Table 1 Summary of the GWAS and replication study

SNP	Allele	Minor allele	Chr.	Chr. location	Gene	Study	No. of samples		MAF		Age and sex adjusted			
							Case	Control	Case	Control	*P*	OR	95% CI	P_{het}
rs13278062	T/G	T	8	23,138,916	*TNFRSF10A-LOC389641*	GWAS	827	3,323	0.417	0.343	2.46×10^{-6}	0.71	0.62–0.82	
						Replication	701	15,565	0.417	0.346	8.19×10^{-8}	0.74	0.66–0.82	
						Combined					1.03×10^{-12}	0.73	0.67–0.80	0.68
rs1713985	T/G	G	4	57,481,207	*REST-C4orf14-POLR2B-IGFBP7*	GWAS	827	3,323	0.333	0.286	9.03×10^{-5}	1.34	1.16–1.56	
						Replication	708	15,569	0.329	0.282	5.71×10^{-5}	1.27	1.13–1.43	
						Combined					2.34×10^{-8}	1.30	1.19–1.42	0.56

The age- and sex-adjusted *P* values were calculated by logistic regression analysis under an additive model. The combined *P* values were calculated by the inverse variance method. The *P* values of heterogeneities (P_{het}) across the population were estimated formally using a Cochran's *Q* test. Chr., chromosome; No., number; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

(**Supplementary Fig. 1c**). When we evaluated the quantile-quantile plot only using the samples in the main (Hondo) cluster, the inflation factor was 1.076 (**Supplementary Fig. 1d**). Therefore, we considered that genotype misclassification or population substructure might not be the cause of the difference in the inflation factor.

In our GWAS, two loci reached a genome-wide significant level of association ($P < 5 \times 10^{-8}$; **Supplementary Fig. 1g**). These two loci have already been reported in previous GWAS[7–12]: *ARMS2* (rs3750847, $P = 8.67 \times 10^{-29}$) and *CFH* (rs800292, $P = 4.23 \times 10^{-15}$). Results from these loci are shown in **Supplementary Figure 2**. We also checked the association of previously reported susceptibility loci for AMD (**Supplementary Table 1**). We found a significant association for three loci (*CFI*, *C2* and *CFB*), whereas we did not replicate susceptibility loci identified in recent GWAS of the European population (*TIMP3* and *LIPC*), probably because of the lower statistical power in our study. Notably, rs2230199, a marker SNP at the *C3* locus, was not polymorphic in the Japanese population. Although exudative AMD is a major type of AMD in Japanese compared to European individuals, these results indicate that the underlying disease mechanisms of AMD are largely similar for both populations, and the differences in genetic modifiers or environmental factors may represent the differences in the prevalence of a specific late-stage AMD type.

To identify additional susceptibility loci, we conducted a replication study using an independent set of 709 Japanese exudative AMD cases and 15,571 controls. Among 146 SNPs that showed $P < 1.0 \times 10^{-4}$ in GWAS, we selected 77 SNPs for the replication study after excluding 47 SNPs within the same locus ($r^2 > 0.8$) and 22 SNPs located at previously reported loci. We successfully genotyped all 77 SNPs using the multiplex PCR-based Invader assay and found significant association in two SNPs after Bonferroni correction (corrected $P < 6.49 \times 10^{-4}$; **Supplementary Table 2**). When we combined the results of the GWAS and a replication study by the inverse variance method, two SNPs reached a genome-wide significance level of association: rs13278062 on chromosome 8p21 (combined $P = 1.03 \times 10^{-12}$, odds ratio (OR) = 0.73, 95% confidence interval (CI) 0.67–0.80) and rs1713985 on chromosome 4q12 (combined $P = 2.34 \times 10^{-8}$, OR = 1.30, 95% CI 1.19–1.42; **Table 1**). The ORs between the GWAS and replication study were quite similar, and we did not observe any heterogeneity across the studies. A previous linkage study has also indicated an association on chromosome 8p21 (ref. 18).

Because exudative AMD is classified into typical AMD (t-AMD) and polypoidal choroidal vasculopathy (PCV), we found both SNPs showed a similar effect on susceptibility to each AMD subtype (**Supplementary Table 3**). Although we performed age-adjusted analysis, there is a possibility that aging or cohort effect might distort

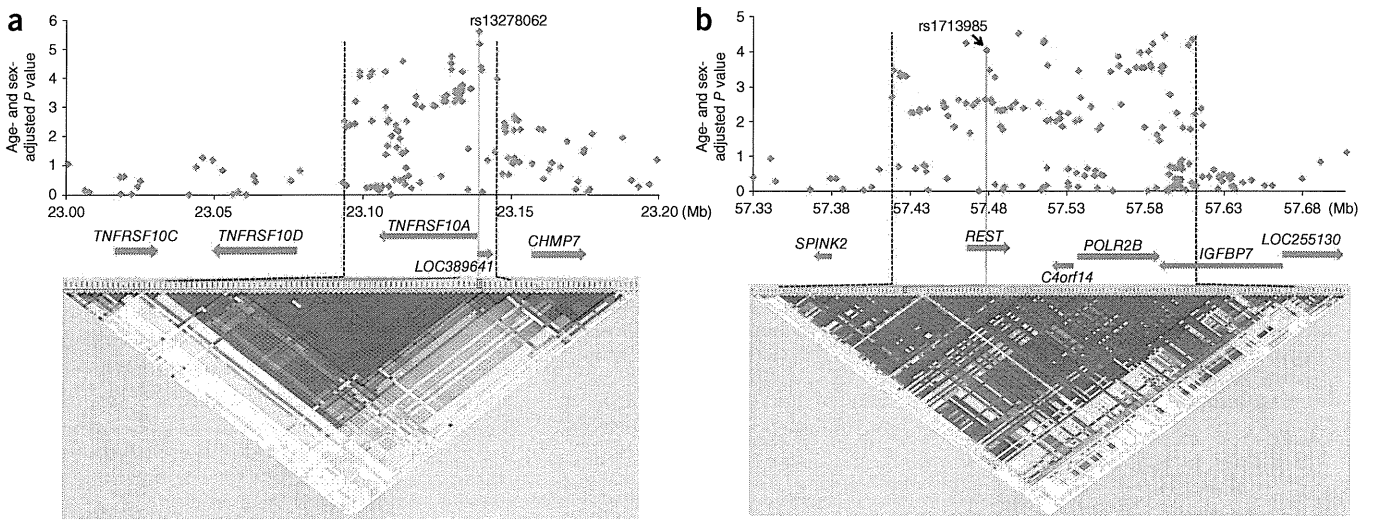

Figure 1 Case-control association plots, LD map and genomic structure of the *TNFRSF10A- LOC389641* region in chromosome 8p21 (**a**) and the *REST-C4orf14-POLR2B-IGFBP7* region in chromosome 4q12 (**b**). The candidate region is shown between the two black dashed lines. We performed fine mapping in the regions from 23.09–23.14 Mb in chromosome 8p21 and 57.42–57.61 Mb in chromosome 4q12. The blue diamonds represent $-\log_{10} P$ values obtained from GWAS and fine mapping. We drew the LD map based on *D'* values using the genotype data from the cases and controls in the GWAS samples. The blue lines indicate the position of the marker SNPs (rs13278062 (**a**) and rs1713985 (**b**)).

the findings of our study because the controls were younger than the cases, overall. However, we did not find marked differences in the minor allele frequencies (MAFs) of the two SNPs in each 10-year age group (**Supplementary Table 4**).

To narrow down the candidate regions and to identify susceptibility genes for exudative AMD, we carried out fine mapping using GWAS case-control samples. We first defined a linkage disequilibrium (LD) block and constructed a $-\log_{10} P$ plot of chromosome 8p21 using GWAS data. We found that the most highly associated SNP (rs13278062) represented an LD block that spanned from 23.078–23.152 Mb. Then, we selected and genotyped 18 SNPs around this LD block based on the data from the HapMap phase 2 Japanese population with a MAF ≥ 0.05. Next, we resequenced a 51-kb region from 23.094–23.145 Mb using 48 individuals with exudative AMD. After excluding repeat sequences, we identified 9 new SNPs in addition to the 88 known SNPs registered in the dbSNP database. After excluding 23 SNPs already genotyped, we genotyped 74 SNPs with MAF ≥ 0.05, and we successfully genotyped 73 of these. However, no SNPs showed stronger association than rs13278062 (**Fig. 1a** and **Supplementary Table 5a**). We also performed haplotype analysis using the four highly associated SNPs (rs2235126, rs7820465, rs13278062 and rs13281363), however, no haplotype showed stronger association than the single-marker association of rs13278062 (**Supplementary Fig. 3**).

rs13278062 is located in *LOC389641* and is also 397 bp upstream of *TNFRSF10A*, the tumor necrosis factor receptor superfamily 10a gene, which encodes one of the TRAIL receptors, TRAILR1. TRAILR1 is broadly expressed in human adult RPE[19] and rod photoreceptors in mice[20], whereas the expression of LOC389641 was low or absent according to the Gene Expression Omnibus (GEO) database. Binding of TRAIL to TRAILR1 is known to induce apoptosis through caspase 8 activation[21]. In addition, the TRAIL-TRAILR1 complex has a nonapoptotic pathway that induces the production of inflammatory cytokines and the promotion of inflammation through activation of nuclear factor B[22,23]. A previous study showed that the activator protein 1 will bind the sequence around rs13278062 and directly regulate TRAILR1 mRNA expression[24]. Moreover, the G allele of rs13278062 has been reported to enhance the transcriptional activity of TRAILR1 by 1.2- to 1.5-fold as compared to the T allele[25]. Although further functional studies are needed, these results speculate that *TNFRSF10A* is the susceptibility gene for exudative AMD and that rs13278062 may be the candidate of functional importance.

We observed the second most significant association at rs1713985 on chromosome 4q12. Based on the LD block and the $-\log_{10} P$ plot of chromosome 4q12 obtained by the GWAS data, we found that rs1713985 represents an LD block that spans from 57.421–57.611 Mb and includes four genes, *REST*, *C4orf14*, *POLR2B* and *IGFBP7* (**Fig. 1b**). Then, we selected and genotyped 120 SNPs around this LD block based on the data from the HapMap phase 2 Japanese population with MAF ≥ 0.05 (**Supplementary Table 5b**). However, this analysis could not narrow down the candidate region because of the long-range LD. According to the global expression profiles in GEO database, all four genes were expressed in human adult RPE[19] and rod photoreceptors in mice[20]. *REST* is a transcriptional repressor that may act as a master negative regulator of neurogeneisis. Overexpression of the *C4orf14* gene product has been reported to induce apoptosis by regulating mitochondrial nitric oxide and calcium[26]. The *IGFBP7* gene product, angiomodulin, is reported to bind chemokines and growth factors including vascular endothelial growth factor A, whose expression is high in RPE cells of AMD.

In conclusion, our data showed that *TNFRSF10A-LOC389641* on chromosome 8p21 and *REST-C4orf14-POLR2B-IGFBP7* on chromosome 4q12 are new susceptibility loci for exudative AMD in the Japanese population. Further functional studies are necessary to clarify the mechanisms of these loci on the susceptibility to exudative AMD.

URLs. GEO, http://www.ncbi.nlm.nih.gov/geo/; R statistical environment version 2.7.0, http://www.r-project.org/; PLINK 1.05, http://pngu.mgh.harvard.edu/~purcell/plink/; Genetic Power Calculator, http://pngu.mgh.harvard.edu/~purcell/gpc/; EIGENSTRAT, http://genepath.med.harvard.edu/~reich/Software.htm.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Accession codes. The expression microarray data on the human adult RPE and rod photoreceptors in mice have been deposited in the GEO database under accession numbers GSE18811 and GSE22317.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

S.A., T.I., Y.N. and M.K. designed the study. S.A., N.H., K.A., T.A. and M.K. performed genotyping. S.A. and M.K. wrote the manuscript. A.T. performed statistical analysis at the genome-wide phase. Y.N. and M.K. managed DNA samples belonging to BioBank Japan. T.I. and Y.N. obtained funding for the study. M.Y., Y.O., S.Y. and H.E. collected GWAS samples. T.T., K.M., S.H., A.N., A.A. and K.K. collected case samples for the replication study. Y.K., N.K., Y.N. and M.K. supervised the study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Samples. Characteristics of the study subjects are shown in **Supplementary Table 6**. For the GWAS, 827 individuals with exudative AMD were collected at Kyushu University. The diagnosis of exudative AMD was based on comprehensive ophthalmic examination, including fluorescein angiography and indocyanine green angiography findings and optical coherence tomography after pupil dilation. We classified exudative AMD into four subtypes under established criteria[27–30]: typical neovascular AMD (t-AMD), polypoidal choroidal vasculopathy (PCV), retinal angiomatous proliferation (RAP) and unclassified. In 827 individuals from the GWAS, we found 298 cases with t-AMD, 480 with PCV, 14 with RAP and 23 unclassified cases. Among these, we found 12 cases that had different subtypes in each eye. Cases with other macular diseases such as high myopia, angioid streaks and central serous chorioretinopathy were excluded. For the control subjects, we used genome-wide screening data of BioBank Japan samples, which consists of 2,421 individuals with thirteen diseases and 902 healthy volunteers recruited from Osaka-Midousuji Rotary Club, Osaka, Japan[31]. Disease status in the control group did not affect the MAFs of the SNPs (**Supplementary Table 7**).

For the replication study, 709 individuals with exudative AMD were recruited at Saitama Medical University ($n = 396$), Kobe University ($n = 212$) and Yokohama City University Medical Center ($n = 101$) under the same criteria as the GWAS cases. The numbers of exudative AMD subtypes were $n = 325$ for t-AMD, $n = 358$ for PCV, $n = 3$ for RAP and $n = 23$ for an unclassified subtype. We also used genome-wide screening data of BioBank Japan data, which consists of 15,571 individuals with one of ten diseases (colorectal cancer, breast cancer, prostate cancer, lung cancer, stomach cancer, diabetes, arteriosclerosis obliterans, atrial fibrillation, cerebral infarction and myocardial infarction) as controls.

All control individuals had not had a recent eye examination, and therefore it is unknown what ocular conditions, including AMD, were present in these individuals. Moreover, controls were significantly younger than cases, which indicates that some of the controls will go onto develop AMD as they age, although the incidence of late AMD is low in the Japanese population[32]. Because these limitations will underestimate the impact of SNPs on the development of late AMD, the true associations may be stronger than those shown in this study.

All participants provided written informed consent to participate in this study. This study was approved by the ethical committees of Kyushu University, Saitama Medical University, Kobe University, Yokohama City University Medical Center, Institute of Medical Science, the University of Tokyo and the RIKEN Yokohama Institute.

SNP genotyping. For the GWAS, we genotyped 832 individuals with exudative AMD using an Illumina Human610-Quad BeadChip, and we genotyped 3,323 controls using the Illumina HumanHap550v3 BeadChip. Although the call rate was ≥0.98 for all cases and controls, five cases were excluded because of paired closely related samples. Among the common SNPs in both BeadChips, 457,489 SNPs in the autosomal chromosomes passed the quality control filters (call rate ≥0.99 in both cases and controls and Hardy-Weinberg equilibrium $P \geq 1.0 \times 10^{-6}$ in controls) and were further analyzed. For the replication study, we selected 146 SNPs that showed an age- and sex-adjusted $P < 1.0 \times 10^{-4}$ in the GWAS. Among these SNPs, 22 were located at previously reported loci[7–12] and were excluded from further study. We calculated the LD coefficient (r^2) between the remaining SNPs and selected the 77 SNPs with the lowest P value within each region of $r^2 \geq 0.8$. In the replication study, we genotyped an additional panel of 709 individuals with exudative AMD using the multiplex PCR-based Invader assay[33] (Third Wave Technologies). We regarded genotyping as having been successful when the number of undetermined samples was less than 10 in a 384-well plate.

Fine mapping and resequencing. For the fine mapping, we used all case and control samples in the GWAS. Tagging SNPs were selected from those with MAF ≥ 5% in the region of interest based on the HapMap phase 2 JPT population. Resequencing of the candidate regions was performed in 48 exudative AMD cases by using an ABI3730 Genetic Analyzer.

Statistical analysis. In all stages, the associations of each SNP were assessed by age- and sex-adjusted logistic regression analysis under an additive model. The combined analysis of the GWAS and the replication study was conducted using the inverse variance method. Heterogeneities across the population were estimated formally by using a Cochran's Q test. To characterize the population structure, we computed genome-wide average identity by state and performed PCA using the EIGENSTRAT program (see URLs). GWAS and replication data were calculated using R statistical environment version 2.7.0 or PLINK 1.05 software[34]. Haploview software was used to analyze LD values[35].

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Original Article

Prevalence and Causes of Functional Disability in an Elderly General Population of Japanese: The Hisayama Study

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ABSTRACT

Background: There are limited data on the prevalence and causes of disability in the elderly general population in Japan.

Methods: In a population-based cross-sectional study of 1550 Japanese aged 65 years or older, we examined the prevalence of functional disability (defined as a Barthel Index score of ≤95) and its causes.

Results: A total of 311 of the participants had a disability (prevalence 20.1%). The prevalence of disability increased with age and doubled with every 5-year increment in age. Prevalence was higher in women than in men, especially among those aged 85 years or older. With respect to the cause of functional disability, dementia accounted for 23.5%, stroke for 24.7%, orthopedic disease for 12.9%, and other disease for 38.9% of cases in men; in women, the respective values were 35.8%, 9.3%, 31.0%, and 23.9%. Regarding age, dementia was the most frequent cause of disability in subjects aged 75 years or older, whereas stroke was most common in subjects aged 65 to 74 years. Approximately two-thirds of cases of total dependence were attributed to dementia in both sexes, whereas the main cause of slight or moderate/severe dependence was stroke in men and orthopedic disease in women. Among participants with total dependence, 94.8% resided in a hospital or health care facility.

Conclusions: Our findings indicate that functional disability is common among Japanese elderly adults and that its major cause is stroke in men and dementia in women.

Key words: functional disability; dementia; stroke; prevalence; Japanese elderly

INTRODUCTION

The elderly population has been rapidly increasing worldwide, especially in developed countries. In Japan, the proportion of adults aged 65 years or older among the whole population has been the highest in the world since 2004, and it reached 23.0% in 2010.[1] Along with this aging population, an increase in functional disability, which causes dependency and institutionalization, is a serious social, medical, and economic concern.[2,3] Studies of the prevalence, causes, and effects of functional disability among the elderly population are therefore needed for appropriate public health policy and planning. Several community-based studies have reported the prevalence of functional disability and its causes in the elderly in Western countries[4–9] and Japan.[10–14] However, participants staying in hospitals or health care facilities were not surveyed in those studies, which likely led to underestimation of the prevalence of disability. Furthermore, information from questionnaires was used to determine causes of disability in those studies. Therefore, it might be valuable to use less-biased community surveys and detailed clinical information to determine the status of functional disability and its causes in Japan. We examined the prevalence and underlying causes of functional disability in an elderly general population of Japanese.

METHODS

Study population

The Hisayama Study is a prospective cohort study of cerebrocardiovascular diseases in the town of Hisayama, a subrural community adjacent to the metropolitan area

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of Fukuoka, Japan.[15] The population of the town has distributions of age, occupational status, and nutrient intake that are almost identical to those for the whole of Japan.[15] Full community surveys of the health status and neurological conditions of residents aged 40 years or older have been repeated since 1961.[15] One characteristic of this study is that all event data on cerebrocardiovascular diseases have been verified by detailed neurological and morphological examinations, including neuroimaging.[15] Additionally, comprehensive surveys of functional disability and dementia in elderly adults have been carried out since 1985.[16] Between October 2005 and August 2006, a total of 1566 residents aged 65 or older (91.5% of the total population in this age group) participated in the examination for the present study. The examination was performed in the public hall of the town or at home. In addition, we visited hospitals and health care facilities to examine institutionalized individuals. After excluding 16 subjects for whom activity of daily living (ADL) status was not available, data from 1550 subjects (601 men and 949 women) were included in the present analysis.

Ethical considerations

This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research. All participants gave written informed consent, which included the purpose and procedures of the research, potential risks and benefits associated with participation, voluntary participation in the study, the right of withdrawal from the research without prejudice or penalty, and the confidentiality and security of personal data.

Questionnaire

In the examination, each participant completed a self-administered questionnaire that inquired about socio-demographic data (including age, sex, marital status, employment status, and place of residence [domicile, hospital, long-term care facility, or nursing home]), Barthel Index items,[17] and past history of diseases (including stroke, coronary heart disease, fracture, head injury, hypertension, diabetes, hyperlipidemia, depression, and other conditions). The completed questionnaires were reviewed by trained nurses or physicians to identify inconsistent answers and unanswered items. To diagnose dementia, all participants took neuropsychological tests (revised version of Hasegawa's Dementia Scale [HDS-R][18] and Mini-Mental State Examination [MMSE][19]), which were performed by trained nurses and physicians. Among the participants, 395 (25.2%) with test scores below the cutoff values (21/30 for the HDS-R and MMSE) underwent an additional comprehensive investigation.

Definition of functional disability

ADL status was determined using the Barthel Index,[17] which estimates the degree of independence in ADL of subjects by using 10 items: feeding (0, 5, or 10 points), bathing (0, 5), dressing (0, 5, 10), grooming (0, 5), bladder control (0, 5, 10), bowel control (0, 5, 10), toileting (0, 5, 10), transferring from bed to a wheelchair (0, 5, 10, 15), walking on a level surface (0, 5, 10, 15), and ascending and descending stairs (0, 5, 10). Functional disability was defined as a Barthel Index score of 95 or lower, in accordance with the definition previously reported in epidemiologic studies.[17,20–22] In addition, the severity of disability was categorized into 3 levels as follows: slight dependence (a Barthel Index score of 95, which corresponds to 1 decrease in an item on the Barthel Index), moderate/severe dependence (a score of 25–90), and total dependence (a score of 0–20, which corresponds approximately to a bedridden state, with at least 8 decreased items).[17]

Cause of disability

To determine the cause of functional disability, all available past clinical information, including medical records and findings from neurologic examination and brain imaging studies, which was gathered by using the follow-up system of the Hisayama Study,[15,23] was reviewed independently by 2 of the authors (D.Y. and T.N.). Any disagreement in cause attribution was resolved by a consensus of a panel of the authors (D.Y., T.N., and Y.K). If a subject had 2 or more conditions that impaired ADL, the disease that contributed to the deterioration of at least 1 category of ADL level (eg, from moderate/severe dependence to total dependence) was defined as the major cause. For instance, if a subject had mild gait disturbance caused by stroke but gradually became bedridden due to subsequent dementia, dementia would be considered the major cause, whereas stroke would be selected if the subject became bedridden soon after a severe stroke event, even if the participant later developed dementia. Among the 311 disability cases, the 2 researchers completely agreed on the cause of functional disability in 242 (77.8%) cases. In the remaining 68 (22.1%) cases, a consensus on the cause was reached after discussion.

Causes of disability were categorized into 4 groups: dementia (vascular dementia, Alzheimer disease, and other dementia), stroke (ischemic stroke and hemorrhagic stroke), orthopedic disease (fracture, arthritis, rheumatoid arthritis, and other orthopedic disease), and other disease. Dementia and its subtypes were diagnosed according to the guidelines of the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R),[24] the criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association,[25] and the criteria of the National Institute of Neurological Disorders and Stroke–Association International pour la Recherche et l'Enseignement en Neurosciences.[26] Stroke was defined as the sudden onset of nonconvulsive and focal neurologic deficits persisting at least 24 hours. A diagnosis of stroke and its subtypes

Table 1. Characteristics of study population by functional disability (Hisayama Study, 2005)

	All subjects (n = 1550)	Subjects without disability (n = 1239)	Subjects with disability (n = 311)	P-value[a]
Age, mean ± SD	75.8 ± 7.3	74.2 ± 6.3	82.1 ± 7.7	<0.001
Women, %	61.1	58.2	72.7	<0.001
Current working status, %				<0.001
Unemployed/retired/housewife	73.1	68.9	90.4	
Working	26.9	31.1	9.6	
Marital status, %				<0.001
Never married	2.5	2.2	3.5	
Married	63.4	68.6	42.8	
Divorced/widowed/separated	34.1	29.2	53.7	
Living arrangement, %				0.04
Living alone	10.9	10.1	14.2	
Living with others	89.1	89.9	85.8	
Place of residence, %				<0.001
Home	91.6	99.3	60.5	
Hospital	5.2	0.6	23.8	
Health care facility	3.2	0.1	15.7	
ADL disability level, %				
Slight dependence	5.0	—	25.4	
Moderate/severe dependence	10.0	—	49.8	
Total dependence	5.1	—	24.8	

[a]P value, comparison between subjects with and without disability.

Table 2. Prevalence of disability by age category (Hisayama Study, 2005)

Age category	Total (n = 1550)		Men (n = 603)		Women (n = 947)		P value between sexes
	No. with disability/ participants	Prevalence, % (95% CI)	No. with disability/ participants	Prevalence, % (95% CI)	No. with disability/ participants	Prevalence, % (95% CI)	
65–69	18/366	4.9 (2.9–7.7)	9/161	5.6 (2.6–10.4)	9/205	4.4 (2.0–8.2)	0.60
70–74	38/393	9.7 (6.9–13.0)	14/171	8.2 (4.6–13.4)	24/222	10.8 (7.1–15.7)	0.38
75–79	53/331	16.0 (12.2–20.4)	18/129	14.0 (8.5–21.2)	35/202	17.3 (12.4–23.3)	0.41
80–84	75/256	29.3 (23.8–35.3)	20/91	22.0 (14.0–31.9)	55/165	33.3 (26.2–41.1)	0.06
85+	127/204	62.3 (55.2–68.9)	24/51	47.1 (32.9–61.5)	103/153	67.3 (59.3–74.7)	0.01
All ages	311/1550	20.1 (18.1–22.2)	85/603	14.1 (11.4–17.1)	226/947	23.9 (21.1–26.7)	<0.001
P for trend		<0.001		<0.001		<0.001	

was determined on the basis of medical records and brain imaging studies.[27] Hemorrhagic stroke included brain hemorrhage and subarachnoid hemorrhage. The diagnosis and classification of orthopedic disease were determined with clinical information available from the questionnaire, medical records, and annual health examinations.

Statistical analysis

The software package SAS (version 9.2; SAS Institute, Cary, NC, USA) was used to perform all statistical analyses. The Student t-test was used to compare continuous variables, and the chi-square test was used to evaluate proportions. We calculated the prevalences of disability with 95% confidence intervals (CIs) by using a binary distribution. Trends in the prevalence of disability across 5-year age categories were tested by means of logistic regression analysis. A 2-sided P value less than 0.05 was considered statistically significant in all analyses.

RESULTS

The characteristics of study subjects according to functional disability status are shown in Table 1. The mean overall age was 76 years, and the proportion of women was 61.1%. A total of 311 subjects (85 men and 226 women) had some type of functional disability, resulting in a prevalence of 20.1%. As compared with those without disability, subjects with disability were more likely to be older, female, unemployed, living alone, and institutionalized. Among those with disability, the proportions of subjects with slight, moderate/severe, and total dependence were 25.4%, 49.8%, and 24.8%, respectively.

As shown in Table 2, the prevalence of functional disability increased with age, with a doubling in prevalence for every 5-year increment. The prevalence of disability was significantly higher in women than in men ($P < 0.001$), especially among participants aged 85 or older ($P = 0.01$). A comparable relationship was observed in subjects with total dependence,

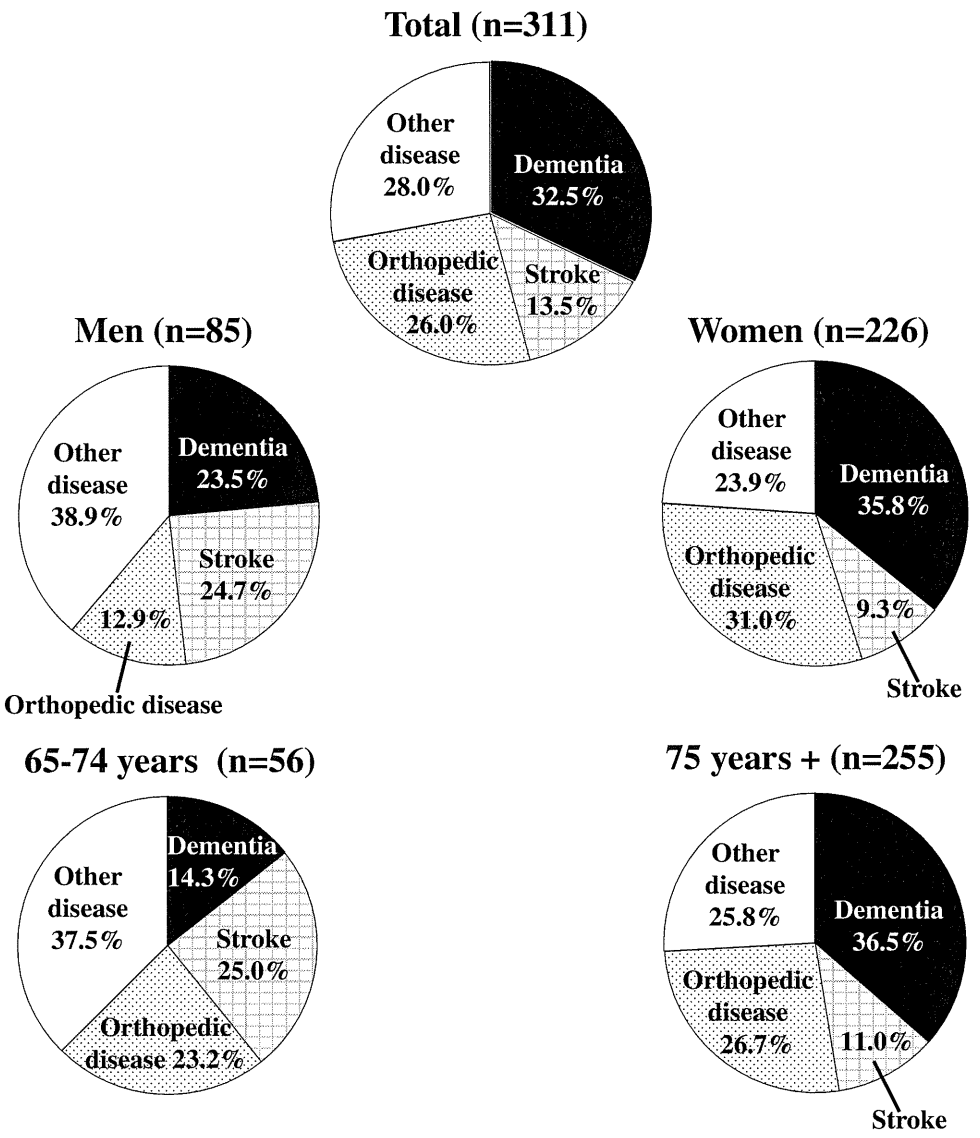

Figure 1. Causes of functional disability by sex and age (Hisayama Study, 2005).

whereas the prevalence of slight and moderate/severe dependence was not significantly different between sexes in any age category (data not shown).

Next, we investigated the causes of functional disability (Figure 1). Among the 311 disability cases, dementia accounted for 32.5%, stroke for 13.5%, orthopedic disease for 26.0%, and other disease for 28.0% of cases. Among the 101 subjects with dementia-related disability, 22 (21.8%) had a history of a stroke events that resulted in slight or moderate/severe dependence. When the results were categorized by sex, dementia accounted for 23.5%, stroke for 24.7%, orthopedic disease for 12.9%, and other disease for 38.9% of cases of functional disability in the 85 disabled men; the respective values were 35.8%, 9.3%, 31.0%, and 23.9% in the 226 disabled women. Stroke was the most common cause of disability in men, whereas dementia and orthopedic disease were more frequent in women. When the findings were analyzed by age category, dementia accounted for 14.3%, stroke for 25.0%, orthopedic disease for 23.2%, and other disease for 37.5% of disability cases in subjects aged 65 to 74 years; the respective proportions were 36.5%, 11.0%, 26.7%, and 25.8% for subjects aged 75 or older; that is, dementia was the most frequent cause of disability in subjects aged 75 or older, whereas stroke was the most common cause in subjects aged 65 to 74 years.

The subtypes of causes of functional disability by sex are shown in Table 3. Among cases of dementia, vascular dementia was most frequent in men (12.9%), whereas Alzheimer disease was most common in women (15.0%). With regard to stroke subtype, ischemic stroke was more frequent in men than in women (17.6% vs 6.2%). With regard to orthopedic disease, the proportions of fracture and arthritis were higher, especially in women (15.0% and 10.2%, respectively).

Figure 2 shows the causes of functional disability among the 311 subjects according to disability severity by sex. In subjects with total dependence, dementia was the most

Table 3. Subtypes of causes of disability by sex (Hisayama Study, 2005)

Disease/condition	Total (n = 311)		Men (n = 85)		Women (n = 226)		P-value[a]
	Number	%	Number	%	Number	%	
Dementia	101	32.5	20	23.5	81	35.8	0.04
Vascular dementia	30	9.6	11	12.9	19	8.4	0.23
Alzheimer disease	40	12.9	6	7.1	34	15.0	0.06
Other dementia	31	10.0	3	3.5	28	12.4	0.02
Stroke	42	13.5	21	24.7	21	9.3	<0.001
Ischemic stroke	29	9.3	15	17.6	14	6.2	0.002
Hemorrhagic stroke	13	4.2	6	7.1	7	3.1	0.20
Orthopedic disease	81	26.0	11	12.9	70	31.0	0.001
Fracture	38	12.2	4	4.7	34	15.0	0.01
Arthritis	25	8.0	2	2.4	23	10.2	0.03
Rheumatoid arthritis	11	3.5	2	2.4	9	4.0	0.73
Other orthopedic disease	7	2.3	3	3.5	4	1.8	0.40
Other disease	87	28.0	33	38.8	54	23.9	0.009

[a]P value for comparison between sexes.

Figure 2. Causes of functional disability by severity of disability in men and women (Hisayama Study, 2005). Total dependence: Berthel Index score = 0–20. Moderate/severe dependence: Berthel Index score = 25–90. Slight dependence: Berthel Index score = 95.

frequent cause in both sexes: the proportion was 62.5% in men and 65.6% in women. In subjects with slight or moderate/severe dependence, stroke was the most common cause of disability in men, whereas orthopedic disease was the most frequent in women.

Finally, we investigated place of residence in the 311 disabled subjects according to functional severity. Among subjects with slight dependence, 91.1% lived at home, 6.3% were hospitalized, and 2.6% stayed in health care facilities; the respective values were 72.3%, 17.4%, and 10.3% for those

with moderate/severe dependence. In contrast, among subjects with total dependence, only 5.2% lived at home, whereas 54.6% and 40.2% stayed in a hospital or health care facility, respectively.

DISCUSSION

The present study demonstrated that the prevalence of functional disability was 20.1% in an elderly general population of Japanese. Additionally, we found that the prevalence of disability increased steeply with age, with a doubling of prevalence for each 5-year increment. Prevalence was higher in women than in men, especially in individuals aged 85 or older. Importantly, in our subjects the major cause of disability was stroke in men and dementia in women. In particular, dementia was the most common cause of disability in subjects with total dependence, most of whom required full-time care in hospitals or health care facilities. These findings highlight the clinical importance of effective strategies for preventing dementia. Such strategies could reduce the social and economic burden of functional disability among elderly Japanese.

Prevalence of disability

There is considerable divergence in the prevalence of disability reported in community-based studies, with values ranging from 6% to 34.5%.[4–13] For aged Japanese populations, these studies have reported a disability prevalence ranging from 8% to 17%,[10–13] which is lower than that obtained in the present study. A possible reason for this discrepancy is the difference in the proportion of old old adults in the studies, as this group is at high risk for functional disability. Among people aged 65 years or older, the proportion of those aged 85 years or older was 4.5% to 8.7% in previous studies, which were conducted from 1977 to 1996,[1,10–13] as compared with 11.4% in the present study, performed in 2005. These findings indicate that the proportion of old old has increased over time in Japan, which has led to a recent increase in the prevalence of functional disability. In addition, some selection bias was likely in previous studies, because subjects staying in hospitals or health care facilities might not have been fully examined. In contrast, the participation rate was high (91%) in our study, and we included institutionalized subjects in the study to minimize selection bias. This bias in previous studies would lead to underestimation of the prevalence of disability. Furthermore, the discrepant findings may have been due to a difference in the definition of disability across studies. The Barthel Index, which was used in our study, has been reported to be more sensitive in detecting disability as compared with other indices with fewer ADL domains (eg, the Katz Index), which were used in other studies.[6,28] Indeed, in a sensitivity analysis using the Katz Index—in which functional disability was defined as need for assistance in 1 or more activities of 6 ADL domains, including feeding, bathing, dressing, toileting, transferring, and continence—the prevalence of disability declined to 18.3% in our study.

Sex differences in disability

In our study, the prevalence of disability was higher in women than in men, especially among persons aged 85 or older. Comparable findings were observed in previous community-based studies in Sweden and Japan.[8,29,30] However, there is no consensus on the interpretation of this sex difference. A possible explanation is that there are sex differences in death rates for underlying diseases; that is, women might survive with some form of disability after developing cardiovascular disease, whereas men might be more likely to die immediately after the incident disease, since the underlying comorbidity may be more severe in men than in women.[31,32] Another possible explanation is that musculoskeletal disease may have a greater influence on functional limitations in women than in men. For example, a population-based study in the United States indicated that musculoskeletal impairments were attributed to disability more frequently in women than in men.[33] In our subjects, disabled women also had a greater incidence than men of orthopedic diseases such as fracture and arthritis.

Cause of disability

In the present study, dementia was the most frequent cause of functional disability in both sexes, especially among those aged 75 or older. In agreement with this finding, the Adult Health Study in Hiroshima, Japan and a community-based study in Stockholm, Sweden showed that dementia had a greater influence on the development of disability and ADL decline than did stroke, orthopedic disease, or other chronic diseases.[34,35] Furthermore, our study found that the proportion of stroke was high in subjects aged 65 to 74 years. Previous community-based prospective studies in Japan and the United States have also shown that stroke was associated with risk of functional disability.[36–38] A systematic review reported that more than one-third of patients with recurrent stroke later developed dementia.[39] We also revealed that 21.8% of subjects with dementia-related disability had a history of stroke events with slight or moderate/severe dependence. These findings indicate that it is important to prevent stroke events to reduce the risk of future dementia and total dependence. Interestingly, orthopedic disease such as fracture and arthritis contributed mainly to slight dependence and moderate/severe dependence in women. Further investigations will be needed to determine the effect of orthopedic disease on subsequent ADL level.

Place of residence and severity of disability

To date, few studies of general populations have classified ADL level according to place of residence. In our study, approximately 95% of subjects with total dependence were institutionalized in hospitals or health care facilities. Most of

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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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

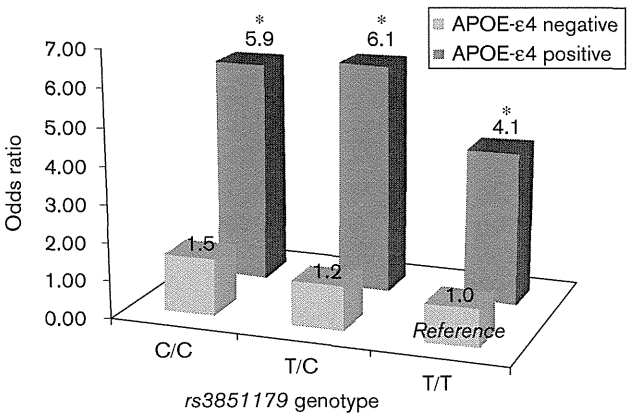
This study was carried out with the approval of the Ethics Committees of the Faculty of Medicine, Kyushu University, and the RIKEN Yokohama Institute. Written informed consent was obtained from all appropriate proxies for LOAD patients and control participants.

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.