development of Type 2 diabetes by a 1-SD increment in log-transformed magnesium intake according to risk factor levels. Obesity was defined as BMI ≥ 25 kg/m^2. Multivariate adjustment was made for age, sex, family history of diabetes, BMI, HDL cholesterol, triglycerides, hypertension, smoking habits, alcohol intake and regular exercise, and intakes of total energy, carbohydrate, crude fibre, saturated and polyunsaturated fatty acids and vitamin C. HOMA-IR (low), homeostasis model assessment of insulin resistance < 1.87; HOMA-IR (high), homeostasis model assessment of insulin resistance ≥ 1.87. hs-CRP (low), high-sensitivity C-reactive protein < 0.40 mg/l; hs-CRP (high), high-sensitivity C-reactive protein ≥ 0.40 mg/l.

who tend to have relatively lower BMI levels, the association between magnesium intake and development of Type 2 diabetes was discordant. Two prospective studies from China and Japan showed the inverse association between magnesium intake and Type 2 diabetes [8,9], but the association was not observed in another large Japanese cohort [10]. While the reason for the inconsistency remains uncertain, it is likely that the methods used for the dietary survey, the criteria used to define diabetes and the different population stratifications affected the association. Our findings suggest that higher intake of magnesium may be a protective factor for incident Type 2 diabetes in Japanese as well as Westerners.

To the best of our knowledge, this is the first prospective cohort study showing the effect modification by insulin resistance and low-grade inflammation on the association between magnesium intake and developing Type 2 diabetes. Although the precise reasons for this finding are not clear, the effect of magnesium on insulin resistance and its related states, such as low-grade inflammatory conditions, may help to explain it. Over 300 enzymes require the presence of magnesium ions for their catalytic action, including all enzymes utilizing or synthesizing adenosine triphosphate (ATP), or those that use other nucleotides to synthesize deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [1]. For example, intracellular magnesium deficiency may lead to decreasing tyrosine kinase activity of insulin receptors and to a post-receptorial impairment in insulin action [2,21], which may result in the development of insulin resistance [22]. The magnesium deficiency in cells may also decrease insulin secretion by interacting with cellular calcium homeostasis [23]. Supplementation of magnesium in a rat model prevented the deterioration of glucose tolerance and delayed the development of diabetes [24]. In addition, a beneficial effect of oral magnesium supplementation on insulin sensitivity was also reported among persons with insulin resistance [25] or Type 2 diabetes [26]. Moreover, the levels of serum magnesium and magnesium intake were inversely associated with inflammatory markers in human [7] and a magnesium-deficient diet in rats led to the occurrence of an inflammatory response [27]. There can be no doubt that magnesium has favourable effects on insulin resistance and low-grade inflammation; thus, the micronutrient appears to act more protectively against the development of Type 2 diabetes in subjects with higher insulin resistance or low-grade inflammation. This may be the reason why the protective association of dietary magnesium with incident Type 2 diabetes was stronger under such conditions.

In the present study, alcohol intake was found to modify the association between magnesium intake and the occurrence of Type 2 diabetes. Chronic consumption of ethanol results in a marked decrease in total tissue magnesium content in rats [28]. In humans, it is well known that hypomagnaesemia occurs in chronic alcoholism [29] and its underlying mechanism is assumed to be a blocking of the tubular reabsorption of magnesium excreted through the kidneys [30]. In addition, a clinical study showed that even moderate alcohol consumption could contribute to the decline of magnesium reabsorption at the tubular level [31]. Thus, it is reasonable to suppose that increased magnesium intake is more effective for prevention of Type 2 diabetes in habitual drinkers, not all of whom would be chronic alcoholics.

The strengths of the present study include its longitudinal population-based design, long-term follow-up period, large number of incident Type 2 diabetes cases, and high participation and follow-up rates, as well as the use of an oral glucose tolerance test for the diagnosis of Type 2 diabetes. However, the study also has several limitations. First, the validity of using the SFFQ to determine magnesium intake was not explored, because the first Standard Tables of Food Composition containing data for magnesium was published after the examination for validity, as was the case in another epidemiological study [5]. However, the SFFQ had already been evaluated and found to reasonably capture habitual diet intake at the time of the study [17]. Thus, we believe that the estimation of magnesium intake was reliable in our study. Second, our measurement of diet was based on a single SFFQ administered at baseline. During the follow-up, the levels of magnesium intake were changed because of lifestyle modification and misclassification of magnesium intake was possible. This could have weakened the association found in this study, biasing the results toward the null hypothesis. Thus, the true association may be stronger than that shown in our study. Third, we lacked detailed data on magnesium supplements and therefore could not discriminate between the effects of dietary and supplemental magnesium on the Type 2 diabetes risk. However, because magnesium supplementation is not widely used in Japan, it is unlikely that supplemental magnesium had a major influence on our findings.

In conclusion, the present analysis clearly showed that elevated dietary magnesium intake was a protective factor against the development of Type 2 diabetes in a general Japanese population. Furthermore, the effect of reducing the risk of Type 2 diabetes interacted with the presence of insulin resistance, low-grade inflammation and drinking habits, which raises the possibility that an intervention to increase magnesium intake will be more effective among subjects with such conditions. Our findings could contribute substantially to the development of an effective strategy for diabetes prevention; for instance, habitual drinkers could be encouraged to shift to a diet that includes magnesium-enriched foods. However, further studies in other ethnic groups will be needed to clarify the protective role of magnesium intake in the development of Type 2 diabetes.

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Competing interests

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Hazard ratios and their 95% confidence intervals for the development of Type 2 diabetes by quartiles of magnesium intake according to risk factor levels, 1988.

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Original Article

Down-regulation of MET in hippocampal neurons of Alzheimer's disease brains

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We found that mRNA of MET, the receptor of hepatocyte growth factor (HGF), is significantly decreased in the hippocampus of Alzheimer's disease (AD) patients. Therefore, we tried to determine the cellular component-dependent changes of MET expressions. In this study, we examined cellular distribution of MET in the cerebral neocortices and hippocampi of 12 AD and 11 normal controls without brain diseases. In normal brains, MET immunoreactivity was observed in the neuronal perikarya and a subpopulation of astrocytes mainly in the subpial layer and white matter. In AD brains, we found marked decline of MET in hippocampal pyramidal neurons and granule cells of dentate gyrus. The decline was more obvious in the pyramidal neurons of the hippocampi than that in the neocortical neurons. In addition, we found strong MET immunostaining in reactive astrocytes, including those near senile plaques. Given the neurotrophic effects of the HGF/MET pathway, this decline may adversely affect neuronal survival in AD cases. Because it has been reported that HGF is also up-regulated around senile plaques, β-amyloid deposition might be associated with astrocytosis through the HGF signaling pathway.

Key words: Alzheimer's disease, HGF, MET, neurotrophic factor, senile plaque.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that accounts for half or more of dementia cases. Since 1961, we have been conducting a long-term prospective cohort study of cerebro-cardiovascular diseases in the town of Hisayama, a suburb of Fukuoka in Japan. Careful surveillance of cognitive impairment was started in 1985. As a part of the Hisayama study, we have shown trends in the prevalence of AD and vascular dementia, and that both insulin resistance and dyslipidemia may be independent risk factors for plaque-type pathology.[1,2] Furthermore, we have extended our observations toward molecular pathological alterations in AD brains by microarray analyses of post mortem human brains donated for the Hisayama study.[3] The full-length study revealed that MET mRNA was most drastically declined in AD hippocampus.

MET is a receptor of hepatocyte growth factor (HGF), a multifunctional protein in hepatocytes. HGF/MET signaling induces glucose metabolism, cell proliferation, neuroprotection and neuroregeneration.[4–7] In the CNS in particular, HGF signaling works as a neuroprotective pathway in ischemic damage,[8–10] neuronal injury,[11] and sporadic and familial ALS with SOD1 gene mutation,[12] and as a recovery pathway from neuronal damage.[13,14] Recent studies revealed that HGF/MET signaling is involved in synaptic plasticity,[15] memory function[16] and neurogenesis in the adult mammalian brain.[17] In recent years, the interaction between diabetes and AD has received much attention.[18,19] AD is related to insulin resistance. In the liver, MET directly engages insulin receptors to form a MET-insulin receptor hybrid complex, culminating in a robust signal output.[20] However, the alteration of MET expression in AD brains is not well understood. In the present study, we performed immunohistochemical staining for MET in AD brains to determine characteristic changes in MET expression in AD brains, especially in the hippocampus.

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MATERIALS AND METHODS

Post mortem brain tissues

We examined 26 autopsy samples, including 12 AD (mean age: 90.3 ± 7.0 years; sex: male = 4, female = 8, mean post mortem interval: 19.1 ± 12.2 h) and 14 controls (74.4 ± 11.7 years; mean post mortem interval: 21.7 ± 16.0 h, sex: male = 7, female = 7) from Hisayama residents. In AD cases, 10 pure AD, one mixed AD and vascular dementia, one mixed AD and dementia with Lewy bodies were included. In all control cases, the concomitant AD-related changes were less than Consortium to Establish a Registry for Alzheimer Disease (CERAD) sparse or Braak stage III. The specimens in each case included middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, hippocampus with entorhinal cortex and transentorhinal cortex (at the level of the lateral geniculate body) and calcarine cortex. The study was approved by the Ethics Committee of the Faculty of Medicine, Kyushu University. Written informed consent was obtained from the families of all subjects. Neuropathologic changes in brain specimens were examined as previously described.[1] Sections were routinely stained using HE, KB stain, modified Bielschowsky silver impregnation and immunohistochemistry for phosphorylated tau protein. AD pathology was assessed according to the CERAD guidelines[21] and Braak staging guidelines.[22]

Immunohistochemistry

We used anti-MET rabbit monoclonal antibody raised against the N-terminal region of MET (EP1454Y; Abcam, Cambridge, UK) at a dilution of 1:250 as a primary antibody, and Envision1 System Labeled Polymer-HRP (horseradish peroxidase) anti-rabbit and anti-mouse (Dako Cytomation, Carpinteria, CA, USA) antibody as the secondary antibody. The specimens were deparaffinized in xylene and rehydrated in ethanol, then autoclaved in 0.01 mol/L citrate buffer, pH 8 to unmask the epitope. Endogenous peroxidase activity was blocked with methanol containing 0.3% H_2O_2. Specimens were incubated with the primary antibody at 4°C overnight. After rinsing, the specimens were incubated with the secondary antibody for 1 h at room temperature. Immunoreactivity was detected using 3, 3′-diaminobenzidine (DAB, Dojindo, Kumamoto, Japan) and specimens were lightly counterstained with hematoxylin.

Immunofluorescence

We performed double immunofluorescence labeling using the anti-MET rabbit monoclonal antibody and an anti-GFAP mouse monoclonal antibody (GA5; Sigma-Aldrich, St Louis, MO, USA). Fluorescein isothiocyanate-labeled anti-rabbit IgG (N1034; Amersham, BKM, Amersham, UK), Alexa488-labeled anti-mouse IgG (Invitrogen, Carlsbad, CA, USA), Alexa546-labeled anti-mouse IgG (Invitrogen), and Alexa546-labeled anti-rabbit IgG (Invitrogen) were used as secondary antibodies. Both primary and secondary antibodies were used at a dilution of 1:50. The specimens were deparaffinized and unmasked as previously described. Specimens were incubated with primary antibodies at 4°C overnight. After rinsing, the specimens were incubated with the appropriate secondary antibodies for 1 h at room temperature. The specimens were counterstained with 4′,6-diamidino-2-phenylindole (Invitrogen). We observed the specimens using a Nikon A1R-A1 Confocal Microscope System (Nikon, Tokyo, Japan).

RESULTS

Immunohistochemical findings for MET in control cases

In the normal brains, we found distinct perikaryal staining for MET in all pyramidal neurons of the hippocampi (Fig. 1). There was no difference in immunoreactivity between CA1 region and CA2 region, but CA4 was weakly stained. Subpopulations of astrocytes, especially those in the subpial layer and white matter, were also immunopositive for MET. In the dentate gyrus, we found MET immunoreactivity in the granule cells and long slender processes extending to the molecular layer, some of which were co-localized with GFAP (Fig. 2). Astrocytes in the granular layer, subgranular layer and CA4 were also MET immunopositive, but those in the molecular layer were negative. In neocortices, we also found similar perikaryal MET immunoreaction, mainly in the pyramidal neurons (Fig. 1).

Immunohistochemical findings for MET in AD cases

In AD brains, we observed marked decline of cytoplasmic MET immunoreactivity in hippocampal neurons compared with normal controls. This decline of neuronal immunoreactivity in pyramidal cells shows a similar tendency of the reduction of MET mRNA (Fig. 3).[3] The extent of the decline of MET immunoreactivity was similar in both CA1 and CA2 regions. The reduction of MET immunoreactivity was also observed in neocortical neurons; however, the reduction appeared milder than that of hippocampal neurons (Fig. 4). In contrast, we observed strong MET immunoreactivity in reactive astrocytes including those near senile plaques in AD

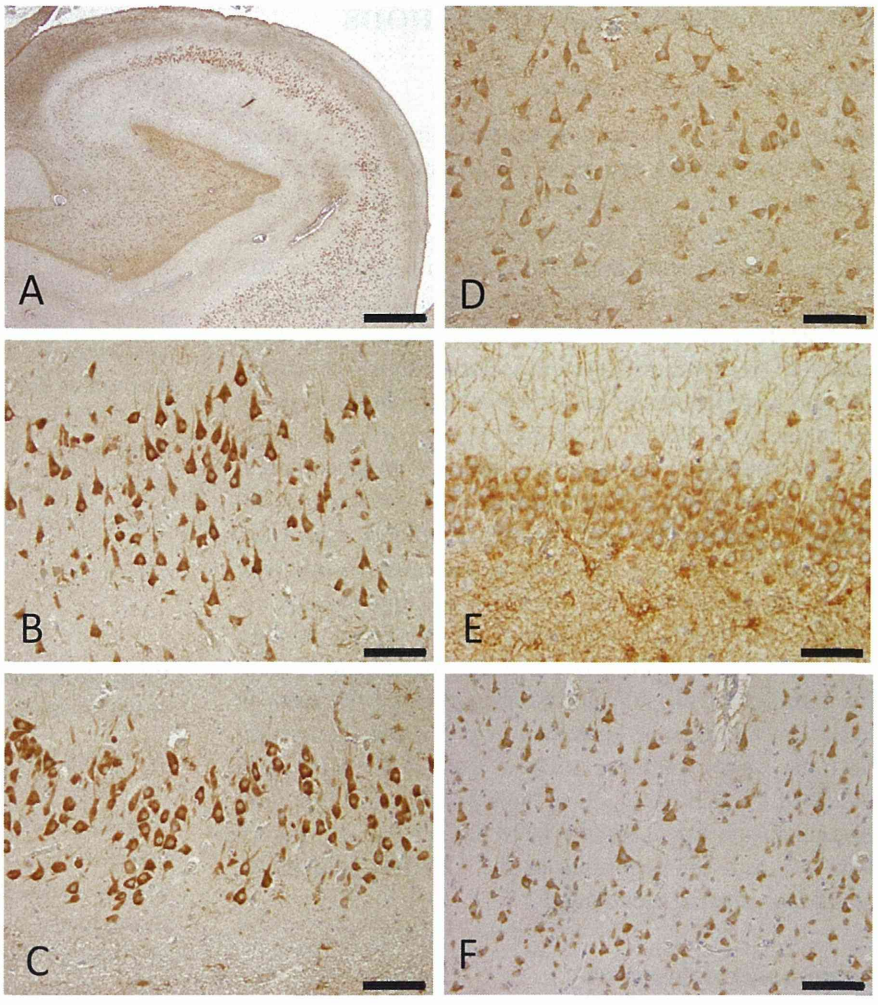

Fig. 1 Cellular distribution of MET immunoreactivity in the hippocampus of a normal control case. (A) Low-power view of the hippocampus shows MET immunoreactivity in the pyramidal layer, the dentate gyrus, and the alveus. Pyramidal neurons show strong MET immunoreactivity in the perikarya (B: cornu ammonis 1 (CA1) region, C: CA2 region, D: CA4 region and E: dentate gyrus, F: frontal cortex) Scale bar = 1 mm (A), 100 μm (B,C,D,F), 50 μm (E).

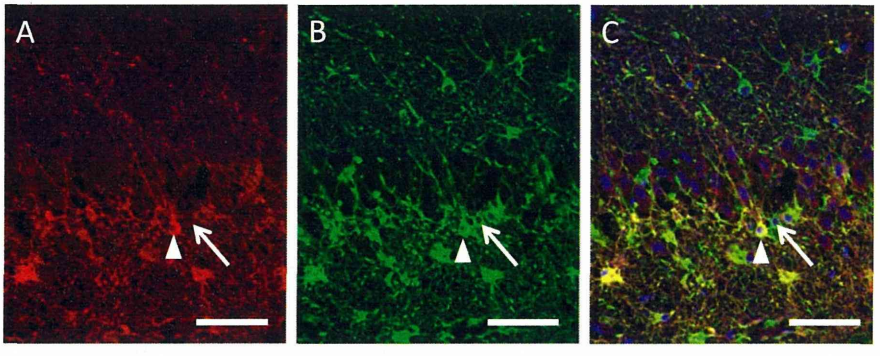

Fig. 2 Immunofluorescence for MET(A) and GFAP(B) in the dentate gyrus of a normal control case. (A) Both dentate granule cells and astrocytes show MET immunoreactivity. (B) Note the long slender cytoplasmic processes from the subgranular zone are also labeled by GFAP immunostaining. (C) Some GFAP positive cells co-express MET (arrowhead) while others do not (arrow). Scale bar = 50 μm.

brains (Figs 5,6). In the dentate gyrus, we found MET immunoreactivity both in granule cells and astrocytes was similar to control cases. The amyloid core, neurofibrillary tangles and Lewy bodies were immunonegative for MET.

DISCUSSION

Previous studies have reported that MET is expressed throughout the brain, preferentially in neurons in the CA1 area of the hippocampus.[23] Most of these neurons are MET positive, and HGF stimulates tyrosine phosphorylation of MET in the neurons.[24] Our study also shows that MET is mainly expressed in neurons and astrocytes of normal brains, while in AD brains neuronal MET is markedly declined, especially in the hippocampal pyramidal cells. The reduction in MET mRNA observed in our previous study may be ascribed to the down-regulation in hippocampal neurons.[3] HGF/MET signaling is an important

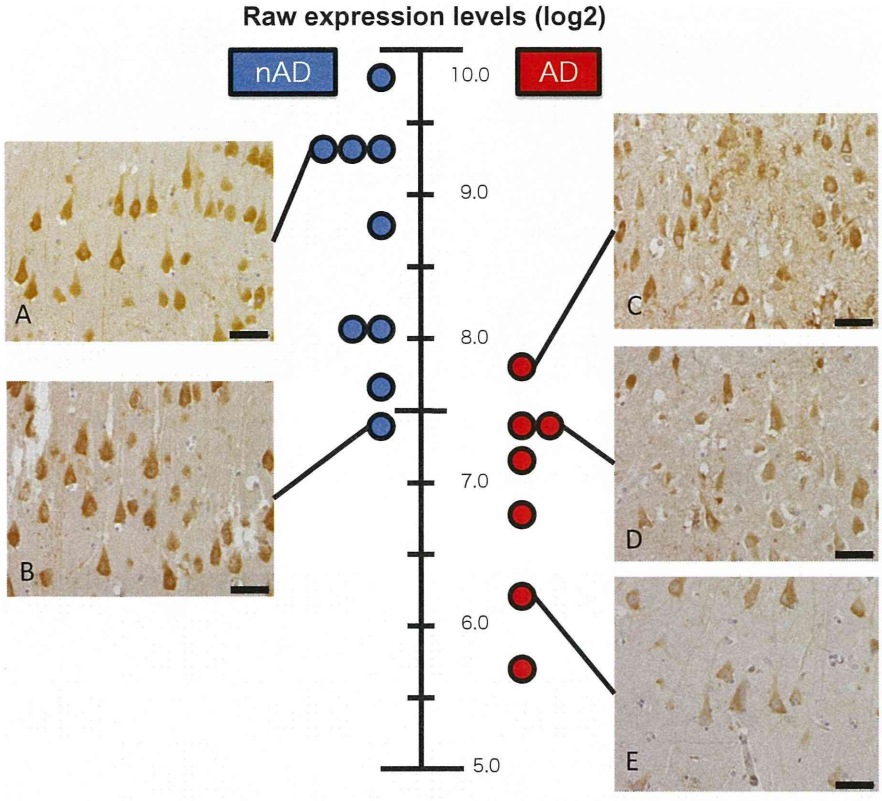

Fig. 3 Comparison of the raw expression levels of MET mRNA and the corresponding MET immunoreactivity in the CA1 region of the hippocampus of non-AD cases (A,B) and AD cases (C,D,E). The vertical bar in the center represents a logarithm of the amount of MET mRNA determined by microarray analysis.[3] Immunohistochemistry reveals decreased labeling in the affected pyramidal neurons of the AD cases (D,E). Scale bar = 50 μm.

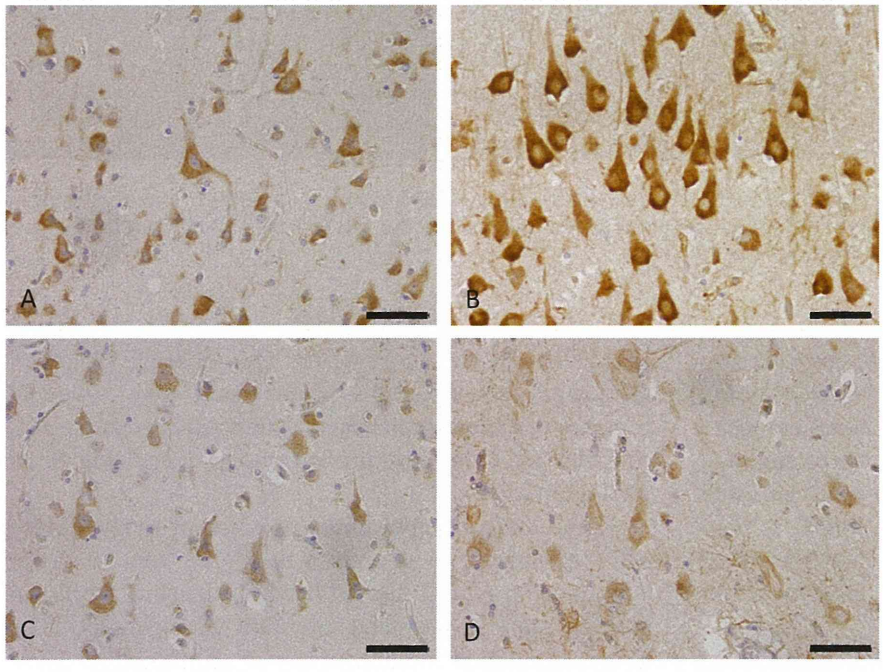

Fig. 4 Immunohistochemistry for MET in the frontal cortex (A,C) and the hippocampus (B,D). In the normal brain (A,B), MET is strongly expressed both in the neocortical neurons and in the pyramidal cells of the hippocampus. In the AD brain (C,D), reduced MET immunoreactivities of the neurons are apparent in both the frontal lobe and hippocampus. Scale bar = 50 μm.

neurotrophic factor in the brain that can help to protect from neuronal death. The decline of MET in pyramidal neurons leads to the dysfunctions of HGF/MET signaling. Our results suggest that dysfunction of HGF/MET signaling in AD cases may adversely affect neuronal survival.

HGF/MET signaling is associated with learning and memory function. According to experiments using a transgenic mouse model, overexpression of HGF in neurons resulted in enhanced memory function.[25] The hippocampus is well known as an important region for learning and

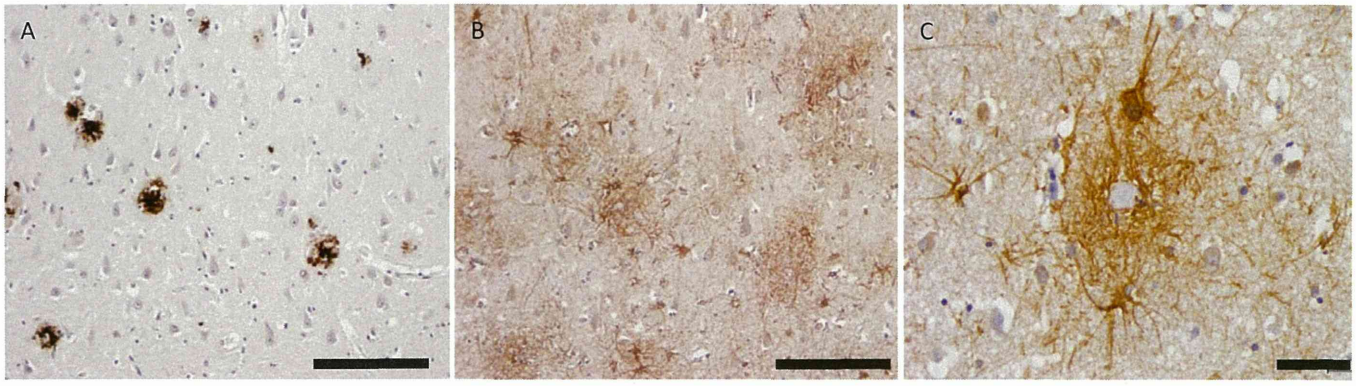

Fig. 5 Elevated MET immunoreactivities of reactive astrocytes near the senile plaques. Low-power views of consecutive specimens show similar patchy distributions of Aβ immunoreactivity (A) and MET immunoreactivity (B). (C) Higher magnification view of the MET immunoreactivity delineates fine processes surrounding the amyloid core as well as astrocyte cell bodies. Scale bar = 200 μm (A,B), 50 μm (C).

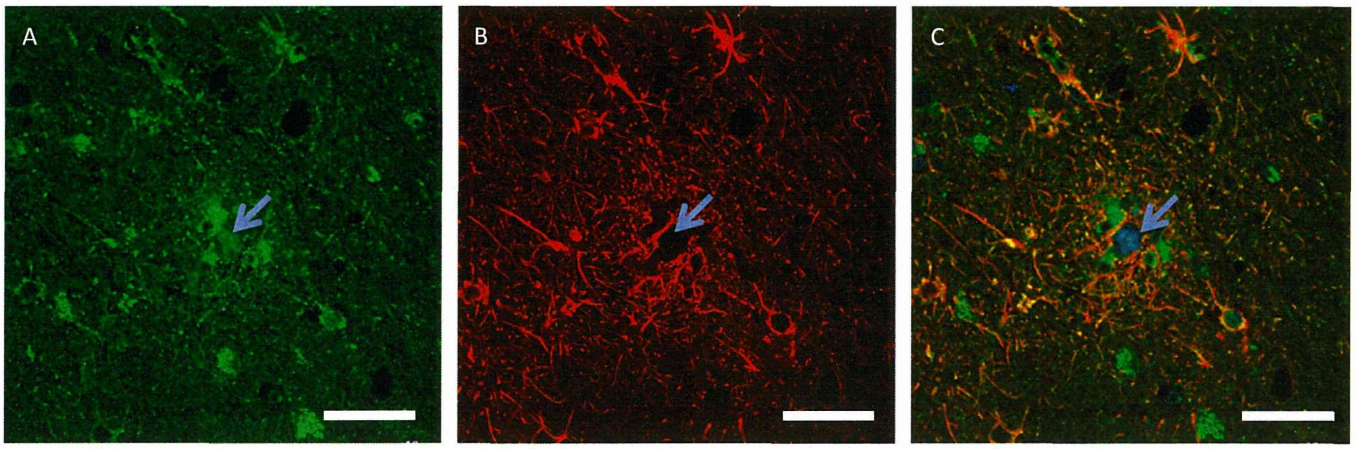

Fig. 6 Double immunofluorescence of a senile plaque for MET (A), GFAP (B) and merged image (C). (A) MET is up-regulated in reactive astrocytes around an amyloid core (arrow). (B) GFAP reactivity is also detected in the astrocytes. (C) MET and GFAP are colocalized in the astrocytes around an amyloid core. Scale bar = 20 μm.

memory, so the depletion of neuronal MET in the hippocampus may be implicated in cognitive impairment and dementia in AD. In the liver, MET is relevant to glycometabolism. MET makes a hybrid complex with insulin receptors to regulate metabolism by promoting hepatic glucose uptake and suppressing hepatic glucose output.[20] Although the function of brain MET is not completely understood, it is plausible that the loss of neuronal MET immunoreactivity may contribute to insulin resistance in AD brains.

We observed increased expression of MET in reactive astrocytes, notably near amyloid deposits. In the mammalian brains, HGF is present not only in hippocampal neurons, but also at high levels in ependymal cells, choroid plexus,[23] astrocytes,[26,27] microglia[28] and oligodendrocyte precursor cells.[29] Previous studies showed that HGF/MET signaling was stimulated in reactive astrocytes in the process of post-damaged regeneration in injury models, ischemic stroke and neuroinflammation.[30] Considering that HGF was also up-regulated around senile plaques,[31] astrocytes near the senile plaques may play a role in reparative defense mechanisms against Aβ deposition, possibly through both paracrine and autocrine signaling of the HGF pathway.

In the human hippocampus, we also observed diffuse MET expression in granule cells of the dentate gyrus. Wang *et al.* reported that HGF/MET signaling works on multiple steps in postnatal forebrain neurogenesis.[17] The hippocampus is known to be one of the neurogenic regions of the adult brain. In the hippocampus, neural stem cells localize in the subgranular zone (SGZ). Ming and Song reported that proliferating radial glia-like precursors and nonradial precursors give rise to intermediate progenitors, which in turn generate neuroblasts. The radial glia-like cell can be identified by GFAP immunostaining in the SGZ.[32] We observed MET- and GFAP-immunopositive SGZ cells

of a similar morphology. We thus suggest that MET is expressed in GFAP-positive radial glia-like cells, and plays a role in neurogenesis. Contrary to our expectation, there was little difference in MET immunopositivity in the dentate gyrus between normal brains and AD brains. Because many factors are involved in adult neurogenesis, further examination of factors related to neurogenesis will be needed.

ACKNOWLEDGMENTS

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総論

病理学から見た認知症の原因疾患と疫学

—久山町研究から—

本田裕之*　岩城　徹**

要　旨

日本人の認知症は65歳以上人口における約1割に達しており，顕著な増加傾向にある．久山町疫学研究では1985年から65歳以上の住民を対象に認知症調査を開始し，剖検による病理学的診断によって病型分類の精度を高めてきた．その結果，アルツハイマー病の増加が際立っていることが明らかとなった．耐糖能異常がアルツハイマー病の老人斑の形成および発症の危険因子となり，その増加傾向に関与している．

認知症有病率の時代的変化と久山町の認知症調査
—増加するアルツハイマー病の有病率—

厚生労働省の発表によると，2012年の認知症の人は305万人（65歳以上人口の9.9%）で，2025年には470万人（同12.8%）まで増えることが推定されている．認知症患者の増加を防ぐためには，基礎研究によって認知症の成因を解明するとともに，正確な疫学研究によって一般住民における認知症の実態を明らかにすることが重要である．久山町は福岡市に隣接する人口約8,400人の都市近郊型の町である．この町の年齢構成・職業構成は全国平均に近似しており，日本国内の現状をほぼそのまま反映していると考えられる．また人口の移動が少なく，長期間の調査が可能である．この町では1961年から心血管病をはじめとする生活習慣病の疫学調査が始まったが，1985年に認知症の調査が新たに開始された．1985年，1992年，1998年，2005年に，久山町の65歳以上の高齢者を対象に認知症の有病率調査を行った[1]．各調査の受診者はそれぞれ887人（受診率95%），1,189人（97%），1,437人（99%），1,567人（92%）であった．医師が面接してDSM-Ⅲ/DSM-ⅢRあるいは柄沢らの「老人ぼけの程度の臨床的判定基準」で認知症の有無，重症度，病型が判定され，認知症の発症率，危険因子，生命予後，およびその時代的変化などが検討されている．

その久山町における断面調査において，65歳以上の住民における認知症の性・年齢調整をした有病率が，1985年6.0%，1992年4.4%，1998年5.3%，2005年8.3%と近年有意に増加している[2]．またアルツハイマー病（AD）の性・年齢調整した有病率も，1985年1.1%，

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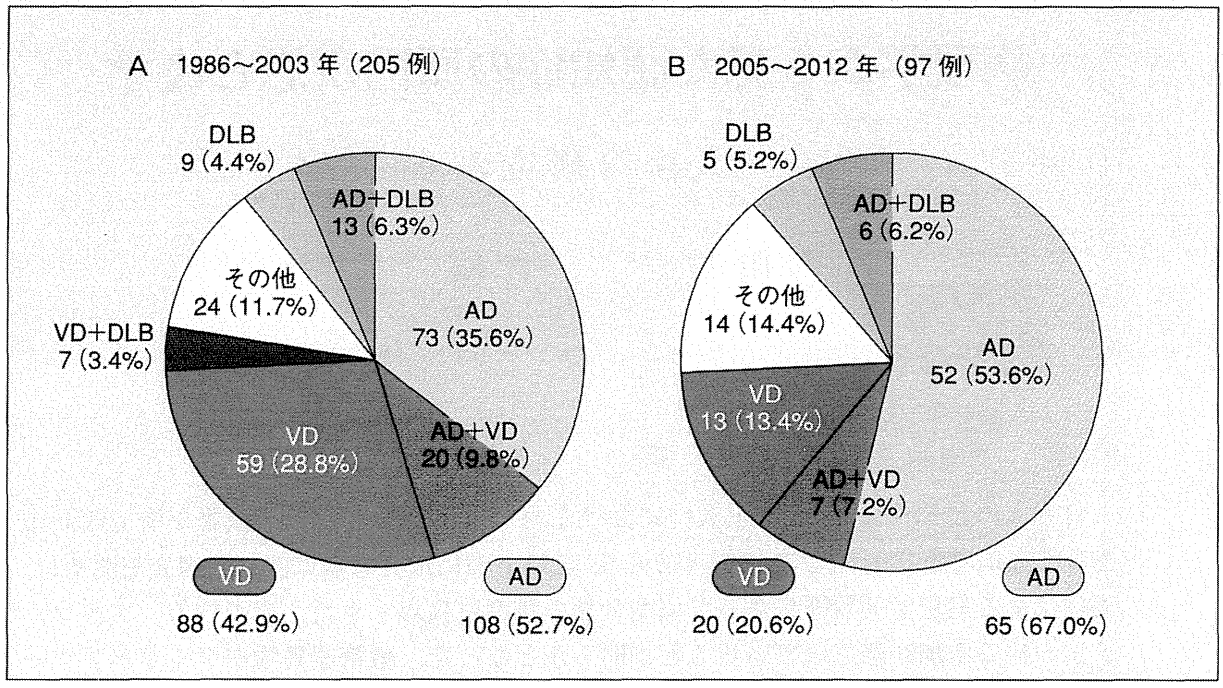
キーワード：病理疫学，認知症，アルツハイマー病，脳血管性認知症，生活習慣病

図1　認知症連続剖検例の病型別分類



A：1986～2003年（205例）：ADが一番多く，VDがこれに次ぐ．他の認知症を混合した場合もADが一番多い病型であった．

B：2005～2012年（97例）：ADが一番多く，VDがこれに次ぐ．Aとの比較においては，ADの増加が著明でありVDの減少も目立つ．

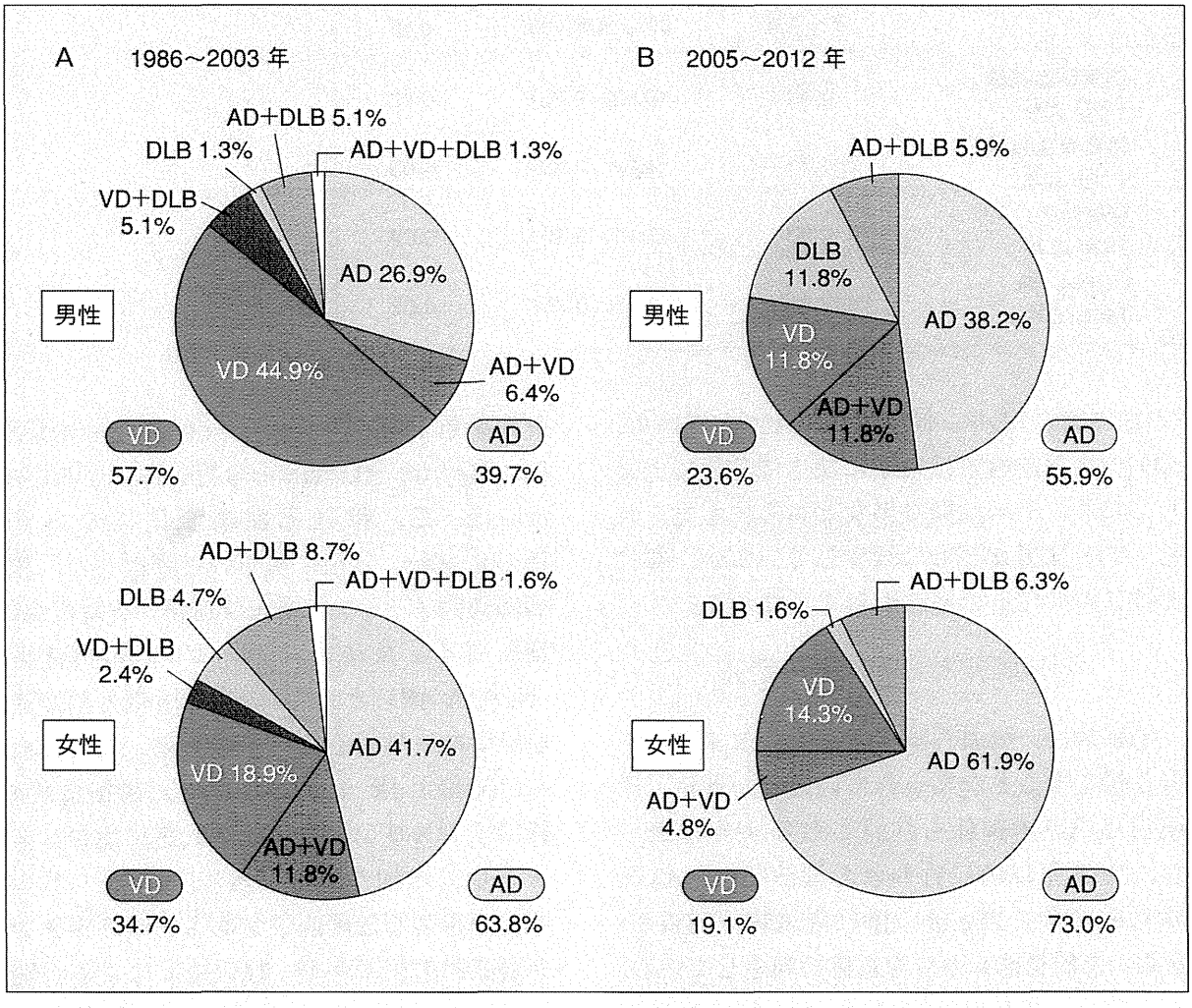
AD：アルツハイマー病，DLB：レビー小体型認知症，VD：脳血管性認知症

1992年1.3%，1998年2.3%，2005年3.8%と有意に増加している．ADを増加させている要因として，遺伝的素因以外に生活習慣病の危険因子が注目されている．一方，脳血管性認知症（VD）の性・年齢調整した有病率は，1985年2.3%，1992年1.5%，1998年1.5%といったんは減少傾向を示したが，2005年には2.5%と上昇傾向に転じた．この上昇傾向は主に80歳以上の高齢者において目立っており，また統計学的な有意差を示すには至っていなかったが，今後の推移が注目される．VD/ADの有病率比を見ると，1985年は2.1であったが，その後1992年1.2，1998年0.65，2005年0.3で，VD優位からAD優位に変化したことがうかがえる．

認知症の病理学的病型分類と動向

近年の認知症病型の動向をより詳細に検討するために，1986年から2003年までの認知症連続剖検205例と，2005年から2012年までの認知症連続剖検97例の病理診断を比較検討した（図1）．1986年から2003年においては，ADが73例（35.6%）で最も多く，次にVD 59例（28.8%）であった．以下，レビー小体型認知症（DLB）が3番目に多く，次にその他の中に含まれる神経原線維変化型認知症と続いた．ADとVDに関しては，それぞれが他の認知症を混合した病理診断を検討しても，ADが52.7%でありVDの42.9%を上回った．2005年から2012年においても，ADは52例（53.6%）であり最も多かった．2003年までと比較すると，ADは35.5%から53.6%と顕著な増加を示していた．一方でVDは13.4%であり，2003年までの28.8%と比較すると大幅に減少していた．ADの上昇，またVDの減少は，それぞれ他の認知症を混合し

図2 認知症病型別分類の性差別



A：1986～2003年：男性ではVDが最も多く，ADがこれに次ぐ．女性においてはADが最も多く，VDが2番目に多い．

B：2005～2012年：男性ではVDが著明に減少し，一方でADが増加しVDを上回っている．女性ではADはさらに増加し，VDは減少している．ADとVDの推移に注目するために，その他の認知症を除外しグラフを作成している．

AD：アルツハイマー病，DLB：レビー小体型認知症，VD：脳血管性認知症

た病理診断においても同様の傾向であった．前述したように，臨床的なADの有病率は1998年の時点ですでに有意な上昇傾向を示していた[2]．2005年から2012年までの近年の剖検診断による検討でADの病理診断率がさらに高くなっており，臨床的に指摘されていたADの有病率の上昇傾向は持続している可能性が高い．

性差別の病理診断の検討も行った（図2）．1986年から2003年において，男性ではVDの病理診断率が44.9%と最も高かった．次いでADの26.9%であった．一方女性においてはADが41.7%と最も高く，次いでVDは18.9%であった．2005年から2012年においては，男性ではADが38.2%と上昇し，VDの11.8%を上回り最も多くを占めていた．VDは2003年までの44.9%から著明に低下していた．女性においてはADが61.9%で最も多く，2003年までの41.7%からさらに上昇していた．VDに関しては2012年までにおい

表1　血糖値，空腹時インスリン値およびHOMA-IRと老人斑との関連

	オッズ比	95%信頼区間	p値
空腹時血糖値 1 SD上昇	1.41	(0.88，2.26)	0.15
糖負荷後血糖値 1 SD上昇	1.71	(1.04，2.80)	0.03
空腹時インスリン 1 log上昇	2.03	(1.11，3.70)	0.02
HOMA-IR 1 log上昇	2.11	(1.18，3.79)	0.01

ては14.3%であり，軽度の低下を示していた．ADの病理診断率は近年になって急速に上昇しており，その傾向は男女どちらにも見られた．VDは男女ともに減少しているが，特に男性においてその減少が目立っていた．

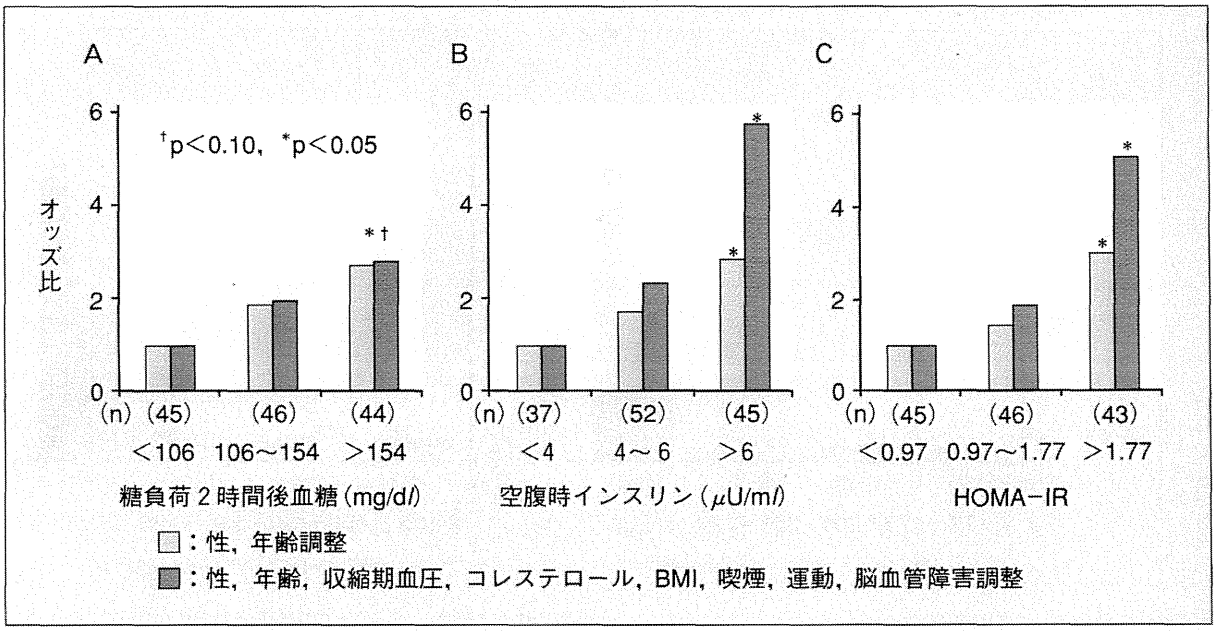
耐糖能異常と認知症の関係

糖尿病は，脳血管障害を進展させてVDを引き起こすことが知られているが，近年，糖尿病とADの関係も注目されるようになった．1998年以降に行われた健診では，40歳以上を対象に75g経口糖負荷試験（OGTT）を用いて耐糖能レベルを正確に判定している．その結果，糖尿病の頻度は1988年では男性15.0%，女性9.9%であったが，2002年ではそれぞれ23.6%，13.4%に増加していた．糖尿病にimpaired fasting glycemia（IFG）およびimpaired glucose tolerance（IGT）の頻度を併せると，地域住民の中高年では男性の約6割，女性の約4割が何らかの耐糖能異常を有すると考えられる[3]．糖尿病とADがともに急速に増加していることに注目し，連続剖検脳データベースを用いて老人斑などの病理変化と耐糖能異常との関連について検討した[4]．

1998年から2003年の約5年間に死亡した住民290人のうち，連続剖検は211例であり（解剖率73%），そのうち1988年に75gOGTTを施行した135人を対象とした．老人斑は平野銀染色を用いたConsortium to Establish a Registry for Alzheimer's Disease（CERAD）criteriaで，神経原線維変化についてはBraak stage分類に基づいて評価した．糖尿病関連因子として，空腹時血糖，負荷後血糖，空腹時インスリン，インスリン抵抗性指標（HOMA-IR）とADの病理所見との関連を統計学的に解析した．その結果，負荷後血糖やHOMA-IRの上昇といった耐糖能異常，特にインスリン抵抗性が老人斑出現のリスクを有意に増加させた（表1，図3）．さらにADの重要な危険因子であるアポリポタンパク質Eの遺伝子多型*APOEε4*による層別解析の結果，*APOEε4*と負荷後血糖が高いなど，インスリン抵抗性を示すグループでは老人斑出現のオッズ比が相乗的に高くなることを見いだした．ホノルルアジア高齢者研究では，216人の糖尿病症例の剖検所見にて，*APOEε4*を有すると海馬領域に老人斑や神経原線維変化が有意に多くなることを報告している[5]．久山町病理疫学研究では，老人斑の蓄積に空腹時血糖上昇よりインスリン抵抗性が関与している点に新規性がある．

さらにAD発症について，糖負荷後2時間値との関連を60歳以上の非認知症住民1,017人を15年間追跡調査した結果，120mg/dl未満のグループに比較して200mg/dl以上のグループではハザード比が3倍以上（臨床診断群で3.42，剖検診断群で3.88．性・年齢調

図3 老人斑の有無に対するオッズ比（文献[4]より改変引用）



老人斑の有無はCERADにおけるnoneをなし，sparse以上をありと定義し，各糖尿病関連因子の値を数値順に並べて，nの数が均等になるように3群に分けた．値が最も低い群を1として，老人斑のリスクについてロジスティック解析によりオッズ比を算出した．

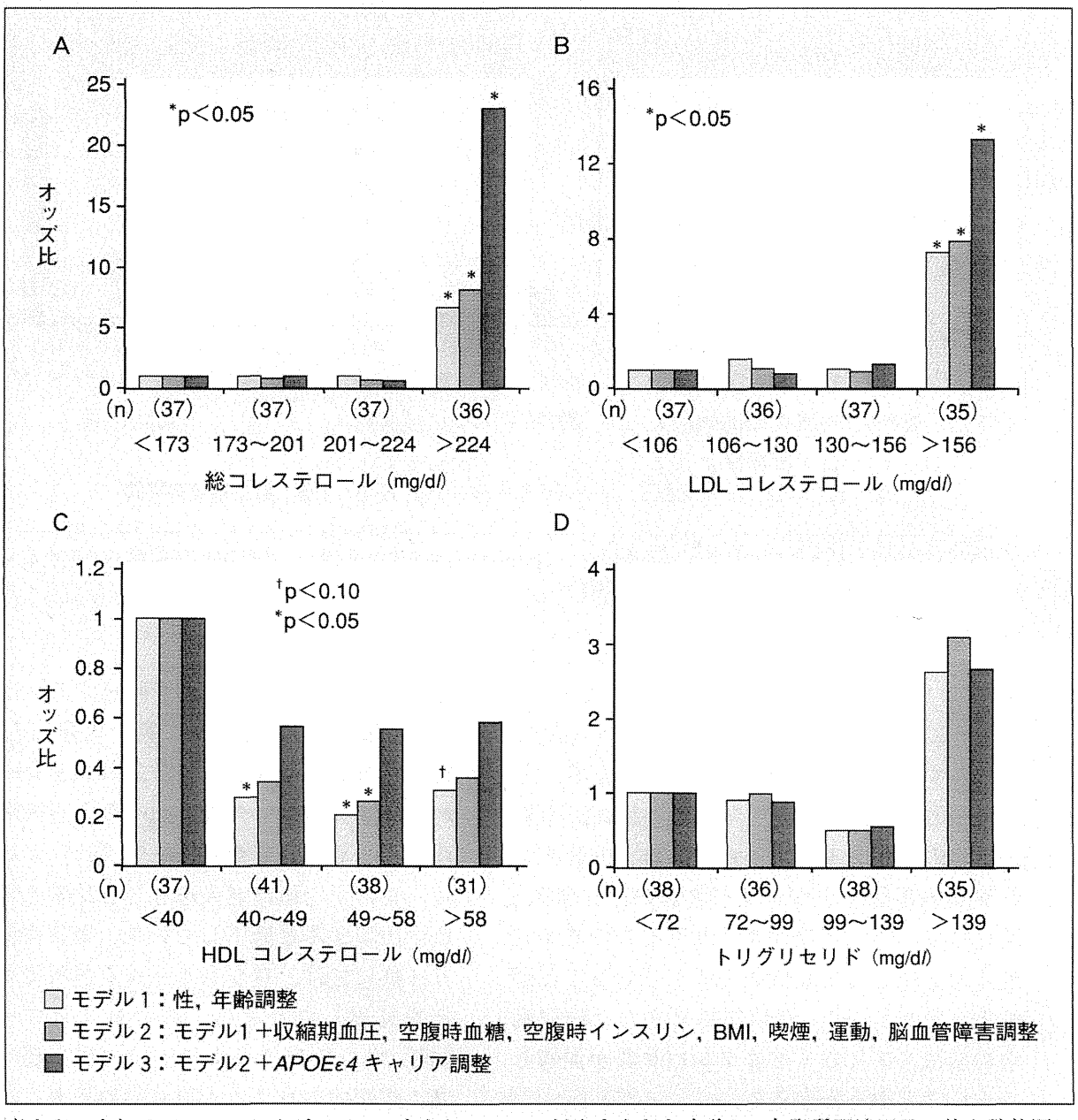
整），有意に上昇することが確認された[6]．このように耐糖能異常または糖尿病は，VDのみならずADの重要な危険因子と言える．その機序として，脳でのインスリンおよびIGFの細胞内シグナルの低下がアミロイドβタンパク質の蓄積，タウタンパク質のリン酸化，酸化ストレス，炎症性サイトカインの産生亢進などを介して認知機能を低下させることが報告されている[7]．耐糖能異常または糖尿病者の脳は，アミロイドβタンパク質が沈着しやすいことがうかがえる．

脂質代謝異常症との関連

これまでコレステロールとADとの関連について，多くの疫学研究が報告されているが，その結果はさまざまで一定の見解が得られていない．さらに，コレステロールとADとの病理所見の関連について報告した研究はほとんどない．そこで耐糖能異常との関連を調べた同様の手法を用いて，脂質代謝異常との関連について統計学的解析を行った．中性脂肪に対する食事の影響を除外するため，空腹時採血を受診した147例を対象とした[8]．総コレステロールとLDLコレステロール平均値は老人斑なしの群に比べると老人斑が出現すると上昇しており，一方HDLコレステロールの平均値は老人斑なしと比較して老人斑が出現すると低下していた．脂質の値により4群に分け，ロジスティック解析でオッズ比を算出したところ，総コレステロールとLDLコレステロールで一定の値を超えると老人斑形成のリスクが有意に高まり，この関連は*APOEε4*アレルとは独立していた（図4）．一方，脂質代謝関連遺伝子と神経原線維変化との関連については有意な関連は認めなかった．

脳内のコレステロール代謝にかかわるアポリポタンパク質Eは老人斑に沈着するとともに，その遺伝子多型がアルツハイマー病の強力な危険因子であることが知られている．しかも血液中と脳内のコレステロール代謝は血液脳関門により隔絶されていて，脳内のコレ

図4　脂質関連因子の4分位ごとの老人斑に対するオッズ比



老人斑の有無はCERADにおけるnoneをなし，sparse以上をありと定義し，各脂質関連因子の値を数値順に並べて，nの数が均等になるように4群に分けた．値が最も低い群を1として，老人斑のリスクについてロジスティック解析によりオッズ比を算出した．

A：総コレステロールでは，値の最も低い群と比べると最も高い群でオッズ比は急上昇し，多変量解析後も有意な差を認めている．

B：LDL コレステロールも同様の傾向が見られる．

C：HDL コレステロールでは，値が40mg/dl以上に上昇した群でオッズ比が低下したが，多変量解析後は有意差がなくなり，*APOEε4* の有無が影響していると考えられる．

D：トリグリセリドでもオッズ比が上昇しているが，有意差は認めていない．

ステロールは血清コレステロールとは別の代謝・輸送系を有している．今後は脂質代謝異常症が脳内の脂質代謝に及ぼす影響を併せて検討することが必要である．

高血圧との関連

高血圧と認知症との関連について，血圧レベルとAD発症率には明らかな関連は認められなかった．一方，VD発症率は正常血圧レベルに比べて軽症高血圧レベル（140～159/90～99mmHg）から有意に高く，中年期高血圧がVDの有意な危険因子であった[9)10)]．日本人に多いVDの予防には，中年期からの適正な高血圧管理が重要と考えられる．

生活習慣病関連因子である耐糖能異常と脂質代謝異常の両方が，老人斑の形成を通してAD病態に関与している可能性を示した．生活習慣病の予防および改善といった介入が，ADの発症リスクを実際にどの程度軽減できるかを疫学研究で明らかにすることが重要な目標となる．

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Epidemiological Pathology of Dementia in a Japanese Community: The Hisayama Study

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