# Glucose tolerance status and risk of dementia in the community

## The Hisayama Study

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ABSTRACT

**Objective:** We investigated the association between glucose tolerance status defined by a 75-g oral glucose tolerance test (OGTT) and the development of dementia.

**Methods:** A total of 1,017 community-dwelling dementia-free subjects aged ≥60 years who underwent the OGTT were followed up for 15 years. Outcome measure was clinically diagnosed dementia.

**Results:** The age- and sex-adjusted incidence of all-cause dementia, Alzheimer disease (AD), and vascular dementia (VaD) were significantly higher in subjects with diabetes than in those with normal glucose tolerance. These associations remained robust even after adjustment for confounding factors for all-cause dementia and AD, but not for VaD (all-cause dementia: adjusted hazard ratio [HR] = 1.74, 95% confidence interval [CI] = 1.19 to 2.53, $p = 0.004$; AD: adjusted HR = 2.05, 95% CI = 1.18 to 3.57, $p = 0.01$; VaD: adjusted HR = 1.82, 95% CI = 0.89 to 3.71, $p = 0.09$). Moreover, the risks of developing all-cause dementia, AD, and VaD significantly increased with elevated 2-hour postload glucose (PG) levels even after adjustment for covariates, but no such associations were observed for fasting plasma glucose (FPG) levels: compared with those with 2-hour PG levels of <6.7 mmol/L, the multivariable-adjusted HRs of all-cause dementia and AD significantly increased in subjects with 2-hour PG levels of 7.8 to 11.0 mmol/L or over, and the risk of VaD was significantly higher in subjects with levels of ≥11.1 mmol/L.

**Conclusions:** Our findings suggest that diabetes is a significant risk factor for all-cause dementia, AD, and probably VaD. Moreover, 2-hour PG levels, but not FPG levels, are closely associated with increased risk of all-cause dementia, AD, and VaD. *Neurology*® 2011;77:1126-1134

GLOSSARY

**AD** = Alzheimer disease; **CI** = confidence interval; ***DSM-III-R*** = *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition, revised; **FPG** = fasting plasma glucose; **HR** = hazard ratio; **IFG** = impaired fasting glycemia; **IGT** = impaired glucose tolerance; **NGT** = normal glucose tolerance; **OGTT** = oral glucose tolerance test; **PG** = postload glucose; **VaD** = vascular dementia.

Diabetes mellitus is one of the most common metabolic disorders, and its prevalence has risen globally in recent years. Some epidemiologic studies have reported that diabetes is independently implicated in the development of dementia.[1–3] However, these findings are inconsistent for its subtypes; one study found an association between diabetes and the risk of both Alzheimer disease (AD) and vascular dementia (VaD),[1] whereas other studies found an association with only AD[2,3] or only VaD,[4–7] and still others showed no association between diabetes and either condition.[8,9] These conflicting results may have been related to differences in the study designs, including the defined criteria for diabetes and dementia subtypes, as well as in the regional characteristics and ethnicities of the settings and subjects. Thus, accurate definitions of diabetes and dementia subtypes are needed to ascertain the true associations between the two, and a 75-g oral glucose tolerance test (OGTT) and morphologic examination of the brain may meet this requirement. However, to date, very few cohort studies have had enough quality data to allow reliable diagnosis using these methods.

Supplemental data at www.neurology.org

Supplemental Data

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

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To resolve these issues, we performed a prospective cohort study of dementia in a Japanese community-dwelling population, all members of which underwent the OGTT. The most important feature of this study is that the subtypes of dementia were verified by detailed neurologic and morphologic examination, including neuroimaging and autopsy. Using data from this cohort study, we investigated the association between glucose tolerance levels defined by the OGTT and the development of dementia and its subtypes.

## METHODS

**Study population.** A population-based prospective study of cerebro-cardiovascular diseases was begun in 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area of Kyushu Island in Japan. In addition, comprehensive surveys of cognitive impairment in the elderly of this town have been conducted since 1985. In 1988, a total of 1,228 residents aged ≥60 years (91.1% of the total population in this age group) participated in a screening examination for the present study. After exclusion of 33 subjects who had dementia, 90 who had already had breakfast, 5 who were on insulin therapy, and 81 who could not complete the OGTT, a total of 1,019 subjects without dementia underwent the OGTT. From a total of 1,019 subjects, 2 who died before starting follow-up were excluded, and the remaining 1,017 subjects (437 men and 580 women) were enrolled in this study.

**Follow-up survey.** The subjects were followed up prospectively for 15 years, from December 1988 to November 2003 (mean 10.9 years; SD 4.1 years). A complete description of the follow-up survey is provided in appendix e-1 on the *Neurology*® Web site at www.neurology.org.

**Diagnosis of dementia.** The diagnosis of dementia was made based on the guidelines of the *DSM-III-R*.[10] Subjects diagnosed with AD met the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria[11] and subjects diagnosed with VaD met the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria.[12] Possible or probable dementia subtypes were diagnosed with clinical information including neuroimaging. Definite dementia subtypes were also determined on the basis of clinical and neuropathologic information. The diagnostic procedure for autopsy cases was reported previously.[13] A neuropathologic diagnosis of AD was made following the National Institute on Aging–Reagan Institute criteria,[14] where the frequency of neuritic plaques and neurofibrillary tangles was evaluated using the Consortium to Establish a Registry for Alzheimer's Disease criteria[15] and Braak stage.[16] Definite VaD cases were confirmed with causative stroke or cerebrovascular change and no neuropathologic evidence of other forms of dementia. Every dementia case was adjudicated by expert psychiatrists.

During the follow-up, 232 subjects (79 men and 153 women) developed dementia. Of these, 201 (86.6%) were evaluated by brain imaging, and 118 (50.9%) underwent brain autopsy; in 110, both were performed. Thus, 209 subjects in all (90.1%) had some kind of morphologic examination. Among the 118 autopsy cases, the clinical diagnosis of 42 cases (35.6%) was changed by the neuropathologic findings. Among all dementia cases, 18 AD cases and 11 VaD cases had other coexisting subtypes of dementia. These cases were counted as events in the analysis for other dementia. In all, 105 cases were categorized as AD, 65 as VaD, and 62 as other dementia.

**Risk factor measurement.** At the baseline examination, we performed the OGTT after an at least 12-hour overnight fast. Plasma glucose levels were determined by the glucose-oxidase method. Glucose tolerance status was defined by the 1998 WHO criteria[17]: normal glucose tolerance (NGT), fasting plasma glucose (FPG) <6.1 and 2-hour postload glucose (PG) <7.8; impaired fasting glycemia (IFG), FPG 6.1 to 6.9 and 2-hour PG <7.8; impaired glucose tolerance (IGT), FPG <7.0 and 2-hour PG 7.8 to 11.0; and diabetes, FPG ≥7.0 mmol/L or 2-hour PG ≥11.1 mmol/L. Each of the FPG and 2-hour PG level was also divided into 4 categories (FPG: <5.6, 5.6 to 6.0, 6.1 to 6.9, and ≥7.0 mmol/L; 2-hour PG: <6.7, 6.7 to 7.7, 7.8 to 11.0, and ≥11.1 mmol/L).

In order to assess the independent effects of glucose tolerance levels on dementia occurrence, the following baseline factors in addition to age and sex were used as confounding factors: 1) information on smoking habits, alcohol intake, and physical activity was obtained by means of a questionnaire administered to each subject; 2) a low education level was defined as ≤6 years of formal education; 3) history of stroke was determined on the basis of all clinical data available in the Hisayama Study; 4) hypertension was defined as blood pressure levels ≥140/90 mm Hg or current treatment with antihypertensive agents; 5) EKG abnormalities were defined as left ventricular hypertrophy (Minnesota Code 3-1), ST depression (4-1, 2, or 3) or atrial fibrillation (8-3); 6) serum total cholesterol levels were measured enzymatically; and 7) body mass index (kg/m$^2$) and waist to hip ratio were used as indicators of obesity.

**Statistical analysis.** The SAS software package, version 9.2 (SAS Institute, Cary, NC), was used to perform all statistical analyses. Age- and sex-adjusted mean values of possible risk factors were calculated by the analysis of covariance method. Frequencies of risk factors were adjusted for age and sex by the direct method. The differences in the mean values and frequencies of risk factors between NGT and other glucose tolerance levels were tested using Fisher least significant difference method and logistic regression analysis, respectively. The incidence of dementia was calculated by the person-years method and was adjusted for age and sex by the direct method using 5-year age groups of the overall study population; the differences among glucose tolerance levels and trends across FPG and 2-hour PG levels were tested using Cox proportional hazards model. The adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) were also calculated using the Cox proportional hazards model. Missing values of waist to hip ratio (n = 27) and education (n = 12) were replaced with the means in the multivariate analysis. The population attributable fraction of combined category of IGT and diabetes for dementia was calculated using the following equation with the observed multivariate-adjusted HR of the combined category and its frequency in event cases (Pe)[18]:

$$PAF = Pe\,(HR - 1)/HR$$

Two-sided $p < 0.05$ was considered statistically significant in all analyses.

**Standard protocol approvals, registrations, and patient consents.** This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Re-

Table 1 Age- and sex-adjusted mean values or frequencies of potential risk factors for dementia according to the 1998 WHO criteria: The Hisayama Study, 1988[a]

| | Normal glucose tolerance (n = 559) | Impaired fasting glycemia (n = 73) | Impaired glucose tolerance (n = 235) | Diabetes (n = 150) | No. of missing values |
|---|---|---|---|---|---|
| Age, y, mean (SD) | 68 (6) | 70 (6)[b] | 69 (6) | 69 (6) | 0 |
| Men, % | 40.8 | 52.1 | 43.8 | 45.3 | 0 |
| Fasting plasma glucose, mmol/L, mean (SD) | 5.3 (0.9) | 6.4 (0.9)[c] | 5.8 (0.9)[c] | 7.7 (0.9)[c] | 0 |
| Two-hour postload glucose, mmol/L, mean (SD) | 5.9 (2.2) | 5.9 (2.2) | 8.9 (2.2)[c] | 14.9 (2.2)[c] | 0 |
| Systolic blood pressure, mm Hg, mean (SD) | 133 (21) | 141 (21)[c] | 143 (21)[c] | 145 (21)[c] | 0 |
| Diastolic blood pressure, mm Hg, mean (SD) | 75 (10) | 76 (10) | 78 (10)[c] | 77 (10)[b] | 0 |
| Hypertension, %[d] | 43.8 | 66.7[c] | 63.2[c] | 62.2[c] | 0 |
| Electrocardiogram abnormalities, % | 20.6 | 31.7 | 18.8 | 21.6 | 0 |
| Body mass index, kg/m$^2$, mean (SD) | 21.8 (3.0) | 22.2 (3.0) | 23.2 (3.0)[c] | 23.2 (3.0)[c] | 0 |
| Waist to hip ratio, cm/cm, mean (SD) | 0.91 (0.07) | 0.93 (0.07)[b] | 0.93 (0.07)[c] | 0.94 (0.07)[c] | 27 |
| Total cholesterol, mmol/L, mean (SD) | 5.3 (1.1) | 5.5 (1.1) | 5.4 (1.1) | 5.7 (1.1)[c] | 0 |
| History of stroke at entry, % | 3.3 | 3.5 | 5.9 | 6.3 | 0 |
| Education ≤6 y, % | 10.3 | 12.5 | 13.9 | 11.3 | 12 |
| Smoking, % | 23.5 | 23.8 | 23.5 | 22.7 | 0 |
| Alcohol intake, % | 23.4 | 29.0 | 27.7 | 34.8[c] | 0 |
| Physical activity, % | 20.2 | 22.8 | 16.8 | 14.7 | 0 |

[a] Mean age was sex adjusted. Percentage of men was age adjusted. Electrocardiogram abnormalities were defined as Minnesota Code 3-1, 4-1, 4-2, 4-3, or 8-3.
[b] $p < 0.05$ vs normal glucose tolerance.
[c] $p < 0.01$ vs normal glucose tolerance.
[d] Hypertension: blood pressure ≥140/90 mm Hg or current use of antihypertensive agents.

search, and written informed consent was obtained from the participants.

**RESULTS** Table 1 shows the age- and sex-adjusted mean values or frequencies of risk factors for dementia by the WHO criteria at baseline. Compared with those with NGT, the mean values of systolic and diastolic blood pressures, body mass index, waist to hip ratio, and total cholesterol, and the frequencies of hypertension and alcohol intake, were higher in subjects with IFG, IGT; or diabetes.

The age- and sex-adjusted incidences and adjusted HRs of all-cause dementia and its subtypes according to glucose tolerance status defined by the WHO criteria are shown in table 2. Compared with those with NGT, the age- and sex-adjusted incidence and HR of all-cause dementia were significantly higher in subjects with IGT as well as those with diabetes. This association remained unchanged in subjects with diabetes even after adjustment for age, sex, hypertension, EKG abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity. In regard to subtypes of dementia, the age- and sex-adjusted incidence and adjusted HRs of AD were significantly higher in subjects with diabetes than in those with NGT. The age- and sex-adjusted incidence and HR of VaD were significantly increased in subjects with IGT or diabetes compared with those with NGT; however, these associations were not significant after multivariable adjustment. No significant associations were observed between glucose tolerance levels and the risk of other dementia. When IGT and diabetes were brought together in one category, this category also had the significantly higher risks of all-cause dementia, AD, and VaD in the age- and sex-adjusted analysis, and these associations remained significant for all-cause dementia and AD even after adjustment for other possible risk factors. The population attributable fraction of this combined category was 14.6% for all-cause dementia, 20.1% for AD, and 17.0% for VaD.

Table 3 presents the associations between FPG levels and adjusted risks of all-cause dementia and its subtypes. The age- and sex-adjusted incidences and HRs of all-cause dementia and any of the dementia subtypes did not differ among FPG levels. This tendency was unchanged even in the multivariate analysis. Conversely, as shown in table 4, the age- and

Table 2 **Age- and sex-adjusted incidence and adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes according to glucose tolerance status defined by WHO criteria**

| Glucose tolerance level | Person-years at risk, n | No. of events, n | Age- and sex-adjusted incidence | Crude HR (95% CI) | p | Age- and sex-adjusted HR (95% CI) | p | Multivariable-adjusted[a] HR (95% CI) | p |
|---|---|---|---|---|---|---|---|---|---|
| **All-cause dementia** | | | | | | | | | |
| Normal | 6,658 | 115 | 20.1 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| IFG | 854 | 13 | 16.0 | 0.89 (0.50–1.58) | 0.70 | 0.74 (0.42–1.31) | 0.30 | 0.63 (0.35–1.13) | 0.12 |
| IGT | 2,611 | 63 | 24.9 | 1.46 (1.07–1.99) | 0.02 | 1.40 (1.03–1.91) | 0.03 | 1.35 (0.98–1.86) | 0.07 |
| DM | 1,544 | 41 | 29.3 | 1.62 (1.14–2.32) | 0.008 | 1.71 (1.19–2.44) | 0.003 | 1.74 (1.19–2.53) | 0.004 |
| IGT + DM | 4,155 | 104 | 26.3 | 1.52 (1.17–1.98) | 0.002 | 1.51 (1.16–1.97) | 0.002 | 1.46 (1.10–1.92) | 0.008 |
| **Alzheimer disease** | | | | | | | | | |
| Normal | 6,658 | 51 | 8.6 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| IFG | 854 | 5 | 6.6 | 0.77 (0.31–1.94) | 0.58 | 0.63 (0.25–1.57) | 0.32 | 0.61 (0.24–1.55) | 0.29 |
| IGT | 2,611 | 29 | 11.7 | 1.53 (0.97–2.41) | 0.07 | 1.46 (0.92–2.30) | 0.11 | 1.60 (0.99–2.59) | 0.05 |
| DM | 1,544 | 20 | 14.2 | 1.81 (1.08–3.03) | 0.03 | 1.94 (1.16–3.26) | 0.01 | 2.05 (1.18–3.57) | 0.01 |
| IGT + DM | 4,155 | 49 | 12.5 | 1.63 (1.10–2.41) | 0.01 | 1.62 (1.10–2.40) | 0.02 | 1.73 (1.15–2.60) | 0.009 |
| **Vascular dementia** | | | | | | | | | |
| Normal | 6,658 | 27 | 5.1 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| IFG | 854 | 6 | 7.1 | 1.76 (0.73–4.26) | 0.21 | 1.40 (0.58–3.41) | 0.46 | 1.01 (0.41–2.52) | 0.98 |
| IGT | 2,611 | 20 | 7.8 | 1.95 (1.09–3.47) | 0.02 | 1.86 (1.05–3.32) | 0.04 | 1.39 (0.76–2.54) | 0.29 |
| DM | 1,544 | 12 | 8.7 | 2.00 (1.01–3.95) | 0.04 | 2.07 (1.05–4.09) | 0.04 | 1.82 (0.89–3.71) | 0.09 |
| IGT + DM | 4,155 | 32 | 7.9 | 1.97 (1.18–3.29) | 0.01 | 1.94 (1.16–3.23) | 0.01 | 1.54 (0.90–2.63) | 0.11 |
| **Other dementia** | | | | | | | | | |
| Normal | 6,658 | 37 | 6.4 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| IFG | 854 | 2 | 2.2 | 0.42 (0.10–1.75) | 0.23 | 0.36 (0.09–1.51) | 0.16 | 0.34 (0.08–1.44) | 0.14 |
| IGT | 2,611 | 14 | 5.5 | 0.99 (0.54–1.84) | 0.99 | 0.96 (0.52–1.78) | 0.90 | 0.94 (0.49–1.78) | 0.84 |
| DM | 1,544 | 9 | 6.5 | 1.08 (0.52–2.24) | 0.83 | 1.10 (0.53–2.28) | 0.80 | 1.19 (0.56–2.52) | 0.66 |
| IGT + DM | 4,155 | 23 | 5.8 | 1.03 (0.61–1.73) | 0.92 | 1.01 (0.60–1.70) | 0.97 | 0.97 (0.57–1.67) | 0.91 |

Abbreviations: CI = confidence interval; DM = diabetes mellitus; HR = hazard ratio; IFG = impaired fasting glycemia; IGT = impaired glucose tolerance.

[a] Multivariate adjustment was made for age, sex, hypertension, electrocardiogram abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity.

sex-adjusted incidences and HRs of all-cause dementia, AD, and VaD significantly increased with rising 2-hour PG levels. Compared with those with 2-hour PG levels of <6.7 mmol/L, the age- and sex-adjusted incidences and HRs of all-cause dementia, AD, and VaD were marginally or significantly higher in subjects with 2-hour PG levels of 7.8 to 11.0 mmol/L and significantly higher in subjects with 2-hour PG levels of ≥11.1 mmol/L. These associations remained robust even after multivariable adjustment; the risks of all-cause dementia and AD were significantly increased in subjects with 2-hour PG levels of 7.8 to 11.0 mmol/L and over, and the risk of VaD was significantly higher in those with 2-hour PG levels of ≥11.1 mmol/L.

Sensitivity analysis in which only definite cases of dementia determined by brain autopsy were used as event cases did not make any material difference in these findings, except with respect to VaD, for which the significant association disappeared, probably due to the few event cases (table 5). When only clinical diagnoses were used for cases with both clinical and neuropathologic diagnoses, the findings were substantially unchanged, though the HRs became slightly lower probably due to the decreased accuracy of diagnosis (tables e-1, e-2, and e-3).

**DISCUSSION** In a long-term prospective study of an elderly Japanese population, we demonstrated that diabetes that was assessed 15 years earlier was a significant risk factor for the development of all-cause dementia, AD, and VaD. Moreover, the risks of developing all-cause dementia and its sub-

Table 3 **Age- and sex-adjusted incidence and adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes according to fasting plasma glucose levels**

| Fasting plasma glucose levels | Person-years at risk, n | No. of events, n | Age- and sex-adjusted incidence | Crude HR (95% CI) | p | Age- and sex-adjusted HR (95% CI) | p | Multivariable-adjusted[a] HR (95% CI) | p |
|---|---|---|---|---|---|---|---|---|---|
| **All-cause dementia** | | | | | | | | | |
| **<5.6** | 5,589 | 101 | 20.7 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **5.6–6.0** | 3,286 | 71 | 25.1 | 1.24 (0.91–1.68) | 0.17 | 1.21 (0.89–1.64) | 0.22 | 1.18 (0.86–1.61) | 0.31 |
| **6.1–6.9** | 1,724 | 39 | 21.6 | 1.13 (0.91–1.91) | 0.14 | 1.13 (0.78–1.64) | 0.52 | 0.96 (0.65–1.41) | 0.82 |
| **≥7.0** | 1,067 | 21 | 22.3 | 1.21 (0.70–1.79) | 0.64 | 1.14 (0.71–1.82) | 0.60 | 1.21 (0.75–1.96) | 0.44 |
| | | | | *p* for trend: 0.23 | | *p* for trend: 0.42 | | *p* for trend: 0.63 | |
| **Alzheimer disease** | | | | | | | | | |
| **<5.6** | 5,589 | 48 | 10.1 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **5.6–6.0** | 3,286 | 30 | 10.3 | 1.11 (0.70–1.74) | 0.67 | 1.14 (0.72–1.80) | 0.58 | 1.11 (0.69–1.77) | 0.68 |
| **6.1–6.9** | 1,724 | 16 | 9.1 | 1.15 (0.65–2.02) | 0.64 | 1.00 (0.57–1.77) | 0.99 | 0.99 (0.49–1.64) | 0.72 |
| **≥7.0** | 1,067 | 11 | 11.9 | 1.23 (0.64–2.37) | 0.53 | 1.29 (0.67–2.48) | 0.45 | 1.41 (0.72–2.76) | 0.32 |
| | | | | *p* for trend: 0.47 | | *p* for trend: 0.56 | | *p* for trend: 0.58 | |
| **Vascular dementia** | | | | | | | | | |
| **<5.6** | 5,589 | 24 | 4.9 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **5.6–6.0** | 3,286 | 19 | 6.7 | 1.38 (0.76–2.52) | 0.29 | 1.29 (0.71–2.36) | 0.41 | 1.19 (0.64–2.19) | 0.58 |
| **6.1–6.9** | 1,724 | 17 | 8.7 | 2.40 (1.29–4.47) | 0.006 | 1.93 (1.03–3.61) | 0.04 | 1.48 (0.77–2.84) | 0.24 |
| **≥7.0** | 1,067 | 5 | 5.2 | 1.12 (0.43–2.93) | 0.82 | 1.10 (0.42–2.89) | 0.84 | 0.99 (0.37–2.69) | 0.99 |
| | | | | *p* for trend: 0.10 | | *p* for trend: 0.19 | | *p* for trend: 0.49 | |
| **Other dementia** | | | | | | | | | |
| **<5.6** | 5,589 | 29 | 5.7 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **5.6–6.0** | 3,286 | 22 | 8.1 | 1.33 (0.76–2.31) | 0.32 | 1.27 (0.73–2.21) | 0.40 | 1.21 (0.68–2.16) | 0.51 |
| **6.1–6.9** | 1,724 | 6 | 3.8 | 0.69 (0.29–1.67) | 0.42 | 0.60 (0.25–1.45) | 0.26 | 0.53 (0.22–1.31) | 0.17 |
| **≥7.0** | 1,067 | 5 | 5.2 | 0.92 (0.36–2.37) | 0.86 | 0.91 (0.35–2.36) | 0.85 | 1.02 (0.39–2.67) | 0.97 |
| | | | | *p* for trend: 0.68 | | *p* for trend: 0.53 | | *p* for trend: 0.52 | |

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a] Multivariate adjustment was made for age, sex, hypertension, electrocardiogram abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity.

types progressively increased with elevating 2-hour PG levels.

In prior prospective epidemiologic studies, there have been conflicting results regarding the associations between diabetes and incidences of all-cause dementia and AD, while the influence of diabetes on the risk of VaD has been positive in most studies.[1,4–7] Cohort studies in which diabetes was defined by nonfasting blood glucose levels or clinical information did not reveal clear associations of diabetes with the development of all-cause dementia and AD,[4–8] while the risks of dementia and its subtypes significantly increased in diabetes in some studies, most of which defined diabetes using the OGTT.[1–3] The latter findings were in accord with ours. This fact suggests that differences in the methods used to define diabetes lead to a discrepancy in the association between diabetes and the risk of dementia, especially AD, and that an OGTT is essential for the definition of diabetes in epidemiologic studies on the diabetes–dementia association.

In our study, the incidence of VaD was significantly higher in subjects with IGT or diabetes than in those with NGT, but this association disappeared after adjustment for other covariates. This might occur due to the few VaD cases. In addition, since other known cardiovascular risk factors, such as hypertension, obesity, and dyslipidemia, accumulate under a prediabetic or diabetic state, as shown in our data (table 1), IGT and diabetes seem to increase the risk of VaD through mediation of these risk factors, especially hypertension.

In the present study, increased 2-hour PG levels including a prediabetic range were significantly

Table 4 **Age- and sex-adjusted incidence and adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes according to 2-hour postload glucose levels**

| 2-Hour postload glucose levels | Person-years at risk, n | No. of events, n | Age- and sex-adjusted incidence | Crude HR (95% CI) | p | Age- and sex-adjusted HR (95% CI) | p | Multivariable-adjusted[a] HR (95% CI) | p |
|---|---|---|---|---|---|---|---|---|---|
| **All-cause dementia** | | | | | | | | | |
| **<6.7** | 5,354 | 85 | 17.6 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **6.7–7.7** | 2,277 | 44 | 20.9 | 1.20 (0.84–1.73) | 0.32 | 1.25 (0.87–1.80) | 0.24 | 1.16 (0.78–1.71) | 0.47 |
| **7.8–11.0** | 2,844 | 67 | 24.7 | 1.53 (1.11–2.11) | 0.009 | 1.54 (1.12–2.12) | 0.009 | 1.50 (1.07–2.11) | 0.02 |
| **≥11.1** | 1,192 | 36 | 32.8 | 2.08 (1.41–3.07) | <0.001 | 2.32 (1.57–3.44) | <0.001 | 2.47 (1.62–3.77) | <0.001 |
| | | | | *p* for trend: <0.001 | | *p* for trend: <0.001 | | *p* for trend: <0.001 | |
| **Alzheimer disease** | | | | | | | | | |
| **<6.7** | 5,354 | 37 | 7.6 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **6.7–7.7** | 2,277 | 20 | 8.8 | 1.25 (0.73–2.16) | 0.41 | 1.23 (0.71–2.12) | 0.46 | 1.49 (0.83–2.67) | 0.17 |
| **7.8–11.0** | 2,844 | 30 | 11.3 | 1.59 (0.98–2.57) | 0.06 | 1.56 (0.96–2.53) | 0.07 | 1.87 (1.13–3.12) | 0.02 |
| **≥11.1** | 1,192 | 18 | 15.8 | 2.44 (1.39–4.29) | 0.002 | 2.75 (1.56–4.85) | <0.001 | 3.42 (1.83–6.40) | <0.001 |
| | | | | *p* for trend: 0.002 | | *p* for trend: <0.001 | | *p* for trend: <0.001 | |
| **Vascular dementia** | | | | | | | | | |
| **<6.7** | 5,354 | 21 | 4.6 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **6.7–7.7** | 2,277 | 12 | 6.3 | 1.33 (0.65–2.70) | 0.43 | 1.49 (0.73–3.04) | 0.27 | 1.14 (0.54–2.41) | 0.73 |
| **7.8–11.0** | 2,844 | 20 | 7.2 | 1.83 (0.99–3.38) | 0.05 | 1.87 (1.01–3.45) | 0.04 | 1.38 (0.72–2.64) | 0.34 |
| **≥11.1** | 1,192 | 12 | 11.2 | 2.75 (1.35–5.60) | 0.005 | 3.15 (1.55–6.43) | 0.002 | 2.66 (1.24–5.70) | 0.01 |
| | | | | *p* for trend: 0.004 | | *p* for trend: 0.002 | | *p* for trend: 0.02 | |
| **Other dementia** | | | | | | | | | |
| **<6.7** | 5,354 | 27 | 5.4 | 1 (referent) | | 1 (referent) | | 1 (referent) | |
| **6.7–7.7** | 2,277 | 12 | 5.8 | 1.04 (0.52–2.04) | 0.92 | 1.08 (0.55–2.15) | 0.82 | 0.86 (0.40–1.84) | 0.70 |
| **7.8–11.0** | 2,844 | 17 | 6.2 | 1.21 (0.66–2.23) | 0.53 | 1.21 (0.66–2.23) | 0.53 | 1.14 (0.60–2.16) | 0.69 |
| **≥11.1** | 1,192 | 6 | 5.8 | 1.05 (0.44–2.55) | 0.91 | 1.12 (0.46–2.71) | 0.81 | 1.21 (0.48–3.04) | 0.69 |
| | | | | *p* for trend: 0.65 | | *p* for trend: 0.59 | | *p* for trend: 0.59 | |

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a] Multivariate adjustment was made for age, sex, hypertension, electrocardiogram abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity.

linked to elevated risks of all-cause dementia, AD, and VaD, but no such associations were observed for FPG. The epidemiologic evidence from Asia has also indicated that 2-hour PG levels are better in detecting prediabetes and diabetes compared with FPG levels.[19] However, very few prospective studies have investigated the associations between FPG as well as 2-hour PG levels and the risks of dementia and its subtypes. Only the Uppsala Longitudinal Study of Adult Men evaluated the associations of FPG levels with the risks of developing AD and VaD,[20,21] and this study concluded that increased FPG levels were not risk factors for these subtypes of dementia. This is in good agreement with our findings. The Uppsala Study[21] and the Honolulu-Asia Aging Study[1] also found no clear associations between 2-hour PG levels and the risks of AD and VaD. These findings are inconsistent with ours. Our recent clinicopathologic study of deceased Hisayama residents revealed that higher levels of 2-hour PG but not of FPG were clearly associated with increased risk for formation of neuritic plaques even after adjustment for confounding factors.[22] This evidence together with the findings of the present study suggests that elevated 2-hour PG levels play an important role in the formation of neuritic plaques, and thereby in the development of AD. Meanwhile, it is well known that increased 2-hour PG levels are closely associated with the development of stroke, which is well established as a main cause of VaD. Thus, it is reasonable to postulate a close association between 2-hour PG levels and the risk of VaD.

Possible pathophysiologic mechanisms through which diabetes or elevated blood glucose levels might

Table 5 Age- and sex-adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes determined by autopsy according to 2-hour postload glucose levels

| 2-Hour postload glucose levels | Person-years at risk, n | No. of events, n | Crude HR (95% CI) | p | Age- and sex-adjusted HR (95% CI) | p |
|---|---|---|---|---|---|---|
| **All-cause dementia** | | | | | | |
| <6.7 | 5,354 | 47 | 1 (referent) | | 1 (referent) | |
| 6.7–7.7 | 2,277 | 23 | 1.14 (0.69–1.88) | 0.61 | 1.24 (0.75–2.05) | 0.39 |
| 7.8–11.0 | 2,844 | 29 | 1.19 (0.75–1.89) | 0.47 | 1.20 (0.76–1.91) | 0.44 |
| ≥11.1 | 1,192 | 19 | 1.94 (1.14–3.31) | 0.01 | 2.24 (1.31–3.83) | 0.003 |
| | | | *p* for trend: 0.04 | | *p* for trend: 0.02 | |
| **Alzheimer disease** | | | | | | |
| <6.7 | 5,354 | 12 | 1 (referent) | | 1 (referent) | |
| 6.7–7.7 | 2,277 | 7 | 1.35 (0.53–3.44) | 0.53 | 1.40 (0.55–3.56) | 0.48 |
| 7.8–11.0 | 2,844 | 12 | 1.94 (0.87–4.33) | 0.10 | 1.92 (0.86–4.26) | 0.11 |
| ≥11.1 | 1,225 | 8 | 3.27 (1.34–8.00) | 0.009 | 3.88 (1.58–9.53) | 0.003 |
| | | | *p* for trend: 0.009 | | *p* for trend: 0.005 | |
| **Vascular dementia** | | | | | | |
| <6.7 | 5,354 | 17 | 1 (referent) | | 1 (referent) | |
| 6.7–7.7 | 2,277 | 8 | 1.09 (0.47–2.54) | 0.83 | 1.23 (0.53–2.86) | 0.63 |
| 7.8–11.0 | 2,844 | 8 | 0.90 (0.39–2.09) | 0.81 | 0.92 (0.40–2.12) | 0.84 |
| ≥11.1 | 1,192 | 7 | 1.98 (0.82–4.77) | 0.13 | 2.32 (0.96–5.61) | 0.06 |
| | | | *p* for trend: 0.36 | | *p* for trend: 0.26 | |
| **Other dementia** | | | | | | |
| <6.7 | 5,354 | 18 | 1 (referent) | | 1 (referent) | |
| 6.7–7.7 | 2,277 | 8 | 1.04 (0.45–2.39) | 0.93 | 1.17 (0.51–2.70) | 0.72 |
| 7.8–11.0 | 2,844 | 9 | 0.96 (0.43–2.14) | 0.92 | 0.98 (0.44–2.19) | 0.97 |
| ≥11.1 | 1,192 | 4 | 1.04 (0.35–3.07) | 0.95 | 1.16 (0.39–3.43) | 0.79 |
| | | | *p* for trend: 0.99 | | *p* for trend: 0.88 | |

Abbreviations: CI = confidence interval; HR = hazard ratio.

affect the initiation and promotion of dementia have been extensively discussed in a number of studies.[23] A recent review summarized 4 major pathways for hyperglycemia-induced dementia: namely, atherosclerosis, microvascular disease, glucose toxicity leading to the accumulation of advanced protein glycation and increased oxidative stress, and changes in insulin metabolism resulting in an insulin-resistant state and distorted amyloid metabolism in the brain.[23] The former 2 pathways are considered to be involved in the development of VaD, while the latter 2 pathways may mainly contribute to the development of AD. Additionally, recent evidence has emerged to imply that vascular factors may be involved in AD.[23] It is reported that 2-hour PG values can be a good marker of oxidative stress levels arising from hyperglycemia[24,25] and correlate with insulin resistance.[26] Higher oxidative stress and insulin resistance may precede the accumulation of amyloid-$\beta$ peptide and neurofibrillary tangles[23,27] and accelerate arteriosclerosis in the brain,[28] resulting in increased risk of AD and VaD. It is known that Asians have lower levels of insulin secretion compared with other ethnic groups[29] and can develop diabetes, insulin resistance, and metabolic syndrome with lower body mass index levels.[30] These findings suggest that hyperglycemia plays a larger role in the development of dementia compared with insulin resistance in Asians including Japanese. Further studies are needed to elucidate the pathogenesis of hyperglycemia and diabetes in the development of dementia.

The strengths of our study include its longitudinal population-based study design, use of OGTT for determination of glucose tolerance levels in all subjects, long duration of follow-up, perfect follow-up of subjects, and morphologic examination of the brains of most dementia cases with autopsy and neuroimaging. Several limitations of our study should be noted. First, the diagnosis of glucose tolerance status was based on a single measurement of glucose levels at baseline, as was the case in most other epidemiologic studies. During the follow-up, risk factor levels were changed due to modifications in lifestyle or medication especially in subjects with diabetes, and

misclassification of glucose tolerance categories was possible. This could have weakened the association found in this study, biasing the results toward the null hypothesis. Therefore, the true association may be stronger than that shown here. Second, some subjects (n = 33 to 65) did not participate in the follow-up surveys of cognitive function performed in 1992, 1998, and 2005, and their cognitive conditions were evaluated only by mail or telephone. This might have resulted in failure to detect dementia cases. However, we also collected information on the development of dementia in another way, namely through the daily monitoring system established in the town. Thus, we believe that we detected almost all dementia cases, and this bias did not affect our findings. Third, the diagnosis of dementia was verified by autopsy only in 50.9% of dementia cases, resulting in a certain degree of subtype misclassification; agreement rate between clinical diagnosis and neuropathologic diagnosis was not high (64.4%) in our autopsy cases of dementia. However, a sensitivity analysis using only definite cases of dementia determined by brain autopsy did not make any material difference in our findings.

Our findings emphasize the need to consider diabetes as a potential risk factor for all-cause dementia, AD, and probably VaD. The other main finding, that elevated 2-hour PG levels are closely associated with increased risks of all-cause dementia and its subtypes, supports the view that postprandial glucose regulation is critical to prevent future dementia. Further investigations are required to clarify the associations between 2-hour PG levels by the OGTT and subtypes of dementia in other ethnic populations.

## AUTHOR CONTRIBUTIONS

Tomoyuki Ohara contributed to the study concept, design, data collection, endpoint adjudication, interpretation of data, statistical analysis, and writing the manuscript. Yasufumi Doi contributed to the study concept, design, interpretation of data, statistical analysis, and writing the manuscript. Toshiharu Ninomiya contributed to the data collection, endpoint adjudication, interpretation of data, and statistical analysis. Yoichiro Hirakawa and Jun Hata contributed to data collection and interpretation of data. Toru Iwaki and Shigenobu Kanba contributed to endpoint adjudication and interpretation of data. Yutaka Kiyohara is a study coordinator and contributed to the study performance, obtaining supporting sources, study concept, design, endpoint adjudication, interpretation of data, and writing of manuscript. All authors critically reviewed the manuscript and approved final version.


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## DISCLOSURE

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## REFERENCES


1. 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.
2. 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.
3. Leibson CL, Rocca WA, Hanson VA, et al. Risk of dementia among persons with diabetes mellitus: a population-based cohort study. Am J Epidemiol 1997;145:301–308.
4. Xu WL, von Strauss E, Qiu CX, Winblad B, Fratiglioni L. Uncontrolled diabetes increases the risk of Alzheimer's disease: a population-based cohort study. Diabetologia 2009;52:1031–1039.
5. Luchsinger JA, Tang MX, Stern Y, Shea S, Mayeux R. Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. Am J Epidemiol 2001;154:635–641.
6. MacKnight C, Rockwood K, Awalt E, McDowell I. Diabetes mellitus and the risk of dementia, Alzheimer's disease and vascular cognitive impairment in the Canadian Study of Health and Aging. Dement Geriatr Cogn Disord 2002;14:77–83.
7. Hassing LB, Johansson B, Nilsson SE, et al. Diabetes mellitus is a risk factor for vascular dementia, but not for Alzheimer's disease: a population-based study of the oldest old. Int Psychogeriatr 2002;14:239–248.
8. Akomolafe A, Beiser A, Meigs JB, et al. Diabetes mellitus and risk of developing Alzheimer disease: results from the Framingham Study. Arch Neurol 2006;63:1551–1555.
9. Irie F, Fitzpatrick AL, Lopez OL, et al. Enhanced risk for Alzheimer disease in persons with type 2 diabetes and APOE $\epsilon$4: the Cardiovascular Health Study Cognition Study. Arch Neurol 2008;65:89–93.
10. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 3rd ed, revised. Washington, DC: American Psychiatric Association; 1987.
11. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.
12. Román GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. report of the NINDS-AIREN International Workshop. Neurology 1993;43:250–260.
13. Fujimi K, Sasaki K, Noda K, et al. Clinicopathological outline of dementia with Lewy bodies applying the revised criteria: the Hisayama Study. Brain Pathol 2008;18:317–325.

14. The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. Neurobiol Aging 1997;18:S1–S2.
15. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD): part II: standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479–486.
16. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–259.
17. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications: part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–553.
18. Rockhill B, Newman B, Weinberg C. Use and misuse of population attributable fractions. Am J Public Health 1998;88:15–19.
19. Qiao Q, Nakagami T, Tuomilehto J, et al. Comparison of the fasting and the 2-h glucose criteria for diabetes in different Asian cohorts. Diabetologia 2000;43:1470–1475.
20. Rönnemaa E, Zethelius B, Sundelöf J, et al. Impaired insulin secretion increases the risk of Alzheimer disease. Neurology 2008;71:1065–1071.
21. Rönnemaa E, Zethelius B, Sundelöf J, et al. Glucose metabolism and the risk of Alzheimer's disease and dementia: a population-based 12 year follow-up study in 71-year-old men. Diabetologia 2009;52:1504–1510.
22. Matsuzaki T, Sasaki K, Tanizaki Y, et al. Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama Study. Neurology 2010;75:764–770.
23. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol 2006;5:64–74.
24. Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006;295:1681–1687.
25. Wolever TMS, Chiasson JL, Csima A, et al. Variation of postprandial plasma glucose, palatability, and symptoms associated with a standardized mixed test meal versus 75 g oral glucose. Diabetes Care 1998;21:336–340.
26. Rendell MS, Jovanovic L. Targeting postprandial hyperglycemia. Metabolism 2006;55:1263–1281.
27. Nunomura A, Perry G, Aliev G, et al. Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 2001;60:759–767.
28. Ceriello A, Taboga C, Tonutti L, et al. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. Circulation 2002;106:1211–1218.
29. Jensen CC, Cnop M, Hull RL, Fujimoto WY, Kahn SE and the American Diabetes Association GENNID study group. β-Cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the US. Diabetes 2002;51:2170–2178.
30. Fukushima M, Usami M, Ikeda M, et al. Insulin secretion and insulin sensitivity at different stages of glucose tolerance: a cross-sectional study of Japanese type 2 diabetes. Metabolism 2004;53:831–835.

# Association of Alzheimer disease pathology with abnormal lipid metabolism

## The Hisayama Study

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ABSTRACT

**Objective:** The relationship between lipid profiles and Alzheimer disease (AD) pathology at the population level is unclear. We searched for evidence of AD-related pathologic risk of abnormal lipid metabolism.

**Methods:** This study included brain specimens from a series of 147 autopsies performed between 1998 and 2003 of residents in Hisayama town, Japan (76 men and 71 women), who underwent clinical examinations in 1988. Lipid profiles, such as total cholesterol (TC), triglycerides, and high-density lipoprotein cholesterol (HDLC), were measured in 1988. Low-density lipoprotein cholesterol (LDLC) was calculated using the Friedewald formula. Neuritic plaques (NPs) were assessed according to the Consortium to Establish a Registry for Alzheimer's Disease guidelines (CERAD) and neurofibrillary tangles (NFTs) were assessed according to Braak stage. Associations between each lipid profile and AD pathology were examined by analysis of covariance and logistic regression analyses.

**Results:** Adjusted means of TC, LDLC, TC/HDLC, LDLC/HDLC, and non-HDLC (defined as TC-HDLC) were significantly higher in subjects with NPs, even in sparse to moderate stages (CERAD = 1 or 2), compared to subjects without NPs in multivariate models including *APOE* $\epsilon$4 carrier and other confounding factors. The subjects in the highest quartiles of these lipid profiles had significantly higher risks of NPs compared to subjects in the lower respective quartiles, which may suggest a threshold effect. Conversely, there was no relationship between any lipid profile and NFTs.

**Conclusion:** The results of this study suggest that dyslipidemia increases the risk of plaque-type pathology. *Neurology*® 2011;77:1068-1075

GLOSSARY

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

To elucidate the association of lifestyle diseases with Alzheimer disease (AD) pathology, a large-scale, population-based clinicopathologic study is required. Since 1961, we have been conducting a long-term prospective cohort study of cerebro-cardiovascular diseases in the town of Hisayama, a suburb of Fukuoka City in Japan. Careful surveillance of cognitive impairment was started from 1985, which was carried out through a daily monitoring system established by the study team, local practitioners, and the town government. In a series of studies, we have reported the incidence and survival of dementia,[1] and trends in the prevalence of AD and vascular dementia.[2] These studies indicate that the prevalence of AD is increasing at an accelerating pace in parallel with an increase of metabolic disorders. Recently, we also reported that insulin

Supplemental data at www.neurology.org

Supplemental Data

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resistance is associated with the plaque-type pathology of AD,[3] even though there are some controversial findings.[4,5]

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

## METHODS

**Subjects.** The design of the Hisayama Study has been described in detail elsewhere.[3,8–10] 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.[9] Briefly, of a total of 3,227 residents aged 40–79 years included in the study registry, 2,587 (participation rate, 80.2%) took part in a clinical examination in 1988. Of the 214 autopsy cases, we excluded 3 subjects whose brain specimens were inadequate for evaluation, and 64 subjects who did not complete the fasting blood protocol in 1988. Finally, 147 subjects who underwent both the fasting blood protocol and brain autopsy were included in the present study. None of the 147 subjects showed signs of dementia at the clinical examination in 1988. The study subjects mostly overlapped with those in our previous study, in which we reported the association of insulin resistance with the plaque-type pathology of AD.[3]

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

**Risk factors.** In the clinical examination performed in 1988, blood samples were collected on the morning after an overnight fast. We used values of total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), triglycerides (TG), TC/HDLC, LDLC/HDLC, and non-HDLC as lipid profiles. Levels of TC, HDLC, and TG were determined enzymatically. LDLC was calculated using the Friedewald formula (LDLC = TC − HDLC − TG/5).[11] Non-HDLC was defined as non-HDLC = TC − HDLC. Other risk factors were also measured as described previously.[3] *APOE* genotyping was determined by direct sequencing. The homozygous ϵ4 genotype was not found among these participants, and those who carried one copy of the ϵ4 allele were categorized as *APOE* ϵ4 carriers.

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

**Statistical analyses.** Mean or geometric mean values of continuous data among the NP or NFT groups were adjusted for age and sex and compared by analysis of covariance. Proportions of categorical data were adjusted for age and sex by direct method and compared by logistic regression analysis. We also used logistic regression analysis to determine relationships between risk factors and pathologic outcome, which are expressed as odds ratios (OR) and 95% confidence intervals (CI). Model 1 was adjusted for age and sex. Model 2 was adjusted for model 1 plus systolic blood pressure, fasting blood glucose levels, fasting insulin levels, body mass index, smoking habit, regular exercise, and cerebrovascular disease. Model 3 was adjusted for model 2 plus *APOE* ϵ4 carrier.

Each lipid profile was divided into 4 groups to compare the risk of NPs among quartiles. Missing values (2 for LDL cholesterol, 1 for fasting insulin levels, 7 for *APOE* ϵ4 carrier, and 1 for the grading of Braak stage) were excluded from the analysis. In addition, subjects were divided into high or low groups at the boundary of the most unfavorable quartile to compare the risk of NPs. Significance was defined as $p < 0.05$.

## RESULTS

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

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

Table 1 **Demographic characteristics of 147 subjects according to the presence of NPs or NFTs[a]**

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

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

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

[b] $p < 0.05$. Male sex is adjusted for age. Age at medical examination is adjusted for sex.

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

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

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

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

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

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

Table 2 Adjusted mean or geometric mean values of each lipid profile according to CERAD score[a]

| | Model 1 | | | | | | Model 2 | | | | | | Model 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CERAD score | | | | | | CERAD score | | | | | | CERAD score | | | | | |
| | 0 (n = 47) | 1 (n = 23) | 2 (n = 22) | 3 (n = 55) | p for trend | p (1–3 vs 0) | 0 (n = 47) | 1 (n = 23) | 2 (n = 22) | 3 (n = 55) | p for trend | p (1–3 vs 0) | 0 (n = 47) | 1 (n = 23) | 2 (n = 22) | 3 (n = 55) | p for trend | p (1–3 vs 0) |
| TC, mmol/L | 4.87 | 5.47[b] | 5.66[b] | 5.32 | 0.07 | 0.006 | 4.85 | 5.47[b] | 5.70[b] | 5.34[b] | 0.05 | 0.005 | 4.82 | 5.42[b] | 5.69[b] | 5.36 | 0.049 | 0.005 |
| LDLC, mmol/L | 3.05 | 3.54 | 3.83[b] | 3.46 | 0.07 | 0.008 | 3.02 | 3.55 | 3.86[b] | 3.47[b] | 0.05 | 0.005 | 3.01 | 3.53 | 3.85[b] | 3.50 | 0.05 | 0.007 |
| HDLC, mmol/L | 1.36 | 1.25 | 1.33 | 1.23 | 0.08 | 0.09 | 1.35 | 1.29 | 1.33 | 1.22 | 0.11 | 0.20 | 1.31 | 1.26 | 1.33 | 1.26 | 0.62 | 0.63 |
| TG, mmol/L | 1.00 | 1.30 | 0.99 | 1.22 | 0.20 | 0.09 | 1.02 | 1.25 | 1.00 | 1.22 | 0.25 | 0.18 | 1.06 | 1.25 | 1.01 | 1.15 | 0.77 | 0.49 |
| TC/HDLC | 3.73 | 4.64[b] | 4.47[b] | 4.56[b] | 0.006 | 0.001 | 3.76 | 4.51[b] | 4.50[b] | 4.59[b] | 0.004 | 0.001 | 3.87 | 4.58[b] | 4.51 | 4.50[b] | 0.05 | 0.009 |
| LDLC/HDLC | 2.39 | 2.93[b] | 3.06[b] | 2.96[b] | 0.01 | 0.003 | 2.38 | 2.87 | 3.07[b] | 2.99[b] | 0.008 | 0.003 | 2.45 | 2.92 | 3.07[b] | 2.94 | 0.06 | 0.02 |
| Non-HDLC, mmol/L | 3.51 | 4.21[b] | 4.33[b] | 4.10[b] | 0.02 | 0.001 | 3.50 | 4.18[b] | 4.37[b] | 4.12[b] | 0.01 | 0.001 | 3.51 | 4.16[b] | 4.36[b] | 4.10[b] | 0.03 | 0.002 |

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

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

[b] $p < 0.05$ vs CERAD score = 0.

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

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

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

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

Table 3 Adjusted mean or geometric mean values of each lipid profile according to Braak and Braak staging[a]

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

Abbreviations: HDLC = high-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglycerides.
[a] Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, and cerebrovascular disease. Model 3 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, cerebrovascular disease, and *APOE* ε4 carrier. Geometric mean of triglycerides is shown due to the skewed distribution.

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

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

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

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

Table 4 **Multivariate-adjusted ORs and 95% CIs for presence of NPs (CERAD score 1–3 vs 0) according to lipid profile levels[a]**

| Quantiles of lipid profiles | Range | No. of subjects with NPs/total (%) | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | p Value | OR (95% CI) | p Value | OR (95% CI) | p Value |
| **TC, mmol/L** | | | | | | | | |
| Q1 | ≤4.48 | 23/37 (62.2) | | | | | | |
| Q2 (vs Q1) | >4.48 and ≤5.20 | 23/37 (62.2) | 1.1 (0.4–3.1) | 0.93 | 0.9 (0.3–2.9) | 0.8302 | 1.1 (0.3–4.4) | 0.93 |
| Q3 (vs Q1) | >5.20 and ≤5.80 | 23/37 (62.2) | 1.0 (0.3–3.1) | 0.96 | 0.7 (0.2–2.6) | 0.62 | 0.7 (0.2–3.1) | 0.65 |
| Q4 (vs Q1) | >5.80 | 31/36 (86.1) | 6.8 (1.8–25.4) | 0.005 | 8.2 (1.9–35.2) | 0.004 | 23.1 (3.8–141.6) | 0.0007 |
| Q4 (vs Q1–3) | | | 6.6 (2.1–20.5) | 0.001 | 9.6 (2.7–34.1) | 0.0005 | 24.8 (4.7–130.5) | 0.0002 |
| **LDLC, mmol/L** | | | | | | | | |
| Q1 | ≤2.75 | 22/37 (59.5) | | | | | | |
| Q2 (vs Q1) | >2.75 and ≤3.35 | 24/36 (66.7) | 1.6 (0.5–5.2) | 0.39 | 1.1 (0.3–3.9) | 0.87 | 1.0 (0.2–4.0) | 0.97 |
| Q3 (vs Q1) | >3.35 and ≤4.02 | 23/37 (62.2) | 1.2 (0.4–3.6) | 0.75 | 1.1 (0.3–3.8) | 0.86 | 1.5 (0.4–6.0) | 0.61 |
| Q4 (vs Q1) | >4.02 | 30/35 (85.7) | 7.5 (1.9–29.0) | 0.004 | 8.1 (1.9–34.0) | 0.005 | 13.5 (2.5–73.1) | 0.003 |
| Q4 (vs Q1–3) | | | 5.8 (1.8–18.4) | 0.003 | 7.5 (2.2–25.3) | 0.001 | 11.6 (2.7–49.4) | 0.0009 |
| **HDLC, mmol/L** | | | | | | | | |
| Q4 | >1.50 | 20/31 (64.5) | | | | | | |
| Q3 (vs Q4) | >1.27 and ≤1.50 | 24/38 (63.2) | 0.7 (0.2–2.1) | 0.49 | 0.7 (0.2–2.5) | 0.63 | 0.9 (0.2–3.8) | 0.94 |
| Q2 (vs Q4) | >1,04 and ≤1.27 | 25/41 (61.0) | 0.9 (0.3–2.7) | 0.86 | 1.0 (0.3–3.3) | 0.94 | 1.0 (0.2–3.8) | 0.95 |
| Q1 (vs Q4) | ≤1.04 | 31/37 (83.8) | 3.2 (0.9–11.5) | 0.07 | 2.8 (0.7–11.0) | 0.15 | 1.7 (0.4–7.8) | 0.49 |
| Q1 (vs Q2–4) | | | 3.8 (1.3–10.9) | 0.01 | 3.1 (1.1–9.2) | 0.04 | 1.8 (0.6–5.6) | 0.34 |
| **TG, mmol/L** | | | | | | | | |
| Q1 | ≤0.81 | 26/38 (68.4) | | | | | | |
| Q2 (vs Q1) | >0.81 and ≤1.11 | 25/36 (69.4) | 0.9 (0.3–2.8) | 0.88 | 1.0 (0.3–3.4) | >0.99 | 0.9 (0.2–3.3) | 0.87 |
| Q3 (vs Q1) | >1.11 and ≤1.56 | 22/38 (57.9) | 0.5 (0.2–1.5) | 0.21 | 0.5 (0.2–1.7) | 0.28 | 0.6 (0.2–2.1) | 0.40 |
| Q4 (vs Q1) | >1.56 | 27/35 (77.1) | 2.7 (0.8–8.9) | 0.11 | 3.1 (0.8–12.4) | 0.10 | 2.7 (0.6–12.2) | 0.19 |
| Q4 (vs Q1–3) | | | 3.5 (1.2–9.6) | 0.02 | 4.0 (1.3–12.8) | 0.02 | 3.5 (1.0–12.3) | 0.05 |
| **TC/HDLC** | | | | | | | | |
| Q1 | ≤3.32 | 21/37 (56.8) | | | | | | |
| Q2 (vs Q1) | >3.32 and ≤4.09 | 23/37 (62.2) | 1.1 (0.4–3.2) | 0.86 | 1.4 (0.4–4.4) | 0.62 | 1.2 (0.3–4.4) | 0.77 |
| Q3 (vs Q1) | >4.09 and ≤5.10 | 24/38 (63.2) | 1.8 (0.6–5.5) | 0.27 | 2.6 (0.7–9.2) | 0.14 | 1.8 (0.4–7.7) | 0.41 |
| Q4 (vs Q1) | >5.10 | 32/35 (91.4) | 13.0 (2.8–59.9) | 0.001 | 18.1 (3.1–105.5) | 0.001 | 19.7 (2.6–149.4) | 0.004 |
| Q4 (vs Q1–3) | | | 9.7 (2.5–37.1) | 0.0009 | 9.7 (2.3–40.1) | 0.002 | 13.1 (2.5–68.6) | 0.002 |
| **LDLC/HDLC** | | | | | | | | |
| Q1 | ≤2.00 | 24/38 (63.2) | | | | | | |
| Q2 (vs Q1) | >2.00 and ≤2.64 | 23/35 (65.7) | 1.1 (0.4–3.1) | 0.90 | 1.0 (0.3–3.3) | >0.99 | 1.0 (0.3–3.5) | 0.96 |
| Q3 (vs Q1) | >2.64 and ≤3.48 | 21/37 (56.8) | 1.1 (0.4–3.2) | 0.92 | 1.3 (0.4–4.3) | 0.68 | 1.2 (0.3–4.9) | 0.75 |
| Q4 (vs Q1) | >3.48 | 31/35 (88.6) | 5.7 (1.4–23.0) | 0.01 | 6.9 (1.4–32.7) | 0.02 | 7.9 (1.2–50.5) | 0.03 |
| Q4 (vs Q1–3) | | | 5.5 (1.7–18.1) | 0.005 | 6.0 (1.7–21.8) | 0.007 | 7.0 (1.5–32.0) | 0.01 |
| **Non-HDLC, mmol/L** | | | | | | | | |
| Q1 | ≤3.29 | 23/38 (60.5) | | | | | | |
| Q2 (vs Q1) | >3.29 and ≤3.86 | 24/37 (64.9) | 1.0 (0.4–3.1) | 0.94 | 0.9 (0.3–3.0) | 0.82 | 0.7 (0.2–2.9) | 0.65 |
| Q3 (vs Q1) | >3.86 and ≤4.61 | 22/37 (59.5) | 1.0 (0.4–3.1) | 0.95 | 1.0 (0.3–3.3) | 0.95 | 0.7 (0.2–2.9) | 0.64 |
| Q4 (vs Q1) | >4.61 | 31/35 (88.6) | 8.5 (2.1–34.6) | 0.003 | 10.1 (2.1–48.2) | 0.004 | 13.1 (2.3–75.9) | 0.004 |
| Q4 (vs Q1–3) | | | 8.2 (2.4–28.2) | 0.0008 | 10.7 (2.8–40.5) | 0.0005 | 16.5 (3.5–77.6) | 0.0004 |

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

[a] Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, cerebrovascular disease, and regular exercise. Model 3 was adjusted for age, sex, systolic blood pressure, fasting blood glucose, fasting insulin, body mass index, current smoking, regular exercise, cerebrovascular disease, and *APOE* $\epsilon$4 carrier.

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

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

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

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

## AUTHOR CONTRIBUTIONS

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

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## DISCLOSURE

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## REFERENCES

1. 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.
2. Sekita A, Ninomiya T, Tanizaki Y, et al. Trends in prevalence of Alzheimer's disease and vascular dementia in a Japanese community: the Hisayama Study. Acta Psychiatr Scand 2010;122:319–325.
3. Matsuzaki T, Sasaki K, Tanizaki Y, et al. Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama Study. Neurology 2010;75:764–770.
4. 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.
5. Arvanitakis Z, Schneider JA, Wilson RS, et al. Diabetes is related to cerebral infarction but not to AD pathology in older persons. Neurology 2006;67:1960–1965.
6. Pappolla MA, Bryant-Thomas TK, Herbert D, et al. Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. Neurology 2003;61:199–205.
7. Launer LJ, White LR, Petrovitch H, Ross GW, Curb JD. Cholesterol and neuropathologic markers of AD: a population-based autopsy study. Neurology 2001;57:1447–1452.
8. Katsuki S. Epidemiological and clinicopathological study on cerebrovascular disease in Japan. Prog Brain Res 1966;21:64–89.
9. Ohmura T, Ueda K, Kiyohara Y, et al. Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: the Hisayama Study. Diabetologia 1993;36:1198–1203.
10. Fujimi K, Sasaki K, Noda K, et al. Clinicopathological outline of dementia with Lewy bodies applying the revised criteria: the Hisayama Study. Brain Pathol 2008;18:317–325.
11. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
12. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD): part II: standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479–486.
13. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–259.
14. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006;112:389–404.
15. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science 1986;232:34–47.
16. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467–1472.
17. Ingelsson E, Schaefer EJ, Contois JH, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007;298:776–785.
18. Hardy J. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. J Alzheimers Dis 2006;9:151–153.
19. Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K. Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. Proc Natl Acad Sci USA 1998;95:6460–6464.
20. Bodovitz S, Klein WL. Cholesterol modulates alpha-secretase cleavage of amyloid precursor protein. J Biol Chem 1996;271:4436–4440.
21. Hayashi H, Kimura N, Yamaguchi H, et al. A seed for Alzheimer amyloid in the brain. J Neurosci 2004;24:4894–4902.
22. Burns MP, Noble WJ, Olm V, et al. Co-localization of cholesterol, apolipoprotein E and fibrillar Abeta in amyloid plaques. Brain Res Mol Brain Res 2003;110:119–125.
23. Kuo YM, Emmerling MR, Bisgaier CL, et al. Elevated low-density lipoprotein in Alzheimer's disease correlates with brain abeta 1–42 levels. Biochem Biophys Res Commun 1998;252:711–715.
24. Wahrle S, Das P, Nyborg AC, et al. Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains. Neurobiol Dis 2002;9:11–23.
25. Anstey KJ, Lipnicki DM, Low LF. Cholesterol as a risk factor for dementia and cognitive decline: a systematic review of prospective studies with meta-analysis. Am J Geriatr Psychiatry 2008;16:343–354.
26. 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.
27. Kivipelto M, Helkala EL, Laakso MP, et al. Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. Ann Intern Med 2002;137:149–155.
28. Whitmer RA, Sidney S, Selby J, Johnston SC, Yaffe K. Midlife cardiovascular risk factors and risk of dementia in late life. Neurology 2005;64:277–281.
29. Solomon A, Kivipelto M, Wolozin B, Zhou J, Whitmer RA. Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. Dement Geriatr Cogn Disord 2009;28:75–80.
30. Mielke MM, Zandi PP, Sjogren M, et al. High total cholesterol levels in late life associated with a reduced risk of dementia. Neurology 2005;64:1689–1695.
31. Mielke MM, Zandi PP, Shao H, et al. The 32-year relationship between cholesterol and dementia from midlife to late life. Neurology 2010;75:1888–1895.

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*Regular Article*

# Nominal association between a polymorphism in *DGKH* and bipolar disorder detected in a meta-analysis of East Asian case–control samples

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*Aim:* Recent genome-wide association studies (GWAS) of bipolar disorder (BD) have detected new candidate genes, including *DGKH*, *DFNB31* and *SORCS2*. However, the results of these GWAS were not necessarily consistent, indicating the importance of replication studies. In this study, we tested the genetic association of *DGKH*, *DFNB31* and *SORCS2* with BD.

*Methods:* We genotyped 18 single-nucleotide polymorphisms (SNP) in *DGKH*, *DFNB31* and *SORCS2* using Japanese samples (366 cases and 370 controls). We also performed a meta-analysis of four SNP in *DGKH*, using the previously published allele frequency data of Han-Chinese case–control samples (1139 cases and 1138 controls).

*Results:* In the association analysis using Japanese samples, a SNP in *SORCS2* (rs10937823) showed nominal genotypic association. However, we could not find any association in an additional analysis of tag SNP around rs10937823. In the meta-analysis of SNP in *DGKH*, rs9315897, which was not significantly associated with BD in the previous Chinese study, showed nominal association.

*Conclusion:* Although the association was not strong, the result of this study would support the association between *DGKH* and BD.

**Key words:** DFNB31, genome-wide association studies, manic–depressive illness, mood disorder, SORCS2.

FAMILY, TWIN AND adoption studies have consistently demonstrated the contribution of inherited genetic variation on risk for bipolar disorder (BD).[1] Therefore, numerous genetic studies, including linkage mapping and candidate gene studies, have been carried out. However, the results of these studies have largely been inconsistent. After the era of linkage study and candidate gene approach, genome-wide association studies (GWAS), which investigate 500 000 to 1 000 000 single-nucleotide polymorphisms (SNP) throughout the genome using DNA microarray, have become popular. For BD, an increasing number of GWAS, including meta-analyses, have been conducted.[2–7,16,17] Meta-analyses of GWAS data of BD and major depressive disorder were also performed.[8,9] These studies identified many previously unsuspected candidate genes.

*DGKH*, *DFNB31* and *SORCS2* are included in these new candidate genes for BD, as well as other promising genes, such as *ANK3*, *CACNA1C*,[3] *PBRM1*[8] and so on. The association of *DGKH*, *DFNB31* and

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*SORCS2* with BD was identified in the first reported GWAS using a DNA pooling strategy followed by individual genotyping.[2] All of these genes are expressed in the brain and have some functional implication with neuropsychiatric disorders. Diacylglycerol kinase (DGK) eta, encoded by *DGKH*, is a member of the DGK family that plays an important role in the inositol pathway, the putative site of action of lithium.[10] *DFNB31* encodes whirlin, which is also known as Cip98. This protein interacts with a calmodulin-dependent serine kinase and is suggested to be involved in the formation of scaffolding protein complex and in synaptic transmission.[11] A mutation in this gene is known to cause Usher syndrome,[12] which is characterized by hearing impairment and progressive vision loss. One study reported frequent comorbidity of bipolar disorder or depressive disorder in Usher syndrome patients.[13] *SORCS2*, encoding a VSP10 domain containing-receptor, is expressed in both the developing and adult brain.[14] While the ligand for SORCS2 is so far unknown, highly homologous other members of the SORCS VSP10 domain-containing-receptor family, SORCS1 and SORCS3, are known to bind several growth factors, such as NGF and PDGF.[15] These findings further make *DGKH*, *DFNB31* and *SORCS2* good candidates for BD. However, GWAS usually investigate hundreds of thousands SNP and always involve the potential for false positive results. In fact, some inconsistent results about these genes were reported in other GWAS for BD.[3–7,16,17]

Several replication studies of *DGKH*, *DFNB31* and *SORCS2* were also reported and they include both positive and negative results. A study in Sardinian samples (197 cases and 300 controls) detected a haplotypic association between BD and *DGKH*.[18] Another study that examined 36 tag SNP from *DGKH* in a Scandinavian population (594 cases and 1421 controls) reported no association.[19] A replication study of GWAS that investigated 26 SNP, including those from *DGKH*, *DFNB31* and *SORCS2* using a Finnish family cohort (723 individuals from 180 families), reported associations of *DFNB31* and *SORCS2* but no association of *DGKH* with BD.[20] Recently, Zeng *et al.* reported a strong haplotypic association (minimum $P = 3.87 \times 10^{-6}$) between *DGKH* and BD in a Han-Chinese case–control cohort.[21]

In this study, we investigated associations of *DGKH*, *DFNB31* and *SORCS2* with BD using Japanese case–control samples (366 cases and 370 controls). Furthermore, we conducted a meta-analysis of four SNP in *DGKH*, which are overlapped with the SNP investigated by Zeng *et al.* In total, 1505 cases and 1508 control samples were used for the meta-analysis.

## METHODS

The Japanese case–control samples consisted of 366 patients with bipolar I disorder (BDI), bipolar II disorder (BDII) or schizoaffective disorder bipolar type (SAB) (257 BDI, 104 BDII and five SAB; 181 men and 185 women, aged 50.1 ± 13.4 years) and 370 control subjects (185 men and 185 women aged 50.6 ± 12.6 years). All subjects were unrelated and ethnically Japanese. The patients were diagnosed by at least two experienced psychiatrists according to the DSM-IV criteria on the basis of unstructured interviews and reviews of their medical records. All healthy control subjects were also psychiatrically screened on the basis of unstructured interviews. The objective of the present study was clearly explained, and written informed consent was obtained from all the participants. The characteristics of the Han-Chinese cohort used for meta-analysis were described elsewhere.[21] This study was approved by the ethics committees of Kyushu University Graduate School of Medicine and RIKEN Brain Science Institute.

For SNP selection, we focused on the SNP that were reported in a previous GWAS by Baum *et al.* and that cause non-synonymous amino acid substitutions and possibly affect the function of encoded proteins. SNP chosen from Baum *et al.* included six SNP whose associations were detected in the follow-up individual genotyping (three in *DGKH*, one in *DFNB31* and two in *SORCS2*, reported *P*-values ranging from 0.0005 to $1.5 \times 10^{-8}$). Three SNP in *DGKH* indicated to be associated in both of the two independent pooling sample sets were additionally selected. The selected non-synonymous SNP consisted of two SNP in *DGKH*, five SNP in *DFNB31* and two SNP in *SORCS2*. The total number of selected SNP was eighteen. Genotyping was performed using TaqMan assay (Applied Biosystems, Foster City, CA, USA). Differences in allele and genotype frequencies between BD and controls were evaluated with Fisher's exact test. Deviations from Hardy–Weinberg equilibrium (HWE), structures of linkage disequilibrium (LD) blocks and haplotypic associations were analyzed using Haploview version 4.1 software,[22] (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/

haploview/haploview). A meta-analysis using the Mantel–Haenszel model and evaluation of sample heterogeneity were performed on Review Manager.[23] The $I^2$ statistics[24,25] were used for the assessment of heterogeneity between the samples.

## RESULTS

The results of genotyping in the Japanese cohort are summarized in Table 1. Three SNP (rs35776153, rs35003670 and rs34058821) registered in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) were not polymorphic in our samples. Genotype frequencies of each SNP were in HWE ($P > 0.05$) except for rs16840892 in the BD group. No SNP displayed significant allelic association with BD. A nominally significant genotypic association was observed with rs10937823 of *SORCS2*. However, the over-represented allele in BD was opposite to the one that was reported in a previous GWAS (T in this study and C in Baum *et al.*). To investigate this locus more intensively, we selected four tag SNP in the region between rs4411993 and rs34058821 (physical chromosomal position spanning 7 517 366 to 7 717 060), using TAG SNP Selection,[26] http://manticore.niehs.nih.gov/), and genotyped them. However, no significant allelic or genotypic association between these four SNP and BD was found (Table 1, with ‡).

In LD structure analysis, two LD blocks in *DGKH* and one block in each of *DFNB31* and *SORCS2* were detected. In haplotype analysis, a total of 14 haplotypes whose frequencies were more than 1% were estimated, but no significant haplotypic associations with BD were detected (data not shown).

The results of the meta-analysis are shown in Table 2. We found a nominal association in rs9315897 ($P = 0.039$), which was not significantly associated with BD in Zeng *et al.* No sample heterogeneity was observed in the four investigated SNP.

## DISCUSSION

In this study, we performed association analyses of *DGKH*, *DFNB31* and *SORCS2* with BD in Japanese case–control samples. We also conducted a meta-analysis of four SNP in *DGKH* using the data from the present Japanese cohort and previously reported Han-Chinese cohort.

The results of association analyses in the Japanese cohort were largely inconsistent with the initial study.[2] Among GWAS, while partly common associations of *DFNB31* (in Sklar *et al.*[4] and WTCCC[6], minimum *P*-value was $8.8 \times 10^{-6}$) and *SORCS2* (in Smith *et al.*,[5] minimum *P*-value was 0.009) were found, largely inconsistent results for these genes were also reported. From the results of GWAS for BD and other complex traits, the effect of a single common variant has been recognized to be relatively low (odds ratio [OR] less than 1.5, often 1.1–1.2[27]), with some exceptional genes containing common high-risk markers, such as *APOE* for Alzheimer's disease and *CFH* and *ARMS2/HTRA1* for age-related macular degeneration. In this study, statistical powers of the Japanese cohort calculated with allele frequencies of our Japanese control samples and OR 1.3 for an alpha level of 0.05 or 0.002 (0.05 divided by the numbers of tested SNP) were 71.7 or 28.9% for *DGKH*, 61.3 or 19.9% for *DFNB31*, and 56.8 or 16.9% for *SORCS2* (calculated with the Genetic Power Calculator,[28] http://statgen.iop.kcl.ac.uk/gpc/). Therefore, the sample number was not sufficient to detect risk-conferring markers with small effects. As a reason for inconsistent results, the adverse effect of population stratification should also be considered. In this study, all participants were recruited in the central area of Japan. Previous studies reported no substantial stratification in this population.[17,29] In particular, samples in Hattori *et al.*[17] were part of the samples used for this study, indicating that false positive results due to the effect of population stratification could be avoided.

In the meta-analysis, we found a nominal but significant association between rs9315897 in *DGKH* and BD. The total number of samples used for meta-analysis was larger than 3000. Thus, this result was more reliable than the result solely from the Japanese cohort. Although the association was not strong as was reported in Baum *et al.*, and did not overcome a correction for multiple testing, this result may indicate this SNP as a risk marker common across ethnicities.

In conclusion, we found a nominal association between a polymorphism in *DGKH* and BD in the meta-analysis using more than 3000 samples from East Asia. However, the association was not strong and further investigations are required to obtain a conclusive result. For polymorphisms in *SORCS2* and *DFNB31*, the statistical power obtained solely from our Japanese samples was apparently insufficient to detect risk markers with weak effect. However, this data also have a particular meaning, because they could be utilized in future meta-analysis.