Lack of Association Between Variations of *PDE4D* and Ischemic Stroke in the Japanese Population

Tomonaga Matsushita, MD; Michiaki Kubo, MD, PhD; Koji Yonemoto, PhD; Toshiharu Ninomiya, MD, PhD; Kyota Ashikawa; Bailing Liang; Jun Hata, MD, PhD; Yasufumi Doi, MD, PhD; Takanari Kitazono, MD, PhD; Setsuro Ibayashi, MD, PhD; Mitsuo Iida, MD, PhD; Yutaka Kiyohara, MD, PhD; Yusuke Nakamura, MD, PhD

Background and Purpose—After the first genomewide association study of ischemic stroke identified *PDE4D* as a susceptible gene, many replication studies have been conducted. However, the validity of the association has remained controversial because of the heterogeneity of both genetic markers and phenotypes.

Methods—We investigated the association between variations of *PDE4D* and ischemic stroke by 3 methods: single-marker, haplotype, and tag-single nucleotide polymorphism (SNP) analyses. In the single-marker analysis, we evaluated the association using 2 large case–control samples (1112 cases and 1112 control subjects in a sample obtained from Kyushu, Japan, and 1711 cases and 1786 control subjects in BioBank Japan) and a prospective cohort with 14 years of follow-up. These samples were analyzed both separately and pooled. Haplotype and tag-SNP analyses were performed using the 2 case–control samples together.

Results—In single-marker association tests, we found no significant association in the same direction among the 6 SNP reported in the initial study and ischemic stroke subtypes. Haplotype analysis revealed no significant association between the region around the 5′-end of the gene and combined atherothrombotic and cardioembolic infarction. Rs7730070, a SNP located around the 3′-end of *PDE4D*, showed the lowest nominal probability value by tag-SNP analysis but was not significant after adjustment for multiple testing (adjusted probability value =0.36).

Conclusions—These results suggest that variations in *PDE4D* are not associated with ischemic stroke risk in the Japanese population. (***Stroke*. 2009;40:1245-1251.**)

Key Words: cerebral infarct ■ genetics ■ PDE4D

Stroke is one of the most common causes of death and long-term disability around the world. Ischemic stroke is the most common form of stroke and is further subdivided into lacunar, atherothrombotic (ATI), and cardioembolic infarction (CEI). As for genetic contributions to the pathogenesis of ischemic stroke, twin and family studies[1,2] suggested that stroke risk was mediated by both environmental and genetic factors. The first genomewide association study of ischemic stroke reported the phosphodiesterase 4D gene (*PDE4D*) as a susceptible gene using 864 cases and 908 control subjects in an Icelandic population.[3] This study showed that the microsatellite marker AC008818–1 and 6 single nucleotide polymorphisms (SNPs) located in the 5′-end of the gene (SNP41, SNP45, SNP56, SNP87, SNP89, and SNP83) were significantly associated with ATI or with the combined ATI and CEI phenotype. Haplotype blocks B and C, which covered 260 kb around the 5′-end of the gene, were also associated, and the combination of the G allele of SNP45, the 0 allele of AC008818–1, and a common haplotype in block C led to the classification of individuals into at-risk, wild-type, and protective groups. Although the authors of the study showed that the affected individuals with the G0 haplotype had lower expression levels of some *PDE4D* isoforms, they could not find causative SNPs or haplotypes. Moreover, the biological role of *PDE4D* in ischemic stroke or the underlying atherosclerosis remained uncertain.

To our knowledge, 15 replication studies have been published on the association between SNPs in *PDE4D* and ischemic stroke.[4–18] However, the results are still controversial. Of the 4 studies that examined associations between the 2 markers (AC008818–1 and SNP45) and combined ATI and

Received June 1, 2008; final revision received September 8, 2008; accepted October 22, 2008.

From the Laboratory for Genotyping Development (T.M., M.K., K.A., B.L.), Center for Genomic Medicine, The Institute of Physical and Chemical Research (RIKEN), Yokohama, Kanagawa, Japan; the Departments of Clinical Sciences (T.M., M.K., T.K., S.I., M.I.) and Environmental Medicine (M.K., K.Y., T.N., J.H., Y.D., Y.K.), Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; and the Laboratory for Molecular Medicine (Y.N.), Human Genome Center, The Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Correspondence to Michiaki Kubo, MD, PhD, Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, 1-7-22, Suehiro-cho, Tsurumi, Yokohama, Kanagawa, 230-0045, Japan. E-mail mkubo@src.riken.jp

Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.108.527408**

CEI, none replicated the original findings.[4–7] Among the 14 studies that examined at least SNP45,[4–17] only one found a nominal association with combined ATI and CEI.[6] Other groups reported significant associations between different phenotypes and different SNPs in the 1.5-Mb region of the gene.[6–13,19] There are thought to be several reasons for these inconsistencies among the results. The sample sizes in most studies were too small and had insufficient power to detect associations.[20] Sampling biases of cases and controls may have distorted true associations. Several positive findings in different SNPs might reflect associations among hidden causative variants linked to the SNPs or to the G0 haplotype. The association between variants in *PDE4D* and ischemic stroke risk might differ among ethnic groups.

According to the recent published criteria, replication studies should examine the same SNP or a SNP in perfect or very high linkage disequilibrium with the prior SNP on the same or a very similar phenotype. They also should show similar magnitude of effect and significance in the same direction.[21] Therefore, we performed single-marker association tests between the 6 SNPs and the same subtypes of ischemic stroke as in the initial study and used a sufficient sample size. We also performed haplotype analyses in blocks B and C using tag-SNPs selected from the same regions. To examine the possibility of hidden causative SNPs, we additionally genotyped 190 tag-SNPs that covered a 2.2-Mb region, including *PDE4D*, and performed association analyses.

Materials and Methods

Study Populations

We used 2 independent Japanese case–control samples and a prospective cohort for this study. One is a Kyushu sample consisting of 1112 cases of ischemic stroke and 1112 age- and sex-matched control subjects. Details on this population were described previously.[22] Briefly, patients with ischemic stroke were recruited from 7 medical centers in and around Fukuoka City, Japan, in 2004. These included 491 cases of lacunar infarction, 369 of ATI, 136 of CEI, and 116 of undetermined subtype. Age (within 5 years) and sex-matched control subjects were selected from the 3328 participants of the Hisayama screening survey between 2002 and 2003. All case subjects were diagnosed by stroke neurologists on the basis of detailed clinical features and ancillary laboratory examinations such as brain imaging. The subtypes of ischemic stroke were determined on the basis of the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke (NINDS-III).[23]

Another case–control sample was selected from the BioBank Japan project.[24] This project was started in 2003 to collect a total of 300 000 cases who have at least one of 47 diseases by a collaborative network of 66 hospitals located throughout Japan. The registration of cases was based on diagnoses made by physicians at the affiliated hospitals. From June 2003 to March 2006, 7974 cases with ischemic stroke were registered. We selected 1711 cases diagnosed with ischemic stroke subtypes by brain imaging, the same as with the Kyushu sample. The subtypes included 1143 with lacunar infarction, 355 with ATI, and 213 with CEI. Control subjects were randomly selected from the subjects who were registered with BioBank Japan for other diseases.

For the prospective cohort study, we used a cohort population of the Hisayama study established in 1988.[25] In this cohort, 2634 Hisayama residents aged ≥40 years and who had no history of stroke or coronary heart disease were enrolled in 1988 and continuously followed up for 14 years until the occurrence of cardiovascular disease or death. Among them, 1656 subjects participated in the examination between 2002 and 2003 and were used in the present study. During the 14-year follow-up, 67 events of first-ever ischemic stroke were observed.

Written informed consent was obtained from all study subjects. The study was approved by the ethics committees of the Graduate School of Medical Sciences at Kyushu University and the Institute of Physical and Chemical Research.

Clinical characteristics of 2 case–control samples are shown in Supplemental Table I, available online at http://stroke.ahajournals.org. In both samples, hypertension was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg or current treatment with hypertensive medication.

SNP Selection and Genotyping

For the association study, we selected 6 SNPs that were significantly associated with ischemic stroke in the initial study: SNP41 (rs12153798), SNP45 (rs12188950), SNP56 (rs702553), SNP83 (rs966221), SNP87 (rs2910829), and SNP89 (rs1396476). In the haplotype analysis, we selected 16 additional tag-SNPs from the regions of blocks B and C defined in the initial study. For tag-SNP analysis, we selected 190 tag-SNPs from the 2.2-Mb region, including *PDE4D*. Tag-SNPs were selected from the Hapmap JPT data by the pairwise tagging method with the following criteria: $r^2>0.8$, minor allele frequency >5%, and call rate >75%.

Genomic DNA was extracted from peripheral blood leukocytes by a standard method. We genotyped SNPs using the multiplex polymerase chain reaction-based Invader assay[26] (Third Wave Technologies) or TaqMan assays (Applied Biosystems) in a blind fashion to the clinical information of study samples. All genotypes were called by visual inspection, and we determined genotype success as <10 undetermined samples in a 384-well plate. When we failed to genotype more than one 384-well plate in a total of 16 plates, we excluded the SNP from further analyses. To validate the genotyping data, we genotyped 10 SNPs in 48 subjects using direct sequencing, and the concordance rate was 99.6%.

Statistical Analysis

We examined the association both by each population and by meta-analysis. We assessed case–control association analysis and Hardy-Weinberg equilibrium by χ^2 test or Fisher exact test, as appropriate. In the association analysis, we mainly used an additive model and also referenced dominant and recessive models. For an easy understanding of the risk direction, we calculated the OR and 95% CI of each SNP according to the risk allele in the initial study. In a meta-analysis of the single-marker association test, pooled estimates of the ORs for 2 case–control studies and one prospective study were obtained using a fixed-effect model. Heterogeneities across the population were estimated formally using Cochran's Q test and the I^2 statistic. Haplotype analysis was performed using Haploview version 4.0 (Broad Institute). For the adjustment for multiple testing, we performed a random permutation test with 10 000 replications. linkage disequilibrium was calculated as D′, and haplotype blocks were defined by Gabriel's criteria.[27]

Results

Single-Marker Association Test

We initially performed single-marker association tests between the 6 SNPs reported in the initial study and the same ischemic stroke subtypes (Table 1). SNP45 and SNP41, which showed the most significant association in

Table 1. Association Between SNPs Reported in the Initial Study and the Subtypes of Ischemic Stroke Among Japanese

Ischemic Stroke Subtype	SNP	Allele 1	Allele 2	Sample	Case AF	Case 11/12/22	Control AF	Control 11/12/22	*P* Value	OR (95% CI)	Meta-Analysis *P* Value	Meta-Analysis OR (95% CI)
ATI												
	SNP83	C	T	Kyushu	0.13	4/84/279	0.14	7/91/269	0.31	0.86 (0.64–1.16)		
				BioBank	0.13	7/79/269	0.15	42/436/1308	0.32	0.88 (0.70–1.12)	0.14	0.87 (0.73–1.05)
				Prospective	0.12	0/4/13	0.14	36/383/1157	0.66	0.79 (0.28–2.25)		
Combined ATI and CEI	SNP41	T	C	Kyushu	1.00	502/0/0	1.00	501/0/0				
				BioBank	1.00	568/0/0	1.00	1779/0/0				
				Prospective	1.00	24/0/0	1.00	1573/0/0				
	SNP45	C	T	Kyushu	1.00	502/0/0	1.00	501/0/0				
				BioBank	1.00	568/0/0	1.00	1779/0/0				
				Prospective	1.00	24/0/0	1.00	1573/0/0				
	SNP56	A	T	Kyushu	0.58	163/252/81	0.54	146/246/104	0.07	1.18 (0.99–1.41)		
				BioBank	0.56	169/290/102	0.56	554/860/352	0.88	1.01 (0.88–1.16)	0.11	1.09 (0.98–1.21)
				Prospective	0.73	14/7/3	0.55	485/766/315	0.02	2.17 (1.14–4.11)		
	SNP87	T	C	Kyushu	0.15	10/126/364	0.15	2/144/352	0.87	0.98 (0.76–1.25)		
				BioBank	0.11	7/116/445	0.13	32/412/1340	0.10	0.84 (0.68–1.03)	0.21	0.91 (0.78–1.06)
				Prospective	0.17	1/6/17	0.14	23/394/1148	0.60	1.22 (0.57–2.63)		
	SNP89	T	G	Kyushu	0.95	450/52/0	0.97	470/33/0	0.03	0.62 (0.40–0.97)		
				BioBank	0.95	516/50/2	0.95	1619/153/8	0.99	1.00 (0.73–1.37)	0.27	0.87 (0.67–1.12)
				Prospective	0.98	23/1/0	0.95	1430/143/3	0.39	2.33 (0.32–17.0)		

Allele 1 indicates the risk allele in the initial study; AF, allele frequency of allele 1; Meta-analysis was performed using a fixed-effect model.

the initial study, were monomorphic, and all individuals were homozygotes of the risk alleles in our population. In all samples, SNP83 showed no significant association with ATI. For the combined ATI and CEI subtypes, we found SNP56 to be significantly associated in the prospective cohort ($P=0.02$; OR, 2.17; 95% CI, 1.14 to 4.11), but it was not associated in the 2 case–control samples. In the meta-analysis, we could not find a significant association between SNP56 and the combined ATI and CEI phenotypes. SNP89 showed a significant association in the Kyushu sample, but its risk was in the opposite direction of the effect ($P=0.03$; OR, 0.62; 95% CI, 0.40 to 0.97). SNP89 was not significantly associated in the BioBank Japan sample and the prospective cohort, and we found no

Table 2. Association Between SNPs Reported in the Initial Study and Subtypes of Ischemic Stroke Among Combined Samples After Stratification by Hypertension

			Hypertension				Without Hypertension			
			Frequency, %				Frequency, %			
Ischemic Stroke Subtype	SNP	RA	Case (n=572)	Control (n=942)	*P* Value	OR (95% CI)	Case (n=130)	Control (n=842)	*P* Value	OR (95% CI)
ATI	SNP83	C	12.9	13.8	0.50	0.93 (0.75–1.15)	11.9	15.6	0.13	0.73 (0.49–1.09)

			Hypertension				Without Hypertension			
			Frequency, %				Frequency, %			
Ischemic Stroke Subtype	SNP	RA	Case (n=822)	Control (n=1017)	*P* Value	OR (95% CI)	Case (n=219)	Control (n=903)	*P* Value	OR (95% CI)
Combined ATI and CEI	SNP41	T	100	100			100	100		
	SNP45	C	100	100			100	100		
	SNP56	A	56.5	55.1	0.38	1.06 (0.93–1.21)	57.4	55.4	0.45	1.08 (0.88–1.34)
	SNP87	T	12.7	13.6	0.45	0.93 (0.77–1.13)	13.1	14.0	0.62	0.93 (0.68–1.26)
	SNP89	T	95.0	95.8	0.23	0.82 (0.60–1.12)	94.7	95.0	0.83	0.95 (0.59–1.52)

RA indicates risk allele in the initial study; Frequency, risk allele frequency; Due to the lack of hypertension status data, 22 ATI cases, 10 CEI cases, and 371 control subjects were excluded in the stratified analysis.

Table 3. Haplotype Analysis of SNPs Selected From the Region of Blocks B and C Among Combined Samples

Haplotype in Block B													
rs4502776	rs13172481	rs6869495	rs1423246	rs1345782	rs6860887	rs10514896	SNP56	rs27222	rs7712662	rs1423473	SNP45	rs153031	SNP41
A	G	A	A	C	C	A	A	C	T	C	C	A	T
G	C	G	G	C	T	G	T	T	T	C	C	G	T
A	G	A	A	C	C	A	A	T	C	T	C	G	T
A	G	A	G	A	T	A	T	T	C	T	C	G	T
G	C	A	G	C	T	G	T	T	T	C	C	G	T
A	G	A	A	C	T	G	T	T	T	C	C	G	T
G	C	A	G	A	T	A	T	T	C	T	C	G	T
G	C	A	A	C	C	A	A	C	T	C	C	A	T
G	C	A	G	C	T	G	T	C	T	C	C	A	T
A	G	A	G	C	C	A	A	C	T	C	C	A	T
A	G	A	A	C	C	G	T	T	T	C	C	G	T

Haplotypes with frequency >2% are shown.

significant association with SNP89 in the meta-analysis. SNP87 was not associated with the combined ATI and CEI phenotypes in any of the samples. We also examined the associations of these SNPs with ischemic stroke or other subtypes in the 2 case–control samples (Supplemental Table II, available online at http://stroke.ahajournals.org). SNP56 showed nominal association with ATI in the Kyushu sample (P=0.02; OR, 1.27; 95% CI, 1.03 to 1.57) but was not associated in the BioBank Japan sample. The meta-analysis showed no significant association between ATI and SNP56. No other SNPs showed a significant association with any phenotype in the same direction as the initial study.

Stratified Analysis by Hypertension Status

Some replication studies showed significant associations between the SNPs in *PDE4D* and ischemic stroke in subjects without hypertension.[11,17] Thus, we evaluated the association between the 6 SNPs and the subtypes of ischemic stroke among the combined samples stratified by hypertension status (Table 2). However, none of the SNPs were associated with ATI or the combined ATI and CEI phenotypes even in the subjects without hypertension.

Haplotype Analysis

Because SNP45 and SNP41, which are key SNPs for haplotype construction in block B, were monomorphic in our population, we constructed haplotypes using SNP56 and 16 additional tag SNPs selected from the regions of blocks B and C (Table 3). In block B, none of the haplotypes were significantly associated with the combined ATI and CEI phenotypes. In block C, the most common haplotype, G-C-C-A-G, showed the lowest probability value, but the association was not significant after adjustment for multiple testing (adjusted P=0.33). There was no significant haplotype in the combined region of blocks B and C (data not shown).

Tag-SNP Analysis

To determine the possibility of a hidden causative SNP, we attempted to examine the associations between tag-SNPs in *PDE4D* and ischemic stroke. We selected 190 additional tag-SNPs from the 2.2-Mb region that included *PDE4D* and genotyped in combined samples of 2823 cases and 2898 control subjects. Because 14 SNPs did not pass our criteria, we finally analyzed 198 SNPs (the 6 reported in the initial study and 192 tag-SNPs). The genomic structure, case–control results, and linkage disequilibrium map of the 2.2-Mb region are shown in the Figure. Although the initial study showed a strong association around the region of blocks A to C, none of the SNPs in this region showed any association. The rs7730070 SNP, located around the 3′-end of *PDE4D*, showed the lowest probability value (OR, 1.21; 95% CI, 1.06 to 1.37; P=0.0037). However, this SNP was not linked to the 5′-end of the gene that was the causative region in the initial study (Figure, C). Moreover, this association was not significant after adjustment for multiple testing (adjusted P=0.36).

Discussion

We examined the association between variations of *PDE4D* and ischemic stroke using 2 independent large case–control samples and a population-based cohort. Using these samples, we tried to replicate the previous reports in 3 ways: a single-marker association test, haplotype analysis in blocks B and C, and tag-SNP analysis, which covered the entire *PDE4D* gene region. Using 2 case–control samples consisting of 2823 cases and 2898 control subjects and a prospective cohort consisting of 1656 subjects, we found no significant association between the same SNPs and the same ischemic stroke subtypes in the single-marker tests. Similarly, no haplotypes in blocks B and C were found to be associated with the combined ATI and CEI phenotypes. Tag-SNP analysis could not find the hidden causative SNP in *PDE4D*. From these results, we suggest that the common variants of *PDE4D* did not confer risk for ischemic stroke, at least in the Japanese population.

Among the replication studies that examined variations of *PDE4D* and ischemic stroke, the most probable reason for the inconsistent findings is that the small sample sizes

Table 3. Continued

Frequency, %			Haplotype in Block C					Frequency, %		
Case	Control	*P* Value	rs35387	rs40512	rs26954	rs26950	rs26948	Case	Control	*P* Value
41.5	40.9	0.63	G	C	C	A	G	34.4	31.7	0.03
14.5	16.0	0.11	C	T	T	G	G	26.0	27.3	0.26
8.1	7.2	0.17	G	C	C	A	A	23.1	24.6	0.16
4.7	5.7	0.10	G	T	C	A	A	7.5	7.0	0.51
4.1	4.7	0.33	G	C	T	G	G	2.7	2.5	0.56
4.1	3.6	0.41								
3.1	3.1	0.87								
3.5	2.7	0.10								
2.9	2.2	0.08								
1.9	2.6	0.07								
2.1	2.1	0.82								

missed true associations of modest effect. Assuming our sample size, the allele frequencies of the SNPs in our control subjects, and the relative risks of the SNPs in the initial study, the power to detect associations at a significance level of 0.05 would be greater than 99% for SNP83 and SNP56, 98.3% for SNP87, and 69.7% for SNP89 in the case–control samples. In contrast, the statistical power of the prospective cohort was <30% for the 6 SNPs. However, a meta-analysis of these samples should increase the statistical power to detect the association. Therefore, if a true association exists, our study could detect the association between SNPs or haplotypes in *PDE4D* and ischemic stroke with high probability. A recent meta-analysis of 5216 cases and 6615 control subjects also showed that allele 0 of AC008818 and haplotype G0 carriers were associated with increased risk of ischemic stroke, but these associations become nonsignificant after exclusion of the initial study.[28] These results indicate that the effect size of *PDE4D* variants on ischemic stroke, if it exists, may be small.

Because the initial study could not determine a causative SNP or haplotype in *PDE4D*, many replication studies have reported positive associations between different SNPs in *PDE4D* and various ischemic stroke subtypes.[19] This indicates the possibility that hidden causative SNPs for ischemic stroke might exist in *PDE4D*. We analyzed a total of 198 tag-SNPs that covered the 2.2-Mb region, including *PDE4D*, but none of the SNPs were significant after adjustment for multiple testing. Because we selected tag-SNPs according to strict criteria, this analysis was able to capture the most common SNPs in *PDE4D*. Therefore, the previous positive findings of different SNPs may be attributable to chance.

One possible reason for the lack of association between *PDE4D* and ischemic stroke in our study was the difference in the ethnic background. Indeed, SNP45 and SNP41, which showed the most significant association with the combined ATI and CEI phenotypes in an Icelandic population, were monomorphic and all of the Japanese populations studied were homozygotes of the risk alleles in both SNPs. If SNP45 or SNP41 or absolutely linked variations are causative, we cannot estimate the effects of these variations on ischemic stroke, because all causative variations are homozygotes of risk alleles in both cases and control subjects.

Several limitations of this study should be discussed. First, we did not genotype the microsatellite marker, AC008818-1, in this study. However, we genotyped 16 tag-SNPs selected

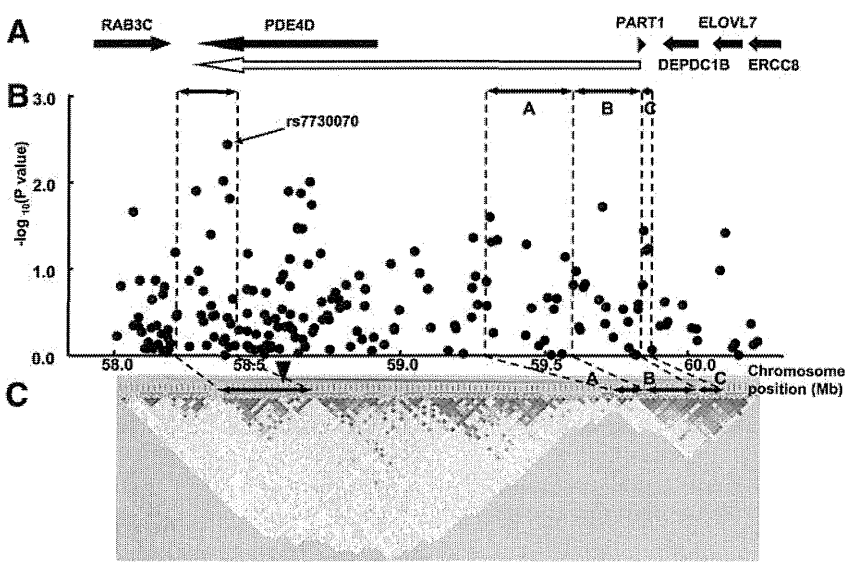


Figure. Genomic structure, case–control results, and linkage disequilibrium map of the 2.2-Mb region, including *PDE4D*. A, Genomic structure around *PDE4D*. The white arrow indicates *PDE4D* reported by the initial study. B, Case–control association results for ischemic stroke among Japanese. The log 10-transformed probability values calculated by the Cochran-Armitage trend test are plotted on the y axis. "A" indicates block A; "B," block B; "C," block C in the initial study. C, Pairwise linkage disequilibrium map between SNPs. The strength of the linkage disequilibrium increases from white to black. A black inverse triangle indicates the location of rs7730070 in the map.

from the regions of blocks B and C according to strict criteria. Therefore, we believe that the effect of AC008818-1 could be sufficiently covered by haplotype analysis using tag-SNPs. Second, we could use only 1656 of 2634 subjects in the prospective cohort. Subjects who developed ischemic stroke would have a higher mortality rate than subjects who did not, and this may have resulted in the lower participation rate in this study. There is a possibility that the results of the prospective cohort might have been distorted by a survivorship bias. Third, the criteria used for classifying ischemic stroke were different between the initial study and ours. For classification of ischemic stroke, the initial study used the Trial of Org 10172 in Acute Stroke Treatment research criteria[29] and we used NINDS-III.[23] However, these 2 classifications are similar to each other, and we diagnosed the subtypes of ischemic stroke by adequate laboratory examinations. We believe that there is no large difference in the phenotype definition.

In conclusion, although we performed a replication study between the variations of *PDE4D* and ischemic stroke risk using 2 independent large case–control samples and a population-based prospective cohort, we failed to replicate the associations. We suggest that variations of *PDE4D* do not confer risk for ischemic stroke in the Japanese population.

Acknowledgments

We thank the residents of Hisayama town and the patients with ischemic stroke for their participation; T. Omae and the staff of the Division of Health and Welfare of Hisayama for their cooperation; many members of the Hisayama study for assistance; T. Ago, H. Ooboshi, M. Kamouchi, H. Sugimori, J. Kuroda, Y. Kumai, N. Hagiwara, S. Yoshimura (Kyushu University Hospital), K. Tamaki, Y. Wakugawa (Hakujyuji Hospital), K. Fujii (Fukuoka Red Cross Hospital), Y. Okada, K. Toyoda (National Hospital Organization, Kyushu Medical Center), T. Nagao (Imazu Red Cross Hospital), H. Nakane (National Hospital Organization, Fukuoka Higashi Medical Center), Y. Yamashita, and K. Kusuda (Seiai Rehabilitation Hospital) for clinical sample collection. We thank all the patients who participated in BioBank Japan project. We also thank all members of BioBank Japan, Institute of Medical Science, the University of Tokyo, and of the Center for Genomic Medicine, The Institute of Physical and Chemical Research, for their contribution to the completion of our study.

Source of Funding

This work was supported in part by Ministry of Education, Culture, Sports, Science and Technology.

Disclosures

None.

References

1. Bak S, Gaist D, Sindrup SH, Skytthe A, Christensen K. Genetic liability in stroke: a long-term follow-up study of Danish twins. *Stroke*. 2002;33:769–774.
2. Kiely DK, Wolf PA, Cupples LA, Beiser AS, Myers RH. Familial aggregation of stroke. The Framingham Study. *Stroke*. 1993;24:1366–1371.
3. Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsdottir HM, Hawkins M, Gudmundsson G, Gudmundsdottir H, Andrason H, Gudmundsdottir AS, Sigurdardottir M, Chou TT, Nahmias J, Goss S, Sveinbjőrnsdottir S, Valdimarsson EM, Jakobsson F, Agnarsson U, Gudnason V, Thorgeirsson G, Fingerle J, Gurney M, Gudbjartsson D, Frigge ML, Kong A, Stefansson K, Gulcher JR. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nat Genet*. 2003;35:131–138.
4. Lőhmussaar E, Gschwendtner A, Mueller JC, Org T, Wichmann E, Hamann G, Meitinger T, Dichgans M. ALOX5AP gene and the *PDE4D* gene in a central European population of stroke patients. *Stroke*. 2005;36:731–736.
5. Kostulas K, Gretarsdottir S, Kostulas V, Manolescu A, Helgadottir A, Thorleifsson G, Gudmundsson LJ, Thorsteinsdottir U, Gulcher JR, Stefansson K, Hillert J. PDE4D and *ALOX5AP* genetic variants and risk for ischemic cerebrovascular disease in Sweden. *J Neurol Sci*. 2007;263:113–117.
6. Meschia JF, Brott TG, Brown RD Jr, Crook R, Worrall BB, Kissela B, Brown WM, Rich SS, Case LD, Evans EW, Hague S, Singleton A, Hardy J. Phosphodiesterase 4D and 5-lipoxygenase activating protein in ischemic stroke. *Ann Neurol*. 2005;58:351–361.
7. Bevan S, Porteous L, Sitzer M, Markus HS. Phosphodiesterase 4D gene, ischemic stroke, and asymptomatic carotid atherosclerosis. *Stroke*. 2005;36:949–953.
8. Nilsson-Ardnor S, Wiklund PG, Lindgren P, Nilsson AK, Janunger T, Escher SA, Hallbeck B, Stegmayr B, Asplund K, Holmberg D. Linkage of ischemic stroke to the *PDE4D* region on 5q in a Swedish population. *Stroke*. 2005;36:1666–1671.
9. Nakayama T, Asai S, Sato N, Soma M. Genotype and haplotype association study of the *STRK1* region on 5q12 among Japanese: a case–control study. *Stroke*. 2006;37:69–76.
10. Song Q, Cole JW, O'Connell JR, Stine OC, Gallagher M, Giles WH, Mitchell BD, Wozniak MA, Stern BJ, Sorkin JD, McArdle PF, Naj AC, Xu Q, Gibbons GH, Kittner SJ. Phosphodiesterase 4D polymorphisms and the risk of cerebral infarction in a biracial population: the Stroke Prevention in Young Women Study. *Hum Mol Genet*. 2006;15:2468–2478.
11. Brophy VH, Ro SK, Rhees BK, Lui LY, Lee JM, Umblas N, Bentley LG, Li J, Cheng S, Browner WS, Erlich HA. Association of phosphodiesterase 4D polymorphisms with ischemic stroke in a US population stratified by hypertension status. *Stroke*. 2006;37:1385–1390.
12. van Rijn MJE, Slooter AJC, Schut AFC, Isaacs A, Aulchenko YS, Snijders PJLM, Kappelle LJ, van Swieten JC, Oostra BA, van Duijn CM. Familial aggregation, the *PDE4D* gene, and ischemic stroke in a genetically isolated population. *Neurology*. 2005;65:1203–1209.
13. Staton JM, Sayer MS, Hankey GJ, Attia J, Thakkinstian A, Yi Q, Cole VJ, Baker R, Eikelboom JW. Association between phosphodiesterase 4D gene and ischaemic stroke. *J Neurol Neurosurg Psychiatry*. 2006;77:1067–1069.
14. Woo D, Kaushal R, Kissela B, Sekar P, Wolujewicz M, Pal P, Alwell K, Haverbusch M, Ewing I, Miller R, Kleindorfer D, Flaherty M, Chakraborty R, Deka R, Broderick J. Association of phosphodiesterase 4D with ischemic stroke: a population-based case–control study. *Stroke*. 2006;37:371–376.
15. Fidani L, Clarimon J, Goulas A, Hatzitolios AI, Evans W, Tsirogianni E, Hardy J, Kotsis A. Association of phosphodiesterase 4D gene G0 haplotype and ischaemic stroke in a Greek population. *Eur J Neurol*. 2007;14:745–749.
16. Kuhlenbäumer G, Berger K, Huge A, Lange E, Kessler C, John U, Funke H, Nabavi DG, Stögbauer F, Ringelstein EB, Stoll M. Evaluation of single nucleotide polymorphisms in the phosphodiesterase 4D gene (*PDE4D*) and their association with ischaemic stroke in a large German cohort. *J Neurol Neurosurg Psychiatry*. 2006;77:521–524.
17. Zee RYL, Brophy VH, Cheng S, Hegener HH, Erlich HA, Ridker PM. Polymorphisms of the phosphodiesterase 4D, cAMP-specific (*PDE4D*) gene and risk of ischemic stroke: a prospective, nested case–control evaluation. *Stroke*. 2006;37:2012–2017.
18. Saleheen D, Bukhari S, Haider SR, Nazir A, Khanum S, Shafqat S, Anis MK, Frossard P. Association of phosphodiesterase 4D gene with ischemic stroke in a Pakistani population. *Stroke*. 2005;36:2275–2277.
19. Rosand J, Bayley N, Rost N, de Bakker PI. Many hypotheses but no replication for the association between *PDE4D* and stroke. *Nat Genet*. 2006;38:1091–1092.
20. Gulcher JR, Kong A, Gretarsdottir S, Thorleifsson G, Stefansson K. Reply to 'Many hypotheses but no replication for the association between *PDE4D* and stroke.' *Nat Genet*. 2006;38:1092–1093.

21. NCI-NHGRI Working Group on Replication in Association Studies. Replicating genotype–phenotype associations. *Nature.* 2007;447:655–670.
22. Kubo M, Hata J, Ninomiya T, Matsuda K, Yonemoto K, Nakano T, Matsushita T, Yamazaki K, Ohnishi Y, Saito S, Kitazono T, Ibayashi S, Sueishi K, Iida M, Nakamura Y, Kiyohara Y. A nonsynonymous SNP in *PRKCH* (protein kinase Cη) increases the risk of cerebral infarction. *Nat Genet.* 2007;39:212–217.
23. Special report from the National Institute of Neurological Disorders and Stroke. Classification of cerebrovascular diseases III. *Stroke.* 1990;21: 637–676.
24. Nakamura Y. The BioBank Japan Project. *Clin Adv Hematol Oncol.* 2007;5:696–697.
25. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Arima H, Tanaka K, Nakamura H, Okubo K, Iida M. Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community. The Hisayama Study. *Stroke.* 2003;34:2349–2354.
26. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. *J Hum Genet.* 2001;46:471–478.
27. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science.* 2002; 296:2225–2229.
28. Bevan S, Dichgans M, Gschwendtner A, Kuhlenbäumer G, Ringelstein EB, Markus HS. Variation in the *PDE4D* gene and ischemic stroke risk. A systematic review and meta-analysis on 5200 cases and 6600 controls. *Stroke.* 2008;39:1966–1971.
29. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE III. Classification of subtype of acute ischemic stroke definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* 1993;24:35–41.

Acta Psychiatr Scand 2010: 122: 319–325
All rights reserved
DOI: 10.1111/j.1600-0447.2010.01587.x

ACTA PSYCHIATRICA SCANDINAVICA

Trends in prevalence of Alzheimer's disease and vascular dementia in a Japanese community: the Hisayama Study

Sekita A, Ninomiya T, Tanizaki Y, Doi Y, Hata J, Yonemoto K, Arima H, Sasaki K, Iida M, Iwaki T, Kanba S, Kiyohara Y. Trends in prevalence of Alzheimer's disease and vascular dementia in a Japanese community: the Hisayama Study.

Objective: To examine secular trends in the prevalence of Alzheimer's disease (AD) and vascular dementia (VD) in a general Japanese population.
Method: Four cross-sectional examinations were conducted among residents of a Japanese community aged ≥65 in 1985, 1992, 1998 and 2005.
Results: The age- and sex-adjusted prevalence of all-cause dementia significantly increased with time (6.0% in 1985, 4.4% in 1992, 5.3% in 1998 and 8.3% in 2005; *P* for trend = 0.002). A similar trend was observed for AD (1.1%, 1.3%, 2.3% and 3.8% respectively; *P* for trend < 0.001), while the age- and sex-adjusted prevalence of VD and other/unclassified dementia showed J-shaped patterns (for VD: 2.3%, 1.5%, 1.5% and 2.5%, respectively, *P* for trend = 0.82; for other/unclassified dementia: 2.6%, 1.7%, 1.5% and 2.0%, *P* for trend = 0.26). The prevalence of AD was likely to increase with time from 1985 to 2005 among subjects aged 75 or older. The ratio of the prevalence of VD to that of AD decreased with time (2.1 in 1985, 1.2 in 1992, 0.7 in 1998 and 0.7 in 2005).
Conclusion: Our findings suggest that the prevalence of all-cause dementia and AD significantly increased over the past two decades in the general Japanese population.

A. Sekita[1,2], T. Ninomiya[3], Y. Tanizaki[1], Y. Doi[3], J. Hata[1], K. Yonemoto[1], H. Arima[1], K. Sasaki[4], M. Iida[3], T. Iwaki[4], S. Kanba[2], Y. Kiyohara[1]

[1]Department of Environmental Medicine, [2]Department of Neuropsychiatry, [3]Department of Medicine and Clinical Science and [4]Department of Neuropathology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Key words: dementia; Alzheimer's disease; vascular dementia; prevalence; secular trend

Yutaka Kiyohara, Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka City 812-8582, Japan.
E-mail: kiyohara@envmed.med.kyushu-u.ac.jp

Accepted for publication June 16, 2010

Significant outcomes

- The prevalence of all-cause dementia significantly increased over the past 20 years in a general population of Japanese elderly.
- The prevalence of Alzheimer's disease in 2005 was approximately threefold higher than that in 1985.
- The ratio of the prevalence of vascular dementia to that of Alzheimer's disease decreased with time.

Limitations

- The diagnosis of dementia and its types was made based only on clinical findings.
- There was a variation in participation rate among the four cross-sectional examinations.
- We have no information regarding factors that contributed to trends in the prevalence of dementia.

Introduction

Approximately 24.3 million people suffer from dementia globally, and this number is expected to double every 20 years to 81.1 million by 2040 because of the rapid increase in the number of the elderly worldwide (1). In Japan, where the elderly population has been increasing faster than in other

countries and the ratio of the elderly to the total population has become the highest in the world, dementia has become a serious social, medical and economic problem. Effective prevention requires a strategy based on information about the morbidity of dementia and its subtypes and its secular trends in general populations. A number of studies have investigated the prevalence of dementia and its subtypes in various populations worldwide (2–8). However, only a few population-based studies have investigated secular trends in the prevalence of dementia in defined populations (9–14), and there were very few studies examining these trends in the 2000s.

Aims of the study

The aim of this analysis was to investigate secular trends in the prevalence of all-cause dementia and dementia subtypes over the past two decades in a general population of Japanese elderly.

Material and methods

Study population

The Hisayama Study is a prospective cohort study of cerebro-cardiovascular diseases in a subrural community, the town of Hisayama, which is adjacent to the metropolitan area of Fukuoka, Japan. The population of the town has distributions of age, occupational status and nutrient intake that are almost identical with those for the whole of Japan (15). The population of the town has been stable for 50 years. As a part of the study, four cross-sectional examinations of dementia have been conducted on Hisayama residents aged 65 or older (10, 16, 17). In 1985, a total of 938 residents in that age group were invited to participate in a cross-sectional examination of dementia. After exclusion of 26 subjects who died, 10 who moved out of the town before the examination and 15 who refused the examination, 887 subjects (353 men and 534 women) underwent the examination (participation rate 94.6%) (Table 1). In a similar manner, we examined 1189 subjects (475 men and 714 women) among 1231 residents (participation rate 96.6%) in 1992, 1437 subjects (571 men and 866 women) among 1442 residents (participation rate 99.7%) in 1998 and 1566 subjects (612 men and 954 women) among 1711 residents (participation rate 91.5%) in 2005. The number of elderly subjects increased during the study period because of aging of the population, which was consistent with the national trend.

Table 1. Demographic characteristics of subjects and diagnostic procedures of dementia in each examination

	Year of examination			
	1985 (n = 887)	1992 (n = 1189)	1998 (n = 1437)	2005 (n = 1566)
Age, years	73.7 ± 6.4	74.2 ± 6.9	74.8 ± 7.2	75.9 ± 7.4
Women, %	60.2	60.1	60.3	60.9
Participation rate, %	94.6	96.6	99.7	91.5
Neuropsychological test	HDS	HDS HDS-R MMSE	HDS-R	HDS-R MMSE
Diagnosis of dementia	DSM-III	DSM-III-R	DSM-III-R	DSM-III-R

HDS, Hasegawa's Dementia Rating Scale; HDS-R, HDS, revised version; MMSE, Mini-Mental State Examination; DSM-III, Diagnostic and Statistical Manual of Mental Disorders, third edition; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders, revised third edition.

Survey of dementia

We carried out a two-phase survey of dementia at each examination. The first screening survey included neuropsychological tests [Hasegawa's Dementia Scale (HDS) (18) in 1985; HDS, HDS revised version (HDS-R) (19) and Mini-Mental State Examination (MMSE) (20) in 1992; HDS-R in 1998; and HDS-R and MMSE in 2005] and questionnaires regarding psychological and medical symptoms, medical conditions and activities of daily living (Table 1). HDS and HDS-R are neuropsychological tests that are widely utilized in Japan and comprised of questions regarding orientation, memory function, common knowledge and calculation capacities. We confirmed the excellent agreement among these tests in 1992 (agreement rate = 95% and kappa coefficient = 0.77 between MMSE and HDS; agreement rate = 96% and kappa coefficient = 0.81 between MMSE and HDS-R). The assessment of neuropsychological tests was performed by investigators who were trained in advance in the use of the tests. For subjects whose test scores were below the cutoff points (22/32.5 for HDS, 21/30 for HDS-R and MMSE), comprehensive investigations, including interviews of the families or attending physicians, physical and neurological examinations and a review of the clinical records, were conducted.

Diagnosis of dementia

The diagnosis of dementia was made clinically based on the guidelines of the Diagnostic and Statistical Manual of Mental Disorders, third edition (DSM-III) (21) in 1985 and those of the DSM-III revised version (DSM-III-R) (22) in 1992, 1998 and 2005 by trained neurologists/psychia-

trists who were supervised by a single neurologist (Y.K.) over the study period (Table 1). We used Karasawa's criteria (23) for the clinical evaluation of dementia as supplementation. The latter has been widely used for epidemiological research on dementia in Japan and divides cases with dementia into four grades of severity according to loss of intellectual abilities, severity of interference with social and occupational functioning and inability to care for oneself. The ischemic score of Hachinski et al. (24) was also used to differentiate vascular dementia (VD) from Alzheimer's disease (AD).

Among a total of 887 subjects screened in 1985, 114 (12.9%) underwent the secondary comprehensive investigation, and of those, 59 (6.7%) were diagnosed as having dementia. Similarly, 194 subjects (16.3%) in 1992, 258 (18.0%) in 1998 and 395 (25.2%) in 2005 underwent comprehensive investigations, and of those, 68 (5.7%), 102 (7.1%) and 195 (12.5%), respectively, were diagnosed as having dementia.

Statistical analysis

Adjusted prevalence of dementia was estimated with 95% confidence interval (CI) by the direct method with 5-year age groupings, where the total population in Japan at the time of the initial examination was used as a standard population. Differences in the adjusted prevalence of dementia were tested, and the adjusted odds ratio (OR) and 95% CI were estimated using the logistic regression model including age taken as a continuous variable and sex.

Ethical considerations

The study protocol was approved by the Human Ethics Review Committee of the Graduate School of Medical Sciences, Kyushu University.

Results

Demographic characteristics of the subjects in the examinations conducted in 1985, 1992, 1998 and 2005 are shown in Table 1. The mean age was slightly increased from 73.7 years in 1985 to 75.9 years in 2005. Women accounted for approximately 60% of total subjects over the four examinations.

The prevalence of all-cause dementia in the four examinations is shown in Table 2. The age- and sex-adjusted prevalence of all-cause dementia significantly increased from 6.0% in 1985 to 8.3% in 2005 (P for trend = 0.002) and was 1.34-fold (P = 0.08) higher in 2005 than in 1985. This trend was observed in the age- and sex-adjusted prevalence of all-cause dementia for both sexes but was only significant for women (P for trend = 0.007).

Table 3 shows the secular trends in the prevalence of dementia by subtypes. The age- and sex-adjusted prevalence of AD significantly increased from 1.1% in 1985 to 3.8% in 2005 (P for trend <0.001) and was 2.00-fold higher in 1998 (P = 0.04) and 3.28-fold higher in 2005 ($P < 0.001$) than in 1985. The age- and sex-adjusted prevalence of VD showed a decreasing trend between 1985 and 1998 (from 2.3% to 1.5%) and then an increasing trend to 2.5% in 2005. A similar trend

Table 2. Secular trends in prevalence of all-cause dementia from 1985 to 2005

	Year of examination				
	1985	1992	1998	2005	P for trend
Total					
Population at risk	887	1189	1437	1566	0.002
No. of cases of dementia	59	68	102	195	
Crude prevalence (%) (95% CI)	6.7 (5.0–8.3)	5.7 (4.4–7.1)	7.1 (5.7–8.5)	12.5 (10.7–14.2)	
Age- and sex-adjusted prevalence (%) (95% CI)	6.0 (4.4–7.6)	4.4 (3.3–5.6)	5.3 (4.2–6.4)	8.3 (7.0–9.5)	
Age- and sex-adjusted odds ratio (95% CI)	1 (reference)	0.70 (0.48–1.03)	0.78 (0.55–1.12)	1.34 (0.97–1.87)	
Women					
Population at risk	534	714	866	954	0.007
No. of cases of dementia	40	51	77	141	
Crude prevalence (%)(95% CI)	7.5 (5.2–9.8)	7.1 (5.2–9.1)	8.9 (6.9–10.9)	14.8 (12.3–17.2)	
Age-adjusted prevalence (%) (95% CI)	6.6 (4.5–8.6)	5.3 (3.8–6.8)	6.4 (4.9–7.9)	9.3 (7.7–10.9)	
Age-adjusted odds ratio (95% CI)	1 (reference)	0.73 (0.46–1.17)	0.83 (0.54–1.29)	1.39 (0.93–2.10)	
Men					
Population at risk	353	475	571	612	0.13
No. of cases of dementia	19	17	25	54	
Crude prevalence (%) (95% CI)	5.4 (3.0–7.8)	3.6 (1.9–5.3)	4.4 (2.7–6.1)	8.8 (6.5–11.2)	
Age- adjusted prevalence (%) (95% CI)	5.4 (3.0–7.8)	3.6 (1.9–5.3)	4.2 (2.6–5.9)	7.2 (5.3–9.2)	
Age-adjusted odds ratio (95% CI)	1 (reference)	0.63 (0.32–1.25)	0.67 (0.36–1.27)	1.25 (0.71–2.20)	

95% CI: 95% confidence interval.

Table 3. Secular trends in prevalence of dementia subtypes from 1985 to 2005

	Year of examination				
	1985 (n = 887)	1992 (n = 1189)	1998 (n = 1437)	2005 (n = 1566)	P for trend
Alzheimer's disease					
No. of cases of dementia	12	21	49	96	<0.001
Crude prevalence (%) (95% CI)	1.4 (0.6–2.1)	1.8 (1.0–2.5)	3.4 (2.5–4.4)	6.1 (4.9–7.4)	
Age- and sex-adjusted prevalence (%) (95% CI)	1.1 (0.4–1.7)	1.3 (0.7–1.9)	2.3 (1.6–3.0)	3.8 (3.0–4.6)	
Age- and sex-adjusted odds ratio (95% CI)	1 (reference)	1.11 (0.53–2.32)	2.00* (1.04–3.87)	3.28** (1.75–6.14)	
Vascular dementia					
No. of cases of dementia	21	22	25	51	0.82
Crude prevalence (%) (95% CI)	2.4 (1.4–3.4)	1.9 (1.1–2.6)	1.7 (1.1–2.4)	3.3 (2.4–4.2)	
Age- and sex-adjusted prevalence (%) (95% CI)	2.3 (1.3–3.3)	1.5 (0.8–2.2)	1.5 (0.9–2.1)	2.5 (1.7–3.2)	
Age- and sex-adjusted odds ratio (95% CI)	1 (reference)	0.70 (0.38–1.29)	0.58 (0.32–1.06)	0.95 (0.56–1.62)	
Other/unclassified dementia					
No. of cases of dementia	26	25	28	48	0.26
Crude prevalence (%) (95% CI)	2.9 (1.8–4.1)	2.1 (1.3–2.9)	1.9 (1.2–2.7)	3.1 (2.2–3.9)	
Age- and sex-adjusted prevalence (%) (95% CI)	2.6 (1.6–3.7)	1.7 (1.0–2.4)	1.5 (0.9–2.2)	2.0 (1.4–2.7)	
Age- and sex-adjusted odds ratio (95% CI)	1 (reference)	0.61 (0.35–1.08)	0.50 (0.29–0.87)	0.69 (0.41–1.14)	

95% CI: 95% confidence interval; *P < 0.05, **P < 0.01 vs. 1985.

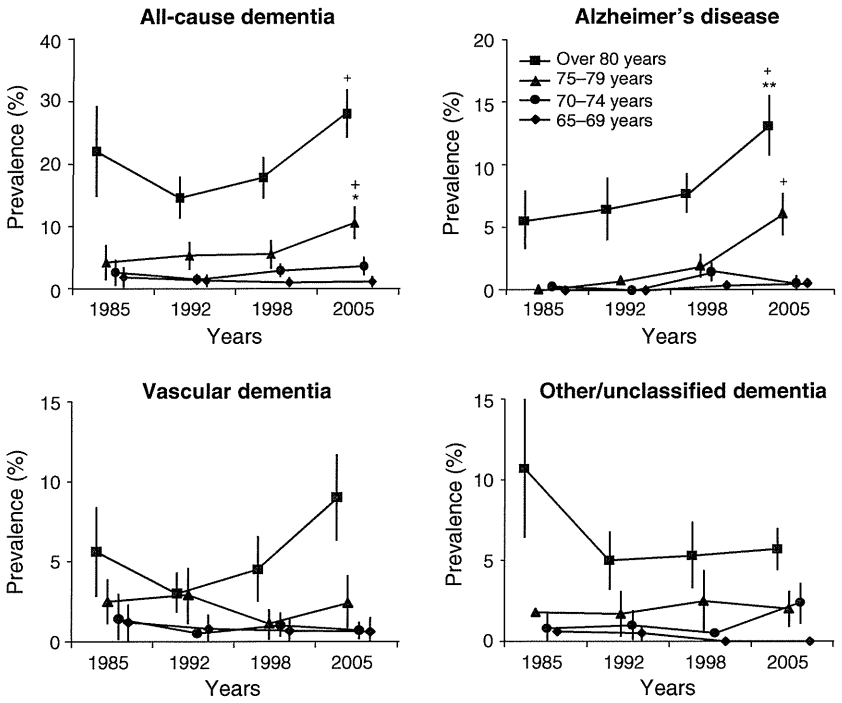


Fig. 1. Secular trends in sex-adjusted prevalence of dementia and its subtypes according to age groups. *P < 0.05, **P < 0.01 vs. 1985, +P for trend < 0.01. The vertical bars of 95% confidence intervals of adjusted prevalence were truncated at zero or more.

was observed for other/unclassified dementia. As a result, the ratio of the prevalence of VD to that of AD decreased with time (2.1 in 1985, 1.2 in 1992, 0.7 in 1998 and 0.7 in 2005).

Figure 1 shows the secular trends in the sex-adjusted prevalence of all-cause dementia and its subtypes according to age groups. The prevalence of all-cause dementia significantly increased from 1985 to 2005 among subjects aged 75 or older (P for trend < 0.01). Such a trend was also observed for the prevalence of AD in the same age group (P for trend < 0.01). The prevalence of VD tended to increase among subjects aged 80 or older in recent years (P for trend = 0.06). There were no clear changes in the prevalence of others/unclassified dementia.

Discussion

The present analysis of repeated cross-sectional examinations in a general population of Japanese elderly demonstrated that the prevalence of all-cause dementia significantly increased from 1985 to 2005. A similar trend was observed for AD but not

for VD. The prevalence of all-cause dementia and AD increased with time among subjects aged 75 or older, while increasing prevalence of VD was observed among subjects aged 80 or older.

Several population-based observational studies have reported secular trends in the prevalence of dementia (9–14). The Lundby Study conducted repeated cross-sectional examinations of dementia in a Swedish community and found no significant changes in the prevalence of senile dementia and multi-infarct dementia from 1945–1957 to 1957–1972 (9). The ZARADEMP project has also found no clear difference in the prevalence of dementia in a Southern European population in 1988–1989 and the prevalence in 1994–1996 (13). In contrast, an observational study from Rochester, Minnesota, in the USA demonstrated that the prevalence of dementia and AD significantly increased from 1975 to 1985 (11). An observational study from Beijing, China, also reported that the prevalence of dementia was slightly higher in 1997 than in 1986 and that AD increased its ranking from the second most common type of dementia (1986) to the most common type (1997) (12). An epidemiological study in the town of Daisen, Japan, has also demonstrated that the prevalence of all-cause dementia, AD and VD increased from 1980 to 2000 (14). In the present study, the prevalence of all-cause dementia and AD increased from 1985 to 2005 in a Japanese community. Although results obtained from Western countries were inconclusive, there may be an increasing burden of dementia in Asian countries.

The ratio of the prevalence of VD to that of AD has been shown to be an effective index for comparing the prevalence of VD and AD in various regions (3). In their recent review, Suh and Shah (3) used this ratio to compare the prevalence of VD and AD in numerous countries and found that AD was more prevalent than VD in USA and Europe. On the other hand, in Asian countries (China, Korea and Japan), there has been a temporal change in the VD/AD ratio. Although VD was more prevalent than AD in Asian countries before 1989, AD has become nearly twice as prevalent as VD since early 1990s (3). The present study confirms the findings of previous observational studies and suggests that AD has become more prevalent than VD in the Asian region in recent years.

The causes of the increase in the prevalence of all-cause dementia and AD observed in our study were not completely resolved. Aging of the study population may be a probable cause of these findings, because age is one of the strongest risk factors for cognitive decline (16, 25). However, the increasing trends in the prevalence of dementia remained significant even after controlling for the confounding effects of age using two different statistical methods, i.e. the direct method using 5-year age groupings and the logistic regression model including age taken as a continuous covariate. Therefore, aging of the study population is not likely to be a leading cause for increasing prevalence of dementia. Another possible cause would be the recent increase in the prevalence of metabolic disorders, such as obesity, hypercholesterolemia and glucose intolerance (15), which have been associated with the risk of AD (26–33).

Another interesting finding of the present study is that the age- and sex-adjusted prevalence of VD decreased from 1985 to 1998 and then increased in 2005, although the trend was not significant. A J-shaped trend in VD was observed among subjects aged 80 or older. VD has not only been shown to be associated with metabolic disorders but also with hypertension. Therefore, the decline in the prevalence of VD in the 1990s may have been ascribable to an improvement in the management of hypertension. In fact, during this period, the incidence and mortality of stroke significantly decreased in Japan, especially among the elderly (34). Without doubt, the popularization of antihypertensive therapy greatly contributed to this welcome trend. However, the steep increase in metabolic disorders and partly insufficient control of hypertension, especially among the elderly, may be responsible for the increasing prevalence of VD in recent years.

In Japan, the number of elderly subjects who lived in old-age homes or were institutionalized in other medical care facilities increased during the study period along with the improvement in the national medical care system for the elderly. Thus, the increase in subjects with dementia in our study may have been attributable to more effective management of these patients in recent years. However, this influence was suggested to be limited because the increase in the prevalence was observed only for AD but not for VD and other/unclassified dementia, and the 10-year survival rates were not significantly different among dementia subtypes in Hisayama residents (17).

The strengths of our study include its long observational period, high participation rates and relatively consistent way to diagnose dementia. The study has three limitations. First, the diagnosis of dementia and its types was made based only on clinical findings. However, we used typical dementia – i.e., AD and VD – as target disease, and the prevalences of all-cause dementia, AD and VD were similar to those obtained from other

observational studies in Asian regions (5, 35–41). Therefore, we believe that this bias is not likely to invalidate the present findings. Second, there was a variation in participation rate among the four cross-sectional examinations. It is generally agreed that an acceptable participation rate in a population-based study, i.e., a rate that practically eliminates the threat of selection bias attributable to non-participants, is above 70% of the target population (42, 43). We enrolled more than 90% of residents in every examination, and, therefore, we believe that the findings of the present study reflect the actual secular trends in prevalence in the Japanese population. Third, we have no information regarding factors that contributed to trends in the prevalence of dementia.

In conclusion, the prevalence of all-cause dementia and AD has increased significantly over the past 20 years in a general population of Japanese elderly. The increasing trend seemed to be observed among subjects aged 75 or older. It is important to establish effective prevention strategies for dementia, particularly for AD, in countries such as Japan, where the elderly population is increasing rapidly.

Declaration of interest

None.

References

1. Ferri CP, Prince M, Brayne C et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005;**366**: 2112–2117.
2. Lobo A, Launer LJ, Fratiglioni L et al. Prevalence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts. Neurology 2000;**54**: S4–S9.
3. Suh GH, Shah A. A review of the epidemiological transition in dementia – cross-national comparisons of the indices related to Alzheimer's disease and vascular dementia. Acta Psychiatr Scand 2001;**104**:4–11.
4. Liu L, Guo XE, Zhou YQ, Xia JL. Prevalence of dementia in China. Dement Geriatr Cogn Disord 2003;**15**:226–230.
5. Zhang ZX, Zahner GE, Roman GC et al. Dementia subtypes in China: prevalence in Beijing, Xian, Shanghai, and Chengdu. Arch Neurol 2005;**62**:447–453.
6. Galasko D, Salmon D, Gamst A et al. Prevalence of dementia in Chamorros on Guam: relationship to age, gender, education, and APOE. Neurology 2007;**68**:1772–1781.
7. Saz P, Lopez-Anton R, Dewey ME et al. Prevalence and implications of psychopathological non-cognitive symptoms in dementia. Acta Psychiatr Scand 2009;**119**:107–116.
8. Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia—meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand 2009;**119**:252–265.
9. Rorsman B, Hagnell O, Lanke J. Prevalence and incidence of senile and multi-infarct dementia in the Lundby Study: a comparison between the time periods 1947–1957 and 1957–1972. Neuropsychobiology 1986;**15**:122–129.
10. Kiyohara Y, Yoshitake T, Kato I et al. Changing patterns in the prevalence of dementia in a Japanese community: the Hisayama Study. Gerontology 1994;**40**(Suppl 2):29–35.
11. Beard CM, Kokmen E, O'Brien PC, Kurland LT. The prevalence of dementia is changing over time in Rochester, Minnesota. Neurology 1995;**45**:75–79.
12. Li S, Yan F, Li G et al. Is the dementia rate increasing in Beijing? Prevalence and incidence of dementia 10 years later in an urban elderly population. Acta Psychiatr Scand 2007;**115**:73–79.
13. Lobo A, Saz P, Marcos G et al. Prevalence of dementia in a southern European population in two different time periods: the ZARADEMP Project. Acta Psychiatr Scand 2007;**116**:299–307.
14. Wakutani Y, Kusumi M, Wada K et al. Longitudinal changes in the prevalence of dementia in a Japanese rural area. Psychogeriatrics 2007;**7**:150–154.
15. Kubo M, Hata J, Doi Y, Tanizaki Y, Iida M, Kiyohara Y. Secular trends in the incidence of and risk factors for ischemic stroke and its subtypes in Japanese population. Circulation 2008;**118**:2672–2678.
16. Yoshitake T, Kiyohara Y, Kato I et al. Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population: the Hisayama Study. Neurology 1995;**45**:1161–1168.
17. Matsui Y, Tanizaki Y, Arima H et al. Incidence and survival of dementia in a general population of Japanese elderly: the Hisayama Study. J Neurol Neurosurg Psychiatry 2009;**80**:366–370.
18. Hasegawa K, Inoue K, Moriya K. An investigation of dementia rating scale for the elderly (in Japanese). Seishin Igaku 1974;**16**:965–969.
19. Katoh S, Simogaki H, Onodera A et al. Development of the revised version of Hasegawa's dementia scale (HDS-R) (in Japanese). Jpn J Geriatr Psychiatry 1991;**2**:1339–1347.
20. Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;**12**:189–198.
21. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 3rd edn. Washington, DC: American Psychiatric Association, 1980.
22. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 3rd edn, revised. Washington, DC: American Psychiatric Association, 1987.
23. Tokyo Metropolitan Geriatric Research Institute, Laboratory of Psychiatry. Social and psychiatric realities of the elderly with senile dementia staying at home in the Tokyo metropolitan area (in Japanese). Tokyo: Government Publication of Tokyo Metropolis, 1981.
24. Hachinski VC, Iliff LD, Zilhka E et al. Cerebral blood flow in dementia. Arch Neurol 1975;**32**:632–637.
25. Luck T, Riedel-Heller SG, Luppa M et al. Risk factors for incident mild cognitive impairment-results from the German Study on Aging, Cognition and Dementia in primary care patients (AgeCoDe). Acta Psychiatr Scand 2010;**121**: 260–272.
26. Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Arch Neurol 2004;**61**:661–666.
27. Peila R, Rodriguez BL, Launer LJ. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia Aging Study. Diabetes 2002;**51**:1256–1262.

28. Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: the Rotterdam Study. Neurology 1999;**53**: 1937–1942.
29. Kivipelto M, Helkala EL, Laakso MP et al. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. BMJ 2001;**322**:1447–1451.
30. Notkola IL, Sulkava R, Pekkanen J et al. Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. Neuroepidemiology 1998;**17**:14–20.
31. Whitmer RA, Gunderson EP, Quesenberry CP Jr, Zhou J, Yaffe K. Body mass index in midlife and risk of Alzheimer disease and vascular dementia. Curr Alzheimer Res 2007;**4**: 103–109.
32. Hayden KM, Zandi PP, Lyketsos CG et al. Vascular risk factors for incident Alzheimer disease and vascular dementia: the Cache County Study. Alzheimer Dis Assoc Disord 2006;**20**:93–100.
33. Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I. An 18-year follow-up of overweight and risk of Alzheimer disease. Arch Intern Med 2003;**163**:1524–1528.
34. Kubo M, Kiyohara Y, Kato I et al. Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community: the Hisayama Study. Stroke 2003;**34**: 2349–2354.
35. Meguro K, Ishii H, Yamaguchi S et al. Prevalence of dementia and dementing diseases in Japan: the Tajiri Project. Arch Neurol 2002;**59**:1109–1114.
36. Yamada T, Hattori H, Miura A, Tanabe M, Yamori Y. Prevalence of Alzheimer's disease, vascular dementia and dementia with Lewy bodies in a Japanese population. Psychiatry Clin Neurosci 2001;**55**:21–25.
37. Ikeda M, Hokoishi K, Maki N et al. Increased prevalence of vascular dementia in Japan: a community-based epidemiological study. Neurology 2001;**57**:839–844.
38. Shibayama H, Kasahara Y, Kobayashi H. Prevalence of dementia in a Japanese elderly population. Acta Psychiatr Scand 1986;**74**:144–151.
39. Jhoo JH, Kim KW, Huh Y et al. Prevalence of dementia and its subtypes in an elderly urban korean population: results from the Korean Longitudinal Study on Health And Aging (KLoSHA). Dement Geriatr Cogn Disord 2008;**26**: 270–276.
40. Ogura C, Nakamoto H, Uema T, Yamamoto K, Yonemori T, Yoshimura T. Prevalence of senile dementia in Okinawa, Japan. Int J Epidemiol 1995;**24**:373–380.
41. Yamada M, Sasaki H, Mimori Y et al. Prevalence and risks of dementia in the Japanese population: RERF's adult health study Hiroshima subjects. Radiation Effects Research Foundation. J Am Geriatr Soc 1999;**47**:189–195.
42. Groves R. Survey errors and survey costs. New York: John Wiley & Sons, 1989.
43. Kasper JD, Shapiro S, Guralnik JM, Bandeen-Roche KJ, Fried LP. Designing a community study of moderately to severely disabled older women: the Women's Health and Aging Study. Ann Epidemiol 1999;**9**:498–507.

Insulin resistance is associated with the pathology of Alzheimer disease

The Hisayama Study

T. Matsuzaki, MD
K. Sasaki, MD, PhD
Y. Tanizaki, MD, PhD
J. Hata, MD, PhD
K. Fujimi, MD, PhD
Y. Matsui, MD, PhD
A. Sekita, MD
S.O. Suzuki, MD, PhD
S. Kanba, MD, PhD
Y. Kiyohara, MD, PhD
T. Iwaki, MD, PhD

Address correspondence and reprint requests to Dr. Kensuke Sasaki, Department of Neuropathology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
ksasaki@np.med.kyushu-u.ac.jp

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-

Editorial, page 758

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

From the Departments of Neuropathology (T.M., K.S., K.F., S.O.S., T.I.), Psychiatry (T.M., K.S., K.F., Y.M., A.S., S.K.), and Environmental Medicine (Y.T., J.H., Y.M., A.S., Y.K.), Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

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-Choujyu-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

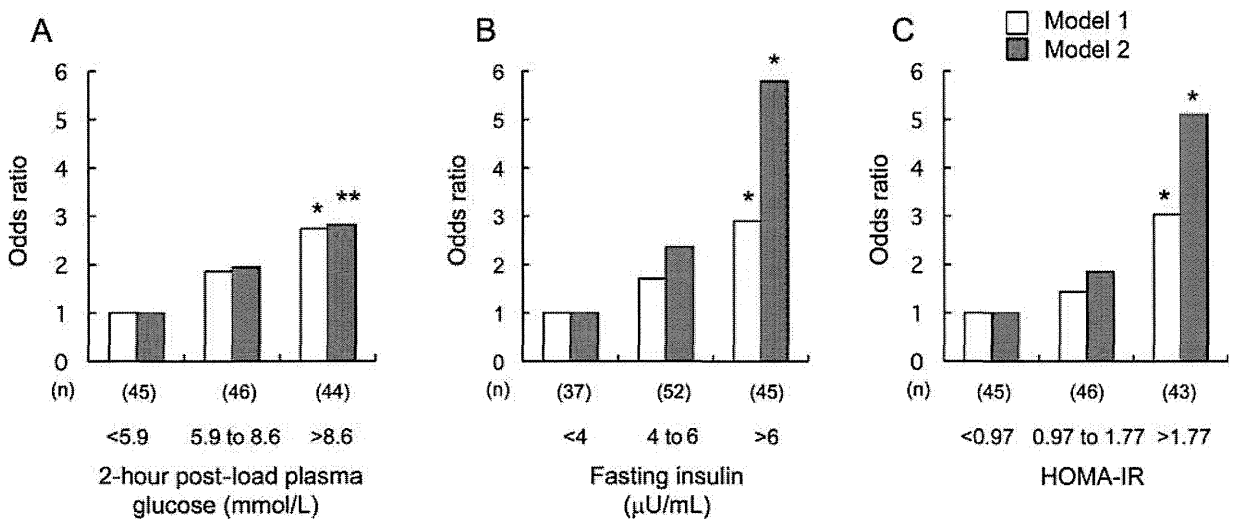
Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; CI = confidence interval; HOMA-IR = homeostasis model assessment of insulin resistance; NP = neuritic plaque; NFT = neurofibrillary tangle; OR = odds ratio.
[a] Model 1: adjusted for age and sex. Model 2: adjusted for 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.

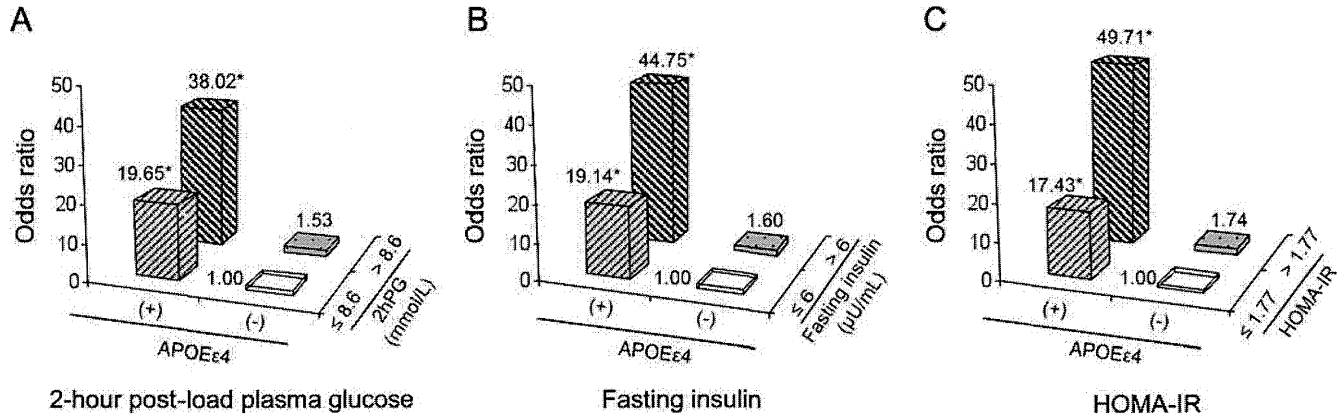
Finally, we examined the combined effects of *APOE* genotype and the magnitude of the diabetes-related factors on the risk of NP pathology (figure 2). For example, the subjects were classified into the following 4 groups according to the 2-hour PG level

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.

REFERENCES

1. Allen KV, Frier BM, Strachan MW. The relationship between type 2 diabetes and cognitive dysfunction: longitudinal studies and their methodological limitations. Eur J Pharmacol 2004;490:169–175.