IRS1 Genotype Modulates Metabolic Syndrome Reversion in Response to 2-Year Weight-Loss Diet Intervention

The POUNDS LOST trial

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OBJECTIVE—Genetic variants near *IRS1* are associated with features of the metabolic syndrome (MetS). We examined whether genetic variants near *IRS1* might modulate the effects of diets varying in fat content on the MetS status in a 2-year weight-loss trial.

RESEARCH DESIGN AND METHODS—Two variants near *IRS1*, rs1522813 and rs2943641, were genotyped in 738 overweight/obese adults (age 60 ± 9 years; BMI 32.7 ± 3.9 kg/m^2) randomly assigned to one of four weight-loss diets (a deficit of 750 kcal/day of caloric intake from baseline) varying in macronutrient contents for 2 years. We compared MetS status of high-fat (40% of caloric intake; *n* = 370) and low-fat (20% caloric intake; *n* = 368) diet groups differentiated by genotypes (rs1522813 A-allele carriers and noncarriers and rs2943641T-allele carriers and noncarriers).

RESULTS—Among rs1522813 A-allele carriers, the reversion rates of the MetS were higher in the high-fat diet group than those in the low-fat diet group over the 2-year intervention (*P* = 0.002), while no significant difference between diet groups was observed among noncarriers (*P* = 0.27). The genetic modulation on dietary effect was independent of weight changes. The odds ratio (OR) for the 2-year reversion of the MetS was 2.88 (95% CI 1.25–6.67) comparing the high-fat and low-fat diets among rs1522813 A-allele carriers, while the corresponding OR was 0.83 (0.36–1.92) in noncarriers. The variant rs2943641 was not observed to modulate dietary effects on the MetS status.

CONCLUSIONS—Our data suggest that high-fat weight-loss diets might be more effective in the management of the MetS compared with low-fat diets among individuals with the A-allele of the rs1522813 variant near *IRS1*.

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The metabolic syndrome (MetS) is a constellation of metabolic abnormalities including abdominal obesity, dyslipidemia (low HDL cholesterol levels, and hypertriglyceridemia), elevated blood pressure, and hyperglycemia (1). It has been well-documented that the MetS increases the risk of diabetes, cardiovascular disease, and all-cause mortality (2). Several clinical trials have suggested that dietary intervention is an effective way to manage the MetS (3–9), though a specific therapeutic diet for the MetS remains to be determined. We have previously shown that weight-loss diets varying in macronutrient components had similar effectiveness in reducing the prevalence of the MetS in a 2-year randomized clinical trial, the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial (10).

Genetic factors may play an important role in the development of the MetS. Insulin receptor substrate 1 (IRS1), encoded by the *IRS1* gene, plays a key role in the insulin signaling pathway (11–13). Recent genome-wide association studies have identified common genetic variants near the *IRS1* gene associated with multiple features of the MetS, such as insulin resistance, abdominal obesity, and dyslipidemia, as well as risk of diabetes and coronary heart disease (14–17). Moreover, our previous gene–diet interaction analysis has shown that the genetic variant rs2943641 near *IRS1* might modulate the effect of diets varying in fat content on weight loss and the improvement of insulin resistance (18). Thus, we hypothesized that genetic variation near *IRS1* may also modify the effect of weight-loss diets varying in fat content on the MetS status.

In the current study, we genotyped another genetic variant, rs1522813, for which occurrence is independent of the previously investigated genetic variant rs2943641 ($r^2 < 0.01$) and is also near *IRS1*, and compared the effects of the high-fat and low-fat diets on the reversion of the MetS according to genotypes of the two variants over a 2-year intervention in 738 overweight or obese adults from the POUNDS LOST trial.

RESEARCH DESIGN AND METHODS

Study participants
The POUNDS LOST trial is a 2-year randomized clinical trial to compare the effects of energy-reduced diets with different compositions of fat, protein, and carbohydrate on weight change. The study design and methods have been described in detail elsewhere (10). A total

of 811 overweight or obese subjects were randomly assigned to one of four diets for 2 years. Participants had to be 30–70 years of age and have a BMI of 25–40 kg/m^2. The target percentages of energy derived from fat, protein, and carbohydrate in the four diets were 20, 15, and 65%; 20, 25, and 55%; 40, 15, and 45%; and 40, 25, and 35%, respectively. Each participant's caloric prescription represented a deficit of 750 kcal/day from baseline, as calculated from the person's resting energy expenditure and activity level. As part of a two-by-two factorial design, two diets were low fat (20%) and two were high fat (40%) and two were average protein (15%) and two were high protein (25%). Major criteria for exclusion were the presence of diabetes or unstable cardiovascular disease, the use of medications that affect body weight, and insufficient motivation. In the current analysis, 738 subjects with genotype data were included (91% of the total subjects in the POUNDS LOST trial). Among them, 61% were women, 80% were white, 15% were African American, 3% were Hispanic, and 2% were Asian or other ethnic groups by self-report. The study was approved by the human subjects committee at the Harvard School of Public Health and Brigham and Women's Hospital, Boston, MA, and the Pennington Biomedical Research Center of the Louisiana State University System, Baton Rouge, LA, and by a data and safety monitoring board appointed by the National Heart, Lung, and Blood Institute. All participants gave written informed consent.

Measurements

Body weight and waist circumference were measured in the morning before breakfast at baseline, 6 months, and 2 years. Height was measured at baseline. BMI was calculated as weight (kg)/height^2 (m^2). Fasting blood samples were collected at baseline, 6 months, and 2 years, and serum glucose, insulin, HDL cholesterol, and triglycerides were measured at the clinical laboratory at the Pennington Biomedical Research Center. Insulin resistance was estimated by homeostasis model assessment of insulin resistance calculated by the following equation: (fasting insulin [μU/mL] × fasting glucose [mg/dL]/18.01)/22.5 (19). Blood pressure was measured at baseline, 6 months, and 2 years with the use of an automated device (HEM-907XL; Omron). To assess the dietary adherence across the intervention, dietary intake was assessed in a random sample of

50% of the participants by a review of the 5-day diet record at baseline and by 24-h recall during a telephone interview on 3 nonconsecutive days at 6 months and 2 years. In addition, food frequency questionnaires were collected in all participants at baseline, 6 months, and 2 years. Participants' physical activity levels were assessed using the Baecke physical activity questionnaire (20) at baseline, 6 months, and 2 years.

Genotyping

DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA). Single nucleotide polymorphisms (SNPs) were genotyped using the Open-Array SNP Genotyping System (BioTrove, Woburn, MA). Replicate quality control samples (10%) were included and genotyped with >99% concordance. In addition to the previously reported SNP, rs2943641 (18), we genotyped another SNP, rs1522813, which showed stronger association with type 2 diabetes than the SNP rs2943641 in our previous analysis (21). The allele frequencies of both SNPs in all participants or in white participants were in Hardy-Weinberg equilibrium ($P > 0.05$).

Definition of the MetS

The MetS was defined based on the National Cholesterol Education Program Adult Treatment Panel III criteria as presenting at least three of the following components: 1) abdominal obesity: waist circumferences ≥102 cm in men or ≥88 cm in women; 2) hyperglyceridemia: triglycerides ≥1.7 mmol/L (150 mg/dL); 3) low HDL cholesterol levels: HDL cholesterol <1.03 mmol/L (40 mg/dL) in men or <1.29 mmol/L (50 mg/dL) in women; 4) high blood pressure: blood pressure ≥130/85 mmHg or current use of antihypertensive medications; or 5) hyperglycemia: fasting plasma glucose ≥6.1 mmol/L (110 mg/dL) (1). Participants who met the criteria for MetS at baseline but not at the 6-month or 2-year assessment were defined as the MetS reversion, while participants who did not meet criteria for MetS at baseline but met the criteria at the 6-month or 2-year assessment were defined as the MetS incidence.

Statistical analysis

General linear models for continuous variables and χ^2 test for categorical variables were applied for the comparison according to genotype groups at baseline. We compared the prevalence of the

MetS and changes in the MetS status (reversion rate and incidence rate) between low-fat and high-fat diet groups by genotype groups at 6 months and 2 years using the χ^2 test. We used logistic regression models to calculate the odds ratios (ORs; [95% CI]) of the reversions of the MetS and individual components by comparing the high-fat diets with the low-fat diets stratified by genotype groups, adjusted for age, sex, ethnicity, and changes in body weight. Gene–diet intervention interactions were tested by including the genotype-by-diet interaction terms in the models. We also used mixed models to test the dietary effects on the reversion of the MetS according to genotype groups over the 2-year intervention. As a result of the relatively small numbers of minor homozygotes, heterozygote and minor homozygotes were combined in the analyses. Because the majority of the participants were white (80%), similar analyses were repeated in white participants. All reported P values are nominal and two-sided, and a P value of 0.05 was considered statistically significant. Statistical analyses were performed with SAS version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS—Table 1 shows the baseline characteristics of participants according to the SNP rs1522813 genotype. The genotype frequencies were similar between men and women, among ethnic groups, and across the diet groups (all $P \geq 0.16$). The prevalence of elevated blood pressure decreased across the genotype groups ($P = 0.02$), but there was no significant differences in the prevalence of the MetS or other individual components of the MetS among the rs1522813 genotype groups (all $P \geq 0.12$). The other SNP, rs2943641, showed weak linkage disequilibrium with the SNP rs1522813 ($r^2 = 0.004$, 0.007, and 0.007; $D' = 0.122$, 0.244, and 0.475, respectively, in the HapMap Centre d'Etude du Polymorphisme Humain from Utah [CEU], Yoruba from Ibadan [YRI], and Japanese population from Tokyo [JPT] + Han Chinese from Beijing [CHB] database [http://hapmap.ncbi.nlm.nih.gov/], respectively). The baseline characteristics of participants according to the SNP rs2943641 genotype have been previously reported (18). No significant differences in the prevalence of the MetS or individual components across the genotype groups were observed (data not shown).

After dietary intervention, the overall prevalence of the MetS was reduced from

Table 1—Baseline characteristics of the study participants

| | All (n = 738) | rs1522813 genotype | | | P value* |
		GG (n = 386)	GA (n = 302)	AA (n = 50)	
Age (years)	51 ± 9	51 ± 9	51 ± 9	50 ± 10	0.23
Sex [n (%)]					0.21
Female	450 (61.0)	236 (61.1)	178 (58.9)	36 (72.0)	
Male	288 (39.0)	150 (38.9)	124 (41.1)	14 (28.0)	
Race or ethnic group [n (%)]					0.42
White	590 (80.0)	318 (82.4)	235 (77.8)	37 (74.0)	
Black	112 (15.2)	51 (13.2)	52 (17.2)	9 (18.0)	
Hispanic	25 (3.4)	11 (2.8)	12 (4.0)	2 (4.0)	
Asian or other	11 (1.5)	6 (1.6)	3 (1.0)	2 (4.0)	
Diet groups [n (%)]					0.16
Low-fat diets (20% of calories)	368 (49.9)	187 (48.4)	161 (53.3)	20 (40.0)	
High-fat diets (40% of calories)	370 (50.1)	199 (51.6)	141 (46.7)	30 (60.0)	
Weight (kg)	93 ± 16	93 ± 14	94 ± 16	91 ± 17	0.74
BMI (kg/m^2)	32.7 ± 3.9	32.6 ± 3.8	32.8 ± 4.0	32.4 ± 3.9	0.76
Baecke physical activity score	1.58 ± 0.11	1.58 ± 0.11	1.58 ± 0.11	1.59 ± 0.12	0.62
MetS [n (%)]	342 (46.3)	185 (47.9)	137 (45.4)	20 (40.0)	0.52
MetS components [n (%)]					
Abdominal obesity	598 (81.0)	314 (81.4)	243 (80.5)	41 (82.0)	0.94
Low HDL cholesterol level	413 (56.0)	203 (52.6)	178 (58.9)	32 (64.0)	0.12
Hypertriglyceridemia	341 (46.2)	183 (47.4)	139 (46.0)	19 (38.0)	0.45
Hyperglycemia	47 (6.4)	24 (6.2)	20 (6.6)	3 (6.0)	0.97
High blood pressure	336 (45.5)	193 (50.0)	126 (41.7)	17 (34.0)	0.02

*P values were calculated by χ^2 test for categorical variables and general linear models for continuous variables.

46.0% at baseline to 33.1% at 6 months and 32.5% at 2 years, and the low-fat and high-fat diets had similar effects on changes in the MetS status (all $P > 0.22$). The prevalence of the MetS was reduced by 11.6 and 14.2% at 6 months and 13.1 and 14.0% at 2 years in the low-fat and high-fat diet groups, respectively.

Table 2 shows the effect of low-fat and high-fat diets on changes in the MetS status stratified by the rs1522813 GG and GA/AA genotype groups. Among participants with GA/AA genotype, the MetS reversion rate (the proportion of participants who had the MetS but reverted at 6 months or 2 years) tended to be higher in the high-fat diet group than in the low-fat diet group at 6 months (42.7 vs. 27.8%; $P = 0.06$) and at 2 years (45.9 vs. 28.1%; $P = 0.04$). Among participants with GG genotype, the low-fat and high-fat diets showed similar effects on the MetS status at 6 months and 2 years (all $P > 0.37$). Results were similar when the analyses were restricted in white participants.

In stratified analysis by the SNP rs2943641, we did not observe any significant dietary effects on the MetS status at 6 months or 2 years among participants with CC genotype or among participants with CT/TT genotype (all $P > 0.28$).

Logistic regression analysis confirmed that high-fat diets were associated with the reversion of the MetS among participants with the rs1522813 A-allele (GA/AA genotypes) (Fig. 1). After adjustment for age, sex, ethnicity, and body weight changes, the ORs (95% CI) for the 6-month MetS reversion for the high-fat diet group compared with low-fat diet group were 2.31 (1.12–4.78) and 0.80 (0.39–1.63) among participants with and without the rs1522813 A-allele, respectively (P for diet–genotype interaction = 0.04). After further adjustment for physical activity, the results remained significant. Further adjustment for changes in insulin resistance, estimated by homeostasis model assessment of insulin resistance, did not change the results. The results were similar at 2 years (ORs [95% CI] 2.88 [1.25–6.67] and 0.83 [0.36–1.92], respectively; P for diet–genotype interaction = 0.04). In our sensitivity analysis, the criterion for waist circumference was excluded in the definition of the MetS. We observed a similar genotype–diet interaction pattern on the reversion of the MetS at 6 months and 2 years (P for interaction = 0.12 and 0.002, respectively).

We then used mixed models to examine the dietary effects on the reversion

of the MetS over the 2-year intervention (Fig. 2). Among participants with GA/AA genotype, the MetS reversion rate was higher in the high-fat diet group than that in the low-fat diet group ($P = 0.002$), while the low-fat and high-fat diets showed similar effects on reversion of the MetS ($P = 0.27$) among participants with GG genotype over the 2-year intervention.

We finally examined the dietary effects on reversion of the components of the MetS by the SNP rs1522813 genotype. Among participants with the rs1522813 GA/AA genotype, the high-fat diets showed directionally consistent effects on reversions of the MetS components compared with the low-fat diets, but the results did not reach a statistically significant level (all $P > 0.05$) (Supplementary Table 1). We observed marginal diet–genotype interactions on the reversion of high blood pressure at 6 months (P for interaction = 0.07) and the reversion of hypertriglyceridemia at 2 years (P for interaction = 0.08).

CONCLUSIONS—In this 2-year weight-loss trial, we examined the effects of variants near the *IRS1* gene on changes in the MetS status in response to

Table 2—*Metabolic syndrome status and weight loss between diet groups by rs1522813 genotype at baseline, 6 months, and 2 years*

	GG			GA/AA		
	Low-fat diets	High-fat diets	P value*	Low-fat diets	High-fat diets	P value*
Baseline						
Participants (n)	187	199		181	171	
Prevalence [n (%)]	97 (51.9)	88 (44.2)	0.13	76 (42.0)	81 (47.4)	0.31
6 months						
Participants (n)	171	177		158	153	
Prevalence [n (%)]	60 (35.1)	58 (32.8)	0.65	57 (36.1)	48 (31.4)	0.38
Reversion [n (%)]†	34 (37.8)	24 (31.2)	0.37	20 (27.8)	32 (42.7)	0.06
Incidence [n (%)]‡	4 (4.9)	5 (5.0)	0.98	5 (5.8)	5 (6.4)	0.87
Weight loss (kg)	−6.7 ± 0.4	−6.6 ± 0.4	0.83	−6.9 ± 0.5	−6.3 ± 0.5	0.33
Weight loss (%)	−7.3 ± 0.4	−7.3 ± 0.4	0.97	−7.3 ± 0.5	−6.7 ± 0.5	0.42
2 years						
Participants (n)	142	150		144	124	
Prevalence [n (%)]	46 (32.4)	48 (32.0)	0.94	50 (34.7)	41 (33.1)	0.78
Reversion [n (%)]†	29 (40.9)	23 (34.9)	0.47	18 (28.1)	28 (45.9)	0.04
Incidence [n (%)]‡	4 (5.6)	5 (6.0)	0.93	4 (5.0)	8 (12.7)	0.10
Weight loss (kg)	−4.3 ± 0.6	−4.1 ± 0.6	0.77	−4.0 ± 0.6	−3.7 ± 0.6	0.70
Weight loss (%)	−4.6 ± 0.6	−4.6 ± 0.6	0.98	−4.1 ± 0.6	−3.9 ± 0.6	0.87

*P values were calculated by χ^2 test for categorical variables and general linear models for continuous variables. †Participants who met the criteria for MetS at baseline but not at the 6-month or 2-year assessment. ‡Participants who did not meet criteria for MetS at baseline but met the criteria at the 6-month or 2-year assessment.

weight-loss diets with two different prescribed levels of fat content. We found a potential interaction between the genetic variant rs1522813 and diet intervention on reversion of the MetS at 6 months and 2 years. Among individuals with the rs1522813 A-allele, the high-fat diets showed greater effect on reversion of the MetS compared with the low-fat diets over the 2-year intervention.

Although a large body of evidence has shown that dietary interventions are beneficial in the management of the MetS, the ideal dietary intervention remains controversial (3–9). In our previous analysis of the POUNDS LOST trial, weight-loss diets with different macronutrient components had similar effectiveness in improving the MetS (10). Our recent gene–diet interaction analyses in the POUNDS LOST trial have suggested that the effects of the diet intervention on body weight and related metabolic traits might be influenced by genetic background (18,22–26). In the current study, our data indicated that high-fat diets were superior to the low-fat diets for reversion of the MetS among individuals with the A-allele of the rs1522813 variant near *IRS1*. The observed genotype–diet interaction on reversion of the MetS might be independent of weight loss or changes in insulin

resistance. This is consistent with the results that we did not find significant genotype–diet interaction on weight loss or changes in insulin resistance. Moreover, the results were similar when we excluded the criterion for waist circumference in the definition of the MetS, further suggesting that the genotype–diet interaction on reversion of the MetS might be less influenced by changes in adiposity. Unlike the weight regain after 6 months, the dietary effect in reducing the prevalence of the MetS persisted at 2 years in the POUNDS LOST trial (10). This may also explain the consistent results at 6 months and 2 years regardless of weight changes during the intervention. Therefore, these results indicate a stable and long-term modification effect of the genetic variant rs1522813 on the MetS reversion in response to diet interventions. Our data may provide useful information for the development of dietary interventions in the management of the MetS based on genetic background.

Another genetic variant near *IRS1*, rs2943641, which has been previously shown to interact with dietary fat intake on the changes in body weight and insulin resistance (18), did not modulate the dietary effects on the MetS status in the current analysis. However, it should be noted that

the currently observed interaction between the genetic variant rs1522813 and diet intervention on reversion of the MetS was independent of changes in body weight and insulin resistance. We did not find any different dietary effects on weight loss according to the rs1522813 genotype (data not shown). The SNPs rs2943641 and rs1522813 fall into two independent linkage disequilibrium blocks with low correlation ($r^2 < 0.01$ for all ethnic groups in the HapMap database) and showed independent associations with risk of type 2 diabetes (21). In addition, there was an interaction between physical activity and the SNP rs1522813, but not the SNP rs2943641, on type 2 diabetes risk in women (21). However, the SNPs in the same linkage disequilibrium block with the rs2943641 appear to show stronger associations with various metabolic traits than the other SNPs near *IRS1* (14). Thus, our findings together with other previous results suggested that different mechanisms might underlie the observed associations and interactions for these two genetic variants. It is possible that the genetic variant rs2943641 might be more related to weight loss and insulin resistance in response to dietary intervention, while the genetic variant rs1522813 is more likely to interact with dietary intervention on changes in blood pressure, plasma HDL cholesterol, and triglycerides. However, the biological function of these genetic variants has not been clarified, and experimental studies are needed in the future.

We did not find significant gene–diet interactions or dietary effects on the reversions of the individual components of the MetS, but the high-fat diet showed directionally consistent improvement on reversions of the MetS components compared with the low-fat diets among participants with the rs1522813 A-allele. These results suggested that the dietary effect on reversion of the MetS might reflect the cumulative effects on the individual components rather than on any single component. This is in line with the fact that genetic variants near *IRS1* were associated with multiple features of the MetS (14–17,21), though the function of these genetic variants has not been clarified. Individuals with the rs1522813 A-allele, which is the risk allele for type 2 diabetes, had a higher reversion rate of the MetS by choosing a high-fat diet compared with those without the A-allele. This is consistent with some previous results that the risk alleles of the genetic variants were associated
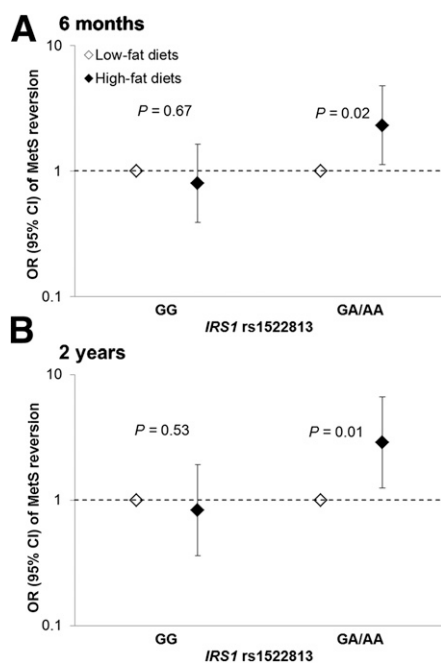
Figure 1—*ORs of 6-month* (A) *and 2-year* (B) *reversion among participants with MetS at baseline in the high-fat diet group compared with the low-fat diet group by rs1522813 genotype. Data were calculated by using logistic regression model after adjustment for age, sex, ethnicity, and body weight change.*

with greater beneficial effects of diet interventions (18,27). The potential mechanisms underlying these findings remain to be investigated. In addition, it is difficult to distinguish the effects of dietary fat and carbohydrate responsible for the observed interactions because the high-fat diets have low carbohydrate content.

To the best of our knowledge, this is the first study to date to investigate the gene–diet interactions on changes in the MetS status in a large and long-term randomized trial. However, several limitations of our study should be considered. Not all participants included in the analysis had MetS at baseline (~50% of the participants met the criteria for the MetS). This may also limit our statistical power of the analysis for the reversion of the MetS, and our study may be underpowered to detect modest effects and interactions. As expected, our findings barely reached the level of statistical significance, and replication of these results is needed in future. Participants had difficulty achieving the goals for macronutrient intake of their assigned group, but mean differences in fat, carbohydrate, or protein intake among the diet groups were significant (10). Because of the relatively small numbers of incident cases of the MetS, we did not
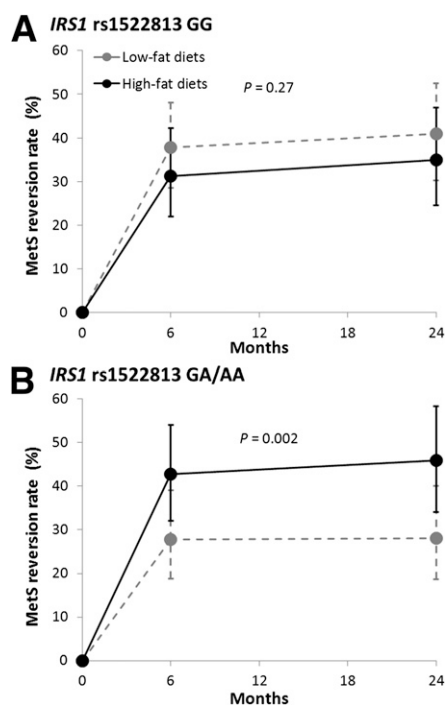
Figure 2—*Reversion rate of MetS in the high-fat diet group compared with the low-fat diet group according to rs1522813 genotype.* A: *Participants with rs1522813 GG genotype.* B: *Participants with rs1522813 GA/AA genotypes. Data are reversion rate (95% CI) of MetS.*

analyze the incidence of the MetS, and it is unknown whether the gene–diet interactions were similar to the prevention of the MetS. The participants of our study were overweight or obese; therefore, further studies are warranted to investigate whether our findings are applicable in people with normal weight. In addition, most of the participants were whites (~80%), and it remains to be determined whether our findings could be generalized to other ethnic groups.

In conclusion, we found that the genetic variant rs1522813 near *IRS1* might modulate the effects of weight-loss diets with different fat content on the MetS status in a 2-year randomized trial. Participants with the rs1522813 A-allele might have a higher reversion rate of the MetS by choosing a high-fat diet compared with a low-fat diet. These novel findings provide supportive evidence for the notion of a personalized dietary intervention in the management of the MetS.

References

1. Expert Panel on Detection, Evaluation, Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NECP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001; 285:2486–2497
2. Gami AS, Witt BJ, Howard DE, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol 2007;49:403–414
3. Azadbakht L, Mirmiran P, Esmaillzadeh A, Azizi T, Azizi F. Beneficial effects of a Dietary Approaches to Stop Hypertension eating plan on features of the metabolic syndrome. Diabetes Care 2005;28:2823–2831
4. Esposito K, Marfella R, Ciotola M, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004; 292:1440–1446
5. Kukkonen-Harjula KT, Borg PT, Nenonen AM, Fogelholm MG. Effects of a weight maintenance program with or without exercise on the metabolic syndrome: a randomized trial in obese men. Prev Med 2005;41:784–790
6. Salas-Salvadó J, Fernández-Ballart J, Ros E, et al.; PREDIMED Study Investigators.

Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. Arch Intern Med 2008; 168:2449–2458

7. Wu H, Pan A, Yu Z, et al. Lifestyle counseling and supplementation with flaxseed or walnuts influence the management of metabolic syndrome. J Nutr 2010;140: 1937–1942

8. Wood RJ, Fernandez ML. Carbohydrate-restricted versus low-glycemic-index diets for the treatment of insulin resistance and metabolic syndrome. Nutr Rev 2009;67: 179–183

9. Riccardi G, Rivellese AA. Dietary treatment of the metabolic syndrome—the optimal diet. Br J Nutr 2000;83(Suppl. 1): S143–S148

10. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. N Engl J Med 2009;360: 859–873

11. Sun XJ, Rothenberg P, Kahn CR, et al. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 1991;352:73–77

12. Araki E, Lipes MA, Patti M-E, et al. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 1994;372:186–190

13. Tamemoto H, Kadowaki T, Tobe K, et al. Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. Nature 1994;372:182–186

14. Kilpeläinen TO, Zillikens MC, Stančáková A, et al. Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. Nat Genet 2011;43:753–760

15. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 2010;466:707–713

16. Rung J, Cauchi S, Albrechtsen A, et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet 2009; 41:1110–1115

17. Samani NJ, Erdmann J, Hall AS, et al.; WTCCC and the Cardiogenics Consortium. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;357:443–453

18. Qi Q, Bray GA, Smith SR, Hu FB, Sacks FM, Qi L. Insulin receptor substrate 1 gene variation modifies insulin resistance response to weight-loss diets in a 2-year randomized trial: the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. Circulation 2011; 124:563–571

19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28: 412–419

20. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36: 936–942

21. He MA, Workalemahu T, Cornelis MC, Hu FB, Qi L. Genetic variants near the IRS1 gene, physical activity and type 2 diabetes in US men and women. Diabetologia 2011;54:1579–1582

22. Qi Q, Bray GA, Hu FB, Sacks FM, Qi L. Weight-loss diets modify glucose-dependent insulinotropic polypeptide receptor rs2287019 genotype effects on changes in body weight, fasting glucose, and insulin resistance: the Preventing Overweight Using Novel Dietary Strategies trial. Am J Clin Nutr 2012;95: 506–513

23. Zhang X, Qi Q, Bray GA, Hu FB, Sacks FM, Qi L. APOA5 genotype modulates 2-y changes in lipid profile in response to weight-loss diet intervention: the Pounds Lost Trial. Am J Clin Nutr 2012;96:917–922

24. Zhang X, Qi Q, Zhang C, et al. FTO genotype and 2-year change in body composition and fat distribution in response to weight-loss diets: the POUNDS LOST Trial. Diabetes 2012;61:3005–3011

25. Mattei J, Qi Q, Hu FB, Sacks FM, Qi L. TCF7L2 genetic variants modulate the effect of dietary fat intake on changes in body composition during a weight-loss intervention. Am J Clin Nutr 2012;96: 1129–1136

26. Zhang X, Qi Q, Liang J, Hu FB, Sacks FM, Qi L. Neuropeptide Y promoter polymorphism modifies effects of a weight-loss diet on 2-year changes of blood pressure: the preventing overweight using novel dietary strategies trial. Hypertension 2012;60:1169–1175

27. Marín C, Pérez-Martínez P, Delgado-Lista J, et al. The insulin sensitivity response is determined by the interaction between the G972R polymorphism of the insulin receptor substrate 1 gene and dietary fat. Mol Nutr Food Res 2011;55:328–335