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High genetic risk scores of *SLIT3*, *PLEKHA5* and *PPP2R2C* variants increased insulin resistance and interacted with coffee and caffeine consumption in middle-aged adults

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Abstract *Backgrounds and Aims:* Insulin resistance is a common feature of metabolic syndrome that may be influenced by genetic risk factors. We hypothesized that genetic risk scores (GRS) of SNPs that influence insulin resistance and signaling interact with lifestyles to modulate insulin resistance in Korean adults.
Methods and results: Genome-wide association studies (GWAS) of subjects aged 40–65 years who participated in the Ansung/Ansan cohorts (8842 adults) in Korea revealed 52 genetic variants that influence insulin resistance. The best gene-gene interaction model was explored using the generalized multifactor dimensionality reduction (GMDR) method. GRS from the best model were calculated and the GRS were divided into low, medium and high groups. The best model for representing insulin resistance included *SLIT3*_rs2974430, *PLEKHA5*_rs1077044, and *PPP2R2C*_rs16838853. The odds ratios for insulin resistance were increased by 150% in the High-GRS group compared to the Low-GRS group. However, ORs for insulin secretion capacity, measured by HOMA-B, were not associated with GRS. Coffee and caffeine intake and GRS had an interaction with insulin resistance: In subjects with high coffee (≥10 cups/week) or caffeine intake (≥220 mg caffeine/day), insulin resistance was significantly elevated in the High-GRS group, but not in the Low-GRS. However, alcohol intake, smoking and physical activity did not have an interaction with GRS. Insulin secretion capacity was not significantly influenced by GRS when evaluating the adjusted odds ratios.
Conclusions: Subjects with High-GRS may be susceptible to increased insulin resistance by 50% and its risk may be exacerbated by consuming more than 10 cups coffee/week or 220 mg caffeine/day.

Introduction

Insulin resistance is a common pathway of metabolic syndrome including dyslipidemia, obesity, and hypertension [1]. Hyperinsulinemia is a major factor in the deterioration of lipid and energy metabolism when insulin resistance increases in Caucasians [1]. However, Asians do not increase serum insulin levels in response to insulin

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resistance as much as Caucasians [2] and the harmful effect of hyperinsulinemia in an insulin resistant state may be different. He et al. [3] have reported that the U-shaped BMI-mortality curve shifts 1–2 kg/m^2 to the left in Chinese compared to white people of European origin. The cutoff of overweight and obesity is 22.5 and 25.9 kg/m^2 for men and 22.8 and 26.6 kg/m^2 for women, after adjusting for fat content and fat distribution in Chinese [3]. This may be related to the lesser insulin secretion response in Asians. Thus, Asians become insulin resistant at a lower BMI compared to Caucasians and dyslipidemia and fatty liver may develop at a lower BMI in those who develop insulin resistance.

The differences between Asians and Caucasians in insulin resistance and insulin secretion are associated with both genetic and environmental factors [4]. In GWAS studies, genetic variants related to type 2 diabetes are mainly involved in insulin secretion in Asians, and some genetic variants of genes known to be involved in insulin signaling do not show a high significance in Asians ($P < 1 \times 10^{-5}$) [5]. These results indicated that a key risk factor for type 2 diabetes is insulin secretion, not insulin resistance, in Asians. However, genetic variants involved in insulin resistance also play an important role in metabolic diseases and are associated with body fat distribution [6]. The genetic variants related to insulin resistance may interact with environmental factors such as nutrients, alcohol, and coffee intake, smoking and physical activity. The genetic variants that directly influence insulin resistance need to be assessed in Asians. Since insulin resistance is involved in multiple factors, it is important to explore multi-genetic variants. The gene–gene interaction is an important component to identify related multi-genetic variants and mechanistic traits. Gene-gene interactions are identified by generalized multifactor dimensionality reduction (GMDR), which is a non-parametric data mining approach to finding the genetic variants of interest [7]. GMDR increases statistical power of gene-gene interactions with adjustment for discrete and continuous covariates in population-based studies compared to the traditional statistical methods for one genetic variant.

Insulin resistance is difficult to measure directly and the best method for measuring insulin resistance is the euglycemic hyperinsulinemic clamp study [8]. However, insulin resistance is most often estimated by calculating homeostatic model assessment for insulin resistance (HOMA-IR), which is calculated by multiplying fasting serum glucose and insulin levels. The HOMA-IR has a strong correlation ($r = 0.88$, $P < 0.0001$) with results from the euglycemic clamp [8,9]. Considering the difficulty of conducting the clamp assays, HOMA-IR is appropriate for measuring insulin resistance.

Insulin resistance is influenced by environmental and dietary factors such as the intake of fats, carbohydrates, coffee and alcohol, smoking status, and physical activity [1,10,11]. However, the effects of coffee and alcohol intakes and smoking status on insulin resistance remain controversial [12–16]. Fat intake is positively associated with metabolic syndrome and increased insulin resistance in Caucasians [17], but it has the opposite relationship in Asians [11]. Moderate alcohol consumption was shown to lower fasting serum insulin concentrations and HbA1c in a systematic review and meta-analysis, indicating improved insulin sensitivity [10], but heavy alcohol consumption exacerbates insulin resistance and causes alcoholic steatosis [16]. Coffee intake also reduces HOMA-IR, especially in people with coffee consumption-associated genetic variants in the POUNDS LOST trial [18]. However, few studies have evaluated how the interaction of genetic variants related to insulin resistance and environmental factors interact to modulate insulin resistance. We hypothesized that genetic variants that influence insulin resistance interact with environmental factors to alter insulin resistance in Korean adults. We tested the hypothesis in subjects who participated in the Korea Association Resources (KARE) project including the Ansan/Ansung cohort.

Methods

Subjects

Subjects were recruited from the population-based Ansan/Ansung prospective cohort, which was part of the Korean Genome Epidemiologic Study (KoGES) in 2001. 10,038 subjects were enrolled in the Ansan/Ansung cohort after initial check-up. This genome-wide association study (GWAS) was designed to assess gene-associated risk factors for chronic diseases, mainly metabolic syndrome and type 2 diabetes. There were 1140 subjects excluded due to a previous history of severe diseases such as cardiovascular diseases and cancers and no participation in follow-up examination. The final number of subjects was 8898 (4241 men and 4657 women) [19].

DNA and blood were collected from the 8898 subjects and the single nucleotide polymorphisms (SNPs) were measured. The final 8842 subjects who were included satisfied all genotyping quality–control processes in the Ansan/Ansung cohort in 2001 [20]. The participants aged 40–69 years old, residing in the rural community of Ansung city and the urban community of Ansan city (4183 men and 4659 women), were included in the present study. Written informed consents were received from all subjects. This study was approved by the institutional review board of the Korean National Institute of Health for the KoGES (KBP-2015-055) and Hoseo University (1041231-150811-HR-034-01).

Basic characteristics of subjects

All of the participants had resided within the survey area for at least 6 months, and they had no serious diseases. Information on age, education, income, smoking history, alcohol consumption, and physical activity was collected during a health interview, and physical characteristics such as height, weight, and body composition were measured as described in the previous studies [21,22]. Subjects were categorized according to smoking habits as current, past (<100 in past 6 months), or never-smokers (<100 cigarettes during

lifetime) [21,22]. Alcohol intake was calculated from the questionnaire about the beverage types, frequencies of drinking, and amount of drinking during the month prior to the interview. Subjects were divided into 3 groups according to grams per day of alcohol consumed, and the drinking status of the subjects were categorized into none (0–1 g), moderate (1–20 g), and heavy (>20 g) of alcohol consumed per day [21]. Coffee intake was estimated by the frequencies of drinking one serving size of coffee during the month prior to the interview. Subjects were separated into 3 groups: none (<3 cup), moderate (3–10 cups/week) and heavy (>10 cups/week).

Biochemical parameters

After at least 8 h fasting, blood samples were collected at 0, 60 and 120 min after a 75 g glucose challenge. Serum glucose and insulin concentrations were measured using an automatic analyzer (ZEUS 9.9; Takeda, Tokyo, Japan) and a gamma counter (1470 Wizard; Perkin–Elmer, San Jose, CA, USA) with a radioimmunoassay kit (DiaSorin, Stillwater, MN, USA), respectively. The area under the curve (AUC) of glucose and insulin concentrations during the oral glucose tolerance test (OGTT) was calculated using the 'trapezoidal rule'. AUC of glucose and insulin concentrations were categorized into two groups by the cut-off points of 970 (75th percentile) and 78.5 (25th percentile), respectively.

Insulin resistance was assessed using the homeostasis model assessment-IR (HOMA for insulin resistance) [fasting insulin concentrations (μIU/ml) × fasting glucose concentrations (mM)/22.5] [21]. Insulin secretion capacity was represented by HOMA-B calculated as (20 × fasting insulin concentrations)/(fasting glucose concentrations − 3.5) [21]. The subjects were categorized into two groups according to the HOMA-IR with the cut-off level of 2.0 (the 75th percentile) and the HOMA-B with the cut-off point of 120 (the 25th percentile).

After the participants rested for at least 5 min in a quiet room while seated, blood pressure was measured in the upper right arm with an appropriately-sized cuff at the heart level using a standard mercury sphygmomanometer (Baumanometer, Copiague, NY, USA). The appropriate cuff size was selected for each subject according to mid-arm circumference. Two measurements were made with at least 5 min intervals in between, and the average of the two measurements was used in the analyses. Serum concentrations of total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides (TG), aspartate aminotransaminases (AST), and alanine aminotransaminases (ALT) were measured by enzymatic methods (Advia 1650, Siemens, Tarrytown, NY, USA). High sensitivity C-reactive protein (hs-CRP) was assessed by the Denka Seiken (Tokyo, Japan) assay. The hs-CRP concentrations were validated against the Dade Behring method.

Assessment of nutrient intake

Usual food intakes were measured by the semi-quantitative food frequency questionnaire (SQFFQ) developed for Korean meals for KoGES [21,23]. The accuracy and reproducibility of the SQFFQ were confirmed by three-day food records, and the results validated that the SQFFQ is reliable for determining usual food intake [21,23]. The SQFFQ includes 103 food items reflecting Korean meals, and the consumption of each food item was calculated by multiplying food frequencies by the amount of food intake in one sitting which was scored as more than, equal to, or less than the standard portion size as previously described [21,23]. Nutrient intake was calculated from the daily food intakes collected by the SQFFQ using the Can-Pro 2.0 nutrient intake assessment software developed by the Korean Nutrition Society.

Genotyping

The genotyping and quality–control processes were described in detail previously [21]. DNA samples from the participants were isolated from their peripheral blood, and genotyping was conducted using the Affymetrix Genome-Wide Human SNP array 5.0 (Affymetrix, Santa Clara, CA). The accuracy of the genotyping was checked using the Bayesian Robust Linear Modeling with Mahalanobis Distance genotyping algorithm [21,22].

SNP imputation and quality control

Imputation of genotypes were carried out with the IMPUTE (v2.644) containing the 1000 Genomes Phase I integrated variant call set release (version 3) and using the NCBI build 37 (hg19) as a reference panel. High imputation quality was included (proper info >0.5) [24]. The exclusion criteria of imputed SNPs were as follows: a posterior probability score <0.90, low genotype information content (info <0.5), Hardy–Weinberg equilibrium (HWE; $P < 0.05$), minor allele frequency (MAF) <0.01, and SNP missing rate >0.1 was calculated for each gene variant.

Identification of the best model for gene-gene interaction by generalized multifactor dimensionality reduction (GMDR)

The study protocol as a flow chart is shown in Fig. 1. Since HOMA-IR results exhibited a non-normal distribution, HOMA-IR was made as a categorical variable for GWAS with a cutoff of 2.0. We selected genetic variants that influence insulin resistance by GWAS with the case and control groups after adjusting for age, gender, area and BMI using GPLINK program version 2.0 (http://pngu.mgh.harvard.edu/~purcell/plink). Among the identified SNPs, we selected 52 SNPs of genes related to insulin resistance ($P < 0.0001$). The GMDR method was used to analyze gene-gene interactions involved in insulin resistance, and 10 SNPs were determined to be involved in insulin resistance by the GMDR program (http://www.ssg.uab.edu/gmdr/) [25–27]. We selected the best model for gene-gene interaction based on trained balance accuracy (TRBA), test balance accuracy (TEBA), and cross-validation consistency (CVC) in the GMDR models [27]. The genetic variants included in the best model had increased insulin resistance the most with gene-gene interactions.

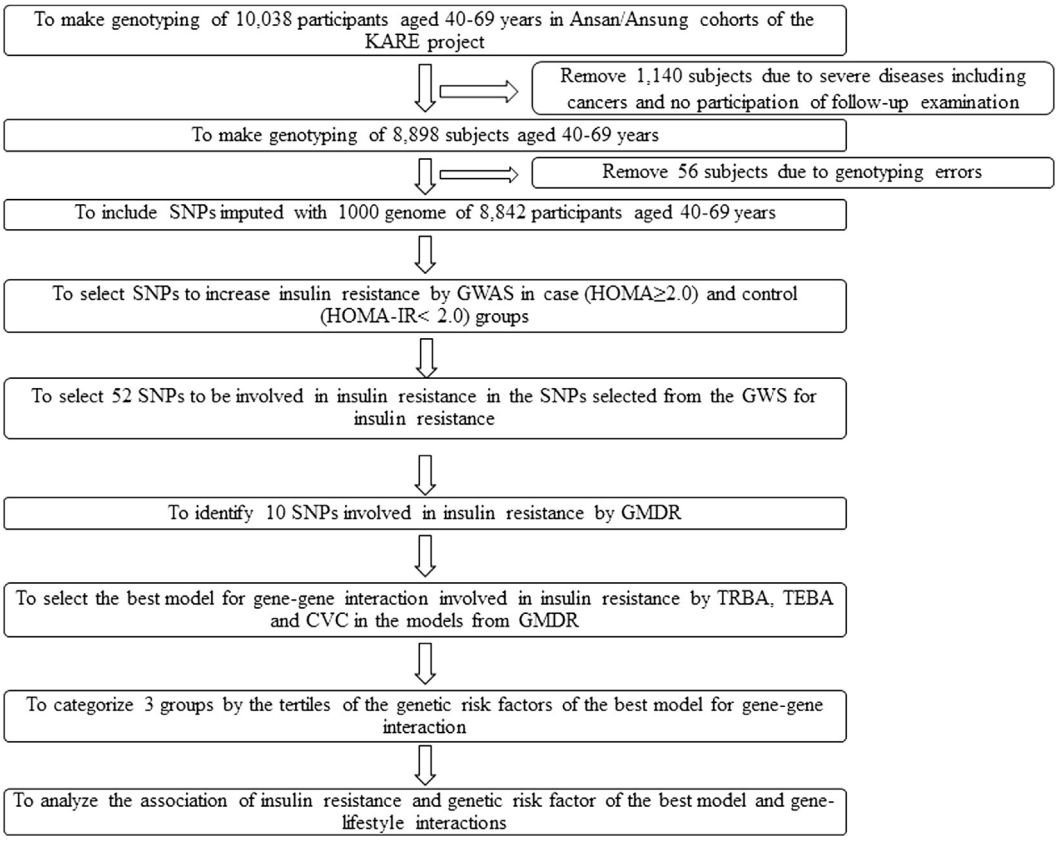

Figure 1 Flowchart to illustrate each step of the study. GWAS, genome-wide association study; GMDR, generalized multifactor dimensionality reduction; TRBA, trained balanced accuracy; TEBA, test balance accuracy; CVC, cross-validation consistency. HOMA-IR, homeostatic model assessment for insulin resistance for insulin resistance.

Statistical analysis

Statistical analyses were conducted using GPLINK version 2.0 and SAS (version 9.3; SAS Institute, Cary, NC, USA). The best model for gene-gene interaction was determined by the GMDR program with sign test of TRBA, TEBA and CVC with and without adjusting covariates of age, gender area, and BMI at $P < 0.05$. Since the number of samples was over 1000, 10-fold cross validation was used. Three SNPs were selected for the best model in the GMDR assay and the risk allele of each SNP was counted as 1 [20]. For example, GG, GT, and TT in each genotype were assigned as 0, 1, and 2, respectively if the T allele was a risk allele. The genetic risk scores (GRS) of the best model were calculated by a summation of the number of risk alleles from each of the 3 SNPs selected in the best model of GMDR. The GRS in the best model was divided into 3 categories by its tertiles of risk alleles (0−1, 2, and ≥3) and a Low-GRS score indicated fewer risk alleles of the SNPs in the best gene-gene-interaction model.

The descriptive statistics for categorical variables, such as gender and lifestyles, were calculated by frequency distributions. Frequency distributions were analyzed according to classification variables using chi-square tests. Descriptive statistics of continuous variables are provided as arithmetic and adjusted means with standard deviations (SDs) according to Low-, Medium- and High-GRS without and with adjusting for covariates. The significant differences among the GRS groups were examined using one-way ANOVA with adjusting for covariates. We performed GWAS to select genetic variants that have a high significance with HOMA-IR after adjusting for covariates such as age, gender, residence area, and BMI with and without alcohol consumption. Odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of elevated HOMA-IR and low HOMA-B associated with the GRS groups were calculated using logistic analysis. The interactions between the GRS groups and alcohol intakes were evaluated in multivariate regression models including the corresponding main effects and interaction terms with the potential confounders. Next, using the multivariate logistic regression method, ORs and CIs for the risk of HOMA-IR and HOMA-B were calculated according to low and high alcohol coffee consumptions, smoking status, and physical activity after controlling for covariates. $P \leq 0.05$ was considered statistically significant.

Results

The best model for gene-gene interactions associated with insulin resistance

GWAS was conducted with the 1000 genomes-imputed data in the KARE study of the Ansan/Ansung cohort to

select 52 genetic variants. We selected 10 genetic variants that were identified as being associated with insulin resistance (HOMA-IR) by GMDR analysis of (Table 1). The ten genetic variants selected for the GMDR are presented in Table 1. From the GMDR results, the model including 3 SNPs was selected with significant TRBA and TEBA at $P < 0.05$ and also CVC was 10/10 with and without adjustment for age, sex, residence area and BMI. Therefore, the best model included 3 SNPs: slit guidance ligand 3 (*SLIT3*)_rs2974430, pleckstrin homology domain containing A5 (*PLEKHA5*)_rs1077044, and protein phosphatase 2 regulatory subunit B-gamma (*PPP2R2C*)_rs16838853 from the GMDR (Table 1). This indicated that the three SNPs had gene-gene interactions that resulted in increased insulin resistance. The GRS was calculated by the summation of the number of the risk alleles of the 3 SNPs in the best model.

Table 2 shows the 10 SNPs that were included in GWAS and GMDR analysis. Each genetic variant met the HWE ($P > 0.05$) standard. OR of each chromosome 4 open reading frame 22 (*C4orf22*)_rs9994008 and dynein cytoplasmic 1 intermediate chain 1 (*DYNC1I1*)_rs10251970 was less than 1, indicating that the subjects with the minor allele of the genetic variant had decreased risk of insulin resistance whereas the ORs of the other genetic variants were greater than 1 ($P < 0.00001$). Minor allele frequency (MAF) of each genetic variant was between 0.079 and 0.4566 (Table 2). Most genetic variants were located in the intron region of the genes, but unc-13 homolog C (*UNC13C*)_ rs7168727 was in the area near the gene 5' region.

Characteristics of subjects based on GRS calculated from the best model of gene-gene interactions

The GRS was calculated by summation of the number of the risk alleles of 3 SNPs in the best model. The GRS of each subject was categorized into 3 groups by tertiles: The scores of the Low-, Medium- and High-GRS of 0−1, 2, ≥3, respectively. Age, gender, and anthropometric characteristics did not differ among the subject based on the GRS groups. There was a small but significant difference in the drinking profiles between the GRS groups with fewer non-drinkers and more light drinkers in the Medium-GRS group (Table 3); however, even though it is statistically significant, the differences are small and may not be meaningful. Coffee consumption, smoking habits, and dietary intakes did not differ among the GRS groups (Table 3).

Metabolic and glucose regulatory parameters according to GRS groups of the best model

Fasting insulin concentrations were significantly higher in the High-GRS group than in the low and Medium-GRS groups ($p < 0.0001$). However, there were no differences among the GRS groups in serum insulin or glucose after glucose load during OGTT. HOMA-IR was significantly higher ($p < 0.0001$) in the High-GRS groups, confirming that subjects with the high genetic risk factor scores for insulin resistance were, in fact, more insulin resistant in this population. Interestingly, HOMA-B was also significantly higher in the High-GRS groups than in the Low-GRS group ($p < 0.0416$; Table 4), indicating that subjects with High-GRS had somewhat greater insulin secretory capacity. As a result, the incidence of type 2 diabetes was not significantly different among GRS groups although it tended to be higher in the High-GRS group than in the Low-GRS group (Table 4).

Likewise, there were no differences in serum cholesterol, blood pressure, C-reactive protein, or circulating ALT and AST liver enzymes among the GRS groups (Table 4).

Adjusted ORs for blood glucose regulation and insulin resistance and secretion of GRS groups from the best model

ORs ratios for elevated insulin resistance (HOMA-IR) were significantly higher in the Medium-GRS, and higher still in

Table 1 Generalized multifactor dimensionality reduction (GMDR) results of multi-locus interaction with genes related to insulin resistance.[a]

	Model	No Adjustment				Adjusted for age, sex, area, BMI			
		TRBA	TEBA	P value	CVC	TRBA	TEBA	P value	CVC
1	*PLEKHA5*_rs1077044	0.5157	0.4992	5 (0.6230)	6/10	0.5156	0.4995	6 (0.3770)	7/10
2	SNP in model 1, *SLIT3* _rs2974430	0.5262	0.5058	1 (0.6230)	6/10	0.5264	0.4952	4 (0.8281)	4/10
3	SNPs in model 2, *PPP2R2C* _rs16838853	0.5392	0.5137	8 (0.0547)	10/10	0.5424	0.5213	9 (0.0107)	10/10
4	SNPs in model 3, *UNC13*C_rs7168727	0.5569	0.5130	9 (0.0107)	8/10	0.5601	0.5257	9 (0.0107)	9/10
5	SNPs in model 4, *NTNG1*_rs7544979	0.5839	0.5180	8 (0.0547)	9/10	0.5876	0.5229	9 (0.0107)	9/10
6	SNPs in model 5, *DYNC1I1*_rs10251970,	0.6171	0.5096	7 (0.1719)	6/10	0.6211	0.5121	7 (0.1719)	7/10
7	SNPs in model 6, *GRIK2*_rs17760780	0.6532	0.5113	6 (0.3770)	9/10	0.6569	0.5101	7 (0.1719)	10/10
8	SNPs in model 7, *C4orf22*_rs9994008	0.6873	0.4979	6 (0.3770)	4/10	0.6895	0.5048	7 (0.1719)	5/10
9	SNPs in model 8, *A2BP1*_rs6500963	0.7179	0.5043	6 (0.3770)	10/10	0.7199	0.5127	6 (0.3770)	10/10
10	SNPs in model 9, *UNC13*C_rs12592703	0.7281	0.5014	5 (0.6230)	10/10	0.7302	0.5159	7 (0.1719)	10/10

Values represented trained balanced accuracy (TRBA), test balance accuracy (TEBA), cross-validation consistency (CVC) and P value for the significance by sign test.

PLEKHA5, pleckstrin homology domain; *SLIT3*, slit guidance ligand 3 containing A5 *PPP2R2C*, protein phosphatase 2 regulatory subunit B-gamma; *UNC13*C, unc-13 homolog C; *NTNG*, netrin G; *DYNC1I1*, dynein cytoplasmic 1 intermediate chain 1; *GRIK2*, glutamate ionotropic receptor kainate type subunit 2; *C4orf22*, chromosome 4 open reading frame 22; *A2BP1*, RNA binding fox-1 homolog 1.

[a] GMDR was used to identify insulin resistance and to determine the strength of the interaction with and without adjusting covariates of age, gender area, and body mass index (BMI).

Table 2 Characteristics of genetic variants selected by the genome-wide association study and generalized multifactor dimensionality reduction analysis to be involved in insulin resistance.

Gene[a]	SNP[b]	Chr.[c]	Position[d]	Functional consequence	Minor/major allele	Minor allele frequency	HWE[e]	OR[f]	P-adjust[g]
NTNG1	rs7544979	1	107823281	intron variant	C/T	0.1778	0.971	1.206	0.0001
C4orf22	rs9994008	4	82026694	intron variant	C/T	0.08428	0.9451	0.7862	0.0004
PPP2R2C	rs16838853	4	6450950	intron variant	A/G	0.4566	0.2651	1.145	0.0004
SLIT3	rs2974430	5	168200254	intron variant	A/G	0.2678	0.4316	1.156	0.0009
GRIK2	rs17760780	6	101988727	intron variant	C/T	0.08891	0.5992	1.248	0.001
DYNC1I1	rs10251970	7	95434768	intron variant	C/T	0.2297	0.4346	0.8578	0.0008
PLEKHA5	rs10770448	12	19235485	intron variant	A/G	0.3605	0.782	1.142	0.0008
UNC13C	rs7168727	15	52091635	near-gene-5	G/T	0.2494	0.8646	1.158	0.0009
UNC13C	rs12592703	15	52099177	intron variant	C/T	0.2484	0.4765	1.157	0.0009
A2BP1	rs6500963	16	7407490	intron variant	C/T	0.07851	0.6065	1.269	0.0008

[a] Genes associated with insulin resistance were selected.
[b] Genetic variants of the gene.
[c] The number of chromosome of the gene SNP.
[d] The position of SNP in the chromosome.
[e] Hardy–Weinberg equilibrium.
[f] Odds ratio for increased insulin resistance by the minor alleles of the SNP.
[g] P value of OR for insulin resistance after adjusted for covariates of age, gender, residence area, BMI.

the High-GRS group after adjusting for age, gender, residence area and anthropometric characteristics (model 1) and after adjusting for model 1 plus dietary and lifestyle factors (model 2). There was no significant difference in the ORs for AUC of glucose during OGTT for Medium or High-GRS groups. AUC of insulin during OGTT was significantly higher for the Medium-GRS group in model 1, but the effect was small (Table 5). These results suggested that subjects with the High-GRS in the best-model of gene-gene interaction were at increased risk of insulin resistance.

Interaction of GRS and HOMA-IR or HOMA-B with lifestyles

There was a significant interaction between GRS and coffee intake and GRS and caffeine intake to increase insulin resistance (Table 6). High, but not low, coffee consumption, significantly increased the ORs for insulin resistance only in the High-GRS group (Table 6; $P < 0.001$). The interaction between coffee and HOMA-IR was shown by the significance of adjusted means of HOMA-IR: the means were significantly higher in the High-GRS only in the high coffee intake ($\geq$10 cups/week; $P = 0.0026$), not in the low coffee intake (Fig. 2A). Consistent with coffee intake, caffeine intake exhibited a significant interaction with GRS. Although there was an interaction between GRS and caffeine intake for insulin resistance, ORs for HOMA-IR increased in the High-GRS compared to the low-GRS in both low and high caffeine intake groups, but the ORs were much higher in the high caffeine intake group ($\geq$220 mg/day) than low caffeine intake (Table 6). The interaction was shown by the differences in adjusted means of HOMA-IR in the low and high caffeine intake. The means were higher in the High-GRS group than the Low-GRS group in high caffeine intake, but not low caffeine intake (Fig. 2B), indicating that GRS had an interaction with caffeine intake to affect HOMA-IR.

Other lifestyle factors including alcohol intake, smoking and physical activity did not exhibit any interaction with GRS to increase insulin resistance (Table 6). Both low and high intakes of alcohol significantly increased the ORs for insulin resistance in the High-GRS compared to the Low-GRS. ORs for insulin resistance were higher in the High-GRS than the Low-GRS in low and high physical activity groups ($>$1 h moderate activity/day; Table 6). The adjusted means of HOMA-IR were higher in the High-GRS than the Low-GRS in the low and high physical activities (Fig. 2C). These results indicated that GRS did not have an interaction with physical activity to influence HOMA-IR. Finally, ORs for HOMA-IR were higher in the High-GRS group than the Low-GRS regardless of smoking status (Table 6).

Discussion

This study identified 3 genetic variations that were associated with increased incidence of insulin resistance and established a dose-related GRS for subjects who carry the SNPs that confer risk of insulin resistance as determined by HOMA-IR. About 37% of the subjects had High-GRS, having the minor alleles of 3 SNPs, which increased their relative risk of having a higher HOMA-IR than those with the lowest GRS by almost 150% (OR = 1.47). These differences were not due to subjects in the High-GRS group having higher BMI or other lifestyle or dietary risk factors for insulin resistance. Therefore, it is reasonable to assume that the one third of individuals who had increased risk, compared to the first third on the scale of GRS, in the Ansan/Ansung cohort was well representative of the Korean population since they included people of various economic and education levels.

Insulin resistance is well known to lead to type 2 diabetes in all populations. However, insulin resistance is managed by the body by increasing insulin secretion to normalize blood glucose concentrations. In this study,

Table 3 Basal characteristics of subjects according to genetic risk score (GRS) of the best model for gene-gene interaction.[1]

	Low-GRS[2] (n = 2556)	Medium-GRS[3] (n = 3043)	High-GRS[4] (n = 3245)	P value[5]
Age (years)	52.1±8.9[6]	52.2 ± 8.9	52.3 ± 9.0	0.8073
Gender (Number, male %)	1050 (43.3)[7]	1749 (47.1)	1260 (47.9)	0.8232
BMI (kg/m^2)	24.7 ± 3.1	24.6 ± 3.1	24.6 ± 3.1	0.2775
Waist circumference (cm)	82.8 ± 9.0	82.6 ± 8.7	82.7 ± 8.8	0.7091
Body fat (%)	27.2 ± 6.9	27.0 ± 7.0	26.9 ± 7.2	0.3446
Total activity (Number, %)				0.1841
Little	1165 (47.5)	1406 (48.0)	1506 (48.3)	
Moderate	634 (25.9)	685 (23.4)	775 (24.8)	
Heavy	653 (26.6)	775 (24.8)	839 (26.9)	
Smoking				0.6763
Never-smoker	1561 (62.1)	1858 (61.8)	1982 (61.9)	
Past-smoker	407 (16.2)	460 (15.3)	487 (15.2)	
Current-smoker,	545 (21.7)	691 (23.0)	734 (22.9)	
Alcohol drinking (g/day)				
Non-drinker (<1)	1479 (59.6)	1698 (57.5)	1865 (59.4)	0.0488
Light drinker (1−15)	558 (22.5)	684 (23.2)	650 (20.7)	
Moderate drinking (15−30)	209 (8.4)	244 (8.3)	298 (9.5)	
Heavy drinker (>30)	234 (9.4)	329 (11.1)	329 (10.5)	
Coffee drinking (cups/week)				
Non-drinker (<3)	662 (25.9)	802 (26.4)	876 (27.0)	0.7027
Moderate drinker (3−9)	343 (13.4)	410 (13.5)	458 (14.1)	
Heavy drinker (≥10)	1551 (60.7)	1831 (60.2)	1911 (58.9)	
Energy intake (kcal/day)	1929 ± 777	1931 ± 697	1910 ± 689	0.4581
Carbohydrate intake (energy percent)	70.8 ± 7.1	70.7 ± 6.8	70.8 ± 6.9	0.7038
Protein (energy percent)	13.5 ± 2.4	13.6 ± 2.4	13.6 ± 2.4	0.1778
Fat intake (energy percent)	14.4 ± 5.5	14.4 ± 5.3	14.4 ± 5.3	0.9225

The values represent means ± standard deviations or number of the subjects (percentage of each group). BMI, body mass index.
[1]GRS of each subject was calculated by the summation of genetic risk alleles in the SNPs of best model (*SLIT3*_rs2974430, *PLEKHA5*_rs1077044, *PPP2R2C*_rs16838853).
[2–4]GRS was divided into 3 categories by tertiles: the Low, Medium and High GRS included the scores of 0−1, 2, ≥3, respectively.
[5]P value for one-way analysis of variance test.
[6] Means ± SD.
[7] Number (% of the number among each GRS group).

Table 4 Parameters related to glucose metabolism according to genetic risk score (GRS) of the best model for gene-gene interaction[1].

	Low-GRS[2] (n = 2556)	Medium-GRS[3] (n = 3043)	High-GRS[4] (n = 3245)	P value	
				model 1[5]	model 2[6]
Serum glucose at fasting (mg/dL)	87.2 ± 21.1	87.2 ± 19.4	89.1 ± 25.1	0.1872	0.2849
Serum glucose at 60 min (mg/dL)	152 ± 53	152 ± 51	156 ± 57	0.1015	0.1491
Serum insulin at fasting (IU/mL)	7.40 ± 4.56[b]	7.50 ± 4.18[b]	7.89 ± 5.34[a]	<0.0001	<0.0001
Serum insulin at 60 min (IU/mL)	32.0 ± 32.8	31.7 ± 31.1	32.4 ± 31.4	0.9175	0.8556
AUC of glucose (mg/dL*min)	859 ± 269	859 ± 274	871 ± 272	0.1632	0.2112
AUC of insulin (IU/mL*min)	167 ± 141	165 ± 130	169 ± 135	0.6212	0.8331
HOMA-IR	1.61 ± 1.11[b]	1.63 ± 1.02[b]	1.75 ± 1.57[a]	<0.0001	<0.0001
HOMA-B	148 ± 128[b]	153 ± 147[ab]	158 ± 162[a]	0.0416	0.0487
Type 2 diabetes (Number, %)	328 (12.8)	416 (13.7)	488 (15.0)	0.1004	
Serum total cholesterol (mg/dL)	192 ± 36.4	192 ± 36	191 ± 36	0.8113	0.8448
Serum HDL (mg/dL)	44.9 ± 10.3	44.7 ± 10.1	44.4 ± 9.9	0.0861	0.0850
Serum LDL (mg/dL)	114 ± 34	115 ± 34	114 ± 34	0.2670	0.1255
Serum TG (mg/dL)	165 ± 104	162 ± 108	162 ± 105	0.3156	0.2289
Systolic blood pressure (mmHg)	118 ± 18	117 ± 18	118 ± 18	0.4660	0.4914
Diastolic blood pressure (mmHg)	75.4 ± 11.4	74.8 ± 11.8	75.0 ± 11.5	0.2627	0.2891
C-reactive protein (mg/L)	0.22 ± 0.74	0.23 ± 0.49	0.23 ± 0.40	0.9625	0.8448
Alanine aminotransferase (U/L)	29.1 ± 37.4	27.8 ± 20.2	27.9 ± 23.4	0.2687	0.2488
Aspartate aminotransferase (U/L)	30.6 ± 24.9	29.4 ± 14.2	29.6 ± 15.6	0.1354	0.1460

The values represent means ± standard deviations.
[1] GRS of each subject was calculated by the summation of genetic risk alleles in the SNPs (*SLIT3*_rs2974430, *PLEKHA5*_rs1077044, *PPP2R2C*_rs16838853) in the best model.
[2–4]GRS was divided into 3 categories by tertiles: the Low, Medium and High GRS were divided by the scores of 0−1, 2, ≥3, respectively.
[5] One-way analysis of variance test after adjusted for age, gender, residence area, and BMI (model 1).
[6] One-way analysis of variance test after adjustment for covariates from model 1 plus smoking and drinking status, income, education, physical activity and daily energy intake (model 2).
HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, Homeostatic model assessment for insulin secretion.
[a,b] Means without a common superscript letter differ in the same row by Tukey test at $P < 0.05$.

Table 5 Adjusted odds ratios for insulin resistance and insulin secretion according to the genetic risk score (GRS) of the best model for gene-gene interaction[1] after covariate adjustments.

	Low[2] (n = 2556)	Model 1[5]		Model 2[6]	
		Medium[3] (n = 3043)	High[4] (n = 3245)	Medium[3] (n = 3043)	High[4] (n = 3245)
HOMA-IR	1[7]	1.214 (1.071−1.376)	1.468 (1.298−1.661)***	1.202 (1.056−1.368)	1.470 (1.293−1.670)***
HOMA-B	1	1.048 (0.919−1.196)	1.122 (0.986−1.277)	1.071 (0.933−1.229)	1.132 (0.989−1.296)
AUC of glucose (mM*min)	1	1.011 (0.876−1.167)	1.063 (0.924−1.223)	0.993 (0.856−1.152)	1.024 (0.885−1.185)
AUC of insulin (nM*min)	1	1.009 (1.003−1.015)**	0.968 (0.900−1.041)	1.060 (0.925−1.216)	1.069 (0.934−1.223)

Values represent odd ratios and 95% confidence intervals.
[1] GRS of each subject was calculated by the summation of genetic risk alleles in the SNPs (*SLIT3*_rs2974430, *PLEKHA5*_rs1077044, *PPP2R2C*_rs16838853) of the best model.
[2–4]GRS was divided into 3 categories by tertiles: the Low, Medium and High GRS were divided by the scores of 0−1, 2, ≥3, respectively.
[5] Adjusted for age, gender, residence area, BMI.
[6] Adjusted for covariate from model 1 plus smoking and drinking status, income, education, physical activity, daily energy intake, coffee intake, caffeine intake and sugar intake.
[7]Low score group was the reference for both model 1 and model 2.
*Significantly different from low score group in logistic regression analysis at *P < 0.05, **P < 0.01, ***P < 0.001.
AUC, area under the curve; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, Homeostatic model assessment for insulin secretion.

Table 6 Adjusted odds ratio for the risk of insulin resistance and insulin secretion by according to the genetic risk score (GRS) of the best model for gene-gene interaction[a] after covariate adjustments according to the levels of parameters related to lifestyles.

	Low intake[b]		High intake	
	Medium[c] (n = 3043)	High[d] (n = 3245)	Medium[c] (n = 3043)	High[d] (n = 3245)
Coffee				
HOMA-IR (GI[e]: P = 0.0214)	1.026 (0.797−1.320)	1.240 (0.972−1.581)	1.339 (1.108−1.618)	1.837 (1.522−2.218)***
HOMA-B (GI: P = 0.7732)	1.100 (0.836−1.446)	1.242 (0.955−1.615)	1.097 (0.891−1.351)	1.129 (0.919−1.388)
Caffeine				
HOMA-IR (GI: P = 0.0483)	1.134 (0.949−1.354)	1.405 (1.180−1.673)**	1.476 (1.062−2.050)	2.268 (1.639−3.140)***
HOMA-B (GI: P = 0.6019)	1.110 (0.949−1.297)	1.163 (0.998−1.357)	0.941 (0.703−1.260)	1.020 (0.766−1.359)
Alcohol				
HOMA-IR (GI: P = 0.1841)	1.247 (1.084−1.434)	1.423 (1.239−1.635)***	1.069 (1.225−2.314)	1.684 (1.225−2.314)***
HOMA-B (GI: P = 0.8945)	1.045 (0.904−1.208)	1.138 (0.987−1.313)	1.247 (0.829−1.874)	1.206 (0.812−1.793)
Total physical activity				
HOMA-IR (GI: P = 0.5341)	1.159 (1.001−1.342)	1.403 (1.214−1.621)***	1.319 (0.993−1.752)	1.706 (1.293−2.251)***
HOMA-B (GI: P = 0.8397)	1.057 (0.905−1.235)	1.136 (0.975−1.324)	1.107 (0.824−1.487)	1.122 (0.840−1.499)
Smoking				
HOMA-IR (GI: P = 0.1755)	1.157 (0.982−1.363)	1.401 (1.192−1.648)***	1.293 (1.043−1.602)	1.640 (1.328−2.025)***
HOMA-B (GI: P = 0.6755)	1.018 (0.862−1.202)	1.128 (0.957−1.329)	1.207 (0.943−1.544)	1.180 (0.926−1.504)

Values represent odd ratios and 95% confidence intervals.
HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, Homeostatic model assessment for insulin secretion.
*Significantly different from major alleles in logistic regression analysis at *P < 0.05;**P < 0.01; ***P < 0.001.
[a] GRS of each subject was calculated by the summation of genetic risk alleles in the SNPs (*SLIT3*_rs2974430, *PLEKHA5*_rs1077044, *PPP2R2C*_rs16838853) of the best model. Reference was the Low risk group (GRS 0−1) but it was not shown in Table 6.
[b] Coffee, caffeine and alcohol intake was divided into low intake group by the cutoff value of less than 10 cups/week, 220 mg/day and 20 g/day, respectively. Past-smoking status was considered as smoking status. The cutoff value of physical activity was 1 h moderate activity per day.
[c] Medium.
[d] High GRS were divided by the scores of 2 and ≥3, respectively.
[e] GRS interactions with main effects. Multivariate regression models include the corresponding main effects, interaction terms of gene and main effects (coffee or caffeine intake), and potential confounders such as age, gender, residence area, BMI, and smoking and drinking status, income, education, physical activity, daily energy intake, coffee intake, caffeine intake and sugar intake. The independent variable was omitted from the covariates.

serum glucose concentration did not differ among GRS groups, however fasting serum insulin was increased with higher GRS. This study revealed that the subjects with a High-GRS for insulin resistance also had an elevated HOMA-B suggesting that they had a greater insulin secretory response, although the ORs for HOMA-B after adjusting for covariates were not affected by GRS. Therefore, in subjects with High-GRS, high insulin resistance may have been partially compensated for with increased insulin secretion, so it did not significantly increase the prevalence of type 2 diabetes. Genetic variants involved in insulin secretion such as cyclin-dependent kinase 5 regulatory subunit associated protein 1 like 1, potassium voltage-gated channel subfamily Q member 1, insulin-degrading enzyme, hematopoietically-expressed homeobox protein and

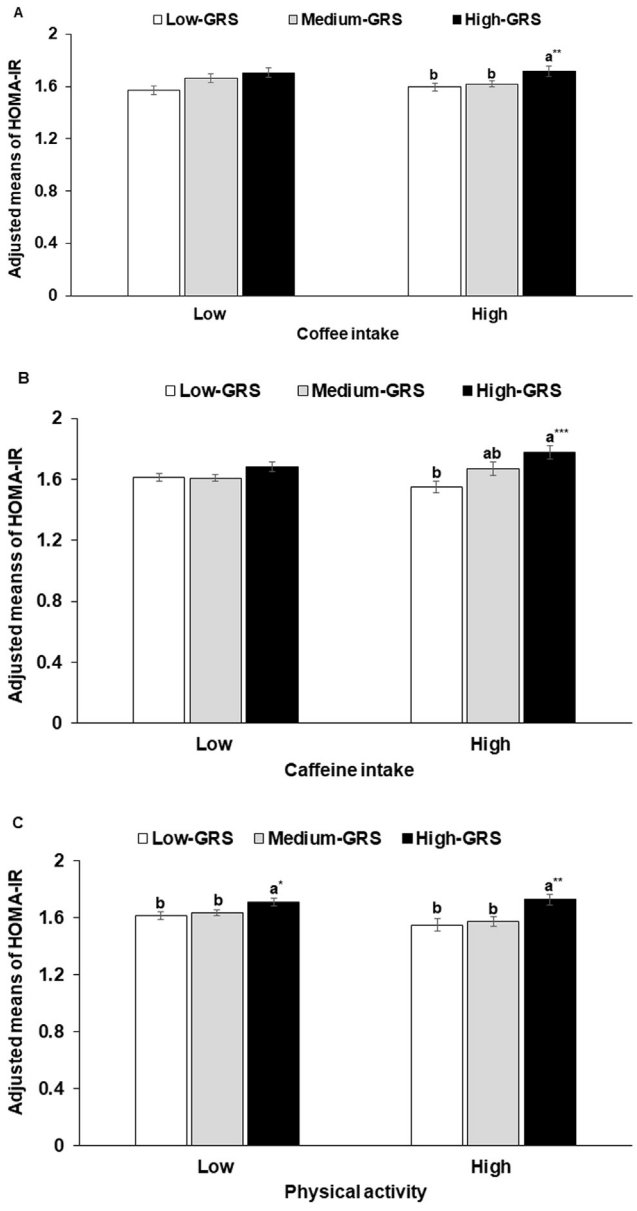

Figure 2 Insulin resistance measured by homeostatic model assessment for insulin resistance (HOMA-IR) in three groups of the genetic variants in genetic risk scores (GRS) of the best model. GRS calculated the summation of genetic risk alleles in the best gene-gene interaction model (*SLIT3*_rs2974430, *PLEKHA5*_rs1077044, *PPP2R2C*_rs16838853) was divided into 3 categories by tertiles: the Low, Medium and High-GRS were divided by the scores of 0–1, 2, ≥3, respectively. (A) Adjusted means of GRS groups in the low- and high-coffee intake; (B) Adjusted means of GRS in the low- and high caffeine intake; C. adjusted means of GRS in the low- and high-physical activity after adjusted with covatiates. Low coffee intake and low caffeine intake were less than 10 cups/week and 220 mg/day, respectively. Physical activity was categorized by 1 h moderate activity per day. Bars represented the means of HOMA-IR. **Significantly different from major alleles in logistic regression analysis at $P < 0.01$; *** at $P < 0.001$. [a,b] Means without a common superscript letter differ in the same row by Tukey test at $P < 0.05$.

ATP-binding cassette transporter 1, are significantly associated with the development of type 2 diabetes especially in Asians [5,28]. These results suggested that insulin secretion may be a primary factor for the development of type 2 diabetes, especially in Asia.

Coffee/caffeine [29], alcohol [30,31] and physical activity [32] have been reported to lower the risk for insulin resistance in some, but not all, studies. It may be associated with the interaction of genetic variants with dietary and lifestyle factors. They may also interact with some genetic variants to increase insulin resistance. The majority of studies have found coffee containing many bioactive compounds to exert beneficial effects on metabolism, including blood glucose regulation. However, decaffeinated coffee was found to improve insulin sensitivity in a recent study, but not coffee with caffeine [33]. However, also the amount roasting of coffee has been shown to have profound effects on the bioactivities of coffee [34]. Therefore, the contradictory results of studies of the effects of coffee on glucose metabolism may be in part due to differences in bioactive compounds relative to caffeine concentrations in coffee, but the present study would suggest that differences in the genetic makeup of individuals may also have an effect. In the Ansan/Ansung cohort coffee and caffeine intake itself did not modulate insulin resistance in middle-aged Koreans. However, coffee and caffeine intake had an interaction with GRS to increase insulin resistance. This indicated that GRS might modulate caffeine and/or polyphenol metabolism or insulin signaling itself. Since the genes involved in GRS are not involved in caffeine metabolism, like aromatase CYP1A2 variants and aryl hydrocarbon receptors [35], the GRS might influence insulin signaling that is counteracted by cAMP activation by caffeine. Carrying high-GRS might, therefore, exacerbate insulin resistance. In low coffee or caffeine intake insulin resistance was not altered by GRS but in high coffee or caffeine intake high-GRS increased insulin resistance more than low-GRS. These results indicate that the controversies surrounding the effects of coffee and caffeine intake on insulin resistance could be partly explained by the differences in genetic background that might be associated with caffeine metabolism.

Of the genes with variants included in the GRS, three genetic variants have not previously been reported to be involved in insulin resistance, but they are related to cell proliferation including cancer, pancreatic β-cells, neruonal cells and myocytes and blood pressure [36–39]. *UNC13C* is involved in skeletal myoblast differentiation through tumor necrosis factor-α and insulin-like growth factor-1 signaling [36] and *DYNC1I1* is associated with RAS-RAF-MEK signaling to protect against neruonal atrophy. These processes may be associated with insulin resistance [37]. The 4q21 locus including *C4orf22* is linked to increased blood pressure [37]. *NTNG1* is a part of Idd18.1 locus which is required to protect against autoimmune diabetes [38]. The *SLIT3* (rs2974430) gene is known to be involved in pancreatic β-cell proliferation and insulin secretion [40]. The *SLIT*3 genes encode proteins that are known to regulate proper cell growth proliferation and are essential for the proper development of many tissues, including pancreatic β-cell survival and function. The other two gene variants (*PLEKHA5*_rs1077044 and *PPP2R2C*_rs16838853) identified as genetic risk factors for insulin resistance were included in the GRS but their functions have not yet been

fully characterized, and are not known to be associated with insulin and/or glucose metabolism. *PLEKHA5* has been linked to more aggressive brain cancer metastasis [41], and its early expression in infants has been identified as one of several early indicators of future seroconversion to pancreatic β-cell autoimmunity, leading to the development of type 1 diabetes [39]. However, its link to insulin resistance remains unclear. *PPP2R2C* appears to function as a tumor suppressor gene [42], and there is no obvious link to insulin at the present time. However, all three of the genes that make up the GRS for insulin resistance are involved in cell growth regulation and tend to be highly expressed in the brain.

This study had several limitations. First, there is the possibility that important genetic risk factors have been missed and that interactions of other genes that were missed would have strengthened the GRS. Second, self-reported dietary and lifestyle questionnaires are subject to error due to reporting errors on the part of the subjects. However, all questionnaires were rigorously validated and shown to be highly reliable to measure gene−nutrient interaction in population studies, including cohort studies. Finally, the practical application of the research is limited because the genes used to calculate the GRS are not well characterized especially for insulin resistance, and there is much to learn about how they can lead to insulin resistance and how the genetic risk of insulin resistance associated with them can be mitigated.

In conclusion, this study demonstrated that 3 genetic variants, when combined, establish a genetic risk factor for insulin resistance that increases the odds of becoming insulin resistant by 50%. Furthermore, high coffee and caffeine consumption (more than 10 cups per week or 220 mg caffeine per day) significantly increased the risk of insulin resistance in subjects with a high genetic risk scores for insulin resistance. This study establishes a basis for genetic testing to identify people who are at increased risk of becoming insulin resistant, and suggests that limiting the consumption of caffeinated beverages may partially mitigate the genetic risk of insulin resistance.

Conflicts of interest

All authors have no conflicts of interest to disclose.

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SP and JWD participated in the experimental design, analyzed the data and wrote the manuscript; ML designed the research and analyzed the data; JWD discussed the results and wrote the manuscript. SP and JWD had primary responsibility for final content. All authors listed in a manuscript have contributed substantially to the work and seen and approved the submitted version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2018.09.009.

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