these subjects had dementia and were bedridden. The increase in patients hospitalized or staying in health care facilities is a major social and economic burden in Japan. Therefore, it is imperative to establish effective strategies for preventing the development of dementia and subsequent deterioration of ADL.

Study strengths and limitations

The strength of our study is that selection bias was minimized by including more than 90% of all Hisayama residents aged 65 years or older and by examining subjects staying in hospitals and health care facilities. In addition, cardiovascular events and dementia were evaluated using not only questionnaires but also detailed clinical information, as these parameters are main endpoints of the ongoing Hisayama Study.[15,23] A limitation is that this was a cross-sectional study. Consequently, causal relationships cannot be inferred between underlying diseases and functional disability.

Conclusion

Our study revealed that functional disability is common among Japanese elderly adults and that dementia is the most frequent cause of disability, especially in persons with total dependence. Stroke is a major cause of disability in men and in individuals aged 65 to 74 years (the young old). In countries such as Japan, where the elderly population is increasing rapidly, it is important to establish effective prevention strategies for dementia and stroke to reduce the risk of disability and extend healthy life expectancy in later life.

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Conflicts of interest: None declared.

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Self-Reported Dietary Intake of Potassium, Calcium, and Magnesium and Risk of Dementia in the Japanese: The Hisayama Study

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OBJECTIVES: To investigate whether higher intake of potassium, calcium, and magnesium reduces the risk of incident dementia.

DESIGN: Prospective cohort study.

SETTING: The Hisayama Study, in Japan.

PARTICIPANTS: One thousand eighty-one community-dwelling Japanese individuals without dementia aged 60 and older.

MEASUREMENTS: A 70-item semiquantitative food frequency questionnaire was used to assess potassium, calcium, and magnesium intakes. Hazard ratios (HRs) for the development of all-cause dementia and its subtypes were estimated using Cox proportional hazards model.

RESULTS: During a 17-year follow-up, 303 participants experienced all-cause dementia; of these, 98 had vascular dementia (VaD), and 166 had Alzheimer's disease (AD). The multivariable-adjusted HRs for the development of all-cause dementia were 0.52 (95% confidence interval [CI] = 0.30–0.91), 0.64 (95% CI = 0.41–1.00), and 0.63 (95% CI = 0.40–1.01) for the highest quartiles of potassium, calcium, and magnesium intake, respectively, compared with the corresponding lowest quartiles. Similarly, the HRs for the development of VaD were 0.20 (95% CI = 0.07–0.56), 0.24 (95% CI = 0.11–0.53), and 0.26 (95% CI = 0.11–0.61) for the highest quartiles of potassium, calcium, and magnesium intake, respectively. There was no evidence of a linear association between these mineral intakes and the risk of AD.

CONCLUSION: Higher self-reported dietary intakes of potassium, calcium, and magnesium reduce the risk of all-cause dementia, especially VaD, in the general Japanese population. **J Am Geriatr Soc 60:1515–1520, 2012.**

Key words: dementia; Alzheimer's disease; vascular dementia; potassium; calcium; magnesium

Recent evidence has emerged to indicate that dietary modification has an important role in preventing life style-related diseases.[1] In several prospective studies, higher intake of potassium, calcium, and magnesium reduced the risk of developing hypertension and stroke.[2–4] These findings raise the possibility that these mineral intakes may be effective at reducing the burden of cardiovascular risk factors and subsequent vascular diseases.

Dementia is one of the causes of disability and premature death in elderly adults[5,6] and is a high-priority public health concern worldwide.[7] Cerebrovascular disease is one of the causes of vascular dementia (VaD).[8] In addition, recent epidemiological studies have suggested that cardiovascular risk factors may play at least a partial role in Alzheimer's disease (AD), which has traditionally been considered a primarily neurodegenerative disorder.[8,9] Therefore, it is reasonable to assume that the intake of these minerals exerts beneficial effects on cerebro- and cardiovascular diseases and their risk factors, leading to a subsequent reduction in the risk of dementia, but few studies have assessed the effects of mineral intake on the risk of dementia. To clarify this issue, a prospective cohort study was performed to evaluate risk factors for the development of dementia in Japanese elderly individuals. The aim of this study was to elucidate the effects of dietary

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intakes of potassium, calcium, and magnesium on the development of dementia and its subtypes in a general population of Japanese elderly adults.

PARTICIPANTS AND METHODS

Study Population

The Hisayama Study is a population-based prospective cohort study of cerebro- and cardiovascular diseases established in the town of Hisayama, located in a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. Full community surveys of health status and neurological conditions of the residents aged 40 and older have been repeated annually since 1961.[10] In 1985, a comprehensive survey of cognitive impairment, including a neuropsychological test (Hasegawa dementia scale[11]) was performed in the elderly adults of the town.[12] In addition, the study team and local physicians or members of the Health and Welfare Office of Hisayama performed annual health examinations and established a daily monitoring system to obtain information on any stroke and dementia that participants developed. In 1988, 1,228 residents aged 60 and older (participation rate 91.1%) underwent a screening examination for the present study. Based on these data, 35 residents who had already had dementia at baseline were identified. After excluding these residents with dementia, 111 residents for whom dietary questionnaires were not available, and one resident with no blood sample, 1,081 participants (457 men and 624 women) were enrolled in this study. This study was conducted with the approval of the Kyushu University institutional review board for clinical research. Written informed consent was obtained from all participants.

Follow-Up Survey

The participants were followed up for 17 years, from December 1988 to November 2005, through the daily monitoring system and annual health examinations.[13] Health status was checked yearly by letter or telephone call for any participant who did not undergo a regular examination or who had moved out of town.[14] Comprehensive screening surveys of cognitive function including neuropsychological tests (the Hasegawa dementia scale,[11] its revised version,[15] or the Mini-Mental State Examination[16]) were conducted in 1992, 1998, and 2005. When new neurological symptoms, including cognitive impairment, were suspected, the physicians and psychiatrists from the study group carefully evaluated the participant. During the follow-up period, there were no participants whose medical condition or vital status could not be ascertained.

Diagnosis of Dementia

The criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised*,[17] were used to define the diagnosis of dementia. Participants diagnosed with AD met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association,[18] and participants diagnosed with VaD met the criteria of the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences.[19] The diagnostic procedure was reported previously.[20] During the 17-year follow-up period, 303 participants (103 men and 200 women) developed dementia, and 518 (47.9%) died. Of those with dementia, 261 (86.1%) were evaluated using brain imaging, and 155 (51.2%) underwent autopsy; both were performed in 143. Thus, 273 participants in all (90.0%) had some kind of morphological examination. Of participants with dementia, 25 with AD and 18 with VaD had other coexisting subtypes of dementia, of whom 14 had mixed AD and VaD. These cases were counted as events in the analyses for each subtype. Finally, 166 participants experienced AD and 98 VaD.

Nutritional Survey

At the baseline examination, a dietary survey was conducted using a 70-item semiquantitative food frequency questionnaire concerning food intake.[21] The validity of this questionnaire has been reported elsewhere.[22] The questionnaire was administered before initiation of this study, and trained dieticians and nutritionists questioned each participant during the examination. Average food intake per day was calculated from the weekly frequency of various foods and the amount of each food portion. Nutritional intake was calculated using the fourth revision of the Standard Tables of Food Composition in Japan.[23] Magnesium intake was calculated from a previously developed magnesium inclusion table.[24] Each nutritional element was adjusted for energy intake using the residual method.[25] The correlation between the food frequency questionnaire and food records was 0.53 for potassium and 0.42 for calcium but was not investigated for magnesium. The validity of magnesium could not be assessed, because there was no standardized table of food compositions for magnesium in Japan in 1988.

Risk Factor Measurement

At baseline, each participant completed a self-administered questionnaire covering medical history, antidiabetic and antihypertensive treatments, educational status, alcohol consumption, smoking habits, and physical activity. History of stroke was determined as a preexisting sudden onset of nonconvulsive and focal neurological deficit persisting for longer than 24 hours on the basis of all available clinical data, including medical records, neurological examination, and brain imaging. A low educational level was defined as <7 years of formal education. Smoking and drinking habits were classified as currently used or not. Regular exercise was defined as engaging in sports or other forms of exertion three or more times a week during leisure time. Blood pressure was measured three times using a standard mercury sphygmomanometer in the sitting position after rest for at least 5 minutes. The mean of three measurements was used for the analysis. Hypertension was defined as blood pressure of 140/90 mmHg or greater or current use of antihypertensive drugs. Body height and weight were measured in light clothing without shoes, and

body mass index (kg/m^2) was calculated. Diabetes mellitus was defined as fasting plasma glucose of 7.0 mmol/L or greater, 2-hour postload glucose concentrations or postprandial glucose concentrations of 11.1 mmol/L or greater, or current use of insulin or oral medication for diabetes mellitus.

Statistical Analysis

Participants were divided into quartiles of potassium, calcium, and magnesium intake. Age- and sex-adjusted mean values or frequencies of potential risk factors for dementia between the lowest and the highest mineral intakes were compared using analysis of covariance for continuous variables and a logistic regression model for dichotomous variables. Participants were censored at date of death or date of the end of follow-up for survival analyses. The age- and sex-adjusted or multivariable-adjusted hazard ratios (HRs) with their 95% confidence intervals (CIs) of intake levels of minerals for the development of dementia were estimated using the Cox proportional hazards model. The assumption of the proportional hazards was checked graphically using the log cumulative hazard plots for outcomes according to the intake levels of each mineral. All measured variables of known or suspected risk factors for dementia were selected as potential confounders. The median values of food intake between the lowest and highest quartiles of mineral intakes were compared using the Student *t*-test. SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina) was used to perform all statistical analyses.

RESULTS

Table 1 compares the age- and sex-adjusted mean values or frequencies of possible risk factors for dementia of the lowest and highest quartiles of dietary potassium, calcium, and magnesium intake at baseline. Participants with the highest intakes of potassium, calcium, and magnesium were more likely to be female and more educated than those with the lowest intakes. The prevalence of diabetes mellitus was higher in participants in the highest quartiles of mineral intakes, and the prevalence of smoking and alcohol intake were lower in the highest-intake quartile for each mineral. The intakes of vitamin C, cholesterol, fatty acids, saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid were all higher in the highest quartile than in the lowest for each mineral. Strong correlations between these mineral intakes were observed (Pearson correlation coefficient (r) = 0.65 between potassium and calcium, r = 0.85 between potassium and magnesium, and r = 0.76 between calcium and magnesium).

The age- and sex-adjusted and multivariable-adjusted HRs and their 95% CIs for the development of all-cause

Table 1. Age- and Sex-Adjusted Potential Risk Factors for Dementia According to Lowest and Highest Quartiles of Self-Reported Dietary Potassium, Calcium, and Magnesium Intake at Baseline

	Potassium Intake		Calcium Intake		Magnesium Intake	
Risk Factors	Q1 (lowest) (n = 270)	Q4 (highest) (n = 270)	Q1 (lowest) (n = 270)	Q4 (highest) (n = 270)	Q1 (lowest) (n = 270)	Q4 (highest) (n = 270)
Age, mean	69	69	69	69	69	69
Female, %	37.1	68.3[a]	44.3	60.1[a]	42.5	69.2[a]
Education ≤6 years, %	16.8	8.8[a]	17.0	9.1[b]	14.7	8.2[b]
History of stroke, %	4.6	3.6	4.1	4.7	4.4	4.0
Systolic blood pressure, mmHg, mean	139	139	141	139	140	140
Diastolic blood pressure, mmHg, mean	76	76	76	77	76	77
Hypertension, %	52.7	53.8	64.7	56.2	53.7	57.7
Diabetes mellitus %	10.3	20.5[a]	9.6	22.6[a]	13.0	21.5[b]
Total cholesterol, mg/dL, mean	207	213	201	218[a]	202	215[a]
Body mass index, kg/m^2, mean	22.0	22.8[a]	22.1	22.5	21.9	22.6[b]
Smoking habits, %	30.3	18.8[a]	27.8	19.0[b]	30.7	16.6[a]
Alcohol intake, %	31.4	22.4[b]	29.7	23.2[b]	30.7	24.7[b]
Regular exercise, %	20.0	19.7[b]	21.2	18.9	21.0	21.9[a]
Dietary intake per day, mean						
Energy, kcal	1,651	1,745[b]	1,716	1,725	1,665	1,718
Vitamin C, mg	51	114[a]	70	91[a]	56	106[a]
Cholesterol, mg	209	251[a]	192	274[a]	208	256[a]
Saturated fatty acid, g	11.2	13.5[a]	10.2	14.5[a]	10.8	13.8[a]
Monounsaturated fatty acid, g	16.8	20.2[a]	16.2	21.1[a]	17.1	20.1[a]
Polyunsaturated fatty acid, g	12.9	18.9[a]	12.5	19.7[a]	13.3	18.5[a]

Age is sex-adjusted; sex is age-adjusted.
P < .01[a], .05[b] vs Q1.

dementia, VaD, and AD according to intakes of potassium, calcium, and magnesium are shown in Table 2. The HR of all-cause dementia decreased significantly with higher intake of each mineral after adjusting for age; sex; low education; history of stroke; hypertension; diabetes mellitus; total cholesterol; body mass index; smoking; alcohol intake; regular exercise; and intakes of energy, vitamin C, cholesterol, saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid (all P for trend <.05). With regard to subtypes of dementia, the multivariable-adjusted HRs of VaD were significantly lower with higher intakes of potassium, calcium, and magnesium (all P for trend <.01), although the multivariate-adjusted HRs of AD were significantly lower in the third quartile of potassium intake and in the second and third quartile of magnesium intake, but there was no evidence of a significant linear association (all P for trend >.09). Because the intakes of the three minerals were strongly correlated, the risks of all-cause dementia and its subtypes in participants with the highest intakes of all three minerals (n = 143) were compared with those with the lowest intakes of these minerals (n = 154). Participants with the highest intakes of all three minerals had 71% (95% CI = 8–91%) lower risk of VaD after adjusting for the above-mentioned potential confounders.

The food intake characteristics of participants in the lowest quartiles of all three mineral intakes were compared with the characteristics of those in the highest quartiles (Table 3). Participants in the highest quartiles tended to eat more potatoes, soybeans and soybean products, vegetables, fruits and fruit juices, algae, fish, eggs, and milk and dairy products and had lower intakes of rice, meat, sugar,

Table 2. Development of All-Cause Dementia, Vascular Dementia, and Alzheimer's Disease According to Quartile of Self-Reported Dietary Potassium, Calcium, and Magnesium Intake

Variable	Q1 (low)	Q2	Q3	Q4 (high)	*P* for trend
All-cause dementia					
Potassium, mg/d	≤1,856	1,857–2,149	2,150–2,559	≥2,560	
Events/participants, n/n	77/270	80/270	76/271	70/270	
HR (95% CI)[a]	1	0.77 (0.56–1.07)	0.70 (0.51–0.97)	0.65 (0.46–0.91)	.01
HR (95% CI)[b]	1	0.69 (0.49–0.99)	0.58 (0.38–0.87)	0.52 (0.30–0.91)	.02
Calcium, mg/d	≤431	432–531	532–638	≥638	
Events/participants, n/n	74/270	78/270	85/271	66/270	
HR (95% CI)[a]	1	0.99 (0.72–1.37)	0.86 (0.63–1.19)	0.77 (0.55–1.07)	.08
HR (95% CI)[b]	1	0.91 (0.64–1.28)	0.77 (0.53–1.11)	0.64 (0.41–1.00)	.04
Magnesium, mg/d	≤147	148–169	170–195	≥196	
Events/participants, n/n	79/270	74/270	72/271	78/270	
HR (95% CI)[a]	1	0.66 (0.48–0.92)	0.56 (0.40–0.77)	0.69 (0.50–0.95)	.02
HR (95% CI)[b]	1	0.61 (0.43–0.86)	0.50 (0.34–0.75)	0.63 (0.40–1.01)	.04
Vascular dementia					
Potassium, mg/d	≤1,856	1,857–2,149	2,150–2,559	≥2,560	
Events/participants, n/n	31/270	29/270	26/271	12/270	
HR (95% CI)[a]	1	0.86 (0.51–1.45)	0.74 (0.43–1.27)	0.36 (0.18–0.70)	.003
HR (95% CI)[b]	1	0.74 (0.41–1.36)	0.48 (0.24–0.98)	0.20 (0.07–0.56)	.003
Calcium, mg/d	≤431	432–531	532–638	≥638	
Events/participants, n/n	32/270	25/270	24/271	17/270	
HR (95% CI)[a]	1	0.81 (0.48–1.38)	0.66 (0.39–1.14)	0.52 (0.29–0.94)	.02
HR (95% CI)[b]	1	0.59 (0.34–1.04)	0.43 (0.23–0.81)	0.24 (0.11–0.53)	<.001
Magnesium, mg/d	≤147	148–169	170–195	≥196	
Events/participants, n/n	35/270	23/270	23/271	17/270	
HR (95% CI)[a]	1	0.55 (0.32–0.94)	0.48 (0.28–0.82)	0.42 (0.23–0.76)	.003
HR (95% CI)[b]	1	0.44 (0.25–0.79)	0.34 (0.17–0.67)	0.26 (0.11–0.61)	.002
Alzheimer's disease					
Potassium, mg/d	≤1,856	1,857–2,149	2,150–2,559	≥2,560	
Events/participants, n/n	34/270	45/270	41/271	46/270	
HR (95% CI)[a]	1	0.82 (0.52–1.30)	0.71 (0.44–1.13)	0.79 (0.50–1.25)	0.3
HR (95% CI)[b]	1	0.69 (0.42–1.14)	0.52 (0.29–0.93)	0.56 (0.26–1.20)	.09
Calcium, mg/d	≤431	432–531	532–638	≥638	
Events/participants, n/n	34/270	43/270	50/271	39/270	
HR (95% CI)[a]	1	1.11 (0.70–1.74)	0.96 (0.61–1.49)	0.89 (0.56–1.42)	.48
HR (95% CI)[b]	1	1.00 (0.61–1.63)	0.92 (0.55–1.54)	0.87 (0.47–1.62)	.61
Magnesium, mg/d	≤147	148–169	170–195	≥196	
Events/participants, n/n	36/270	39/270	42/271	49/270	
HR (95% CI)[a]	1	0.67 (0.42–1.07)	0.62 (0.39–0.97)	0.80 (0.52–1.25)	.45
HR (95% CI)[b]	1	0.58 (0.35–0.95)	0.53 (0.31–0.92)	0.72 (0.38–1.37)	0.4

[a] Adjusted for age and sex.

[b] Adjusted for age; sex; low education; history of stroke; hypertension; diabetes mellitus; total cholesterol; body mass index; smoking; alcohol intake; regular exercise; and energy, vitamin C, cholesterol, saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid intake.

HR = hazard ratio; CI = confidence interval.

Table 3. Comparisons of the Amounts of Each Food Group Consumed Between the Lowest and Highest Quartiles for Intake of All Three Minerals (Potassium, Calcium, and Magnesium)

Food Group	Median (Interquartile Range)		*P*-Value
	Lowest Quartile (n = 143)	Highest Quartile (n = 154)	
Rice	235.8 (196.2–281.4)	144.4 (103.3–170.5)	<.001
Breads	1.05 (−2.5–17.7)	1.36 (−0.8–40.9)	.32
Noodles and other cereals	1.78 (−3.4–14.0)	2.77 (−0.96–25.8)	.31
Potatoes	9.46 (3.96–718.9)	20.2 (9.9–42.1)	<.001
Soybeans and soybean products	44.7 (16.4–62.3)	123.2 (84.6–171.8)	<.001
Miso	13.9 (9.3–15.7)	14.4 (11.8–15.3)	.50
Pickles	25.9 (8.6–44.9)	29.0 (8.94–59.3)	.11
Green vegetables	41.7 (27.8–59.3)	124.2 (91.2–147.4)	<.001
Other vegetables	93.9 (71.0–141.8)	255.9 (182.4–295.4)	<.001
Fruits and fruit juices	34.4 (12.2–64.4)	80.1 (49.6–146.5)	<.001
Algae	0.48 (0.21–0.93)	1.39 (0.88–1.98)	<.001
Fish	20.4 (10.7–33.6)	41.5 (29.7–60.8)	<.001
Meat	20.1 (10.6–30.8)	13.9 (6.8–24.7)	.01
Eggs	26.0 (13.6–45.9)	38.6 (20.7–48.8)	.01
Milk and dairy products	25.7 (−5.0–68.9)	197.3 (121.3–250.5)	<.001
Fats and oils	19.7 (15.6–35.8)	18.3 (14.4–23.2)	.13
Sugar and confectioneries	23.1 (15.5–36.3)	18.6 (12.4–26.3)	<.001
Alcoholic beverages	47.8 (−18.0–202.6)	8.1 (−18.8–68.2)	.01
Salt	12.4 (9.1–15.2)	11.2 (8.8–14.4)	.29

confectioneries, and alcoholic beverages. Comparable patterns of food intakes were found when food intakes of participants in the highest and lowest quartiles of each mineral were compared separately.

DISCUSSION

The present study demonstrated that higher self-reported dietary intakes of potassium, calcium, and magnesium reduced the risk of all-cause dementia and VaD but not of AD. Several longitudinal studies have reported the preventive effects of dietary intakes of these minerals on the risk of stroke,[3,4] but to the best of the knowledge of the authors of the current study, this is the first prospective cohort study showing that higher self-reported dietary intakes of potassium, calcium, and magnesium are associated with a lower risk of dementia. The separate effects of each mineral on dementia were not distinguished because these minerals were strongly correlated with one another. Furthermore, the possibility that some other factors contained in the foods than the minerals themselves caused the favorable effects on dementia cannot be excluded. Nevertheless, these findings may provide intriguing information on the beneficial effects of a diet rich in these minerals against dementia in Japanese.

The mechanism through which the risk of VaD decreased with higher intakes of these minerals is unclear. Hypertension has been recognized as a strong risk factor for vascular diseases, including VaD.[26] There is some evidence of the antihypertensive effects of these mineral intakes,[2] but the adjustment for hypertension had little effect on the association between each mineral intake and the risk of VaD in the present study. As alternative mechanisms, it has been reported that these minerals may have some favorable effects against vascular diseases through inhibition of free radical formation and platelet aggregation, improvement of dyslipidemia, and an increase in insulin sensitivity.[27–29] Further investigation will be needed to clarify this issue.

In the present study, the risk of AD tended to decrease with higher self-reported dietary mineral intakes, but there was no clear evidence of a significant linear association. As was discussed, the self-reported dietary mineral intakes are likely to have some type of favorable effects on atherosclerotic cardiovascular diseases such as stroke and VaD, but AD has been considered a primarily neurodegenerative disorder caused by amyloid deposition, although recent epidemiological studies have suggested the partial involvement of cardiovascular risk factors in AD development.[8,9] Therefore, these self-reported dietary mineral intakes may have had only a modest benefit in reducing the risk of AD.

Some potential limitations of this study should be noted. First, information regarding dietary nutrient intake derived from a semiquantitative food frequency questionnaire may be not be fully valid. Additionally, dietary intake was assessed only once, at baseline. These

limitations could lead to misclassification of mineral intake to some extent. Such misclassification would weaken the association found in the present study, biasing the results toward the null hypothesis. Second, the validity of magnesium intake estimation made using a semiquantitative food frequency questionnaire has not been explored, although given the high correlations between magnesium intake and calcium (0.76), potassium (0.65), and fiber intakes (0.63), it is likely that the findings on magnesium are meaningful. Finally, the lack of information about the use of supplements containing potassium, calcium, or magnesium may have reduced the accuracy of the findings to some extent.

In conclusion, the present study demonstrated that self-reported dietary intakes of potassium, calcium, and magnesium were associated with lower risks of all-cause dementia and VaD in the general Japanese elderly population. Although plausible mechanisms to account for these associations remain unclear, these findings imply that consuming foods high in potassium, calcium, and magnesium may reduce the risk of late-life onset of dementia, especially VaD. Further epidemiological and clinical studies are warranted to determine whether a diet rich in these minerals can lessen the future risk of dementia.

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Author Contributions: Mio Ozawa: study concept, design, interpretation of data, statistical analysis, and writing of the manuscript. Toshiharu Ninomiya: data collection, interpretation of data, and writing of the manuscript. Tomoyuki Ohara, Yoichiro Hirakawa, Yasufumi Doi, and Jun Hata: data collection and interpretation of data. Kazuhiro Uchida and Tomoko Shirota: nutritional data collection and interpretation of data. Takanari Kitazono: interpretation of data. Yutaka Kiyohara was the study coordinator and contributed to securing of funds, study concept, interpretation of data, and writing of the manuscript.

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Association study of susceptibility genes for late-onset Alzheimer's disease in the Japanese population

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APOE is an established susceptibility gene for late-onset Alzheimer's disease (LOAD). Recent genome-wide association studies have identified many additional susceptibility genes for LOAD in populations of European descent. However, there is little information on whether or not genetic variants in these genes are associated with other ethnicities. To investigate the association of seven genes identified by genome-wide association studies, we carried out a case–control study using 825 LOAD cases and 2934 controls in the Japanese population. For the _APOE_ gene, _APOE_-ε4 carriers had a 4.54-fold higher risk than _APOE_-ε4 noncarriers after adjusting for age and sex ($P=4.6\times10^{-27}$). For other genes, the single-nucleotide polymorphism in the _PICALM_ gene was significantly associated with LOAD ($P=0.02$, odds ratio = 1.23). There was no significant interaction between _PICALM_ and _APOE_-ε4 carrier status (P for interaction = 0.68). Our data indicate that _PICALM_ is also a susceptibility gene for LOAD in the Japanese population. _Psychiatr Genet_ 00:000–000 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Late-onset Alzheimer's disease (LOAD) is the most common form of dementia. *APOE* is an established susceptibility gene for LOAD (Slooter *et al.*, 1998). With the rapid advance in genetic research, the genome-wide association study (GWAS) has identified eight additional susceptibility genes for LOAD in populations of European descent (Reiman *et al.*, 2007; Beecham *et al.*, 2009; Carrasquillo *et al.*, 2009; Harold *et al.*, 2009; Lambert *et al.*, 2009; Seshadri *et al.*, 2010). Among these, the associations of four genes (*CR1*, *CLU*, *PICALM*, and *GAB2*) have already been confirmed by meta-analysis (Ikram *et al.*, 2009; Jun *et al.*, 2010). However, these results were from populations of only European descent; it is unclear whether these genes are also associated with the risk of LOAD in other ethnicities that have different genetic backgrounds. In Japan, no study has studied the associations between these susceptibility genes, except for *APOE* and *GAB2* and LOAD (Miyashita *et al.*, 2009). Here, we carried out a case–control study to elucidate the associations between the susceptibility genes identified by GWAS and LOAD in the Japanese population.

Methods

Study participants

LOAD cases were collected at Kyushu University and 21 affiliated hospitals and institutes ($n=825$, women 77.1%; mean age 83.2±6.5 years). LOAD was diagnosed using clinical information, including neuroimaging results, according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (McKhann *et al.*, 1984). All LOAD cases in this study fulfilled the criteria for probable Alzheimer's disease. Control individuals were recruited from the participants of the Hisayama study. In 2002–2003, a total of 3196 residents of the town of Hisayama aged 40 years or older participated in a health examination (78% participation rate) (Kubo *et al.*, 2007). All participants were followed up prospectively until 2007. A complete description of the follow-up survey on dementia has been published recently (Ohara *et al.*, 2011). The diagnosis of dementia was made on the basis of the guidelines of the *Diagnostic and Statistical Manual of Mental Disorders, Revised 3rd Ed.* (American Psychiatric Association, 1987). All dementia cases were adjudicated by expert psychiatrists. After excluding 262 patients who had developed dementia by 2007, the remaining 2934 dementia-free ~~patients~~ were used as controls (women 56.0%; mean age 60.2±11.5 years).

Single-nucleotide polymorphism selection and genotyping

From the published GWAS for LOAD, we initially selected eight single-nucleotide polymorphisms (SNPs) that showed

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genome-wide significant levels of association ($P < 5.0 \times 10^{-7}$). Among these, we could not genotype one SNP (rs5984894), located at the *PCDH11X* gene on chromosome X (Carrasquillo *et al.*, 2009), because the flanking sequence (1 kb) around it showed extremely high sequence homology (99.3%) to the Y chromosome. In addition, we selected two SNPs (rs429358 and rs7412) to determine *APOE*-ε2/ε3/ε4 alleles (Belbin *et al.*, 2007). Therefore, we genotyped nine SNPs (seven SNPs identified by GWAS and two for the *APOE* allele) using the multiplex PCR-based Invader assay (Third Wave Technologies, Madison, Wisconsin, USA) (Ohnishi *et al.*, 2001). The overall call rate was 99.6%.

Statistical analysis

The Hardy–Weinberg equilibrium for genotype distribution in control individuals was tested using the χ^2-test. Because there was a difference in the mean age between LOAD cases and controls, ~~age-adjusted~~ and sex-adjusted association analysis was carried out by the logistic regression analysis under an additive genetic model to calculate the odds ratio (OR) and the 95% confidence interval (CI) of each SNP according to the risk allele in the initial study. The software package SAS (version 9.2; SAS Institute, Cary, North Carolina, USA) was used to carry out the statistical analysis. Two-sided P value less than 0.05 was considered statistically significant in all analyses. Assuming our sample size, the allele frequencies of the SNPs of the HapMap Japanese in Tokyo, the relative risks of the SNPs in the initial study, and an α error level of 0.05, the statistical power of each SNP was estimated using Purcell's method (Purcell *et al.*, 2003).

Ethical considerations

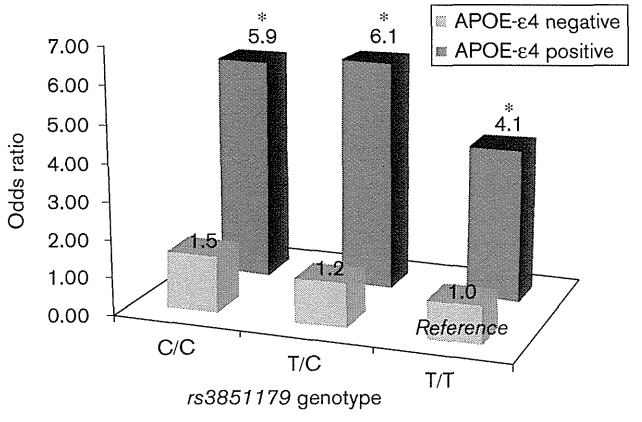
This study was carried out with the approval of the Ethics Committees of the Faculty of Medicine, Kyushu University, and the RIKEN Yokohama Institute. Written informed consent was obtained from all appropriate proxies for LOAD patients and control participants.

Results

All SNPs in the control individuals were in Hardy–Weinberg equilibrium ($P > 0.01$). For the *APOE* gene, *APOE*-ε4 carriers had a 4.54-fold (95% CI 3.43–6.00, $P = 4.6 \times 10^{-27}$) higher risk of LOAD than *APOE*-ε4 noncarriers in the ~~age-adjusted~~ and sex-adjusted analysis. For other genes, we found a significant association with LOAD in one SNP after adjustment for age and sex (Table 1): rs3851179, located at 88.5 kb upstream from the *PICALM* gene (OR 1.23, 95% CI 1.03–1.47). This tendency was unchanged even after adjustment for age, sex, and the *APOE*-ε4 genotype. Marginal associations were observed in rs11610206, located at 9.1 kb downstream from *FAM113B*, and rs744373, 29.8 kb upstream from *BIN1*, probably because of our insufficient sample size.

We also analyzed the combined effect of rs3851179 and *APOE*-ε4 carrier status on the risk of LOAD (Fig. 1). When the *APOE*-ε4 noncarriers with the rs3851179 nonrisk (T/T) genotype were used as a reference, the risks of LOAD were significantly higher in all *APOE*-ε4 carriers irrespective of the rs3851179 genotype. When stratified by *APOE*-ε4 carrier status, the LOAD risks tended to increase with the rs3851179 genotype in *APOE*-ε4 noncarriers (P for trend = 0.053), whereas no significant trend was observed in *APOE*-ε4 carriers (P for

Fig. 1



Impact of rs3851179 genotype on the risk of late-onset Alzheimer's disease stratified by the *APOE*-ε4 allele. The odds ratio was calculated using a logistic regression model after adjustment for age and sex. *P<0.01 versus reference.

Table 1 Associations between susceptibility genes and late-onset Alzheimer's disease in a Japanese population

					Case				Control					Age and sex adjusted			
~~SNPs~~	Gene	Chr.	Position	Allele[a] [1/2]	11	12	22	Total	MAF	11	12	22	Total	MAF	OR (95% CI)	*P*	Power
rs6656401	*CR1*	1	205758672	[G/A]	756	69	0	825	0.04	2706	223	4	2933	0.04	1.21 (0.78–1.89)	0.39	0.32
rs744373	*BIN1*	2	127611085	[A/G]	354	380	91	825	0.34	1397	1256	280	2933	0.31	1.20 (0.99–1.44)	0.06	0.79
rs11136000	*CLU*	8	27520436	[T/C]	60	295	469	824	0.25	242	1156	1535	2933	0.28	1.06 (0.88–1.29)	0.54	0.76
rs3851179	*PICALM*	11	85546288	[T/C]	121	394	310	825	0.39	518	1434	982	2934	0.42	1.23 (1.03–1.47)	0.02	0.87
rs2373115	*GAB2*	11	77768798	[T/G]	177	382	266	825	0.45	540	1405	989	2934	0.42	0.85 (0.72–1.01)	0.06	1.00
rs11610206	*FAM113B*	12	45925793	[C/T]	10	127	688	825	0.09	33	506	2395	2934	0.10	1.33 (0.99–1.77)	0.053	NA
rs597668	*EXOC3L2*	19	50400728	[T/C]	265	418	142	825	0.43	937	1449	547	2933	0.43	0.93 (0.78–1.11)	0.44	0.88

The odds ratio was calculated using logistic regression analysis under an additive genetic model after adjustment for age and sex.
We could not calculate the statistical power of rs11610206 because information about this SNP was insufficient in the initial report.
Chr., chromosome; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]Allele 2 was the risk allele in the initial study.

trend = 0.38). There was no significant effect of the interaction between *APOE*-ϵ4 carrier status and rs3851179 genotype on the risk of LOAD (*P* for interaction = 0.68).

Discussion

In the present study, we found that the marker SNP for the *PICALM* gene (rs3851179) was significantly associated with LOAD in the Japanese population. Although the association of this SNP has already been replicated in populations of European descent (Carrasquillo *et al.*, 2010; Corneveaux *et al.*, 2010; Jun *et al.*, 2010; Kamboh *et al.*, 2010), this is, to our knowledge, the first association study to show significant associations between *PICALM* and LOAD in other ethnicities.

Although rs3851179 is located at 88.5 kb upstream from the *PICALM* gene and 87 kb upstream from the *EED* gene, the HapMap data indicate that this SNP is located in the linkage disequilibrium block, which includes the *PICALM* gene, but not the *EED* gene, in populations of both European and Japanese descent (data not shown). Besides, the precise role of the *PICALM* gene product in LOAD development is unclear. This product is expressed in all tissue types, with a prominent expression in neurons, and plays an important role in the trafficking of vesicle-associated membrane protein 2, which is crucial to neuronal function and memory formation (Harel *et al.*, 2008; Bettens *et al.*, 2010). Meanwhile, the *PICALM* gene product might play a role in amyloid precursor protein processing through the endocytic pathway (Harold *et al.*, 2009; Bettens *et al.*, 2010). Therefore, functional variants in *PICALM* may possibly affect the changes in synaptic function or endocytosis of the amyloid precursor protein, resulting in the susceptibility for LOAD. Further functional studies of *PICALM* could lead toward a better understanding of this issue.

In this study, we could not find significant associations between six susceptibility genes and LOAD. There is a large difference in the minor allele frequency (MAF) of rs6656401 (*CR1*) between European and Japanese populations (MAF = 0.23 for Europeans and 0.04 for Japanese). This low MAF in Japanese markedly decreased the statistical power to 0.32 in this study. Meanwhile, our study had a marginal statistical power of two SNPs [0.79 for rs744373 (*BIN1*) and 0.76 for rs11136000 (*CLU*)], but no significant associations after adjustment for age and sex. One reason for this may be our relatively small case–control samples. Another is that, because the LOAD cases and controls were all individuals of Japanese descent, the genetic heterogeneity among different ethnicities could have weakened the associations with these two SNPs.

In contrast, although our samples had sufficient statistical power (> 0.80), we found no significant associations in two SNPs: rs237311 (*GAB2*) and rs597668 (*EXOC3L2*). This might be because of genetic differences between populations. On the basis of the HapMap data, linkage disequilibrium blocks surrounding these SNPs differ between Europeans and Japanese. Miyashita *et al.* (2009) also reported a difference in the linkage disequilibrium block of the *GAB2* gene between European and Japanese populations. Therefore, it is possible that the unknown causative variants and the marker SNPs are tightly linked in populations of European descent, whereas such linkages may be weak or nonexistent in the Japanese population. Another possibility is that the *GAB2* association is detectable only with stratification by *APOE*-ϵ4 carrier status (Liang *et al.*, 2011). However, we could not find a significant association between rs237311 and LOAD in either *APOE*-ϵ4 carriers or noncarriers (OR 0.93, 95% CI 0.68–1.28 for *APOE*-ϵ4 carriers; OR 0.85, 95% CI 0.69–1.05 for *APOE*-ϵ4 noncarriers). Meanwhile, we failed to find a significant association between rs597668 and LOAD after adjustment for age, sex, and APOE genotype (OR 0.97, 95% CI 0.81–1.17). Lambert *et al.* (2011) and Carrasquillo *et al.* (2011) also reported a nonsignificant association between the *EXOC3L2* gene and LOAD after adjustment for age, sex, and the *APOE*-ϵ4 genotype.

Conclusion

We found the genetic variants in *APOE* and *PICALM* to be associated with LOAD in the Japanese population. Together with published data on populations of European descent, our data indicate that *APOE* and *PICALM* could be the susceptibility genes for LOAD in several ethnic populations. Further investigations are required to establish more reliable associations between these genes and LOAD.

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Conflicts of interest

There are no conflicts of interest.

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Risk factors for anxiety and depression in patients with glaucoma

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ABSTRACT

Aim To assess the risk factors for anxiety and depression in patients with glaucoma.

Methods Anxiety and depression in 408 patients with glaucoma were evaluated using the hospital anxiety and depression scale (HADS) questionnaire, which consists of two subscales, representing HADS-anxiety (HADS-A) and HADS-depression (HADS-D). To identify the risk factors for anxiety and depression, the stepwise and multiple linear regression analyses were carried out with the HADS-A and HADS-D subscores as dependent variables and demographic and clinical features as independent variables.

Results A stepwise linear regression analysis revealed the significantly related factors to be age for HADS-A (β=−0.046, p=0.0007) and HADS-D (β=0.035, p=0.011) and the mean deviation of the Humphrey Visual Field Analyzer 30-2 (HFA30-2) in the better eye for HADS-D (β=−0.095, p=0.0026). Based on multiple linear regression analyses, significant relationships were confirmed between age and the HADS-A subscore (β=−0.046, p=0.0008). Significant relationships were also confirmed between age (β=0.037, p=0.0077) or the mean deviation of HFA30-2 in the better eye (β=−0.094, p=0.0036) and the HADS-D subscore.

Conclusion A younger age was thus found to be a risk factor for anxiety, while an older age and increasing glaucoma severity were risk factors for depression in patients with glaucoma.

INTRODUCTION

Glaucoma is one of the most common chronic eye diseases that can potentially result in bilateral blindness, and glaucoma has previously been reported to associate with anxiety[1–4] and depression,[2 5–7] which are the two most common forms of psychological disturbances. We previously evaluated these psychological disturbances in patients with primary open angle glaucoma (POAG) and sex- and age-matched reference subjects using the hospital anxiety and depression scale (HADS), and also reported that the prevalence of POAG patients with anxiety or depression was higher than that in the reference subjects, which supports that glaucoma is related to anxiety and depression.[8] These psychological disturbances affect the quality of life in patients with glaucoma.[9] Additionally, it was also reported that the presence of depressive symptoms in patients led to poor glaucoma medication use.[10] Providing glaucoma patients with appropriate psychological care is therefore essential to improving their quality of life and drug adherence. In order to detect, prevent and treat the emotional problems that develop in patients with glaucoma, it is important to understand the risk factors for these psychological disturbances. However, only a few studies about the risk factors for these conditions were reported previously, and further studies with a larger sample size, using different and reliable instruments for evaluating these psychological disturbances, are desirable to more fully elucidate their risk factors. In this study, we aimed to identify the predictors of anxiety and depression in patients with glaucoma.

PARTICIPANTS AND METHODS

Glaucoma patients, including those with POAG, exfoliation glaucoma (XFG), primary angle closure glaucoma (PACG) and secondary glaucoma (SG), were enrolled from ophthalmology practices in University of Yamanashi and 14 general hospitals in Japan. A questionnaire including a checklist of systemic diseases and prescribed medications and a Japanese version of the HADS for psychiatric evaluation were distributed to the participants, and they submitted the questionnaire voluntarily. All participants had a complete fundus examination and a typical glaucomatous cupping of the optic disc with compatible visual field defects detected by automated static perimetry (Humphrey Visual Field Analyzer 30-2, Humphrey Instruments, San Leandro, California, USA; HFA30-2). Mean deviation (MD) in the last HFA30-2 taken within 3 years of enrolment in this study was used to evaluate the visual field loss. Only 'reliable' visual fields, defined by false-positive results, false-negative results or fixation losses not exceeding 33%, were used. The glaucoma patients with other ocular diseases that can cause visual field defect were not eligible to participate in this study. No patients with the following conditions were also eligible because of the high possibility that central nervous disorders may have caused psychological disturbances: patients with cerebrovascular disease,[11] primary neurodegenerative diseases[12] or schizophrenia.[13] The patients prescribed β-blocker eye drops or oral carbonic anhydrase inhibitors were eligible to investigate whether these medicines cause depression. All participants gave a written informed consent prior to enrolment and our research protocol was approved by the Ethics Committee of University of Yamanashi. The study was conducted in accordance with the Declaration of Helsinki.

Evaluation for psychological disturbance

The HADS was used in this study. This questionnaire was developed by Zigmond and Snaith[14] to identify and quantify the two most common forms

of psychological disturbances (anxiety and depression) in physically ill patients. Data on the test–retest reliability and validity of HADS have been previously reported.[15] Additionally, the original HADS was translated into Japanese and back-translated to English to ensure that the original meaning was retained. Factor analysis has provided reasonable confirmation of the Japanese version of the HADS scales.[16] This scale contains 14 questions graded on a 4-point Likert scale (0–3) and consists of two subscales, thus representing HADS-anxiety (HADS-A) and HADS-depression (HADS-D). The minimum sum score of each of the seven item subscales is 0 and the maximum is 21, and higher scores are indicative of a higher level of depression and anxiety. In this study, scores higher than 10 on the HADS-A and HADS-D were defined as anxiety and depression respectively, as previously described.[8]

Statistical analysis

Data were analysed using SAS statistical software (V.9.1, SAS Institute Inc.). The mean subscores of the HADS-A and HADS-D were compared between the patients with POAG and non-POAG, and among the patients with POAG, XFG, PACG and SG, using Student t test. To identify the predictive factors for psychological disturbances (anxiety and depression) in patients with glaucoma, a stepwise linear regression analysis was carried out with HADS-A and HADS-D subscores as dependent variables and demographic and clinical features as independent variables. Age, gender, MD of the HFA30-2 in the better and worse eyes, the number of different types of eye drops used daily, and a history of glaucoma surgery (use of β-blocker eye drops or oral carbonic anhydrase inhibitors for the HADS-D subscore) were tested as independent variables. A multiple linear regression model was used to confirm the relationship between age and anxiety (HADS-A subscore), adjusted with or without demographic and clinical variables. The same model was also used to confirm the relationships between age or MD of the HFA30-2 in the better eye and depression (HADS-D subscore). We divided the samples into 10-year cohorts and 10 dB cohorts of the MD of the HFA30-2 in the better eye to graph the relationship between age or MD and these psychological disturbances. The mean subscores of the HADS-A and HADS-D were compared between the MD groups using an analysis of variance, and the prevalence of anxiety and depression was compared between the MD groups using the χ^2 test. For statistical analysis, visual acuity was converted into a logarithm of minimum angular resolution (logMAR) visual acuity. For the eyes that could not be examined by HFA30-2 because of poor visual function, the vision level of these eyes was assigned an MD value of −34.0 dB. p Value <0.05 was considered to be statistically significant.

RESULTS

Four hundred and eight glaucoma patients, including 318 POAG, 43 XFG, 32 PACG and 15 SG patients, participated in this study. The demographic data for the participants are shown in table 1. The mean age of the participants was 66.2±11.8 (mean ± SD) years, and ranged from 25 to 89 years. The participants consisted of 194 male and 214 female patients. The mean subscores of the HADS-A and HADS-D in the participants were 5.5±3.2 (ranged from 0 to 14) and 4.9±3.2 (ranged from 0 to 15), respectively. The mean subscores of the HADS-A in patients with POAG, XFG, PACG and SG were 5.5±3.3, 5.0±3.1, 5.5±2.8 and 6.6±3.7, respectively, and the mean subscores of the HADS-D in patients with POAG, XFG, PACG and SG were 4.9±3.2, 4.8±2.7, 4.8±3.3 and 5.1±4.0, respectively. There were no significant differences in the mean subscores of the HADS-A and HADS-D between the patients with POAG and non-POAG or among the patients with POAG, XFG, PACG and SG.

Table 1 Demographic and clinical characteristics of participants

	n (%)	Mean ± SD	Range
Age (years)		66.2±11.8	25–89
Male gender	194 (47.5)		
LogMAR best corrected visual acuity			
Better eye		−0.025±0.14	−0.70–0.30
Worse eye		−0.25±0.57	−3.2–0.18
Mean deviation of HFA30-2 (dB)			
Better eye		−6.7±7.1	−30.9–2.6
Worse eye		−13.2±9.2	−34.0–1.2
Number of different types of eye drops		−2.0±1.3	0–7
History of glaucoma surgery	95 (23.3)		
Use of β-blocker eye drops	257 (63.0)		
Use of oral carbonic anhydrase inhibitors	18 (4.4)		
HADS-A subscores		5.5±3.2	0–14
HADS-D subscores		4.9±3.2	0–15

HADS-A, hospital anxiety and depression scale-anxiety; HADS-D, hospital anxiety and depression scale-depression; HFA30-2, Humphrey Visual Field Analyzer 30-2; LogMAR, logarithm of minimum angular resolution.

To identify the variables that were independently associated with the HADS-A or HADS-D subscores, we conducted a stepwise linear regression analysis. This analysis revealed age to be significantly associated with the HADS-A (β=−0.046, p=0.0007, table 2) and HADS-D (β=0.035, p=0.011, table 3), and MD of the HFA30-2 in the better eye was related to the HADS-D (β=−0.095, p=0.0026, table 3). To confirm the association between the HADS subscores and these identified risk factors, multiple linear regression analyses were performed. Significant relationships were found between age and the HADS-A subscore both with and without adjusting for demographic and clinical variables (β=−0.046, p=0.0008 and β=−0.043, p=0.0022, respectively, table 4). Significant relationships were also found, both with and without adjusting for demographic and clinical variables, between the age (β=0.037, p=0.0077 and β=0.036, p=0.0076 respectively) or MD of the HFA30-2 in the better eye (β=−0.094, p=0.0036 and β=−0.065, p=0.0033 respectively) and the HADS-D subscore (table 5). These relationships were graphically illustrated in figures 1–4. The HADS-D subscores and the prevalence of depression were still significantly different between the MD groups (p=0.012, analysis of variance and p=0.0019, χ^2 test respectively), even when the patients were limited to those aged 70–80 years to exclude the influence of age.

Table 2 Factors predicting anxiety (HADS-A subscore) in patients with glaucoma as determined by a stepwise linear regression analysis

Variables	β	SE	p Value
Age	−0.046	0.013	0.0007
Male gender	–	–	–
Mean deviation of HFA30-2 in the better eye	–	–	–
Mean deviation of HFA30-2 in the worse eye	–	–	–
Number of different types of eye drops	–	–	–
History of glaucoma surgery	0.58	0.37	0.12

No variables other than age and history of glaucoma surgery met the 0.15 significance level for entry into the model.
β, unstandardised regression coefficient; HADS-A, hospital anxiety and depression scale-anxiety; HFA30-2, Humphrey Visual Field Analyzer 30-2, F change=7.0, p Value=0.0010. SE, standard error

Table 3 Factors predicting depression (HADS-D subscore) in patients with glaucoma as determined by a stepwise linear regression analysis

Variables	β	SE	p Value
Age	0.035	0.014	0.011
Male gender	–	–	–
Mean deviation of HFA30-2			
In the better eye	−0.095	0.031	0.0026
In the worse eye	0.041	0.025	0.096
Number of different types of eye drops	–	–	–
History of glaucoma surgery	–	–	–
Use of β-blocker eye drops	–	–	–
Use of oral carbonic anhydrase inhibitor	–	–	–

No variables other than age and mean deviation of HFA30-2 met the 0.15 significance level for entry into the model.
β, unstandardised regression coefficient; HADS-D, hospital anxiety and depression scale-depression; HFA30-2, Humphrey Visual Field Analyzer 30-2, F change=5.6, p Value=0.0008.

DISCUSSION

In this study, age had a negative correlation with the HADS-A subscore in patients with glaucoma. To our knowledge, this is the first report to show that younger glaucoma patients tend to be more anxious compared with older patients. A younger age as a predictor for anxiety has also been reported for other chronic physical diseases, such as cardiovascular diseases[17] and cancer.[18] Ramsawh *et al*[19] described that the severity of anxiety disorders declined over time by a naturalistic, longitudinal, short-interval follow-up performed to elucidate the course of anxiety disorders over 14 years in a population of 453 largely middle-aged patients recruited from outpatient psychiatry and primary care facilities. Orgeta[20] reported that younger adults had greater emotional regulation difficulties compared with older ones, which may support the fact that younger age is a risk factor for anxiety in patients with glaucoma. As glaucoma can potentially result in bilateral blindness, younger glaucoma patients may have been more anxious about maintaining their visual function, because of their longer remaining lifespan. Bechetoille *et al*[4] described that anxiety scores in a glaucoma-specific health-related quality of life questionnaire dropped noticeably when patients were first diagnosed with glaucoma. It has also been reported that most glaucoma patients showed an anxious reaction to their announcement of the diagnosis, and that they were dissatisfied with the information provided by their doctor and sought other sources of information.[3] Patients with little information about their disease also have higher HADS scores,[15] and so it is important for ophthalmologists to provide accurate and appropriate information about glaucoma to prevent patients, especially younger patients, from developing anxiety.

Table 4 Results of a multiple regression analysis using the HADS-A subscale as the dependent variable in patients with glaucoma

	Non-adjusted		Adjusted with demographic and clinical variables	
Independent variables	β	p Value	β	p Value
Age	−0.046	0.0008	−0.043	0.0022
Male gender	–	–	−0.13	0.68
Mean deviation of HFA30-2				
In the better eye	–	–	−0.046	0.15
In the worse eye	–	–	0.045	0.093
Number of different types of eye drops	–	–	0.16	0.22
History of glaucoma surgery			0.60	0.14

β, unstandardised regression coefficient; HADS-A, hospital anxiety and depression scale-anxiety; HFA30-2, Humphrey Visual Field Analyzer 30-2.

Table 5 Results of a multiple regression analysis using the HADS-D subscale as the dependent variable in patients with glaucoma

	Non-adjusted		Adjusted with demographic and clinical variables	
	β	p Value	β	p Value
Age	0.036	0.0076	0.007	0.0077
Male gender	–	–	0.24	0.44
Mean deviation of HFA30-2	–	–	−0.094	0.0036
In the better eye				
In the worse eye	–	–	0.048	0.078
Number of different types of eye drops	–	–	0.15	0.32
History of glaucoma surgery	–	–	0.058	0.89
Use of β-blocker eye drops	–	–	0.13	0.74
Use of oral carbonic anhydrase inhibitor			−0.88	0.26

β, unstandardised regression coefficient; HADS-D, hospital anxiety and depression scale-depression; HFA30-2, Humphrey Visual Field Analyzer 30-2.

It was previously reported that anxious personality traits and anxiety disorders were more prevalent in a severe POAG group (n=48) than control (n=37) or early POAG (n=48) groups using the Vocabulaire Binois Pichot, Cattel's 16 Personality Factors, Thematic Apperception Test and the Rorschach Test,[1] and that the risk of anxiety in female patients with glaucoma (n=68) was found to be higher than in male subjects (n=53) using the HADS.[9] However, there were no relationships between anxiety and the severity of glaucoma or gender in this study, although the severity of the visual field defect in the better eye was associated with depression. Further studies using other instruments should be performed to elucidate whether the severity of glaucoma and gender are associated with anxiety.

With regard to depression, older age and a decreasing MD of the HFA30-2 in the better eye were associated with depression in this study. Skalicky and Goldberg[6] reported that depression was more prevalent with increasing glaucoma severity in patients aged 70–79 years using the Nelson Glaucoma Severity Scale and the Geriatric Depression Scale-15 questionnaire, and that older age was a risk factor for depression. Our results supported their report, and this finding was confirmed in patients with a wider range of ages (ranging from 25 to 89 years) by a multiple linear regression analysis. Erb *et al*[2] reported that the POAG inpatients (n=21), not outpatients, had higher scores for depression compared with the cataract patients (n=18) using the Beck Depression Inventory. This might have been because the POAG inpatients might suffer from more severe glaucoma compared

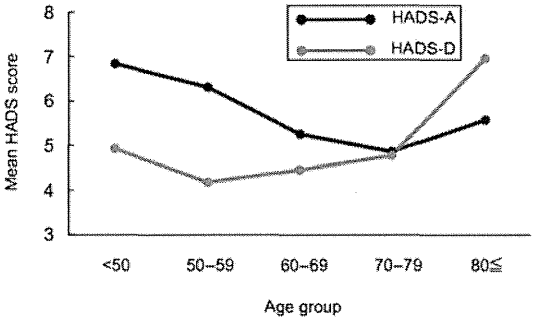

Figure 1 The association between age and the hospital anxiety and depression score (HADS) in patients with glaucoma. The HADS-anxiety (HADS-A) subscores in younger glaucoma patients tended to be higher compared with those in older patients. On the other hand, the HADS-depression (HADS-D) subscores in older glaucoma patients tended to be higher compared with those in younger patients.

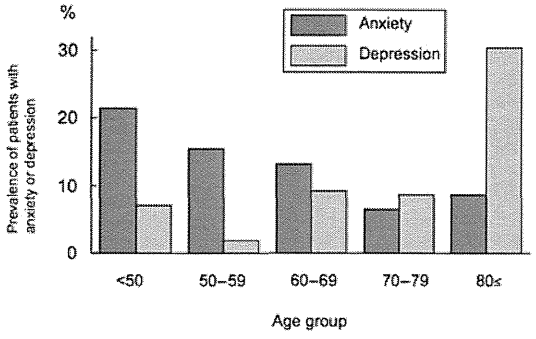

Figure 2 The association between age and the prevalence of anxiety (a score higher than 10 on the hospital anxiety and depression score-anxiety) or depression (a score higher than 10 on the hospital anxiety and depression score-depression) in patients with glaucoma. The prevalence of anxiety in younger glaucoma patients tended to be higher compared with that in older patients. On the other hand, the prevalence of depression in older glaucoma patients tended to be higher compared with that in younger patients. The prevalence of anxiety was higher than that of depression in glaucoma patients younger than 70 years of age.

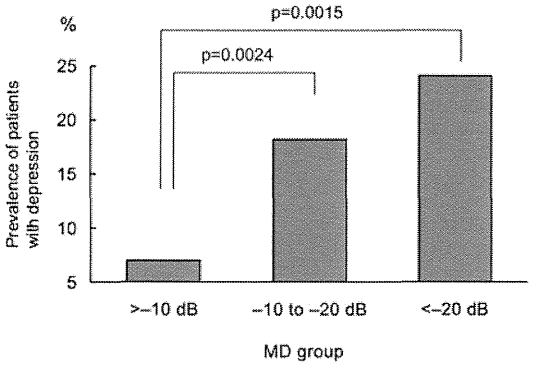

Figure 4 The association between the MD of the HFA30-2 in the better eye and depression (a score higher than 10 on the hospital anxiety and depression score-depression) in patients with glaucoma. There was a significant difference (p=0.0006, χ^2 test) in the prevalence of depression between the MD groups. The prevalence of depression in patients with an MD ranging from −10 to −20 dB or an MD −20 dB was significantly higher than that in patients with an MD −10 dB. HFA30-2, Humphrey Visual Field Analyzer 30-2; MD, mean deviation.

with the POAG outpatients. Cumurcu *et al*[21] reported that the scores of the Hamilton Depression Rating Scale and Montgomery-Asberg Depression Rating Scale of the XFG group (n=41) were significantly higher than those of the control (n=40) and POAG (n=32) groups, although there were no differences between the control and POAG groups regarding depression. In this study, there were no differences between the patients with POAG and XFG regarding depression. Tastan *et al*[9] reported that the risk of anxiety and depression was higher in unmarried compared with married participants, including 121 patients with glaucoma and 64 control subjects. Unfortunately, we did not investigate whether the participants were married or unmarried, and so this factor was not tested as an independent variable in our stepwise linear regression model.

It remains controversial with regard to whether the clinical use of a β-blocker is responsible for depression.[22 23] We previously reported that the prevalence of patients with depression was not different between the POAG patients being treated with and without β-blocker eye drops.[8] This study also did not find any relationship between the use of β-blocker eye drops and depression, although a stepwise linear regression analysis was performed with a larger number of glaucoma patients. The use of β-blocker eye drops does not seem to be the principal cause of depression in patients with glaucoma. However, it has been reported that depression in patients with glaucoma while using timolol maleate (non-selective β-blocker) eye drops was improved by withdrawal or switching to betaxolol hydrochloride (β-1 selective β-blocker) eye drops.[24] Thus, patients with suspected depression should be evaluated by using survey instruments for depression or by consulting a psychiatrist before the prescription of β-blocker eye drops. Depression has also been reported as one of the side-effects of oral carbonic anhydrase inhibitors,[25] although there have been no studies that have performed a statistical analysis to evaluate the relationship between the use of oral carbonic anhydrase inhibitors and depression. In this study, as was the case with the β-blocker eye drops, there was no statistically significant relationship found between oral carbonic anhydrase inhibitor use and depression. However, further studies in a larger number of patients should be performed to more fully evaluate the relationship between them, because the number of patients who used oral carbonic anhydrase inhibitors in this study was small (18 patients).

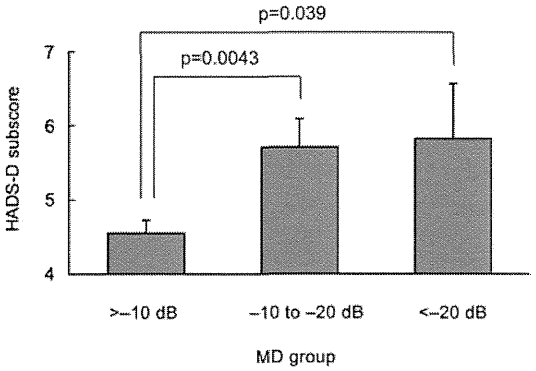

Figure 3 The association between the MD of the HFA30-2 in the better eye and the HADS-D subscore in patients with glaucoma. There was a significant difference (p=0.0040, analysis of variance) in the HADS-D subscores between the MD groups. The HADS-D subscores in patients with an MD ranging from −10 to −20 dB or an MD −20 dB were significantly higher than those in patients with an MD −10 dB. HADS-D, hospital anxiety and depression score-depression; HFA30-2, Humphrey Visual Field Analyzer 30-2; MD, mean deviation.

As potential limitations of this study, the population sample was based on voluntary participation, and both anxiety and depression might affect an individual's participation in filling out a questionnaire. This selection bias might have had some influence on the investigated outcomes. In addition, these psychological disturbances might affect an individual's ability to perform a visual field test. Although the HADS is easy and convenient for trial purposes, a questionnaire is not comparable with a formal psychiatric diagnosis of depression or anxiety. Moreover, other ethnic populations may have different psychological reactions to glaucoma.

In conclusion, a younger age was found to be a risk factor for anxiety, while an older age and increasing glaucoma severity were risk factors for depression in patients with glaucoma. It is therefore essential for physicians and co-medical staff to be aware of the risk factors for anxiety and depression in patients with glaucoma, and to provide glaucoma patients with appropriate psychological care as well as ophthalmological care to prevent them from developing anxiety and depression.

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Risk factors for anxiety and depression in patients with glaucoma

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Gamma Band Neural Synchronization Deficits for Auditory Steady State Responses in Bipolar Disorder Patients

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Abstract

Periodic auditory click stimulation has been reported to elicit an auditory steady state response (ASSR). The ASSR has been suggested to reflect the efficiency of γ-amino butyric acid (GABA) inhibitory interneuronal activity. Although a potential role for GABAergic dysfunction has been previously proposed, the role of neural synchronization in the ASSR in people with bipolar disorder (BD) has received little attention. In the current study, we investigated ASSRs to 20 Hz, 30 Hz, 40 Hz and 80 Hz click trains in BD patients. A total of 14 (4 males) BD patients and 25 (10 males) healthy controls participated in this study. ASSRs were obtained using whole-head 306-channel magnetoencephalography to calculate, ASSR power values and phase locking factors (PLF). BD patients exhibited significantly reduced mean ASSR power and PLF values bilaterally at frequencies of 30, 40, and 80 Hz ($p<0.05$ for these frequencies). At 20 Hz, bipolar patients showed no significant reduction in mean ASSR power and PLF values. There was a significant negative correlation between 80 Hz-ASSR-power values obtained from the right hemisphere and scores on the Hamilton Depression Rating Scale (rho = −0.86, $p=0.0003$). The current study showed reduced low and high gamma band ASSR power and PLF bilaterally with no significant beta band ASSR reduction in BD patients. BD patients are characterized by deficits in gamma band oscillations, which may be associated with GABA inhibitory interneuronal activity dysfunction.

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Introduction

Periodic auditory click stimulation elicits an auditory steady state response (ASSR) that synchronizes to both the phase and frequency of the click stimulus. Several magnetoencephalography (MEG) studies have reported that source generators of ASSR are restricted to the primary auditory cortex [1], [2]. Neural circuitry functioning in the primary auditory cortex can be assessed using MEG-ASSR. The ASSR can reveal information about neural activity with respect to phase synchronization and response magnitude. The ASSR exhibits resonant frequencies in response to click trains at approximately 40 Hz and 80 Hz, although 40 Hz click trains produce responses of a larger magnitude [3].

Responses between 14 and 30 Hz are categorized as beta band activity, and rhythms >30 Hz are categorized as gamma band activity [4]. In addition, gamma band activity is subdivided into low (30–70 Hz) and high gamma band (>70 Hz) oscillations [5]. It has been suggested that the ASSR reflects the efficiency of γ-amino butyric acid (GABA) inhibitory interneuronal activity, which control the timing of pyramidal neurons in layer II/III of the cortex [6], [7]. Additionally, interactions between pyramidal neurons and inhibitory neurons have been found to produce emergent oscillations [8]. Emrich et al. proposed that GABAergic dysfunction plays a role in bipolar disorder (BD), based on the efficacy of valproate in the treatment of patients with this disorder [9]. Moreover, a post-mortem study of BD patients reported down-regulation in the expression of GABAergic genes (e.g., glutamic acid decarboxylase) [10]. Since ASSR is linked to GABA activity, investigations of ASSR are important in understanding BD.

In an MEG study of ASSR in BD, Maharajh et al. reported that patients exhibited a reduced right 40-Hz ASSR [11]. An electroencephalography (EEG) study by O'Donnell et al. reported reduced 20-, 30-, 40-, and 50-Hz ASSR in BD patients [12]. In addition, Rass et al. reported reduced ASSR power at 40 Hz and reduced ASSR synchronization at 40 Hz- and 50 Hz- stimulation in BD patients [13]. Studies of ASSR in schizophrenia (SZ) have consistently reported reduced gamma band ASSR [14–18]. For example, Light et al. reported that SZ patients exhibited reductions in both the evoked power and phase synchronization of ASSR to 30- and 40- Hz stimulation, but exhibited normal responses to 20- Hz stimulation [16]. Uhlhaas et al. suggested that

GABA is involved in the generation and synchronization of beta and gamma oscillations [4]. One computational modeling study (assuming that reduction of GABAergic interneurons increases the variability of GABA time constants) showed reduced 40 Hz responses and increased 20 Hz responses [19].

As discussed above, BD and SZ patients show similar patterns of ASSR deficits. Moreover, a post-mortem study reported a reduction in the numerical density of inhibitory interneurons in both BD and SZ [20]. Taken together, these findings indicate that neural circuitry dysfunction may exhibit similarities between these disorders at least to some extent. Recently, high gamma band oscillations have become a subject of increasing research interest [21], [22]. However, to our knowledge, only two studies have examined high gamma band ASSR (i.e., ASSR to 80 Hz click trains) in SZ [17], [18], with no studies of high gamma band ASSR in patients with BD. Overall, ASSR has received less attention in BD than in SZ research.

The current study used MEG to examine beta (ASSR to 20 Hz click trains), low (ASSR to 30 and 40 Hz click trains) and high gamma ASSR in BD patients. The present study was designed to test the hypothesis that BD patients exhibit reduced low and high gamma ASSR and no significant beta ASSR reduction.

Results

Demographic Characteristics

There were no significant group differences in age, handedness, self or parental SES or years of education (Table 1). There was no significant correlation between the dose of neuroleptic medication or lithium and ASSR power or PLF ($-0.48 \leq rho \leq 0.63$, $0.06 \leq p \leq 0.97$ for neuroleptics; $-0.65 \leq rho \leq 0.35$, $0.08 \leq p \leq 1.0$ for lithium). ASSR variables did not correlate significantly with valproate dosage, with the exception of significant negative correlations between right hemisphere 40 Hz-ASSR and the dosage (rho = −0.75, $p = 0.02$ for PLF; rho = −0.66, $p = 0.05$ for power).

To exclude the effects of transient gamma band responses [9], we also performed the analyses of the ASSR using a 200–500 ms window. The statistically significant results reported below remained the same. ASSR variables did not correlate significantly with demographic data or clinical scale scores ($-0.008 \leq rho \leq 0.54$, $0.07 \leq p \leq 0.93$) in either group, with the exception of a significant negative correlation between right hemisphere 80 Hz-ASSR-power and the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D) scores (rho = −0.86, $p = 0.0003$) in participants with BD.

Mean ASSR Power

Figure 1 shows group-averaged time-frequency maps of ASSR power for each hemisphere. Values of mean±SD ASSR power are shown in Table 2. A repeated measures analysis of variance (ANOVA) demonstrated significant main effects of group ($F[1,37] = 7.0$, $p = 0.01$), frequency ($F[3,35] = 34.7$, $p < 0.0001$), and hemisphere ($F[1,37] = 10.9$, $p = 0.002$), and significant frequency-by-group ($F[3,35] = 3.4$, $p = 0.03$) and frequency-by-hemisphere ($F[3,35] = 3.4$, $p = 0.03$) interactions, with no other significant interactions ($0.50 \leq p \leq 0.64$). To delineate the significant frequency-by-group interaction, group differences were compared with *t*-tests using the average of both hemispheres for each frequency. Participants with BD showed significantly reduced ASSR power at 30-Hz ($t[37] = 3.1$, $p = 0.004$), 40-Hz ($t[37] = 2.6$, $p = 0.01$), and 80-Hz ($t[37] = 2.2$, $p = 0.03$), while no significant group differences were observed at 20-Hz ($t[37] = 0.38$, $p = 0.71$).

Table 1. Demographic and Clinical Characteristics of Participants.

	HC	BD	df	*t* or x^2	*p*
Sex, M/F, No	10/15	4/10	1	0.51	0.50
Age (years)	37.6±15.8	40.8±13.0	37	−0.68	0.50
Handedness	96.4±7.1	96.4±9.5	37	−0.01	0.99
SES	2.3±0.7	2.6±1.1	37	−0.85	0.40
Parental SES	2.8±1.0	3.1±1.1	37	−0.65	0.52
Education (years)	14.5±2.1	13.6±2.3	37	1.2	0.22
Symptom onset (years)		28.6±13.8			
Duration of illness (years)		11.6±9.9			
Medication dose (CPZ equiv., mg)		314±201			
YMRS		1.9±3.9			
SIGH-D		8.6±5.0			

Values are mean ± SD unless otherwise noted. HC: healthy controls, BD: patients with bipolar disorder,
SES = socioeconomic status, YMRS = Young Mania Rating Scale, SIGH-D = Structured Interview Guide for the Hamilton Depression Rating Scale.
Patients with BD were administered the following medications : N = 2 lithium & valproate; N = 1 lithium, quetiapine & zotepine; N = 1 lithium & quetiapine; N = 1 quetiapine, amoxapine & paroxetine; N = 1 valproate, amoxapine, trazodone & paroxetine; N = 1 valproate & quetiapine, N = 1 quetiapine & paroxetine; N = 1 lithium, valproate, quetiapine & amitriptyline; N = 1 valproate & trazodone; N = 1 lithium, valproate, olanzapine & risperidone; N = 1 valproate & quetiapine; N = 1 lithium, valproate & quetiapine; N = 1 lithium, quetiapine & levomepromazine.
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For 40 Hz harmonic response to 20 Hz stimulation, a repeated measures ANOVA demonstrated a significant main effect of hemisphere ($F[1,37] = 4.91$, $p = 0.033$), a trend-level significant group effect ($F[1,37] = 2.91$, $p = 0.096$), and no significant hemisphere-by-group interaction ($F[1,37] = 1.69$, $p = 0.20$), indicating trend-level reductions of 40 Hz harmonic powers to 20 Hz stimulation in BD patients.

Mean ASSR PLF

Figure 2 shows group averaged time-frequency maps of ASSR PLF for each hemisphere. The mean±SD of ASSR PLF values are shown in Table 3. A repeated measures ANOVA demonstrated significant main effects of group ($F[1,37] = 12.0$, $p = 0.001$), frequency ($F[3,35] = 49.8$, $p < 0.0001$), and hemisphere ($F[1,37] = 10.7$, $p = 0.002$), and significant frequency-by-group ($F[3,35] = 4.3$, $p = 0.02$) and frequency-by-hemisphere ($F[3,35] = 6.3$, $p = 0.002$) interactions, with no other significant interactions ($0.15 \leq p \leq 0.62$). To delineate the significant frequency-by-group interaction, group differences were compared with *t*-tests, using the average of both hemispheres for each frequency. Participants with BD exhibited significantly reduced ASSR PLF at 30-Hz ($t[37] = 3.1$, $p < 0.0001$), 40-Hz ($t[37] = 3.0$, $p = 0.005$), and 80-Hz ($t[37] = 2.3$, $p = 0.03$), while no significant group differences were observed for 20-Hz ($t[37] = 1.5$, $p = 0.17$).

For 40 Hz harmonic response to 20 Hz stimulation, a repeated measures ANOVA demonstrated no significant main effects of group ($F[1,37] = 2.33$, $p = 0.14$) or hemisphere ($F[1,37] = 3.36$, $p = 0.075$) and no significant hemisphere-by-group interaction ($F[1,37] = 1.21$, $p = 0.28$).