Genetic Variants at the PDZ-Interacting Domain of the Scavenger Receptor Class B Type I Interact with Diet to Influence the Risk of Metabolic Syndrome in Obese Men and Women1-3

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Abstract
The scaffolding protein PDZ domain containing 1 (PDZK1) regulates the HDL receptor scavenger receptor class B type I. However, the effect of PDZK1 genetic variants on lipids and metabolic syndrome (MetS) traits remains unknown. This study evaluated the association of 3 PDZK1 single nucleotide polymorphisms (SNP) i33968C > T, i15371G > A, and i19738C > T with lipids and risk of MetS and their potential interactions with diet. PDZK1 SNP were genotyped in 1000 participants (481 men, 519 women) included in the Genetics of Lipid Lowering Drugs and Diet Network study. Lipoprotein subfractions were measured by proton NMR spectroscopy and dietary intake was estimated using a validated questionnaire. The PDZK1_i33968C > T polymorphism was associated with MetS (P = 0.034), mainly driven by the association of the minor T allele with higher plasma triglycerides (P = 0.004) and VLDL (P = 0.021), and lower adiponectin concentrations (P = 0.022) than in participants homozygous for the major allele (C). We found a significant gene × BMI × diet interaction, in which the deleterious association of the i33968T allele with MetS was observed in obese participants with high PUFA and carbohydrate (P-values ranging from 0.004 to 0.020) intakes. Conversely, a there was a protective effect in nonobese participants with high PUFA intake (P < 0.05). These findings suggest that PDZK1_i33968C > T genetic variants may be associated with a higher risk of exhibiting MetS. This gene × BMI × diet interaction offers the potential to identify dietary and other lifestyle changes that may obviate the onset of MetS in individuals with a specific genetic background. J. Nutr. 139: 842-848, 2009.

Introduction
Metabolic syndrome (MetS),10 resulting from the clustering of abdominal obesity, hypertension, dyslipidemia, and hyperglycemia, is a common risk factor for atherosclerotic cardiovascular diseases in populations exposed to overnutrition (1). The prevalence of MetS in the United States has been estimated at 26% and its worldwide prevalence has steadily increased over the recent years as a consequence of the global weight gain and unhealthy behaviors adopted by many populations (2).

The etiology of MetS is complex, being defined by a genetic susceptibility and environmental influences, as well as their

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3 Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
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5 Abbreviations used: BP, blood pressure; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; DHQ, diet history questionnaire; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; HDL-C, HDL-cholesterol; IDL, intermediate-density lipoprotein; LDL-C, LDL-cholesterol; LD, linkage disequilibrium; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; PDZK1, PDZ domain containing 1; RCT, reverse cholesterol transport; SCARB1, scavenger receptor class B type I; SNP, single nucleotide polymorphism; TG, triglyceride.
interactions (3–5). Given the number of metabolic pathways involved in MetS, many loci have been associated with individual susceptibility to MetS (3). Moreover, several twin and familial aggregation studies have also revealed a substantial genetic component for each of the MetS traits (3) and several genome-wide analyses reported suggested regions of significant linkage with the individual components of MetS at various chromosomes (6–8). However, the identification of reliable genetic markers to identify MetS risk has not been very successful, partly due to the rather incomplete knowledge of the constellation of genes implicated in this syndrome. Therefore, identification of new genes is paramount to the generation of diagnostic tools for MetS risk detection and the implementation of early prevention strategies.

The hallmarks of the dyslipidemia associated with MetS are high circulating triglyceride (TG) and low HDL-cholesterol (HDL-C) concentrations. A major player in lipoprotein metabolism is the scavenger receptor class B type I (SCARB1). Originally reported as an HDL receptor, it became evident that SCARB1 was, in fact, a multiligand receptor involved in the reverse cholesterol transport (RCT) by mediating the selective uptake of cholesteryl esters from HDL, as well as LDL and VLDL particles (9–11). In the liver, normal expression of SCARB1 protein is controlled by its adaptor PDZ domain containing 1 (PDZK1) (12,13), a 70-kDa protein composed of 4 modular PDZ-interacting domains that bind at the C terminus of SCARB1 (9). Evidence from mice (9,10,14) has shown that the loss of hepatic PDZK1 expression promotes atherosclerosis, probably due to dramatic reductions of SCARB1 activity and RCT as well as significant increases in atherogenic apoB-containing lipoproteins such as LDL and VLDL particles. Overall, current evidence supports the hypothesis that PDZK1 is a likely candidate gene related to lipoprotein metabolism and MetS traits in humans.

PDZK1 gene has been mapped to human chromosome 1q21, a chromosomal region that has been linked repeatedly with multiple metabolic abnormalities, such as abdominal obesity, hypertension, and MetS risk in several genome-wide linkage analyses (7,8). There are no reports, to our knowledge, examining the potential associations between single nucleotide polymorphisms (SNP) at the PDZK1 gene with lipoprotein levels and MetS risk. Therefore, our goals in this study were to assess the association of novel polymorphisms at the PDZK1 gene with lipoprotein levels and the MetS-related phenotypes and investigate whether PDZK1 SNP interact with dietary factors to modulate MetS risk.

Methods

Subjects. The study population consisted of 1000 participants (481 men and 519 women, age 49 ± 16 y) included in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study. Participants were recruited from 3-generational pedigrees from 2 National Heart, Lung, and Blood Institute Family Heart Study field centers (Minneapolis, MN and Salt Lake City, UT) (15). The study population was all of Caucasian origin. The detailed design and methodology of the study has been described previously (16). The protocol was approved by the Institutional Review Boards at the University of Alabama, the University of Minnesota, the University of Utah, and Tufts University. Written informed consent was obtained from each participant.

Data collection. For GOLDN participants, clinical examinations at the baseline visit included anthropometrical and blood pressure (BP) measurements. Weight was measured with a beam balance and height with a fixed stadiometer. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the umbilicus. BP was measured twice with an oscillometric device (Dinamap Pro Series 100, GE Medical Systems) while subjects were seated and had rested for 5 min. Reported systolic and diastolic BP values were the mean of 2 measurements. Questionnaires were administered to assess demographic information and medical and medication history. Physical activity was expressed as metabolic equivalent task hours based on self-reported types and durations of activities over a period of 24 h.

The habitual dietary food intake was assessed by the Diet History Questionnaire (DHQ) developed by the National Cancer Institute (17). It consisted of 124 food items and included portion size and dietary supplement questions. The nutrient and food group database, created for analyzing the DHQ, was based on national dietary intake data from the 1994–1996 USDA's Continuing Survey of Food Intake by Individuals. These 24-h dietary recall data were used to decide which foods to include on the DHQ and what the portion sizes should be. The DHQ was administered once, when the first screening took place. Two studies have confirmed its validity (18,19).

Laboratory methods. Blood samples were drawn from participants after an overnight fast. Plasma was prepared and all samples stored and analyzed together after the study was complete. Glucose was measured using the method of a hexokinase-mediated reaction and total cholesterol using a cholesterol esterase cholesterol oxidase reaction on a Hitachi 911 autoanalyzer (Roche Diagnostics). The same reaction was used to measure HDL-C after precipitation of non-HDL-C with magnesium/dextran. LDL cholesterol (LDL-C) was measured by use of a homogeneous direct method (LDL Direct Liquid Select Cholesterol Reagent; Equal Diagnostics). TG were measured by glycerol-blanked enzymatic method on the Roche COBAS PARA centrifugal analyzer (Roche Diagnostics). Plasma insulin and total adiponectin concentrations were measured using specific RIA kits (Linco Research).

Lipoprotein particle concentrations and size were measured in 995 participants by proton NMR spectroscopy (20,21). Data were obtained from the measured amplitudes of the spectroscopically distinct lipid methyl group NMR signals. We measured the concentrations of the following subfractions: large HDL (8.8–13.0 nm), medium HDL (8.2–8.8 nm), small HDL (7.3–8.2 nm), intermediate-density lipoprotein (IDL; 23.0–27.0 nm), small LDL (diameter 18.0–21.2 nm), large LDL (21.2–23.0 nm), large VLDL (>60 nm), medium VLDL (35.0–60.0 nm), and small VLDL (27.0–35.0 nm). The small LDL subfraction encompassed both medium small (19.8–21.2 nm) and very small (18.0–19.0 nm) particles.

Genetic analyses. DNA was extracted from blood samples and purified using commercial Puregene reagents (Gentra Systems) following the manufacturer's instructions. Three PDZK1 intrinsic SNP (i33968C>T, rs3912316; i5371G>A, rs11576685; and i9738C>T, rs1284300) were genotyped. SNP were selected using 2 criteria: bioinformatics functional assessment and linkage disequilibrium (LD) structure. Computational analysis of PDZK1 SNP ascribed potential functional characteristics to each variant allele. Upstream SNP analyzed by MAPPER (22) identified allele-specific transcription factor binding sites. Intron SNP were also analyzed with MAPPER and manually checked for altered mRNA splice donor and acceptor sites and transversions affecting the poly-pyrimidine tract near splice acceptors. Minor allele frequencies of coding sequence SNP were too low to provide adequate statistical power. At the time of genotyping, the PDZK1 reference mRNA was 64 residues shorter at the 5' end. Mapping rs11576685 to the updated PDZK1 mRNA (accession NM_002614.3) showed the SNP “moving” from the near promoter region to intron 1. Nonetheless, this SNP was in a putative CAAT-box weakened by the minor allele. Assessing LD structure at the PDZK1 locus facilitated the selection of tag SNP representing different LD blocks. Of 25 blocks of tag SNP from 10 kb upstream to 5 kb downstream of the 36-kb PDZK1 gene, we selected proxy SNP from those 3 blocks that, based on our bioinformatics analysis, have a putative function. Genotyping more SNP across such a relatively small gene is not likely to add value to the phenotype-genotype association analysis. Genotyping was performed using a TaqMan assay with allele-specific probes on the ABI Prism 7900 HT Sequence Detection system (Applied Biosystems) according to routine laboratory protocols (23). The pairwise LD between SNP was estimated as the correlation coefficient (R) in...
unrelated participants using the Helixtree software package (Golden Helix).

**Statistical analyses.** SPSS software (version 15.0) was used for statistical analyses. A logarithmic transformation was applied to measure of plasma TG, insulin, and adiponectin to normalize the distribution of the data. On the basis of the National Cholesterol Education Program-Adult Treatment Panel III guidelines (1), participants were diagnosed with MetS if they exhibited ≥ 3 of the following components: 1) a waist circumference ≥102 cm for men and ≥88 cm for women; 2) a plasma TG concentration of ≥1.65 mmol/L (150 mg/dL); 3) a plasma concentration of HDL-C <1.04 mmol/L (40 mg/dL) for men and <1.30 mmol/L (50 mg/dL) for women; 4) a systolic BP ≥130 mm Hg and diastolic BP ≥85 mm Hg; and 5) a fasting plasma glucose concentration >6.10 mmol/L (110 mg/dL).

Data are presented as means ± SD for continuous variables and as frequencies or percentages for categorical variables. Differences in mean values were assessed using ANOVA and unpaired *t* tests. Categorical variables were compared by using the Pearson chi-square or Fisher's exact tests. Potential confounding factors were age, sex, physical activity, smoking habit (current vs. never and past smokers), alcohol consumption (current vs. nondrinkers), medications (treatment for hypertension, hypercholesterolemia, diabetes, and self-reported use of hormone therapy by women), prior coronary heart disease (CHD), presence of type 2 diabetes, and family relationships. Corrections for multiple comparisons were made using the Bonferroni technique (*P*-values were multiplied by the number of analyses performed). Further adjustment for BMI was used to assess the association between PDZKI polymorphisms and particle fractions. We fitted logistic regression models to estimate the OR and 95% CI of MetS across PDZKI genotypes stratified by dietary intakes (as dichotomous variables) and to control for the effect of covariates and total energy intake. We analyzed these data both in overall participants and also subdivided using the BMI threshold value of 30 kg/m^2^, based on the standard definition of obesity. As a measure of the goodness of fit of the models, the square of the correlation coefficient among diet components was calculated. Further adjustment for saturated fat, monounsaturated fatty acid (MUFA), PUFA, protein, and carbohydrates (as continuous variables) was used to mutually adjust intake for each nutrient. Two-sided *P*-values < 0.05 were considered significant.

**Results**

Characteristics of the GOLDN participants and genotype frequencies by sex are shown (Table 1). Although BMI did not differ by sex, average weight was higher in men than in women. BP and plasma glucose and TG concentrations were higher in men, whereas HDL-C and adiponectin concentrations were higher in women. The other variables examined did not differ. For all PDZKI polymorphisms, there was no departure from the Hardy-Weinberg equilibrium (*P* > 0.05). The pairwise LD in correlation coefficients of all 3 SNP is presented in Supplemental Table 1. Considering the lack of a significant PDZKI SNP × gender interaction for all variables examined, data from men and women were pooled for subsequent analyses. Because of the low genotype frequencies of homozygotes for the minor alleles, we analyzed all SNP in 2 genotype categories using the dominant model to maximize the statistical power.

In the multivariate-adjusted logistic regression model, T allele carriers at the PDZKI_i33968C > T SNP had a higher risk of MetS (OR 1.47; *P* = 0.034), mainly driven by the association of the minor T allele with higher TG (OR 1.50; *P* = 0.010), and a trend toward increased abdominal obesity (OR 1.22; *P* = 0.211) and hypertension (OR 1.42; *P* = 0.123) compared with CC participants (Table 2). For the PDZKI_i15371G > A SNP, minor A allele carriers had more abdominal obesity than GG participants, but MetS prevalence did not differ between genotypes. We did not observe any significant association with the individual components of MetS for the PDZKI_i19738C > T SNP (Table 2). Given that PDZKI_i33968C > T was the only SNP related to MetS, results for this SNP were examined in more detail.

**Anthropometrical variables did not differ between genotype groups.** However, T allele carriers had a higher log plasma TG concentration (0.24 ± 0.01 mmol/L) than CC participants (vs. 0.17 ± 0.01 mmol/L; *P* = 0.004). Conversely, log adiponectin was lower in T allele carriers (3.83 ± 0.01 µg/L) compared with CC participants (3.86 ± 0.01 µg/L; *P* = 0.022) (Table 3). After the Bonferroni test, T allele carriers still had higher log TG than CC participants (*P* = 0.036), whereas genotypes did not differ for log adiponectin concentrations (data not shown). Moreover, T allele carriers also tended to have higher systolic BP and higher plasma total cholesterol, glucose, and insulin concentrations than CC participants (*P* = 0.074–0.169). We also examined the effect of the PDZKI_i33968C > T SNP on lipoprotein concentrations and particle size (Table 4). The only associations were found for large and medium VLDL concentrations that were higher in T allele carriers (0.41 ± 0.04 and 0.61 ± 0.03 g/L, respectively) than in CC participants (0.31 ± 0.02 g/L; *P* = 0.021; and 0.52 ± 0.02 g/L, *P* = 0.019, respectively). After multiple comparisons testing, these associations were marginally significant (*P* = 0.147 and 0.133, respectively) (data not shown).

We next examined whether the associations between PDZKI_i33968C > T SNP and MetS prevalence were related to dietary habits in this population. Because there were no significant differences in the dietary intake according to genotype groups (data not shown), we investigated whether PDZKI gene × diet interactions could modulate the observed association with MetS risk. We dichotomized dietary intakes according

**Table 1** General characteristics of the GOLDN participants

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y, range)</td>
<td>49 (18–68)</td>
<td>49 (18–92)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>89.8 ± 16.0</td>
<td>76.1 ± 17.5*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.5 ± 4.8</td>
<td>28.3 ± 4.6</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>100 ± 14</td>
<td>93 ± 18*</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>38 (8)</td>
<td>43 (8)</td>
</tr>
<tr>
<td>Current alcohol drinkers, n (%)</td>
<td>243 (51)</td>
<td>240 (48)</td>
</tr>
<tr>
<td>Physical activity, h/d</td>
<td>3.0 ± 1.3</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>119 ± 15</td>
<td>113 ± 18*</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>71 ± 6</td>
<td>66 ± 9*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>5.83 ± 1.17</td>
<td>5.44 ± 0.89*</td>
</tr>
<tr>
<td>Log (plasma insulin), pmol/L</td>
<td>1.93 ± 1.05</td>
<td>1.91 ± 1.05</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.95 ± 0.98</td>
<td>5.04 ± 1.06</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>3.21 ± 0.78</td>
<td>3.11 ± 0.65*</td>
</tr>
<tr>
<td>Log (TG, mmol/L)</td>
<td>0.24 ± 0.10</td>
<td>0.16 ± 0.09*</td>
</tr>
<tr>
<td>Log (plasma adiponectin), µg/L</td>
<td>3.74 ± 0.24</td>
<td>3.96 ± 0.23*</td>
</tr>
<tr>
<td>MetS, n (%)</td>
<td>154 (32)</td>
<td>150 (29)</td>
</tr>
<tr>
<td>PDZKI SNP, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i33968C &gt; T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>354 (74%)</td>
<td>385 (74%)</td>
</tr>
<tr>
<td>CT+TT</td>
<td>127 (26%)</td>
<td>134 (26%)</td>
</tr>
<tr>
<td>i1 5371G &gt; A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>448 (93%)</td>
<td>481 (93%)</td>
</tr>
<tr>
<td>AA+AA</td>
<td>32 (7)</td>
<td>36 (7)</td>
</tr>
<tr>
<td>i19738C &gt; T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>401 (83%)</td>
<td>444 (86%)</td>
</tr>
<tr>
<td>CT+TT</td>
<td>80 (17)</td>
<td>75 (14)</td>
</tr>
</tbody>
</table>

1 Values are means ± SD unless noted otherwise. Symbols indicate different from men: *P* < 0.001, †*P* < 0.05.
to the median value for each nutrient. Our analysis showed that T allele carriers consuming diets containing low MUFA (OR 1.92; \( P = 0.015 \)), high protein (OR 1.81, \( P = 0.018 \)), or high carbohydrate (OR 1.89, \( P = 0.013 \)) intakes were associated with a higher risk of MetS than CC participants. However, no significant gene \( \times \) diet interactions were found (Supplemental Table 2). Based on previous evidence shown for other loci (24,25), we investigated whether BMI played a significant role in modifying the observed associations (Table 5). For these analyses, we dichotomized BMI above and below 30 kg/m\(^2\). Interestingly, food intake for each nutrient and total energy intake did not differ between nonobese and obese participants (data not shown). A significant gene \( \times \) BMI \( \times \) diet interaction was found (\( P = 0.004 \)) in which the deleterious association of the i33968T allele with MetS was observed in obese subjects with high dietary intake from PUFA (\( P = 0.015 \)), whereas a protective effect of this SNP was seen in nonobese participants (\( P = 0.024 \)). This significant gene \( \times \) BMI \( \times \) diet interaction was still observed regardless of the PUFA family investigated: (n-3) (\( P = 0.004 \)) or (n-6) (\( P = 0.006 \)). Moreover, the deleterious effect of the i33968T allele with MetS was observed only in obese participants with high-carbohydrate intake (\( P = 0.005 \)). Although differences were marginally significant, the association of the minor T allele with MetS was also seen in obese participants with high-protein (\( P = 0.057 \)) and saturated fat (\( P = 0.133 \)) intakes and in nonobese participants with low-MUFA intake (\( P = 0.057 \)) (Table 5). Because PUFA intake was correlated with intakes from total fat, saturated fat, MUFA, protein, and carbohydrates (\( r = 0.759 - 0.941 \); \( P < 0.001 \)), models were additionally adjusted for each nutrient. Further adjustment weakened significant associations only for PUFA (\( P = 0.060 \)) and for PUFA and carbohydrate components in obese participants (\( P = 0.016 \) and \( P = 0.006 \), respectively), whereas the interaction with dietary protein was no longer significant (\( P = 0.060 \)) (data not shown). No significant gene \( \times \) diet interactions were found for total fat either in obese or nonobese participants.

### Discussion

In the current study, we found a significant association between PDZK1_i33968C > T SNP and risk of MetS and MetS-related phenotypes. Moreover, we found a significant gene \( \times \) BMI \( \times \) diet interaction for total energy intake (\( P = 0.003 \)). These findings suggest that the PDZK1 gene may play a role in the modulation of MetS risk and that the interaction with dietary intake is particularly important in obese individuals. Further studies are needed to investigate the biological mechanisms underlying these associations and to explore the potential implications for personalized nutrition and lifestyle interventions.
diet interaction in which the deleterious association of the i33968T allele with MetS was observed in obese participants with high dietary intake from carbohydrates and marginally significant association of the i33968T allele with MetS was observed in obese participants with high-saturated fat and -protein intakes. Overall, these findings suggest that dietary habits may modulate the genetic susceptibility toward developing MetS, particularly in obese participants.

It is noteworthy that this is the first study, to our knowledge, that examines in a relatively large population the effects of PDZK1_i33968C > T SNP on lipids and MetS traits. We found that i33968T allele carriers had higher TG concentrations than CC homozygotes, supporting a key role for PDZK1 in TG metabolism. The role of PDZK1 in the metabolism of the apoB-containing lipoproteins is unclear. There is currently no evidence that this receptor is directly involved in the internalization of TG from the lipoprotein particles. However, animal studies indicated that TG concentrations were modified by PDZK1 expression (14). More specifically, mice overexpressing the receptor had lower TG concentrations, whereas PDZK1 knock-out (KO) mice had higher TG concentrations (14).

Although a number of studies from mice (9-11,14) have reported that hepatic PDZK1 plays a key role in the HDL metabolism through its interaction with SCARB1, PDZK1_i33968C > T SNP did not modulate HDL-C concentrations in our study population. The apparently lesser role of PDZK1 in HDL metabolism in humans may be due to the normal expression of SCARB1 in extrahepatic tissues and cells (e.g. macrophages) despite hepatic PDZK1/SCARB1 deficiency (9,10,26), contributing to the normal lipid transport activity. Moreover, the unaffected expression of other genes involved in the RCT pathway, such as the cholesteryl ester transfer protein (CETP), may explain the lack of association between PDZK1 genotypes and HDL-C. In this regard, Zhou et al. (27) did not find differences in HDL-C levels between PDZK1/CETP double KO mice and single CETP KO control mice, concluding that CETP expression was independent of PDZK1. Likewise, the reported uptake of cholesterol esters from LDL particles independently of SCARB1 expression (28) supports the lack of association between PDZK1 genotypes and LDL-C levels.

Importantly, i33968T allele carriers had lower adiponectin levels than CC homozygotes, suggesting a more inflammatory state that may predispose individuals toward the cluster of risk factors associated with MetS (29,30). Although hypertriglyceridemia was the only individual MetS trait significantly associated with the i33968T SNP, we also found trends toward abdominal obesity and hypertension as well as borderline effects on glucose and insulin levels, suggesting a combination of minor effects collectively contributing to MetS. Moreover, hypoadiponectinemia has been closely related with the clinical phenotype of MetS (29), reinforcing the role of PDZK1_i33968C > T SNP in the development of MetS. The mechanism by which this polymorphism may contribute to the observed associations is unknown. Given its location in a noncoding region, the likelihood that this SNP represents a functional mutation is low. However,
the presence of transcriptional enhancers and other regulatory elements, observed frequently in intronic regions (31), may explain our findings.

We also examined the association between the PDZK1_i33968C > T SNP and lipoprotein concentrations and particle size. Previous studies in mice have reported increased concentrations of large HDL and VLDL particles in hepatic PDZK1 deficiency (11,15,26,32). However, we did not find significant associations between PDZK1 and HDL size. Normal expression of SCARB1 in extraneoplastic tissues is likely to have compensated for PDZK1 deficiency by preventing the build-up of excess unesterified cholesterol in the plasma and limiting the size of HDL particles. As reported previously in mice (11,32), the increased concentrations of VLDL particles in i33968T allele carriers compared with CC homozygotes may be explained by their reduced clearance as a direct effect of an impaired uptake of VLDL by the liver as a result of SCARB1 deficiency.

Overall, these metabolic abnormalities have been reported previously in KO mice (14,26,32) in which PDZK1 expression was inactivated. Moreover, 2 prior studies have demonstrated that PDZK1 deficiency promotes atherosclerosis in murine models (26,32). In this regard, Kocher et al. (26) reported that the inactivation of PDZK1 expression in mice increased aortic atherosclerosis through the suppression of the HDL-dependent activation of endothelial nitric oxide synthase with a reduction of nitric oxide levels. Given the MetS-related phenotypes observed in i33968T allele carriers, we speculate a potential downregulation of the PDZK1 gene in those participants.

In addition to the genetic susceptibility, environmental factors such as dietary habits may contribute to MetS. Growing evidence supports the possibility that diets with low PUFA (33–36) and moderate-to-high carbohydrate contents (37,38) are associated with increased risk of MetS through mechanisms involving dyslipidemia, vascular dysfunction, and insulin resistance. Moreover, whereas epidemiological data support the role of SCARBI gene in those participants.

In conclusion, this study conducted in a relatively large North American Caucasian population supports the hypothesis that PDZK1_i33968C > T SNP is associated with risk of MetS, which is driven primarily by the association of the i33968T allele carriers with higher TG and lower adiponectin concentrations. This association was particularly evident in obese participants with high dietary intake from PUFA and carbohydrates. Interestingly, this gene × BMI × diet interaction offers the potential to identify dietary and other lifestyle changes that, when implemented, may obviate the onset of MetS in individuals with a specific genetic background.

**Literature Cited**


43. Speakman JR. Thrifty genes for obesity and the metabolic syndrome: time to call off the search? Diab Vasc Dis Res. 2006;3:7-11.