Insulin resistance is associated with the pathology of Alzheimer disease

The Hisayama Study

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ABSTRACT

Objective: We examined the association between diabetes-related factors and pathology of Alzheimer disease (AD) to evaluate how diabetes affects the pathogenic process of AD.

Methods: This study included specimens from a series of 135 autopsies of residents of the town of Hisayama in Fukuoka prefecture (74 men and 61 women) performed between 1998 and 2003, who underwent a 75-g oral glucose tolerance test in clinical examinations in 1988. We measured diabetes-related factors including fasting glucose, 2-hour post-load plasma glucose, fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) in 1988. Neuritic plaques (NPs) were assessed according to the Consortium to Establish a Registry for Alzheimer's Disease guidelines and neurofibrillary tangles (NFTs) were assessed according to Braak stage. The associations between each factor and AD pathology were examined by analysis of covariance and logistic regression analyses.

Results: Higher levels of 2-hour post-load plasma glucose, fasting insulin, and HOMA-IR were associated with increased risk for NPs after adjustment for age, sex, systolic blood pressure, total cholesterol, body mass index, habitual smoking, regular exercise, and cerebrovascular disease. However, there were no relationships between diabetes-related factors and NFTs. Regarding the effects of *APOE* genotype on the risk of AD pathology, the coexistence of hyperglycemia and *APOE* ϵ4 increased the risk for NP formation. A similar enhancement was observed for hyperinsulinemia and high HOMA-IR.

Conclusion: The results of this study suggest that hyperinsulinemia and hyperglycemia caused by insulin resistance accelerate NP formation in combination with the effects of *APOE* ϵ4.

Neurology® 2010;75:764–770

GLOSSARY

AD = Alzheimer disease; **BMI** = body mass index; **CERAD** = Consortium to Establish a Registry for Alzheimer's Disease; **CI** = confidence interval; **FPG** = fasting plasma glucose; **GSK3** = glycogen synthase kinase 3; **HOMA-IR** = homeostasis model assessment of insulin resistance; **IDE** = insulin-degrading enzyme; **NFT** = neurofibrillary tangle; **NP** = neuritic plaque; **OGTT** = oral glucose tolerance test; **OR** = odds ratio; **PG** = post-load plasma glucose.

The prevalence of diabetes is growing at epidemic proportions worldwide, and is becoming a major health problem. Several large longitudinal population-based studies have shown that the rate of cognitive decline is accelerated in elderly people with type 2 diabetes compared with the general population.[1-3] Similarly, other epidemiologic studies have revealed that diabetes increases the risk of dementia,[2,4-7] including Alzheimer disease (AD), which is the most common cause of dementia in late life.[2,4,5,8,9] Therefore, the effect of diabetes on cognitive function in the elderly has significant public health implications.

Several lines of evidence indicate a role of insulin and glucose metabolism on the risk of developing dementia, including AD.[10-14] Many mechanisms through which diabetes could increase the risk of dementia have been postulated, and include glucose toxicity, insulin resis-

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e-Pub ahead of print on August 25, 2010, at www.neurology.org.

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Study funding: Supported by a Grant-in-Aid for Scientists from the Ministry of Health, Labour and Welfare, Japan (H19-nanchi-ippan-006), a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare (Comprehensive Research on Aging and Health: H20-Choujuu-004), and a Grant-in-Aid for Scientists from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 19300125).

Disclosure: Author disclosures are provided at the end of the article.

tance, oxidative stress, advanced glycation end products, inflammatory cytokines, and microvascular and macrovascular disease.[15] However, the determinant pathway, which is more critical to AD pathogenesis, is less clear. Understanding the role of disease-related risk factors for AD pathogenesis may help to identify specific modifiable risk factors that could enable the prevention of AD.[16] Therefore, identifying the dominant pathway through which diabetes influences the pathogenic process of AD may have benefits for public health.

To clarify the relationship between diabetes and AD, we searched for evidence of AD-related pathologic risk by examining the associations between diabetes-related factors and typical AD-related pathologic outcomes, neuritic plaques (NPs) and neurofibrillary tangles (NFTs).

METHODS

Subjects. Since 1961, we have been conducting a long-term prospective cohort study of cerebro-cardiovascular diseases in the town of Hisayama, a suburb of the city of Fukuoka in southern Japan. The design of the Hisayama Study has been described in detail elsewhere.[17-19] 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.[19] Briefly, of a total of 3,390 residents aged over 40 years included in this registry, 2,742 (participation rate, 80.9%) took part in a clinical examination in 1988. Of these, a 75-g oral glucose tolerance test (OGTT) was performed in 2,520 subjects. Of the 214 autopsy cases, we excluded 3 subjects whose brain specimens were inadequate for evaluation, and 76 subjects who did not complete the OGTT in 1988. Finally, 135 subjects who underwent both the OGTT and brain autopsy were included in the present study. None of the 135 subjects showed signs of dementia at the clinical examination in 1988. Careful surveillance of cognitive impairment was carried out through a daily monitoring system established by the study team, local practitioners, and the town government.[9,18]

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, the 75-g OGTT was performed after at least a 12-hour overnight fast and the following 3 diabetes-related factors were determined: fasting plasma glucose (FPG), 2-hour post-load plasma glucose (2-hour PG), and fasting insulin. Glucose was determined by the glucose oxidase method and fasting insulin was determined by a radioimmunoassay. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: FPG (mmol/L) × fasting insulin (μU/mL)/22.48155.[20] Blood pressure was measured 3 times at the right upper arm using a mercury sphygmomanometer after at least 5 minutes of rest in a sitting position; the mean of the 3 measurements was used in the analysis. Total cholesterol levels were determined enzymatically. Height and weight were measured in light clothes without shoes, and body mass index (BMI; weight/height squared, kg/m^2) was calculated. Information on exercise and smoking habits was obtained via a standard questionnaire, and these factors were classified as being habitual or not. Regular exercise means engaging in sports or other forms of exertion regularly more than 3 times per week during leisure time. *APOE* genotyping was determined by direct sequencing at Takara Bio Inc., Japan. No homozygous ϵ4 genotype was found among these participants, and those who carried 1 copy of the ϵ4 allele were categorized as *APOE* ϵ4 carriers.

Assessment of neuropathologic changes. The assessment of AD pathology was conducted according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) guidelines and Braak stage established by Braak and Braak.[21-23] Brains were fixed in 10% buffered formalin for at least 2 weeks. Brain specimens in each case included the middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, anterior cingulated gyrus, amygdala, hippocampus with entorhinal and transentorhinal cortex (at the level of the lateral geniculate body), calcarine cortex, basal ganglia including the nucleus basalis of Meynert, thalamus, substantia nigra, locus ceruleus, and dorsal vagal nucleus. Sections were embedded in paraffin and were routinely stained using hematoxylin-eosin, Klüver-Barrera, and a modified Bielschowsky method. Specimens from each subject were immunostained with antibodies against phosphorylated tau (AT8, mouse monoclonal, 1:500; Innogenetics, Belgium). Immunolabeling was detected using a standard indirect immunoperoxidase method and visualized using diaminobenzidine (Dojindo, Japan) as a chromogen. The frequency of NPs defined by the CERAD criteria were semiquantitatively categorized into the following 4 groups: none (score 0), sparse (score 1), moderate (score 2), and frequent (score 3). The extent of NFTs according to Braak stage was semiquantitatively classified into the following 4 groups: stage 0, stage I to II, stage III to IV, and stage V to VI. For the pathologic assessment of cerebrovascular diseases, any types of cerebral infarctions and hemorrhages were registered according to gross examination and microscopic assessment, regardless of clinical features. This factor was classified as being present or not.

Statistical analyses. Statistical analyses were conducted using SAS software version 9 (SAS Institute, Cary, NC). Mean or geometric mean values of the diabetes-related factors among the groups of NPs or NFTs were calculated and compared by analysis of covariance, with adjustment for age at clinical examination and sex. We used logistic regression analysis to determine relationships between the risk factors (diabetes-related factors, *APOE* genotype, and their interaction) and pathologic outcome (presence or absence of NPs and NFTs) and are expressed as odds ratios (OR) and 95% confidence intervals (CI). Continuous variables (FPG, fasting insulin, and HOMA-IR) were divided into 3 groups to compare the risk of NPs among tertiles. Missing values (1 for fasting insulin, 1 for HOMA-IR, 6 for *APOE* ϵ4 carrier, and 1 for the grading of Braak stage) were excluded from the analysis. Age at clinical examination was used for adjustment in the present study; adjustment for age at death resulted in equivalent statistical outcomes. Significance was de-

Table 1 Demographic characteristics of the study subjects (n = 135)[a]

Variables	Values
Male sex	54.8
Age at medical examination, y	67.0 ± 9.5
Fasting plasma glucose, mmol/L	5.9 ± 1.2
2-hour post-load plasma glucose, mmol/L	8.3 ± 4.3
Fasting insulin, μU/mL[b,c]	5.2 (2.0–13.6)
HOMA-IR[b,c]	1.3 (0.5–4.0)
Systolic blood pressure, mm Hg	138.7 ± 23.6
Diastolic blood pressure, mm Hg	76.5 ± 12.1
Serum total cholesterol, mmol/L	5.2 ± 1.1
BMI, kg/m^2	22.0 ± 3.2
Current smoking	32.6
Regular exercise[d]	11.1
APOE ε4 carrier[c]	19.4

Abbreviations: BMI = body mass index; HOMA-IR = homeostasis model assessment of insulin resistance.
[a] Values are means ± SD or percentage.
[b] Geometric means and 95% prediction intervals are shown for fasting insulin and HOMA-IR due to their skewed distributions.
[c] Missing values: 1 for fasting insulin, 1 for HOMA-IR, and 6 for *APOE* ε4 carrier.
[d] Engaging in sports or other forms of exertion regularly more than 3 times per week during leisure time.

fined as $p < 0.05$, and marginal significance was defined as $0.05 \leq p < 0.10$ in statistical analysis.

RESULTS The characteristics of the study subjects at clinical examination in 1988 (n = 135) are described in table 1. Mean ± SD age at clinical examination was 67.0 ± 9.5 and mean ± SD age at death was 79.5 ± 9.3 years, and 54.8% (n = 74) of the subjects were male. Overall, 19.4% (n = 25) of subjects were carrying *APOE* ε4. There was no 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). Out of the 135 subjects, 15.6% (n = 21) developed Alzheimer-type dementia. Based on the assessment of AD pathology, the frequencies of NPs were categorized into the following 4 groups by CERAD criteria: 34.8% (n = 47) for none (score 0), 17.0% (n = 23) for sparse (score 1), 14.1% (n = 19) for moderate (score 2), and 34.1% (n = 46) for frequent (score 3). The frequencies of NFTs were classified into the following 4 groups by Braak stage: 14.2% (n = 19) for stage 0, 18.7% (n = 25) for stage I to II, 44.0% (n = 59) for stage III to IV, and 23.1% (n = 31) for stage V to VI. Prevalence of cerebrovascular disease at autopsy was 59.3% (n = 80), which included any types of infarctions (n = 73) and hemorrhages (n = 10).

As shown in table 2, we compared the age- and sex-adjusted mean (or geometric mean) values of diabetes-related factors among groups according to CERAD score for NPs or Braak stage for NFTs. The subjects with NPs (CERAD score 1 to 3) showed significantly higher levels of 2-hour PG, fasting insulin, and HOMA-IR than those without NPs (CERAD score 0). However, there was no obvious dose-response relationship between these variables and CERAD score. The FPG levels remained broadly constant irrespective of CERAD score. Regarding the frequencies of NFTs, we found no relationship between any diabetes-related factor and Braak stage.

As shown in table 3, we estimated the effect of each diabetes-related factor on the presence of AD pathology using logistic regression analysis. As for NPs, elevated 2-hour PG significantly increased the risk of NPs in the age- and sex-adjusted analysis (model 1). Similarly, hyperinsulinemia and high HOMA-IR were also significant positive risk factors

Table 2 Age- and sex-adjusted means of glucose, insulin, and HOMA-IR according to CERAD score and Braak stage[a]

	Frequency of NPs (CERAD score)				*p* Value (CERAD score 1–3 vs 0)	Frequency of NFTs (Braak stage)				*p* Value (Braak stage I–IV vs 0)
	0	1	2	3		0	I, II	III, IV	V, VI	
Fasting plasma glucose, mmol/L	5.7	6.0	6.2	5.9	0.22	5.7	6.1	5.8	6.0	0.38
2-hour post-load plasma glucose, mmol/L	7.2	9.0[c]	9.6[b]	8.7	0.03	7.0	9.2[c]	8.4	8.5	0.13
Fasting insulin, μU/mL	4.6	6.1[b]	5.2	5.6[c]	0.03	5.1	5.0	5.2	5.7	0.81
HOMA-IR	1.2	1.6[b]	1.4	1.4[c]	0.02	1.3	1.4	1.3	1.5	0.62

Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; HOMA-IR = homeostasis model assessment of insulin resistance; NP = neuritic plaque.
[a] Geometric means for fasting insulin and HOMA-IR are shown due to their skewed distributions.
[b] $p < 0.05$, [c] $p < 0.10$ vs CERAD score = 0 or Braak stage = 0.

Table 3 Odds ratios and 95% confidence intervals for the presence vs absence of neuritic plaques and neurofibrillary tangles[a]

	OR for presence of NPs (CERAD score 1–3 vs 0)				OR for presence of NFTs (Braak stage I–VI vs 0)			
	Model 1		Model 2		Model 1		Model 2	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Fasting plasma glucose, mmol/L	1.33 (0.86–2.04)	0.20	1.41 (0.88–2.26)	0.15	1.31 (0.72–2.37)	0.38	1.35 (0.74–2.47)	0.33
2-hour post-load plasma glucose, mmol/L	1.66 (1.04–2.63)	0.03	1.71 (1.04–2.80)	0.03	1.58 (0.85–2.93)	0.15	1.67 (0.88–3.17)	0.12
Fasting insulin, μU/mL	1.61 (1.04–2.48)	0.03	2.03 (1.11–3.70)	0.02	1.05 (0.62–1.79)	0.85	1.06 (0.55–2.04)	0.86
HOMA-IR	1.67 (1.08–2.59)	0.02	2.11 (1.18–3.79)	0.01	1.14 (0.66–1.98)	0.64	1.19 (0.62–2.30)	0.60

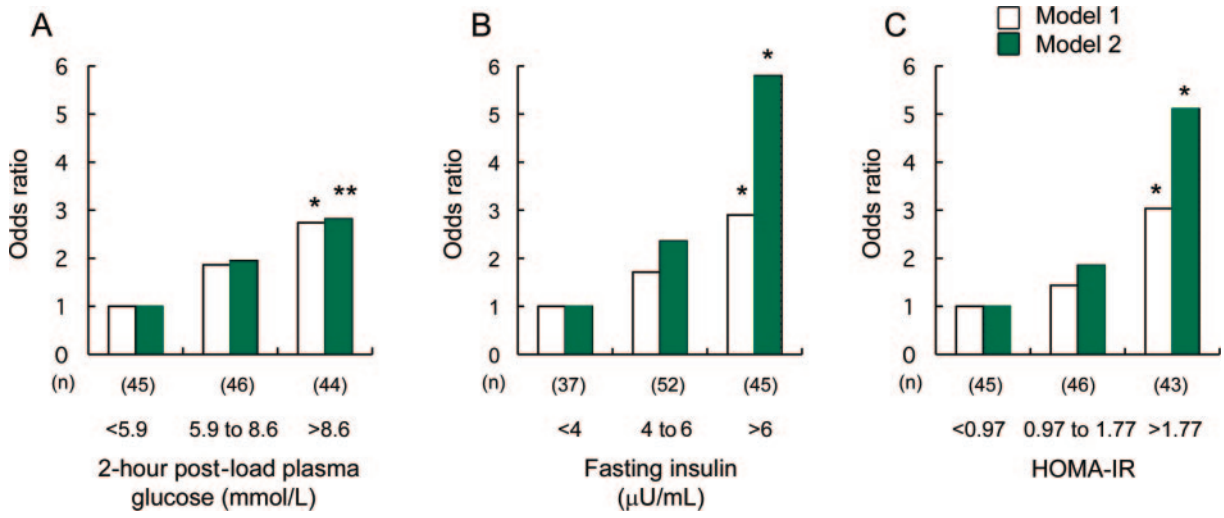
Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; CI = confidence interval; HOMA-IR = homeostasis model assessment of insulin resistance; NP = neuritic plaque; NFT = neurofibrillary tangle; OR = odds ratio.
[a] Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, total cholesterol, body mass index, current smoking, regular exercise, and cerebrovascular disease. ORs are given for each 1-SD increase in glucose, or log fasting insulin and HOMA-IR values.

for NPs. However, there was no relationship between FPG and NPs. These results were almost the same in the multivariate analyses after adjustment for age, sex, systolic blood pressure, total cholesterol, BMI, current smoking, regular exercise, and cerebrovascular disease (model 2). We repeated analyses after excluding the 21 cases with cognitive impairment, and the associations remained unchanged. On the other hand, we found no significant association between diabetes-related factors and NFT pathology (Braak stage I to VI vs stage 0).

To confirm the association between diabetes-related factors and NPs, we compared the risk of NPs among tertiles of 2-hour PG, fasting insulin, and HOMA-IR (figure 1). Compared with the lowest tertile of 2-hour PG (<5.9 mmol/L), the risk of NPs was significantly increased in the highest tertile (>8.6 mmol/L) after adjustment for age and sex (model 1). After adjustment for the aforementioned confounding factors (model 2), this relationship was marginally significant. On the other hand, the highest tertiles of fasting insulin (>6 μU/mL) and HOMA-IR (>1.77) showed increased risk for NPs compared with the lowest tertiles (<4 μU/mL for insulin, <0.97 for HOMA-IR) in models 1 and 2.

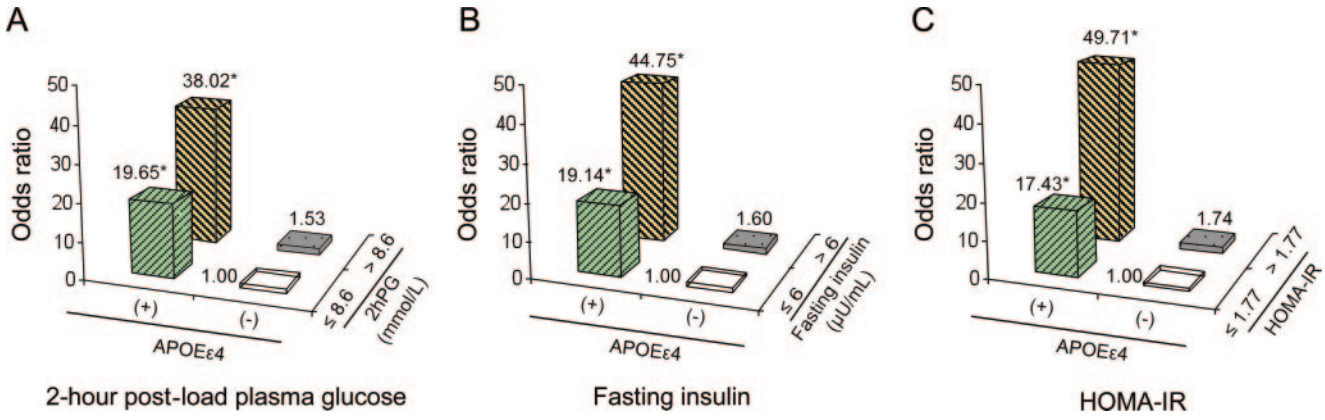
Finally, we examined the combined effects of *APOE* genotype and the magnitude of the diabetes-related factors on the risk of NP pathology (figure 2). For example, the subjects were classified into the following 4 groups according to the 2-hour PG level

Figure 1 Odds ratios for each tertile of glucose (A), insulin (B), and HOMA-IR (C) vs the lowest tertile for the presence of neuritic plaques



Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, total cholesterol, body mass index, current smoking, regular exercise, and cerebrovascular disease. *$p < 0.05$, **$p < 0.10$ vs the lowest tertile. HOMA-IR = homeostasis model assessment of insulin resistance.

Figure 2 Odds ratios for the presence of neuritic plaques according to diabetes-related risk factors and *APOE* genotype



Adjusted for age, sex, and total cholesterol. The numbers in the figure are odds ratios vs the reference group (*APOE* ε4 noncarrier and lower level of glucose [A], insulin [B], or HOMA-IR [C]). *$p < 0.05$ vs reference group. 2hPG = 2-hour post-load plasma glucose; HOMA-IR = homeostasis model assessment of insulin resistance.

and *APOE* status: low 2-hour PG (lowest and second tertiles, ≤8.6 mmol/L) and noncarriers of *APOE* ε4 (group 1), high 2-hour PG (highest tertile, >8.6 mmol/L) and noncarriers of *APOE* ε4 (group 2), low 2-hour PG and *APOE* ε4 carriers (group 3), and high 2-hPG and *APOE* ε4 carriers (group 4). The ORs for the presence of NPs in these 4 groups were 1.0 in group 1 (reference), 1.5 in group 2, 19.7 in group 3, and 38.0 in group 4. As a result, the coexistence of hyperglycemia and *APOE* ε4 genotype (group 4) was associated with the greatest risk for NPs. We performed similar analyses with fasting insulin and HOMA-IR, and similar patterns were observed.

DISCUSSION We suggest that hyperglycemia, hyperinsulinemia, and insulin resistance are risk factors for NP pathology in AD, and might affect the initiation of NP formation. The lack of a dose-response relationship, and the absence of a significant association between the diabetes-related factors and NFT pathology, might be due to an epidemiologic competing effect, indicating that subjects with very high diabetes-related factors at the clinical examination in 1988 probably died earlier as a result of cardiovascular disease, for example. Nevertheless, NFT pathology was less associated with diabetes-related factors, and NFT pathology is considered to be a consequence of β-amyloid deposition in the amyloid cascade hypothesis.[24] The diabetes-related factors may act upstream of the cascade, and might trigger the AD pathogenesis.

Type 2 diabetes is based on insulin resistance and involves chronic compensatory hyperinsulinemia and hyperglycemia. Insulin itself may affect amyloid metabolism, which leads to NP formation. An impaired insulin signaling may exacerbate β-amyloid accumulation by a weakened inhibition on glycogen synthase kinase 3 (GSK3), which is thought to be critically involved in AD pathogenesis.[25] Activated GSK3 triggers γ-secretase activity[26] and increases β-amyloid production.[27] Alternatively, excessive β-amyloid can be cleared by endocytosis or through direct extracellular proteolytic degradation by insulin-degrading enzyme (IDE).[28] Insulin seems to inhibit the extracellular degradation of β-amyloid by competition for IDE.[29] Furthermore, several lines of evidence suggest that the toxic effects of hyperglycemia can lead to slowly progressive functional and structural abnormalities in the brain.[30] It is possible that vascular factors induced by metabolic disturbance may modify the AD-related pathology, however, the positive association between diabetes-related factors and NP pathology still remained even after the adjustment for cerebrovascular lesions in our study.

On the contrary, insulin is known to facilitate memory in normal physiology, as demonstrated when administered at optimal doses and in the context of sufficient glucose availability.[31] The formation of NPs, as described above, is a hallmark of AD, which refers to the pathologic entity; meanwhile, Alzheimer dementia, which refers to clinical dementia, may also be caused in part by deficiencies in intracellular and intercellular signaling.[32] Insulin resistance affects insulin signaling, which might lead to a decline in cognitive function. In this study, the subjects who developed Alzheimer dementia were far less than those who manifested NPs (n = 21 vs 88); therefore, the present pathology-based study should overlap, but is also distinct from the previously reported clinicoepidemiologic studies.[2,4,5,8,9] Our target in this study was to evaluate how diabetes affects the neuropathologic process of AD, which would precede the cognitive decline.

Four previous studies have examined the association between diabetes and AD-related pathology, but their results are inconsistent.[5,33-35] Of these, the Honolulu-

Asia Aging Study was the only population-based study and reported that participants with type 2 diabetes and the *APOE* ε4 allele had a higher number of hippocampal NPs and NFTs in the cortex and hippocampus than those without diabetes and the ε4 allele.[5] In our study, the combination of the unfavorable status afforded by the diabetes-related factors and the presence of the ε4 allele was associated with NP formation, but not with NFT formation (data not shown). The discrepancy in these studies may reflect differences in design of these studies. One possibility is the difference in the observation period between the evaluation of diabetes and the 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 that the presence of AD might affect lifestyle of the subjects and the severity of glucose intolerance. Another possibility is the difference in the study subjects. Both studies were population-based and included Asian subjects; however, the mean age at clinical examination of the Honolulu-Asia Aging Study (78 years) was greater than that in our study. The other 3 studies[33-35] reported controversial or statistically insignificant results between diabetes status and AD pathology, probably due to the facility-based design and different races.

Our study suggests that the combination of each diabetes-related factor and the *APOE* ε4 genotype may have a synergistic effect on the risk of NPs, even though we failed to show a statistically positive interaction (p for interaction = 0.90 [2-hour PG], 0.84 [fasting insulin], 0.79 [HOMA-IR]). The Honolulu-Asia Aging Study[5] also showed synergistic effects of diabetes and the *APOE* ε4 genotype on AD pathology; however, that study did not account for some diabetes-related factors such as insulin levels and HOMA-IR. It was found that apolipoprotein E2 and E3, but not E4, may be involved in β-amyloid clearance.[36] Additionally, apolipoprotein E is commonly colocalized with β-amyloid in NPs,[37] which led to the hypothesis that apolipoprotein E may be involved in β-amyloid aggregation and plaque formation. Because the apolipoprotein E4 isoform stimulates the nucleation and aggregation of β-amyloid in an isoform-specific manner and does not significantly affect the accumulation of β-amyloid deposits,[38] both apolipoprotein E4 and diabetes-related factors may act synergistically on the initiation of β-amyloid aggregation. We consider that a future study using a larger sample size is needed to investigate the interaction between each diabetes-related factor and the *APOE* genotype on the risk of AD pathology.

There are some limitations to our present study. First, the crude, semiquantitative evaluation of NPs (CERAD) and NFTs (Braak stage) could affect the statistical analyses. Second, the medical history of diabetes, such as disease duration, glucose control, and complications, were not considered in this study. Despite these limitations, our study has several strengths. We included community-based subjects, who had detailed metabolic characterization at midlife based on comprehensive blood testing, which included 75-g OGTT profiles and fasting insulin levels, and we systematically assessed AD pathology. Accordingly, the data included in this study are of value to examine the 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.

Further studies are required to determine if there is a causal link between insulin resistance and the development of NPs or other AD-related neuropathologies. In the future, adequate control of diabetes might contribute to a strategy for the prevention of AD.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. T. Matsuzaki.

ACKNOWLEDGMENT

The authors thank S. Nagae and K. Sato for technical assistance.

DISCLOSURE

Dr. Matsuzaki reports no disclosures. Dr. Sasaki receives research support from the Ministry of Health, Labor and Welfare of Japan. Dr. Tanizaki, Dr. Hata, Dr. Fujimi, Dr. Matsui, Dr. Sekita, and Dr. Suzuki report no disclosures. Dr. Kanba has served on scientific advisory boards for Eli Lilly and Company, GlaxoSmithKline, Pfizer Inc., Mitsubishi Tanabe Pharma Corporation, Ono Pharmaceutical Co. Ltd., Astellas Pharma Inc., Asahi Kasei Corporation, Shionogi & Co., Ltd., and Otsuka Pharmaceutical Co., Ltd.; has served on the editorial boards of *Molecular Psychiatry*, the *Journal of Neuroscience and Psychiatry*, the *Asian Journal of Psychiatry*, and the *Asia Pacific Journal of Psychiatry*; has received speaker honoraria from Eli Lilly and Company, GlaxoSmithKline, Pfizer Inc., Asahi Kasei Corporation, Janssen, Tsumura & Co., Ajinomoto Co., Inc., Mitsubishi Tanabe Pharma Corporation, Meiji Techno Co. Ltd., Kyowa Hakko Kirin Pharma, Inc., Dainippon Sumitomo Pharma Co., Ltd., Organon International (Schering-Plough Corp.), Otsuka Pharmaceutical Co., Ltd., and Astellas Pharma Inc.; and has received research support from Eli Lilly & Co., GlaxoSmithKline, Pfizer Inc., Asahi Kasei Corporation, Janssen, Tsumura & Co., Ajinomoto Co., Inc., Mitsubishi Tanabe Pharma Corporation, Meiji Techno Co. Ltd., Kyowa Hakko Kirin Pharma, Inc., Dainippon Sumitomo Pharma Co., Ltd., Organon International (Schering-Plough Corp.), Otsuka Pharmaceutical Co., Ltd., and the Ministry of Health, Labor and Welfare of Japan and the Ministry of Education, Culture, Sports, Science and Technology of Japan. Dr. Kiyohara receives research support from the Ministry of Health, Labor and Welfare of Japan. Dr. Iwaki receives research support from the Ministry of Education, Culture, Sports, Science and Technology of Japan and serves on the editorial boards of *Neuropathology*, *Brain Tumor Pathology*, *Pathology—Research and Practice*, and *Pathology International*.

Received October 5, 2009. Accepted in final form February 19, 2010.

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