ACC2 gene polymorphisms, metabolic syndrome, and gene-nutrient interactions with dietary fat

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Abstract Acetyl-CoA carboxylase β (ACC2) plays a key role in fatty acid synthesis and oxidation pathways. Disturbance of these pathways is associated with impaired insulin responsiveness and metabolic syndrome (MetS). Gene-nutrient interactions may affect MetS risk. This study determined the relationship between ACC2 polymorphisms (rs2075263, rs2268387, rs2284685, rs2284689, rs2300453, rs3742023, rs3742026, rs4766587, and rs6606667) and MetS risk, and whether dietary fatty acids modulate this in the LIPGENE-SU. VLMAX study of MetS cases and matched controls (n = 1754). Minor A allele carriers of rs4766587 had increased VI.MAX study of MetS cases and matched controls (n = 3500–3507). Lower ACC2 rs4766587 carrier status was associated with higher odds of MetS (OR 1.82 [CI 1.14, 2.94], P = 0.01; OR 1.62 [CI 1.05, 2.50], P = 0.0064). This was exacerbated in individuals with a high-fat intake (>35% energy) (OR 1.62 [CI 1.05, 2.50], P = 0.027), particularly a high intake (>5.5% energy) of n-6 polyunsaturated fatty acid (PUFA) (OR 1.82 [CI 1.14, 2.94], P = 0.01; OR = 0.05 for gene-nutrient interaction). Saturated and monounsaturated fatty acid intake did not modulate MetS risk. Importantly, we replicated some of these findings in an independent cohort. In conclusion, the ACC2 rs4766587 polymorphism influences MetS risk, which was modulated by dietary fat, suggesting novel gene-nutrient interactions.

Supplementary key words genetic polymorphisms • insulin resistance • fatty acid metabolism • polyunsaturated fatty acids

Abbreviations: ACC2, acetyl-CoA carboxylase β; BMI, body mass index; CVD, cardiovascular disease; FDR, false discovery rate; HOMA, homeostasis model assessment; HWE, Hardy-Weinberg Equilibrium; LC n-3 PUFA, long chain n-3 PUFA; MetS, metabolic syndrome; OR, odds ratio; QUICKI, quantitative insulin-sensitivity check index; SFA, saturated fatty acid; SNP, single nucleotide polymorphism; SREBP, sterol regulatory element binding protein; TAG, triacylglycerol; T2DM, type 2 diabetes mellitus.

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Acetyl-CoA carboxylase α (ACC1) and β (ACC2) catalyze the carboxylation of acetyl-CoA to malonyl-CoA. The level of malonyl-CoA regulates the rate of fatty acid entry into mitochondria and fatty acid oxidation by modulating carnitine palmitoyltransferase-1. Thus, ACC1 and ACC2 are key regulators of the fatty acid synthesis and oxidation pathways (1–5). Much of the work on ACC to date has focused on animal studies, where cellular distribution of ACC and mRNA tissue expression patterns suggested separate functional roles for ACC1 and ACC2 (1, 6–9). However, recent examination of human ACC expression indicates greater expression of ACC2 in both oxidative and lipogenic tissues (10).

Excess lipid accumulation is an important factor contributing to the development of insulin resistance, metabolic syndrome (MetS), and type 2 diabetes (T2DM) (11–14). MetS is a common, multicomponent condition characterized by abdominal obesity, insulin resistance, dyslipidemia, and hypertension, and it is associated with increased risk of T2DM and cardiovascular disease (CVD) (15). The association of disturbed fatty acid metabolism with such conditions suggests ACC as an attractive therapeutic target. Experiments using ACC knockout mice models and treatment with ACC inhibitors confirm the potential of ACC-targeted treatment to reduce risk factors associated with insulin resistance, obesity, MetS, and T2DM (6, 7, 16, 17).

The current global epidemic in the incidence of MetS and T2DM is an important illustration of the interaction between environmental and genetic factors to diet-related polygenic disorders. Dietary fat is an important environmental factor, where excessive exposure (high-fat, obeseogenic, insulin-desensitizing diets) and interaction with genetic factors play a key role in the development of MetS (18–23). ACC gene expression is under hormonal and nutritional control (9). Polyunsaturated fatty acids (PUFA) can decrease ACC expression in vitro (24), and feeding studies in rats have demonstrated that dietary fat composition influences hepatic ACC expression and activity (25). In humans, lifestyle intervention, including dietary advice and physical exercise, has reduced ACC2 expression (26). While ACC2 clearly plays an important role in lipid metabolism, insulin resistance, and obesity, no studies to date have examined the potential association between genetic variants of ACC2 and those traits. Therefore, we investigated the potential relationship between common genetic polymorphisms of ACC2 and MetS and its phenotypes, and whether this is modulated by dietary fatty acid intake.

**MATERIALS AND METHODS**

**Subjects, MetS classification, and study design**

This study is part of a prospective case control candidate gene study of LIPGENE, a European Union (EU) Sixth Framework Integrated Programme project entitled “Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis.” Participants were selected from an existing French SU.VI.MAX cohort including 13,000 adults studied over 7.5 years from 1994 to 2002 (27). The LIPGENE-SU.VI.MAX study is a nested case control study of MetS including women aged 35–60 years and men aged 45–60 years recruited from SU.VI.MAX (28). Additional ethical approval from the ethics committee (CCPPRB of Paris-Cochin Hospital) included an additional clause (N° Am 2840-12-706) to perform biochemical analysis and genetic analyses required for the LIPGENE study. LIPGENE participants were informed of the study objectives and signed a consent form. Participants were invited to provide a 24 h dietary record every two months, for a total of six records per year. Information was collected with the use of computerized questionnaires that were transmitted during a brief telephone connection via the Minitel Telematic Network (France Télécom, Paris, France), a small terminal that was widely used in France as an adjunct to the telephone. Participants were guided by the software’s interactive facilities and by a previously validated instruction manual for coding food portions, including more than 250 foods presented in three portion sizes. Two intermediate and extreme portions could also be chosen, yielding a total of seven choices for estimating quantities consumed (29). Baseline daily dietary intake data was estimated by using food composition tables validated for the French population (30).

Baseline and 7.5 year follow-up data, including full clinical examination records, were made available to LIPGENE. These data were used to identify cases (i.e., individuals who developed elements of MetS) during the 7.5 y follow up period and controls. MetS cases were selected according to the NCEP-ATP III criteria for MetS (31). Participants were required to fulfill at least three of the following five criteria: increased waist circumference (>94 cm (men) or >80 cm (women)); elevated fasting blood glucose (>5.5 mmol/l or treatment for diabetes); elevated triacylglycerol (TAG) (>1.5 mmol/l or treatment for dyslipidemia); low high-density lipoprotein cholesterol (<1.04 mmol/l (men) or <1.29 mmol/l (women)); or elevated systolic/diastolic blood pressure (>130/85 mm Hg or antihypertensive treatment). MetS cases were defined as men or women with ≥3 abnormalities; controls were defined as men with ≤1 abnormality or women with no abnormalities. Cases and controls (n = 1754) were matched according to age (±5 years), gender, and number of dietary records available. For replication purposes, we analyzed data from a separate, independent LIPGENE MetS case-only cohort of 464 subjects participating in a 12-week dietary intervention to alter the quantity and quality of dietary fatty acid intake (32).

**Biochemical analysis**

Fasting glucose, TAG, HDL, and total cholesterol were measured as previously described (35). Insulin and C-peptide were determined by electrochemiluminescence immunoassays (Roche Diagnostics, France). NEFA and LDL cholesterol were measured by enzymatic colorimetric methods (Randox Laboratories, UK, and Roche Diagnostics, France). Homeostasis model assessment (HOMA), a measure of insulin resistance, was calculated as:

\[
\text{HOMA} = \frac{(\text{fasting plasma glucose} \times \text{fasting serum insulin})}{22.5}
\]

(34). Quantitative insulin-sensitivity check index (QUICKI), a measure of insulin sensitivity, was calculated as:

\[
\text{QUICKI} = \frac{1}{\log \text{fasting insulin} + \log \text{fasting glucose} + \log \text{fasting NEFA}}
\]

(35).

**DNA extraction and genotyping**

DNA extraction from buffy coats and whole genome amplification of low yielding samples (<10 ng) was performed as previously described (36). ACC2 genotype data from HapMap v1.1 (www.hapmap.org) was uploaded into HITAGENE, a web-based combined database and genetic analysis software suite developed by Hitachi Dublin Laboratory. Haplotype frequencies were estimated by implementation of the expectation maximization algorithm. Using a 5% cut-off for individual haplotype frequency and >70% for the sum of all haplotype frequencies, haplotype-tagged ACC2 genotype, dietary fat, and metabolic syndrome 3501
SNPs (rs2075263, rs2268387, rs2284685, rs2284689, rs2300453, rs3742023, rs3742026, rs4766587, and rs6606697) were identified using SNP Tagger (37). SNPs were genotyped as part of the entire genotyping component of the LIPGENE study by Illumina Inc. (San Diego, CA) using the Golden Gate Assay on a BeadStation 500G genotyping system. We achieved an average genotyping success rate of 99% and call rate of 99%. Linkage disequilibrium between SNPs and departure of genotype distributions from Hardy-Weinberg Equilibrium (HWE) were assessed in HITAGENE.

Statistical analysis

Statistical analysis was performed using SAS for Windows™, version 9.0 (SAS Institute, Cary, NC). Data are expressed as means ± SEM. After checking for skew and kurtosis, glucose, insulin, NEFA, TAG, QUICKI, and HOMA were normalized by logarithmic transformation. Genotype frequencies were compared between cases and controls in HITAGENE using Fisher’s exact test. Conditional logistic regression determined associations between genotypes and continuous MetS phenotypes. Estimating equation (GEE) linear regression (38) investigated associations below and above the fatty acid median. Generalized t-tests were used to compare associations between genotype and anthropometric measures and insulin-related phenotypes in the LIPGENE MetS case-only replication study (32). P < 0.05 was considered significant.

RESULTS

Genetic variation at the ACC2 locus and MetS risk

Table 1 details the ACC2 polymorphisms studied. All SNPs were in HWE (P > 0.01). Genotype frequencies were different between MetS cases and controls for rs4766587 (P = 0.0018, FDR = 0.025); rs3742023 (P = 0.025, FDR = 0.03); and rs2284685 (P = 0.042, FDR = 0.19). Examination of allele distributions revealed differences between MetS cases and controls for rs4766587 (OR 1.31 [CI 1.12, 1.54], P = 0.0008, FDR = 0.01), where the minor A allele was more frequent in the MetS cases. Allele frequency differences failed to reach statistical significance for rs4766587 (OR 1.14 [CI 0.99, 1.31], P = 0.06, FDR = 0.09) and rs3742023 (OR 1.13 [CI 0.98, 1.30], P = 0.09, FDR = 0.12). Only the association between rs4766587 and MetS risk remained significant following correction for multiple testing (FDR < 0.05) and in the multivariate logistic regression.
analysis, where MetS risk conferred by possession of the A allele was almost 30% higher relative to the GG homozygotes (OR 1.29 [CI 1.08, 1.58], P = 0.0064, FDR = 0.02). Following further adjustment for insulin and glucose concentrations, this association remained significant (OR 1.41 [CI 1.14, 1.98], P = 0.015); thus, we focused our analyses on this polymorphism. Homogeneity of the genotype effect of ACC2 rs4766587 on MetS was assessed by stratifying according to gender. This analysis revealed that the association with MetS primarily derived from the male subjects (OR 2.31 [CI 1.20, 4.44], P = 0.06). Formal tests of heterogeneity (Breslow-Day) between effect was in the same direction in the female subjects, it was not significant (OR 1.22 [CI 0.69, 3.01], P = 0.66). Clinical characteristics according to gender and found no gender differences between genotypes.

**Gene-nutrient interactions modulate MetS risk**

We examined the influence of dietary fat intake on MetS risk by stratifying according to the control median fat intake. Dietary intake of all types of fatty acids (SFA, PUFA, and MUFA) was higher among the high-fat consumers (>35% energy) compared with low-fat consumers (<35% energy) (P<0.0001). Interestingly MetS risk was modulated by dietary fat status, where risk conferred by being an A allele carrier for rs4766587 was exacerbated among individuals with a high-fat intake (>35% energy) (OR 1.66 [CI 1.04, 2.70], P = 0.027). Interaction analysis confirmed this gene-nutrient interaction (P = 0.04). Conversely, MetS risk was abolished among individuals with a low-fat intake (Fig. 1).

Examination of the individual fatty acid classes identified a gene-nutrient interaction with PUFA (P = 0.035 for gene-nutrient interaction), where A allele carriers with high PUFA intake (>5.5% energy) had increased MetS risk (OR 1.53 [CI 1.02, 2.60], P = 0.04). The interaction observed between rs4766587 and PUFA on MetS risk was reflected by both n-6 PUFA (OR 1.80 [CI 1.12, 2.83], P = 0.01; P = 0.05 for gene-nutrient interaction) and n-3 PUFA (OR 1.75

### Clinical characteristics according to ACC2 rs4766587 genotype

The clinical characteristics and dietary fatty acid intakes of subjects according to ACC2 rs4766587 genotype are presented in Table 2. In terms of their phenotype, A allele carriers had greater BMI (P = 0.03) and waist circumference (P = 0.03) compared with the GG homozygotes. Examination of the metabolic parameters revealed that A allele carriers had impaired insulin sensitivity as assessed by QUICKI (P = 0.04). No differences were noted with respect to the subjects’ lipid profiles, with the exception of NEFA, which were lower in the A allele carriers (P = 0.03). Age, gender distribution, medication use, and alcohol and dietary fat intake were not different between groups. We also examined the clinical characteristics across genotypes according to gender and found no gender differences between genotypes.

### TABLE 2. Clinical characteristics and dietary fat intakes of all subjects according to ACC2 rs4766587 genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GG</th>
<th>AG</th>
<th>AA</th>
<th>AG + AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>994</td>
<td>659</td>
<td>108</td>
<td>760</td>
</tr>
<tr>
<td>Male/female (%)</td>
<td>60/40</td>
<td>61/39</td>
<td>59/41</td>
<td>60/40</td>
</tr>
<tr>
<td>Age (y)</td>
<td>58 ± 0.17</td>
<td>58 ± 0.19</td>
<td>58 ± 0.33</td>
<td>58 ± 0.20</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 ± 0.14</td>
<td>26.1 ± 0.17</td>
<td>27.1 ± 0.47</td>
<td>26.8 ± 0.16</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>88 ± 0.42</td>
<td>89 ± 0.49</td>
<td>90 ± 1.3</td>
<td>90 ± 0.46</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.24 ± 0.03</td>
<td>5.27 ± 0.04</td>
<td>5.29 ± 0.02</td>
<td>5.28 ± 0.03</td>
</tr>
<tr>
<td>Insulin (mmol/l)</td>
<td>7.27 ± 0.19</td>
<td>7.46 ± 0.21</td>
<td>8.13 ± 0.70</td>
<td>7.55 ± 0.21</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.34 ± 0.00</td>
<td>0.32 ± 0.00</td>
<td>0.31 ± 0.00</td>
<td>0.32 ± 0.00</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>131 ± 0.50</td>
<td>131 ± 0.63</td>
<td>135 ± 1.63</td>
<td>132 ± 0.59</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>82 ± 0.30</td>
<td>82 ± 0.38</td>
<td>84 ± 0.89</td>
<td>82 ± 0.35</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.71 ± 0.03</td>
<td>5.70 ± 0.03</td>
<td>5.83 ± 0.10</td>
<td>5.72 ± 0.03</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.46 ± 0.01</td>
<td>1.48 ± 0.02</td>
<td>1.54 ± 0.04</td>
<td>1.49 ± 0.02</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.56 ± 0.04</td>
<td>3.48 ± 0.05</td>
<td>3.62 ± 0.11</td>
<td>3.50 ± 0.04</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1.27 ± 0.02</td>
<td>1.28 ± 0.03</td>
<td>1.17 ± 0.06</td>
<td>1.26 ± 0.03</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>0.94 ± 0.03</td>
<td>0.87 ± 0.03</td>
<td>0.88 ± 0.08</td>
<td>0.87 ± 0.03</td>
</tr>
<tr>
<td>Lipid lowering medication (%)</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Anti-diabetic medication (%)</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>Hypertensive medication (%)</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Dietary fat intake (% energy)</td>
<td>35.27 ± 0.23</td>
<td>35.09 ± 0.35</td>
<td>36.02 ± 0.10</td>
<td>35.19 ± 0.26</td>
</tr>
<tr>
<td>PUFA intake (% energy)</td>
<td>5.63 ± 0.07</td>
<td>5.65 ± 0.09</td>
<td>5.79 ± 0.25</td>
<td>5.67 ± 0.08</td>
</tr>
<tr>
<td>n-6 PUFA intake (% energy)</td>
<td>5.05 ± 0.07</td>
<td>5.10 ± 0.09</td>
<td>5.23 ± 0.05</td>
<td>5.12 ± 0.08</td>
</tr>
<tr>
<td>n-3 PUFA intake (% energy)</td>
<td>0.57 ± 0.01</td>
<td>0.55 ± 0.01</td>
<td>0.55 ± 0.03</td>
<td>0.55 ± 0.01</td>
</tr>
<tr>
<td>SFA intake (% energy)</td>
<td>15.46 ± 0.13</td>
<td>15.23 ± 0.14</td>
<td>15.65 ± 0.46</td>
<td>15.29 ± 0.14</td>
</tr>
<tr>
<td>MUFA intake (% energy)</td>
<td>14.19 ± 0.11</td>
<td>14.16 ± 0.13</td>
<td>14.71 ± 0.40</td>
<td>14.24 ± 0.13</td>
</tr>
<tr>
<td>Alcohol intake (% energy)</td>
<td>6.58 ± 0.25</td>
<td>6.82 ± 0.35</td>
<td>6.78 ± 0.54</td>
<td>6.80 ± 0.40</td>
</tr>
</tbody>
</table>

Values are means ± SEM. ACC2, acetyl-CoA carboxylase β; BMI, body mass index; DBP, diastolic blood pressure; QUICKI, quantitative insulin-sensitivity check index; SBP, systolic blood pressure; SFA, saturated fatty acid; TAG, triacylglycerol.

*Indicates P < 0.05 compared with GG homozygotes for linear regression adjusted for potential confounding factors.

*P < 0.01 obtained using ANOVA.

*P < 0.05 obtained using ANOVA.

*P < 0.05 for post hoc multiple comparisons as assessed by Bonferroni compared with the GG homozygotes.
take, these genetic differences persisted in the high-fat but not the low-fat consumers, in keeping with our original findings that a high-fat diet exacerbated and a low-fat diet abolished MetS risk. Furthermore, consistent with the original gene-PUFA interaction where A allele carriers with high PUFA intake had increased MetS risk, examination of this cohort also identified a gene-PUFA interaction. Among the high PUFA consumers, AA homozygotes had greater waist circumference ($P = 0.028$) compared with the GG homozygotes.

**DISCUSSION**

To our knowledge, this is the first study to report an association between ACC2 rs4766587 and the risk of having MetS. We demonstrated that a common genetic variant at the ACC2 locus, rs4766587, was associated with approximately 30% higher MetS risk, which may in part be explained by increased risk of abdominal obesity and impaired insulin sensitivity. There is no functional data on ACC2 rs4766587; thus, we can only speculate about mechanisms underlying our findings. The intronic location of this SNP has the potential to affect mRNA stability or mod-

![Fig. 1. MetS risk related to interactions between ACC2 rs4766587 and dietary fat intake. Odds ratios and 95% confidence intervals for the associations between ACC2 rs4766587 and the MetS, stratified according to dietary fat intake, were determined by logistic regression analyses comparing A allele carriers to the GG homozygotes. Potential confounding factors included in the analyses were age; gender; BMI; plasma insulin and glucose concentrations; smoking status; physical activity; alcohol intake; and treatment for lipid lowering, hypertension, and diabetes. MetS risk conferred by possession of the rs4766587 A allele was approximately 40% higher relative to the GG homozygotes ($P = 0.015$). MetS risk conferred by being an A allele carrier was exacerbated among the high-fat consumers (>35% energy) ($P = 0.027$) and was abolished among the low-fat consumers (<35% energy) ($P = 0.111$). ACC2, acetyl-CoA carboxylase β; BMI, body mass index; MetS, metabolic syndrome.]

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Controls (N = 877)</th>
<th>MetS Cases (N = 877)</th>
<th>OR [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCG</td>
<td>36.96%</td>
<td>35.65%</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GGA</td>
<td>36.21%</td>
<td>35.48%</td>
<td>0.98 [0.84, 1.15]</td>
<td>0.79</td>
</tr>
<tr>
<td>ACG</td>
<td>20.10%</td>
<td>25.26%</td>
<td>1.34 [1.12, 1.61]</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

Odds ratios, 95% CI, and P values for the association between ACC2 haplotypes (represented by rs4766587, rs3742023, and rs2284685) and the risk of having MetS. GCG, the most common haplotype, was used as the reference haplotype. ACC2, acetyl-CoA carboxylase β; MetS, metabolic syndrome; OR, odds ratio.

**Table 3. Haplotype frequencies and OR for MetS among all subjects**

**Replcation of genetic associations and gene-nutrient interaction**

We attempted to replicate our findings in a separate, independent LIPGENE MetS case-only cohort ($n = 464$) (32). We replicated the finding that rs4766587 AA homozygotes had increased BMI ($P = 0.006$), body weight ($P = 0.019$), and waist circumference ($P = 0.014$) relative to the GG homozygotes. Furthermore, in this MetS case-only cohort, AA homozygotes were more insulin-resistant ($P = 0.05$) (as assessed by HOMA) compared with the GG homozygotes. Interestingly, when stratified by dietary fat intake, these genetic differences persisted in the high-fat but not the low-fat consumers, in keeping with our original findings that a high-fat diet exacerbated and a low-fat diet abolished MetS risk. Furthermore, consistent with the original gene-PUFA interaction where A allele carriers with high PUFA intake had increased MetS risk, examination of this cohort also identified a gene-PUFA interaction. Among the high PUFA consumers, AA homozygotes had greater waist circumference ($P = 0.028$) compared with the GG homozygotes.
ulate ACC2 gene transcriptional activity. Cellular ACC2 activity is known to be dependent on mRNA translational rates and degree of protein phosphorylation states (41–44). However, there is a lack of studies in which ACC mRNA expression or ACC protein levels have been measured together with ACC2 activity, leaving the question of whether changes in ACC mRNA or protein actually translate into alterations of enzymatic activity. Nevertheless, work by Pape et al. demonstrated that starvation and diabetes reduced ACC mRNA as well as ACC activity (i.e., changes in the level of mRNA correspond to changes in the activity and amount of acetyl-CoA carboxylase), leading the authors to conclude that physiological conditions that affect fatty acid synthesis do so, in part, by modulating ACC gene expression (45). More recently Castle et al. demonstrated that overexpression of ACC2 isoforms [the usual ACC2v.1 and a novel isoform (ACC2v.2)] increase ACC2 enzymatic activity to a similar extent, indicating that changes at the protein level translate to alterations in activity (10). It is also possible that this SNP may be a surrogate marker for other functional SNPs of ACC2 or other genes in the region. Thus, our results require further investigation and validation, and functional studies are needed to ascertain their biological significance. We noted gender differences for the association between rs4766587 and MetS risk. Whereas the effect was in the same direction among female subjects, it did not reach statistical significance, which may reflect lack of statistical power. Studies in mice have shown that gender influences ACC expression (46); thus, our findings may also be linked to gender-specific differences in adipose tissue mass distribution or hormonal status.

Dietary fat status and composition modulated the genetic association with MetS, where high habitual dietary fat intake (>35% energy) accentuated the risk conferred by being an A allele carrier for rs4766587. Importantly, the greatest MetS risk was observed among individuals with the top 50th percentile dietary PUFA intake. These novel gene-nutrient interactions suggest that dietary fat intake may have the potential to modify genetic predisposition to MetS. An individual’s phenotype represents a complex interaction between genetic background and environmental factors during the individual’s lifetime. Environmental factors, such as lifestyle intervention (including dietary advice and physical exercise) leading to improved glycemic control and reduced adiposity, have been shown to down-regulate ACC2 expression in skeletal muscle (26). Other studies have demonstrated that ACC gene expression is under hormonal and nutritional control (9), and n-3 and n-6 PUFA (but not SFA or MUFA) have been shown to reduce ACC expression in vitro (24). Whereas these data support our findings in that comparable effects of n-3 and n-6 PUFA intake and lack of effect of saturated or monounsaturated fat intake were observed on MetS risk, the underlying mechanisms are unknown. Animal feeding studies have demonstrated the comparative effects of dietary fatty acid composition on ACC expression and activity (25). The authors report that hepatic ACC expression was higher following supplementation with saturated fat and also MUFA-rich olive oil compared with both n-6 PUFA-rich sunflower oil and n-3 PUFA-rich linseed oil supplementation. Furthermore, ACC expression was higher following sunflower oil feeding compared with either linseed or LC n-3 PUFA-rich sardine oil supplementation (25). Although this previous study (25) provides evidence that dietary fat quality can influence ACC expression, the authors did not examine whether the quantity of dietary fat alters ACC expression. Subsequent treatment of high-fat fed rats with ACC inhibitors demonstrated enhanced fatty acid oxidation, reduced TAG, and improved insulin sensitivity (17), supporting the potential of ACC-targeted treatment to reduce MetS risk factors. Indeed, ACC2 knockout mice are protected from diet-induced obesity and diabetes (6, 7, 16).

In our current study, MetS risk was subject to a significant effect modification by dietary fat intake, with the deleterious effect conferred by the A allele exacerbated among individuals consuming a high-fat diet, particularly one high in PUFA (both n-3 and n-6 PUFA). One interpretation could be that A allele carriers (43% of this population) may be more sensitive to dietary fat, so that high total fat or PUFA intake exacerbates this specific genetic susceptibility of developing MetS. This gene-PUFA interaction is somewhat surprising and needs confirmation in other populations, considering that high n-6 PUFA have been mostly associated with decreased risk of MetS and T2DM (21–23, 47). Interestingly, saturated and monounsaturated fat intake did not modulate MetS risk, suggesting a PUFA-specific effect. Previous work has shown that n-3 and n-6 PUFA can decrease expression of both ACC and sterol regulatory element binding protein (SREBP) (24, 48). Note that SREBP may regulate ACC expression (49–51). In addition, cellular ACC2 activity depends on phosphorylation status (41–44), and insulin is known to activate ACC activity by promoting dephosphorylation (52). Recent data has shown reduced ACC phosphorylation in conjunction with increased insulin-stimulated glucose disposal in T2DM patients following insulin-sensitizing therapy (53). The authors of that study hypothesized that increased expression of genes involved in fat oxidation and mitochondrial function, such as ACC, may lead to reduced production of lipotoxic metabolites that have the potential to improve insulin signaling and augment insulin sensitivity. Whether PUFA may exert similar effects via ACC is unknown. Similarly, the mechanisms underlying interactions between PUFA and ACC2 genotype have not been elucidated and warrant further investigation.

Due to the limited number of SNPs (n = 1, rs4766587) studied, hypothesis-driven statistical tests performed, and lack of consensus regarding the best strategy for correction for multiple testing (54, 55), we decided to present the unadjusted P values for this single SNP analysis. It is worth noting that in the current study, the phenotypic data substantiate the genetic data, where risk genotypes were associated with greater BMI, waist circumference, and impaired insulin sensitivity and, as such, should be given more credence. However replication of these gene-nutrient interactions in an independent cohort would be
understanding of the molecular mechanisms underlying habitual dietary fat intake, in particular PUFA. A better
appreciation of the genetic predisposition to MetS conferred by genetic and MetS risk. The novel gene-nutrient interac-
tion hypothesis is important for developing personalized dietary recommendations to reduce MetS risk among subjects with different genetic back-
grounds.

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