Calpain-10 interacts with plasma saturated fatty acid concentrations to influence insulin resistance in individuals with the metabolic syndrome\textsuperscript{1–4}

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ABSTRACT

Background: Calpain-10 protein (intracellular Ca\textsuperscript{2+}-dependent cysteine protease) may play a role in glucose metabolism, pancreatic \(\beta\) cell function, and regulation of thermogenesis. Several \textit{CAPN10} polymorphic sites have been studied for their potential use as risk markers for type 2 diabetes and the metabolic syndrome (MetS). Fatty acids are key metabolic regulators that may interact with genetic factors and influence glucose metabolism.

Objective: The objective was to examine whether the genetic variability at the \textit{CAPN10} gene locus is associated with the degree of insulin resistance and plasma fatty acid concentrations in subjects with MetS.

Design: The insulin sensitivity index, glucose effectiveness, insulin resistance [homeostasis model assessment of insulin resistance (HOMA-IR)], insulin secretion (disposition index, acute insulin response, and HOMA of \(\beta\) cell function), plasma fatty acid composition, and 5 \textit{CAPN10} single nucleotide polymorphisms (SNPs) were determined in a cross-sectional analysis of 452 subjects with MetS participating in the LIPGENE dietary intervention cohort.

Results: The rs2953171 SNP interacted with plasma total saturated fatty acid (SFA) concentrations, which were significantly associated with insulin sensitivity (\(P < 0.031\) for fasting insulin, \(P < 0.028\) for HOMA-IR, and \(P < 0.012\) for glucose effectiveness). \(G/G\) genotype was associated with lower fasting insulin concentrations, lower HOMA-IR, and higher glucose effectiveness in subjects with low SFA concentrations (below the median) than in subjects with the minor \(A\) allele (\(G/A\) and \(A/A\)). In contrast, subjects with the \(G/G\) allele with the highest SFA concentrations (above the median) had higher fasting insulin and HOMA-IR values and lower glucose effectiveness than did subjects with the \(A\) allele.

Conclusion: The rs2953171 polymorphism at the \textit{CAPN10} gene locus may influence insulin sensitivity by interacting with the plasma fatty acid composition in subjects with MetS. This trial was registered at clinicaltrials.gov as NCT00429195. Am J Clin Nutr doi: 10.3945/ajcn.110.010512.

INTRODUCTION

In the era of rapid development of diabetes and obesity, the in-depth study of the metabolic syndrome (MetS) is highly relevant. MetS represents a combination of cardiometabolic risk determinants, including central obesity, insulin resistance, dyslipidemia, and hypertension (1). The expression of these major factors may be the result of complex interactions between genetic and environmental factors. Moreover, evidence suggests that some people are genetically predisposed to insulin resistance (2)—a possible underlying mechanism for these metabolic alterations. Acquired factors, such as excess body fat and physical inactivity, can elicit insulin resistance and MetS in these people. Observational and intervention studies suggest that the quality of dietary fats may influence insulin resistance. For example, saturated fat worsens and monounsaturated and polyunsaturated fats improve insulin sensitivity (3, 4), although some individuals may be more insensitive (hyporesponders) to dietary intervention than others (hyperresponders) (5, 6). Thus, general recommendations may not be beneficial for all individuals. In support of this hypothesis, nutrigenetics has emerged as a multidisciplinary field focusing on interactions between nutritional and genetics factors and health outcomes. Greater insight into the mechanisms behind MetS may improve our understanding of how to prevent and manage this complex condition.

The calpain-10 gene (\textit{CAPN10}) is located at 2q37 and encodes a ubiquitously expressed member of the calpain-like cysteine protease family. Genetic and functional data indicate that

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calpain-10 has an important role in insulin resistance and intermediate phenotypes, including those associated with adipocytes (7, 8). Calpain-10 may facilitate the translocation of GLUT4 by reorganization of the cytoskeleton. Furthermore, the CAPN10 gene has been associated with several aspects of MetS, such as elevated body mass index (BMI) (9), plasma cholesterol concentrations (10), hypertension (11, 12), and hypertriglyceridemia (13).

Most gene markers for type 2 diabetes and MetS are not determinants of disease, but are predisposing factors in combination with unhealthy environmental exposures. Thus, it is important to understand the cross-talk between genes and the environment and more specifically with dietary factors. In view of the physiologic role of calpain-10 in glucose homeostasis and the link between insulin resistance and dietary fat, we hypothesized that genetic variations in calpain in humans might be associated with variability in insulin responses to different fatty acids in MetS patients from the LIPGENE dietary intervention cohort.

SUBJECTS AND METHODS

The design of the current study is described in recent articles from the LIPGENE cohort (5, 14, 15).

Subjects

A total of 452 participants aged 35–70 y with a body mass index (BMI; in kg/m²) of 20 to 40 were recruited for the LIPGENE dietary intervention study from 8 European countries (Ireland, United Kingdom, Norway, France, the Netherlands, Spain, Poland, and Sweden) in accordance with the Helsinki Declaration of 1975 as revised in 1983. The study was registered with The US National Library of Medicine Clinical Trials registry (NCT00429195). Subject eligibility was determined by using a modified version of the National Cholesterol Education Program criteria for MetS (16), by which subjects were required to fulfill ≥3 of the following 5 criteria: waist circumference >102 cm (men) or >88 cm (women), fasting plasma glucose concentration of 5.5 to 7.0 mmol/L, triglyceride concentration to fulfill program criteria for MetS (16), by which subjects were required to fulfill ≥3 of the following 5 criteria: waist circumference >102 cm (men) or >88 cm (women), fasting plasma glucose concentration of 5.5 to 7.0 mmol/L, triglyceride concentration ≥1.5 mmol/L, HDL cholesterol concentration <1.0 mmol/L (men) or <1.3 mmol/L (women), and blood pressure ≥130/85 mm Hg or treatment of previously diagnosed hypertension. The preintervention data for the LIPGENE dietary intervention cohort are provided elsewhere (17, 18). Briefly, subjects completed 3-d weighed food diaries and a food-frequency questionnaire to assess habitual dietary intake. On the basis of these data, nutritionists advised subjects individually on recommended food choices for their allocated diet. Fortnightly, 24-h dietary recalls were completed, and the frequency of study food consumption was monitored. Anthropometric measurements were recorded according to a standardized protocol for the LIPGENE study, and blood pressure was measured according to the guidelines of the European Society of Hypertension (19).

Biochemical measurements

Plasma, serum, and buffy coat were prepared from 12-h fasting blood samples in each subject. Serum insulin was measured by 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). Homeostasis model assessment of insulin resistance (HOMA-IR) was derived from fasting glucose and insulin concentrations as follows: (fasting plasma glucose × fasting serum insulin)/22.5 (20). Because HOMA-IR takes into account both insulin and glucose concentrations, it may be a more complete index than plasma insulin. HOMA of β cell function (HOMA-B) was calculated as follows: (20 × fasting serum insulin)/(fasting plasma glucose − 3.5). An insulin-modified intravenous glucose-tolerance test (IVGTT) was performed (21). Insulin sensitivity (sensitivity index; SI) and glucose effectiveness were determined by using the MINMOD Millenium Program (version 6.02; Richard N Bergman) (22). The acute insulin response to glucose (AIRg = first-phase insulin response) was defined as the incremental area under the curve from time 0 to 8 min. The disposition index was calculated as the product of AIRg and insulin sensitivity.

Cholesterol and triglycerides were quantified by using the IL Test Cholesterol kit and IL Test Triglycerides kit (Instrumentation Laboratories). Plasma nonesterified fatty acid composition was measured on a GC 2010 gas-liquid chromatograph (Shimadzu, Kyoto, Japan) (23).

Single nucleotide polymorphism selection and genotyping

CAPN10 genotype data from HapMap v1.1 (www.hapmap.org) was uploaded into HITAGENE—a web-based combined database and genetic analysis software suite. Haplotype frequencies were estimated by implementation of the expectation maximization algorithm. With the use of a 5% cutoff for individual haplotype frequency and >70% for the sum of all haplotype frequencies, haplotype tagged single nucleotide polymorphisms (SNPs) were identified by using SNP tagger (www.broad.mit.edu/mpg/tagger/server.html). Together with 1 SNP from the literature (rs5030952) (24), 4 SNPs (rs2953171, rs2953166, rs2953161, and rs2975776) were genotyped by Illumina Inc (San Diego, CA). DNA was extracted from buffy coat samples by using the AutoPure LS automated system (Gentra 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). Adherence to Hardy-Weinberg equilibrium at each SNP locus was determined by using the chi-square test with 1 df.

Statistical analysis

Biochemical variables were assessed for normality of distribution, and skewed variables were normalized by log10 or square root transformation as appropriate. Statistical analysis was carried out by using SPSS version 18.0 for Windows (SPSS Inc, Chicago, IL). Data are presented as means ± SEs for continuous variables and as frequencies or percentages for categorical variables. Differences in mean values were assessed by analysis of variance and unpaired t tests. Furthermore, comparisons of frequencies between qualitative variables were carried out by using the chi-square test. Potential confounding factors were age, sex, BMI, and LIPGENE center of origin. 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 was investigated by using the median of all different groups of plasma fatty acids to dichotomize the sample and by using the resulting groups (above the median or below the median) as a fixed factor in combination with the SNP genotypes in a univariate analysis of variance. Bonferroni’s test was used when post hoc analysis was required. A linear regression model including the original covariates was applied to create predicted values for homeostasis model assessment of insulin resistance (HOMA-IR) for the genotype of rs2953171. A difference was seen between the genotype groups, with the major allele homozygote group (○; n = 331) appearing to be “high responders” to plasma concentrations of saturated fatty acids (SFA) and the minor allele genotype group (Δ; n = 121) appearing to be “low responders.”

RESULTS

Characteristics of study participants

Baseline demographic and biochemical characteristics according to the rs2953171, rs2953166, rs2953161, rs2975776, and rs5030952 SNPs at the CAPN10 gene locus are presented in Table 1 and elsewhere (see supplemental Table 1 under “Supplemental data” in the online issue). Genotype distributions did not deviate from Hardy-Weinberg expectations. Moreover, distribution of the minor allele frequency between the different countries did not differ. Given the low genotype frequencies of individuals homozygous for the minor alleles, and because the analysis did not suggest a recessive mode of action, we analyzed the 4 following SNPs (rs2953171, rs2953166, rs2975776, and rs5030952) using 2 genotype categories. However, the rs2953161 SNP was analyzed according to the 3 genotypes. For the CAPN10 rs2953171 SNP, carriers of the minor allele A had higher plasma triglyceride concentrations than did those homozygous for the major allele G (Table 1). No other significant baseline differences were observed in relation to age, BMI, fasting lipids, and glucose and insulin concentration by genotype. In addition, no significant associations were found between the other 4 SNPs explored (see supplemental Table 1 under “Supplemental data” in the online issue).

Insulin sensitivity

All SNPs were used in the analysis; however, the results are presented only for rs2953171, which shows the most interesting and significant findings. Given that the aim of this study was to investigate potential gene-nutrient interactions, we examined the effect of the 5 CAPN10 SNPs on glucose metabolism according to plasma fatty acid status.

Gene-nutrient interactions between the rs2953171 CAPN10 and plasma concentration of total saturated fatty acids were found. In the whole cohort, this SNP interacted with plasma saturated fatty acids to influence insulin sensitivity. Thus, in subjects with low SFA concentrations (below the median of 30.92), the G/G genotype was associated with lower HOMA-IR

| TABLE 1 | Characteristics of participants at baseline according to the rs2953171 single nucleotide polymorphism
<table>
<thead>
<tr>
<th>rs2953171</th>
<th>G/G (n = 331)</th>
<th>G/A + A/A (n = 121)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54.14 ± 0.5</td>
<td>55.34 ± 0.8</td>
<td>0.209</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.35 ± 0.2</td>
<td>32.77 ± 0.3</td>
<td>0.362</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.29 ± 0.5</td>
<td>5.48 ± 0.8</td>
<td>0.064</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.27 ± 0.05</td>
<td>3.26 ± 0.10</td>
<td>0.932</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.09 ± 0.01</td>
<td>1.12 ± 0.02</td>
<td>0.471</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.72 ± 0.04</td>
<td>1.98 ± 0.09</td>
<td>0.005</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>1.01 ± 0.01</td>
<td>1.04 ± 0.02</td>
<td>0.262</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/L)</td>
<td>1.38 ± 0.01</td>
<td>1.42 ± 0.02</td>
<td>0.164</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.96 ± 0.04</td>
<td>5.92 ± 0.07</td>
<td>0.620</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>10.02 ± 0.31</td>
<td>10.37 ± 0.50</td>
<td>0.561</td>
</tr>
</tbody>
</table>

All values are means ± SEs. P < 0.05 was considered significant.

FIGURE 1. A linear regression model including the original covariates was applied to create predicted values for homeostasis model assessment of insulin resistance (HOMA-IR) for the genotype of rs2953171. A difference was seen between the genotype groups, with the major allele homozygote group (○; n = 331) appearing to be “high responders” to plasma concentrations of saturated fatty acids (SFA) and the minor allele genotype group (Δ; n = 121) appearing to be “low responders.”
subjects carrying the minor genotype. There were no significant interactions between other nutrient effects and plasma concentrations of saturated fatty acids (SFA) above (G/G = 158 and G/A + A/A = 66) or below (G/G = 173 and G/A + A/A = 55) the median within the same genotype group on glucose effectiveness (A) and fasting insulin (B). Values are means ± SDs. P values were adjusted for age, sex, BMI, and lipid genotype of origin.

**FIGURE 2.** Interaction between the rs2953171 single nucleotide polymorphism at the CAPN10 gene locus and plasma concentrations of saturated fatty acids (SFA) above (G/G = 158 and G/A + A/A = 66) or below (G/G = 173 and G/A + A/A = 55) the median within the same genotype group on glucose effectiveness (A) and fasting insulin (B). Values are means ± SDs. P values were adjusted for age, sex, BMI, and LIPGENE center of origin.

\( P < 0.028 \) (Figure 1), lower fasting insulin \( P < 0.031 \) (Figure 2), and higher glucose effectiveness \( P < 0.012 \) (Figure 2) than in subjects carrying the minor A allele (G/A and A/A). In contrast, subjects with the G/G genotype with the highest concentration of saturated fatty acids (above the median of 30.93) had higher fasting insulin and HOMA-IR and lower glucose effectiveness than did subjects carrying the A allele. Although we explored the genetic component, independently of the nutrient effect, no differences were observed. The insulin sensitivity index did not differ between participants with different genotypes. There were no significant interactions between other groups of plasma fatty acids and CAPN10 SNPs on glucose metabolism. A linear regression model including the original covariates was applied to create predicted values of HOMA-IR according to genotype at the rs2953171 SNP (Figure 1). The genotype groups exhibited striking differences in the predicted changes in HOMA-IR in relation to plasma saturated fatty acid concentrations. Thus, from baseline data, the model predicts that in individuals carrying the G/G genotype for rs2953171, an increase in plasma saturated fatty acids would elicit a considerable increase in HOMA-IR. This increase would not be seen in subjects carrying the minor A allele.

**Insulin secretion**

We examined the effect of the 5 SNPs on insulin secretion according to plasma fatty acid status. For that purpose, we measured the AIRg, disposition index, and HOMA-B. In contrast with the insulin sensitivity findings, we did not observe any gene-nutrient interactions for these variables.

**DISCUSSION**

Our findings support the hypothesis that the rs2953171 CAPN10 genetic variant influences insulin sensitivity by interacting with plasma saturated fatty acids in MetS patients. Thus, the G/G genotype was associated with lower fasting insulin concentrations, lower HOMA-IR, and higher glucose effectiveness in subjects with low plasma saturated fatty acid concentrations than was the minor A allele (G/A and A/A). In contrast, higher fasting insulin and HOMA-IR and lower glucose effectiveness were observed in subjects with the G/G genotype with the highest concentration of plasma saturated fatty acids than in subjects with the A allele.

The number of studies investigating gene-nutrient interactions related to cardiovascular disease and MetS continues to grow and holds the potential for reducing disease risk at the level of the individual genotype. Although we cannot perform interventions to change genetic constitution, environmental interventions may reduce insulin resistance, and individuals with the risk allele might attenuate the harmful effects by dietary changes. Dietary fat is an important environmental factor, with excessive exposure playing a key role in the development of MetS (25–27). Many aspects of the diet have been considered important in the modulation of insulin resistance; however, in the past decade more attention has been given to the effect of the quality of dietary fat, independent of total amount. We have shown an association between several variants of candidate genes and insulin sensitivity in response to dietary fat in healthy populations (6, 28). In the present study we observed a gene–fatty acid interaction between the rs2953171 polymorphism of CAPN10 and the proportion of saturated fatty acids in plasma. This SNP interacted with plasma saturated fatty acids to determine insulin sensitivity, which suggests the potential sensitivity of this SNP to dietary factors. In contrast, no differences were observed with other types of fat in plasma, such as MUFA, or PUFA. It should be noted, however, that plasma saturated fatty acids only partly reflect the dietary intake, because it also reflects the net endogenous synthesis of saturated fatty acids. Both genetic and functional data indicate that CAPN10 has an important role in insulin metabolism and intermediate phenotypes, including those associated with adipose tissue. Emerging evidence suggests that CAPN10 facilitates GLUT4 translocation and promotes the reorganization of the cytoskeleton. Subsequent to the initial identification of CAPN10 as a diabetes predisposition factor, numerous genetic studies have focused on the strength of individual CAPN10 SNPs in diabeticogenesis. A previous study showed that genetic variations in the CAPN10 gene at SNP-43 and SNP-63 were associated with elevated plasma free fatty acid concentrations and insulin resistance (29). However, the influence of CAPN10 genetic variants in response to dietary fat remains unknown. To our knowledge, our study was the first to explore the interactions between SNPs at the CAPN10 gene locus, plasma fatty acids, and glucose metabolism in subjects with MetS. Moreover, this was the first study to show that the rs2953171SNP is related to metabolic traits, such as insulin sensitivity. Measurements of HOMA-IR and fasting insulin showed that, in subjects with high plasma saturated fatty acid concentrations, the G/G genotype was associated with higher insulin resistance than was the A allele. On the basis of the results observed for the rs2953171 SNPs in the LIPGENE cohort,
we speculate that endogenous fatty acids modulate the involvement of the CAPN10 gene in downstream pathways, and insulin resistance may be linked to dietary patterns, such as a link between high saturated fat consumption and higher lipogenic activity (30).

Although we did not perform functional studies, previous data suggest that some CAPN10 SNPs, such as UCSNP-43 and -44, act as a regulator of CAPN10 expression (31). Thus, in Pima Indians with normal glucose tolerance, carriers of the CAPN10 SNP-43 G/G genotype had lower expression levels of CAPN10 mRNA than did carriers of the A allele in skeletal muscle (32). This agrees with the common variant hypothesis, which suggests that polymorphisms increasing susceptibility for a complex polygenic disease do so via effects on gene expression (33). Despite the evidence, interpretation of our results should be made with caution, because no data exist on the functionality of the rs2953171 SNP.

CAPN10 is an important molecule in the β cell. It may be a fuel sensor and a determinant of insulin exocytosis, with actions in the mitochondria and plasma membrane, respectively. However, in our study, we found no differences in the measurements of HOMA-B, AIRg, or glucose disposition index, which provides a useful measure of β cell function in the presence of the rs2953171 SNP. Our findings suggest that this gene-nutrient interaction modifies only insulin sensitivity but not insulin secretion. This is interesting because insulin resistance is believed to be the most important factor that links metabolic abnormalities in patients with MetS. Also, many authors have suggested that insulin resistance is the primary abnormality and that β cell dysfunction is a late event that arises from the prolonged increased secretory demand placed on the β cell by insulin resistance (34). The results of the current study suggest an enhanced beneficial effect of decreasing the amount of saturated fatty acids in the diet of persons with the G/G genotype in comparison with persons with the A allele.

The LIPGENE cohort is a carefully characterized population, and the multicenter origin of the patients allows extrapolation of the results to the European population. However, the main limitation of our present study was that it was cross-sectional and cannot prove causality. Nevertheless, identified gene-nutrient interactions may lay the groundwork for future studies. In addition to investigations of newly discovered gene variants, continuing efforts must focus on the confirmation of previously reported genetic associations and interactions in additional populations. In conclusion, our results support the notion that saturated fatty acids may play a contributing role in triggering insulin resistance by interacting with genetic variants at the CAPN10 gene locus in patients with MetS. Additional investigations of these novel associations and gene-nutrient interactions may help to improve the therapeutic efficacy of dietary recommendations with a “personalized nutrition” approach, wherein the genetic profile may determine the choice of dietary therapy to aid responsiveness to dietary fatty acid interventions.

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The authors’ responsibilities were as follows—HMR and JL-M: had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; PPM, JD-L, AG-R, and JL-M: conceived and designed the study; HLG, CMW, BK, BK-W, EEB, OH, MM-M, CD, UR, WHS, JAL, and CAD: collected and assembled data; PP-M, JD-L, HR, and JL-M: analyzed and interpreted the data; JD-L and AG-R: provided statistical expertise; PP-M, JD-L, HMR, and JL-M: drafted the manuscript; and HLG, CMW, BK, BK-W, EEB, OH, MM-M, CD, UR, WHS, JAL, and CAD: critically reviewed the manuscript for important intellectual content. None of the authors had any conflicts of interest that could affect the performance of the work or the interpretation of the data.

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