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ORIGINAL ARTICLE

# Effect of dietary energy and polymorphisms in *BRAP* and *GHRL* on obesity and metabolic traits

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**KEYWORDS**
Obesity;
Single nucleotide polymorphism;
Energy intake;
*BRAP*;
*GHRL*

**Summary**
*Objective:* Obesity, a risk factor for all-cause and cardiovascular mortality, is a major health concerns among middle-aged men. The aim of this study was to investigate a possible association of dietary habits and obesity related single nucleotide polymorphisms (SNPs) with obesity and metabolic abnormalities.
*Methods:* We conducted a retrospective cohort study using annual health examination data of 5112 male workers, obtained between 2007 and 2011. Average dietary energy was estimated using electronically collected meal purchase data from cafeteria. We examined 8 SNPs related to obesity: *GHRL* rs696217, *PPARG* rs1175544, *ADIPOQ* rs2241766, *ADIPOQ* rs1501299, *PPARD* rs2016520, *APOA5* rs662799, *BRAP* rs3782886, and *ITGB2* rs235326. We also examined whether SNPs that were shown to associate with obesity affect other metabolic abnormalities such as blood pressure (BP), glucose, and lipid profile.
*Results:* Average dietary energy significantly associated with increased abdominal circumference (AC) and body mass index (BMI). The odds ratios (ORs) of overweight and obesity also increased. The major allele of rs696217 significantly increased BMI and an increased OR with obesity, while the minor allele of rs3782886 was associated with significantly decreased AC and the decreased ORs with overweight and obesity. The minor allele of rs3782886 was also associated with significantly decreased systolic BP (SBP), triglyceride (TG), high-density lipoprotein (HDL), and fasting blood sugar (FBS), while rs696217 was not associated with other metabolic abnormalities.
*Conclusions:* Average dietary energy in lunch, rs3782886, and rs696217 were associated with obesity, and rs3782886 was associated with other metabolic abnormalities.
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## Introduction

Obesity is one of the most common nutritional disorders and there is extensive evidence that mortality is higher among obese individuals [1—3], mostly due to cardiovascular diseases and malignant diseases. Lately, midlife obesity has been recognised as a risk for future disability and frailty [4]. The prevalence of obesity in Japan, defined as BMI $\geqq$25 kg/m$^2$, is about 30% in men and 20% in women [5]. It is especially common in middle-aged men. Obesity is also a risk factor for hypertension [6], type 2 diabetes [7], and coronary heart disease [8] in the Japanese population.

Genetic factors contribute to the development of obesity. Based on genome wide association studies [9,10] and candidate gene studies [11—18], to date, a number of single nucleotide polymorphisms (SNPs) have been associated with obesity. Several candidate gene studies have shown to associate with obesity among Japanese or Eastern Asian population which included SNPs in genes that encode energy balance-regulating peptides (i.e., leptin and ghrelin) [11], members of the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors [12,15], adiponectin [13,14], adipoproteins [16], and breast cancer suppressor protein (BRCA1)-associated protein (BRAP)) [17], and adhesion molecules [18]. Many post-genome wide association studies have examined the interaction between SNPs associated with obesity and food intake, or other food behaviour-related traits [19—21].

Environmental factors, such as dietary habits, are also important risk for obesity and metabolic syndrome. Most studies of dietary intakes use a dietary self-report instrument such as food frequency questionnaire (FFQ) and 24-h recall, and dietary intake recovery biomarkers [22]. However, data from self-report instrument contain reporting bias. Dietary intake recovery biomarkers can provide accurate assessments of short-term intakes of dietary components, but these biomarkers are expensive or inconvenient to measure. In addition, these biomarkers are notoriously limited in their use because biomarkers are not available for all nutrients and many factors besides dietary intake influence nutrition biomarkers such as competitive uptake, nutrient absorption.

Here, in order to estimate daily energy intake among participants, we examined meal purchase data that were automatically recorded when an employee purchased lunch in the cafeteria of Toyota Motor Corporation. The meal purchase data included the individual food contents and dietary elements, such as energy, salt, carbohydrate,

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protein, and fat. The data were available for up to 6 months before the health examination, so they reflect the unbiased energy intake of each individual. It is essential to investigate the relation between meal data and obesity in the cohort of male workers, which is a population at-risk for obesity.

The aim of this study was to evaluate the contribution of high dietary energy and obesity related genetic polymorphisms to the development of obesity among Japanese male workers. Furthermore, we investigated whether there was significant relation between metabolic traits and SNPs that showed a significant relation to obesity.

## Methods

### Study population

In this retrospective observational study, the participants were all male employees of Toyota Motor Corporation, Limited. in Japan. Only those who consented to DNA sampling were included, and the DNA samples were obtained between 2011 and 2014 from 5112 individuals. We did not provide any exclusion criteria. Any individuals who received the annual health examination were included in this research regardless of health problem. The study protocol was approved by the ethics committee of Nagoya University School of Medicine (No. 1089-4) and conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent. This trial was registered at umin.ac.jp as UMIN000016266.

### Lifestyle evaluation

The lifestyle of each individual was evaluated using self-administered questionnaires and included an assessment of smoking status, exercise habits, and drinking habits. Smoking status was defined as the current smoking status of the participants. Responses for drinking habits were divided into four categories (none, <2 days per week, 3—6 days per week, and every day). Exercise habits were also divided into three categories (none, <6 days per week, and every day).

The meal purchase data, which included automatically recorded dietary elements, such as energy, salt, carbohydrate, protein, and fat when an employee purchased lunch in the cafeteria, were available to allow estimation of the individual eating habits. In the cafeteria, employees can purchase variety of foods, which change regularly. Average energy, salt, carbohydrate, protein, and fat consumption were calculated using data from 6 months before the health examination. ''Average energy'' refers to the total amount of energy during the period divided by usage count (number of meals). Food frequency refers to the usage counts of cafeteria divided by months. Preparation of meals in the cafeteria has been entrusted to the subcontractor, and the nutritional values of the meal is evaluated by registered dietitians that belongs to the cafeteria management company.

### Clinical parameters

The health examinations performed from 2007 through 2011 included physical measurements and serum biochemical measurements. Physical measurements included the measurement of the height, weight, body mass index (BMI), and abdominal circumference (AC) of the participants in the fasting state. Those measurements were performed by trained medical staffs. BMI was calculated as the weight in kilograms divided by the square of the height in meters. The abdominal circumference at the umbilical level is measured in the late exhalation phase while standing [23]. Obesity is usually diagnosed as BMI $\geqq$ 25.0 kg/m$^2$ in Japan [24], while in other countries, BMI $\geqq$ 25.0 kg/m$^2$ is defined as overweight. In this study, we defined BMI $\geqq$ 25.0 kg/m$^2$ as overweight, and BMI $\geqq$ 30.0 kg/m$^2$ as obesity in accordance with the 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults [25].

Blood pressure (BP) was measured in the sitting position using an automatic sphygmomanometer (Kentaro HBP-9020; Omron, Tokyo, Japan).

The biochemical measurements included measurement of the total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), fasting blood sugar (FBS), and uric acid (UA).

A substantial proportion of the study participants between 2007 and 2008 lacked serum LDL data as measurement of serum LDL was not common in Japan at that time. However, since 2009, measurement of serum LDL has been considered one of the essential factors in health examinations. After 2009, the measurement was added to the health examinations of all participants.

### SNP selection

In our previous study, we developed an SNP measuring system including 99 candidate SNPs that were associated with coronary heart disease,

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hypertension, dyslipidemia, diabetes mellitus, hyperuricemia, renal disease, or obesity [26]. Here we selected eight candidate SNPs that were associated with obesity: *GHRL* rs696217 (Leu72Met)[11], *PPARG* rs1175544[12], *ADIPOQ* rs2241766 (T45G)[13], *ADIPOQ* rs1501299 (G276T) [14], *PPARD* rs2016520 (T294C) [15], *APOA5* rs662799 (T−1131C)[16], *BRAP* rs3782886 [17], and *ITGB2* rs235326 [18].

## Genotyping the SNPs

DNA was extracted from blood samples obtained from the participants (0.2 mL each) at DNA Chip Research Incorporated (QIAamp® series; QIAGEN K.K., Tokyo, Japan). All SNP genotyping was performed using the DigiTag2 assay [27].

Primers, probe sequences, and PCR conditions for SNPs from the original cohort were as described previously [26]. The laboratory technicians were blinded to the participants' identity, demographic characteristics, and study outcomes.

### Statistical analysis

The clinical characteristics of the participants from 2007 to 2011 were summarised in medians and interquartile ranges (IQRs) for quantitative variables (i.e., age, BMI, AC, systolic blood pressure (SBP), and diastolic blood pressure (DBP)), and in numbers and percentages for qualitative variables (i.e., smoking status, exercise habits, and drinking habits).

Allele frequencies were estimated using the gene-counting method, and the Pearson's chi-square test was used to identify any significant deviation from Hardy−Weinberg equilibrium (HWE).

For each SNP, a score of 0, 1, or 2 was assigned depending on the number of minor alleles. The SNP score was treated as a continuous variable.

A linear mixed model for longitudinal analysis was applied to the health examination data to determine the relation between the dependent (BMI and AC) and independent variables. A generalised estimation equation was applied for binary outcomes such as overweight and obesity.

Multivariable models were adjusted for potential confounders. Variable selection was based on the literature review [15,17] and clinical knowledge. In order to improve accuracy of reflecting dietary habits of participants, we added the analysis of the limited population in which the eating frequency in the cafeteria over 10 times a month.

In the first step, we examined the relation between obesity trait (BMI, AC, overweight, and obesity) and the mean dietary energy adjusted for age, current smoking status, exercise habits, and drinking habits. In the second step, we estimated the effect size (regression coefficient for BMI and AC and odds ratios (ORs) for overweight and obesity) adjusted for age, current smoking status, exercise habits, drinking habits, and average dietary energy. In the third step, we examined whether the obesity-related SNPs that identified in the second step associated with other metabolic traits such as SBP, TG, HDL, LDL, and FBS. We performed univariate analysis and three multivariate analyses, and those were, adjusted for BMI in model one; age, smoking habits, sport habits, drinking habits, mean dietary energy in model two; and values in model two plus BMI in model three.

All data were treated as fixed effects, and the within-subject covariance structure was compound symmetric. $P < 0.05$ was considered statistically significant. The data were analysed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2014).

# Results

## Clinical characteristics

Clinical characteristics (clinical parameters, lifestyle data, and meal purchase data) of the study participants in each year are shown in Table 1. DNA samples were obtained from 5112 individuals, and the number of participants in the annual health examinations conducted from 2007 through 2011 differed yearly. The baseline characteristics, except smoking status and exercise habits, were similar across the 5 years. The number of participants who smoked declined, while the proportion of participants who habitually exercised increased. The prevalence individuals that met the criteria for overweight (BMI $\geqq 25\,kg/m^2$) in each year was approximately 30% and those for obesity (BMI $\geqq 30\,kg/m^2$) was about 5% in this population.

## Characteristics of SNPs

The SNPs examined in this study are shown in Table 2. Genotype frequencies did not significantly deviate from HWE.

## Effects of dietary energy on obesity

The average dietary energy was significantly associated with AC, BMI, and obesity in both univariate and multivariate analysis. The multivariate analysis was adjusted for age, smoking status, exercise

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**Table 1** Clinical characteristics of participants in annual health examinations.[a]

| Characteristics | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|
| Number of participants | 4623 | 4366 | 5111 | 4817 | 5018 |
| Age | 45 (39, 49) | 46 (42, 51) | 46 (41, 51) | 48 (42, 52) | 48 (43, 53) |
| BMI | 23.2 (21.3, 25.5) | 23.3 (21.5, 25.6) | 23.3 (21.3-25.6) | 23.2 (21.3-25.5) | 23.4 (21.4-25.7) |
| AC | 83 (77.5, 89) | 83.4 (78, 89) | 83 (77.5, 89) | 82.8 (77.5, 88.7) | 83 (77.5, 89) |
| SBP | 121 (111, 131) | 121 (112, 131) | 121 (112, 131) | 121 (112, 130) | 122 (113, 131) |
| DBP | 76 (68, 83) | 77 (70, 84) | 77 (70, 84) | 77 (70, 84) | 77 (70, 84) |
| FBS | 93 (87, 100) | 93 (87, 100) | 92 (87, 100) | 92 (87, 99) | 92 (87, 100) |
| TC | 204 (181, 227) | 206 (183, 229) | 206 (184, 229) | 202 (180, 225) | 202 (181, 226) |
| TG | 103 (71, 156) | 107 (74, 161) | 106 (72, 163) | 102 (70, 153) | 99 (70, 149) |
| HDL | 58 (49,69) | 56 (48, 66) | 55 (47, 66) | 56 (47, 67) | 57 (48, 68) |
| LDL[b] | 129 (108, 151) | 124 (103, 144) | 124 (103, 146) | 121 (102, 143) | 123 (103, 144) |
| UA | 6 (5.2, 6.9) | 6.1 (5.2, 6.9) | 6 (5.2, 6.9) | 6 (5.3, 6.9) | 6 (5.2, 6.8) |
| Overweight (BMI ≧ 25) | 1392, 30.1% | 1351, 30.9% | 1579, 30.9% | 1432, 29.7% | 1598, 31.8% |
| Obesity (BMI ≧ 30) | 208, 4.5% | 199, 4.6% | 241, 4.7% | 228, 4.7% | 263, 5.2% |
| Lifestyle | | | | | |
| Exercise habits | | | | | |
| None | 1955, 58% | 1716, 39% | 1793, 35% | 1563, 33% | 1636, 33% |
| <6 days per week | 801, 24% | 2028, 46% | 2500, 49% | 2427, 50% | 2572, 51% |
| Every day | 620, 18% | 622, 14% | 810, 16% | 818, 17% | 804, 16% |
| Drinking habits | | | | | |
| None | 1155, 25% | 1039, 24% | 1222, 24% | 1130, 23% | 1217, 24% |
| <2 days per week | 956, 21% | 895, 20% | 1224, 24% | 1170, 24% | 1249, 25% |
| 3—6 days per week | 648, 14% | 631, 14% | 764, 15% | 780, 16% | 797, 16% |
| Every day | 1864, 40% | 1801, 41% | 1892, 37% | 1729, 36% | 1750, 35% |
| Smoking | 2015, 44% | 1841, 42% | 2027, 40% | 1785, 37% | 1729, 34% |
| Meal purchase data | | | | | |
| Number of participants | 2751 | 4015 | 4691 | 4383 | 4531 |
| Eating times (times) | 103 (83, 110) | 103 (83, 110) | 104 (84, 110) | 106 (88, 111) | 99 (81, 108) |
| Eating frequency (/month) | 17.2 (14.2, 18.3) | 17.2 (14.2, 18.3) | 17.3 (14.4, 18.3) | 17.7 (15, 18.5) | 16.8 (14, 18.2) |
| Energy (kcal/times) | 832 (740, 925) | 810 (732, 897) | 780 (703, 873) | 756 (678, 837) | 753 (679, 839) |
| Salt (g/times) | 5.3 (4.5, 6.2) | 5.3 (4.5, 6.2) | 5.2 (4.3, 6.2) | 5 (4.2, 5.9) | 5 (4.2, 5.8) |
| Fat (g/times) | 28.5 (24, 33) | 27.3 (22.9, 31.4) | 26 (21.7, 30.3) | 24 (20, 28.1) | 24.3 (20.2, 28.4) |
| Carbohydrate (g/times) | 108 (98, 122) | 107 (97, 120) | 104 (95, 117) | 103 (94, 115) | 102 (93, 114) |
| Protein (g/times) | 29.8 (26.6, 33.1) | 28.9 (25.9, 32) | 28 (25, 31.4) | 27.1 (24.1, 30) | 27 (24.1, 30.1) |

BMI = body mass index; AC = abdominal circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein; FBS = fasting blood sugar; UA = uric acid.

[a] Continuous data represent medians (1st quartile, 3rd quartile). Categorical data indicate n values (%).

[b] Number of participants for whom serum LDL was measured was 1405 and 3839 in 2007 and 2008, respectively.

habits, and drinking habits. The regression coefficient of average dietary energy per 100 kcal increase was 0.43 cm (±0.027 cm) for AC and 0.12 kg/m$^2$ (±0.0086 kg/m$^2$) for BMI. The OR of the average dietary energy per 100 kcal increase was 1.21 [95% confidence interval (CI), 1.16—1.26] for overweight and 1.35 [95% CI, 1.25—1.45] for obesity.

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**Table 2** Characteristics of SNPs.

| SNP | Chr | Position (GRCh37) | Gene | A/a | Genotype | | | Minor allele frequency | HWE (*P* value) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | AA | Aa | aa | | | |
| rs696217 | 3 | 10,331,457 | *GHRL* | T/G | 3251 (63.6%) | 1645 (32.2%) | 212 (4.2%) | 0.203 | 0.829 | 11 |
| rs1175544 | 3 | 12,467,044 | *PPARG* | A/G | 1829 (35.8%) | 2452 (48.0%) | 825 (16.2%) | 0.402 | 0.947 | 12 |
| rs2241766 | 3 | 186,570,892 | *ADIPOQ* | G/T | 2522 (49.4%) | 2133 (41.8%) | 446 (8.7%) | 0.297 | 0.868 | 13 |
| rs1501299 | 3 | 186,571,123 | *ADIPOQ* | A/C | 2575 (50.5%) | 2096 (41.1%) | 432 (8.5%) | 0.29 | 0.851 | 14 |
| rs2016520 | 6 | 35,378,778 | *PPARD* | G/A | 3232 (63.3%) | 1669 (32.7%) | 206 (4.0%) | 0.204 | 0.605 | 15 |
| rs662799 | 11 | 116,663,707 | *APOA5* | G/A | 2187 (42.8%) | 2276 (44.6%) | 641 (12.6%) | 0.349 | 0.197 | 16 |
| rs3782886 | 12 | 112,110,489 | *BRAP* | G/A | 2786 (54.7%) | 1957 (38.4%) | 353 (6.9%) | 0.261 | 0.711 | 17 |
| rs235326 | 21 | 46,311,813 | *ITGB2* | T/C | 2801 (55.0%) | 1928 (37.9%) | 360 (7.1%) | 0.26 | 0.258 | 18 |

rs# = rs number, Chr = chromosome, A = major allele, a = minor allele, HWE = Hardy–Weinberg equilibrium; MAF, minor allele frequency; HWE *P* values were calculated by Pearson's chi-square test.

## Effects of SNPs on obesity

Multivariate analysis of each SNP versus BMI, AC, overweight, and obesity are shown in Table 3.

*GHRL* rs696217 was significantly associated with BMI and obesity. The coefficient of the minor allele was 0.18 kg/m$^2$ ($\pm$0.083 kg/m$^2$) for BMI, while the OR of the minor allele was 1.40 [95% CI, 1.13–1.74] for obesity. *BRAP* rs3782886 was significantly associated with AC, overweight, and obesity in multivariate analysis. The coefficient of the minor allele was -0.66 kg/m$^2$ ($\pm$0.200 kg/m$^2$) for AC, and the OR of the minor allele was 0.74 [95% CI, 0.67–0.83] for overweight and 0.62 [95% CI, 0.50–0.77] for obesity. *PPARG* rs1175544 was significantly associated with overweight. That result was similar in the limited population in which the eating frequency in the cafeteria over 10 times a month (Supplementary Table 1).

## Effects of SNPs on other metabolic traits

Univariate and multivariate analyses of each SNP versus metabolic traits are shown in Table 4. The minor allele of *BRAP* rs3782886 had significantly decreased SBP, TG, HDL, LDL, and FBS in univariate analysis and multivariate analysis of the model 1. In the model 2, which was adjusted for the same variables as obesity related traits, the minor allele of *BRAP* rs3782886 was significantly associated to decrease SBP, TG, HDL and FBS. In model 3, which was adjusted for the variables in model 2 and BMI, the SNP was significantly associated with HDL and FBS. On the other hand, *GHRL* rs696217 was not associated with other metabolic abnormalities.

## Discussion

In the present study, we showed that the average dietary energy in lunch, as estimated by meal purchase data, was a significant risk factor for obesity. Additionally, *BRAP* rs3782886 and *GHRL* rs696217 were found to be significant risk factors for obesity after adjustment for age and lifestyle confounders, including dietary energy.

This study was unique in that the average dietary energy estimated from meal purchase data was found to be a significant longitudinal risk factor for obesity. Dietary energy intake is traditionally assessed based on self-reported estimates. A study by Dhurandhar et al. highlighted significant limitations of such self-reported estimates of energy intake and proposed the need for more objective measures of energy balance [28]. The advantage

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**Table 3** Multivariate analysis of SNPs and obesity-related traits adjusted for lifestyle confounders.

| | AC | | | BMI | | | Overweight (BMI ≥ 25) | | | Obesity (BMI ≥ 30) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | SE | *P* value | Coefficient | SE | *P* value | OR | 95% CI | *P* value | OR | 95% CI | *P* value |
| rs696217 | 0.37 | 0.21 | 0.083 | 0.18 | 0.083 | 0.033* | 1.07 | 0.97–1.19 | 0.20 | 1.40 | 1.13–1.74 | 0.002* |
| rs1175544 | 0.27 | 0.18 | 0.13 | 0.091 | 0.068 | 0.18 | 1.09 | 1.01–1.19 | 0.037* | 0.95 | 0.79–1.14 | 0.58 |
| rs2241766 | −0.18 | 0.19 | 0.35 | −0.093 | 0.074 | 0.21 | 0.97 | 0.88–1.06 | 0.44 | 0.90 | 0.73–1.10 | 0.31 |
| rs1501299 | 0.16 | 0.19 | 0.40 | 0.091 | 0.074 | 0.22 | 1.05 | 0.96–1.15 | 0.27 | 1.15 | 0.94–1.39 | 0.17 |
| rs2016520 | −0.10 | 0.22 | 0.65 | −0.047 | 0.084 | 0.58 | 0.96 | 0.86–1.06 | 0.43 | 0.99 | 0.79–1.24 | 0.93 |
| rs662799 | 0.20 | 0.18 | 0.27 | 0.075 | 0.070 | 0.29 | 1.00 | 0.92–1.09 | 0.96 | 1.04 | 0.86–1.25 | 0.69 |
| rs3782886 | −0.66 | 0.20 | 0.001* | −0.15 | 0.077 | 0.054 | 0.74 | 0.67–0.83 | <0.001* | 0.62 | 0.50–0.77 | <0.001* |
| rs235326 | 0.12 | 0.20 | 0.54 | 0.081 | 0.076 | 0.29 | 1.00 | 0.91–1.10 | 0.96 | 0.89 | 0.72–1.10 | 0.29 |

AC = abdominal circumference; BMI = body mass index; SE = standard error; OR = odds ratio.
Adjusted for age, smoking status, sport habits, drinking habits, mean dietary energy.
* $P < 0.05$.

of our study is that these objective measurements are available because meal purchase data provides daily dietary lunch data for a period of up to 6 months.

This study is also unique because it considered BMI and AC as repeated measured outcomes in a linear mixed model and generalised estimating equation. This model is robust, even with missing data, and allows for the analysis of repeated data with a variable number of measurements per subject, as in our data set. Because obesity and metabolic syndrome are reversible conditions, with repeating rounds of morbidity and remission, the analysis presented in this study, based on the linear mixed model, is particularly suitable for probing these diseases.

To the best of our knowledge, this was the first to demonstrate that *BRAP* rs3782886 is significantly associated with obesity and metabolic traits among Japanese male workers. The minor allele of *BRAP* rs3782886 was shown to be a significant risk factor for coronary artery disease in Asian populations [29]; however, in Chinese adolescents, a low OR indicates that this allele confers protection against metabolic syndrome [17], despite the fact that high BMI and abdominal adiposity are known risk factors for cardiovascular disease. BRAP was shown to activate inflammatory cascades by regulating NF-κB [30]. Inflammation in the central nervous system, especially hypothalamic IKKβ/NF-κB activation, can lead to obesity [31]. Thus, the minor allele of *BRAP* rs3782886 may be protective against obesity by the reduction of the transcriptional activity of *BRAP*. However, it is unclear why the minor allele is the risk factor for cardiovascular diseases.

We also considered an alternative explanation for the relation between *BRAP* and metabolic abnormalities. *ALDH2* rs671 and *BRAP* rs3782886 are in strong linkage disequilibrium (LD) according to the HapMap database [32]. Meta-analysis of genome-wide association studies in East Asian populations has shown associations between single polymorphisms of *ALDH2* rs671 and obesity, various cardiovascular risk factors and coronary artery disease [10,33]. Drinking habits strongly affect appetite and body weight, so we observed this association in a multivariate analysis adjusted for dietary and drinking habits. *ALDH2* rs671 is also associated with coronary artery disease (CAD) and metabolic traits. The minor allele of this SNP was associated with CAD, high LDL, and low HDL, while the major allele was associated with high blood pressure, and high glucose [33]. In the present study, the relation was similar that reported in prior studies. So far, we cannot explain this contradictory

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**Table 4** Associations of rs696217 and rs3782886 with metabolic traits.

| | | rs696217 | | | rs3782886 | | |
|---|---|---|---|---|---|---|---|
| | | Coefficient | SE | *P* value | Coefficient | SE | *P* value |
| SBP | Univariate | 0.18 | 0.28 | 0.52 | −1.39 | 0.25 | <0.001* |
| | Model 1 | −0.056 | 0.26 | 0.83 | −1.17 | 0.23 | <0.001* |
| | Model 2 | 0.25 | 0.28 | 0.37 | −0.83 | 0.26 | 0.0025* |
| | Model 3 | 0.021 | 0.26 | 0.94 | −0.35 | 0.26 | 0.18 |
| TG | Univariate | 0.62 | 1.97 | 0.75 | −8.16 | 1.80 | <0.001* |
| | Model 1 | −1.29 | 1.85 | 0.49 | −6.37 | 1.69 | <0.001* |
| | Model 2 | 0.77 | 2.04 | 0.7 | −7.15 | 1.99 | <0.001* |
| | Model 3 | −1.07 | 1.92 | 0.58 | −3.47 | 1.88 | 0.066 |
| HDL | Univariate | −0.34 | 0.35 | 0.32 | −3.04 | 0.32 | <0.001* |
| | Model 1 | −0.019 | 0.32 | 0.95 | −3.35 | 0.29 | <0.001* |
| | Model 2 | −0.33 | 0.34 | 0.34 | −1.61 | 0.32 | <0.001* |
| | Model 3 | −0.02 | 0.32 | 0.95 | −1.99 | 0.30 | <0.001* |
| LDL | Univariate | 0.42 | 0.67 | 0.53 | 1.43 | 0.61 | 0.02* |
| | Model 1 | −0.13 | 0.65 | 0.85 | 1.96 | 0.60 | 0.001* |
| | Model 2 | 0.60 | 0.68 | 0.38 | 0.046 | 0.66 | 0.94 |
| | Model 3 | 0.072 | 0.66 | 0.91 | 0.97 | 0.64 | 0.13 |
| FBS | Univariate | 0.12 | 0.42 | 0.78 | −1.43 | 0.38 | <0.001* |
| | Model 1 | −0.14 | 0.40 | 0.74 | −1.19 | 0.37 | 0.0012* |
| | Model 2 | 0.17 | 0.41 | 0.68 | −1.32 | 0.40 | <0.001* |
| | Model 3 | −0.061 | 0.40 | 0.88 | −0.95 | 0.38 | 0.013* |

SE = standard error; SBP = systolic blood pressure; TG = triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein; FBS = fasting blood sugar.
Model 1 adjusted for BMI.
Model 2 adjusted for age, smoking status, sport habits, drinking habits, mean dietary energy.
Model 3 adjusted for the variables in model 2, plus BMI.
* $P < 0.05$.

relation sufficiently. Additional studies are necessary to determine how the SNP rs3782886 affects cardiovascular diseases.

We also firstly demonstrated the association between *GHRL* rs696217 (Leu72Met) and obesity traits by using dietary data in larger cohort of Japanese male workers. In a prior study, the authors didn't examine the association with dietary data [11]. *GHRL* rs696217 is a single nucleotide substitution of C214A, with Met replacing Leu at codon 72 in the preproghrelin amino-acid sequence. Ghrelin, which is abundantly produced by the stomach, stimulates food intake [34] and is involved in the regulation of energy homeostasis [35]. The frequency of the 72Met allele is approximately 20% among Japanese [11], while the frequency is approximately 8% among Caucasians and approximately 2% among Africans [36]; hence, this SNP is relatively common among Japanese. A recent review of the literature uncovered several case-control studies in Japan that indicate a significant association *between GHRL* rs696217 and obesity [37]. Therefore we suggest that this SNP should be carefully considered when examining obesity related health problems among the Japanese population.

One of the limitations of this study is that we can only collect data on meals served for lunch, using electronic meal purchase data. In the present study, we didn't use the self-report instruments, such as FFQ or dietary intake recovery biomarkers for validation. In addition, these data reflected the only food purchased in the cafeteria, not necessarily real-consumed food. If workers purchased food elsewhere, meal purchase data may report considerably less food than they purchased. The second limitation is that the data were limited to dietary elements, such as salt, energy, carbohydrate, protein, and fat, so we are unaware of the type of food these individuals consume, the timing of meal, or the order of eating at each meal. One type of fatty acid present in fish or specific types of food were reported to have positive effects on disease prevention. The third, preparation of meals in the cafeteria has been entrusted to the subcontractor, and the nutritional values of the meal are evaluated by registered dietitians belonging to the cafeteria management company. So we have

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no way to confirm the nutritional values provided for each food, this may influence validity of the nutritional values we used for the analysis and that was considered as one of the limitations. Nevertheless, unbiased data for a particular period were available in meal purchase data. Finally, the purpose was to study obesity and metabolic traits, but those are surrogate endpoints. We were more interested in determining whether we can stratify the risk of obesity to the development of more serious illness in the future. Aging society, changes in the prevalence of obese population, and dietary habits among Japanese might increase the chance of developing such diseases. Long term observation of this cohort will help us to understand and identify the population, that is at a high risk of developing cardiovascular diseases.

In conclusion, we demonstrated that Japanese male workers consuming high energy diets and harboring high-risk SNP alleles of *GHRL* rs696217 or *BRAP* rs3782886 were obese and that rs3782886 was also associated with metabolic traits such as HDL and FBS. Further studies are necessary to determine whether the minor allele of rs3782886 is associated with cardiovascular diseases. Prospective observations or lifestyle interventions for these high risk populations are also necessary to confirm such association.

## Author's contribution

TI, MA, MN, YY, and SM designed the study, MI, TN, HY, HT, and HO acquired the data, TI, MA, MN, and YK performed the statistical analyses, YY acquired the funding, TI drafted the manuscript, MA, MN, YY, HH, SK, TK, and SM provided critical revisions to the writing of the manuscript. All authors read and approved the final manuscript.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orcp.2016.05.004.

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