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%; $P=.01$) 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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GENOMICS IS REVOLUTIONIZING biomedical research and providing great expectations with regard to disease prevention and treatment.[1] The classic candidate gene approach and the newer genome-wide association studies[2] have identified genetic variants that predispose patients to common diseases. If the current trends continue, most common disease-predisposition polymorphisms will soon be identified. Thus, a major remaining research challenge will be to characterize gene-environment interactions because these are essential for the translation of genomics into clinical medicine and improved public health.[3] Diet is one of the most important environmental factors that interacts with the genome to modulate disease risk,[4] and better understanding of these interactions has the potential to support disease prevention via modification of dietary recommendations. However, progress in this area has been slow because of the low evidence level achieved so far. Although studies[4-7] reported enticing gene-diet interactions, their level of replication has been extremely low. Thus, a vast proportion of gene-diet interactions has not been replicated. Only a small number of interactions have been replicated twice, and, to the best of our knowledge, none has been replicated in 3 or more independent populations. This problem has plagued classic genotype-phenotype association studies and, currently, consistency

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is thought to be a crucial causal criterion of credibility of genome-wide association studies.[8] Therefore, the National Cancer Institute–National Human Genome Research Institute Working Group on Replication in Genotype-Phenotype Associations[9] 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)[10,11] 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 FOS[18] 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 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 45 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.[12,17,20,22] 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.[21] 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 Survey[23] was estimated. Fasting glucose, triglycerides, total cholesterol, and high-density lipoprotein cholesterol levels were measured by standard methods.[12,19,20,22] For the FOS samples, plasma *APOA1* and *APOA2* concentrations were determined by means of turbidimetric immunoassays (Wako Chemicals USA Inc, Richmond, Virginia).

DIETARY INTAKE

Diet was measured by validated questionnaires in each specific population[24-26]: the Willett[24] semiquantitative food frequency questionnaire, administered at examination 5, in the FOS; the Diet History Questionnaire in the GOLDN Study[12,25]; and a specially designed and validated food frequency questionnaire[26] 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; Qiagen, 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 χ^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,[12] in which similar effects were found for individuals with TT and TC genotypes, recessive effects for 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.[12] 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[27] 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.[28]

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 *APOA1* 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 *APOA1*, 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

Table. General Characteristics of the Studied Populations Dependent on the *APOA2* –265T>C Polymorphism[a]

Characteristics	Framingham Offspring Study		GOLDN Study		Boston–Puerto Rican Centers on Population Health and Health Disparities Study	
	TT+TC (n=1217)	CC (n=237)	TT+TC (n=913)	CC (n=165)	TT+TC (n=832)	CC (n=98)
Men/women, No.	606/611	110/127	439/474	75/90	231/601	32/66
Age, y	55.4 (9.3)	55.7 (9.8)	48.7 (16.3)	49.8 (15.4)	57.7 (7.7)	57.9 (7.5)
Weight, kg	78.5 (16.6)	78.8 (16.9)	82.2 (18.0)	86.1 (19.2)[b]	80.2 (17.4)	81.5 (19.7)
BMI	27.6 (4.9)	27.5 (4.9)	28 (5.5)	29.2 (6.2)[b]	32.1 (6.8)	32.1 (7.2)
Waist, m	1.0 (0.1)	1.0 (0.1)	0.95 (0.2)	0.99 (0.2)[b]	1.0 (0.1)	1.0 (0.2)
Cholesterol, mg/dL	204.9 (36.1)	208.3 (36.9)	190.8 (39.3)	189.8 (26.7)	184.1 (43.0)	182.8 (38.3)
LDL-C, mg/dL	126.9 (30.8)	129.1 (32.9)	121.4 (31.5)	121.4 (30.1)	107.2 (34.9)	109.0 (34.4)
HDL-C, mg/dL	49.4 (14.8)	50.1 (16.1)	47.2 (13.1)	47.3 (13.2)	44.5 (12.2)	44.6 (12.4)
Triglycerides, mg/dL	149.7 (109.9)	150.5 (97.7)	138.4 (101.1)	131.0 (74.5)	164.5 (123.7)	157.8 (87.4)
Fasting glucose, mg/dL	101.5 (29.2)	101.5 (29.2)	101.4 (18.7)	101.4 (15.7)	122.8 (53.1)	117.8 (40.2)
Total energy intake, kcal/d	1837.8 (611.7)	1940.9 (649.6)[b]	2021.7 (827.4)	2203.9 (973.2)[b]	2120.1 (868.0)	2076.9 (853.4)
Total fat, g/d	60.5 (25.3)	62.8 (25.6)	80.6 (39.8)	89.8 (44.2)[b]	74.0 (34.9)	71.9 (35.1)
Saturated fat, g/d	21.3 (9.5)	22.1 (9.7)	27.2 (14.1)	29.9 (15.9)[b]	23.1 (11.9)	22.3 (12.2)
Monounsaturated fatty acids, g/d	23.1 (10.1)	23.9 (10.1)	30.3 (15.1)	34.1 (17.1)[b]	26.8 (13.0)	26.0 (12.9)
Polyunsaturated fatty acids, g/d	12.1 (5.4)	12.5 (5.7)	17.1 (8.5)	19.2 (9.7)[b]	17.8 (8.6)	17.7 (8.6)
Proteins, g/d	69.4 (25.7)	72.0 (27.2)	79.5 (35.2)	87.0 (40.1)[b]	91.6 (40.7)	89.8 (43.1)
Carbohydrates, g/d	234.7 (89.5)	252.1 (96.1)[b]	245.6 (101.2)	259.3 (123.3)	272.1 (112.8)	269.1 (109.8)
Physical activity[c]	37.1 (7.3)	36.6 (6.7)	34.2 (6.2)	34.7 (6.4)	31.5 (4.5)	31.1 (4.7)
Current smoking, No. (%)	214 (17.6)	42 (17.6)	67 (7.3)	14 (8.5)	197 (23.7)	24 (24.7)
Current drinking, No. (%)	853 (70.1)	169 (71.3)	450 (49.3)	96 (58.2)[b]	320 (38.5)	36 (37.1)
Lipid medication use, No. (%)	105 (8.6)	18 (7.6)	45 (4.9)	8 (5.1)	310 (37.3)	42 (42.9)
Diabetes mellitus, No. (%)	106 (8.7)	24 (10.1)	70 (7.7)	16 (9.7)	337 (40.5)	46 (46.9)
Obesity, No. (%)	308 (25.3)	61 (25.7)	293 (32.1)	67 (40.6)[b]	472 (56.8)	56 (57.1)

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 0.0113; and fasting glucose to millimoles per liter, multiply by 0.0555.

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

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

[c] 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.

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 with those same characteristics could reflect a greater intake of fruit and cereal in the FOS participants to satisfy their greater appetite. Possibly this is the case for individuals from the FOS, despite their following a healthy diet, because they possess the CC genotype. Further adjustment for physical activity did not affect the statistical significance of results.

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 1**A), 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

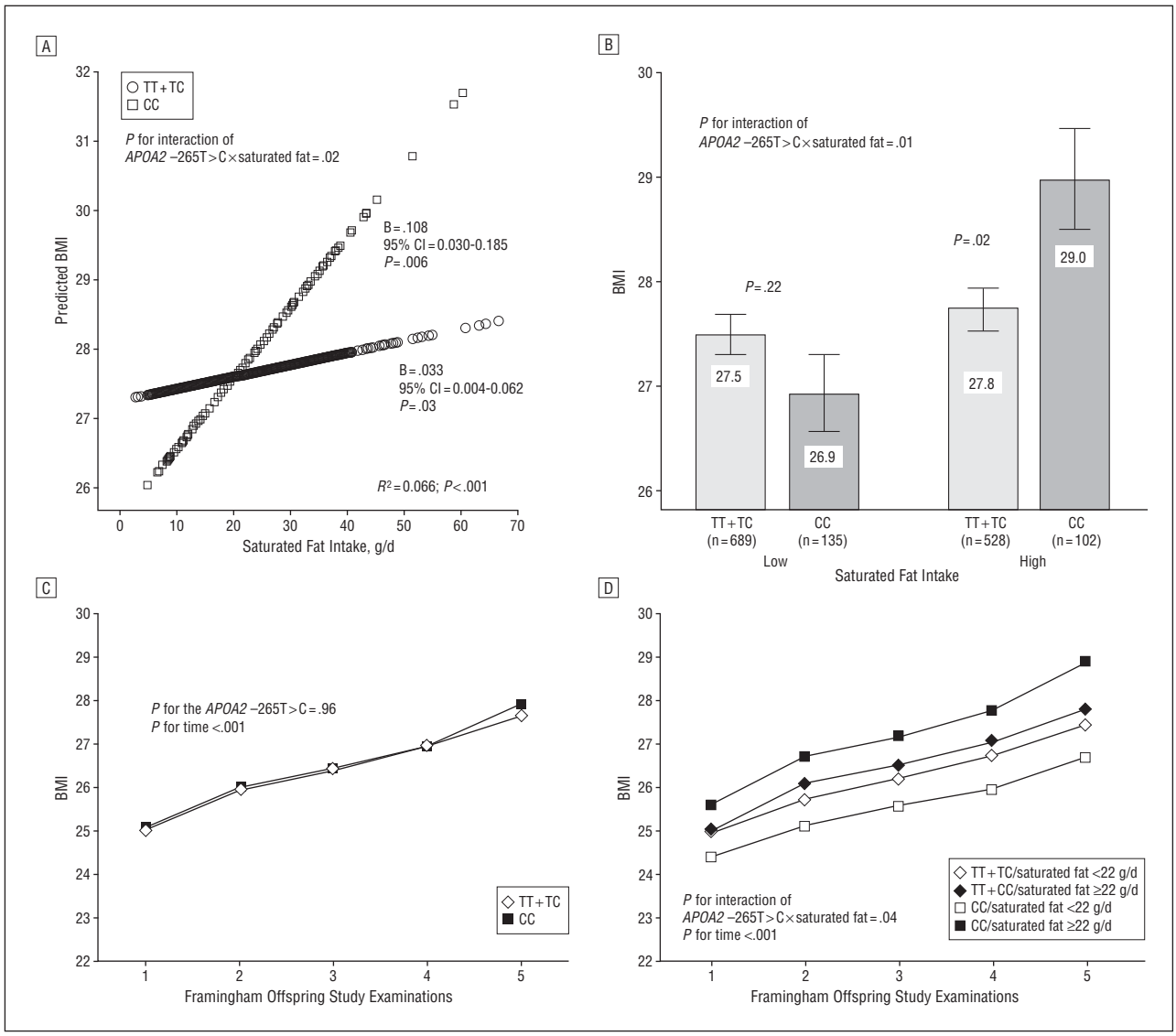

Figure 1. Interaction between the *APOA2* –265T>C polymorphism and saturated fat intake with regard to body mass index (BMI) in the Framingham Offspring Study. A, Predicted values of BMI at examination 5 by the *APOA2* –265T>C polymorphism (n=1217 carriers of the T allele and n=237 individuals with the CC genotype), dependent on the saturated fat consumed (as continuous) in men and women. Predicted values were calculated from the regression models that contain 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. The *P* value for the interaction term was obtained in the multivariate interaction model. All the variables in the model are referred to by R^2 (r^2=0.016, *P*=.01 for the interaction variables). In the stratified analysis by genotype, multivariate-adjusted regression coefficients (B), 95% confidence intervals (CIs), and the corresponding *P* values were estimated after adjustment for the described covariates. B, Means of BMI values at examination 5 in both men and women according to the *APOA2* –265T>C polymorphism in accordance with the strata of saturated fat intake (< 22 g/d [low] and ≥22 g/d [high]). Estimated means were adjusted for the same factors as in panel A. The *P* values for the interaction terms between saturated fat intake (as dichotomous) and the *APOA2* polymorphism were obtained in the hierarchical multivariate interaction model. In the stratified analysis by saturated fat intake levels, *P* values were estimated after multivariate adjustment for the covariates indicated for panel A. Bars indicate SEMs. C and D, Longitudinal analysis of BMI values dependent on the *APOA2* –265T>C polymorphism across 20 years of follow-up in the Framingham Offspring Study. We included 1087 study participants who attended each of the first 5 examinations. C, Model did not include the interaction term between the polymorphism and saturated fat and was adjusted for sex and age. D, Model also included the interaction term with saturated fat (as dichotomous, 2 strata, as in examination 5).

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.

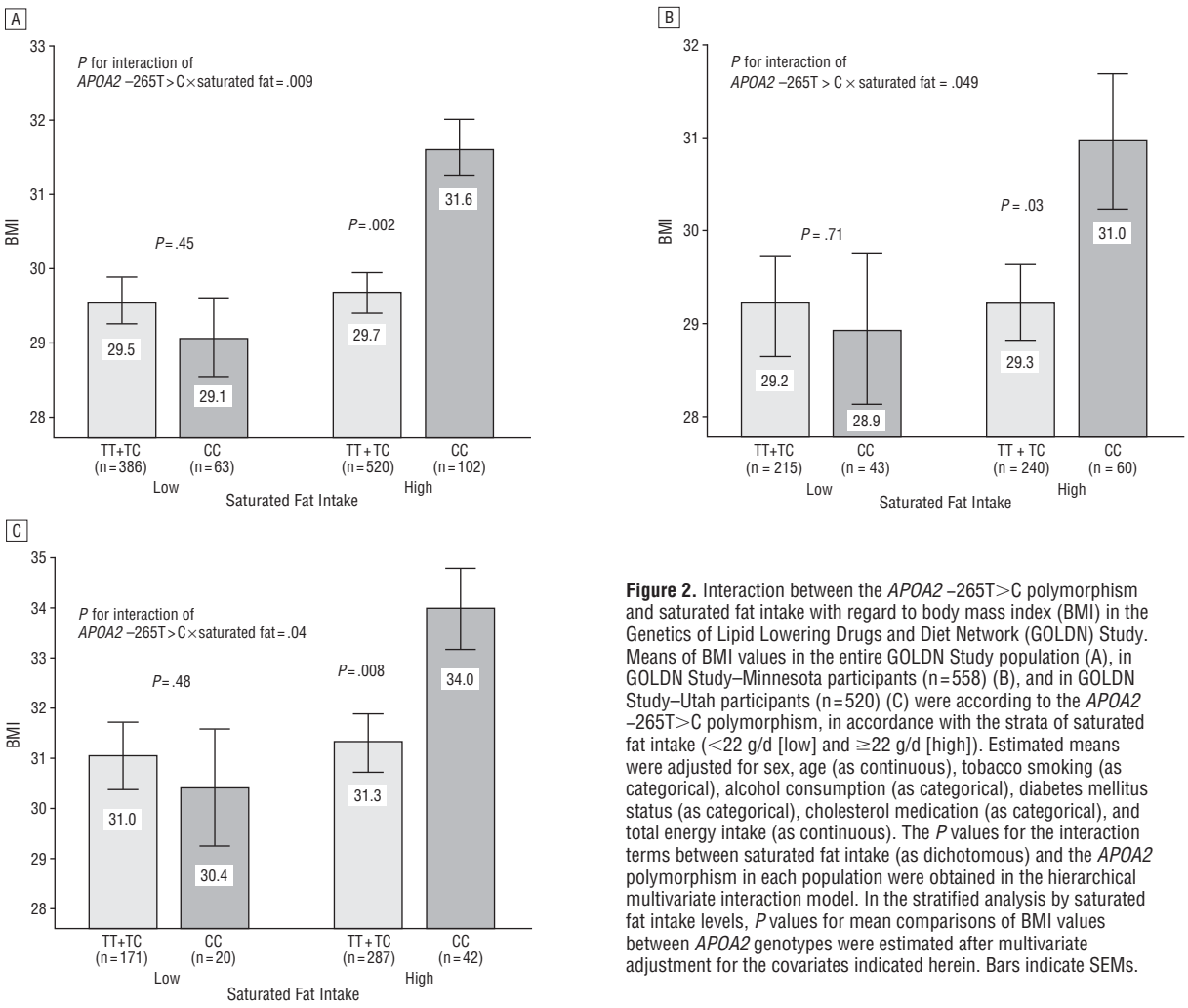

Figure 2. Interaction between the *APOA2* –265T>C polymorphism and saturated fat intake with regard to body mass index (BMI) in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study. Means of BMI values in the entire GOLDN Study population (A), in GOLDN Study–Minnesota participants (n=558) (B), and in GOLDN Study–Utah participants (n=520) (C) were according to the *APOA2* –265T>C polymorphism, in accordance with the strata of saturated fat intake (<22 g/d [low] and ≥22 g/d [high]). Estimated means were adjusted for 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). The *P* values for the interaction terms between saturated fat intake (as dichotomous) and the *APOA2* polymorphism in each population were obtained in the hierarchical multivariate interaction model. In the stratified analysis by saturated fat intake levels, *P* values for mean comparisons of BMI values between *APOA2* genotypes were estimated after multivariate adjustment for the covariates indicated herein. Bars indicate SEMs.

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 2**A). 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[27] 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

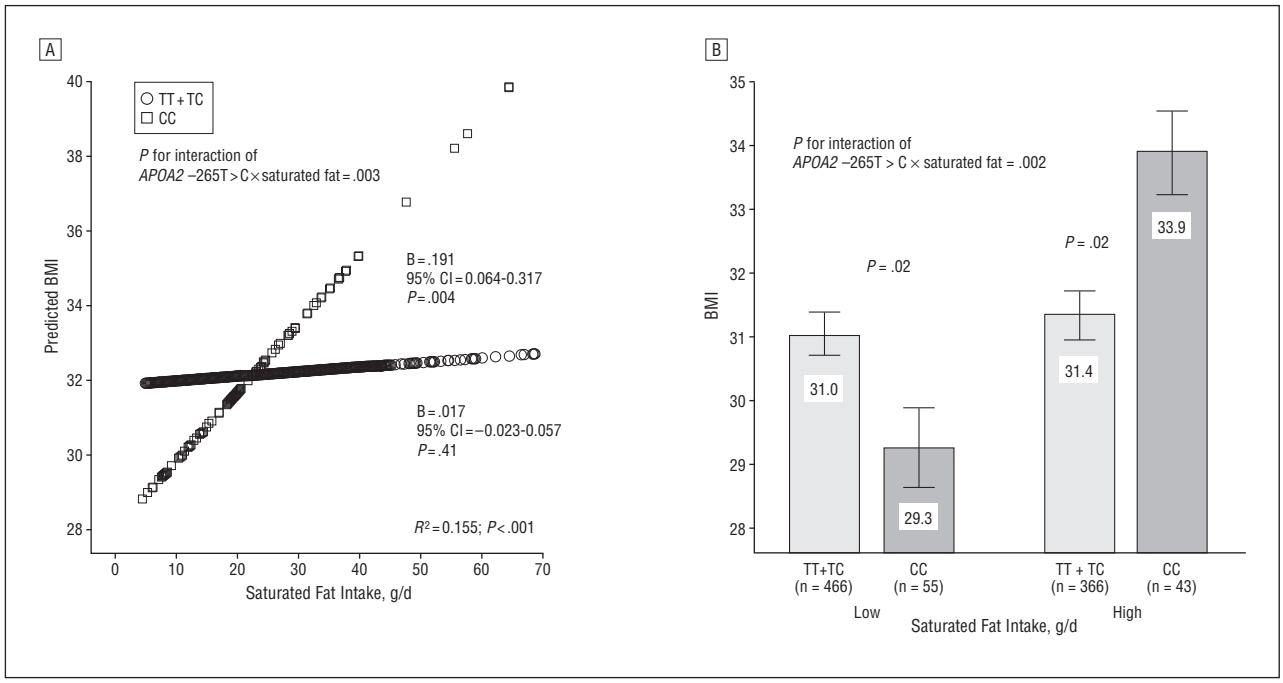

Figure 3. Interaction between the *APOA2* –265T>C polymorphism and saturated fat intake with regard to body mass index (BMI) in the Boston–Puerto Rican Centers on Population Health and Health Disparities Study. A, Predicted values of BMI by the *APOA2* –265T>C polymorphism (n=832 carriers of the T allele and n=98 individuals with the CC genotype) were according to the saturated fat consumed (as continuous) in both men and women. Predicted values were calculated 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^2 (r^2=0.012, P=.008 for the interaction variables). In the stratified analysis by genotype, multivariate adjusted regression coefficients (B), 95% confidence intervals (CIs), and the corresponding P values were estimated after adjustment for the described covariates. B, Means of BMI values in both men and women were according to the *APOA2* –265T>C polymorphism in accordance with the strata of saturated fat intake (<22 g/d and ≥22 g/d). Estimated means were adjusted for the same factors in panel A. In the stratified analysis by saturated fat intake levels, P values for mean comparisons of BMI values between *APOA2* genotypes were estimated after multivariate adjustment for the covariates indicated for panel A. Bars indicate SEMs.

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 (I^2=0%, P=.90) or for the low–saturated fat stratum (I^2=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

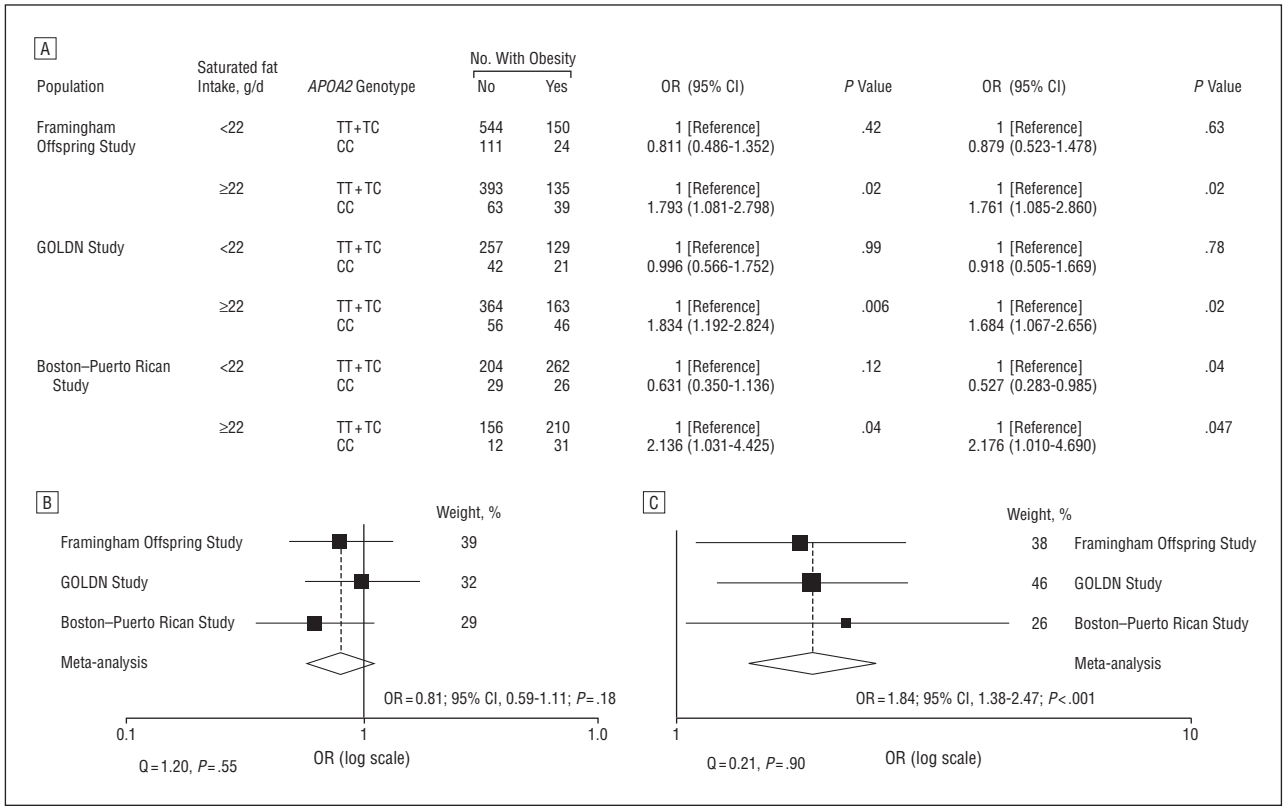

Figure 4. Interaction between the *APOA2* –265T>C polymorphism and saturated fat intake in determination of obesity risk in 3 independent populations (Framingham Offspring Study, the Genetics of Lipid Lowering Drugs and Diet Network [GOLDN] Study, and the Boston–Puerto Rican Centers on Population Health 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 χ^2-based Q statistic.

regard to obesity prevalence. Furthermore, the magnitude of the association with obesity was homogeneous across populations and higher (OR, 1.84 in the meta-analysis) than usually reported in relevant genetic studies.[30] Our findings also demonstrated a good internal replication within each population. Of note is the interesting longitudinal observation found in the FOS during the 20-year follow-up. One limitation of this analysis is the assumption of a similar classification of participants into lower–saturated fat or higher–saturated fat strata for the whole period. However, in previous work that analyzed diets of FOS participants at examinations 3 and 5, we demonstrated stable patterns of consumption over time, specifically for intakes of saturated fat.[31]

On the other hand, the association between saturated fat intake and obesity risk is controversial and has been the subject of intense debate.[32,33] One explanation may be the different response to saturated fat, depending on the individual genotype. We demonstrate herein that the effect of saturated fat on BMI and obesity is highly dependent on the *APOA2* –265T>C genotype. Furthermore, although this gene-diet interaction only applies to 10% to 15% of the population (those with the CC genotype), other genes could have similar interactions, which would contribute to the diversity and complexity of obesity. With consideration that efforts to manage obesity have not proven to be as widely successful as many had hoped, new perspectives to reenergize prevention and treatment are needed. Thus, study of the interaction effects of dietary factors and genes with regard to obesity may define the new challenge of this field. Moreover, recent studies[34] have outlined the fact that Mediterranean, low-carbohydrate, or other types of diet may be effective alternatives to low-fat diets for weight loss. However, these studies did not analyze genetic factors and so did not identify which individuals would respond better to each type of diet. Such information will be crucial in the new era of obesity research.

These results should stimulate more mechanistic research 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[10,13,14,35] 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 dietary interactions.[36] In addition, our further in silico analysis of the –265 *APOA2* region indicates the possibility of

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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