Gene-nutrient interactions in the metabolic syndrome: single nucleotide polymorphisms in \textit{ADIPOQ} and \textit{ADIPOR1} interact with plasma saturated fatty acids to modulate insulin resistance\textsuperscript{1–3}

Jane F Ferguson, Catherine M Phillips, Audrey C Tierney, Pablo Pérez-Martínez, Catherine Defoort, Olfa Helal, Denis Lairon, Richard Planells, Danielle I Shaw, Julie A Lovegrove, Ingrid MF Gjelstad, Christian A Drevon, Ellen E Blaak, Wim HM Saris, Iwona Leszczyńska-Goląbek, Beata Kiec-Wilk, Ulf Risérus, Brita Karlström, José Lopez-Miranda, and Helen M Roche

\textbf{ABSTRACT}

\textbf{Background:} Progression of the metabolic syndrome (MetS) is determined by genetic and environmental factors. Gene-environment interactions may be important in modulating the susceptibility to the development of MetS traits.

\textbf{Objective:} Gene-nutrient interactions were examined in MetS subjects to determine interactions between single nucleotide polymorphisms (SNPs) in the adiponectin gene (\textit{ADIPOQ}) and its receptors (\textit{ADIPOR1} and \textit{ADIPOR2}) and plasma fatty acid composition and their effects on MetS characteristics.

\textbf{Design:} Plasma fatty acid composition, insulin sensitivity, plasma adiponectin and lipid concentrations, and \textit{ADIPOQ}, \textit{ADIPOR1}, and \textit{ADIPOR2} SNP genotypes were determined in a cross-sectional analysis of 451 subjects with the MetS who participated in the LIPGENE (Diet, Genomics, and the Metabolic Syndrome: an Integrated Nutrition, Agro-food, Social, and Economic Analysis) dietary intervention study and were repeated in 1754 subjects from the LIPGENE-SU.VILMAX (SUPplementation en Vitamines et Minéraux AntioXydants) case-control study (http://www.ucd.ie/lipgene).

\textbf{Results:} Single SNP effects were detected in the cohort. Triacylglycerols, nonesterified fatty acids, and waist circumference were significantly different between genotypes for 2 SNPs (rs266729 in \textit{ADIPOQ} and rs10920533 in \textit{ADIPOR1}). Minor allele homozygotes for both of these SNPs were identified as having degrees of insulin resistance, as measured by the homeostasis model assessment of insulin resistance, that were highly responsive to differences in plasma saturated fatty acids (SFAs). The SFA-dependent association between \textit{ADIPOR1} rs10920533 and insulin resistance was replicated in cases with MetS from a separate independent study, which was an association not present in controls.

\textbf{Conclusions:} A reduction in plasma SFAs could be expected to lower insulin resistance in MetS subjects who are minor allele carriers of rs266729 in \textit{ADIPOQ} and rs10920533 in \textit{ADIPOR1}. Personalized dietary advice to decrease SFA consumption in these individuals may be recommended as a possible therapeutic measure to improve insulin sensitivity. This trial was registered at clinicaltrials.gov as NCT00429195. \textit{Am J Clin Nutr} 2010;91:794–801.

\textbf{INTRODUCTION}

The metabolic syndrome (MetS) is a complex disorder characterized by abdominal obesity, insulin resistance, hypertension, dyslipidemia, and inflammation\textsuperscript{1}. It is associated with severe health complications, such as an increased risk of type 2 diabetes and cardiovascular disease (CVD). Development of the MetS is influenced by genetic as well as environmental factors, with dietary fat being a major environmental factor\textsuperscript{(2, 3)}. Adiponectin is the most quantitatively abundant adipokine secreted by adipocytes\textsuperscript{(4)}. Concentrations of circulating adiponectin are reduced in obese and type 2 diabetes subjects\textsuperscript{(5)}. It is proposed that this hormone is a key player in the etiology of the MetS, because it may be an important regulator of insulin sensitivity and inflammation. Thus, polymorphisms in the \textit{ADIPOQ} gene and its receptors, \textit{ADIPOR1} and \textit{ADIPOR2}, may play a role in the pathogenesis of the MetS\textsuperscript{(6)}. Functional studies in animal models have shown that adiponectin attenuates insulin resistance by reducing the triacylglycerol content in muscle and liver\textsuperscript{(7)}. Adiponectin knockout mice develop insulin resistance in response to a high-fat, high-sucrose diet\textsuperscript{(8)}, whereas mice with increased plasma adiponectin exhibit improved insulin sensitivity\textsuperscript{(9)}. The precise roles of the adiponectin receptors are not yet fully known, and they may have diverse and even contradictory effects. The mouse \textit{Adipor1} receptor appears to act via the AMPK pathway, whereas the \textit{Adipor2} receptor has greater involvement in peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \)) signaling\textsuperscript{(10)}. \textit{Adipor1} knockout mice demonstrated a role in the pathogenesis of the MetS (6).

\textsuperscript{1} From the University College Dublin, Dublin, Ireland (JFF, CMP, ACT, and HMR); the University of Reading, Reading, United Kingdom (DIS and JAL); the University of Oslo, Oslo, Norway (IMFG and CAD); INSERM, University Méditerranée Aix-Marseille, Faculty of Medicine, Marseille, France (CD, DL, OH, and RP); the Research Institute Maastricht, Maastricht, Netherlands (EEB and WHMS); the University of Cordoba, Cordoba, Spain (PPM and JL-M); Jagiellonian University Medical College, Krakow, Poland (IL-G and BK-W); and Uppsala University, Uppsala, Sweden (UR and BK).

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\textsuperscript{3} Address correspondence to HM Roche, Nutrigenomics Research Group, UCD School of Public Health and Population Science, UCD Conway Institute, University College Dublin, Ireland. E-mail: helen.roche@ucd.ie.

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velop obesity and decreased glucose tolerance, whereas Adipor2 knockout mice appear protected against diet-induced obesity and have better glucose tolerance and reduced cholesterol (11). However, Adipor2 deletion leads to inadequate pancreatic β cell function and type 2 diabetes in the longer term (12). In humans, associations have been found between many polymorphisms in ADIPOQ, ADIPOR1, and ADIPOR2 and adiponectin concentrations, insulin resistance, and MetS phenotypes (13, 14). Inconsistencies in the reported associations of polymorphisms in these genes and metabolic measures could be due to environmental interactions, particularly dietary factors. Gene-nutrient interactions can modulate the effect of certain polymorphisms in MetS-related traits (15). Although, thus far, there has been little focus on gene-nutrient interactions with ADIPOQ and its receptors, one study conducted in obese females found that there was an interaction between the rs266729 polymorphism of ADIPOQ and the percentage of dietary-derived energy from fat with the development of obesity (16).

Our present study addressed the hypothesis that interactions between adiponectin and adiponectin receptor gene variants and fatty acids may modulate metabolism of subjects with the MetS, particularly insulin resistance. Thus, we examined interactions between polymorphisms in ADIPOQ, ADIPOR1, and ADIPOR2 and plasma fatty acid composition in relation to metabolic indexes of insulin resistance, dyslipidemia, hypertension, obesity, and plasma adiponectin in 451 subjects with the MetS. A second MetS case-control population (n = 1754) was used to attempt to replicate the gene-nutrient interactions.

SUBJECTS AND METHODS

Subjects

Subjects aged 35–70 y with a body mass index (BMI; in kg/m²) of 20 to 40 were recruited for the LIPGENE (Diet, Genomics, and the Metabolic Syndrome: an Integrated Nutrition, Agro-food, Social, and Economic Analysis) dietary intervention study, details of which have been published (17). In the present study, the preintervention data for 451 subjects were analyzed. Subjects were recruited from 8 European countries: Ireland (n = 59), United Kingdom (n = 58), Norway (n = 63), France (n = 39), the Netherlands (n = 45), Spain (n = 70), Poland (n = 67), and Sweden (n = 50). See Supplemental Table 1 under "Supplemental data" in the online issue for the clinical characteristics of the subjects. All centers conformed to the Helsinki Declaration of 1975 as revised in 1983. Subject eligibility was determined by using a modified version of the National Cholesterol Education Program (NCEP) criteria for MetS (18), and subjects were required to fulfill ≥3 of the following 5 criteria: waist circumference >102 cm (men) or >88 cm (women), fasting glucose 5.5–7.0 mmol/L, triacylglycerol ≥1.5 mmol/L, HDL cholesterol <1.0 mmol/L (men) or <1.3 mmol/L (women), and blood pressure ≥130/85 mm Hg or treatment of previously diagnosed hypertension. Anthropometric measurements and samples taken in the supine position were recorded according to a common standardized protocol for the LIPGENE study, and blood pressure was measured according to the European Society of Hypertension Guidelines (19). Subjects for the LIPGENE-SU.VI.MAX (SUPplementation en VItamines et Minéraux AntiOxydants) population were selected from the existing French SU.VI.MAX cohort, a prospective population-based study (13,000 subjects studied over 7 y, 1995–2002) (20). Baseline and 7-y follow-up data were made available to LIPGENE and were used to identify individuals who developed elements of the MetS over the 7-y follow up period and matched control subjects (n = 1754). MetS cases and controls were selected according to screening criteria and a scoring system, based on World Health Organization and the NCEP-Adult Treatment Panel III criteria for the MetS. Additional ethical approval from the ethical committee (of Paris-Cochin Hospital) included an additional clause (n° Am 2840–12–706) to perform the biochemical analysis and genetic analysis required for the LIPGENE study. LIPGENE-SU.VI.MAX subjects (women aged 35–60 y and men aged 45–60 y) were informed of the study objectives and signed a consent form. Plasma fatty acids, homeostasis model assessment of insulin resistance (HOMA-IR), and SNP genotyping data were analyzed here, and there were complete data for 818 cases and matched controls. There was no overlap in participants between the LIPGENE dietary intervention study and the LIPGENE-SU.VI.MAX population.

Biochemical measurements

In the LIPGENE dietary intervention cohort, plasma, serum, and buffy coat were prepared from 12-h fasting blood samples from each subject. Serum insulin was measured with a solid-phase, 2-site fluoroimmunometric assay on a 1235 automatic immunoassay system (AutoDELFIA kits; Wallac Oy, Turku, Finland). Plasma glucose concentrations were measured by using the IL Test Glucose Hexokinase Clinical Chemistry kit (Instrumentation Laboratories, Warrington, United Kingdom). HOMA-IR was derived from fasting glucose and insulin concentrations [(fasting plasma glucose × fasting serum insulin)/22.5] as determined previously (21). Plasma concentrations of adiponectin were measured by enzyme-linked immunosorbent assay (Duoset ELISA Development System; R&D Systems, Minneapolis, MN). Cholesterol and triacylglycerol were quantified by using the IL Test Cholesterol kit and the IL Test Triglycerides kit (Instrumentation Laboratories, Warrington, United Kingdom). The IL Test HDL-C Kit (Instrumentation Laboratories) was used for direct quantification of HDL cholesterol. The WAKO NEFA C enzymatic color kit (Alpha Laboratories, Hampshire, United Kingdom) was used to quantify plasma nonesterified fatty acid (NEFA) concentrations. The plasma fatty acid composition for both study populations was measured on a GC 2010 gas-liquid chromatograph (Shimadzu, Japan), as previously described (22). In the LIPGENE-SU.VI.MAX population, fasting glucose was measured as previously described (20). Fasting insulin concentrations were measured by electrochemiluminescence immunoassays (Roche Diagnostics, Meylan, France), and HOMA-IR was calculated from these concentrations as described previously.

SNP selection and genotyping

Genotype data from HapMap (http://hapmap.org) (ADIPOQ, ADIPOR1, and ADIPOR2) were uploaded into HITAGENE, a combined database and set of genetic analysis tools developed by Hitachi Dublin Laboratory. Haplotype frequencies in each
gene were estimated by implementation of the expectation maximization algorithm. Using a 5% cutoff for individual haplotype frequency and >70% for the sum of all haplotype frequencies, genotype data were then transferred from HITAGENE to SNP tagger (http://www.broad.mit.edu/mpg/tagger/server.html) to identify haplotype tagged (Ht) SNPs. A combination of Ht SNPs and SNPs previously mentioned in the literature were genotyped providing a total of 11 SNPs in 3 genes: ADIPOQ (rs266729, rs822395, rs17366568, rs2241766, and rs1063538), ADIPOR1 (rs2275737, rs10753929, and rs10920533), and ADIPOR2 (rs6489323, rs1058322, and rs10848571). DNA was extracted from buffy coat samples by using the AutoPure LS automated system (Genta Systems Inc, Minneapolis, MN), and low-yielding samples (<10 ng) were subjected to whole genome amplification by using the REPLI-g kit (Qiagen Ltd, West Sussex, United Kingdom). Genotyping was conducted by Illumina Inc (San Diego, CA). Adherence to Hardy-Weinberg equilibrium. Linkage disequilibrium between SNPs was assessed by using THESIAS version 3.1 (23). Because there was no significant linkage disequilibrium, all SNPs were retained for further analysis.

Statistical analysis

Biochemical variables were assessed for normality of distribution, and skewed variables were normalized by log_{10} or square root transformation as appropriate. Analysis of variance-based models were used to test for associations between individual SNPs and biomarkers of the MetS. Gene-nutrient interactions were tested for by using an SNP–fatty acid interaction term in a univariate general linear model. The effect of each SNP interacting with plasma saturated fatty acids (SFAs) on each biochemical variable was investigated using SFA as a continuous variable with an SNP-SFA interaction term and with SNP and SFA entered individually, controlling for age, sex, BMI, and LIPGENE center of origin. If a significant interaction was found, the result was verified by analysis of variance in the cohort dichotomized by the median level of SFA by using Tukey’s post hoc test. To correct for multiple testing, the false discovery rate (FDR) procedure was used for each SNP on all variables tested. Statistical analysis was carried out by using SPSS version 14.0 for Windows (SPSS Inc, Chicago, IL) and SAS for Windows (version 9.0, SAS Institute, Cary, NC).

RESULTS

Genotype frequencies and metabolic characteristics

Details of the 11 SNPs characterized in this study and the genotype frequencies in the LIPGENE cohort are presented in Table 1. There were no significant differences in genotype frequencies across the 8 European countries. All SNPs were used in the analysis; however, the results are presented for only the 2 SNPs (rs266729 and rs10920533) that showed a number of interesting significant associations. Characteristics of the subjects with different alleles for ADIPOQ rs266729 and ADIPOQ1 rs10920533 are summarized in Table 2. Triacylglycerol (P = 0.009) and NEFA (P = 0.003) concentrations were significantly different between the genotype groups for ADIPOQ rs266729, and waist circumference varied significantly for ADIPOQ1 rs10920533 (P = 0.021). The association with NEFA remained significant (FDR: P = 0.039) when the FDR was used to correct for multiple testing, whereas the association with triacylglycerol was reduced to borderline significance (FDR: P = 0.059), and the association with waist circumference was no longer significant (FDR: P = 0.27).

Effect of SFA-SNP interactions on insulin sensitivity

Because this study was primarily interested in potential interactions between genotypes and nutritional status, the effect of the 2 previously mentioned SNPs on metabolic markers was examined in combination with plasma fatty acid status. Statistically significant gene-nutrient interaction terms between SNPs and plasma SFAs were detected for effects on insulin sensitivity. Similar results were obtained for both fasting insulin and HOMA-IR. HOMA-IR was used for further analysis, because it takes into account both fasting insulin and fasting glucose. From the initial analyses using a univariate general linear model to assess statistical interaction, the overall interaction terms for the effect on HOMA-IR were as follows: rs266729 in ADIPOQ (P = 0.01; adjusted FDR: P = 0.059) and rs10920533 in ADIPOQ1.

TABLE 1

Details of all single nucleotide polymorphisms (SNPs) in ADIPOQ, ADIPOR, and ADIPOR2 genotypes and the observed frequencies in the LIPGENE (Diet, Genomics, and the Metabolic Syndrome: an Integrated Nutrition, Agro-food, Social, and Economic Analysis) dietary intervention cohort.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>SNP rs number</th>
<th>Location within gene</th>
<th>Genotype frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADIPOQ</td>
<td>3</td>
<td>rs266729</td>
<td>5’ near-gene</td>
<td>CC/CG/ GG = 41/162/248</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs822395</td>
<td>Intron 1</td>
<td>AA/AG/ GG = 191/206/54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs17366568</td>
<td>Intron 1</td>
<td>AA/AG/ GG = 787/357</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2241766</td>
<td>Exon 2</td>
<td>AA/AC/CC = 340/101/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1063538</td>
<td>3’ UTR</td>
<td>GG/GT/TT = 170/203/78</td>
</tr>
<tr>
<td>ADIPOR1</td>
<td>1</td>
<td>rs2275737</td>
<td>Intron 1</td>
<td>AA/AC/CC = 782/224/154</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs10753929</td>
<td>Intron 1</td>
<td>AA/AG/ GG = 894/351</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs10920533</td>
<td>Intron 1</td>
<td>AA/AG/ GG = 371/192/224</td>
</tr>
<tr>
<td>ADIPOR2</td>
<td>12</td>
<td>rs6489323</td>
<td>Intron 1</td>
<td>AA/AG/ GG = 232/182/39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1058322</td>
<td>Intron 1</td>
<td>AA/AG/ GG = 40/182/230</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs10848571</td>
<td>Intron 3</td>
<td>GG/GT/TT = 199/150/87</td>
</tr>
</tbody>
</table>

1 UTR, untranslated region.
TABLE 2
Clinical characteristics of subjects in the LIPGENE (Diet, Genomics, and the Metabolic Syndrome: an Integrated Nutrition, Agro-food, Social, and Economic Analysis) dietary intervention study related to ADIPOQ rs266729 and ADIPOIR rs10920533 genotypes1

<table>
<thead>
<tr>
<th>ADIPOQ</th>
<th>ADIPOIR rs10920533</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CC</strong></td>
<td><strong>CG</strong></td>
</tr>
<tr>
<td><strong>AA</strong></td>
<td><strong>AG</strong></td>
</tr>
</tbody>
</table>

| Sex (M/F) | Age (y) | Insulin (μIU/mL) | HOMA-IR | Glucose (mmol/L) | Adiponectin (μg/mL) | TAG (mmol/L) | Cholesterol (mmol/L) | LDL (mmol/L) | HDL (mmol/L) | TAG (μmol/L) | NEFA (μmol/L) | BMI (kg/m²) | Waist circumference (cm) | Diastolic BP (mm Hg) | Systolic BP (mm Hg) | Plasma SFA (% of total lipids) | Plasma MUFA (% of total lipids) | Plasma PUFA (% of total lipids) |
|----------|---------|-----------------|---------|-----------------|-------------------|----------|---------------------|------------|-------------|-------------|---------------|-------------|------------------|----------------------|-------------------|-----------------------|----------------------|------------------------|------------------------|
| 20/17    | 53.7 ± 1.3   | 11.0 ± 1.3      | 2.2 ± 0.6 | 3.4 ± 0.3       | 5.6 ± 0.1         | 3.5 ± 0.1 | 1.2 ± 0.1           | 1.2 ± 0.1  | 2.2 ± 0.2   | 713.1 ± 41.2 | 578.2 ± 16.5 | 32.6 ± 0.7  | 110.3 ± 2.6           | 87.3 ± 1.9          | 140.5 ± 2.5         | 32 ± 0.9            | 29.3 ± 0.7           | 38.8 ± 1.0       |
| 72/88    | ± 1.3     | ± 0.7           | ± 0.6    | ± 0.3           | ± 0.1             | ± 0.1    | ± 0.1               | ± 0.1     | ± 0.2       | ± 0.3       | ± 0.5         | ± 0.3        | ± 0.7             | ± 0.8                | ± 0.2              | ± 0.3                | ± 0.7               | ± 0.1                 |
| 106/143  | 9.8 ± 0.3   | 2.6 ± 0.1       | 3.9 ± 0.2 | 3.2 ± 0.1       | 3.1 ± 0.1         | 3.1 ± 0.1 | 1.1 ± 0.0           | 1.1 ± 0.0  | 1.8 ± 0.1   | 622.6 ± 15.2 | 626.2 ± 15.2 | 32.4 ± 0.3  | 105.6 ± 0.9           | 86.8 ± 0.8           | 139.6 ± 1.2         | 33.1 ± 0.6          | 27.8 ± 0.4           | 39.7 ± 0.4         |
| 0.789    | 0.596     | 0.766           | 0.137    | 0.286           | 0.303             | 0.009    | 0.003               | 0.009      | 0.02        | 0.003       | 0.003         | 0.938        | 0.180             | 0.499               | 0.739              | 0.137                | 0.192                |
| 20/17    | 55.2 ± 1.7  | ± 1.3           | ± 0.6    | ± 0.3           | ± 0.1             | ± 0.1    | ± 0.1               | ± 0.1     | ± 0.2       | 594.6 ± 41.5 | 612.9 ± 16.2 | 32.6 ± 0.7  | 105.6 ± 0.9           | 86.8 ± 0.8           | 139.6 ± 1.2         | 33.1 ± 0.6          | 27.8 ± 0.4           | 39.7 ± 0.4         |
| 72/88    | ± 1.3     | ± 0.7           | ± 0.6    | ± 0.3           | ± 0.1             | ± 0.1    | ± 0.1               | ± 0.1     | ± 0.2       | ± 0.3       | ± 0.5         | ± 0.3        | ± 0.7             | ± 0.8                | ± 0.2              | ± 0.3                | ± 0.7               | ± 0.1                 |
| 104/143  | 10.3 ± 1.2  | 2.9 ± 0.3       | 5.9 ± 0.1 | 3.2 ± 0.1       | 3.1 ± 0.1         | 3.1 ± 0.1 | 1.1 ± 0.0           | 1.1 ± 0.0  | 1.8 ± 0.1   | 619.9 ± 15.9 | 619.9 ± 15.9 | 32.4 ± 0.3  | 105.9 ± 0.7           | 86.1 ± 0.6           | 138.7 ± 1.0         | 33.2 ± 0.4          | 28.8 ± 0.8           | 37.5 ± 0.4         |
| 0.396    | 0.139     | 0.096           | 0.341    | 0.715           | 0.405             | 0.659    | 0.079               | 0.021      | 0.18        | 0.17        | 0.18          | 0.396        | 0.180             | 0.499               | 0.739              | 0.18                 | 0.25                 |
| 0.331    | 0.341     | 0.079           | 0.18     | 0.174           | 0.188             | 0.187    | 1.05               | 0.425      | 0.17        | 0.17        | 0.17          | 0.425        | 0.188             | 0.499               | 0.739              | 0.18                 | 0.17                 |

1 HOMA-IR, homeostasis model assessment of insulin resistance; TAG, triacylglycerol; NEFA, nonesterified fatty acid; BP, blood pressure; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Superscript letters indicate significant differences between groups (Tukey’s post hoc test).

2 ANOVA for differences between genotype groups.

3 Mean ± SEM (all such values).

ADIPORI (P = 0.004; adjusted FDR P = 0.026). There were no significant interactions between other plasma fatty acid variables (monounsaturated fatty acids and n−6 or n−3 polyunsaturated fatty acids) and SNPs on insulin sensitivity.

Effect of individual SNPs on insulin sensitivity at plasma SFA concentrations above or below the median of the cohort

The statistical interactions were probed further for their specific biological effects by dichotomizing the cohort with respect to the median plasma SFA concentrations. A difference was observed between genotype groups. The minor allele homozygotes displayed phenotypic variation in response to differences in plasma SFA concentrations, whereas in the major allele carriers insulin sensitivity remained relatively stable despite variations in SFAs. As shown in Figure 1, A and B, there were significant differences in HOMA-IR between genotype groups for rs266729 and rs10920533 depending on plasma SFA concentrations. From this, it is apparent that the degree of insulin sensitivity in individuals homozygous for the major allele (CC and AA) is sensitive to the concentrations of plasma SFAs, whereas the heterozygotes appear to be insensitive (CG and AG), and the major allele homozygotes (GG and GG) only showed a small response.

Combining SNPs into multilocus genotypes can improve the strength of the association

These 2 SNPs (rs266729 in ADIPOQ and rs10920533 in ADIPOIR) were investigated further to determine whether a combination of alleles into a multilocus genotype, interacting with plasma SFAs, increased the association with HOMA-IR. A combined interaction term of the 2 SNPs and plasma SFAs appeared to have a highly significant association with HOMA-IR (P < 0.001). Whereas this does not prove a biological interaction, the increased strength of association resulting from using both SNPs combined rather than individually could indicate that considering combined genotypes at both loci for individuals would be more informative than analyzing either locus individually. For both SNPs, the minor allele homozygotes displayed significant increases in HOMA-IR with high plasma SFA concentrations. Therefore, these individuals were pooled in a multilocus genotype and were compared with individuals who were heterozygous or major allele homozygous for either SNP. Application of analysis of variance on this multilocus genotype showed a highly significant effect of plasma SFAs on insulin sensitivity in individuals who were minor allele homozygotes for either of the SNPs in the multilocus genotype but not in those carrying a major allele at both loci, which indicated a significant gene-nutrient interaction in this multilocus genotype (Figure 2).

Using multilocus SNP combinations to strengthen the predictive power of the effects of genotype and plasma SFAs on insulin sensitivity

The predicted HOMA-IR values based on the model were determined by using a linear regression model for each of the 2 multilocus genotype groups and used for the graphic representation (Figure 3). A clear difference in HOMA-IR in response to plasma SFAs can be seen between the 2 multilocus genotype groups. Such information could theoretically be applied in the context of nutrigenomics, where an individual’s genotype at
these 2 SNPs might predict whether their degree of insulin resistance may be modulated by alterations in plasma SFAs.

Repliation of the effect of the rs10920533 SNP and plasma SFAs on HOMA-IR in a second population

To determine whether the gene-nutrient interactions observed in the LIPGENE dietary intervention cohort could be replicated in an independent population, data from the LIPGENE-SU.VI. MAX MetS case-control cohort were analyzed. As presented in Figure 4, the rs10920533 SNP in ADIPOR1 had a significant plasma SFA-dependent effect on HOMA-IR within MetS cases only. This association was not seen when looking at both cases and controls combined or in the control population alone. Specifically, the minor allele homozygotes (AA) again showed significant variation in HOMA-IR, depending on whether plasma SFA concentrations were above or below the median, whereas carriers of the major allele (AG and GG) appeared less sensitive to differences in plasma SFAs. The association with rs266729 was not replicated in the second population.

DISCUSSION

Single SNP associations with MetS traits

Adiponectin is an important insulin-sensitizing adipokine. Thus, polymorphisms in the adiponectin gene and its receptors are likely candidates for playing a role in the development of insulin resistance and the MetS. In obese subjects, SNPs in ADIPOQ were associated with adiponectin concentrations and type 2 diabetes (24–26). Adiposity-dependent associations with insulin resistance have also been reported (27–29). Data from previous studies suggest a possible involvement of many SNPs in the adiponectin receptors with potential importance to the MetS (31–36); however, there remains a lack of reliable replication of associations. In the present study, an association with waist circumference was also observed, albeit with a different SNP in ADIPOR1 (rs10920533) to those mentioned in the literature. The SNPs investigated have not been the same in each population, making direct comparisons with previous work more difficult.

SFA is an important mediator of the association between SNPs in adiponectin and its receptors and insulin sensitivity and plasma adiponectin

Few studies have investigated gene-nutrient interactions involving SNPs in ADIPOQ, ADIPOR1, or ADIPOR2. One study showed reduced adiponectin concentrations after x-linolenic acid supplementation in overweight individuals, although polymorphisms in 2 ADIPOQ SNPs did not appear to have any effect (37). However, this study was conducted in a small number of individuals, and adiponectin concentrations were different in the intervention group and controls before dietary intervention. A potential gene-nutrient interaction was found between the rs266729 polymorphism of ADIPOQ and the percentage of energy derived from fat in the diet for the development of obesity (16). In the present study, this SNP interacted with plasma SFAs to determine HOMA-IR, thus replicating the potential sensitivity of this SNP to dietary factors. In subjects with nonalcoholic fatty liver disease, an interesting association was observed between the rs2241766 and rs1501299 SNPs in ADIPOQ with postprandial adiponectin and lipoprotein profiles (38). Individuals with susceptible genotypes were more sensitive to an oral fat load, which might suggest greater susceptibility to suboptimal dietary fat intakes. Many studies reported associations with rs2241766 dependent on adiposity (27, 28), and it is
significant differences at the same plasma SFA category. Bars not sharing a common letter or symbol are used for comparisons between different genotype groups in the above or below the median within the same genotype group. Symbols (*) and (#) are used for comparisons between SFA concentrations and below the median and above the median within the same genotype group. Bars not sharing a common letter or symbol are significantly different at \( P < 0.05 \) (ANOVA and Tukey's post hoc test). The group comprising the CC or AA allele homozygotes from both single nucleotide polymorphisms showed significant differences in HOMA-IR depending on whether their plasma SFA concentration was above or below the median. The individuals with this "high responder" genotype and below-median fatty acid profile had a significantly lower HOMA-IR than did individuals with a below-median fatty acid profile combined with a CC or AA genotype and plasma SFA concentration above the median had a significantly higher HOMA-IR than did individuals with a "low-responder" genotype and an above-median SFA concentration. For CC or AA, \( n = 39 \) for below the median and \( n = 31 \) for above the median. For CG+GG and AG +GG, \( n = 181 \) for below the median and \( n = 180 \) for above the median.

possible that, in these patients, obesity may be linked to dietary patterns, for example, by high saturated fat consumption linked to higher lipogenic activity. A recent study reported a significant interaction between an ADIPOQ SNP and insulin resistance in an integrated nutrition, agro-food, social, and economic analysis cohort in relation to the multilocus genotype of ADIPOQ rs266729 and ADIPOQ rs10920533 and stratified by preintervention median plasma saturated fatty acid (SFA) concentrations. Values are expressed as group means ± SEs. Letters (a and b) are used for comparisons between SFA concentrations above or below the median within the same genotype group. Symbols (*) and (#) are used for comparisons between different genotype groups in the same plasma SFA category. Bars not sharing a common letter or symbol are significantly different at \( P < 0.05 \) (ANOVA and Tukey's post hoc test). The group comprising the CC or AA allele homozygotes from both single nucleotide polymorphisms showed significant differences in HOMA-IR depending on whether their plasma SFA concentration was above or below the median. The individuals with this "high responder" genotype and below-median fatty acid profile had a significantly lower HOMA-IR than did individuals with a below-median fatty acid profile combined with a CG/GG and AG/GG "low responder" genotype. Individuals with a "high responder" genotype and plasma SFA concentration above the median had a significantly higher HOMA-IR than did individuals with a "low-responder" genotype and an above-median SFA concentration. For CC or AA, \( n = 39 \) for below the median and \( n = 31 \) for above the median. For CG+GG and AG +GG, \( n = 181 \) for below the median and \( n = 180 \) for above the median.

SNP combination into multilocus genotypes improves the strength of association and predictive capacity

Evaluation of the interactions between SNPs may be advantageous, and detection of these interactions can indicate significant associations with a far greater effect than any one SNP individually. Combining SNPs that each contribute a small effect to the same trait can have a cumulative effect, greatly increasing predictive power, even in the absence of a direct interaction between the genes (40). In our present study, the combination of 2 SNPs, interacting with the same fatty acid variable to influence a shared trait, facilitated a greater predictive capacity to identify individuals likely to be more responsive to modification of dietary saturated fatty acid intake. In this case, sample size constraints limited the multilocus genotype to 2 SNPs; however, larger sample sizes would allow for additional SNPs to be included in a similar way, inviting the possibility to generate a database of robust multilocus-SNP-nutrient associations.

Effect of the rs10920533 SNP interacting with plasma SFA was replicated in MetS subjects from a separate population

Many reported associations are notoriously difficult to replicate in different study populations; therefore, data from a second population were analyzed to determine whether similar effects could be observed. The association with the rs266729 SNP was not observed in this population; however, the association with the rs10920533 SNP was replicated within MetS cases only. Differences in population stratification and environmental factors
AG (human evolutionary history (44). Multiple testing is a recurrent issue in association studies and one that has not yet been successfully resolved. Whereas both unadjusted P values and P values corrected for by using FDR were considered here, standard multiple correction methods are still ill-suited to hypothesis-driven studies such as this one. Thus, at present, replication in other cohorts remains the most reliable method for discerning genuine associations from false-positive ones.

In conclusion, this study suggests that 2 SNPs in genes encoding adiponectin (rs266729) and its receptor 1 (rs10920533) interact with plasma SFAs to alter insulin sensitivity as measured by HOMA-IR. Individuals homozygous for the minor allele and additionally having a high SFA concentration appear to be particularly susceptible. The association with rs10920533 was replicated in a separate population of MetS cases. Within the context of personalized nutrition, the genotype at these polymorphisms may play a role in the responsiveness to dietary fatty acid modification in subjects with the MetS.

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ADIPONENTIN GENES INTERACT WITH SFA ON HOMA-IR


