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Association between the *APOA2* promoter polymorphism and body-weight in Mediterranean and Asian populations. Replication of a gene-saturated fat interaction

Dolores Corella[1,2,3], **E Shyong Tai**[4], **Jose V Sorlí**[2,3], **Suok Kai Chew**[5], **Oscar Coltell**[1,6], **Mercedes Sotos-Prieto**[2,3], **Antonio García-Rios**[3,7], **Ramón Estruch**[3,8], and **Jose M. Ordovas**[1,3,9,*]

[1]Nutrition and Genomics Laboratory, JM-USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA

[2]Genetic and Molecular Epidemiology Unit School of Medicine. University of Valencia, Valencia, Spain

[3]CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain

[4]Department of Endocrinology, Singapore General Hospital, Singapore

[5]Epidemiology and Disease Control Division, Ministry of Health Singapore, Singapore

[6]Department of Computing Languages and Systems. Universitat Jaume I. Castellon, Spain.

[7]Reina Sofia University Hospital, Lipids and Atherosclerosis Research Unit, University of Cordoba, Cordoba, Spain

[8]Department of Internal Medicine. Hospital Clinic, Barcelona, Spain

[9]Department of Cardiovascular Epidemiology and Population Genetics, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Abstract

Objective—The *APOA2* gene has been associated with obesity and insulin resistance (IR) in animal and human studies with controversial results. We have reported an *APOA2*-saturated fat interaction determining body mass index (BMI) and obesity in American populations. This work aims to extend our findings to European and Asian populations.

Methods—Cross-sectional study in 4602 subjects from 2 independent populations: A high cardiovascular risk Mediterranean population (n=907 men and women; aged 67+/−6 years) and a multiethnic Asian population (n=2506 Chinese, n=605 Malays and n=494 Asian Indians; aged 39+/−12 years), participating in a Singapore National Health Survey. Anthropometric, clinical,

[*]**Correspondence to**: Jose M. Ordovas. Director, Nutrition and Genomics Laboratory, JM-USDA Human Nutrition, Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111-1524, USA. Tel: (617) 556-3102. Fax: (617) 556-3211, jose.ordovas@tufts.edu.

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biochemical, lifestyle and dietary variables were determined. Homeostasis model assessment of IR (HOMA-IR) was used in Asians. We analyzed gene-diet interactions between the *APOA2* −265T>C polymorphism and saturated fat intake (<or>=22 g/d) on anthropometric measures and IR.

Results—Frequency of CC subjects differed among populations (1%–15%). We confirmed a recessive effect of the *APOA2* polymorphism, and replicated the *APOA2*–saturated fat interaction on body-weight. In Mediterranean individuals, the CC genotype was associated with a 6.8% greater BMI in those consuming a high (P=0.018), but not a low (P=0.316) saturated fat diet. Likewise, the CC genotype was significantly associated with higher obesity prevalence in Chinese and Asian Indians only with a high-saturated fat intake (P=0.036). We also found a significant *APOA2*-saturated fat interaction in determining IR in Chinese and Asian Indians (P=0.026).

Conclusion—The influence of the *APOA2* −265T>C polymorphism on body-weight-related measures was modulated by saturated fat in Mediterranean and Asian populations.

Keywords

Obesity; gene-diet interaction; insulin resistance; saturated fat; APOA2

INTRODUCTION

For over two decades, the search for the genetic background underlying common forms of obesity has yielded scores of associations that, for the most part, could not be successfully validated in replication studies (1). Genome-wide association (GWA) studies have led to the discovery of new obesity genes (2–4). Among them, the obesity-associated gene (FTO) has now become the first gene with associations thoroughly replicated (2,5,6). Nevertheless, common interactions with environmental factors may have contributed to mask the effect of other single nucleotide polymorphisms (SNPs) on obesity (7). Progress in the investigation and replication of gene-environment interactions for obesity has been slow. However, some steps forward have been accomplished, such as those involving the FTO with physical activity (8–10), and the *APOA2* with saturated fat intake (11). In both cases, significant interactions were found and replicated in independent populations. Regarding the *APOA2* locus, several associations with obesity, insulin-resistance (IR) and diabetes have been reported both in animal (12–15) and human studies (16–18). Specifically, the −265T>C promoter polymorphism (rs5082), which has proved to be functional in two independent investigations (19,20), has been significantly associated with obesity-related measures in some human studies (11,19,21–23). In a previous investigation carried out on White Americans participating in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study (21), we observed recessive effects for the −265T>C polymorphism. Homozygous individuals for the −265C allele had higher body mass index (BMI) and obesity risk than did carriers of the T allele. However, we did not find significant associations between this SNP and body-weight measures in the Framingham Study or in the Boston Puerto Rican study, but we did find significant interactions between this SNP and saturated fat intake (11). When saturated fat intake was high (higher than 22 g/d; approx 10% of energy), individuals homozygous for the −265C allele had higher mean BMI than

carriers of the T allele (an association which was statistically significant), but no differences in BMI were found when saturated fat intake was low.

Therefore, our objectives were to extend previous findings by: 1) analyzing the association between the *APOA2* −265T>C SNP and body-weight-related variables in a Mediterranean and in a multiethnic Asian population; 2) examining the replication of the previously reported interaction between this SNP and saturated fat intake on body-weight in these populations and 3) exploring the association of this SNP with IR and diabetes and its potential modulation by saturated fat.

PATIENTS AND METHODS

We analyzed 4,602 subjects from two independent populations. All participants gave their informed consent. The ethics committee of the University of Valencia and the ethics committee of the Singapore General Hospital approved the studies.

Mediterranean population

We studied 907 unrelated Whites (325 men and 582 women), aged 55–80 years who participated in the PREDIMED (Prevención con Dieta Mediterránea) study and were consecutively recruited in the Valencia Region (Spain) from October 2003 to September 2008. Details of this study have been reported elsewhere (24). Briefly, high-risk participants were selected by physicians in primary care centers. Eligible subjects were elderly community-dwelling persons who fulfilled at least 1 of 2 criteria: type 2 diabetes or 3 or more cardiovascular disease risk factors (current smoking, hypertension, dyslipidemia, overweight, or a family history of premature cardiovascular disease). Here, we included data from the baseline cross-sectional examination.

Asian population

We studied 3,695 individuals (1,714 males and 1,981 females) who participated in the 1998 Singapore National Health Survey and had genetic data. The detailed methodology of this survey on a nationally representative household sample has been described elsewhere (25). Briefly, the survey protocol was based on the WHO-recommended model for field surveys on diabetes and the WHO MONICA protocol. Individuals representing the Singapore housing population (Chinese, Malays and Indians) aged 18–69 years were selected by disproportionate stratified and systematic sampling. Dietary intake was only assessed in a random sub-sample (1 in 2) of the participants. In the present study, dietary data were available for 1,923 subjects (1,204 Chinese, 412 Malays and 307 Asian Indians). These subjects did not differ in the main variables examined from subjects without dietary data.

Anthropometric, biochemical, clinical and lifestyle determinations

Anthropometric variables including height, weight and waist were measured in both populations by standard techniques (24,25). Body mass index (BMI) was calculated as weight (kg)/height^2(m). Obesity was defined as BMI>=30kg/m^2 for the Mediterranean population and as BMI>=27kg/m^2 for the Asian population (26,27). Fasting triglycerides and HDL-C were measured by standard methods (24,25). In the Asian population, fasting

glucose (Boehringer Manheim, Mannheim, Germany) and fasting insulin (immunoassay using an Abbott AxSYM; Abbott Laboratories, Chicago, IL) were determined in all participants (25). IR (fasting glucose × fasting insulin/22.5) was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) method (28), as a biomarker for IR. In the Asian population, previously diagnosed diabetes was assessed by questionnaire (25). After the fasting sample collection, a 75-g oral glucose tolerance test was taken for all subjects except for previously diagnosed diabetic subjects. After 2 h, glucose (2-h glucose) and insulin (2-h insulin) were measured. Following biochemical determinations, subjects with fasting glucose ≥126 mg/dL or 2-h glucose ≥200 mg/dL were also classified as having diabetes (29). In the PREDIMED Study, IR was not determined. Diagnosis of type 2 diabetes was based on at least one of the following criteria: i) Current treatment with insulin or oral hypoglycemic drugs; ii) Fasting blood glucose >=126 mg/dl in two measurements; iii) Casual fasting blood glucose >= 200mg/dl; iv) 2-h glucose >=200 mg/dL (after the oral glucose tolerance test). In the PREDIMED Study, practically all the diabetic subjects (>99%) had previously been diagnosed by the doctor (based on the above criteria) before entering the study. However, in the Asian population an important number of previously unknown diabetes cases (more than 55% of total diabetics) were detected.

In the PREDIMED Study, the baseline examination included assessment of standard cardiovascular risk factors, medication use, socio-demographic factors and lifestyle variables, as previously detailed (24). Physical activity was estimated by the Minnesota Leisure Time Physical Activity questionnaire (30). In Asians, an interviewer-administered questionnaire was used to capture data on sociodemographic factors, smoking, alcohol consumption and physical activity as previously reported (25).

Dietary intake

In the Mediterranean population food consumption and nutrient intake was determined by a validated food frequency questionnaire (FFQ) (31) and Spanish food composition tables. In Singapore, a validated FFQ was used (32). It comprised 159 individual food items, grouped into 23 main food types and 25 food subtypes to ensure that foods consumed by the three ethnic groups were represented. The food composition database of the Singapore Ministry of Health was used to estimate macronutrient intake.

Genetic analyses

We performed the *APOA2*−265T>C genotyping (rs5082) using a Taqman assay with allele-specific probes in the ABIPrism 7900HT Sequence Detection System (Applied Biosystems) (21). Quality control measures including positive and negative controls as well as replicated samples (10% at random) were employed. Genotype frequencies were consistent with Hardy-Weinberg equilibrium (P=0.10, P=0.79, P=0.59, P=0.20 for Mediterranean subjects, Chinese, Malays and Asian Indians, respectively).

Statistical analyses

The Chi square test was used to compare proportions. We applied ANOVA or Student's *t*-test to compare crude means. Multivariate adjustment of continuous variables was carried out by linear regression. Triglycerides and IR were log-transformed. Recessive effects of the

APOA2 SNP were tested and considered in the models. To study gene-diet interactions in determining BMI and body-weight, we used multivariate linear regression models including main effects and interaction terms and control for the same variables that we reported in our previous study (11). We fitted separate models for each population. Saturated fat intake was considered as categorical (low or high). 22g/d was established as the cut-off point based on our previous work (11). We adjusted interaction models for sex, age, ethnicity (when including more than one population), smoking, drinking, diabetes, lipid medication and total energy intake (basic models). The adjustment for lipid medication was not included in the Asian populations because this information was not available. Further adjustments of basic models for physical activity or hypertension were also considered. In addition, adjustment for total fat, MUFA and carbohydrates were carried out when indicated.

Logistic regression models, including main effects and interaction terms, were fitted to test the *APOA2*-SATFAT interaction in determining the odds ratio (OR) of obesity and diabetes. OR and 95% confidence interval (CI) were estimated for each stratum of saturated fat. Multivariate adjustments were undertaken as indicated. Standard regression diagnostic procedures were used to ensure the appropriateness of the fitted models. All reported probability tests were 2-sided. Differences were considered significant at $P < 0.05$.

RESULTS

Characteristics of the populations

We studied subjects from a Mediterranean and a multiethnic Asian population (Table 1). By design, Mediterranean subjects were older and had a higher prevalence of cardiovascular risk factors. In Singapore, prevalence of diabetes was higher in Asian Indians (19.6%). Chinese had lower BMI and diabetes. Prevalence of CC subjects was higher in the Mediterranean population (15%). Saturated fat intake was lower in Chinese. We did not find significant associations between the *APOA2* SNP and saturated fat intake either in Chinese or Asian Indians, but a borderline significant association was observed in Malays (P=0.08 Fisher exact test). 100% of the Malay CC subjects (n=6) consumed more than 22 g/d of saturated fat vs 59% in T-allele carriers. In the Mediterranean population, we found a significant association (P=0.014) in males (84% of the CC males consumed >=22 g/d of saturated fat vs 65% in T-allele carriers). No significant association was detected in females.

Associations between the *APOA2* −265T>C SNP and anthropometric variables

Consistent with our previous findings (11), the effects of this SNP in body-weight were recessive in both Mediterranean subjects and Asian Indians (Figure 1). No associations were found in Chinese or Malays (Table 2). In Asian Indians, homozygous CC subjects had higher means of weight, BMI and waist circumference than T-allele carriers. Prevalence of obesity (BMI>=27 Kg/m2) was also statistically higher in CC Indian subjects (OR for the CC in comparison with TT+CT subjects was 3.21; 95%CI: 1.45–7.12, P=0.004), after multivariate adjustment for sex, age, smoking, drinking and diabetes. Further adjustment for physical activity did not change the estimation (P=0.005). In the Mediterranean population, the *APOA2* SNP was significantly associated with weight and BMI after multivariate adjustment for sex, age, smoking, drinking and diabetes. No significant association was

found with waist circumference (P=0.375). Obesity was not significantly associated with the CC genotype, either unadjusted or after adjustment for sex, age, smoking, drinking and diabetes (OR: 1.28; 95%CI, 0.83–1.96, P=0.264).

Interactions between the *APOA2* −265T>C SNP and saturated fat intake in determining anthropometric variables

According to the interaction model previously reported (11), two categories of saturated fat intake were considered (<22g/d and >=22 g/d). In the Mediterranean population (Figure 2 A), we found a statistically significant interaction between the *APOA2* SNP and saturated fat on BMI in the multivariate adjusted model (P for interaction: 0.030). Among those within the lower saturated fat strata (<22g/d), the *APOA2* SNP was not significantly associated with BMI (P=0.316). In contrast, the CC genotype was associated with greater BMI (P=0.018) in the higher saturated fat strata. Further adjustment for physical activity, total fat intake, MUFA and carbohydrates did not change the statistical significance (P: 0.045). In the sub-group analysis by gender, we found that the magnitude of the interaction was higher in women than in men. However, taking into account the lower sample size for men, we cannot confirm differential effects.

In the Asian population, the *APOA2*-saturated fat interactions were not analyzed in Malays because there were no CC subjects that had a saturated fat intake lower than 22g/d. Firstly, the interaction analysis was undertaken in Chinese and Asian Indians jointly (Figure 2B). Although a greater mean BMI was observed in CC subjects consuming a high saturated fat, the differences did no reach the statistical significance, and the interaction term between the *APOA2* genotype and saturated fat was not statistically significant (P=0.531). Taking into account the low prevalence of the CC genotype in Chinese, and the possible heterogeneity of the effects compared with Asian Indians, we analyzed the *APOA2*-saturated fat interaction in Asian Indians. Although the interaction term was not statistically significant (P=0.445), we observed similar interaction effects to those of the Mediterranean population. A higher saturated fat intake increased the differences in BMI between the *APOA2* genotypes (about 7.1% in the high saturated fat strata: 25.2+/−0.5 Kg/m2 in TT+TC versus 27.1+/−1.2 Kg/m2 in CC; P=0.155 and less than 1% in the low saturated fat: 25.4+/−0.6 Kg/m2 in TT+TC versus 25.7+/−1.5 Kg/m2 in CC subjects; P =0.758). These results could reflect a lack of statistical power due to the small sample size (only 18 CC Indian subjects with dietary data). Considering our limitations in sample size in the Asian population for detecting as statistically significant the *APOA2*-saturated fat interaction in determining the continuous variable BMI, we focused on the dichotomic variable of obesity (BMI>=27 Kg/m^2). For this analysis, our sample size had sufficient power to detect statistically significant differences. Consistent with our previous results, we found that the CC genotype was only associated with a higher OR of obesity in subjects in the high-saturated fat stratum (Table 3). If saturated fat consumption was low, the CC genotype was not associated with obesity. These results were statistically significant for Asian Indians and also reached the statistical significance when the Chinese and Asian Indians were analyzed jointly (OR for obesity in CC homozygotes: 3.31; 95%CI:1.08–10.18; P=0.036 in the high fat strata).

Association between the *APOA2* −265T>C SNP, IR and diabetes. Modulation by saturated fat intake

In Asian Indians, the *APOA2* SNP was significantly associated with higher IR in the unadjusted analysis (3.7+/−1.9 in CC vs 2.8+/−2.8 in T-allele carriers; P=0.012). No statistically significant associations were found in Chinese (1.9+/−1.3 in CC vs 1.8+/−1.3 in T-allele carriers; P=0.611) or in Malays (2.0+/−1.3 in CC vs 2.4+/−2.3 in T-allele carriers; P=0.484). Next, we tested the *APOA2*-saturated fat interaction in determining IR in Chinese and Asian Indians (Figure 3) and found statistically significant results (P for interaction: 0.026). The CC genotype was associated with higher IR only when saturated fat intake was high (>=22g/d). The *APOA2*-saturated fat interaction remained statistically significant even after additional adjustment for BMI (P=0.039).

We further analyzed the association between the *APOA2* SNP and diabetes in Chinese and Asian Indians. In comparison with T-allele carriers, CC subjects had a higher risk of diabetes (OR: 2.14; 95%CI: 1.01–4.56, P=0.048) after adjustment for sex, age and ethnic group. However additional adjustment for smoking, drinking and BMI, attenuated the association (P=0.167). We tested if this association was modulated by saturated fat and found that, in the low saturated fat strata (<22 g/d), the CC genotype was not associated with higher diabetes risk (OR: 0.72; 95%CI: 0.08–6.72; P=0.770). However, in the high-saturated fat strata, the CC genotype was borderline (P=0.080) associated with higher diabetes risk (OR: 3.10; 95%CI: 0.87–11.02), after adjustment for sex, age, ethnic group, smoking, drinking and total energy intake. Further adjustment for other macronutrients and physical activity did not change the statistical significance of results.

In the Mediterranean population, we did not measure IR. In terms of diabetes, the *APOA2* SNP was not associated with diabetes in the whole population (P=0.927). However, in females, prevalence of diabetes was higher in CC women consuming less than 22g/d of saturated fat (P=0.045), suggesting a potential situation of reverse causation.

DISCUSSION

We have previously reported in three American populations that the *APOA2* −265T>C SNP was associated with BMI or obesity only in the presence of high-saturated fat intake (11). Our current data are consistent with this gene-diet interaction and extends the findings to other geographical areas (Europe and Asia). When saturated fat intake is low (<22g/d), this SNP does not have any effect on BMI or obesity. However, when saturated fat intake is high (>=22g/d), significant differences in anthropometric variables were detected between CC individuals and T-allele carriers. Further adjustment for other macronutrients did not change the significance of our findings, supporting the specificity of saturated fat as a driver of this interaction.

Current recommendations to increase the consistency of gene-disease associations underline the importance of replication in independent samples (33). Our results, supporting the previous findings on anthropometric variables in the Framingham, the GOLDN and the Boston-Puerto Rican studies (11), contribute to increase the external validity of this gene-diet interaction. Moreover, we have reported for the first time a gene-diet interaction

between the *APOA2* SNP and saturated fat intake in determining IR in Chinese and Asian Indians.

The mechanisms behind these novel interactions are still unknown; however, there are some lines of evidence supporting our findings. APOA2, the second most abundant apolipoprotein in HDL, plays a complex and relatively undefined role in lipoprotein metabolism, IR, obesity and atherosclerosis susceptibility (34). Castellani et al (12), found that APOA2 transgenic mice had increased body-weight and body fat content in comparison to control mice. They also found that overexpression of APOA2 in these mice increased IR as demonstrated by 2–3 fold higher fasting insulin levels and a delayed clearance of glucose bolus (12). These results were consistent with the previous observation of Breslow et al (35) in homozygous *APOA2* knockout mice. APOA2 deficiency was associated with lower HDL, free fatty acid, glucose, and insulin levels, suggesting an insulin hypersensitivity state. More targeted mechanistic studies using these experimental models have confirmed the role of APOA2 in IR (13,15,36). However, some controversial results regarding APOA2 overexpression and IR and atherosclerosis have also been reported (34,37,38,39). One of the sources of discrepancies is that overexpression of human *APOA2* in mice does not always exert effects similar to those of murine *APOA2* (38). Interestingly, the other source of discrepancy and particularly relevant to our findings, is the demonstrated difference in APOA2 expression depending on the type of diet administered (34,38). Thus, Escola-Gil et al (39) reported an APOA2-high fat diet interaction in determining the atherosclerotic effects of the APOA2 transgenic mice. After an atherogenic high-fat (cocoa butter) diet, human APOA2 transgenic mice developed more extensive aortic atherosclerosis than control mice. In contrast to the atherogenic diet-fed animals, there was no significant difference in the area of atherosclerotic lesion when they were fed a regular chow diet.

Taken together, the strong influence of a high-fat diet on the effects of the APOA2 in transgenic animal models could help to explain our epidemiological gene-diet interaction. Although the −265T>C SNP has been associated with ~30% drop in basal transcription activity (19,20) and lower plasma APOA2 concentrations (11) in standard conditions, a high-saturated fat diet could mask or reverse these effects resulting in a metabolic situation more favorable to increased body-weight and even IR. One possible hypothesis that we can suggest for integrating observations on animal and human models would be as follows: The C allele, associated in general with a lower APOA2 expression would give rise to lower plasma concentrations of APOA2, found in our previous work (11). If the APOA2 acted as a satiety signal, as is suggested for the APOA4 (40), a lower plasma APOA2 concentration would give rise to greater appetite. This appetite would preferably be for foods rich in saturated fat, and this higher fat intake would lead to greater weight and/or IR. In CC individuals who did not consume more saturated fat (maintaining it below 22 g/d), no weight gain or greater susceptibility to IR would be observed.

Previous human studies did not investigate the influence of diet on the effects of the −265T>C SNP, but controversial results on the association between this SNP and anthropometric measures have been reported. This SNP has been associated with lower waist circumference in men (19); with higher abdominal fat depots in women (22); with

higher waist-to-hip ratio in French (23) and with higher BMI in White Americans (21). No association was also reported (41).

Type 2 diabetes has been linked to chromosome 1q21–24 in several populations (16–18), supporting a role for the APOA2 gene (located in 1q23) as a potential candidate; however, this association is still controversial. A recent case-control cohort comprising 3,093 French Caucasian subjects, did not find significant association between APOA2 SNPs and type 2 diabetes (23). However, significant gene-gene interactions with SNPs in the adjacent calsequestrin 1 gene, so increasing diabetes risk, were found in Utah subjects (42). Likewise, Elbein et al (43) evaluated the association between APOA2 SNPs and diabetes in Northern European subjects. Although they did not find strong associations with diabetes, some variants (including the promoter polymorphism) were significantly associated with 2-h glucose and insulin secretion. However, none of these studies examined the potential modulation by dietary fat. We observed a statistically significant interaction of the −265T>C SNP with saturated fat intake in Chinese and Asian Indians. CC subjects had higher IR than T-allele carriers only with a high-saturated fat consumption. Similarly, we observed a higher OR for diabetes in the high-saturated fat strata (borderline significant). In the Mediterranean population, the *APOA2* SNP was not associated with diabetes risk in the whole population. In the analysis stratified by saturated fat intake, we observed a higher prevalence of female diabetic subjects in the low saturated fat strata. Taking into account the characteristics of our high-risk Mediterranean population, in which the majority of individuals (>99%) with diabetes were previously diagnosed by their physician before the inclusion in the study, this observation could reflect a reverse causation situation due to dietary recommendations for treating diabetes, this being a limitation of our study. In contrast, the Asian population was a representative general population aged 18–69 years, in which a high percentage of individuals with diabetes (>55% of total) were newly detected. However, in the Asian population, the main limitation was the low prevalence of CC subjects in comparison with Caucasian populations. This observation, apart from affecting the statistical significance of the results, contributes to the fact that the population impact of this interaction in determining obesity and IR in Asians in low.

In conclusion, despite some sample size limitations and population specific results, we have replicated a gene-diet interaction involving the *APOA2* −265T>C SNP and saturated fat intake in determining body-weight in a Mediterranean and an Asian population, so increasing the external validity of our previously reported results in American populations (11). In addition, our results suggest that this interaction may also play a role in IR and diabetes. However, prospective studies are required to better characterize this dietary modulation.

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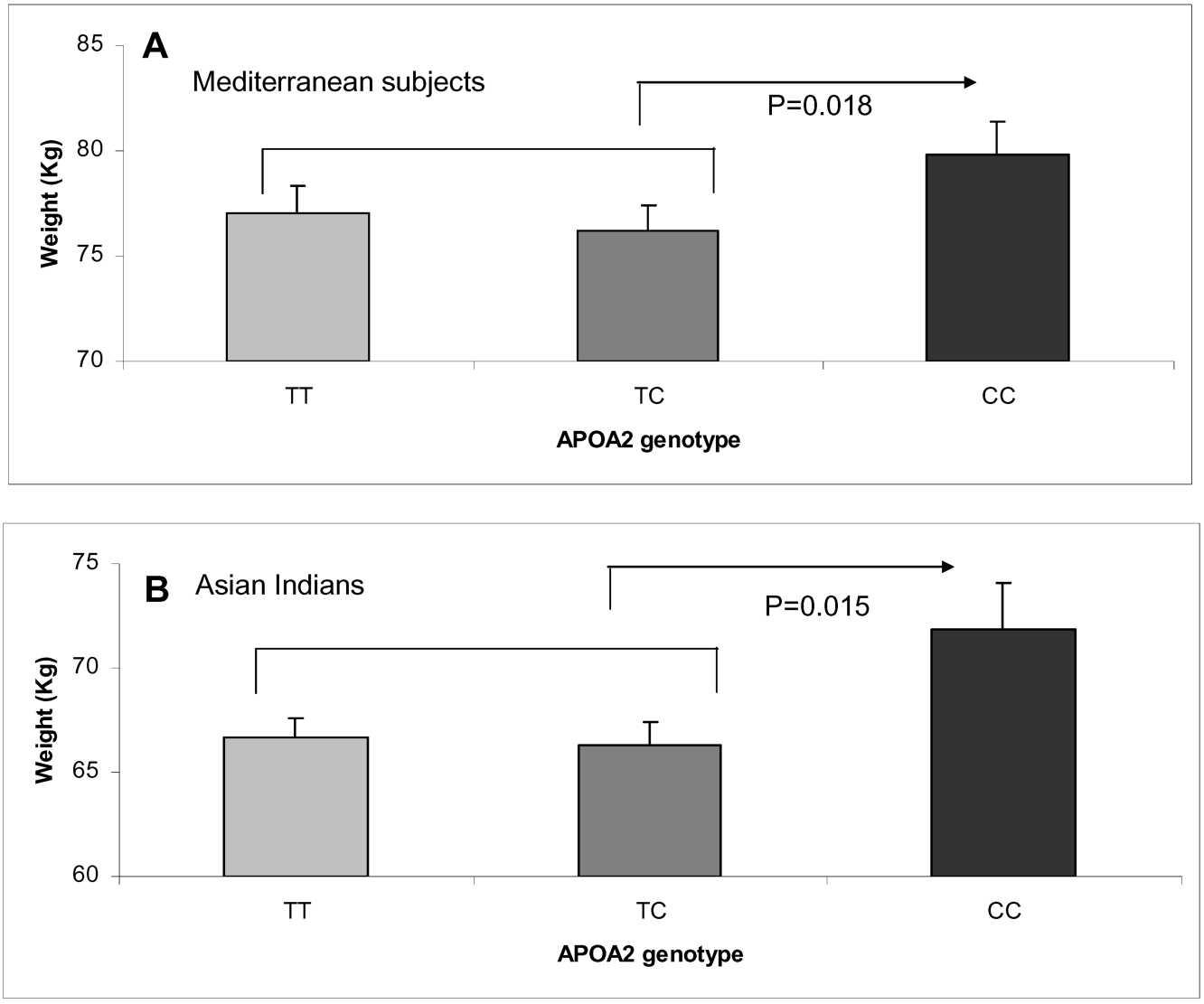

Figure 1.

Association between the *APOA2* −265T>C polymorphism and body-weight in (**A**) Mediterranean subjects (n= 374 TT, 398 TC and 135 CC) and in (**B**) Asian Indians (n= 305 TT, 160 TC and 29 CC). Adjusted means and error bars by genotype. Means were adjusted for sex, age, tobacco smoking and alcohol consumption. * P values for mean comparison between CC and T allele carriers obtained in the multivariate adjusted models. Error bars: SE of means.

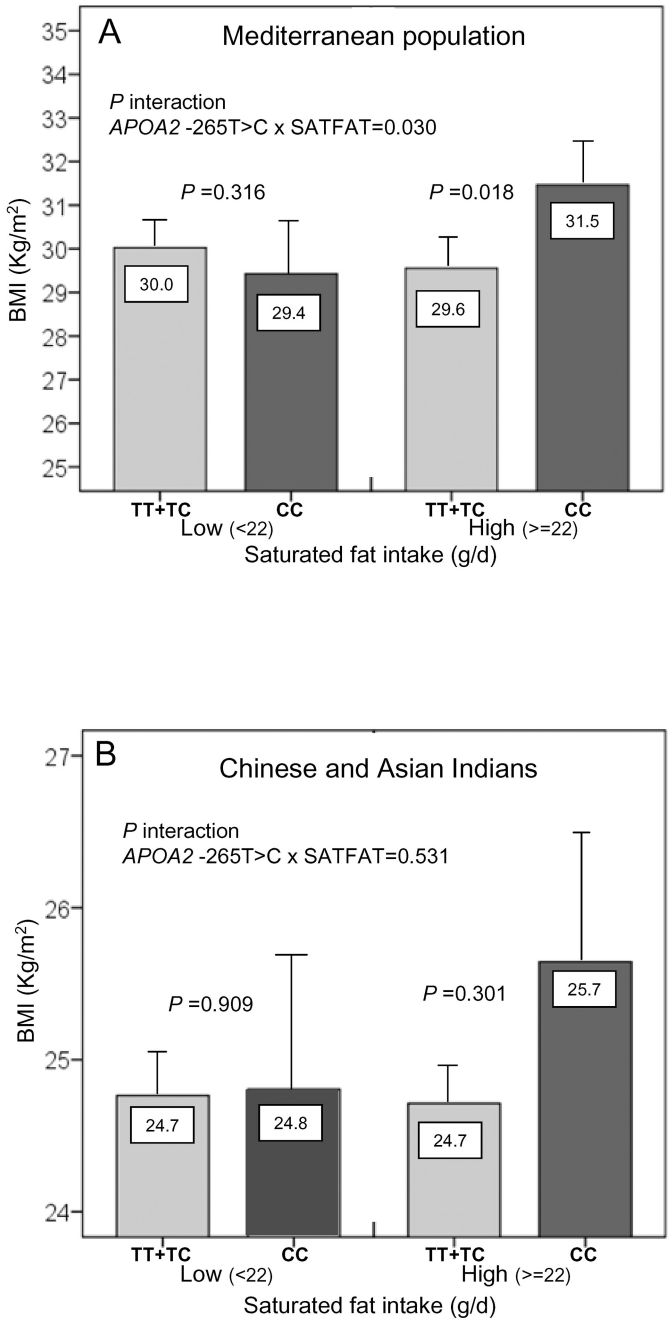

Figure 2.

Interaction between the *APOA2* −265T>C polymorphism and saturated fat intake on BMI in (**A**) Mediterranean subjects (n=772 T-allele carriers and n= 135CC) and in (**B**) Chinese and Asian Indians (n= 1483T-allele carriers and 28 CC sucjects). Adjusted means of BMI are shown depending on the *APOA2* −265T>C polymorphism according to the strata of saturated fat intake (below and above 22g/d). Means were adjusted for sex, age (as continuous), tobacco smoking (as categorical), alcohol consumption (as categorical), diabetes status (as categorical), lipid medication (only in the Mediterranean population),

ethnicity (in the Asian population) and total energy intake (as continuous). 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 between APOA2 genotypes were estimated after multivariate adjustment for the covariates indicated above. Bars indicate standard error (SE) of means.

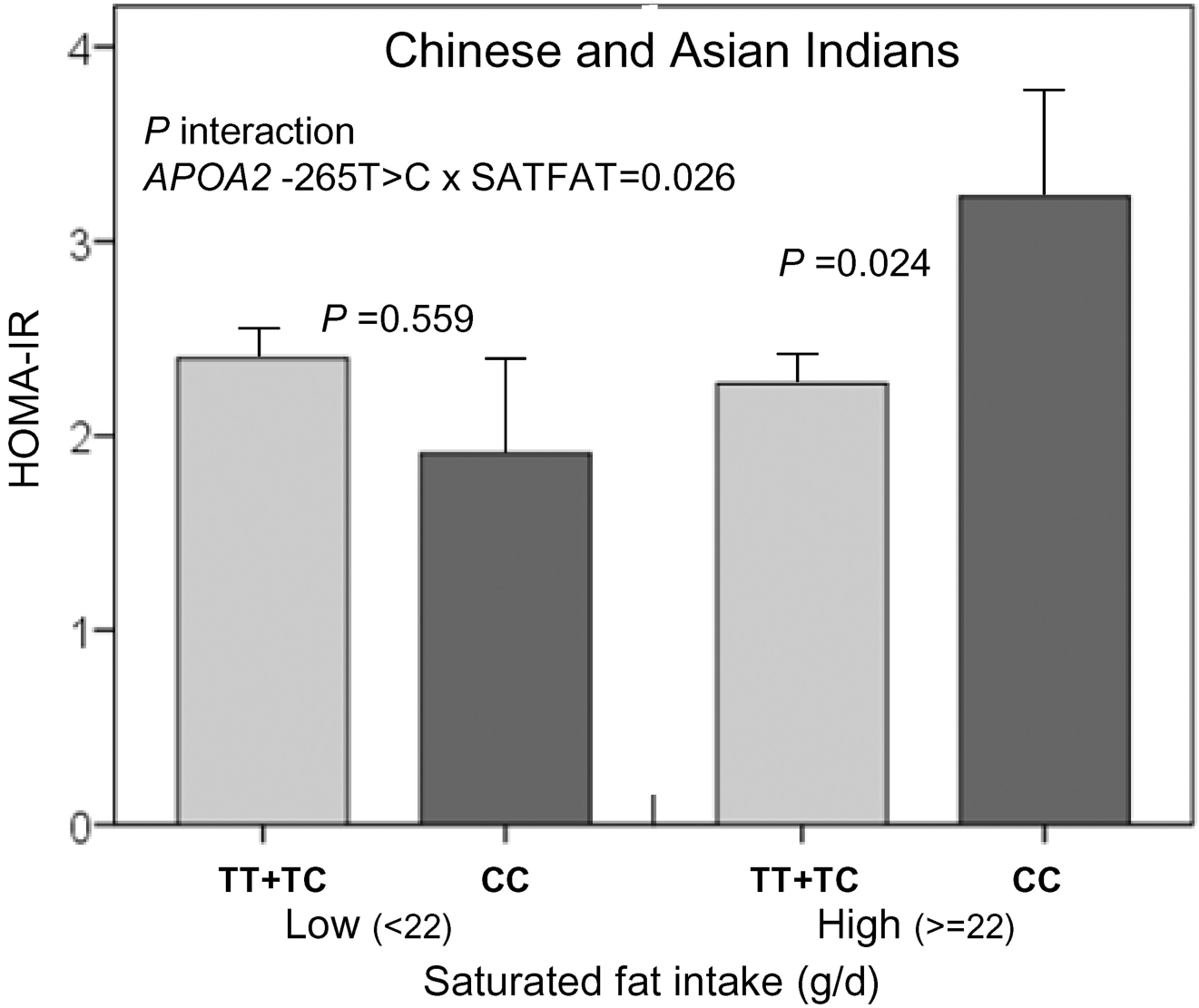

Figure 3.

Interaction between the APOA2 −265T>C polymorphism and saturated fat intake in determining IR in Chinese (n=1204) and Asian Indians (n=307). Adjusted means of IR are shown depending on the APOA2 −265T>C polymorphism (n=1493 T-allele carriers and 28 CC) according to the strata of saturated fat intake (below and above 22g/d). Means were adjusted for sex, age (as continuous), ethnicity, tobacco smoking (as categorical), alcohol consumption (as categorical), and total energy intake (as continuous). 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 between *APOA2* genotypes were estimated after multivariate adjustment for the covariates indicated above. All statistical analyses were carried out with the ln transformed IR. Bars indicate standard error (SE) of means.

Table 1

General characteristics of the studied populations

	Mediterranean population (n=907)	Multiethnic Asian population		
		Chinese (n=2506)	Malays (n=695)	Asian Indians (n=494)
	Mean SD	Mean SD	Mean SD	Mean SD
Male/Female	325/582	1143/1363	335/360	236/258
Age (years)	67 (6)	38 (12)	39 (13)	40 (12)
Weight (kg)	77.5 (13.1)	60.5 (12.3)	65.8 (13.5)	66.4 (13.0)
BMI (Kg/m^2)	30.9 (5.1)	22.7 (3.7)	25.6 (5.0)	25.1 (4.6)
Waist (cm)	104 (12)	78 (11)	83 (12)	85 (12)
HDL-C (mg/dL)	53.2 (14.1)	55.4 (14.8)	50.3 (12.9)	44.1 (11.3)
Fasting triglycerides (mg/dL)	130.9 (80.9)	124.6 (106.8)	148.6 (101.5)	151.3 (127.3)
Fasting glucose (mg/dL)	122.7 (40.2)	100.8 (23.4)	109.8 (37.8)	113.4 (39.6)
HOMA-IR	ND	1.8 (1.3)	2.4 (2.2)	2.9 (2.8)
Energy intake (Kcal/d)*	2253 (669)	2045 (744)	2177 (931)	2220 (771)
Total fat intake (g/d)*	97.4 (32.9)	61.8 (29.3)	69.2 (39.5)	69.2 (29.3)
Total fat intake (% energy)*	39.1 (7.1)	26.6 (5.3)	27.9 (6.0)	27.7 (5.6)
Saturated fat intake (g/d)*	25.7 (10.0)	24.2 (12.7)	29.8 (17.8)	28.9 (15.2)
Saturated fat (% energy)*	10.2 (2.3)	10.3 (2.5)	11.8 (3.2)	11.4 (2.9)
Monounsaturated fat (% energy)*	19.2 (4.8)	9.2 (2.3)	9.0 (2.5)	8.1 (2.2)
Polyunsaturated fat (% energy)*	6.3 (2.3)	5.2 (2.0)	4.8 (2.0)	5.8 (2.6)
Saturated fat < 22g/d*; n (%)	362 (39.9)	614 (51.0)	165 (40.0)	115 (37.5)
Drinkers; n (%)	467 (51.5)	1132 (45.2)	55 (7.9)	162 (32.8)
Current smokers; n (%)	106 (11.7)	316 (12.6)	162 (23.3)	73 (14.8)
BMI>=30 Kg/m2	478 (52.7)	93 (3.7)	112 (16.1)	61 (12.3)
BMI>=27 Kg/m2	711 (78.4)	310 (12.4)	234 (33.7)	153 (31.0)
Diabetes; n (%)	443 (48.8)	182 (7.3)	103 (14.8)	97 (19.6)
Hypertension; n (%)	724 (79.8)	209 (8.3)	62 (8.9)	56 (11.3)
APOA2−265T>C genotype; n (%)				
TT	374 (41.2)	2080 (83.0)	558 (80.3)	305 (61.7)
TC	398 (43.9)	405 (16.2)	128 (18.4)	160 (32.4)
CC	135 (14.9)	21 (0.8)	9 (1.3)	29 (5.9)

*Dietary variables were only determined in a random sample (1 in 2) of the Asian population (n=1,923 subjects: 1204 Chinese, 412 Malays and 307 Asian Indians)

HOMA-IR: Homeostasis model assessment of insulin resistance
ND: Not determined

Table 2

Association of the *APOA2* polymorphism with anthropometric variables in the Mediterranean and the Asian populations

	Mediterranean population				Asian Indians				Chinese				Malays			
	TT+TC (n=772)	CC (n=135)			TT+TC (n=465)	CC (n=29)			TT+TC (n=2485)	CC (n=21)			TT+TC (n=686)	CC (n=9)		
	Mean SD	Mean SD	p*1*	p*2*	Mean SD	Mean SD	p*1*	p*2*	Mean SD	Mean SD	p*1*	p*2*	Mean SD	Mean SD	p*1*	p*2*
Weight (kg)	77.0 (12.8)	79.5 (14.9)	0.047	0.018	65.9 (12.2)	70.6 (12.2)	0.049	0.021	60.5 (12.3)	59.8 (14.4)	0.772	0.951	65.8 (13.6)	64.9 (9.7)	0.841	0.969
BMI (Kg/m^2)	30.6 (5.1)	31.9 (3.7)	0.049	0.038	24.9 (4.6)	27.4 (4.6)	0.006	0.012	22.7 (3.7)	22.6 (3.7)	0.816	0.759	25.6 (5.0)	25.1 (4.6)	0.769	0.774
Waist (cm)	103.8 (12.1)	104.5 (12.6)	0.605	0.375	84.9 (11.8)	89.1 (11.7)	0.067	0.036	78.1 (10.7)	77.7 (11.4)	0.864	0.987	82.8 (12.1)	81.1 (8.8)	0.665	0.792
Obesity*; n (%)	404 (52.3)	74 (54.8)	0.594	0.264	136 (29.2)	17 (58.6)	0.001	0.004	308 (12.4)	2 (9.5)	0.864	0.590	231 (33.7)	3 (33.3)	0.983	0.967

1 P values for the comparion of unadjusted means (for continuous variables) or percentages (for categorical variables) between APOA2 genotypes

2 P values obtained in the multivariate adjusted (for sex, age, tobaco smoking, alcohol and diabetes) models

* Obesity was defined as BMI>= 30 Kg/m^2 in the Mediterranean and as BMI>=27 Kg/m^2 in the Asian population

SD: Standard devitation

Table 3

Associations between the APOA2 −265C>T polymorphism and obesity depending on the saturated fat intake category (lower or higher than 22 g/d) in the Asian population. Multivariate logistic regression analysis

	Saturated fat	*APOA2* Genotype	Obesity* (n)							
			No	Yes	OR[1]	(95% CI)	p*1*	OR[2]	(95% CI)	p*2*
Asian Indians	<22g/d	TT+TC	78	30	1			1		
		CC	5	2	0.913	(0.151–5.525)	0.921	1.048	(0.158–6.956)	0.962
	>=22g/d	TT+TC	132	49	1			1		
		CC	4	7	4.502	(1.085–18.579)	0.038	4.834	(1.172–19.940)	0.029
Chinese	<22g/d	TT+TC	540	68	1			1		
		CC	6	0	0.000	-	0.493	0.000	-	0.999
	>=22g/d	TT+TC	505	81	1			1		
		CC	3	1	2.141	(0.207–22.180)	0.523	2.009	(0.186–21.677)	0.565
Chinese and Asian Indians**	<22g/d	TT+TC	618	98	1			1		
		CC	11	2	0.557	(0.101–3.063)	0.501	0.599	(0.101–3.546)	0.572
	>=22g/d	TT+TC	637	130	1			1		
		CC	7	8	3.310	(1.076–10.182)	0.036	3.332	(1.074–10.337)	0.037

*BMI>=27 Kg/m2

1Models were adjusted for sex, age, tobacco smoking, alcohol consumption and diabetes

2Model were adjusted for sex, age, tobacco smoking alcohol consumption, diabetes, total energy intake, total carbohydrates and proteins

**Additional adjustment for ethnic group was carried out

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