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Original Article

Associations Between *hOGG1* Ser326Cys Polymorphism and Increased Body Mass Index and Fasting Glucose Level in the Japanese General Population

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ABSTRACT

Background: Evidence suggests that Ser326Cys, a genetic polymorphism of human 8-oxoguanine glycosylase 1 (*hOGG1*), is associated with insulin resistance and type 2 diabetes; however, the underlying mechanism is unclear. Recently, an animal study showed a significant association between the *hOGG1* genotype and obesity, although evidence for such an association in humans is limited. The purpose of this study was to examine the association between the *hOGG1* genotype and body mass index (BMI) and fasting blood glucose (FBG) levels.

Methods: Cross-sectional analysis was conducted using the baseline survey data from a Japan Multi-Institutional Collaborative Cohort Study, which included 1793 participants aged 40–69 years. The *hOGG1* polymorphism was detected using a multiplex polymerase chain reaction-based invader assay. Multiple linear regression, analysis of covariance, and logistic regression were used to control for confounding variables.

Results: The Cys allele was significantly associated with increased BMI, FBG level, and total cholesterol (TC) level, even after adjustment for gender, age, energy intake, alcohol, smoking, physical activity, and family history of diabetes. An association with BMI was still observed after further adjustment for FBG and TC, but not for the study area (Amami or the mainland). The Cys/Cys genotype was significantly more prevalent in the participants with higher BMI (>27.5 kg/m^2). However, the impact of genotype decreased and significance disappeared after adjusting for the study area.

Conclusions: The present results suggest that the study area being inside Japan confounds the association between *hOGG1* genotype and obesity.

Key words: human 8-oxoguanine glycosylase 1 (*hOGG1*); obesity; body mass index (BMI); fasting blood glucose (FBG); polymorphism; study area

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INTRODUCTION

Reactive oxygen species (ROS) are known to play an essential role in the pathogenesis of diabetes.[1] Several studies have reported that oxidative stress associated with insulin resistance, β cell dysfunction, impaired glucose tolerance, and mitochondrial dysfunction can ultimately lead to the diabetes disease state.[2–4] ROS also cause strand breaks and base modifications in DNA, including the oxidation of guanine residues to 8-hydroxy-2′-deoxyguanine (8-OHdG). These ROS-induced mutations alter the function of various genes and influence the pathogenesis of several diseases, such as cancer, cardiovascular disease, neurodegenerative diseases, and diabetes.[1] Base-excision repair (BER) plays an important role in preventing such disease, and human 8-oxoguanine glycosylase 1 (*hOGG1*) is one of the key glycosylases involved in the BER system.[5] The Ser326Cys polymorphism of the highly polymorphic OGG1 gene has been studied the most because this polymorphism is associated with functional differences in enzyme activity[6] and loss of function.[7] However, most epidemiological studies of this polymorphism have focused on cancer susceptibility.[8–12]

In the past decade, several studies have reported that the Ser326Cys *hOGG1* polymorphism is associated with insulin resistance[13] and type 2 diabetes[14–17]; however, the underlying mechanism has not been elucidated. Obesity-associated insulin resistance is a major risk factor for type 2 diabetes,[18] and fat accumulation has been reported to be associated with systemic oxidative stress[19,20]; therefore, it may be possible to assess the risk of diabetes based on the association between the Ser326Cys *hOGG1* polymorphism and body mass index (BMI). Recently, an animal study found that *hOGG1* deficiency alters cellular substrate metabolism, which favors a sparing phenotype and increased susceptibility to obesity[21]; however, evidence for such association between *hOGG1* polymorphism and BMI in humans is limited.[14]

The purpose of this study was to determine whether the *hOGG1* Cys allele is associated with BMI and fasting glucose level. We also studied whether this association is modified by the effects of other factors.

METHODS

Study participants

The purpose of the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study is to confirm and detect gene-environment interactions for lifestyle-related diseases using a large genome cohort, as previously described.[22] Briefly, the J-MICC Study, which was initiated in 2005, included volunteers aged 35–69 years from 10 areas of Japan: Chiba, Shizuoka, Okazaki, Aichi, Takashima and Kyoto, which are located in Honshu Island; Tokushima, which is located in Shikoku Island; Fukuoka and Saga, which are located in Kyushu Island; and Amami, which is located 380 km southwest of Kyushu Island. Throughout this paper, we refer to Honshu Island, Shikoku Island, and Kyushu Island as "the mainland". In this cross-sectional study, data from 4512 participants throughout these areas were collected during the period of 2005–2008.[23] Written informed consent was obtained from all participants. The study protocol was approved by the Nagoya University School of Medicine ethics committees and other participating institutions.

Questionnaire and measurements

A self-administered questionnaire was used to collect data on alcohol consumption, smoking, dietary habits, physical activity, current medication, disease history, and first-degree family history of diabetes. Details of the dietary assessment and estimation of physical activity were reported elsewhere.[24–27]

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). We defined a BMI of $>27.5\ kg/m^2$ as obese; increased mortality has been reported above this point among East Asians.[28] The HbA1c (%) and fasting blood glucose (FBG), triglyceride, total cholesterol, and HDL cholesterol levels were measured in laboratories in each study area, and the results of these measurements were collected. The HbA1c (%) value was converted from the Japan Diabetes Society (JDS) to the National Glycohemoglobin Standardization Program (NGSP) by using the following equation published by the JDS: NGSP (%) = 1.02 × JDS (%) + 0.25%.[29]

Genotyping

Genotyping was performed as described previously.[23] Single nucleotide polymorphisms, including the *hOGG1* Ser326Cys (rs1052133), were genotyped using a multiplex polymerase chain reaction-based Invader assay (Third Wave Technologies, Madison, WI, USA)[30] at the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN.

Statistical analysis

In the analysis, we excluded 2719 participants based on any of the following conditions: missing data of *hOGG1* polymorphism ($n = 11$); missing FBG ($n = 2627$) data; taking type 2 diabetes medication ($n = 89$); or a dietary energy intake greater than 4000 kcal/day ($n = 2$). Consequently, data for 976 men and 817 women aged 35–69 years were retained for analysis. Among these participants, data on alcohol consumption (17 men and 23 women) or physical activity (5 men and 5 women) were missing for some participants.

All analyses were performed with the SAS statistical software package (Ver. 9.3 for Windows; SAS Institute, Cary, NC, USA). A P value of less than 0.05 was considered statistically significant.

Table 1. Characteristics according to the *hOGG1* Ser326Cys genotype among 1793 subjects

	Ser/Ser		Ser/Cys		Cys/Cys		*P*
n (%) *n* = 1793	365	(20.4)	866	(48.3)	562	(31.3)	
Gender, women (%)	180	(49.3)	393	(45.4)	244	(43.4)	0.209
Age (y) (SD)	55.0	(8.6)	54.5	(8.9)	55.1	(8.7)	0.354
Study area, Amami area (%)	60	(16.4)	185	(21.4)	204	(36.3)	<0.001
Total energy intake (kcal/d) (SD)	1729.7	(348.4)	1752.9	(371.6)	1730.0	(376.3)	0.406
BMI (kg/m^2) (SD)	23.2	(3.0)	23.2	(3.3)	23.7	(3.5)	0.021
Physical activity level (METs·h) (SD)	13.6	(12.1)	14.7	(13.9)	15.5	(14.9)	0.775
Current alcohol drinkers, *n* (%)	192	(53.6)	485	(57.2)	333	(60.9)	0.001
Current smoking, *n* (%)	63	(17.3)	155	(17.9)	98	(17.4)	0.981
Family history of diabetes, *n* (%)	58	(15.9)	142	(16.4)	85	(15.1)	0.451
HbA1c (NGSP) (%)	5.61	(0.55)	5.54	(0.44)	5.58	(0.46)	0.199
FBG (mg/dL) (SD)	96.9	(13.3)	96.6	(14.6)	99.4	(16.9)	<0.001
TG (mg/dL) (SD)	110.8	(69.1)	114.0	(80.9)	116.7	(82.4)	0.368
TC (mg/dL) (SD)	207.1	(33.0)	210.9	(34.0)	212.3	(34.2)	0.077
HDL-C (mg/dL) (SD)	64.7	(16.5)	64.1	(16.4)	63.3	(16.5)	0.278

BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.
P values for Chi-square test or Kruskal-Wallis test.

To compare the characteristics of participants according to the *hOGG1* genotype, we used the Kruskal-Wallis test for continuous variables and χ^2 tests for categorical variables. Adjusted means and their 95% confidence intervals (CIs) of BMI, FBG, and total cholesterol according to *hOGG1* genotype were evaluated by least-squares general linear regression, and linear trends were assessed by the statistical significance of the regression coefficient of an ordinal variable for the factor under the following considerations: gender; age (continuous); energy intake (continuous); physical activity level (continuous); alcohol consumption status (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46.0 g ethanol/day); smoking status (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/day); first-degree family history of diabetes (positive, negative, or unknown); study area (Amami or the mainland); BMI (continuous, for the evaluation of FBG and total cholesterol); FBG (continuous, for BMI and total cholesterol); and total cholesterol (continuous, for BMI and FBG). Odds ratios (ORs) and 95% CIs of *hOGG1* genotype for excessive BMI (>27.5 kg/m^2) were estimated using logistic regression models adjusted for potential confounders (age, BMI, energy intake, alcohol consumption, smoking, physical activity, family history of diabetes, and study area).

RESULTS

The characteristics of study participants according to the *hOGG1* Ser326Cys genotype are shown in Table 1. The genotype distributions of the *hOGG1* Ser326Cys gene among all participants followed the Hardy-Weinberg equilibrium ($\chi^2 = 0.511$, $P = 0.475$). Genotype frequency was significantly different in the Amami area ($P < 0.001$). The Cys allele carriers had significantly higher mean BMI ($P = 0.021$) and FBG ($P < 0.001$) levels, and a higher proportion were current alcohol drinkers ($P < 0.001$). TC level tended to be higher in Cys allele carriers, although this difference was not statistically significant ($P = 0.077$).

After adjusting for possible confounding factors, such as gender, age, energy intake, physical activity level, ethanol intake, smoking, and family history of diabetes, the Cys allele was found to be significantly associated with higher BMI, FBG, and TC levels in a dose-dependent manner (all $P < 0.05$, Table 2, Model 2). The association with BMI was still significant after further adjustment for FBG and TC ($P = 0.02$, Model 3). However, the significance disappeared after adjusting for study area ($P = 0.23$, Model 4).

Data for the evaluation of the association between obesity (BMI > 27.5 kg/m^2) and *hOGG1* genotype using logistic regression analysis are shown in Table 3. The prevalence of obesity in the Cys/Cys genotype was significantly greater after adjusting for gender, age, energy intake, physical activity level, ethanol intake, smoking, family history of diabetes, FBG, and TC (Model 3). Although Cys allele carriers tended to have a higher proportion of obesity, the significance of this association disappeared after adjusting for the study area (Model 4). The OR of the Amami area for obesity was 2.44 (95% CI 1.67–3.56), which was greater than that of the *hOGG1* genotype.

DISCUSSION

In this cross-sectional study, we observed significant associations between the *hOGG1* Cys/Cys genotype and higher BMI and incidence of obesity, after adjustment for possible confounding factors other than the study area. After adjusting for study area, however, this significance disappeared, suggesting that study area is a confounding factor. Despite this lack of association, Cys allele carriers tended to have a higher proportion of obesity than Ser/Ser.

Table 2. Adjusted means of BMI, FBG, and TC according to *hOGG1* Ser326Cys genotype

	Ser/Ser		Ser/Cys		Cys/Cys		*P* for trend
	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Model 1[a]							
BMI (kg/m^2)	23.2	(22.8–23.5)	23.2	(23.0–23.4)	23.7	(23.4–24.0)	0.013
FBG (mg/dL)	97.1	(95.6–98.6)	96.6	(95.6–97.6)	99.2	(98.0–100.4)	0.013
TC (mg/dL)	206.6	(203.2–210.0)	210.9	(208.6–213.1)	212	(209.5–215.0)	0.025
Model 2[b]							
BMI (kg/m^2)	23.2	(22.9–23.5)	23.2	(23.0–23.4)	23.7	(23.4–24.0)	0.018
FBG (mg/dL)	97.3	(95.8–98.8)	96.3	(95.4–97.2)	99.1	(97.9–100.2)	0.025
TC (mg/dL)	207.0	(203.5–210.4)	210.8	(208.5–213.0)	212	(209.1–214.6)	0.040
Model 3[c]							
BMI (kg/m^2)	23.2	(22.8–23.5)	23.3	(23.1–23.5)	23.6	(23.4–23.9)	0.020
FBG (mg/dL)	97.7	(96.4–99.1)	96.6	(95.7–97.5)	98.4	(97.3–99.5)	0.260
TC (mg/dL)	207.1	(203.6–210.5)	211.0	(208.7–213.2)	212	(208.7–214.3)	0.071
Model 4[d]							
BMI (kg/m^2)	23.3	(22.9–23.6)	23.3	(23.1–23.5)	23.5	(23.2–23.8)	0.230
FBG (mg/dL)	98.1	(96.7–99.4)	96.7	(95.8–97.6)	98.1	(97.0–99.2)	0.769
TC (mg/dL)	207.1	(203.7–210.6)	211.0	(208.7–213.2)	211	(208.6–214.3)	0.078

BMI, body mass index; CI, confidence interval; FBG, fasting blood glucose; TC, total cholesterol.
[a]Adjusted for gender and age (continuous).
[b]Adjusted for Model 1 and further adjusted for energy intake (continuous), physical activity level (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/day), smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/day), and family history of diabetes (positive, negative, or unknown).
[c]Adjusted for all variables in Model 2 and further adjusted for BMI (for FBG and TC), FBG (for BMI and TC), and TC (for BMI and FBG).
[d]Adjusted for all variables in Model 3 and further adjusted for the study area (Amami or the mainland).

Table 3. Odds ratios and 95% CIs for obesity (BMI > 27.5 kg/m^2) according to *hOGG1* Ser326Cys genotype among 1793 subjects

BMI (kg/m^2)	>27.5	≤27.5	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
	n	*n*	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Ser/Ser	24	341	1.00	reference	1.00	reference	1.00	reference	1.00	reference
Ser/Cys	84	782	1.50	(0.94–2.41)	1.44	(0.90–2.32)	1.55	(0.94–2.57)	1.45	(0.87–2.41)
Cys/Cys	62	500	1.77	(1.08–2.90)	1.74	(1.06–2.86)	1.74	(1.03–2.94)	1.46	(0.86–2.48)
			P_{trend} = 0.026		P_{trend} = 0.029		P_{trend} = 0.049		P_{trend} = 0.225	

BMI, body mass index; CI, confidence interval; OR, odds ratio.
[a]Adjusted for gender and age (continuous).
[b]Adjusted for Model 1 and further adjusted for energy intake (continuous), physical activity level (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/d), smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/d), and family history of diabetes (positive, negative, or unknown).
[c]Adjusted for all variables in Model 2 and further adjusted for FBG and TC.
[d]Adjusted for all variables in Model 3 and further adjusted for the study area (Amami or the mainland).

Several epidemiological studies have examined the associations between the Ser326Cys *hOGG1* polymorphism and insulin resistance[13] and type 2 diabetes.[14–17] Wang et al reported that the Cys/Cys variant significantly decreases insulin sensitivity, even after adjustment for possible confounders, including BMI, among the Taiwanese.[13] Three studies detected significant association between Ser326Cys *hOGG1* polymorphism and diabetes.[14,16,17] Specifically, Daimon et al reported that decreased insulin secretion is associated with being a Cys allele carrier, measured by homeostatic model assessment beta cell function (HOMA-β) in a Japanese population,[14] while Gönül et al reported a significant association between the Cys allele and insulin resistance in a Turkish population, measured by HOMA-R.[17] On the other hand, one case-control study conducted in a Polish population failed to detect an association between this polymorphism and diabetes[15] due to limited sample size. Regarding obesity, an animal study showed significant association between the *hOGG1* genotype and obesity,[21] and one epidemiological study reported a positive association between BMI and this polymorphism.[14] Our study showed no evidence of an association between this polymorphism and increased BMI and FBG.

In the present study, the study area had a significant impact on the prevalence of obesity. Among the studies reporting genetic differences between the Amami and the mainland populations,[23,31,32] two used data from the J-MICC study.[23,32] Nishiyama et al found a low but significant level of genetic

differentiation between the mainland population and the population of the Amami Islands,[32] while Wakai et al reported that some polymorphisms showed a substantial difference in minor allele frequency among the participating cohorts.[23] They proposed that genetic variation among the study areas should be considered when analyzing the data from the J-MICC study. According to the Japanese Single Nucleotide Polymorphisms (JSNP) database, the frequency of *hOGG1* genotypes of Ser/Ser, Ser/Cys, and Cys/Cys was reported for 18%, 59%, and 23% of participants, respectively.[33] In this study, the genotype frequency of Ser/Ser, Ser/Cys, and Cys/Cys polymorphisms in mainland Japan was 22.7%, 50.7%, and 26.6%, while those on Amami Island were 13.4%, 41.2%, and 45.4%, respectively. We found that the variation in the *hOGG1* genotype Cys/Cys frequency between the Amami and the mainland could lead to a false-positive result if the study area was not considered. This is known as confounding by population stratification,[34] which needs to be carefully considered in genetic epidemiology, even in the relatively homogeneous Japanese population. A significantly higher BMI in the Amami area may reflect population differences in genetic or environmental factors; therefore, further investigation is needed.

This study has several methodological limitations. First, the cross-sectional nature of our study limits our ability to determine causation, even though we excluded participants who were on medication for type 2 diabetes. In addition, we did not have appropriate replication data accompanying this study. Second, although measuring fat accumulation using computed tomography scans or echograms is ideal, we used BMI to evaluate obesity. Misclassification of obesity may have therefore occurred; however, misclassification of obesity would be expected to lower estimations for the association. Third, there may be intrinsic information bias in our assessments of lifestyle-related factors, such as dietary and family history. However, if any misclassification were present, it would be non-differential by the *hOGG1* genotype and would likely underestimate the true associations. Finally, although we adjusted for potential confounding factors in the multivariate analysis, residual confounding factors by known or unknown risk factors may have been present.

In conclusion, these results suggest that the *hOGG1* Ser/Cys genotype may have some influence on obesity, although its contribution is smaller than the influence of the study area. While our study found no associations of this genotype with BMI or FBG levels, we did find evidence of confounding by population stratification for these associations. This report may provide important information for genetic association analysis in the Japanese population.

ONLINE ONLY MATERIAL

Abstract in Japanese.

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ENDOCRINE GENETICS/EPIGENETICS

A polymorphism near *MC4R* gene (rs17782313) is associated with serum triglyceride levels in the general Japanese population: the J-MICC Study

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Abstract Previously reported associations of a common polymorphism near melanocortin-4 receptor (*MC4R*) gene (rs17782313) with BMI/obesity were inconsistent, especially in East Asia, and the associations of the polymorphism with serum lipid levels have not been fully elucidated. This study evaluated the association between rs17782313 and obesity-related traits and serum lipid levels in the general Japanese population. A total of 2,035 subjects (aged 35–69 years, 1,024 males and 1,011 females) enrolled in the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study. We examined the associations between near *MC4R* polymorphism (rs17782313) and obesity-related traits [height, weight, body mass index (BMI), weight change from 20 years old], serum lipid levels (triglycerides, total and HDL-cholesterol), and intake of nutrients (total energy and macronutrients). Polymorphism of rs17782313 (minor C allele) was positively associated with serum triglyceride levels (*P* for trend = 0.020) adjusted for age and sex. Analysis using a general linear model revealed that the number of minor C alleles was positively associated with serum triglyceride levels after adjustment for age, sex, and potential

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confounders (P for trend = 0.004). Statistical significance did not change after further adjustment for total energy intake and BMI. There was no significant association between rs17782313 and obesity-related traits including BMI. Interactions between rs17782313 and sex, BMI, or total energy intake for triglyceride levels were not significant. To our knowledge, this study demonstrated for the first time that rs17782313 was associated with serum triglyceride levels in Asian population. Further studies are needed to confirm this result.

Keywords *MC4R* · Polymorphism · Triglycerides · Body mass index

Introduction

Recently, obesity has become an important issue worldwide, because it causes various lifestyle-related diseases such as cardiovascular disease and type 2 diabetes (T2DM) [1]. Obesity is known to be a complex disorder caused by both genetic and environmental factors [2–4].

A meta-analysis of genome-wide association studies (GWAS) in European ancestry revealed that a common polymorphism the near *melanocortin 4 receptor* (*MC4R*) gene (rs17782313) had the second strongest association signal following *FTO* rs9939609 for BMI/obesity [5]. *MC4R* is located on chromosome 18q22 and encodes a protein which is expressed mainly in the brain and is involved in appetite regulation [6]. Rare mutations in the *MC4R* gene were previously associated with monogenic obesity in humans [7, 8]. *MC4R* rs17782313 was also reported to be associated with waist circumference [9] and T2DM [10]. The results of the association between rs17782313 and obesity were confirmed in various ethnic groups [11]; however, it is not consistent in East Asians, especially Japanese [12–17].

In addition, *MC4R* was reported to associate with lipid metabolism, especially triglycerides [18, 19]. An association between obesity-reducing mutation in the *MC4R* gene Val103Ile and lower triglyceride levels in humans [18], and the promotion of triglyceride synthesis in the *MC4R* inhibition or disruption in rodents [19] were observed. However, few reports have focused on the associations of rs17782313 with serum triglyceride levels, aside from those related to low HDL-cholesterol [20] and high LDL-cholesterol levels [14].

The aim of this study was to confirm the association between near *MC4R* rs17782313 and obesity-related traits including body mass index (BMI) in the general Japanese population. We also investigated the association between the polymorphism and serum lipid levels.

Subjects and methods

Study subjects

The Japan Multi-Institutional Collaborative Cohort (J-MICC) Study is a large genome cohort followed to confirm and detect gene-environment interactions for lifestyle-related diseases; the details of this cohort were described elsewhere [21]. Briefly, the J-MICC Study was started in 2005, and subjects aged 35–69 years were enrolled voluntarily from 10 areas of Japan. In the present cross-sectional study, we used the data of 4,512 subjects in a baseline survey, who were recruited from each area for the period 2005–2008 [22]. Written informed consent was obtained from each subject and the study protocol was approved by the ethics committees of Nagoya University School of Medicine (the affiliation of the former principal investigator Nobuyuki Hamajima), Aichi Cancer Center Research Institute (the affiliation of the present principal investigator Hideo Tanaka), and each participating institution.

Questionnaires and anthropometric and clinical measurements

A self-administered questionnaire included items on smoking and drinking habits, dietary habit, current medication, and past history of diseases, and data were checked by trained staff. For the dietary assessment, a validated food-frequency questionnaire asked about the intake frequency of 47 foods and beverages over the past year, and the intake of total energy (kcal), protein (g), fat (g), and carbohydrates (g) per day was calculated [23–25]. Intake of dietary protein, fat, and carbohydrates was divided by total energy intake and expressed as % energy.

According to the number of cigarettes per day, smoking habit was classified into four categories: never, former, and current smokers (<20 or ≥20 cigarettes/day). Drinking habit was classified into three groups: never, former, and current drinkers (≥one time/week). Physical activity during leisure time was estimated by multiplying the frequency and average duration of light (walking, hiking, etc., 3.4 metabolic equivalents [METs]), moderate (light jogging, swimming, etc., 7.0 METs), and vigorous intensity (marathon running, combative sports, etc., 10.0 METs) exercise, and MET-hours/week of leisure time activity were calculated by summing the exercise at each level. Subjects were divided into four groups by quartiles of MET-hours/week.

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. BMI was calculated as weight (kg)/[height (m)]^2. Weight change (kg) from 20 years old was calculated as baseline weight minus weight at 20 years old. Venous blood samples were obtained from each

subject, and serum triglycerides, total cholesterol, and HDL-cholesterol were measured by laboratories in each research area.

Genotyping of polymorphism

DNA from buffy coat was extracted using the Qiagen BioRobot M48 Workstation (Qiagen, Hilden, Germany). Details of genotyping were described previously [22]. Briefly, single nucleotide polymorphisms, including the polymorphism near *MC4R* rs17782313, were genotyped using a multiplex PCR-based Invader assay (Third Wave Technologies, Madison, WI, USA) at the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN. Genotype distributions were tested for Hardy–Weinberg equilibrium.

Statistical analysis

From 4,512 subjects, those who met any of the following criteria were excluded: (i) lack of data on genotype ($n = 21$), serum lipids ($n = 1{,}234$), BMI ($n = 1$), smoking habit ($n = 7$), drinking habit ($n = 1$), and MET-hours/week ($n = 52$), (ii) history of cerebrovascular disease ($n = 99$), ischemic heart diseases ($n = 150$), and diabetes ($n = 190$, including those under treatment), (iii) taking cholesterol-lowering medication ($n = 40$), (iv) eating within 3 h before blood drawing (not fasting, $n = 885$), and (v) implausible values of estimated total energy intake (<1,000 kcal/day, $n = 78$ or >4,000 kcal/day, $n = 2$), leaving 2,035 subjects eligible for the analyses.

To analyze sex differences in subject characteristics, t tests or Wilcoxon's rank sum tests were used for continuous variables, and the χ^2 test was used for categorical variables. A general linear model tested variables of quantitative traits for differences and linear trends by 3 genotype groups (wild, hetero, and homo) of rs17782313, adjusted for age (category: 35–39, 40–49, 50–59, and 60–69 years old), sex, and potential confounders, such as research area, leisure time physical activity (METs; quartiles), smoking habit, drinking habit, and additionally adjusted for total energy intake (quartiles), and BMI (quartiles). Linear trends were assessed using ordinal variables of 0 (wild), 1 (hetero), and 2 (homo of minor allele) for the polymorphism in each statistical model. Gene-environment interactions were evaluated by including interaction terms of 3 genotypes (TT, TC, and CC) * sex or genotype * BMI (<median, ≥median) or genotype * each nutrient intake (total energy, protein, fat, and carbohydrate; <median, ≥median) in the model. All analyses were performed using the SAS software package (Ver. 8.2 for Windows; SAS Institute, Cary, NC, USA), and $P < 0.05$ was considered significant.

Results

Table 1 shows the characteristics of study subjects, including anthropometric measures, serum lipid levels, total energy and macronutrient intake, and rs17782313 genotype distributions by sex. The mean age was 55.7 years for men and 55.1 years for women. Allele frequency of the polymorphism rs17782313 was not significantly different between sex ($P = 0.581$). Frequency of the polymorphism was similar to other Japanese populations [13, 17] and was in agreement with Hardy–Weinberg equilibrium (62.3 % in TT, 32.9 % in TC, and 4.8 % in CC, $\chi^2 = 0.649$, $P = 0.421$).

We examined associations between rs17782313 and obesity-related traits (height, weight at baseline and 20 years old, BMI at baseline and 20 years old, weight change from 20 years old), serum lipid levels (triglycerides, total and HDL-cholesterol), and total energy and macronutrients intake (energy % of protein, fat, and carbohydrate) (Table 2). Among them, only serum triglyceride levels were significantly associated with rs17782313 after adjustment for age and sex (P for trend = 0.020). Weight change from 20 years old was associated with rs17782313 but not significant ($P = 0.065$) among obesity-related traits, while other traits including BMI were not associated with the polymorphism.

Next, we assessed the relationships of serum triglyceride levels with obesity-related traits and total energy and macronutrients intake (Table 3). Serum triglyceride levels were significantly associated with weight at baseline ($P < 0.0001$), BMI ($P < 0.0001$) at baseline, and weight change from 20 years old ($P < 0.0001$).

Furthermore, we analyzed associations between rs17782313 and serum triglyceride levels with consideration of potential confounders (Table 4). A general linear model adjusted for age, sex, and potential confounders, such as research area, physical activity, smoking habit, and drinking habit revealed that the number of rs17782313 minor C alleles was significantly associated with serum triglyceride levels (Model 1: P for trend = 0.004). After further adjustment for total energy intake in Model 2, the results did not change (P for trend = 0.004). Additional adjustment for BMI (Model 3) did not substantially change the association (P for trend = 0.011). When we adjusted for weight change from 20 years old instead of BMI, the results did not change: adjusted means were 105.6 (95 % CI 98.3–113.5) for TT, 110.6 (95 % CI 102.5–119.3) for TC, and 117.3 (95 % CI 104.4–131.8) for CC (P for trend = 0.009). Analysis using multiple logistic regression also confirmed a significant association between hypertriglyceridemia (serum triglycerides ≥150 mg/dL) and rs17782313 genotypes.

In addition, we evaluated the associations of rs17782313 and obesity (BMI ≥25: Japan Society for the Study of

Table 1 Characteristics of the subjects by sex

	Men ($n = 1,024$)	Women ($n = 1,011$)	P value
Age (years)[a]	55.7 ± 9.0	55.1 ± 8.7	0.088
Height (cm)[a]	166.8 ± 6.3	153.8 ± 5.8	<0.0001
Weight (kg)[a]			
Baseline	66.2 ± 9.9	53.7 ± 8.1	<0.0001
20 years old	58.8 ± 7.1	49.7 ± 7.5	<0.0001
Body mass index (kg/m^2)[a]			
Baseline	23.7 ± 3.0	22.7 ± 3.2	<0.0001
20 years old	21.1 ± 2.1	21.0 ± 3.2	0.706
Weight change from 20 years old (kg)[a]	7.4 ± 7.5	4.0 ± 8.5	<0.0001
Triglycerides (mg/dL)[b]	111.5 (80.0, 157.5)	84.0 (60.0, 119.0)	<0.0001
Total cholesterol (mg/dL)[a]	205.6 ± 31.7	217.8 ± 35.3	<0.0001
HDL-cholesterol (mg/dL)[a]	59.0 ± 15.7	68.2 ± 15.2	<0.0001
Energy (kcal/day)[a]	1,947 ± 355	1,576 ± 236	<0.0001
Protein (energy %)[a]	11.7 ± 1.9	13.3 ± 1.9	<0.0001
Fat (energy %)[a]	19.9 ± 5.2	26.0 ± 5.8	<0.0001
Carbohydrate (energy %)[a]	57.5 ± 6.5	55.9 ± 5.0	<0.0001
Physical activity level (MET-hours/week)[b]	7.7 (1.2, 20.3)	7.7 (0, 17.9)	0.024
Smoking habit[c]			
Never	300 (29.3)	922 (91.2)	<0.0001
Former	426 (41.6)	29 (2.9)	
Current (<20 cigarettes/day)	94 (9.2)	41 (4.1)	
Current (≥20 cigarettes/day)	204 (19.9)	19 (1.9)	
Drinking habit[c]			
Never	217 (21.2)	630 (62.3)	<0.0001
Former	14 (1.4)	14 (1.4)	
Current	793 (77.4)	367 (36.3)	
Near *MC4R* rs17782313[c]			
T/T	631 (61.6)	637 (63.0)	0.581
T/C	339 (33.1)	330 (32.6)	
C/C	54 (5.3)	44 (4.4)	

Variables are presented as the mean ± SD[a] or median (25 %, 75 %)[b] for continuous variables and as a number (%)[c] for categorical variables
Sex differences were analyzed by t test[a], Wilcoxon rank-sum test[b], or chi-square test[c]
MET metabolic equivalent

Obesity criteria for obesity) or weight change from 20 years old (≥5.1 kg: median). However, there were no significant associations between rs17782313 and higher BMI or weight change from 20 years (data not shown).

Finally, potential gene-environment interaction between the genotype and sex, BMI, and total energy intake on hypertriglyceridemia was investigated. We did not find significant interaction of genotype * sex ($P = 0.184$), genotype * BMI ($P = 0.729$), or genotype * energy ($P = 0.845$) with hypertriglyceridemia. Energy % of macronutrient intakes (protein, fat, and carbohydrate) did not show significant interaction with rs17782313 for hypertriglyceridemia (data not shown).

Discussion

To the best of our knowledge, we demonstrated for the first time, the association between near *MC4R* polymorphism (rs17782313) and serum triglyceride levels in Asian population. This significant association did not change after adjusting for lifestyle factors including physical activity, smoking habit, and drinking habit. Moreover, further adjustment for total energy intake and BMI did not alter the results, suggesting that the associations were independent of, or not mediated by, an increase in food intake or obesity. In addition, the polymorphism was not associated with BMI/obesity.

Table 2 Anthropometric characteristics, serum lipid levels, and nutrient intakes according to near *MC4R* genotype

rs17782313 (N)	Genotype						Adjusted *P* for trend
	T/T (1268)		T/C (669)		C/C (98)		
	Adjusted mean	(95 % CI)	Adjusted mean	(95 % CI)	Adjusted mean	(95 % CI)	
Height (cm)	161.6	(161.2–162.0)	161.9	(161.4–162.4)	161.9	(160.8–163.0)	0.328
Weight (kg)							
Baseline*	59.5	(58.9–60.1)	59.9	(59.1–60.6)	60.7	(58.9–62.4)	0.178
20 years old*	53.5	(53.1– 53.9)	53.8	(53.3–54.4)	53.8	(52.5–55.2)	0.320
Body mass index (kg/m^2)							
Baseline*	22.9	(22.7–23.1)	22.9	(22.7–23.2)	23.2	(22.6–23.8)	0.329
20 years old*	20.9	(20.8–21.0)	20.9	(20.8–21.1)	20.9	(20.4–21.3)	0.932
Weight change from 20 years old (kg)	5.6	(5.0–6.1)	5.9	(5.2–6.6)	7.3	(5.7–9.0)	0.065
Triglycerides (mg/dL)*	93.3	(90.0–96.7)	97.2	(92.9–101.7)	103.3	(93.1–114.5)	0.020
Total cholesterol (mg/dL)	206.5	(204.2–208.8)	204.3	(201.4–207.2)	205.6	(199.0–212.2)	0.253
HDL-cholesterol (mg/dL)	64.1	(63.0–65.2)	63.5	(62.1–64.8)	61.4	(58.3–64.5)	0.105
Energy (kcal/day)	1,750	(1,729–1,771)	1,746	(1,719–1,772)	1,761	(1,700–1,822)	0.995
Protein (energy %)	12.4	(12.3–12.6)	12.4	(12.3– 12.6)	12.3	(12.0–12.7)	0.847
Fat (energy %)	23.4	(23.1–23.8)	23.5	(23.1–24.0)	23.6	(22.5–24.7)	0.633
Carbohydrate (energy %)	56.0	(55.6–56.4)	56.3	(55.8–56.8)	56.0	(54.9–57.2)	0.515

Data are adjusted means (95 % CI). Each analysis was performed by a general linear model

Adjusted for age and sex (weight at 20 years old and body mass index at 20 years old are not adjusted by age)

CI confidence interval

* Calculated after log-transformed

Table 3 Associations of anthropometric characteristics and nutrient intakes with serum triglyceride levels (mg/dL)

	β	(95 % CI)	Adjusted P value
Height (cm)	−0.001	(−0.003 to 0.001)	0.262
Weight (kg)			
Baseline*	0.851	(0.699 to 1.004)	<0.0001
20 years old*	−0.038	(−0.222 to 0.146)	0.683
Body mass index (kg/m^2)			
Baseline*	1.069	(0.904 to 1.233)	<0.0001
20 years old*	0.135	(−0.073 to 0.342)	0.202
Weight change from 20 years old (kg)	0.007	(0.006 to 0.008)	<0.0001
Energy (kcal/day)	−0.00003	(−0.00006 to 0.000003)	0.080
Protein (energy %)	0.001	(−0.004 to 0.006)	0.715
Fat (energy %)	−0.0002	(−0.002 to 0.002)	0.818
Carbohydrate (energy %)	−0.001	(−0.003 to 0.001)	0.167

Each analysis was performed by a general linear model adjusted for age and sex (weight at 20 years old and body mass index at 20 years old are not adjusted by age)

Triglyceride levels were log-transformed before analysis

β unstandardized regression coefficients, *CI* confidence interval

* Calculated after log-transformed

Minor C allele carriers of rs17782313 (T/C or C/C) had higher adjusted means of serum triglyceride levels compared with the T/T genotype. Several population-based studies have evaluated the associations between near *MC4R* rs17782313 and serum triglyceride levels, but no study obtained significant results [9, 14, 20, 26]. Kring et al. [20] reported that rs17782313 was associated with only lower HDL-cholesterol among plasma lipids in Caucasian men. Tao et al. [14] indicated that rs17782313 had an impact on plasma levels of LDL-cholesterol and total cholesterol in a BMI-adjusted model, but not on triglycerides, in Chinese. Other researchers reported that there were no associations between rs17782313 and circulating levels of lipids, including triglycerides [9, 26]. In a meta-analysis of 46 GWAS studies on 96,598 European descent, Teslovich et al. [27] found significant 32 loci ($P < 5 \times 10^{-8}$), including 16 novel loci, associated with triglyceride levels. In this paper, rs17782313 was not included in these significant loci, however, rs17782313 minor C allele was potentially inversely associated with triglyceride levels ($P = 2.62 \times 10^{-5}$) in European population. This inverse association in European population was opposite to our finding. The reason of this discrepancy between races is unclear. Recently, several studies reported significant associations between near *MC4R* rs17782313 and metabolic syndrome [28, 29]. However, these reports did not show the result for each component of metabolic syndrome, including serum triglyceride levels. The results of our study revealed that rs17782313 was also associated with hypertriglyceridemia (triglyceride ≥150 mg/dL) which is one component of metabolic syndrome, thus we suggested that the near *MC4R* polymorphism is associated with an abnormal status of serum triglycerides, as well as the serum level itself. In the present study, the association between near *MC4R* rs17782313 and serum triglyceride levels was independent of lifestyle factors such as physical activity, smoking habit, and drinking habit. This association was also not affected by increased food intake or obesity, as suggested by the results adjusting for total energy intake and BMI. Instead of BMI, we adjusted for weight change from 20 years old, which was marginally associated with rs17782313 ($P = 0.065$), however, the associations between serum triglyceride levels and rs17782313 were not affected. Furthermore, the interactions of genotype and total energy intake or BMI with triglyceride levels were not significant. The biological mechanism of the associations between rs17782313 and triglyceride levels has not been fully elucidated. However, a missense mutation in the *MC4R* gene Val103Ile, which has a function to lower obesity risk, was associated with lower triglyceride levels in cardiovascular patients [18]. In *MC4R* inhibition or disruption in rodents, promotion of lipid uptake, triglyceride synthesis, and fat accumulation in white adipose tissue was observed [19]. The report also concluded that the central nervous system-melanocortin receptor (CNS-Mcr), which directly and rapidly controls triglyceride synthesis, lipid deposition, and lipid mobilization in white adipose tissue, was largely independent of changes in food intake [19]. It has been reported that rs17782313 was not in linkage disequilibrium (LD) with Val103Ile ($r^2 = 0.001$; $D' = 0.48$) [5]. Thus, although the function of rs17782313 is not clear, the polymorphism

Table 4 Multivariate adjusted associations of near *MC4R* polymorphisms (rs17782313) with serum triglyceride levels (mg/dL)

Characteristics	Genotype						Adjusted *P* for trend
rs17782313 (N)	T/T (1268)		T/C (669)		C/C (98)		
	Adjusted mean	(95 % CI)	Adjusted mean	(95 % CI)	Adjusted mean	(95 % CI)	
Model 1[a]	106.1	(98.5–114.2)	111.7	(103.3–120.8)	119.1	(105.7–134.1)	0.004
Model 2[b]	105.5	(98.0–113.6)	111.2	(102.8–120.2)	118.3	(105.0–133.2)	0.004
Model 3[c]	107.2	(99.8–115.1)	111.9	(103.7–120.6)	118.9	(106.0–133.4)	0.011

Data are adjusted means (95 % CI). Each analysis was performed by a general linear model

CI confidence interval

[a] Adjusted for age, sex, area, physical activity, smoking habits, and drinking habits

[b] Adjusted for age, sex, area, physical activity, smoking habits, drinking habits, and total energy intake

[c] Adjusted for age, sex, area, physical activity, smoking habits, drinking habits, total energy intake, and BMI

might change the triglyceride synthesis by altering *MC4R*-related function.

In this study, we could not confirm the association between near *MC4R* rs17782313 and BMI as a continuous variable and obesity (BMI ≥25; JASSO criteria for obesity). Loos et al. [5] conducted a meta-analysis of seven genome-wide association studies for BMI, including 16,876 individuals of white-European descendants, and established the second obesity-susceptibility locus of *MC4R* rs17782313 after replicating the association in an independent sample of 60,352 individuals. This association was confirmed in various ethnic groups [11]; however, it was inconsistent in East Asians, especially in Japanese [12, 13, 17]. A case-control study of 2,865 Japanese individuals found a marginally significant association ($P = 0.049$) between rs17782313 and the risk of obesity [12]. In 1,142 Japanese adults, the TC + CC genotype showed significantly greater BMI ($P = 0.039$) [17]. In addition, the minor allele of rs17782313 tended to show the increased BMI in a population-based study of 2,806 middle-aged to elderly Japanese, although the association did not reach statistical significance [13]. It was reported that rs17782313 was associated with long-term weight change. Carriers of C allele had a 0.2 kg/m^2 greater 10-year increase in BMI ($P = 0.028$) than non-carriers [30]. In our study, weight change from 20 years old was associated with rs17782313, but not significant ($P = 0.065$). We also investigated binary variables of weight change from 20 years old (<5.1 kg or ≥5.1 kg) by a multiple logistic regression model; however, it did not reach statistical significance. Ile251Leu in the near *MC4R* gene, which has a lower obesity risk, as well as Val103Ile, was reported to be not in LD with rs17782313 ($r^2 = 0.001$; $D' = 0.49$) [5]. One reason for the discrepancy of the association between rs17782313 and obesity is that the minor C allele frequency of rs17782313 was different among races. The minor allele frequency of rs17782313 polymorphism was 0.283 in Europeans (HapMap database), but was slightly lower in Asians (0.222 in Japanese, 0.144 in Chinese). In the current study, minor allele frequency (0.213) was similar to the HapMap database and previous reports [13, 17], and slightly lower than Europeans, so the difference in allele frequency might be one reason for the different results among races. Another reason may be the differences in study design, frequency of obesity, dietary habits, lifestyle, and other characteristics among studies.

This study has several strengths. First, we recruited subjects of reasonable sample size from various areas of Japan. Second, we obtained information on many potential confounders, including lifestyle factors, so we could adjust for them in statistical analysis. Circulating level of triglycerides is affected by foods, however, we obtained information on the final meal time and could exclude subjects who had eaten within 3 h before blood sampling. To the best of our knowledge, this study was the first population-based study that revealed a significant relationship between near *MC4R* rs17782313 and serum triglyceride levels in Asian population.

Also, this study has several limitations. First, it was cross-sectional, so causal relationships cannot be argued. Second, information on lifestyle factors, such as physical activity, smoking habit, drinking habit, and total energy intake, was obtained from a self-reported questionnaire; thus, non-differential misclassification may have occurred. Third, our results may not apply to other ethnic groups, because this study was performed in only Japanese.

In conclusion, our study found for the first time that near *MC4R* rs17782313 polymorphism was associated with serum triglyceride levels in Asian population independent of food intake and obesity. Further studies are needed to confirm this result and to clarify the underlying mechanisms.

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Conflict of interest The authors declare no conflict of interest.

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Original Article

Obesity/Weight Gain and Breast Cancer Risk: Findings From the Japan Collaborative Cohort Study for the Evaluation of Cancer Risk

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ABSTRACT

Background: We analyzed data from the Japan Collaborative Cohort Study (36 164 women aged 40–79 years at baseline in 1988–1990 with no previous diagnosis of breast cancer and available information on weight and height) to examine the association between baseline body mass index (BMI)/weight gain from age 20 years and breast cancer risk in a non-Western population.

Methods: The participants were followed prospectively from enrollment until 1999–2003 (median follow-up: 12.3 years). During follow-up, breast cancer incidence was mainly confirmed through record linkage to population-based cancer registries. A Cox proportional hazards model was used to calculate hazard ratios (HRs) and 95% CIs for the association between breast cancer risk and body size.

Results: In 397 644.1 person-years of follow-up, we identified 234 breast cancer cases. Among postmenopausal women, the adjusted HR increased with BMI, with a significant linear trend ($P < 0.0001$). Risk was significantly increased among women with a BMI of 24 or higher (HR: 1.50, 95% CI: 1.09–2.08 for BMI of 24–28.9, and 2.13, 1.09–4.16 for BMI ≥ 29) as compared with women with a BMI of 20 to 23.9. Weight gain after age 20 years and consequent overweight/obesity were combined risk factors for postmenopausal breast cancer risk. This combined effect was stronger among women aged 60 years or older. However, the HRs were not significant in premenopausal women.

Conclusions: Our findings support the hypothesis that weight gain and consequent overweight/obesity are combined risk factors for breast cancer among postmenopausal women, particularly those aged 60 years or older.

Key words: breast cancer; obesity; weight gain; cohort study

INTRODUCTION

Since the early 1990s, breast cancer has been the most frequently diagnosed cancer in Japanese women.[1] Among women, the mortality rate of breast cancer is second only to that of stomach cancer. The recent continuous increase in breast cancer incidence has been an important public health concern in Japan, and the attention devoted to obesity/weight gain as a risk factor for breast cancer has also increased.

Obesity is a well-known risk factor for postmenopausal breast cancer.[2–4] Numerous epidemiologic studies have reported positive associations between obesity and breast cancer risk among white,[5–10] African-American,[11–13] and East Asian women.[14–17] Furthermore, weight gain has been reported as an independent risk factor.[8,9,11,17–21] Several studies have reported an inverse association between body weight in early adulthood and breast cancer incidence.[17,19,20] However, the association has been somewhat inconsistent among

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premenopausal women. Obesity is associated with a decreased risk of breast cancer among white women,[4,10,22–24] although accumulating evidence suggests that the inverse association is limited to women with estrogen receptor- and progesterone receptor-positive tumors.[25–28] Studies of non-white racial/ethnic groups are more limited, and the results are mixed.

To assist in cancer prevention, we analyzed data from a large cohort study—the Japan Collaborative Cohort (JACC) Study—which included 64 327 Japanese women, to examine the association of baseline body mass index (BMI)/weight gain with breast cancer risk, considering menopausal status at baseline. We also investigated the interaction of age on this association.

METHODS

Study population

We analyzed data from the JACC Study, a prospective cohort study that evaluated cancer risk associated with lifestyle factors among the Japanese population. The study has been described in detail previously.[29,30] In brief, the JACC Study was initiated in 1988–1990 and included 110 792 individuals (46 465 men and 64 327 women) aged 40 to 79 years from 45 areas throughout Japan. All participants were subsequently followed for all-cause mortality. In addition, study participants living in 24 areas with cancer registry systems were followed for cancer incidence.

Of the 64 327 women in the baseline cohort, 38 720 lived in the 24 areas where data on cancer incidence were available. The present study excluded 248 women who reported a previous diagnosis of breast cancer and 2308 women who did not provide information on height or weight at baseline. Thus, 36 164 women were included in the present analysis.

Informed consent was obtained from the participants in the form of signatures on the cover pages of the questionnaires, with the exception of those in a few study areas where informed consent was provided at the group level after the aims and data confidentiality had been explained to community leaders. The Ethics Board of Sapporo Medical University approved our study.

Exposure assessment

As a relative indicator of body weight, BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Information regarding weight and height was obtained from the self-reported questionnaire. Change in weight from age 20 years to the baseline measurement was calculated as the difference in the reported values at baseline among 20 418 women whose information on weight at age 20 years was available. We did not use BMI for age 20 years because we did not have access to height information at that age.

Information on other potential breast cancer risk factors such as family history of breast cancer, tobacco and alcohol use, age at menarche, marital status, parity, age at first birth, menopausal status, hormone use, and physical activity was collected in the baseline questionnaire. We have no information after baseline, including information on body size or menopausal status.

Follow-up and identification of breast cancer cases

We followed the study participants from enrollment until 1999–2003. During this period, a population registry was used in each municipality to ascertain the residential status and vital status of the participants. In Japan, the Family Registration Law requires registration of all deaths, which theoretically provides complete mortality data. Breast cancer incidence was confirmed mainly through record linkage to population-based cancer registries in each area. To complete the incidence data, we also conducted a systematic review of death certificates and medical records at major local hospitals in some areas.

During the study period, 1799 (5.0%) participants were lost to follow-up due to moving out of their designated study areas. Among the 234 breast cancer cases, no information on diagnosis was available for 13 (5.6%), ie, they were identified with death certification only (DCO). The world standard for DCO in cancer registration is less than 10%. The mortality-to-incidence ratio for breast cancer was 0.262 (58/221) in the cohort covered by cancer registries, which was within the range calculated using available data from population-based cancer registries in Japan (0.20–0.30). We estimated that 36.5 cases of incident breast cancer were not included in the cancer registries.

Statistical analysis

For each cohort subject, person-years of follow-up were counted as time from enrollment to diagnosis of breast cancer, death from any cause, or end of follow-up (1999–2003), whichever occurred first. For breast cancer cases ascertained only by death certificates, person-years of follow-up were calculated from enrollment to death from breast cancer. Those who died from causes other than breast cancer or who moved out of the study areas were treated as censored cases. We used a Cox proportional hazards model to estimate hazard ratios (HRs) and 95% CIs for the association of breast cancer risk with baseline BMI/weight change. Women were divided into 5 categories, using baseline BMI (in accordance with the World Health Organization classification)[31]: less than 18.5, 18.5–19.9, 20–23.9, 24–28.9, and 29 kg/m^2 or higher. Furthermore, BMI was entered directly to evaluate the linear trend of relative weight. The effect of age on the association between BMI and breast cancer risk was examined by analyzing the relationship between age and BMI. Finally, to investigate the combined effect of baseline BMI and weight change from age 20 years, we recategorized the participants into 4 groups using the following cutoff points: baseline BMI less than 24 kg/m^2 and weight gain of less than 10 kg from age 20 years to the baseline measurement.

Table 1. Baseline characteristics associated with BMI in the JACC Study

Characteristics	BMI at baseline				
	<18.5	18.5–19.9	20–23.9	24–28.9	≥29
Number, *n* (row%)	2373 (6.6%)	3654 (10.1%)	18231 (50.4%)	10737 (29.7%)	1169 (3.2%)
Height (cm)	152.0 ± 7.0	151.0 ± 5.8	151.3 ± 5.5	150.7 ± 5.6	149.3 ± 6.4
BMI	17.4 ± 1.0	19.3 ± 0.4	22.0 ± 1.1	25.8 ± 1.3	31.0 ± 2.0
Weight at age 20 years (kg)	46.5 ± 6.1	47.8 ± 5.7	49.6 ± 6.2	51.0 ± 6.6	52.2 ± 6.8
Weight change[a] (kg)	−6.3 ± 5.9	−3.7 ± 5.4	1.1 ± 6.3	7.8 ± 7.0	17.1 ± 8.3
Age at inclusion (years)	61.3 ± 10.8	58.5 ± 10.7	57.1 ± 10.0	57.9 ± 9.3	58.3 ± 9.3
Age at menarche (years)	15.2 ± 1.8	15.0 ± 1.8	14.9 ± 1.8	14.8 ± 1.8	14.9 ± 1.9
Age at first birth (years)	25.4 ± 3.5	25.2 ± 3.3	25.0 ± 3.2	24.9 ± 3.2	25.0 ± 3.5
Age at menopause (years)	48.2 ± 4.9	48.5 ± 4.5	48.8 ± 4.6	48.7 ± 4.8	48.5 ± 5.1
Years of education	16.5 ± 2.2	16.6 ± 2.1	16.7 ± 2.1	16.3 ± 2.0	16.0 ± 2.1
Nulliparous, *n* (%)	144 (6.6%)	175 (5.2%)	700 (4.1%)	404 (4.0%)	53 (4.9%)
Not married, *n* (%)	69 (3.4%)	61 (1.9%)	227 (1.4%)	111 (1.2%)	20 (2.0%)
Exogenous female hormone use, *n* (%)	124 (6.2%)	160 (5.2%)	792 (5.1%)	471 (5.2%)	61 (6.1%)
Family history of breast cancer, *n* (%)	30 (1.3%)	42 (1.2%)	269 (1.5%)	167 (1.6%)	13 (1.1%)
Current smoker, *n* (%)	162 (7.6%)	201 (6.2%)	779 (4.7%)	470 (4.8%)	81 (7.7%)
Current drinker, *n* (%)	453 (20.4%)	790 (23.1%)	4250 (24.8%)	2444 (24.2%)	223 (20.5%)

BMI, body mass index.
Mean (SD) or %, calculated from subjects with no missing data for any variable.
[a]Difference in body weight at age 20 years and baseline.

We evaluated the association using age-adjusted and multivariable models with adjustment for age (using 10-year age groups), tobacco smoking (never, past, current, or unknown), alcohol consumption (never, past, current, or unknown), age at menarche (<15, 15–16, ≥17 years, or unknown), education level (attended school until age <16, 16–18, ≥19 years, or unknown), parity (nulliparous, 1, 2–3, ≥4 births, or unknown), age at first birth (<22, 22–23, 24–25, ≥26 years, or unknown), menopausal status (premenopausal at baseline, <45, 45–49, or ≥50 years), use of exogenous female hormone (yes, no, or unknown), first-degree family history of breast cancer (yes, no, or unknown), and physical activity categories[32] (4 groups using the following cutoff points of physical activity: daily walking <1 h and exercise time <1 h a week, or unknown). All analyses were performed with regard to menopausal status and stratified by 6 study areas (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku, and Kyushu).

We repeated the analysis after excluding the first 2 years of follow-up, during which 38 cases of breast cancer were diagnosed. All *P* values were 2-sided, and a *P* value less than 0.05 was considered to indicate statistical significance. All regression analyses were performed using the PROC PHREG procedure of SAS Version 9.1 (SAS Institute, Cary, NC, USA). Study areas were not incorporated in the Cox model with other potential confounders but were adjusted for using the strata option in the PHREG procedure.

RESULTS

Average age and BMI (SD) at baseline of the 36 164 women were 57.8 (10.0) years and 22.9 (3.1) kg/m^2, respectively. In 397 644.1 person-years of follow-up (median follow-up time, 12.3 years), we identified 234 breast cancer cases. Table 1 shows the distribution of risk factors for breast cancer in association with BMI. Women with a BMI less than 18.5 were older and more likely to be nulliparous and unmarried. The 2 extreme BMI groups had higher percentages of smokers and lower percentages of drinkers. Groups with higher BMI at baseline had increased weights at age 20 years and greater weight gain from age 20 years to baseline. However, the difference in weight at age 20 years between the 2 extreme BMI groups was relatively small (46.5 kg vs 52.2 kg), and weight change from age 20 years (−6.3 kg vs 17.1 kg) was a stronger contributor to body size at baseline. The average (SD) overall change in weight during the period was 2.7 (8.2) kg.

Table 2 shows breast cancer risk associated with baseline BMI in relation to menopausal status. After adjustment for potential confounding factors, neither a significant HR nor a linear trend was observed among the 8131 premenopausal women. In contrast, among 28 033 postmenopausal women, the adjusted HR increased with BMI and showed a significant linear trend ($P < 0.0001$). Furthermore, significantly increased risk was observed among women with a BMI of 24 or higher (HR: 1.50, 95% CI: 1.09–2.08 for BMI of 24–28.9; 2.13, 1.09–4.16 for BMI ≥29) as compared with those with a BMI of 20 to 23.9. The adjusted HRs per 5-kg/m^2 increment in BMI among pre- and postmenopausal women were 0.95 (95% CI: 0.60–1.50) and 1.68 (95% CI: 1.34–2.01), respectively.

To observe the effect of age on the association between BMI and breast cancer risk among postmenopausal women, we calculated the HR for a 5-kg/m^2 increment in BMI in younger (40–59 years) and older (60–79 years) age groups. The older group had a higher HR (2.00, 95% CI: 1.48–2.70) than the younger group (1.37, 95% CI: 0.96–1.96) for a