# Variations in the preproghrelin gene correlate with higher body mass index, fat mass, and body dissatisfaction in young Japanese women[1–3]

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**ABSTRACT**

**Background:** Ghrelin is an endogenous peptide that stimulates growth hormone secretion, enhances appetite, and increases body weight and may play a role in eating disorders.

**Objective:** The purpose was to determine whether any preproghrelin gene variants are associated with anthropometric measures, circulating ghrelin, lipid concentrations, insulin resistance, or psychological measures relevant to eating disorders in young women.

**Design:** This cross-sectional study compared outcome measures between preproghrelin genotypes. The participants in the study included 264 Japanese women [university students with a mean (±SD) age of 20.4 ± 0.7] with no history of eating disorders. The main outcomes were responses to the Eating Disorder Inventory-2 (EDI-2), anthropometric measures, measures of depression and anxiety, and fasting blood concentrations of acylated or desacyl ghrelin, lipids, glucose, and insulin.

**Results:** Two single nucleotide polymorphisms (SNPs) whose minor allele frequencies were >0.05—the Leu72Met (408 C→A) SNP in exon 2 and the 3056 T→C SNP in intron 2—were used for association analysis. The *3056C* allele was significantly associated with a higher acylated ghrelin concentration ($P = 0.0021$), body weight ($P = 0.011$), body mass index ($P = 0.007$), fat mass ($P = 0.012$), waist circumference ($P = 0.008$), and skinfold thickness ($P = 0.011$) and a lower HDL-cholesterol concentration ($P = 0.02$). Interestingly, the *3056C* allele was related to elevated scores in the Drive for Thinness–Body Dissatisfaction (DT-BD) subscale of the EDI-2 ($P = 0.003$).

**Conclusion:** Our findings suggest that the preproghrelin gene 3056T→C SNP is associated with changes in basal ghrelin concentrations and physical and psychological variables related to eating disorders and obesity. *Am J Clin Nutr* 2007;86:25–32.

**KEY WORDS** Eating disorders, ghrelin, body mass index, body dissatisfaction, polymorphisms, obesity, HDL cholesterol, Eating Disorder Inventory-2

## INTRODUCTION

Eating disorders are characterized by severe alterations in eating behavior, body shape perception, and body weight regulation. Genetic factors play an important role in the susceptibility to eating disorders such as anorexia nervosa (AN) and bulimia nervosa (BN) (1–3). In addition, psychopathologies relevant to eating disorders, such as the drive for thinness, body dissatisfaction, and body mass index (BMI), have a considerable genetic component (4, 5). Studies of risk factors (6) found that childhood or parental obesity promotes dieting behavior, and these risk factors are more prominent among persons with BN (7). Thus, candidate genes related to appetite control, energy expenditure, and obesity have been studied in search of a predisposition to eating disorders (8).

Ghrelin is an orexigenic peptide ligand that stimulates growth hormone secretion when it binds to the growth hormone secretagogue receptor (9). Ghrelin is primarily produced by neuroendocrine cells in the stomach fundus (9–11) and induces appetite and increases food intake in rodents and humans (12, 13). Ghrelin secretion is up-regulated under conditions of negative energy balance, such as emaciation, and is down-regulated under conditions of positive energy balance, such as obesity (14, 15). The plasma ghrelin concentration rises during fasting and falls quickly after meal (16). Thus, ghrelin plays a role in the long-term as well as the short-term regulation of feeding.

Early studies on the role of ghrelin in eating disorders indicate that underweight AN patients have elevated plasma ghrelin concentrations and that the concentration returns to normal after weight gain (10, 15). A more recent investigation, however, has indicated that the fasting plasma concentration of biologically

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active ghrelin (acylated ghrelin) is not elevated, whereas a degraded form of ghrelin (desacyl ghrelin) is elevated in AN (17). It was also reported that the normal meal-induced decrease in plasma ghrelin concentrations is blunted in BN (18). Thus, impaired ghrelin regulation may play a role in the etiology and pathology of AN and BN.

The gene encoding preproghrelin, the ghrelin precursor, is located on chromosome 3at 3p26-25 and comprises 4 exons and 3 introns (19, 20). Three nonsynonymous single nucleotide polymorphisms (SNPs) in the preproghrelin gene have been reported: Arg51Gln SNP is associated with lower ghrelin and insulin-like growth factor I concentrations (21, 22). Leu72Met SNP has been linked to obesity-related phenotypes, but the findings are controversial (19, 22–25). Leu90Gln SNP also has been linked to obesity (26). Recently, we found that the 3056 T→C SNP in intron 2 and the Leu72Met SNP of the preproghrelin gene were significantly associated with purging-type BN (27).

The purpose of the present study was to determine whether any preproghrelin gene polymorphisms are associated with anthropometric, biochemical and psychological measures relevant to eating disorders or obesity in non-clinical young women who are at the ages vulnerable to eating disorders.

## SUBJECTS AND METHODS

### Subjects

Unrelated woman Japanese volunteers were recruited among university students after excluding those who reported a history of eating disorders or any other psychiatric, digestive, endocrine, or metabolic diseases. We thus obtained data from 264 nonclinical woman subjects. Their mean (±SD) age and current BMI (in kg/m$^2$) were 20.4 ± 0.7 y (range: 19–23 y) and 20.6 ± 2.3 (range: 16.2–33.1), respectively (**Table 1**).

The menstrual phase of each subject on the day of blood sampling was specified based on the date of the last menses and the subject's usual menstrual cycle. Seventy subjects were estimated to be in the follicular phase, 94 were in the luteal phase, and 39 were near ovulation. The phases of 61 subjects were not specified because of an irregularity of the cycle or incomplete information.

The ethics committees of the National Center of Neurology and Psychiatry and the Tokyo Kasei University approved the investigation. All subjects gave their written informed consent before participation in the study. Parental consent was obtained for subjects aged <20 y.

### Anthropometric measurements

BMI was calculated from measurements of height and weight. Fat mass and lean mass were determined by bioelectrical impedance analysis with a TBF-410 Body Composition Analyzer (TANITA, Tokyo, Japan) according to the manufacturer's internal algorithm. Waist and hip circumferences were measured by trained personnel using a tape measure, and the waist-to-hip ratio was then calculated. Triceps and subscapular skinfold thicknesses were measured by using a skinfold caliper, and the skinfold thicknesses were summed. Each subject was asked for her lowest and highest weights after she had reached adult height, and minimum and maximum BMI values were calculated.

**TABLE 1**
General data and characteristics of the subjects[1]

| Variable | Healthy young women ($n$ = 264) |
|---|---|
| Age (y) | 20.4 ± 0.7 (19–23) |
| Height (cm) | 159.2 ± 5.4 (147.2–176.8) |
| Weight (kg) | 52.3 ± 6.7 (39.8–82.3) |
| BMI (kg/m$^2$)[2] | 20.6 ± 2.3 (16.2–33.1) |
| Underweight ($n$ = 36) | 17.7 ± 0.6 (16.2–18.4) |
| Healthy weight ($n$ = 214) | 20.7 ± 1.5 (18.5–24.9) |
| Overweight ($n$ = 12) | 26.4 ± 1.3 (25.1–29.4) |
| Obese ($n$ = 2) | 31.7 ± 2.1 (30.2–33.1) |
| Fat mass (kg) | 13.2 ± 4.3 (6.3–39.1) |
| Lean mass (kg) | 39.1 ± 3.4 (31.8–49.0) |
| Waist circumference (cm) | 65.0 ± 5.1 (54–87) |
| Waist-to-hip ratio | 0.71 ± 0.04 (0.64–0.85) |
| Sum of skinfold thicknesses (mm) | 31.1 ± 12.1 (11–75) |
| Maximum BMI (kg/m$^2$)[3] | 21.8 ± 2.3 (17.0–33.1) |
| Minimum BMI (kg/m$^2$)[3] | 19.4 ± 1.9 (15.2–25.7) |
| Acylated ghrelin (fmol/mL) | 20.3 ± 9.8 (4.2–66.2) |
| Desacyl ghrelin (fmol/mL) | 148.5 ± 106.4 (32.1–740.0) |
| Acylated/desacyl ghrelin | 0.16 ± 0.09 (0.008–0.58) |
| Total cholesterol (mg/dL) | 181.1 ± 28.6 (115–305) |
| HDL cholesterol (mg/dL) | 70.3 ± 13.1 (31–110) |
| Triacylglycerol (mg/dL) | 62.2 ± 24.6 (26–173) |
| Free fatty acids (mEq/L) | 0.68 ± 0.31 (0.09–1.95) |
| Glucose (mg/dL) | 92.7 ± 7.1 (54–120) |
| Insulin (μIU/L) | 7.09 ± 3.43 (0.96–31.1) |
| HOMA-IR | 1.64 ± 0.84 (0.21–7.67) |

[1] All values are $\bar{x}$ ± SD; range in parentheses. HOMA-IR, homeostasis model assessment of insulin resistance.
[2] Underweight, BMI <18.5; healthy weight, 18.5 ≤ BMI < 25, overweight, 25 ≤ BMI <30; obese, BMI > 30.
[3] Minimum and maximum values were calculated on the basis of self-reported lowest and highest weights after reaching adult height.

### Blood sampling

Blood was obtained by venipuncture between 0900 and 1100 after an overnight fast of >12 h. Blood for the ghrelin assay was collected into a tube containing 500 KIU aprotinin and 1.25 mg sodium EDTA/mL whole blood, chilled immediately on ice, and centrifuged (1500 × $g$ for 15 min at 4 °C) within 30 min after collection. The plasma was collected, acidified with 1/10 volume of 1 mol HCl/L (28), and then stored at −80 °C until assayed. The blood cell component was stored separately for genetic analysis. The samples for the serum lipids assay and the plasma glucose assay were collected in separate tubes, prepared by standard methods, and stored at −80 °C.

### Fasting plasma acylated and desacyl ghrelin measurements

The fasting concentrations of the intact acylated form of ghrelin (acylated ghrelin) and of the degraded desacyl form of ghrelin (desacyl ghrelin) were measured by using 2 commercially available enzyme-linked immunosorbent assay (ELISA) kits, an Active Ghrelin ELISA Kit and a Desacyl-Ghrelin ELISA Kit, respectively, according to the manufacturer's protocol (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan) (29, 30). The minimal detection limits of acylated and desacyl ghrelin in this assay system were 2.5 and 12.5 fmol/mL, respectively. The intra- and interassay CVs were 4.3% and 3.5%, respectively, for acylated ghrelin and 3.5% and 6.7%, respectively, for desacyl ghrelin.

### Fasting blood biochemical measurements

Fasting concentrations of serum total cholesterol, HDL-cholesterol, triacylglycerol, free fatty acids, and plasma glucose and insulin were measured by using standard enzymatic methods with an automated analyzer (SRL, Ltd, Tachikawa, Tokyo, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting plasma insulin ($\mu$U/L) × fasting plasma glucose (mmol/L)/22.5.

### SNP selection

We selected 3 nonsynonymous SNPs in the coding region of the preproghrelin gene (OMIM: 605353): in exon 2, the SNPs Arg51Gln (346 G→A) (Celera database ID = hCV25607739) and Leu72Met (408 C→A) (NCBI dbSNP database ID = rs696217); in exon 3, the SNP Leu90Gln (3412 T→A) (rs4684677). We also selected 3 SNPs in the noncoding region of the gene that are reported to be polymorphic in the Japanese population according to the JSNP database (31) or the international HapMap project (32): 3056 T→C (rs2075356) and 3083 A→G (rs35682) in intron 2 and 3615 A→C (rs35683) in intron3 (Internet: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi? db = SNP).

### Genotyping

Genomic DNA was extracted from peripheral blood by using a standard procedure. The genotypings of the Arg51Gln, Leu72Met, 3056 T→C, 3083 A→G, Leu90Gln, and 3615 A→C polymorphisms were performed by using TaqMan SNP Genotyping Assays with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) as described by the manufacturer.

### Interviews and questionnaires

A clinical history was taken from each participant by a physician with expertise in eating disorders. Participants who had a history of eating disorders or other psychiatric, digestive, endocrine, or metabolic diseases were eliminated from the study. Each participant was administered a questionnaire on the course of body weight change as well as the Japanese version of the Eating Disorder Inventory-2 (EDI-2) (33), the Beck Depression Inventory-2 (BDI-2) (34), and the State-Trait Anxiety Inventory (STAI) (35). Depression and anxiety are symptoms often accompanied with eating disorders (6, 7).

EDI-2 is a self-rating scale that assesses the multidimensional symptomatology commonly associated with AN and BN (33). A Japanese version of this instrument, which has been translated and validated (M Shoji, personal communication, 2006), consists of 6 factors resulting in a corresponding 6 subscales named Bulimia, Drive for Thinness–Body Dissatisfaction (DT-BD), Interoceptive Awareness, Impulse Regulation, Ineffectiveness, and Interpersonal Difficulty. In the original English version of EDI-2, drive for thinness and body dissatisfaction are separate subscales. All the factors indicated satisfactory internal-consistency reliability, with Cronbach's $\alpha$ coefficients between 0.70 and 0.89. The EDI-2 measurement uses a 6-point Likert scale (5 = always, 0 = never).

### Statistical analysis

The chi-square test was used to determine whether the observed genotype frequencies deviated from Hardy-Weinberg equilibrium. Linkage disequilibrium (LD) among the different markers was analyzed with Haploview software (36). For the cross-sectional analysis, all anthropometric variables were compared by one-factor analysis of variance (ANOVA), and all biochemical and psychological variables were compared after adjustment for BMI by analysis of covariance. Values for acylated ghrelin, desacyl ghrelin, the ratio of acylated ghrelin to desacyl ghrelin, insulin, and HOMA-IR were analyzed after logarithmic transformation because of their skewed distribution. A Fisher's exact test was used to compare frequencies of underweight, normal weight, overweight, and obesity between groups. Correlations between variables were assessed by using Spearman's correlation coefficients. A $P$ value $<0.05$ in the 2-tailed test was considered significant. The statistical software was the SAS System for WINDOWS (version 8; SAS Institute Japan, Tokyo, Japan).

## RESULTS

### General measurements and correlation among variables

The demographic, anthropometric, and metabolic variables are shown in Table 1. There was a good correlation between acylated and desacyl ghrelin concentrations ($r = 0.35$, $P < 0.0001$). Correlations of acylated ghrelin, desacyl ghrelin, and the ratio of acylated to desacyl ghrelin with anthropometric and blood biochemical measures are shown in **Table 2**. Plasma acylated ghrelin concentrations correlated negatively and modestly with BMI, waist circumference, and fat mass, but not with lean mass, waist-to-hip ratio, or skinfold thickness. The acylated ghrelin concentration also correlated negatively with insulin and HOMA-IR. The desacyl ghrelin concentration showed negative and weak correlations with BMI, fat mass, waist circumference, waist-to-hip ratio, and sum of skinfold thicknesses. Desacyl ghrelin correlated positively and weakly with serum free fatty acid and negatively with insulin and HOMA-IR. The ratio of acylated to desacyl ghrelin showed a weak positive correlation with skinfold thickness and a negative correlation with free fatty acids.

Of the psychometric measures, the Bulimia subscale scores of the EDI-2 showed positive correlations with BMI, fat mass, lean mass, skinfold thickness, waist circumference, and waist-to-hip ratio and a weak negative correlation with acylated ghrelin concentration (**Table 3**). The DT-BD scores correlated positively with BMI, fat mass, lean mass, waist circumference, waist-to-hip ratio, and skinfold thickness and negatively with HDL-cholesterol concentrations (Table 3). DT-BD did not correlate with the plasma acylated ghrelin concentration. The other EDI-2 subscale scores—BDI-2 and STAI scores—did not correlate with any anthropometric or blood measures (data not shown).

### Estimation of genotype and allele frequencies and LD among SNPs in the sample set

Four SNPs (Arg51Gln, 3083 A→G; Leu90Gln, and 3615 A→C) that had a low (<0.05) minor allele frequency in our samples were excluded from further association analysis to avoid a genotype that occurs at a very low frequency (data not shown). MAFs for the remaining 2 SNPs, Leu72Met and 3056 T→C were 0.168 and 0.267, respectively. The 2 SNPs were in strong LD (pairwise D' = 0.827, $r^2 = 0.368$). Genotype frequencies [n (%)] of the Leu72Met SNPs were as follows: *Met/Met*, 10 (3.8); *Leu/Met*, 69 (26.1); and *Leu/Leu*, 185 (70.1). The frequencies of

**TABLE 2**
Spearman's correlation coefficients for the relation of acylated ghrelin, desacyl ghrelin, and the ratio of acylated to desacyl ghrelin with anthropometric and blood measures[1]

| | Acylated ghrelin | | Desacyl ghrelin | | Acylated/desacyl ghrelin | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| BMI | −0.21 | 0.0009 | −0.17 | 0.007 | −0.03 | 0.61 |
| Fat mass | −0.22 | 0.0005 | −0.16 | 0.008 | −0.04 | 0.54 |
| Lean mass | −0.04 | 0.57 | −0.02 | 0.77 | −0.005 | 0.94 |
| Waist circumference | −0.20 | 0.001 | −0.18 | 0.004 | −0.005 | 0.93 |
| Waist-to-hip ratio | −0.11 | 0.07 | −0.16 | 0.010 | 0.04 | 0.54 |
| Sum of skinfold thicknesses | −0.06 | 0.34 | −0.20 | 0.001 | 0.14 | 0.027 |
| Total cholesterol | −0.003 | 0.96 | 0.07 | 0.24 | −0.12 | 0.056 |
| HDL cholesterol | 0.04 | 0.54 | 0.07 | 0.28 | −0.03 | 0.61 |
| Triacylglycerol | −0.07 | 0.25 | 0.03 | 0.64 | −0.09 | 0.13 |
| Free fatty acids | −0.10 | 0.10 | 0.14 | 0.030 | −0.22 | 0.0003 |
| Glucose | −0.04 | 0.48 | −0.09 | 0.17 | −0.01 | 0.87 |
| Insulin | −0.16 | 0.008 | −0.17 | 0.007 | 0.02 | 0.80 |
| HOMA-IR | −0.17 | 0.006 | −0.17 | 0.008 | 0.003 | 0.96 |

[1] HOMA-IR, homeostasis model assessment of insulin resistance. *P* values ≤ 0.05 indicate significance.

the 3056 T→C genotypes were as follows: *C/C*, 23 (8.7); *T/C*, 95 (36.0); and *T/T*, 146 (55.3). The genotype distributions of both the Leu72Met and 3056 T→C SNPs followed Hardy-Weinberg equilibrium ($P > 0.05$, chi-square test).

### Comparison of anthropometric measures between preproghrelin genotypes

Comparisons of anthropometric data among the preproghrelin genotypes are shown in **Table 4**. The Leu72Met SNP was not related to any differences in the anthropometric values. In contrast, the subjects with the *3056C* allele (*C3056C* and *T3056C* genotypes) had a significantly higher mean current body weight (one-factor ANOVA), BMI, fat mass, waist circumference, sum of skinfold thicknesses, and self-reported past minimum and maximum BMIs than did the *T3056T* genotype. There was a significant difference in the frequencies of subgroups based on the BMI between 3056 T→C genotypes (Fisher's exact test). There were more overweight or obese and less underweight subjects in the *3056C* allele group than in the *T3056T* genotype.

### Comparison of fasting concentrations of acylated and desacyl ghrelins, lipids, and glucose and insulin resistance between preproghrelin genotypes

Plasma concentrations of acylated ghrelin, desacyl ghrelin, and acylated-desacyl ratios; serum lipids, glucose, and insulin, and the HOMA-IR are shown in **Table 5**. Subjects with the *3056C* allele had significantly higher acylated ghrelin concentrations ($P = 0.0021$, $P = 0.020$, and $P = 0.019$ after adjustment for BMI, insulin and, HOMA-IR, respectively; ANCOVA) and lower HDL-cholesterol concentrations than did those with the *T3056T* genotype. The *72Met* carriers (*Met72Met* and *Leu72Met* genotypes) also had higher acylated ghrelin concentrations than

**TABLE 3**
Spearman's correlation coefficients for the relation of bulimia with the drive for thinness–body dissatisfaction scores on the Eating Disorder Inventory-2 (EDI-2) with anthropometric and blood measures[1]

| | Bulimia | | Drive for thinness–body dissatisfaction | |
|---|---|---|---|---|
| | *r* | *P* | *r* | *P* |
| BMI | 0.38 | < 0.0001 | 0.51 | < 0.0001 |
| Fat mass | 0.39 | < 0.0001 | 0.48 | < 0.0001 |
| Lean mass | 0.25 | < 0.0001 | 0.20 | 0.002 |
| Waist circumference | 0.39 | < 0.0001 | 0.38 | < 0.0001 |
| Waist-to-hip ratio | 0.25 | < 0.0001 | 0.16 | 0.014 |
| Sum of skinfold thicknesses | 0.32 | < 0.0001 | 0.32 | < 0.0001 |
| Acylated ghrelin | −0.14 | 0.032 | −0.10 | 0.14 |
| Desacyl ghrelin | −0.04 | 0.50 | −0.05 | 0.47 |
| Acylated/desacyl ghrelin | −0.08 | 0.23 | −0.04 | 0.52 |
| Total cholesterol | 0.06 | 0.38 | 0.05 | 0.47 |
| HDL cholesterol | −0.11 | 0.09 | −0.17 | 0.009 |
| Triacylglycerol | 0.06 | 0.38 | 0.06 | 0.33 |
| Free fatty acids | −0.12 | 0.071 | −0.01 | 0.86 |
| Glucose | −0.10 | 0.10 | −0.07 | 0.28 |
| Insulin | 0.08 | 0.23 | 0.13 | 0.04 |
| HOMA-IR | 0.07 | 0.27 | 0.14 | 0.03 |

[1] HOMA-IR, homeostasis model assessment of insulin resistance. *P* values ≤ 0.05 indicate significance.

**TABLE 4**
Comparisons of age and anthropometric measures between the genotypes of the preproghrelin single nucleotide polymorphisms

| | Leu72Met (408 C→A) | | | 3056 T→C | | |
|---|---|---|---|---|---|---|
| Variable | *AA + CA* ($n$ = 79) | *CC* ($n$ = 185) | $P^1$ | *CC + TC* ($n$ = 118) | *TT* ($n$ = 146) | $P^1$ |
| Age (y) | 20.5 ± 0.7[2] | 20.3 ± 0.7 | 0.24 | 20.4 ± 0.6 | 20.3 ± 0.8 | 0.44 |
| Weight (kg) | 53.5 ± 7.1 | 51.8 ± 6.5 | 0.056 | 53.4 ± 6.8[3] | 51.3 ± 6.4[3] | 0.011[3] |
| BMI (kg/m$^2$) | 20.9 ± 2.6 | 20.5 ± 2.2 | 0.13 | 21.0 ± 2.6[3] | 20.3 ± 2.0[3] | 0.007[3] |
| Underweight [$n$ (%)] | 8 (10.1) | 28 (15.1) | 0.24[4] | 10 (8.5)[3] | 26 (17.8)[3] | 0.041[4] |
| Healthy weight [$n$ (%)] | 64 (81.0) | 150 (81.1) | | 99 (83.9)[3] | 115 (78.8)[3] | |
| Overweight [$n$ (%)] | 6 (7.6) | 6 (3.2) | | 7 (5.9)[3] | 5 (3.4)[3] | |
| Obese [$n$ (%)] | 1 (1.3) | 1 (0.5) | | 2 (1.7)[3] | 0 (0)[3] | |
| Fat mass (kg) | 13.8 ± 4.8 | 12.9 ± 4.1 | 0.12 | 13.9 ± 4.9[3] | 12.6 ± 3.8[3] | 0.012[3] |
| Lean mass (kg) | 39.7 ± 3.2 | 38.9 ± 3.4 | 0.068 | 39.5 ± 3.2 | 38.8 ± 3.5 | 0.063 |
| Waist circumference (cm) | 65.5 ± 5.1 | 64.8 ± 5.1 | 0.33 | 65.9 ± 5.3[3] | 64.2 ± 4.9[3] | 0.008[3] |
| Waist-to-hip ratio | 0.71 ± 0.03 | 0.71 ± 0.04 | 0.74 | 0.72 ± 0.04 | 0.71 ± 0.04 | 0.15 |
| Sum of skinfold thicknesses (mm) | 32.4 ± 13.2 | 30.6 ± 11.6 | 0.28 | 33.3 ± 12.5[3] | 29.4 ± 11.6[3] | 0.011[3] |
| Maximum BMI (kg/m$^2$)[5] | 22.3 ± 2.6 | 21.6 ± 2.1 | 0.051 | 22.2 ± 2.4[3] | 21.5 ± 2.2[3] | 0.016[3] |
| Minimum BMI (kg/m$^2$)[5] | 19.7 ± 1.9 | 19.3 ± 1.9 | 0.10 | 19.7 ± 1.8[3] | 19.2 ± 1.9[3] | 0.031[3] |

[1] $P$ values refer to group differences in means by one-factor ANOVA.
[2] $\bar{x}$ ± SD (all such values).
[3] $P$ values ≤ 0.05 indicate significance.
[4] $P$ value refers to group differences in frequencies by Fisher's exact test.
[5] Calculated on the basis of self-reported lowest and highest weights after adult height was reached. Underweight, BMI < 18.5; healthy weight, 18.5 ≤ BMI < 25; overweight, 25 ≤ BMI <30; obese, BMI ≥ 30.

did those with *Leu72Leu* genotype ($P$ = 0.015, $P$ = 0.047 and $P$ = 0.035 after adjustment for BMI, insulin and HOMA-IR, respectively). When subjects were subdivided by BMI, elevation of acylated ghrelin was significant in the underweight group for both the *3056C* and *72Met* variants.

There was no difference in desacyl ghrelin concentrations or acylated:desacyl ratios between the 3056T→C SNPs genotypes or the Leu72Met genotypes, whether the comparison was made in all subjects or in each subgroup divided by BMI. Also, no difference was found in fasting blood total cholesterol, triacylglycerol, free fatty acids, glucose, and insulin or HOMA-IR between the preproghrelin genotypes.

The concentrations of fasting acylated ghrelin did not differ among the subgroups on the basis of menstrual phase, and there

**TABLE 5**
Comparisons of blood biochemical measures between the genotypes of the preproghrelin single nucleotide polymorphisms[1]

| | Leu72Met (408 C→A) | | | 3056 T→C | | |
|---|---|---|---|---|---|---|
| Variable | *AA + CA* ($n$ = 79) | *CC* ($n$ = 185) | $P^2$ | *CC + TC* ($n$ = 118) | *TT* ($n$ = 146) | $P^2$ |
| Acylated ghrelin (fmol/mL)[3] | 21.9 ± 9.6[4] | 19.7 ± 9.9[4] | 0.015[4,5] | 21.8 ± 9.7[4] | 19.1 ± 9.8[4] | 0.0021[4,6] |
| BMI (kg/m$^2$)[7] | | | | | | |
| Underweight | 29.0 ± 12.0[4] | 19.2 ± 6.9[4] | 0.028[4] | 27.8 ± 11.5[4] | 19.0 ± 6.9[4] | 0.029[4] |
| Healthy weight | 21.6 ± 9.1 | 20.2 ± 10.3 | 0.17 | 21.7 ± 9.5 | 19.7 ± 10.3 | 0.069 |
| Overweight or obese | 16.2 ± 7.5 | 9.6 ± 6.3 | 0.50 | 16.8 ± 7.1 | 7.4 ± 3.7 | 0.12 |
| Desacyl ghrelin (fmol/mL)[3] | 147.3 ± 85.2 | 149.0 ± 114.5 | 0.56 | 146.5 ± 89.2 | 150.1 ± 118.9 | 0.37 |
| Acylated/desacyl ghrelin[3] | 0.172 ± 0.091 | 0.160 ± 0.084 | 0.19 | 0.171 ± 0.087 | 0.158 ± 0.085 | 0.075 |
| Total cholesterol (mg/dL) | 180.5 ± 26.2 | 181.3 ± 29.7 | 0.91 | 181.4 ± 30.3 | 180.9 ± 27.3 | 0.75 |
| HDL cholesterol (mg/dL) | 69.1 ± 11.7 | 70.8 ± 13.6 | 0.58 | 67.6 ± 12.1[4] | 72.5 ± 13.5[4] | 0.021[4] |
| Triacylglycerol (mg/dL) | 60.5 ± 22.7 | 63.0 ± 25.4 | 0.26 | 64.3 ± 26.1 | 60.6 ± 23.3 | 0.53 |
| Free fatty acids (mEq/L) | 0.71 ± 0.35 | 0.66 ± 0.29 | 0.27 | 0.70 ± 0.34 | 0.66 ± 0.28 | 0.27 |
| Glucose (mg/dL) | 92.9 ± 5.8 | 92.7 ± 7.6 | 0.92 | 92.8 ± 6.2 | 92.7 ± 7.8 | 0.88 |
| Insulin (μIU/L)[3] | 7.46 ± 3.93 | 6.93 ± 3.20 | 0.50 | 7.07 ± 3.64 | 7.10 ± 3.27 | 0.20 |
| HOMA-IR[3] | 1.72 ± 0.97 | 1.61 ± 0.79 | 0.59 | 1.63 ± 0.90 | 1.65 ± 0.81 | 0.16 |

[1] All values are $\bar{x}$ ± SD. HOMA-IR, homeostasis model assessment of insulin resistance.
[2] $P$ values refer to group differences in means after adjustment for BMI by ANCOVA.
[3] Values were analyzed after logarithmic transformation because of skewed distribution.
[4] $P$ values ≤ 0.05 indicate significance.
[5] $P$ = 0.047 and 0.035 after adjustment for insulin and HOMA-IR, respectively.
[6] $P$ = 0.020 and 0.019 after adjustment for insulin and HOMA-IR, respectively.
[7] Underweight, BMI < 18.5; healthy weight, 18.5 ≤ BMI < 25; overweight, 25 ≤ BMI <30; obese, BMI ≥ 30.

**TABLE 6**
Scores on the Eating Disorder Inventory-2 (EDI-2), Beck Depression Inventory-2 (BDI-2), and State-Trait Anxiety Inventory (STAI) by preproghrelin single nucleotide polymorphisms[1]

| | Leu72Met (408 C→A) | | | 3056 T→C | | |
|---|---|---|---|---|---|---|
| | *AA + CA* ($n = 79$) | *CC* ($n = 185$) | $P^2$ | *CC + TC* ($n = 118$) | *TT* ($n = 146$) | $P^2$ |
| EDI-2 | | | | | | |
| Bulimia | 20.1 ± 9.8 | 18.5 ± 9.4 | 0.48 | 20.1 ± 9.2 | 18.1 ± 9.8 | 0.51 |
| Drive for Thinness–Body Dissatisfaction | 26.1 ± 7.7 | 23.4 ± 8.1 | 0.066 | 26.5 ± 6.8[3] | 22.4 ± 8.6[3] | 0.0030[3] |
| Interoceptive Awareness | 8.8 ± 5.9 | 9.2 ± 5.0 | 0.65 | 8.4 ± 5.5 | 9.7 ± 5.0 | 0.070 |
| Impulse Regulation | 9.3 ± 5.1 | 8.5 ± 4.4 | 0.23 | 8.8 ± 4.6 | 8.6 ± 4.7 | 0.73 |
| Ineffectiveness | 12.4 ± 5.3 | 11.6 ± 4.9 | 0.27 | 11.8 ± 5.0 | 11.9 ± 5.1 | 0.75 |
| Interpersonal Difficulty | 9.3 ± 3.8 | 8.8 ± 4.0 | 0.32 | 8.7 ± 3.4 | 9.1 ± 4.4 | 0.37 |
| BDI-2 | 12.4 ± 8.0 | 11.5 ± 7.9 | 0.43 | 11.7 ± 7.8 | 11.9 ± 8.0 | 0.77 |
| STAI-state | 44.0 ± 12.2 | 42.7 ± 10.4 | 0.46 | 43.2 ± 10.7 | 43.0 ± 11.3 | 0.96 |
| STAI-trait | 47.9 ± 11.5 | 46.6 ± 11.0 | 0.45 | 46.4 ± 10.7 | 47.4 ± 11.4 | 0.36 |

[1] All values are $\bar{x}$ ± SD.
[2] *P* values refer to group differences in means after adjustment for BMI by ANCOVA.
[3] *P* values ≤ 0.05 indicate significance.

was no difference in frequencies of the menstrual phases between the preproghrelin genotypes (data not shown).

### Comparison of eating disorder–related psychopathologies between preproghrelin genotypes

Mean scores on the EDI-2 subscales for the Leu72Met and 3056 T→C SNPs genotypes are shown in **Table 6**. Subjects with the *72Met* allele had higher scores on the DT-BD subscale than did those with the *Leu72Leu* genotype ($P = 0.018$, ANOVA), but the association diminished after adjustment for BMI ($P = 0.066$). The *3056C* allele carriers of the 3056 T→C SNP also had significantly higher DT-BD scores, even after adjustment for BMI. The remaining EDI-2 subscale, depression scale (BDI-2), state anxiety scale (STAI-state), and trait anxiety scale (STAI-trait) did not differ significantly between the genotypes.

## DISCUSSION

In this study, we provided evidence that the minor *3056C* allele of the 3056 T→C SNP in intron 2 of the preproghrelin gene is associated with higher obesity-related anthropometric measures, elevated fasting acylated ghrelin concentrations, and lower serum HDL-cholesterol concentrations. Moreover, the subjects with the *3056C* allele had higher scores on the DT-BD subscale of the EDI-2, which is one of the psychopathologies characteristic of eating disorders. The Leu72Met SNP in exon 2, which is in strong LD with the 3056 T→C SNP, showed an association with acylated ghrelin concentration, but to a lesser extent.

We previously found that the 3056 T→C and the Leu72Met SNPs, and the haplotype formed by the 2 SNPs, are associated with the susceptibility to purging-type BN in Japanese (27). Our current findings are generally consistent with these findings in BN patients, because premorbid obesity and body dissatisfaction are widely recognized as important risk factors for BN (37).

The role of the common Leu72Met SNP has been studied intensively with regard to obesity-related phenotypes, but the findings have been controversial. The *72Met* allele has been associated with an earlier onset of obesity (19, 25) and with a positive family history for obesity (24). Obese children carrying the *72Met* allele have higher BMIs than do those carrying only the *72Leu* allele (23). Studies with normal-weight, healthy individuals, however, have yielded opposite results. The *Met72Met* genotype was associated with lower BMI, fat mass, and abdominal visceral fat in white individuals, and the *72Met* allele was associated with lower fat mass and higher insulin-like growth factor 1 concentrations in individuals of African descent (22).

In the current study, the *3056C* allele was associated consistently with most of the anthropometric variables related to obesity. Thus, the *3056C* allele is more likely to be the actual risk-conferring allele than is the *72Met* allele, and this might be the reason for the controversial findings concerning the Leu72Met SNP and obesity-related phenotypes.

We showed for the first time that the 3056 T>C SNP is related to fasting acylated ghrelin concentration. When the subjects were subdivided by BMI, the difference in acylated ghrelin was evident in the underweight subjects. On the other hand, we found no difference in desacyl ghrelin or in the ratio of acylated to desacyl between the preproghrelin genotypes. Previous studies, in which only total ghrelin was measured, found no changes in basal ghrelin concentrations because of the Leu72Met SNP (22, 24). *N*-Octanoylation of the Ser-3 hydroxyl group is thought to be essential for the biological activity of ghrelin (acylated ghrelin) (38). The acylated ghrelin is quite unstable and is rapidly degraded to the *des*-octanoyl form (desacyl ghrelin) (39). Desacyl ghrelin, however, was recently reported to have some functions, such as the stimulation of food intake (40) and the inhibition of isoproterenol-induced lipolysis (41) in rodents.

In our sample, both acylated and desacyl ghrelin concentrations correlated negatively with BMI, insulin, and insulin resistance in agreement with previous studies (14, 15, 42). However, the difference in acylated ghrelin concentrations due to the *3056C* allele remained significant even after adjustment for BMI, insulin, and HOMA-IR, which suggests that the effect of the *3056C* allele was not secondary to changes in BMI or insulin resistance. HDL cholesterol correlated negatively with BMI, but not with acylated or desacyl ghrelin, and its association with the *3056C* allele was at least partially dependent on changes in body size and fat measures.

The physiologic significance for the 2.8-fmol/mL difference in plasma acylated ghrelin concentrations is unclear. It has been

indicated that the gastric vagal afferent, rather than blood circulation, is the major pathway conveying ghrelin's signals for starvation and GH secretion to the brain (43). If so, the plasma concentration is not a direct index of actual ghrelin action but rather an indicator of the status of ghrelin secretion and metabolism.

The Leu72Met SNP of the preproghrelin gene is outside the region encoding the mature ghrelin product, and its functional significance remains unclear (19). The molecular mechanisms of the effect of the 3056 T→C SNP has not yet been studied; however, an intronic SNP possibly affects gene expression and mRNA stability (44).

The EDI-2 DT-BD scores were elevated in subjects with the *3056C* allele. The DT-BD factor consists of items such as fear of fatness, desire for thinness, and dissatisfaction with one's own body size and shape. Considerable heritability for the BD (52∼59%) and *DT* (44∼51%) scores and also relatively high heritability for perfectionism (43%) and present BMI (64%) have been reported in twin studies (4, 5). Our current findings suggest that the preproghrelin gene may contribute to the heritability of the BD and DT scores and BMI.

The nature of the direction of causality among the ghrelin concentrations, anthropometric measures, and psychological variables is unclear. We speculate that a slight, but long-lasting, increment in ghrelin action due to the *3056C* allele in the preproghrelin gene results in a larger BMI and higher body fat by enhancing appetite, growth hormone release, and fat deposition (9, 12, 13). Our current data and previous studies showed positive correlations among DT and BD scores and body size measures (5). In addition to the elevated body size and adiposity, physical factors such as weight fluctuation and poor physical form and psychosocial factors such as severe life stress, negative affection, and perceived peer pressure to be thin are risk factors for body dissatisfaction (45, 46). Although the ghrelin/growth hormone secretagogue receptor system is involved in the modulation of the hypothalamic-pituitary-adrenal axis response to stress in humans (47), the relation between ghrelin and these psychosocial risk factors is not known.

We recruited the women for the current study from healthy university students in the Tokyo metropolitan area who were of similar age (≈20 y) and who presumably shared similar social values and lifestyles. The subjects were relatively free of long-term effects of lifestyle habits and secondary changes due to diseases. These characteristics of the sample subjects, in theory, allow for better detection of small genetic effects on anthropometric and blood-composition variables. Although the expression of ghrelin may be affected by estrogen or menstrual phase (48), we found no differences in ghrelin concentrations between the menstrual phases nor in the frequencies of the menstrual phases between the preproghrelin genotypes. Because the estrogen concentration fluctuates within each menstrual phase, adjustment for blood estrogen concentrations will be needed to exclude the possible confounding effect of estrogen on basal ghrelin concentrations. Studies in a male population will be necessary to validate the current findings and to test possible sex differences in the effect of preproghrelin variants.

The mean BMI of Japanese women aged 20–24 was 20.39 ± 2.70 in 1996–2000 (49). Therefore, the constitution of our subjects (BMI: 20.4 ± 0.7) was average for the Japanese. Nevertheless, the desire of young women for thinness is greater than other age groups (50). The *3056C* allele carriers were relatively larger and more dissatisfied with their body than were noncarriers. Further studies are needed to determine whether *3056C* allele carriers are more inclined to develop eating disorders or obesity in the future than are noncarriers.

In conclusion, the *3056C* allele was associated with higher body size and fat measures, basal ghrelin concentrations, and drive for thinness and body dissatisfaction in young women. Our current findings should be confirmed by further studies and in other populations. The molecular mechanisms of the effect of the *3056C* allele on these variables, which are related to eating disorders and obesity, remain to be elucidated.

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The authors' responsibilities were as follows—TA: conceived of and designed the study, directed the laboratory analyses, performed the statistical analysis, and wrote the manuscript; YI and FK: participated in designing the study and helped recruit the participants, conduct the anthropometric measurements, collect the blood samples; MS: performed the psychological assessments; GK: supervised the study and helped design the study and write the manuscript. None of the authors had any financial or personal conflicts of interest.

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