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 rs6606697) and MetS risk, and whether dietary fatty acids modulate this in the LIPGENE-SU.VI.MAX study of MetS cases and matched controls (n = 1754). Minor A allele carriers of rs4766587 had increased MetS risk (OR 1.29 [CI 1.08, 1.58], *P* = 0.0064) compared with the GG homozygotes, which may in part be explained by their increased body mass index (BMI), abdominal obesity, and impaired insulin sensitivity ($P < 0.05$). MetS risk was modulated by dietary fat intake (*P* = 0.04 for gene-nutrient interaction), where risk conferred by the A allele was exacerbated among 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 fat (PUFA) (OR 1.82 [CI 1.14, 2.94], *P* = 0.01; *P* = 0.05 for gene-nutrient interaction). Saturated and monounsaturated fat 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.**—Phillips, C. M., L. Goumidi, S. Bertrais, M. R. Field, L. Adrienne Cupples, J. M. Ordovas, J. McMonagle, C. Defoort, J. A. Lovegrove, C. A. Drevon, E. E. Blaak, B. Kiec-Wilk, U. Riserus, J. Lopez-Miranda, R. McManus, S. Hercberg, D. Lairon, R. Planells, and H. M. Roche. ***ACC2*** **gene polymorphisms, metabolic syndrome, and gene-nutrient interactions with dietary fat.** ***J. Lipid Res.*** **2010.** 51: **3500–3507.**

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

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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 (33). 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: [(fasting plasma glucose × fasting serum insulin) / 22.5] (34). Quantitative insulin-sensitivity check index (QUICKI), a measure of insulin sensitivity, was calculated as = [1 / (log fasting insulin + log fasting glucose + log 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

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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 MetS. Three genotype groups were first considered to check different inherent models (additive, dominant, and recessive). Where a dominant or recessive effect existed, analyses were repeated comparing carriers versus noncarriers of that particular allele. Where gender differences were observed, formal tests of heterogeneity (Breslow-Day) between the odds ratios (OR) of the gender-specific estimates were performed. To determine modulation by dietary fatty acids, logistic analyses were repeated using the median concentration of control subjects to dichotomise fatty acids, and associations were examined below and above the fatty acid median. Generalized estimating equation (GEE) linear regression (38) investigated associations between genotypes and continuous MetS phenotypes. Potential confounding factors used in the adjusted multivariate analysis included age; gender; body mass index (BMI); insulin and glucose concentrations; smoking status; physical activity; energy intake; and use of medications for lipid lowering, hypertension, and diabetes. Where a significant association was found, the result was verified by ANOVA using the Bonferroni post hoc test. To account for multiple testing, false discovery rates (FDR) (39) were computed. FDR-adjusted P values ≤ 0.05 were considered significant. Haplotype analysis was conducted using the THESIAS program (http://ecgene.net/genecanvas) (40). Paired 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 = 0.023); 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 rs2284685 (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

TABLE 1. *ACC2* polymorphisms investigated and comparison of their genotype frequencies in MetS cases and controls

Locus	Location	Genotype	MetS Cases		Controls		Fisher's P	Dominant Model	Recessive Model	Additive Model
			N	% Frequency	N	% Frequency				
rs4766587	Intron 38	A/A	67	0.076	41	0.046	**0.0025**	**0.0032**	**0.0109**	**0.0018**
		A/G	344	0.393	308	0.352				
		G/G	466	0.531	528	0.602				
rs3742023	Exon 44	A/A	110	0.125	113	0.128	0.0706	0.8836	**0.0256**	0.0651
		A/G	388	0.442	432	0.493				
		G/G	379	0.433	332	0.379				
rs2284685	Intron 39	C/C	342	0.390	299	0.341	0.1195	**0.0424**	0.4211	**0.0449**
		C/G	404	0.460	433	0.494				
		G/G	131	0.150	145	0.165				
rs6606697	Intron 5	A/A	121	0.136	96	0.142	0.9400	0.7778	0.8015	0.9988
		A/G	409	0.482	407	0.482				
		G/G	347	0.382	374	0.376				
rs2268387	Intron 18	A/A	292	0.334	303	0.346	0.6880	0.6424	0.6497	0.9712
		A/G	439	0.500	420	0.479				
		G/G	146	0.166	154	0.175				
rs2300453	Intron 23	A/A	604	0.689	595	0.679	0.2575	0.6749	0.1205	0.3585
		A/C	252	0.287	249	0.284				
		C/C	21	0.024	33	0.038				
rs3742026	Intron 28	C/C	121	0.138	96	0.110	0.1704	0.0882	0.2143	0.1175
		C/G	409	0.467	407	0.464				
		G/G	347	0.396	374	0.427				
rs2284689	Intron 39	A/A	40	0.046	25	0.029	0.1585	0.0705	0.3271	0.0772
		A/G	266	0.304	261	0.298				
		G/G	571	0.651	591	0.674				
rs2075263	Exon 52	A/A	565	0.645	586	0.669	0.2789	0.3052	0.1530	0.0879
		A/G	274	0.312	265	0.302				
		G/G	38	0.043	26	0.030				

Genotype frequencies were compared between cases and controls in HITAGENE using Fisher's exact test. ACC2, acetyl-CoA carboxylase β; MetS, metabolic syndrome. Boldface type indicates statistically significant p values.

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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], $P = 0.012$, A allele carriers relative to the GG homozygotes). Although the effect was in the same direction in the female subjects, it was not significant (OR 1.22 [CI 0.69, 3.01], $P = 0.66$). Formal tests of heterogeneity (Breslow-Day) between the odds ratios of the gender-specific estimates confirmed the existence of a gender-specific association with MetS ($P = 0.03$).

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.

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

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

Characteristic	GG	AG	AA	AG + AA
N	994	652	108	760
Male/female (%)	60/40	61/39	59/41	60/40
Age (y)	58 ± 0.17	58 ± 0.19	58 ± 0.33	58 ± 0.20
BMI (kg/m^2)	25.3 ± 0.14	26.1 ± 0.17	27.1 ± 0.47[b d]	26.8 ± 0.16[a]
Waist (cm)	88 ± 0.42	89 ± 0.49	90 ± 1.3[c]	90 ± 0.46[a]
Glucose (mmol/l)	5.24 ± 0.03	5.27 ± 0.04	5.29 ± 0.02	5.28 ± 0.03
Insulin (mmol/l)	7.27 ± 0.19	7.46 ± 0.21	8.13 ± 0.70[c,d]	7.55 ± 0.21
QUICKI	0.34 ± 0.00	0.32 ± 0.00	0.31 ± 0.00[c,d]	0.32 ± 0.00[a]
SBP (mm Hg)	131 ± 0.50	131 ± 0.63	135 ± 1.63[c,d]	132 ± 0.59
DBP (mm Hg)	82 ± 0.30	82 ± 0.38	84 ± 0.89	82 ± 0.35
Total cholesterol (mmol/l)	5.71 ± 0.03	5.70 ± 0.03	5.83 ± 0.10	5.72 ± 0.03
HDL (mmol/l)	1.46 ± 0.01	1.48 ± 0.02	1.54 ± 0.04	1.49 ± 0.02
LDL (mmol/l)	3.56 ± 0.04	3.48 ± 0.05	3.62 ± 0.11	3.50 ± 0.04
TAG (mmol/l)	1.27 ± 0.02	1.28 ± 0.03	1.17 ± 0.06	1.26 ± 0.03
NEFA (mmol/l)	0.94 ± 0.03	0.87 ± 0.03	0.88 ± 0.08[c]	0.87 ± 0.03[a]
Lipid lowering medication (%)	19	20	20	20
Anti-diabetic medication (%)	4	3	4	3.7
Hypertensive medication (%)	21	21	23	22
Dietary fat intake (% energy)	35.27 ± 0.23	35.09 ± 0.35	36.02 ± 0.10	35.19 ± 0.26
PUFA intake (% energy)	5.63 ± 0.07	5.65 ± 0.09	5.79 ± 0.25	5.67 ± 0.08
n-6 PUFA intake (% energy)	5.05 ± 0.07	5.10 ± 0.09	5.23 ± 0.05	5.12 ± 0.08
n-3 PUFA intake (% energy)	0.57 ± 0.01	0.55 ± 0.01	0.55 ± 0.03	0.55 ± 0.01
SFA intake (% energy)	15.46 ± 0.13	15.23 ± 0.14	15.65 ± 0.46	15.29 ± 0.14
MUFA intake (% energy)	14.19 ± 0.11	14.16 ± 0.13	14.71 ± 0.40	14.24 ± 0.13
Alcohol intake (% energy)	6.58 ± 0.25	6.82 ± 0.35	6.78 ± 0.54	6.80 ± 0.40

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.

[a] Indicates $P < 0.05$ compared with GG homozygotes for linear regression adjusted for potential confounding factors.

[b] $P < 0.01$ obtained using ANOVA.

[c] $P < 0.05$ obtained using ANOVA.

[d] $P < 0.05$ for post hoc multiple comparisons as assessed by Bonferroni compared with the GG homozygotes.

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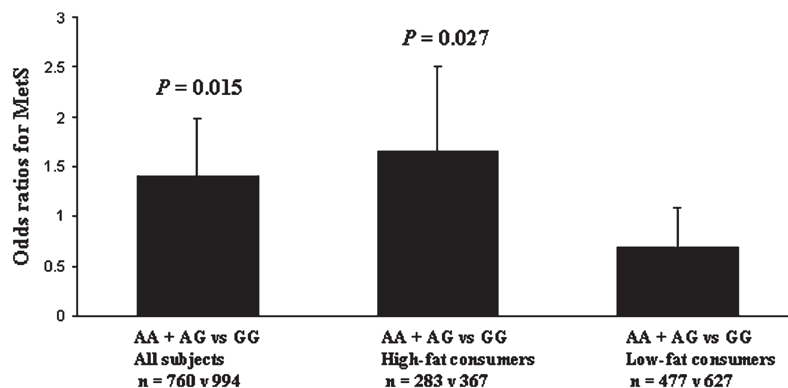

Fig. 1. MetS risk related to interactions between *ACC2* rs4766587 and dietary fat intake. Odds ratios and 95% confidence intervals for the associations between *ACC2* rs4766587 and the MetS, stratified according to dietary fat intake, were determined by logistic regression analyses comparing A allele 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.

[CI 1.02, 3.12], $P = 0.03$; $P = 0.08$ for gene-nutrient interaction). Dietary intake of saturated and monounsaturated fat did not modulate MetS risk (data not shown).

ACC2 haplotypes and MetS risk

Haplotype analysis revealed the combined effect of *ACC2* rs4766587 with rs3742023 and rs2284685 on MetS risk. After haplotypes with frequencies < 5% were excluded from the analysis, three haplotypes were identified (GCG, GGA, and ACG). **Table 3** shows the ACG haplotype, representing that > 20% of the observed haplotypes were associated with increased risk of MetS compared with GCG, the most common reference haplotype. When the rs4766587 G allele was replaced by the A allele in the GCG haplotype, risk of MetS was > 30% higher (OR 1.34 [CI 1.12, 1.61], $P = 0.0018$). Due to the limited number of haplotypes of sufficient frequency generated in this analysis, it was not possible to examine the influence of haplotypic background on the impact of the rs4766587 polymorphism on MetS risk. Moreover, we investigated whether dietary fat intake influenced the haplotype association with MetS. We did not find any evidence that fatty acid composition or amount altered the MetS risk associated with the ACG haplotype.

Replication 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.

DISCUSSION

To our knowledge, this is the first study to report an association between *ACC2* polymorphisms 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-

TABLE 3. Haplotype frequencies and OR for MetS among all subjects

Haplotype	Controls (N = 877)	MetS Cases (N = 877)	OR [95% CI] P
GCG	36.96%	35.65%	1
GGA	36.21%	33.48%	0.98 [0.84, 1.15] $P = 0.79$
ACG	20.10%	25.26%	1.34 [1.12, 1.61] $P = 0.0018$

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.

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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 (ACC2.v2)] 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-6 and n-3 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

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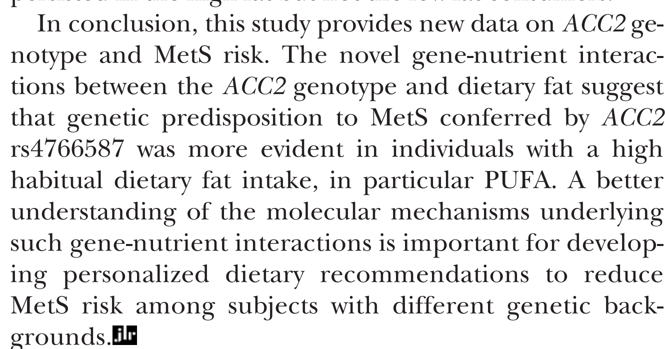
valuable in validating these new findings. Thus, we analyzed a separate independent LIPGENE MetS case only cohort (n = 464) (32). Importantly, we replicated the genetic associations with increased BMI, body-weight, waist circumference, and insulin resistance. Consistent with our original findings, we also determined that these genetic differences persisted in the high-fat but not the low-fat consumers.



In conclusion, this study provides new data on *ACC2* genotype and MetS risk. The novel gene-nutrient interactions between the *ACC2* genotype and dietary fat suggest that genetic predisposition to MetS conferred by *ACC2* rs4766587 was more evident in individuals with a high habitual dietary fat intake, in particular PUFA. A better understanding of the molecular mechanisms underlying such gene-nutrient interactions is important for developing personalized dietary recommendations to reduce MetS risk among subjects with different genetic backgrounds.

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REFERENCES

1. Abu-Elheiga, L., A. Jayakumar, A. Baldini, S. S. Chirala, and S. J. Wakil. 1995. Human acetyl-CoA carboxylase: characterization, molecular cloning, and evidence for two isoforms. *Proc. Natl. Acad. Sci. USA.* **92:** 4011–4015.
2. Barber, M. C., N. T. Price, and M. T. Travers. 2005. Structure and regulation of acetyl-CoA carboxylase genes of metazoa. *Biochim. Biophys. Acta.* **1733:** 1–28.
3. McGarry, J. D. 1992. What if Minkowski had been ageusic? An alternative angle on diabetes. *Science.* **258:** 766–770.
4. Munday, M. R. 2002. Regulation of mammalian acetyl-CoA carboxylase. *Biochem. Soc. Trans.* **30:** 1059–1064.
5. Wakil, S. J., E. B. Titchener, and D. M. Gibson. 1958. Evidence for the participation of biotin in the enzymic synthesis of fatty acids. *Biochim. Biophys. Acta.* **29:** 225–226.
6. Abu-Elheiga, L., M. M. Matzuk, K. A. Abo-Hashema, and S. J. Wakil. 2001. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science.* **291:** 2613–2616.
7. Abu-Elheiga, L., W. Oh, P. Kordari, and S. J. Wakil. 2003. Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. *Proc. Natl. Acad. Sci. USA.* **100:** 10207–10212.
8. Bianchi, A., J. L. Evans, A. J. Iverson, A. C. Nordlund, T. D. Watts, and L. A. Witters. 1990. Identification of an isozymic form of acetyl-CoA carboxylase. *J. Biol. Chem.* **265:** 1502–1509.
9. Kim, K. H. 1997. Regulation of mammalian acetyl-coenzyme A carboxylase. *Annu. Rev. Nutr.* **17:** 77–99.
10. Castle, J. C., Y. Hara, C. K. Raymond, P. Garrett-Engele, K. Ohwaki, Z. Kan, J. Kusunoki, and J. M. Johnson. 2009. ACC2 is expressed at high levels in human white adipose and has an isoform with a novel N-terminus. [title corrected] *PLoS ONE.* **4:** e4369.
11. Boden, G. 1997. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes.* **46:** 3–10.
12. Kelley, D. E., B. Goodpaster, R. R. Wing, and J. A. Simoneau. 1999. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am. J. Physiol.* **277:** E1130–E1141.
13. Seppala-Lindroos, A., S. Vehkavaara, A. M. Hakkinen, T. Goto, J. Westerbacka, A. Sovijarvi, J. Halavaara, and H. Yki-Jarvinen. 2002. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J. Clin. Endocrinol. Metab.* **87:** 3023–3028.
14. Unger, R. H. 2002. Lipotoxic diseases. *Annu. Rev. Med.* **53:** 319–336.
15. Moller, D. E., and K. D. Kaufman. 2005. Metabolic syndrome: a clinical and molecular perspective. *Annu. Rev. Med.* **56:** 45–62.
16. Oh, W., L. Abu-Elheiga, P. Kordari, Z. Gu, T. Shaikenov, S. S. Chirala, and S. J. Wakil. 2005. Glucose and fat metabolism in adipose tissue of acetyl-CoA carboxylase 2 knockout mice. *Proc. Natl. Acad. Sci. USA.* **102:** 1384–1389.
17. Savage, D. B., C. S. Choi, V. T. Samuel, Z. X. Liu, D. Zhang, A. Wang, X. M. Zhang, G. W. Cline, X. X. Yu, J. G. Geisler, et al. 2006. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J. Clin. Invest.* **116:** 817–824.
18. Kabagambe, E. K., M. Y. Tsai, P. N. Hopkins, J. M. Ordovas, J. M. Peacock, I. B. Borecki, and D. K. Arnett. 2008. Erythrocyte fatty acid composition and the metabolic syndrome: a National Heart, Lung, and Blood Institute GOLDN study. *Clin. Chem.* **54:** 154–162.
19. Phillips, C., J. Lopez-Miranda, F. Perez-Jimenez, R. McManus, and H. M. Roche. 2006. Genetic and nutrient determinants of the metabolic syndrome. *Curr. Opin. Cardiol.* **21:** 185–193.
20. Szabo de Edelenyi, F., L. Goumidi, S. Bertrais, C. Phillips, R. Macmanus, H. Roche, R. Planells, and D. Lairon. 2008. Prediction of the metabolic syndrome status based on dietary and genetic parameters, using Random Forest. *Genes & Nutrition.* **3:** 173–176.
21. Vessby, B. 2003. Dietary fat, fatty acid composition in plasma and the metabolic syndrome. *Curr. Opin. Lipidol.* **14:** 15–19.
22. Warensjo, E., U. Riserus, and B. Vessby. 2005. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia.* **48:** 1999–2005.
23. Warensjo, E., J. Sundstrom, L. Lind, and B. Vessby. 2006. Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. *Am. J. Clin. Nutr.* **84:** 442–448.
24. Field, F. J., E. Born, S. Murthy, and S. N. Mathur. 2002. Polyunsaturated fatty acids decrease the expression of sterol regulatory element-binding protein-1 in CaCo-2 cells: effect on fatty acid synthesis and triacylglycerol transport. *Biochem. J.* **368:** 855–864.
25. Takeuchi, H., T. Nakamoto, Y. Mori, M. Kawakami, H. Mabuchi, Y. Ohishi, N. Ichikawa, A. Koike, and K. Masuda. 2001. Comparative effects of dietary fat types on hepatic enzyme activities related to the synthesis and oxidation of fatty acid and to lipogenesis in rats. *Biosci. Biotechnol. Biochem.* **65:** 1748–1754.
26. Mensink, M., E. E. Blaak, H. Vidal, T. W. De Bruin, J. F. Glatz, and W. H. Saris. 2003. Lifestyle changes and lipid metabolism gene expression and protein content in skeletal muscle of subjects with impaired glucose tolerance. *Diabetologia.* **46:** 1082–1089.
27. Astorg, P., S. Bertrais, F. Laporte, N. Arnault, C. Estaquio, P. Galan, A. Favier, and S. Hercberg. 2008. Plasma n-6 and n-3 polyunsaturated fatty acids as biomarkers of their dietary intakes: a cross-sectional study within a cohort of middle-aged French men and women. *Eur. J. Clin. Nutr.* **62:** 1155–1161.
28. Phillips, C. M., L. Goumidi, S. Bertrais, M. R. Field, G. M. Peloso, J. Shen, R. McManus, S. Hercberg, D. Lairon, R. Planells, et al. 2009. Dietary saturated fat modulates the association between STAT3 polymorphisms and abdominal obesity in adults. *J. Nutr.* **139:** 2011–2017.
29. Le Moullec, N., M. Deheeger, P. Preziosi, P. Monteiro, P. Valeix, M-F. Rolland-Cachera, G. Potier de Courcy, J-P. Christides, F. Cherouvrier, P. Galan, et al. 1996. Validation du manuel-photos utilisé pour l'enquête alimentaire de l'étude SU.VI.MAX. *Cahiers de Nutrition et de Diététique.* **31:** 158–164.
30. Feinberg, M., J. C. Favier, J. Ireland-Ripert, Fondation Française pour la Nutrition (FFN), and Centre Informatique sur la Qualité des Aliments (CIQUAL). 1987. Répertoire général des aliments: Tome 1 - Table de composition des Corps Gras. Technique et Documentation-Lavoisier, Paris.
31. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). 2001. *Jama.* **285:** 2486–2497.
32. Tierney, A. C., J. McMonagle, D. I. Shaw, H. L. Gulseth, O. Helal, W. H. M. Saris, J. A. Paniagua, C. Defoort, C. M. Williams, B. Karlström, et al. 2010. Effects of dietary fat modification on insulin sensitivity and other risk factors of the metabolic syndrome – LIPGENE: an European randomized dietary intervention study. *Int. J. Obes.* In press.
33. Hercberg, S., P. Galan, P. Preziosi, S. Bertrais, L. Mennen, D. Malvy, A. M. Roussel, A. Favier, and S. Briancon. 2004. The SU.VI.

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MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. *Arch. Intern. Med.* **164:** 2335–2342.

34. Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* **28:** 412–419.
35. Perseghin, G., A. Caumo, M. Caloni, G. Testolin, and L. Luzi. 2001. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. *J. Clin. Endocrinol. Metab.* **86:** 4776–4781.
36. Phillips, C. M., L. Goumidi, S. Bertrais, J. F. Ferguson, M. R. Field, E. D. Kelly, G. M. Peloso, L. A. Cupples, J. Shen, J. M. Ordovas, et al. 2009. Complement component 3 polymorphisms interact with polyunsaturated fatty acids to modulate risk of metabolic syndrome. *Am. J. Clin. Nutr.* **90:** 1665–1673.
37. de Bakker, P. I., R. Yelensky, I. Pe'er, S. B. Gabriel, M. J. Daly, and D. Altshuler. 2005. Efficiency and power in genetic association studies. *Nat. Genet.* **37:** 1217–1223.
38. Liang, K. Y., and S. L. Zeger. 1993. Regression analysis for correlated data. *Annu. Rev. Public Health.* **14:** 43–68.
39. Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B.* **57:** 289–300.
40. Tregouet, D. A., and V. Garelle. 2007. A new JAVA interface implementation of THESIAS: testing haplotype effects in association studies. *Bioinformatics.* **23:** 1038–1039.
41. Chen, Z. P., T. J. Stephens, S. Murthy, B. J. Canny, M. Hargreaves, L. A. Witters, B. E. Kemp, and G. K. McConell. 2003. Effect of exercise intensity on skeletal muscle AMPK signaling in humans. *Diabetes.* **52:** 2205–2212.
42. Thampy, K. G., and S. J. Wakil. 1988. Regulation of acetyl-coenzyme A carboxylase. II. Effect of fasting and refeeding on the activity, phosphate content, and aggregation state of the enzyme. *J. Biol. Chem.* **263:** 6454–6458.
43. Vavvas, D., A. Apazidis, A. K. Saha, J. Gamble, A. Patel, B. E. Kemp, L. A. Witters, and N. B. Ruderman. 1997. Contraction-induced changes in acetyl-CoA carboxylase and 5′-AMP-activated kinase in skeletal muscle. *J. Biol. Chem.* **272:** 13255–13261.
44. Winz, R., D. Hess, R. Aebersold, and R. W. Brownsey. 1994. Unique structural features and differential phosphorylation of the 280-kDa component (isozyme) of rat liver acetyl-CoA carboxylase. *J. Biol. Chem.* **269:** 14438–14445.
45. Pape, M. E., F. Lopez-Casillas, and K. H. Kim. 1988. Physiological regulation of acetyl-CoA carboxylase gene expression: effects of diet, diabetes, and lactation on acetyl-CoA carboxylase mRNA. *Arch. Biochem. Biophys.* **267:** 104–109.
46. Morise, A., C. Thomas, J. F. Landrier, P. Besnard, and D. Hermier. 2009. Hepatic lipid metabolism response to dietary fatty acids is differently modulated by PPARalpha in male and female mice. *Eur. J. Nutr.* **48:** 465–473.
47. Riserus, U., W. C. Willett, and F. B. Hu. 2009. Dietary fats and prevention of type 2 diabetes. *Prog. Lipid Res.* **48:** 44–51.
48. Xu, J., M. T. Nakamura, H. P. Cho, and S. D. Clarke. 1999. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J. Biol. Chem.* **274:** 23577–23583.
49. Eberle, D., B. Hegarty, P. Bossard, P. Ferre, and F. Foufelle. 2004. SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie.* **86:** 839–848.
50. Foufelle, F., and P. Ferre. 2002. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochem. J.* **366:** 377–391.
51. Oh, S. Y., S. K. Park, J. W. Kim, Y. H. Ahn, S. W. Park, and K. S. Kim. 2003. Acetyl-CoA carboxylase beta gene is regulated by sterol regulatory element-binding protein-1 in liver. *J. Biol. Chem.* **278:** 28410–28417.
52. Mabrouk, G. M., I. M. Helmy, K. G. Thampy, and S. J. Wakil. 1990. Acute hormonal control of acetyl-CoA carboxylase. The roles of insulin, glucagon, and epinephrine. *J. Biol. Chem.* **265:** 6330–6338.
53. Coletta, D. K., A. Sriwijitkamol, E. Wajcberg, P. Tantiwong, M. Li, M. Prentki, M. Madiraju, C. P. Jenkinson, E. Cersosimo, N. Musi, et al. 2009. Pioglitazone stimulates AMP-activated protein kinase signalling and increases the expression of genes involved in adiponectin signalling, mitochondrial function and fat oxidation in human skeletal muscle in vivo: a randomised trial. *Diabetologia.* **52:** 723–732.

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