APOA2, Dietary Fat, and Body Mass Index

Replication of a Gene-Diet Interaction in 3 Independent Populations

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Background: Nutrigenetics studies the role of genetic variation on interactions between diet and health, aiming to provide more personalized dietary advice. However, replication has been low. Our aim was to study interaction among a functional APOA2 polymorphism, food intake, and body mass index (BMI) in independent populations to replicate findings and to increase their evidence level.

Methods: Cross-sectional, follow-up (20 years), and case-control analyses were undertaken in 3 independent populations. We analyzed gene-diet interactions between the APOA2 −265T>C polymorphism and saturated fat intake on BMI and obesity in 3462 individuals from 3 populations in the United States: the Framingham Offspring Study (1454 whites), the Genetics of Lipid Lowering Drugs and Diet Network Study (1078 whites), and Boston–Puerto Rican Centers on Population Health and Health Disparities Study (930 Hispanics of Caribbean origin).

Results: Prevalence of the CC genotype in study participants ranged from 10.5% to 16.2%. We identified statistically significant interactions between the APOA2 −265T>C and saturated fat regarding BMI in all 3 populations. Thus, the magnitude of the difference in BMI between the individuals with the CC and TT+TC genotypes differed by saturated fat. A mean increase in BMI of 6.2% (range, 4.3%-7.9%; P=0.01) was observed between genotypes with high (~22 g/d) but not with low-saturated fat intake in all studies. Likewise, the CC genotype was significantly associated with higher obesity prevalence in all populations only in the high-saturated fat stratum. Meta-analysis estimations of obesity for individuals with the CC genotype compared with the TT+TC genotype were an odds ratio of 1.84 (95% confidence interval, 1.38-2.47; P<.001) in the high-saturated fat stratum, but no association was detected in the low-saturated fat stratum (odds ratio, 0.81; 95% confidence interval, 0.59-1.11; P=.18).

Conclusion: For the first time to our knowledge, a gene-diet interaction influencing BMI and obesity has been strongly and consistently replicated in 3 independent populations.

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is thought to be a crucial causal criterion of credibility of genome-wide association studies.9 Therefore, the National Cancer Institute—National Human Genome Research Institute Working Group on Replication in Genotype-Phenotype Associations10 supports replication as the most reliable approach to increase evidence level and subsequent clinical applications.

Consistent with these recommendations, our major aim was to conduct a replication study in nutrigenetics. For this purpose, we focused on our recently reported association between the functional −265T>C single nucleotide polymorphism (SNP)11 in the APOA2 gene promoter, food intake, and obesity risk in non-Hispanic white Americans.12 The second major high-density lipoprotein apolipoprotein, APOA2, is an enigmatic protein in search of a function.13 Although animal models have found that overexpression of APOA2 results in hypertriglyceridemia, obesity, and insulin resistance,14,15 its role in humans remains controversial.10,11,16,17 Therefore, our goals were (1) to analyze the association between the APOA2 −265T>C SNP and obesity-related variables in the Framingham Offspring Study, with a focus on gene-diet interactions with fat intake and (2) to study the replication of these gene-diet interactions in other American populations.

METHODS

We studied 3462 individuals from 3 independent populations. The populations are from the Framingham Offspring Study (FOS), the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study, and the Boston–Puerto Rican Centers on Population Health and Health Disparities (Boston–Puerto Rican) Study. All participants provided written informed consent.

THE FRAMINGHAM OFFSPRING STUDY

We included 1454 genetically unrelated non-Hispanic whites (716 men and 738 women), aged 26 to 80 years, who participated in the fifth-examination visit of the FOS18 and had complete data for the genetic, clinical, dietary, and anthropometric variables analyzed. These individuals were obtained from an FOS cohort that had yielded a standard previously plated set of unrelated DNA strands in which only 1 individual from each pedigree was randomly selected. The institutional review boards (IRBs) for human research at Boston University and Tufts University/New England Medical Center approved the protocol. Alcohol, tobacco smoking, diabetes mellitus status, and physical activity were defined previously.19-21 For longitudinal analysis, we included 1087 unrelated individuals (540 men and 547 women) who attended each of the first 5 examinations: examination 1, August 30, 1971, to September 3, 1975; examination 2, October 9, 1979, to October 27, 1983; examination 3, December 20, 1984, to September 30, 1987; examination 4, April 22, 1987, to September 11, 1991; and examination 5, January 3, December 20, 1984, to September 30, 1987; examination 4, April 22, 1987, to September 11, 1991; and examination 5, January 23, 1991, to June 29, 1995. Anthropometric and demographic variables were measured at each cycle.

THE GENETICS OF LIPID-LOWERING DRUGS AND DIET NETWORK STUDY

For the GOLDN Study, a total of 1200 adults of European ancestry were recruited from 2 National Heart, Lung, and Blood Institute Family Heart Study field centers (Minneapolis, Minnesota, and Salt Lake City, Utah), as previously reported.12 We included 1078 adults (514 men and 564 women) for whom data for all examined variables were complete. The protocol was approved by the institutional review boards at the University of Alabama, University of Minnesota, University of Utah, and Tufts University.

THE BOSTON–PUERTO RICAN CENTERS ON POPULATION HEALTH AND HEALTH DISPARITIES STUDY

Comprising approximately 1200 free-living ethnic Puerto Rican (Hispanics of Caribbean origin) individuals, aged 43 to 75 years, in the greater Boston, Massachusetts, area,22 the Boston–Puerto Rican Study is one of the National Institutes of Health–funded Centers on Population Health and Health Disparities. We analyzed the 930 (263 men and 667 women) individuals for whom we had complete data. The protocol was approved by the institutional review board at Tufts University. In these populations, individuals included did not differ from those excluded because of incomplete data with regard to the variables analyzed.

ANTHROPOMETRIC, PHYSICAL ACTIVITY, AND BIOCHEMICAL DETERMINATIONS

Anthropometric variables, such as height, weight, and waist circumference, were measured in all cohorts by standard techniques.12,17,22 Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Obesity was defined as a BMI of 30 or greater. Physical activity in the FOS was assessed with the physical activity index calculated at examination 4 from the number of hours each day spent sleeping, sedentary, performing slightly physical activities, moderately physical activities, or highly physical activities, weighted in accordance with the estimated oxygen consumption required.22 In the GOLDN Study, a nonvalidated questionnaire was used that contains questions with regard to the number of hours per day dedicated to different activities.22 In the Boston–Puerto Rican Study, a physical activity score based on the Paffenbarger questionnaire of the Harvard Alumni Activity Survey23 was estimated. Fasting glucose, triglycerides, total cholesterol, and high-density lipoprotein cholesterol levels were measured by standard methods.22,24-26 For the FOS samples, plasma APOA1 and APOA2 concentrations were determined by means of turbidimetric immunonassays (Wako Chemicals USA Inc, Richmond, Virginia).

DIETARY INTAKE

Diet was measured by validated questionnaires in each specific population24-26, the Willett24 semiquantitative food frequency questionnaire, administered at examination 5, in the FOS; the Diet History Questionnaire in the GOLDN Study21,23, and a specially designed and validated food frequency questionnaire26 in the Boston–Puerto Rican Study. Nutrient data were derived from the Harvard University food composition database, the United States Department of Agriculture database, and the Minnesota Nutrient System.

GENETIC ANALYSES

We isolated DNA from blood by means of an examination kit (QIAamp DNA Blood Maxi Kit; Qagen, Hilden, Germany). We performed the APOA2 −265T>C genotyping (rs5082) by means of a Taqman assay with allele-specific probes on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc, Foster City, California).12 Quality control measures were ap-
plied. Genotype frequencies were consistent with Hardy-Weinberg equilibrium in all populations.

STATISTICAL ANALYSES

We used $\chi^2$ tests to verify percentages. Normality of continuous variables was examined. Triglycerides were log transformed, and alcohol and polyunsaturated fatty acids were square root transformed. We applied analysis of variance and the t test to compare crude means. Because of the results obtained in the previous GOLDN Study, in which similar effects were found to compare crude means. Because of the results obtained in the previously described. In the Boston–Puerto Rican Study, further adjustment of basic models for physical activity were formed, and alcohol and polyunsaturated fatty acids were square root transformed. We applied analysis of variance and the t test to compare crude means. Because of the results obtained in the previous GOLDN Study, in which similar effects were found to compare crude means. Because of the results obtained in the previously described association between the APOA2−265T>C polymorphism were considered in this analysis after having checked the validity of this model in the other populations. We also tested the statistical homogeneity by sex, and men and women were analyzed together. To study gene-diet interactions in determination of BMI, we used multivariate linear regression models, including main effects and interaction terms. We fitted separate models for each population, including the same variables for the interaction terms and the multivariate adjustments. Saturated fat intake was considered continuous and categorical (low or high). A level of 22 g/d was established as the cutoff point to classify the low-saturated fat or high-saturated fat intake based on the FOS results. In addition to the unadjusted models, we adjusted analyses for sex, age, tobacco smoking, alcohol consumption, diabetes, lipid medication, and total energy intake (basic models). Additional adjustments of basic models for physical activity were considered for each population. In the GOLDN Study, additional adjustments for family relationships were undertaken as previously described. In the Boston–Puerto Rican Study, further adjustment for admixture by means of the first component variable derived from the analysis of 100 ancestry-informative markers was undertaken. To study the specificity of the effect, we sequentially adjusted for other nutrients (total fat, carbohydrates, and proteins) as indicated.

When the APOA2−saturated fat interaction was considered to be continuous, it was depicted by the computation of the predicted values for each individual from the adjusted regression model and the plotting of these values against saturated fat intake by the APOA2 genotype. Regression coefficients were estimated in stratified analyses by genotype. When saturated fat was considered as categorical (<22 g/d or ≥22 g/d), stratified analyses were conducted. To increase the consistency, we have also undertaken internal replication analysis on the same population. The GOLDN Study was stratified by study center and the Boston–Puerto Rican Study by diabetes status. In the FOS population we also analyzed the APOA2−saturated fat interaction with regard to BMI across 20-year follow-up in a general linear multivariate model for repeated measures with interaction terms. Five direct measures of BMI were considered at examinations 1-5) as dependent variables. The APOA2 polymorphism, saturated fat (as dichotomous), and age at baseline were covariates. Main effects and interaction terms were tested.

In all populations, logistic regression models, including main effects and interaction terms, were fitted to test the APOA2−saturated fat interaction in determination of the odds ratio (OR) of obesity. Study-specific ORs and 95% confidence intervals (CIs) were estimated for each stratum of saturated fat. Multivariate adjustments were performed as indicated.

We also performed a meta-analysis of study-specific estimates of ORs for the 2 strata of saturated fat intake. Heterogeneity was tested by use of the Cochran Q Association statistic and quantified by $I^2$. We pooled study-specific estimates in accordance with the inverse-variance fixed effect. Statistical analyses were conducted with SAS statistical software, version 9.1 (SAS Institute Inc, Cary, North Carolina); SPSS statistical software, version 15.0 (SPSS Inc, Chicago, Illinois); and MIX software, version 1.7 (Kitasato University, Tokyo, Japan), for meta-analysis. Standard regression diagnostic procedures were used to ensure the appropriateness of the fitted models. All reported probability tests were 2-sided. Differences were considered statistically significant at $P<.05$. With consideration of the magnitude of the effect, the allele frequency, and the standard type 1 error (5%), our study has a power of 80% or higher to detect statistically significant interactions in each population.

RESULTS

We studied 3462 individuals from 3 independent cohorts in the United States (the FOS, the GOLDN Study, and the Boston–Puerto Rican Study). The Table gives the demographic, anthropometric, clinical, biochemical, dietary, and lifestyle characteristics of participants in accordance with the APOA2−265T>C SNP for each population. Prevalence of individuals with the CC genotype did not differ between the FOS (16.2%) and the GOLDN Study (15.3%). A statistically significant lower prevalence (10.5%) was found in the Boston–Puerto Rican Study. Demographic characteristics and physical activity did not differ significantly between CC and T allele carriers in any of the 3 populations (Table). Among white populations, prevalence of obesity was higher in the GOLDN Study cohort than in the FOS. Likewise, mean fat intake, mainly saturated fat, was higher in the GOLDN Study population than in the FOS. No significant association of the APOA2−265T>C SNP with high-density lipoprotein cholesterol level was found in any of the 3 populations. Concentrations of APOA2 and APOAI were only determined in the FOS population. In this cohort, plasma APOA2 concentrations (eTable 1; http://www.archinternmed.com) were significantly lower in individuals with the CC genotype, whereas no effects were observed for APOAI, a result that supports the functionality and specificity of this SNP.

We next examined in the FOS population our previously described association between the APOA2 SNP, food intake, and body weight. In the FOS, we also found that individuals with the CC genotype had higher energy intake than T allele carriers ($P=.02$) (Table). These results were consistent with our previous finding in the GOLDN Study, which showed that daily energy intake was approximately 200 kcal/d higher in individuals with the CC genotype than in T allele carriers ($P=.005$). However, the magnitude of the genotype effect was lower in FOS (approximately 100 kcal/d), and differences in total fat intake, saturated fat, and monounsaturated fatty acids did not reach the statistical significance that they did in the GOLDN Study. This difference could be owing to the higher prevalence of obesity and total fat and saturated fat intake in the GOLDN Study population (Table). Therefore, we hypothesized that the APOA2 SNP would have a greater influence in determining food intake in individuals with obesity. Consistent with this notion, we found that individuals with obesity who have the CC genotype from the FOS had statistically
higher intakes of calories, total fat, saturated fat, monounsaturated fatty acids, protein, carbohydrates, and fructose than those who carry the T allele carriers (eTable 2). The greater carbohydrate and fructose intake in individuals from the FOS with obesity who have the CC genotype compared with those from the GOLDN Study (Table) reflects a greater intake of fruit and cereal in the FOS individual with the CC genotype, with the crossing point between the 2 regression lines at 22 g/d of saturated fat, which was approximately the population mean. We next assessed the impact of increased saturated fat intake with regard to BMI increase was most noticeable for individuals with the CC genotype, with the crossing point between the 2 regression lines at 22 g/d of saturated fat, which was approximately the population mean. We next assessed the impact of increased saturated fat intake with regard to BMI increase was most noticeable for individuals with the CC genotype, with the crossing point between the 2 regression lines at 22 g/d of saturated fat, which was approximately 2.2 g/d. Moreover, in the FOS population as a whole, the CC genotype was not associated with higher BMI or obesity as previously observed in the GOLDN Study population (Table). In view of the different dietary fat intakes among these populations, we focused on gene–dietary fat interactions. We found a statistically significant interaction between total fat and the APOA2 SNP (P = .04). However, on analysis of the different fat types, the interaction was stronger and more significant for saturated fat, which indicates a more specific effect of this variable; therefore, we focused on saturated fat. When we considered saturated fat as continuous in the FOS population (Figure 1A), the individuals with the CC genotype exhibited a higher association (B = 0.108 kg/m^2; P = .006) than carriers of the T allele (B = 0.033 kg/m^2; P = .03) between saturated fat intake and BMI (P for interaction = .02). Thus, the impact of increased saturated fat intake with regard to BMI increase was most noticeable for individuals with the CC genotype, with the crossing point between the 2 regression lines at 22 g/d of saturated fat, which was approximately the population mean. We next assessed the relationship of the APOA2 SNP with BMI, stratified according to these levels of saturated fat (Figure 1B). We also detected a statistically significant interaction term (P = .01) between the APOA2 SNP and saturated fat intake as categorical. Among those within the lower saturated fat strata (<22 g/d), the APOA2 SNP was not significantly associated with BMI (P = .22). In contrast, the CC genotype was associated with greater BMI (approximately 4.3%, P = .02) in the higher–saturated fat strata. Further adjustment of this interaction for physical activity did not alter the statistical significance of results (P = .03). Furthermore, in the FOS population we examined whether the APOA2–saturated fat interaction is influenced by plasma APOA2 concentrations. Thus, the basic model was adjusted for APOA2, and we found

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### Table. General Characteristics of the Studied Populations Dependent on the APOA2 –265T>C Polymorphism

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Framingham Offspring Study</th>
<th>GOLDN Study</th>
<th>Boston–Puerto Rican Centers on Population Health and Health Disparities Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT + TC (n=1217)</td>
<td>CC (n=237)</td>
<td>TT + TC (n=913)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CC (n=165)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TT + TC (n=832)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CC (n=98)</td>
</tr>
<tr>
<td>Men/women, No.</td>
<td>606/611</td>
<td>110/127</td>
<td>439/474</td>
</tr>
<tr>
<td>Age, y</td>
<td>55.4 (9.3)</td>
<td>55.7 (9.6)</td>
<td>48.7 (16.3)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.5 (16.6)</td>
<td>78.8 (16.9)</td>
<td>82.2 (18.0)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.6 (4.9)</td>
<td>27.5 (4.9)</td>
<td>28.5 (5.5)</td>
</tr>
<tr>
<td>Waist, m</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.2)</td>
<td>0.95 (0.2)</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>204.9 (36.1)</td>
<td>208.3 (36.9)</td>
<td>190.8 (39.3)</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>126.9 (30.8)</td>
<td>128.1 (32.9)</td>
<td>121.4 (21.5)</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>49.4 (14.8)</td>
<td>50.1 (16.1)</td>
<td>47.2 (13.1)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>149.7 (109.9)</td>
<td>150.5 (97.7)</td>
<td>138.4 (101.1)</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>101.5 (29.2)</td>
<td>101.5 (29.2)</td>
<td>101.4 (18.7)</td>
</tr>
<tr>
<td>Total energy intake, kcal/d</td>
<td>1837.8 (611.7)</td>
<td>1940.9 (849.6)</td>
<td>2021.7 (827.4)</td>
</tr>
<tr>
<td>Total fat, g/d</td>
<td>60.9 (25.3)</td>
<td>62.8 (25.6)</td>
<td>80.6 (39.8)</td>
</tr>
<tr>
<td>Saturated fat, g/d</td>
<td>21.3 (9.5)</td>
<td>22.1 (9.7)</td>
<td>27.2 (14.1)</td>
</tr>
<tr>
<td>Monounsaturated fatty acids, g/d</td>
<td>23.1 (10.1)</td>
<td>23.9 (10.1)</td>
<td>30.3 (15.1)</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids, g/d</td>
<td>12.1 (5.4)</td>
<td>12.5 (5.7)</td>
<td>17.1 (8.5)</td>
</tr>
<tr>
<td>Proteins, g/d</td>
<td>69.4 (25.7)</td>
<td>72.0 (27.2)</td>
<td>79.5 (35.2)</td>
</tr>
<tr>
<td>Carbohydrates, g/d</td>
<td>234.7 (89.5)</td>
<td>252.5 (96.1)</td>
<td>245.6 (101.2)</td>
</tr>
<tr>
<td>Physical activity&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.1 (7.3)</td>
<td>36.6 (6.7)</td>
<td>34.2 (6.2)</td>
</tr>
<tr>
<td>Current smoking, No. (%)</td>
<td>214 (17.6)</td>
<td>42 (17.6)</td>
<td>67 (7.3)</td>
</tr>
<tr>
<td>Current drinking, No. (%)</td>
<td>853 (70.1)</td>
<td>169 (71.3)</td>
<td>450 (49.3)</td>
</tr>
<tr>
<td>Lipid medication use, No. (%)</td>
<td>105 (8.6)</td>
<td>18.6 (7.6)</td>
<td>45 (4.9)</td>
</tr>
<tr>
<td>Diabetes mellitus, No. (%)</td>
<td>106 (8.7)</td>
<td>24 (10.1)</td>
<td>70 (7.7)</td>
</tr>
<tr>
<td>Obesity, No. (%)</td>
<td>308 (25.3)</td>
<td>61 (25.7)</td>
<td>293 (32.1)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

SI conversion factors: To convert cholesterol, LDL-C, and HDL-C to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0555.

Data are presented as mean (SD) unless otherwise indicated.

<sup>a</sup>Measured by different physical activity scores in the Framingham Offspring, GOLDN, and Boston–Puerto Rican Centers on Population Health and Health Disparities Study, as described in the “Methods” section.

<sup>b</sup>Statistically significant differences (P < .05) between carriers of the T allele and CC genotype for the corresponding variable in each population.

<sup>c</sup>Current smoking, No. (%) = (Current smoking, No. / Total No.) × 100.
that the significance of the interaction term remained practically unchanged ($P = .01$).

To verify the internal replication of this gene-diet interaction, we analyzed BMI data from 1087 individuals who attended FOS examinations 1 through 5 (20 years of follow-up). When the interaction with saturated fat was not considered, no differences in BMI were observed, depending on the APOA2 SNP at any examination (Figure 1C). However, if 2 strata of saturated fat intake were considered (with the assumption of a similar fat intake strata across the examinations), a statistically significant interaction between the APOA2 SNP and saturated fat ($P = .04$) with regard to BMI across the 20-year follow-up was noted (Figure 1D). Thus, consistent with the results observed for examination 5, individuals with the CC genotype have a higher BMI than the other genotypes throughout the 20-year follow-up period only when they have a high–saturated fat consumption.
Because of the relevance and novelty of this gene-diet interaction, we examined its replication in the GOLDN Study. With consideration of the 2 categories of saturated fat (<22 g/d and ≥22 g/d) that we examined in the FOS, we consistently found the same statistically significant interaction whether considering the unadjusted model or the multivariate basic model (P = .01) (Figure 2A). Further adjustment of this basic model for family relationships (P = .04) or physical activity (P = .01) did not alter the statistical significance of results. Given that this GOLDN Study population consumes a higher–saturated fat diet, the APOA2 SNP was generally associated with higher BMI. However, this observation was not present in GOLDN Study participants with a low–saturated fat intake (P = .45). In contrast, the CC genotype was strongly associated with greater BMI in individuals with a high–saturated fat intake (approximately 6.4%, P = .002). Further internal replication of this interaction was obtained from separate analyses of the Minnesota-based and Utah-based study participants (Figure 2B and C).

Furthermore, we investigated the replication of this gene-diet interaction in an ethnically different population of Hispanics of Caribbean origin living in Boston. We consistently found a statistically significant interaction between the APOA2 SNP and saturated fat with regard to BMI (basic models) whether saturated fat was considered as continuous (P = .003) or categorical (P = .002) (Figure 3). After additional adjustment for physical activity, the interaction terms remained statistically significant (P = .004 and P = .001, respectively). These results were totally in accordance with our previous findings in whites. Thus, in the Boston–Puerto Rican study, when saturated fat intake was high, individuals with the CC genotype also had significantly higher BMI than carriers of the T allele (approximately 7.9%; P = .02). Moreover, further adjustment of basic models for admixture did not change the statistical significance of the interaction terms (P = .006 and P = .003 for continuous and categorical saturated fat variables, respectively).

With the consideration that in the Boston–Puerto Rican Study population prevalence of diabetes was high (42%), we analyzed whether the APOA2–saturated fat interaction was present in individuals with and without diabetes. The internal replication of this interaction was also obtained (P for interaction < .05 in each group: P = .04 for
APOA2 (B, Means of BMI values in both men and women were according to the APOA2 adjusted regression coefficients (B), 95% confidence intervals (CIs), and the corresponding P from the regression models that contain the saturated fat intake, the APOA2 polymorphism, their interaction term, and the potential confounders (sex, age [as continuous], tobacco smoking [as categorical], alcohol consumption [as categorical], diabetes mellitus status [as categorical], cholesterol medication [as categorical], and total energy intake [as continuous]). Circles and squares represent estimated values for T allele carriers and individuals with the CC genotype, respectively. All the variables in the model are referred to by R categorical], and total energy intake [as continuous]).

In 3 independent US populations, we have replicated a gene-diet interaction that influences body weight. This is the first time, to our knowledge, that such consistent replication is found in nutrigenetic studies. This novel and reliable interaction involves influence of the APOA2−265T>C SNP and saturated fat intake on BMI and obesity. When saturated fat intake is low, the APOA2−265T>C SNP does not affect BMI. However, when saturated fat intake is high, this SNP is strongly associated with BMI and obesity. Therefore, this APOA2−saturated fat interaction may clarify previous controversial associations reported for this promoter polymorphism.10,12,16,17 The APOA2 SNP can be considered as a thrifty genotype because, depending on the presence of an obesogenous (high–saturated fat diet) or restrictive (low–saturated fat diet) environment, the phenotypic expression is different. We have selected the cutoff point of 22 g/d to define the 2 saturated fat strata based on the results of the FOS and with consideration that this amount of fat represents 10% of daily energy intake in a standard 2000-kcal/d diet. This figure has been largely reported as the threshold between low–saturated fat and high–saturated fat diets.29 Moreover, we have demonstrated a linear dose effect in the interaction that contributes to its independence from a fixed cutoff level. Another strength of this study is the replication of the interaction, not only in white Americans but also in Hispanics of Caribbean origin living in Boston, with a lower C allele prevalence, which contributes to its external validity and reinforces the notion that individuals with the CC genotype are especially susceptible to the detrimental effect of high–saturated fat diets with individuals with and P=.01 for individuals without diabetes mellitus, results not shown).

Finally, we examined the APOA2–saturated fat interaction to determine obesity in the 3 populations independently and pooled in a meta-analysis (Figure 4). We found consistent gene-diet interactions across all 3 populations. The CC genotype was only associated with a higher prevalence of obesity in individuals in the high–saturated fat stratum. If saturated fat consumption was low, the CC genotype was not associated with obesity. In the meta-analysis, we observed no significant heterogeneity either for the high–saturated fat (I²=0%, P=.90) or for the low–saturated fat stratum (I²=0%, P=.55) group. The overall association meta-analysis in the high–saturated fat group showed a statistically higher OR of obesity for CC homozygotes (OR, 1.84; 95% CI, 1.38-2.47; P<.001), by means of the fixed-effect model. However, in the low–saturated fat group, no increased OR for obesity was found for CC homozygotes in comparison with carriers of the T allele (OR, 0.81; 95% CI, 0.59-1.11; P=.18).

Comment

In 3 independent US populations, we have replicated a gene-diet interaction that influences body weight. This is the first time, to our knowledge, that such consistent replication is found in nutrigenetic studies. This novel and reliable interaction involves influence of the APOA2−265T>C SNP and saturated fat intake on BMI and obesity. When saturated fat intake is low, the APOA2−265T>C SNP does not affect BMI. However, when saturated fat intake is high, this SNP is strongly associated with BMI and obesity. Therefore, this APOA2–saturated fat interaction may clarify previous controversial associations reported for this promoter polymorphism.10,12,16,17 The APOA2 SNP can be considered as a thrifty genotype because, depending on the presence of an obesogenous (high–saturated fat diet) or restrictive (low–saturated fat diet) environment, the phenotypic expression is different. We have selected the cutoff point of 22 g/d to define the 2 saturated fat strata based on the results of the FOS and with consideration that this amount of fat represents 10% of daily energy intake in a standard 2000-kcal/d diet. This figure has been largely reported as the threshold between low–saturated fat and high–saturated fat diets.29 Moreover, we have demonstrated a linear dose effect in the interaction that contributes to its independence from a fixed cutoff level. Another strength of this study is the replication of the interaction, not only in white Americans but also in Hispanics of Caribbean origin living in Boston, with a lower C allele prevalence, which contributes to its external validity and reinforces the notion that individuals with the CC genotype are especially susceptible to the detrimental effect of high–saturated fat diets with...
would contribute to the diversity and complexity of obe-
type), other genes could have similar interactions, which
to 15% of the population (those with the CC geno-
more, although this gene-diet interaction only applies to
ing on the individual genotype. We demonstrate herein
may be the different response to saturated fat, depend-
lyzed diets of FOS participants at examinations 3 and 5,
the whole period. However, in previous work that ana-
assumptions of a similar classification of participants
research to explain such epidemiologic interactions. On this
regard to obesity prevalence. Furthermore, the magni-
tude of the association with obesity was homogeneous
populations and higher (OR, 1.84 in the meta-
Our findings also demonstrated a good internal
across populations and higher (OR, 1.84 in the meta-
for other macronutrients, such as carbohydrates (as continuous), proteins (as continuous), and total fat (as continuous). B and C, Study-specific estimates of
findings. Thus, genetic linkages between body weight and
These results should stimulate more mechanistic re-
search to explain such epidemiologic interactions. On this
issue, there are some lines of evidence that support our
findings. Thus, genetic linkages between body weight and
lipoprotein metabolism in mice are strongly suggested
by a quantitative trait locus for body weight that points
to APOA2. Moreover, studies in mice have revealed a
role of the APOA2 gene expression with regard to insulin resistance, obesity, and atherosclerosis but with
controversial results that have been attributed to di-
etary interactions. Moreover, studies in mice have re-
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etary interactions. Moreover, studies in mice have re-

<table>
<thead>
<tr>
<th>Population</th>
<th>Saturated fat intake, g/d</th>
<th>APOA2 Genotype</th>
<th>No. With Obesity</th>
<th>OR (95% CI)</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Framingham Offspring Study</td>
<td>&lt;22 TT + TC</td>
<td>544</td>
<td>150</td>
<td>1 (Reference)</td>
<td>.42</td>
<td>1 (Reference)</td>
<td>.63</td>
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<tr>
<td></td>
<td>CC</td>
<td>111</td>
<td>24</td>
<td>0.811 (0.486-1.352)</td>
<td>.55</td>
<td>0.879 (0.523-1.478)</td>
<td>.90</td>
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<tr>
<td>≥22 TT + TC</td>
<td>CC</td>
<td>393</td>
<td>135</td>
<td>1 (Reference)</td>
<td>.02</td>
<td>1 (Reference)</td>
<td>.02</td>
</tr>
<tr>
<td>GOLDN Study</td>
<td>&lt;22 TT + TC</td>
<td>257</td>
<td>129</td>
<td>1 (Reference)</td>
<td>.99</td>
<td>1 (Reference)</td>
<td>.78</td>
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<tr>
<td></td>
<td>CC</td>
<td>42</td>
<td>21</td>
<td>0.996 (0.566-1.752)</td>
<td>.91</td>
<td>0.918 (0.505-1.669)</td>
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<tr>
<td>Boston–Puerto Rican Study</td>
<td>&lt;22 TT + TC</td>
<td>364</td>
<td>163</td>
<td>1 (Reference)</td>
<td>.006</td>
<td>1 (Reference)</td>
<td>.02</td>
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<tr>
<td></td>
<td>CC</td>
<td>56</td>
<td>46</td>
<td>1.834 (1.192-2.824)</td>
<td>.18</td>
<td>1.684 (1.067-2.656)</td>
<td>.04</td>
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<tr>
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<td>CC</td>
<td>204</td>
<td>262</td>
<td>1 (Reference)</td>
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<td>.04</td>
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<tr>
<td></td>
<td></td>
<td>29</td>
<td>26</td>
<td>0.631 (0.350-1.136)</td>
<td>.52</td>
<td>0.527 (0.283-0.985)</td>
<td>.03</td>
</tr>
</tbody>
</table>

Figure 4. Interaction between the APOA2 –265T>C polymorphism and saturated fat intake in determination of obesity risk in 3 independent populations (Framingham Offspring Study, the Genetics of Lipid Lowering Drugs and Diet Network [GOLDN] Study, and the Boston–Puerto Rican Centers on Population Health and Health Disparities [Boston–Puerto Rican Study]). Separated and pooled analyses were according to the saturated fat intake strata (<22 g/d and ≥22 g/d). A, Logistic regression estimation in determination of obesity risk in each independent population was according to the saturated fat intake strata (<22 g/d and ≥22 g/d). Study-specific odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each strata of saturated fat intake. Two separate multivariate adjustments were performed. Model 1 was adjusted for sex, age (as continuous), tobacco smoking (as categorical), alcohol consumption (as categorical), diabetes mellitus status (as categorical), and cholesterol medication (as categorical). Model 2 was also adjusted for energy intake (as continuous) and for other macronutrients, such as carbohydrates (as continuous), proteins (as continuous), and total fat (as continuous). B and C, Study-specific estimates of ORs and the pooled estimation of obesity risk in individuals with the CC genotype were according to the 2 strata of saturated fat intake (low and high, respectively) in comparison with carriers of the T allele. Heterogeneity was tested by the Cochran χ²-based Q statistic.
allele-specific binding of the transcription factor CCAAT/enhancer binding protein α (CEBPA), which has been involved in adipogenesis.37 Our results also suggest that APOA2 acts as a satiety signal, as described for APOA4 in other studies,38 given the significant associations between the APOA2 −265T>C SNP and food intake in the FOS and GOLDN studies.12

In conclusion, we have consistently replicated a gene-diet interaction with regard to BMI and obesity in 3 US populations by which individuals with the APOA2 CC genotype seem more susceptible to increased BMI and obesity when they consume a high−saturated fat diet. Therefore, if no unmeasured confounders exist, and these results are replicated in subsequent trials, personalized nutritional recommendations in terms of specific reductions of saturated fat intake in individuals with the CC genotype may be a future nutrigenetics application.

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REFERENCES


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