# Interactions of single nucleotide polymorphisms with dietary calcium intake on the risk of metabolic syndrome[1–3]

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**ABSTRACT**

**Background:** Gene-nutrient interactions may be important in modulating susceptibility to metabolic disorders.

**Objectives:** The objectives of this study were to assess the association of dietary calcium intake with the risk of metabolic syndrome and to investigate the interaction effects between dietary calcium intake and candidate gene polymorphisms.

**Design:** Subjects were participants in the Korea Association Resource project, which was initiated in 2007 as a large-scale, genomewide association analysis. A total of 8031 subjects were included in the study. Associations were assessed by using multivariable-adjusted logistic regression analyses.

**Results:** High calcium intake appeared to be associated with a low risk of metabolic syndrome after covariates in both men (*P*-trend = 0.03) and women (*P*-trend = 0.0002) were controlled for. Among 27 single nucleotide polymorphisms (SNPs) selected as possible candidate gene polymorphisms affecting the risk of metabolic syndrome, 3 SNPs [rs6445834 in Rho guanine nucleotide exchange factor 3 (*ARHGEF3*), rs10850335 in T-box 5 (*TBX5*), rs180349 in *BUD13* homolog (*Saccaromyces cerevisiae*) (*BUD13*)] showed significant interaction effects with calcium intake tertiles or sufficiency in both men and women. Subjects with major allele homozygotes of these gene polymorphisms and high calcium intakes generally had a lower risk of metabolic syndrome than did those with minor allele homozygotes and low calcium intakes.

**Conclusion:** Dietary calcium intake appears to be inversely associated with the risk of metabolic syndrome and may modulate susceptibility to the syndrome in subjects who are minor allele carriers of rs6445834 in *ARHGEF3*, rs10850335 in *TBX5*, or rs180349 in *BUD13*. *Am J Clin Nutr* 2012;95:231–40.

## INTRODUCTION

Metabolic syndrome, a clustering phenomenon of metabolic phenotypes such as abdominal obesity, dyslipidemia, hypertension, and insulin resistance, is an important precursor of cardiovascular disease and type 2 diabetes (1). Metabolic syndrome is a major public health challenge in both developed and developing countries, and the prevalence of diseases related to metabolic syndrome shows an increasing trend (2, 3). The prevalence of metabolic syndrome in Korea has also been steadily increasing over recent years, from 24.9% in 1998 to 31.3% in 2007. This prevalence is relatively high compared with those of other Asian countries (4, 5).

The progression of metabolic syndrome is influenced by genetic susceptibility and environmental factors, including diet, and the interactions between them (6–9). Recently, published genomewide association studies have described candidate genes that contribute to metabolic syndrome and its phenotypes around the world (10–19). Some of these studies have also tried to investigate the interaction effects between the identified gene polymorphisms and dietary intake. Among dietary factors, interactions between fat intake and genes on the risk of metabolic syndrome have been studied and been found to be potential modulators of genetic susceptibility to metabolic syndrome (7, 20–22).

A growing body of research shows an inverse association between dairy consumption and the prevalence of metabolic syndrome (23). The effects of dairy consumption on the risk of metabolic syndrome can be partially explained by calcium intake in dairy foods (24), and several studies have found that people with higher calcium intakes have a lower prevalence of metabolic syndrome and its components (25–27). Dietary calcium is known to play a key role in blood pressure control and adipocyte metabolism, and thus its effects on metabolic syndrome could be partially mediated by its effects on body fat, blood pressure, and insulin sensitivity (28).

Given the substantial influence of gene-diet interactions on metabolic disorders, calcium intake could modify genetic susceptibility to metabolic syndrome similar to the way in which fat intake has an effect on the risk of metabolic syndrome conferred by gene polymorphisms (11–14). To our knowledge, there is no study investigating an interaction effect between calcium intake and gene polymorphisms on the risk of metabolic syndrome. A finding of gene-calcium interactions could improve our understanding of the mechanisms of metabolic alterations and could eventually lead to effective, personalized prevention of metabolic syndrome. Thus, the objectives of this study were to investigate the association of dietary calcium intake with the risk of metabolic syndrome and its phenotypes and to study the interaction

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effects between dietary calcium intake and candidate gene polymorphisms.

## SUBJECTS AND METHODS

### Subjects

Our study subjects were participants in the KARE[4] project, which was initiated in 2007 as a large-scale, genomewide association analysis. The KARE population was recruited from 2 community-based cohorts that had been established as part of the KoGES in 2001. The cohorts were taken from the Ansung and Ansan areas in the Gyeonggi Province of South Korea and were a source of extensive phenotypic data for >260 traits. Data were collected through epidemiologic surveys, physical examinations and laboratory tests. The initial cohort included 10,038 participants (5018 for Ansung and 5020 for Ansan) between 39 and 70 y of age. After performing genotype calling and quality-control processes, a total of 8842 individuals were included in the KARE study. Further description of the study population may be found in a previous publication (10). Among those 8842 subjects, we excluded subjects who did not complete the food-frequency questionnaire ($n$ = 674) or who reported implausibly low or high energy intakes (<500 kcal/d or >4000 kcal/d) ($n$ = 137). Thus, the total number of study subjects used in our final analyses was 8031 (3846 men and 4185 women). The institutional review board of Hanyang University Medical Center approved this study.

### General characteristics, anthropometric measurements, and biochemical variables

Information on demographic characteristics, education (less than high school or high school or greater), smoking status (current smoker or non–current smoker), alcohol intake (g/d), exercise (metabolic equivalent task hours/d), and family history of disease (hypertension, type 2 diabetes, or myocardial infarction) was collected by using an interview-administered questionnaire. Height was measured with a stadiometer to the nearest 0.1 cm, and weight was measured with a metric scale to the nearest 0.01 kg while the subjects were wearing light clothing with no shoes. BMI was calculated as weight (kg)/height ($m^2$). WC was measured half-way between the lowest rib margin and the iliac crest. BP was measured by using a standard protocol after each subject had been sitting for ≥5 min. Systolic BP and diastolic BP measurements were recorded at least twice at 30-s intervals, and then an average value was used. Blood samples were collected after ≥8 h of fasting, and plasma concentrations of total cholesterol, HDL cholesterol, triacylglycerol, and glucose were quantified by using biochemical assays performed by a central laboratory (Seoul Clinical Laboratories, Seoul, Republic of Korea). For subjects with triacylglycerol concentrations <400 mg/dL, LDL cholesterol was calculated as described by Friedewald et al (29).

### Metabolic syndrome

Metabolic syndrome was defined by using modified criteria proposed by the Third Report of the National Cholesterol Education Program Adult Treatment Panel. A metabolic syndrome diagnosis was made when a subject fulfilled 3 of the following 5 criteria: WC ≥90 cm in men and ≥85 cm in women (30), triacylglycerol ≥150 mg/dL or treatment of dyslipidemia, HDL cholesterol <40 mg/dL in men and <50 mg/dL in women or treatment of dyslipidemia, systolic and diastolic BP ≥130 and 85 mm Hg or antihypertensive treatment, and fasting blood glucose ≥100 mg/dL or treatment of type 2 diabetes (31).

### Dietary measurements

Well-trained interviewers collected dietary data by using a semiquantitative food-frequency questionnaire that asked each participant to provide his or her usual intake of 103 food items over a period of 12 mo. A semiquantitative food-frequency questionnaire was developed and validated by KoGES (Korean Genome Epidemiologic Information Management System) (32, 33). It consisted of 9 frequency categories ranging from "never" to ≥3 servings/d and 3 portion-size categories ["less" (0.5 serving), a standard amount (1 serving), or "more" (1.5 servings)] for each food item. All frequencies were standardized into "times per day" by using a conversion factor of 30.4 d/mo or 4.3 wk/mo. Food and nutrient intakes per day were calculated by using a weighted frequency per day, a portion size per unit, and a recipe and nutrient database that was provided by KoGES (32).

### Genotyping and imputation

Genomic DNA samples were isolated from peripheral blood drawn from the participants and were genotyped on the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix). Bayesian robust linear modeling with the Mahalanobis distance (BRLMM) Genotyping Algorithm (Affymetrix) was used for the genotype calling of 500,568 SNPs. The genotype calling and quality-control processes are described in more detail in a previous study (10). The missing genotypes were imputed by using PLINK software and the JPT/CHB reference panel in HAPMAP (http://hapmap.ncbi.nlm.nih.gov). From the sample and SNP quality controls, a total of 8842 individuals and 327,872 SNPs were ultimately included in the analyses. Note that there was no evidence for possible population stratification in these data.

### Statistical analysis

Before examining the interaction effects of calcium intake and gene polymorphism on metabolic syndrome, we conducted a genomewide association analysis of 327,872 SNPs to select candidate SNPs to be included in the interaction analysis. For each SNP, association with metabolic syndrome was tested on the basis of logistic regression. We adjusted for age, sex, recruitment area, and BMI, and an additive model was assumed for the genetic mode. First, we screened out SNPs with a weak association to metabolic syndrome ($P > 0.001$). Then, we screened out SNPs with minor allele frequency (<0.05) and SNPs that are not mapped to an exon/intron or that were within the 5-kbp upstream/0.5-kbp downstream regions of known genes. Finally, among the SNPs that passed the above screening, we selected 27 SNPs that showed strong statistical evidence from our data (ie, $P < 0.0001$) and/or have been reported to have significant

[4] Abbreviations used: BP, blood pressure; KARE, Korea Association Resource; kbp, kilobase pair; KoGES, Korean Genome Epidemiology Study; SNP, single nucleotide polymorphism; WC, waist circumference.

**TABLE 1**
Description of SNPs included in the interaction analyses[1]

| SNP | Chromosome | Position | Locus | Gene symbol | Location | Minor allele | MAF | RAF | OR | Test statistic | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| rs2013196 | 1 | 208035034 | 1q32.2b | *IRF6* | Intron | A | 0.118 | 0.118 | 1.237 | 4.168 | $3.08 \times 10^{-5}$ |
| rs3753513 | 1 | 208066413 | 1q32.2b | *C1orf107* | Upstream (5 kbp) | A | 0.117 | 0.117 | 1.236 | 4.108 | $3.99 \times 10^{-5}$ |
| rs11119354 | 1 | 208090720 | 1q32.2b | *C1orf107* | Intron | A | 0.118 | 0.118 | 1.232 | 4.064 | $4.81 \times 10^{-5}$ |
| rs6445834 | 3 | 56890759 | 3p14.3b | *ARHGEF3* | Within cytoband | C | 0.361 | 0.361 | 1.147 | 3.897 | $9.72 \times 10^{-5}$ |
| rs12650219 | 4 | 148960608 | 4q31.23b | *ARHGAP10* | Intron | G | 0.230 | 0.770 | 0.850 | −3.937 | $8.25 \times 10^{-5}$ |
| rs628857 | 5 | 177947274 | 5q35.3c | *COL23A1* | Intron | G | 0.276 | 0.724 | 0.860 | −3.935 | $8.32 \times 10^{-5}$ |
| rs6923338 | 6 | 52657281 | 6p12.1d | *LOC100129499, TMEM14A* | Upstream (5 kbp), intron | G | 0.445 | 0.555 | 0.869 | −4.104 | $4.06 \times 10^{-5}$ |
| rs9692157 | 7 | 131545695 | 7q32.3c | *PLXNA4* | Intron | C | 0.061 | 0.061 | 1.342 | 4.340 | $1.42 \times 10^{-5}$ |
| rs2880160 | 7 | 131551243 | 7q32.3c | *PLXNA4* | Intron | C | 0.072 | 0.072 | 1.286 | 3.993 | $6.52 \times 10^{-5}$ |
| rs17482753 (13) | 8 | 19876926 | 8p21.3c | *LPL* | Within cytoband | T | 0.124 | 0.876 | 0.816 | −3.896 | $9.79 \times 10^{-5}$ |
| rs10503669 (17) | 8 | 19891970 | 8p21.3c | *LPL* | Within cytoband | T | 0.121 | 0.879 | 0.817 | −3.855 | $1.16 \times 10^{-4}$ |
| rs17410962 (15) | 8 | 19892360 | 8p21.3c | *LPL* | Within cytoband | A | 0.124 | 0.876 | 0.817 | −3.890 | $1.00 \times 10^{-4}$ |
| rs11595663 (18) | 10 | 12709525 | 10p13e | *CAMK1D* | Intron | G | 0.197 | 0.197 | 1.151 | 3.320 | $9.01 \times 10^{-4}$ |
| rs11259039 | 10 | 14462815 | 10p13c | *FAM107B* | Within cytoband | A | 0.159 | 0.841 | 0.829 | −3.971 | $7.14 \times 10^{-5}$ |
| rs180349 (14) | 11 | 116117037 | 11q23.3b | *BUD13* | Within cytoband | A | 0.227 | 0.227 | 1.186 | 4.221 | $2.43 \times 10^{-5}$ |
| rs11216126 (14) | 11 | 116122450 | 11q23.3b | *BUD13* | Within cytoband | C | 0.203 | 0.797 | 0.832 | −4.288 | $1.80 \times 10^{-5}$ |
| rs6589566 (14) | 11 | 116157633 | 11q23.3b | *ZNF259* | Intron | C | 0.218 | 0.218 | 1.172 | 3.909 | $9.28 \times 10^{-5}$ |
| rs10850335 (16) | 12 | 113297491 | 12q24.21a | *TBX5* | Intron | G | 0.285 | 0.285 | 1.142 | 3.457 | $5.47 \times 10^{-4}$ |
| rs2296189 | 13 | 27791642 | 13q12.2b | *FLT1* | Nonsynonymous | C | 0.170 | 0.830 | 0.832 | −3.989 | $6.64 \times 10^{-5}$ |
| rs951304 (19) | 14 | 75775603 | 14q24.3c | *C14orf118* | Within cytoband | C | 0.236 | 0.764 | 0.872 | −3.43 | $6.03 \times 10^{-4}$ |
| rs4903393 (19) | 14 | 75803941 | 14q24.3c | *C14orf118* | Within cytoband | A | 0.315 | 0.686 | 0.880 | −3.516 | $4.39 \times 10^{-4}$ |
| rs17252387 | 15 | 66398543 | 15q23a | *ITGA11* | Intron | G | 0.074 | 0.926 | 0.758 | −4.109 | $3.97 \times 10^{-5}$ |
| rs11636199 | 15 | 73612392 | 15q24.2 | *PTPN9* | Intron | G | 0.498 | 0.502 | 0.877 | −3.904 | $9.44 \times 10^{-5}$ |
| rs4319862 | 18 | 57644314 | 18q21.33a | *RNF152* | Intron | T | 0.191 | 0.191 | 1.190 | 4.118 | $3.83 \times 10^{-5}$ |
| rs41377151 (15) | 19 | 50114786 | 19q13.32 | *APOC1* | Nearby_gene | C | 0.112 | 0.112 | 1.208 | 3.640 | $2.72 \times 10^{-4}$ |
| rs6108327 | 20 | 9605563 | 20p12.2b | *PAK7* | Intron | C | 0.376 | 0.624 | 0.870 | −3.955 | $7.67 \times 10^{-5}$ |
| rs2904324 (15) | 20 | 38137600 | 20q12b | *MAFB* | Nearby_gene | C | 0.160 | 0.840 | 0.855 | −3.317 | $9.10 \times 10^{-4}$ |

[1] MAF, minor allele frequency; RAF, Risk allele frequency; SNP, single nucleotide polymorphism.

**TABLE 2**
General subject characteristics by calcium intake[1]

| | Low | Medium | High | *P*-trend | Insufficiency[2] | Sufficiency | *P* |
|---|---|---|---|---|---|---|---|
| Men | | | | | | | |
| *n* (%) | 1282 (33.3) | 1282 (33.3) | 1282 (33.3) | | 2829 (73.6) | 1017 (26.4) | |
| Calcium intake (mg) | 282.9 (69.0–345.4)[3] | 404.0 (345.5–473.0) | 567.2 (473.1–1386.2) | | 364.1 (69.7–599.9) | 716.5 (561.4–2131.9) | |
| Age (y) | 52.6 ± 0.2[4] | 50.9 ± 0.2 | 51.0 ± 0.2 | 0.0055[5] | 51.6 ± 0.2 | 51.3 ± 0.3 | 0.2912[5] |
| High school education (%) | 53.0 | 60.1 | 63.8 | <0.0001[6] | 56.6 | 66.3 | <0.0001[6] |
| Exercise (MET-h/d) | 3.3 ± 0.03 | 3.1 ± 0.03 | 3.1 ± 0.03 | 0.0008[5] | 3.1 ± 0.02 | 3.1 ± 0.04 | 0.8511[5] |
| Family history (%) | 30.2 | 29.4 | 28.6 | 0.3864[6] | 29.0 | 30.4 | 0.4076[6] |
| Current smoker (%) | 51.7 | 47.6 | 46.7 | 0.0147[6] | 48.3 | 49.9 | 0.3541[6] |
| Alcohol intake (g/d) | 24.1 ± 1.1 | 23.8 ± 1.1 | 26.4 ± 1.1 | 0.1064 | 24.4 ± 0.7 | 25.8 ± 1.2 | 0.3481[5] |
| BMI (kg/m$^2$) | 24.2 ± 0.1 | 24.3 ± 0.1 | 24.2 ± 0.1 | 0.8543 | 24.2 ± 0.1 | 24.4 ± 0.1 | 0.1525[5] |
| Daily dietary intake | | | | | | | |
| Total energy (kcal) | 1934.5 ± 14.6 | 1975.2 ± 14.5 | 1967.6 ± 14.5 | 0.1379[5] | 1799.4 ± 8.4 | 2403.7 ± 14.0 | <0.0001[5] |
| Carbohydrate (g) | 321.0 ± 0.8 | 314.3 ± 0.8 | 307.6 ± 0.8 | <0.0001[5] | 319.2 ± 0.5 | 300.9 ± 0.9 | <0.0001[5] |
| Fat (g) | 26.7 ± 0.2 | 30.2 ± 0.2 | 32.5 ± 0.2 | <0.0001[5] | 29.5 ± 0.1 | 30.9 ± 0.2 | <0.0001[5] |
| Protein (g) | 54.9 ± 0.2 | 61.8 ± 0.2 | 67 ± 0.2 | <0.0001[5] | 59.9 ± 0.1 | 65.1 ± 0.3 | <0.0001[5] |
| Fiber (g) | 4.9 ± 0.05 | 6.2 ± 0.05 | 7.2 ± 0.05 | <0.0001[5] | 5.8 ± 0.04 | 7.0 ± 0.06 | <0.0001[5] |
| Glycemic load | 191.1 ± 0.5 | 182.3 ± 0.5 | 174 ± 0.5 | <0.0001[5] | 186.9 ± 0.3 | 170.1 ± 0.6 | <0.0001[5] |
| Cholesterol (mg) | 118.2 ± 2.3 | 156.4 ± 2.3 | 191.9 ± 2.3 | <0.0001[5] | 148.4 ± 1.6 | 175.6 ± 2.7 | <0.0001[5] |
| Carbohydrate (% of energy) | 71.8 ± 0.1 | 69 ± 0.1 | 67.3 ± 0.1 | <0.0001[5] | 70.7 ± 0.1 | 65.8 ± 0.1 | <0.0001[5] |
| Fat (% of energy) | 13.8 ± 0.1 | 15.7 ± 0.1 | 16.6 ± 0.1 | <0.0001[5] | 14.5 ± 0.1 | 18.1 ± 0.1 | <0.0001[5] |
| Protein (% of energy) | 12.3 ± 0.1 | 13.8 ± 0.1 | 14.9 ± 0.1 | <0.0001[5] | 13.1 ± 0.04 | 15.3 ± 0.1 | <0.0001[5] |
| WC (cm) | 83.6 ± 0.2 | 83.9 ± 0.2 | 83.5 ± 0.2 | 0.6011[5] | 84.1 ± 0.2 | 83.5 ± 0.1 | 0.0206[5] |
| Triacylglyceride (mg/dL) | 180.1 ± 3.4 | 178.8 ± 3.4 | 174.4 ± 3.4 | 0.2262[5] | 180.3 ± 3.8 | 176.9 ± 2.3 | 0.4499[5] |
| HDL cholesterol (mg/dL) | 43.3 ± 0.3 | 43.6 ± 0.3 | 44.0 ± 0.3 | 0.0948[5] | 43.5 ± 0.3 | 43.7 ± 0.2 | 0.5510[5] |
| Systolic BP (mm Hg) | 122.1 ± 0.5 | 121.7 ± 0.5 | 121.6 ± 0.5 | 0.4905[5] | 121.8 ± 0.5 | 121.8 ± 0.3 | 0.9625[5] |
| Diastolic BP (mm Hg) | 81.6 ± 0.3 | 81.7 ± 0.3 | 81.7 ± 0.3 | 0.8685[5] | 81.7 ± 0.3 | 81.7 ± 0.2 | 0.9199[5] |
| Fasting blood glucose (mg/dL) | 86.1 ± 0.8 | 89.6 ± 0.8 | 89.9 ± 0.8 | 0.0007[5] | 90.2 ± 0.9 | 87.9 ± 0.5 | 0.0208[5] |
| Women | | | | | | | |
| *n* (%) | 1395 (33.3) | 1395 (33.3) | 1395 (33.3) | | 2923 (69.8) | 1262 (30.2) | |
| Calcium intake (mg) | 287.6 (43.4–360.8) | 431.4 (360.9–516.1) | 628.7 (516.3–1715.4) | | 332.5 (21.7–590.0) | 685.5 (510.3–2251.9) | |
| Age (y) | 54.1 ± 0.2 | 51.6 ± 0.2 | 52.1 ± 0.2 | <0.0001[5] | 53.8 ± 0.1 | 49.6 ± 0.2 | <0.0001[5] |
| High school education (%) | 25.5 | 35.0 | 36.3 | <0.0001[6] | 29.3 | 39.1 | <0.0001[6] |
| Exercise (MET-h/d) | 3.37 ± 0.03 | 3.09 ± 0.03 | 3.05 ± 0.03 | 0.0036[5] | 3.1 ± 0.02 | 3.1 ± 0.04 | 0.4197[5] |
| Family history (%) | 30.6 | 32.3 | 32.8 | 0.2079[6] | 30.9 | 34.1 | 0.0094[6] |
| Current smoker (%) | 3.1 | 3.8 | 3.6 | 0.5160[6] | 3.3 | 4.0 | 0.2396[6] |
| Alcohol (g/d) | 1.6 ± 0.19 | 1.5 ± 0.19 | 1.8 ± 0.19 | 0.2537[5] | 1.8 ± 0.1 | 1.5 ± 0.2 | 0.3654[6] |
| BMI (kg/m$^2$) | 25 ± 0.1 | 24.9 ± 0.1 | 24.8 ± 0.1 | 0.0855[5] | 24.9 ± 0 | 24.8 ± 0 | 0.3486[5] |
| Daily dietary intake | | | | | | | |
| Total energy | 1733.1 ± 13.9 | 1820.7 ± 13.9 | 1771.2 ± 13.9 | 0.1267[5] | 1606.8 ± 8.5 | 2164.7 ± 13 | <0.0001[5] |
| Carbohydrate (g) | 328.1 ± 0.8 | 319.5 ± 0.8 | 306.3 ± 0.8 | <0.0001[5] | 321.5 ± 0.6 | 309.9 ± 0.9 | <0.0001[5] |
| Fat (g) | 22 ± 0.2 | 27.6 ± 0.2 | 32 ± 0.2 | <0.0001[5] | 26.0 ± 0.1 | 30.2 ± 0.2 | <0.0001[5] |
| Protein (g) | 52.8 ± 0.2 | 60.8 ± 0.2 | 67.6 ± 0.2 | <0.0001[5] | 58.1 ± 0.1 | 66.0 ± 0.2 | <0.0001[5] |
| Fiber (g) | 5.5 ± 0.06 | 6.8 ± 0.06 | 7.7 ± 0.06 | <0.0001[5] | 6.3 ± 0.04 | 7.5 ± 0.06 | <0.0001[5] |
| Glycemic load | 197.5 ± 0.6 | 187.3 ± 0.6 | 173 ± 0.6 | <0.0001[5] | 190.2 ± 0.4 | 176.2 ± 0.7 | <0.0001[5] |
| Cholesterol | 107.2 ± 2.5 | 158.5 ± 2.5 | 203.5 ± 2.5 | <0.0001[5] | 144.3 ± 1.8 | 184.6 ± 2.8 | <0.0001[5] |

*(Continued)*

**TABLE 2** (*Continued*)

| | Low | Medium | High | *P*-trend | Insufficiency[2] | Sufficiency | *P* |
|---|---|---|---|---|---|---|---|
| Carbohydrate (% of energy) | 75.1 ± 0.1 | 71.3 ± 0.1 | 68.5 ± 0.1 | <0.0001[5] | 73.3 ± 0.1 | 67.9 ± 0.1 | <0.0001[5] |
| Fat (% of energy) | 11.0 ± 0.1 | 13.8 ± 0.1 | 15.7 ± 0.1 | <0.0001[5] | 12.3 ± 0.1 | 16.4 ± 0.1 | <0.0001[5] |
| Protein (% of energy) | 11.9 ± 0.1 | 13.5 ± 0.1 | 15.0 ± 0.1 | <0.0001[5] | 12.8 ± 0.04 | 15.0 ± 0.1 | <0.0001[5] |
| WC (cm) | 82.5 ± 0.2 | 81.2 ± 0.2 | 80.7 ± 0.2 | <0.0001[5] | 81.3 ± 0.3 | 81.5 ± 0.2 | 0.3454[5] |
| Triacylglyceride (mg/dL) | 152.2 ± 2.3 | 149.1 ± 2.3 | 143.2 ± 2.3 | 0.0045[5] | 144.5 ± 2.4 | 149.7 ± 1.6 | 0.0720[5] |
| HDL cholesterol (mg/dL) | 45.1 ± 0.3 | 45.8 ± 0.3 | 46.0 ± 0.3 | 0.0212[5] | 46.3 ± 0.3 | 45.3 ± 0.2 | 0.0029[5] |
| Systolic BP (mm Hg) | 122.3 ± 0.5 | 120.1 ± 0.5 | 120.2 ± 0.5 | 0.0049[5] | 120.7 ± 0.5 | 121.0 ± 0.3 | 0.6382[5] |
| Diastolic BP (mm Hg) | 79.7 ± 0.3 | 78.1 ± 0.3 | 78.3 ± 0.3 | 0.0021[5] | 78.4 ± 0.3 | 78.8 ± 0.2 | 0.3437[5] |
| Fasting blood glucose (mg/dL) | 82.2 ± 0.7 | 82.5 ± 0.7 | 83.4 ± 0.7 | 0.1739[5] | 83.4 ± 0.7 | 82.4 ± 0.5 | 0.2396[5] |

[1] All values were adjusted for age. BP, blood pressure; MET-h, metabolic equivalent task hours; WC, waist circumference.
[2] Defined as calcium intake of <600 mg/d in men aged 30–49 y, <570 mg/d in men aged 50–64 y, <560 mg/d in men aged ≥65 y, <510 mg/d in women aged 30–49 y, <590 mg/d in women aged 50–64 y, and <570 mg/d in women aged ≥65 y.
[3] Median; range in parentheses (all such values).
[4] Mean ± SEM (all such values).
[5] General linear model analysis.
[6] Cochran-Mantel-Haenszel analysis.

associations with metabolic-syndrome–related traits (eg, triglyceride or HDL-/LDL-cholesterol concentrations, BP, type 2 diabetes) in previous genomewide association studies (**Table 1**).

All analyses were performed separately for male and female subjects. Nutrient intakes were adjusted for total energy intake by using the residual method. Subjects were categorized into tertiles for relative comparison of calcium intakes or into 2 groups (sufficiency/insufficiency) according to the Estimated Average Requirement criteria of the Dietary Reference Intake for assessment of absolute dietary calcium intake status. The general linear model and the Cochran-Mantel-Haenszel analysis with adjustment for age were used to determine differences in means and distribution of general characteristics and to test the linear trends according to calcium intake. Variables that showed significantly different means or distributions between calcium intake groups were considered as potential confounders and were adjusted in analyses. Multivariate logistic regression analysis was applied to obtain ORs and corresponding 95% CIs for the risk of metabolic syndrome. All statistical analyses were performed by using SAS software (version 9.2; SAS Institute Inc), and $P$ values $<0.05$ were considered significant.

## RESULTS

General subject characteristics sorted by calcium intake are shown in **Table 2**. In comparing calcium intake tertiles, we noted that men with high calcium intakes tended to be slightly younger, more educated, and less likely to smoke and exercise. Women with high calcium intakes also tended to be younger, more educated, and less likely to exercise. Men with sufficient calcium intakes were more educated, and women with sufficient calcium intakes were younger, more educated, and more likely to have a family history of diseases. Dietary factors significantly related to calcium intake included intakes of carbohydrate, fat, protein, fiber, and cholesterol, as well as glycemic load. Calcium intake was positively related to fat, protein, fiber, and cholesterol intakes and inversely related to carbohydrate intake and glycemic load in both men and women. A significant relation between calcium intake and components of metabolic syndrome could be mostly found in women.

The age-adjusted prevalence of metabolic syndrome, including each of the 5 individual components, and multivariate-adjusted ORs according to calcium intake tertile and calcium sufficiency are shown in **Table 3**. The prevalence of metabolic syndrome was not significantly different between calcium intake groups in men. For women, the prevalence of metabolic syndrome was lower in the highest calcium intake tertile than in the lowest tertile. The group with sufficient calcium intakes had a higher prevalence of metabolic syndrome than did the group with insufficient calcium intakes in women. In multivariate analyses, men in the highest calcium intake tertile had a significantly lower risk of metabolic syndrome, high WC, and hypertriglyceridemia compared with those in the lowest tertile. For women, being in the highest calcium intake tertile was significantly related to a decreased risk of metabolic syndrome, as well as to a decreased risk of 4 of the 5 components of metabolic syndrome (low HDL cholesterol was the exception). Men with sufficient calcium intakes had significantly lower risks of high WC and high BP, and women with sufficient calcium intakes had significantly lower risks of metabolic syndrome, high WC,

**TABLE 3**
Associations between calcium intake and risk of metabolic syndrome[1]

| | Low | Middle | High | *P*-trend | Insufficiency | Sufficiency | *P* |
|---|---|---|---|---|---|---|---|
| Men (*n*) | 1282 | 1282 | 1282 | | 2829 | 1017 | |
| High WC | | | | | | | |
| Prevalence (%) | 21.2 | 21.9 | 20.6 | 0.69[3] | 22.4 | 20.8 | 0.28[3] |
| OR (95% CI)[2] | 1.00 | 0.86 (0.69, 1.06) | 0.72 (0.56, 0.93) | 0.01[4] | 1.00 | 0.67 (0.53, 0.85) | |
| Hypertriglyceridemia | | | | | | | |
| Prevalence (%) | 49.7 | 49.5 | 46.3 | 0.09[3] | 50.4 | 47.8 | 0.17[3] |
| OR (95% CI) | 1.00 | 0.91 (0.76, 1.08) | 0.76 (0.62, 0.93) | 0.01[4] | 1.00 | 0.97 (0.80, 1.18) | |
| Low HDL cholesterol | | | | | | | |
| Prevalence (%) | 38.3 | 37.3 | 34.3 | 0.03[3] | 38.2 | 36.1 | 0.23[3] |
| OR (95% CI) | 1.00 | 0.93 (0.78, 1.11) | 0.84 (0.68, 1.03) | 0.10[4] | 1.00 | 1.14 (0.93, 1.39) | |
| High BP | | | | | | | |
| Prevalence (%) | 44.9 | 46.8 | 46.8 | 0.40[3] | 46.3 | 46.1 | 0.91[3] |
| OR (95% CI) | 1.00 | 0.97 (0.81, 1.16) | 0.86 (0.70, 1.06) | 0.14[4] | 1.00 | 0.81 (0.66, 0.98) | |
| High blood glucose | | | | | | | |
| Prevalence (%) | 16.4 | 17.2 | 20.3 | 0.01[3] | 18.9 | 17.6 | 0.36[3] |
| OR (95% CI) | 1.00 | 0.93 (0.73, 1.17) | 1.06 (0.81, 1.37) | 0.53[4] | 1.00 | 0.99 (0.78, 1.27) | |
| Metabolic syndrome | | | | | | | |
| Prevalence (%) | 26.8 | 27.9 | 25.9 | 0.55[3] | 28.0 | 26.5 | 0.35[3] |
| OR (95% CI) | 1.00 | 0.92 (0.76, 1.13) | 0.77 (0.61, 0.98) | 0.03[4] | 1.00 | 0.83 (0.67, 1.03) | |
| Women (*n*) | 1395 | 1395 | 1395 | | 2923 | 1262 | |
| High WC | | | | | | | |
| Prevalence (%) | 41.1 | 34.9 | 32.2 | <0.0001[3] | 33.7 | 37.1 | 0.03[3] |
| OR (95% CI) | 1.00 | 0.73 (0.61, 0.88) | 0.61 (0.49, 0.76) | <0.0001[4] | 1.00 | 0.69 (0.56, 0.84) | |
| Hypertriglyceridemia | | | | | | | |
| Prevalence (%) | 36.8 | 36.7 | 34.0 | 0.08[3] | 33.6 | 36.8 | 0.04[3] |
| OR (95% CI) | 1.00 | 0.92 (0.77, 1.10) | 0.78 (0.63, 0.96) | 0.02[4] | 1.00 | 0.77 (0.64, 0.93) | |
| Low HDL cholesterol | | | | | | | |
| Prevalence (%) | 72.4 | 69.7 | 68.5 | 0.03[3] | 66.7 | 71.7 | 0.001[3] |
| OR (95% CI) | 1.00 | 0.88 (0.73, 1.06) | 0.81 (0.65, 1.00) | 0.07[4] | 1.00 | 0.78 (0.65, 0.95) | |
| High BP | | | | | | | |
| Prevalence (%) | 44.6 | 38.8 | 38.8 | 0.0003[3] | 39.5 | 41.3 | 0.25[3] |
| OR (95% CI) | 1.00 | 0.74 (0.62, 0.9) | 0.72 (0.58, 0.90) | 0.01[4] | 1.00 | 0.86 (0.71, 1.05) | |
| High blood glucose | | | | | | | |
| Prevalence (%) | 12.1 | 9.4 | 11.2 | 0.39[3] | 10.9 | 10.9 | 0.95[3] |
| OR (95% CI) | 1.00 | 0.62 (0.47, 0.82) | 0.68 (0.50, 0.92) | 0.03[4] | 1.00 | 0.77 (0.57, 1.03) | |
| Metabolic syndrome | | | | | | | |
| Prevalence (%) | 36.3 | 33.0 | 29.8 | <0.0001[3] | 30.4 | 34.2 | 0.01[3] |
| OR (95% CI) | 1.00 | 0.81 (0.67, 0.98) | 0.65 (0.52, 0.81) | 0.0002[4] | 1.00 | 0.68 (0.55, 0.84) | |

[1] Prevalences were adjusted for age. BP, blood pressure; WC, waist circumference.
[2] ORs and 95% CIs by calcium intake tertile were adjusted for age, educational level, smoking status, exercise, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber for men and for age, educational level, exercise, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber for women. The ORs and 95% CIs for the subjects grouped by calcium intake (insufficiency/sufficiency) were adjusted for age, educational level, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber for men and for age, educational level, family history of disease, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber for women.
[3] Cochran-Mantel-Haenszel analysis.
[4] Logistic regression analysis.

hypertriglyceridemia, and low HDL cholesterol compared with the risks of those with insufficient calcium intakes.

As shown in **Tables 4** and **5**, the gene-nutrient interaction effects between calcium intake and SNPs were significantly associated with the risk of metabolic syndrome. Among 27 SNPs, 3 SNPs [Rho guanine nucleotide exchange factor 3 (*ARHGEF3*) rs6445834, T-box 5 (*TBX5*) rs10850335, *BUD13* homolog (*Saccharomyces cerevisiae*) (*BUD13*) rs180349] had a significant interaction effect with calcium intake tertile or sufficiency in both men or women. In men, *ARHGEF3* rs6445834 and *BUD13* rs180349 had significant dietary calcium intake–dependent effects on the risk of metabolic syndrome (*P*-interaction = 0.03 with tertiles and *ARHGEF3* rs6445834; 0.05 with sufficient status and *BUD13* rs180349). In women, *TBX5* rs10850335 had a significant effect (*P*-interaction = 0.04 with sufficient calcium intake status), and *ARHGEF3* rs6445834 and *BUD13* rs180349 had marginally significant effects (*P*-interaction = 0.06 with sufficient calcium intake status and *ARHGEF3* rs6445834; 0.06 with tertiles and *BUD13* rs180349). Generally, the groups with major allele homozygotes and high calcium intakes had the lowest risk of metabolic syndrome compared with groups with minor allele homozygotes and low calcium intakes.

We examined which components of metabolic syndrome appeared to be related to calcium intake (Tables 4 and 5). Among the phenotypes of metabolic syndrome, significant interaction effects between calcium intake and the 3 investigated SNPs were found with high BP in men and with high WC and high BP in women. In men, all 3 SNPs had a significant dietary calcium intake–dependent

**TABLE 4**
Interactions between calcium intake and gene polymorphism for the risk of MetS in men[1]

| | Low[2] ($n$ = 1282) | Middle[2] ($n$ = 1282) | High[2] ($n$ = 1282) | $P$-interaction[3] | Insufficiency[3] ($n$ = 2829) | Sufficiency[3] ($n$ = 1017) | $P$-interaction[3] |
|---|---|---|---|---|---|---|---|
| MetS | | | | | | | |
| rs6445834 (*ARHGEF3*) | | | | | | | |
| *CC* | 1.00 | 1.28 (0.77, 2.12) | 0.77 (0.45, 1.30) | 0.03 | 1.00 | 0.63 (0.38, 1.05) | 0.34 |
| *TC* | 1.05 (0.71, 1.57) | 0.81 (0.54, 1.22) | 0.89 (0.58, 1.35) | | 0.86 (0.66, 1.12) | 0.72 (0.51, 1.02) | |
| *TT* | 0.84 (0.56, 1.26) | 0.88 (0.58, 1.33) | 0.56 (0.36, 0.87) | | 0.70 (0.53, 0.92) | 0.61 (0.43, 0.87) | |
| rs10850335 (*TBX5*) | | | | | | | |
| *GG* | 1.00 | 0.79 (0.42, 1.50) | 0.65 (0.34, 1.24) | 0.96 | 1.00 | 0.52 (0.28, 0.96) | 0.29 |
| *AG* | 0.64 (0.40, 1.04) | 0.57 (0.35, 0.94) | 0.49 (0.29, 0.81) | | 0.61 (0.44, 0.85) | 0.53 (0.36, 0.79) | |
| *AA* | 0.58 (0.36, 0.94) | 0.54 (0.33, 0.87) | 0.46 (0.28, 0.75) | | 0.57 (0.41, 0.79) | 0.47 (0.31, 0.70) | |
| rs180349 (*BUD13*) | | | | | | | |
| *AA* | 1.00 | 1.26 (0.58, 2.73) | 1.04 (0.47, 2.32) | 0.76 | 1.00 | 0.51 (0.25, 1.03) | 0.05 |
| *AG* | 1.02 (0.55, 1.87) | 1.02 (0.55, 1.87) | 0.75 (0.40, 1.41) | | 0.80 (0.55, 1.16) | 0.59 (0.37, 0.92) | |
| *GG* | 0.86 (0.47, 1.57) | 0.76 (0.41, 1.39) | 0.68 (0.37, 1.26) | | 0.66 (0.46, 0.95) | 0.46 (0.30, 0.69) | |
| WC | | | | | | | |
| rs6445834 (*ARHGEF3*) | | | | | | | |
| *CC* | 1.00 | 1.28 (0.77, 2.12) | 0.77 (0.45, 1.30) | 0.56 | 1.00 | 0.73 (0.42, 1.27) | 0.25 |
| *TC* | 1.05 (0.71, 1.57) | 0.81 (0.54, 1.22) | 0.89 (0.58, 1.35) | | 1.09 (0.81, 1.48) | 0.67 (0.46, 0.99) | |
| *TT* | 0.84 (0.56, 1.26) | 0.88 (0.58, 1.33) | 0.56 (0.36, 0.87) | | 0.99 (0.73, 1.35) | 0.71 (0.47, 1.05) | |
| rs10850335 (*TBX5*) | | | | | | | |
| *GG* | 1.00 | 0.60 (0.30, 1.20) | 0.45 (0.22, 0.92) | 0.69 | 1.00 | 0.61 (0.32, 1.15) | 0.34 |
| *AG* | 0.55 (0.33, 0.92) | 0.55 (0.33, 0.92) | 0.37 (0.22, 0.64) | | 0.73 (0.50, 1.05) | 0.46 (0.30, 0.72) | |
| *AA* | 0.60 (0.36, 1.00) | 0.48 (0.29, 0.80) | 0.48 (0.28, 0.81) | | 0.75 (0.52, 1.08) | 0.52 (0.34, 0.81) | |
| rs180349 (*BUD13*) | | | | | | | |
| *AA* | 1.00 | 0.59 (0.25, 1.38) | 0.46 (0.18, 1.11) | 0.69 | 1.00 | 1.29 (0.60, 2.78) | 0.35 |
| *AG* | 0.71 (0.38, 1.31) | 0.62 (0.33, 1.17) | 0.53 (0.28, 1.01) | | 1.18 (0.74, 1.89) | 0.76 (0.44, 1.29) | |
| *GG* | 0.70 (0.38, 1.28) | 0.61 (0.33, 1.13) | 0.51 (0.27, 0.96) | | 1.16 (0.73, 1.83) | 0.72 (0.43, 1.19) | |
| BP | | | | | | | |
| rs6445834 (*ARHGEF3*) | | | | | | | |
| *CC* | 1.00 | 1.34 (0.83, 2.17) | 0.84 (0.52, 1.35) | 0.44 | 1.00 | 0.55 (0.34, 0.87) | 0.05 |
| *TC* | 1.1 (0.76, 1.60) | 0.95 (0.66, 1.39) | 0.92 (0.62, 1.35) | | 0.87 (0.68, 1.11) | 0.69 (0.50, 0.95) | |
| *TT* | 0.89 (0.61, 1.29) | 0.90 (0.62, 1.32) | 0.81 (0.54, 1.20) | | 0.74 (0.57, 0.95) | 0.67 (0.48, 0.94) | |
| rs10850335 (*TBX5*) | | | | | | | |
| *GG* | 1.00 | 0.92 (0.49, 1.73) | 0.57 (0.30, 1.06) | 0.25 | 1.00 | 0.49 (0.27, 0.87) | 0.01 |
| *AG* | 0.59 (0.37, 0.95) | 0.62 (0.38, 1.00) | 0.61 (0.37, 1.00) | | 0.63 (0.45, 0.86) | 0.60 (0.41, 0.88) | |
| *AA* | 0.67 (0.42, 1.07) | 0.63 (0.39, 1.01) | 0.52 (0.32, 0.84) | | 0.66 (0.48, 0.91) | 0.48 (0.33, 0.70) | |
| rs180349 (*BUD13*) | | | | | | | |
| *AA* | 1.00 | 1.81 (0.86, 3.81) | 1.14 (0.53, 2.44) | 0.26 | 1.00 | 1.54 (0.77, 3.07) | 0.01 |
| *AG* | 1.23 (0.69, 2.19) | 1.20 (0.67, 2.15) | 1.18 (0.65, 2.14) | | 1.06 (0.73, 1.54) | 1.01 (0.66, 1.56) | |
| *GG* | 1.37 (0.78, 2.41) | 1.25 (0.71, 2.21) | 1.05 (0.59, 1.88) | | 1.17 (0.81, 1.69) | 0.79 (0.52, 1.19) | |

[1] Values are ORs; 95% CIs in parentheses. All analyses were conducted by logistic regression analysis. BP, blood pressure; MetS, metabolic syndrome; WC, waist circumference.
[2] Adjusted for age, educational level, smoking status, exercise, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber.
[3] Adjusted for age, educational level, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber.

effect on the risk of high BP ($P$-interaction = 0.05 with sufficient calcium intake status in *ARHGEF3* rs6445834; 0.01 in *TBX5* rs10850335; 0.01 in *BUD13* rs180349). For women, *TBX5* rs10850335 and *BUD13* rs180349 had a significant dietary calcium intake–dependent effect on the risk of high WC ($P$-interaction = 0.009 with calcium intake tertile in *TBX5* rs10850335; 0.01 in *BUD13* rs180349). *ARHGEF3* rs6445834 had a significant dietary calcium intake–independent effect on the risk of high BP ($P$-interaction = 0.02 with calcium intake tertile).

## DISCUSSION

In this large-scale genomewide association study, we observed a significantly lower risk of metabolic syndrome and its phenotypes among subjects with higher calcium intakes. We also observed that the extent of calcium intake appears to modify the effects of gene polymorphisms rs6445834 in *ARHGEF3*, rs180349 in *BUD13*, and rs10850335 in *TBX5*, which are associated with the risk of metabolic syndrome. Among the phenotypes of metabolic syndrome, the interaction effects between calcium intake and the 3 gene polymorphisms were especially significant with high WC and high BP.

Koreans have considerably lower calcium intakes (548.1 mg/d for men and 434.8 mg/d for women, according to the 2009 Korean NHANES) than do Americans (766–896 mg/d for adults aged ≥31 y; US NHANES, 1999–2004) (34). The calcium intake in this study population was also low (473.6 mg/d for men and 459.9 mg/d for women), thus yielding a high prevalence of insufficiency in calcium intake (73.6% for men and 69.8% for women).

**TABLE 5**
Interaction between calcium intake and gene polymorphism for the risk of MetS in women[1]

| | Low[2] ($n$ = 1395) | Middle[2] ($n$ = 1395) | High[2] ($n$ = 1395) | $P$-interaction[3] | Insufficiency[3] ($n$ = 2923) | Sufficiency[3] ($n$ = 1262) | $P$-interaction[3] |
|---|---|---|---|---|---|---|---|
| MetS | | | | | | | |
| rs6445834 (*ARHGEF3*) | | | | | | | |
| *CC* | 1.00 | 1.05 (0.65, 1.68) | 1.01 (0.62, 1.62) | 0.06 | 1.00 | 0.80 (0.51, 1.24) | 0.50 |
| *TC* | 0.89 (0.62, 1.29) | 0.84 (0.57, 1.22) | 0.58 (0.39, 0.87) | | 0.76 (0.59, 0.97) | 0.57 (0.41, 0.80) | |
| *TT* | 0.96 (0.67, 1.39) | 0.61 (0.41, 0.91) | 0.53 (0.35, 0.81) | | 0.73 (0.57, 0.95) | 0.44 (0.31, 0.63) | |
| rs10850335 (*TBX5*) | | | | | | | |
| *GG* | 1.00 | 0.62 (0.33, 1.16) | 0.42 (0.21, 0.83) | 0.08 | 1.00 | 0.48 (0.25, 0.92) | 0.04 |
| *AG* | 0.6 (0.37, 0.96) | 0.51 (0.31, 0.83) | 0.52 (0.31, 0.85) | | 0.71 (0.51, 0.99) | 0.64 (0.44, 0.94) | |
| *AA* | 0.65 (0.41, 1.04) | 0.52 (0.32, 0.84) | 0.37 (0.23, 0.61) | | 0.75 (0.54, 1.03) | 0.44 (0.30, 0.65) | |
| rs180349 (*BUD13*) | | | | | | | |
| *AA* | 1.00 | 0.64 (0.31, 1.31) | 0.26 (0.12, 0.57) | 0.12 | 1.00 | 0.80 (0.55, 1.16) | 0.06 |
| *AG* | 0.58 (0.35, 0.98) | 0.55 (0.33, 0.92) | 0.44 (0.25, 0.75) | | 0.66 (0.46, 0.95) | 0.51 (0.25, 1.03) | |
| *GG* | 0.52 (0.32, 0.85) | 0.41 (0.25, 0.68) | 0.35 (0.21, 0.59) | | 0.59 (0.37, 0.92) | 0.46 (0.30, 0.69) | |
| WC | | | | | | | |
| rs6445834 (*ARHGEF3*) | | | | | | | |
| *CC* | 1.00 | 1.19 (0.66, 2.12) | 0.94 (0.52, 1.69) | 0.10 | 1.00 | 0.75 (0.49, 1.16) | 0.50 |
| *TC* | 1.28 (0.80, 2.03) | 1.03 (0.65, 1.66) | 0.97 (0.60, 1.57) | | 0.79 (0.61, 1.01) | 0.54 (0.39, 0.74) | |
| *TT* | 1.27 (0.79, 2.02) | 1.06 (0.66, 1.71) | 0.79 (0.48, 1.30) | | 0.78 (0.60, 1.00) | 0.56 (0.40, 0.78) | |
| rs10850335 (*TBX5*) | | | | | | | |
| *GG* | 1.00 | 0.56 (0.30, 1.03) | 0.39 (0.20, 0.76) | 0.009 | 1.00 | 0.64 (0.35, 1.18) | 0.06 |
| *AG* | 0.6 (0.38, 0.97) | 0.45 (0.28, 0.72) | 0.39 (0.24, 0.65) | | 0.74 (0.54, 1.02) | 0.58 (0.40, 0.85) | |
| *AA* | 0.63 (0.40, 1.00) | 0.46 (0.29, 0.74) | 0.38 (0.23, 0.62) | | 0.79 (0.57, 1.08) | 0.52 (0.35, 0.75) | |
| rs180349 (*BUD13*) | | | | | | | |
| *AA* | 1.00 | 0.52 (0.25, 1.09) | 0.63 (0.30, 1.30) | 0.01 | 1.00 | 0.90 (0.46, 1.75) | 0.06 |
| *AG* | 0.94 (0.57, 1.57) | 0.70 (0.41, 1.17) | 0.53 (0.31, 0.92) | | 1.09 (0.74, 1.59) | 0.72 (0.46, 1.13) | |
| *GG* | 0.97 (0.59, 1.59) | 0.77 (0.46, 1.26) | 0.63 (0.38, 1.05) | | 1.17 (0.80, 1.69) | 0.84 (0.56, 1.28) | |
| BP | | | | | | | |
| rs6445834 (*ARHGEF3*) | | | | | | | |
| *CC* | 1.00 | 0.80 (0.50, 1.29) | 0.86 (0.54, 1.39) | 0.02 | 1.00 | 0.70 (0.45, 1.09) | 0.22 |
| *TC* | 0.79 (0.54, 1.13) | 0.77 (0.53, 1.12) | 0.68 (0.46, 1.00) | | 0.74 (0.58, 0.96) | 0.78 (0.56, 1.07) | |
| *TT* | 1.12 (0.77, 1.61) | 0.62 (0.42, 0.91) | 0.64 (0.43, 0.96) | | 0.84 (0.65, 1.09) | 0.65 (0.47, 0.91) | |
| rs10850335 (*TBX5*) | | | | | | | |
| *GG* | 1.00 | 0.66 (0.35, 1.24) | 0.68 (0.35, 1.33) | 0.20 | 1.00 | 0.81 (0.44, 1.49) | 0.11 |
| *AG* | 0.71 (0.44, 1.16) | 0.64 (0.39, 1.04) | 0.70 (0.43, 1.16) | | 0.83 (0.59, 1.15) | 0.88 (0.60, 1.29) | |
| *AA* | 0.84 (0.53, 1.35) | 0.57 (0.35, 0.92) | 0.51 (0.31, 0.85) | | 0.84 (0.61, 1.17) | 0.62 (0.42, 0.91) | |
| rs180349 (*BUD13*) | | | | | | | |
| *AA* | 1.00 | 0.41 (0.19, 0.86) | 0.46 (0.22, 0.96) | 0.21 | 1.00 | 0.56 (0.28, 1.14) | 0.10 |
| *AG* | 0.8 (0.47, 1.34) | 0.67 (0.40, 1.14) | 0.66 (0.38, 1.14) | | 1.02 (0.70, 1.49) | 0.98 (0.63, 1.53) | |
| *GG* | 0.78 (0.47, 1.28) | 0.56 (0.34, 0.93) | 0.54 (0.32, 0.91) | | 0.91 (0.63, 1.31) | 0.81 (0.53, 1.22) | |

[1] Values are ORs; 95% CIs in parentheses. All analyses were conducted by logistic regression analysis. BP, blood pressure; MetS, metabolic syndrome; WC, waist circumference.
[2] Adjusted for age, educational level, exercise, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber.
[3] Adjusted for age, educational level, family history of disease, glycemic load, and intakes of energy, protein, fat, cholesterol, and fiber.

Our findings on the association between higher dietary calcium intake and lower risk of metabolic syndrome are in agreement with those of other studies (25–27). The potential beneficial effects of calcium intake on the risk of metabolic syndrome appear to be mediated by calcium effects on body fat, BP, and insulin sensitivity (28). A low calcium intake has been found to be related to an increase in the calcium content of tissues, such as adipocytes and vascular smooth muscle cells, which leads to a stimulation of fatty acid synthase activity, a decrease in lipolysis in adipocytes, and increases in vascular smooth muscle tone and BP (28).

The inverse association between calcium intake and metabolic syndrome, including phenotypes of metabolic syndrome, was more significant in women than it was in men. According to previous studies, this inverse association has been especially observed in postmenopausal women (25, 26). Several studies have reported that menopausal status is a major determinant of efficient calcium absorption, and that serum calcium concentrations decrease until perimenopause and then increase abruptly, reaching a new plateau (35–37). These findings may explain sex differences with respect to the effects of calcium intake on the risk of metabolic syndrome. Because menopausal status data were not available for this study, we were unable to examine any associations with menopausal status; however, because the mean age of subjects in this study corresponded to the average age of menopause in Korean women (49.0 y) (38), we infer that this may partly explain the noted sex difference.

This study identified gene polymorphisms that are correlated with metabolic syndrome and also found interaction effects with calcium intake on the risk of metabolic syndrome and its individual components. Three gene polymorphisms—*ARHGEF3* rs6445834,

*TBX5* rs10850335, and *BUD13* rs180349—had significant interaction effects with calcium intake in both men or women. Two of these genes were previously confirmed to be associated with phenotypes of metabolic syndrome: *TBX5* is associated with BP (16) and *BUD13* with triacylglyceride concentrations (14). Gene polymorphism *ARHGEF3* has been identified as being associated with bone mineral density in a previous study (39); in the present study, it was found to be associated with the risk of metabolic syndrome and was further shown to have an interaction effect with calcium intake. Bone mineral density has been found to be influenced by obesity (40, 41), hyperlipidemia (42, 43), high BP (44), and hyperglycemia (45), all of which are components of metabolic syndrome. A recent study also showed that metabolic syndrome is associated with lower bone mineral density (46). We infer that the gene polymorphism *ARHGEF3* may be related to the risk of metabolic syndrome as well as to bone mineral density. Several studies have reported that several allelic variants of a major putative gene could collectively affect the correlated phenotypes (47, 48).

We noted that the effects of calcium intake on metabolic syndrome risk conferred by gene polymorphisms were also seen in the risk of high WC and high BP (both phenotypes of metabolic syndrome) in both men and women. One possible interpretation is that high calcium intake could affect both BP and adiposity control by inhibiting lipogenesis and by stimulating lipolysis in adipocytes and decreasing BP in vascular smooth muscle cells through regulation of the 1,25-dihydroxyvitamin D response (28).

When interpreting these study results, we must consider some limitations of the study. Because the relation between calcium intake and metabolic syndrome risk was analyzed with a cross-sectional study design, it was not possible to establish a cause-effect relation. Another limitation is that we could not fully control for possible confounding factors, such as vitamin D status, because such data were not available. This is significant because vitamin D is an important factor in the calcium regulation mechanism and in metabolic diseases (25, 27, 28). Despite these limitations, this is the first study to our knowledge to show that dietary calcium intake may modulate the risk of metabolic syndrome conferred by genetic polymorphisms. Gene-diet interaction replication studies in various populations will be needed to confirm these results. Nutritional intervention studies may also provide evidence to support these findings. If substantiated, these findings could be used to deliver more precise dietary advice to those patients with genetic susceptibility and inappropriate dietary behavior. In conclusion, this study suggests that high calcium intake is associated with a decreased risk of metabolic syndrome in both men and women. It further suggests that a genetic predisposition to metabolic syndrome through the *ARHGEF3*, *TBX5*, and *BUD13* genotypes may be modified by calcium intake amount. These findings may lead to an effective approach for reducing the risk of metabolic syndrome through dietary therapy or recommendations on the basis of individual genetic profiles.

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The authors' responsibilities were as follows—KRK: designed the study, conducted statistical analyses, and wrote the manuscript; YJY: managed the dietary data set and assisted with statistical analyses; KAK: assisted in study design and conducted genotyping analyses; and MKK: conceived and designed the study and supervised all aspects of its implementation. All of the authors read and approved the final manuscript. None of the authors had a conflict of interest.

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