Interactions Between Variants in the β_3-Adrenergic Receptor and Peroxisome Proliferator–Activated Receptor-γ2 Genes and Obesity

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OBJECTIVE — Previous studies have reported modest associations between measures of obesity and the Trp64Arg variant of the β_3-adrenergic receptor (ADRβ3) and the Pro12Ala variant of the peroxisome proliferator–activated receptor (PPAR)-γ2. We hypothesized that these single gene variants may mark mutations that act through convergent pathways to produce synergistic effects on obesity.

RESEARCH DESIGN AND METHODS — The sample included 453 subjects from 10 large Mexican-American families participating in the population-based San Antonio Family Heart Study. The effects of each gene variant singly and jointly were estimated as fixed effects using the measured genotype approach framework. Analyses were conditioned on the pedigree structures to account for the correlations among family members. Statistical significance was evaluated by the likelihood ratio test with adjustment for age, sex, and diabetes status.

RESULTS — The allele frequencies for the ADRβ3 Trp64Arg and PPARγ2 Pro12Ala variants were 18 and 12%, respectively. The ADRβ3 variant was not significantly associated with any of the obesity-related traits, but subjects with the PPARγ2 variant ($n = 98$) had significantly higher levels of fasting insulin ($P = 0.03$), leptin ($P = 0.009$), and waist circumference ($P = 0.03$) than those without. Subjects with both gene variants ($n = 32$) had significantly higher BMI, insulin, and leptin levels than those with only the PPARγ2 variant ($n = 66$) (P for interaction: 0.04, 0.02, and 0.01 for BMI, fasting insulin, and leptin, respectively).

CONCLUSIONS — Our results suggest that epistatic models with genes that have modest individual effects may be useful in understanding the genetic underpinnings of typical obesity in humans.

Diabetes Care 24:672–677, 2001

Human obesity is a multifactorial syndrome influenced by both genetic and behavioral factors (1). Efforts to identify variants in specific genes and to understand how they interact with each other to cause obesity have been accelerated in recent years to incorporate our growing understanding of the physiological and molecular pathways involved in this disorder. Variation at multiple genetic loci contribute to the etiology of typical obesity, and it is likely that allelic effects at some loci may be amplified in the presence of variants at other loci. In fact, it has been demonstrated in rodents that a combination of genetic defects increases the level of obesity significantly compared with that attributed by the independent gene defects alone (2). In humans, however, it may be more difficult to detect such interactions due to underlying genetic heterogeneity and our current lack of information regarding which loci to test.

In our previous studies of Mexican-Americans, we have implicated a role for variants in two obesity candidate genes, the β_3-adrenergic receptor (*ADRβ3*) and the peroxisome proliferator–activated receptor-γ2 (*PPARγ2*), in the etiology of obesity (3–6). Both variants are associated with amino acid substitutions that may result in altered function of their respective gene products (7–9). *ADRβ3* is expressed in adipocytes and mediates the rate of lipolysis in response to catecholamines, whereas *PPARγ2* is a nuclear receptor that acts as a transcription factor to regulate adipocyte differentiation and insulin sensitivity. Among our study participants, we observed an association between the Trp64Arg variant of *ADRβ3* and obesity (as have many others [10,11]), although the association in our population could be detected only by matching siblings for a previously detected obesity quantitative trait locus on chromosome 2 (4). We have subsequently detected an association between the Pro12Ala variant of *PPARγ2* and obesity (6), which has also been reported by others (12–14).

The *ADRβ3* Arg and *PPARγ2* Ala variants are reasonably common in diverse populations, and thus, the frequency of individuals who harbor both variants is appreciable. Because both of these gene products influence fat accumulation, we evaluated evidence for interaction between these two variants to determine whether subjects having variants in both *ADRβ3* and *PPARγ2* are at increased risk

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Received for publication 30 October 2000 and accepted in revised form 2 January 2001.

Abbreviations: ADRβ3, β_3-adrenergic receptor; BAT, brown adipose tissue; FFA, free fatty acid; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

for obesity beyond the expected risk based on the additive effects associated with each individual variant. In addition, we have estimated the combined contribution of these two variants (both independent and interaction effects) to the total variation of obesity-related traits.

RESEARCH DESIGN AND METHODS

Subjects and data collection

Subjects for this study were participants of the San Antonio Family Heart Study, a population-based family study designed to identify the genetic factors of atherosclerosis and its determinants in Mexican-Americans (15). The families included the first-, second-, and third-degree relatives aged ≥16 years of participating probands who were ascertained without regard to the proband's medical condition. This report is based on 453 subjects comprising the initial set of 10 families who were genotyped for both the *ADRβ3* Trp64Arg and the *PPARγ2* Pro12Ala variants. Informed consent was obtained from all participants, and all protocols were approved by the institutional review board.

The examination procedure included measurements of blood pressure, anthropometry, and an oral glucose tolerance test. Serum glucose concentrations were measured using an Abbott V/P Analyzer (Abbott Laboratories, Abbott Park IL), and serum concentrations of insulin and leptin were measured using commercial radioimmunoassay kits (insulin, Diagnostic Products, Los Angeles, CA; leptin, Linco, St. Louis, MO), as previously described (15,16). The coefficient of variation for fasting insulin and leptin were 6.5 and 7.5%, respectively. Diabetes was diagnosed according to World Health Organization criteria (17). BMI was calculated as weight (kilograms) divided by height (meters squared). Related obesity measures included waist circumference, measured to the nearest centimeter, and total body fat (or fat mass, in kilograms), assessed by bioimpedance. In addition, we accessed smoking habits, physical activity by 7-day recall, and daily dietary caloric intake by food frequency questionnaire (15).

Genotyping

DNA was isolated from lymphocytes using standard methods. The Trp64Arg variant of the *ADRβ3* and the Pro12Ala variant of *PPARγ2* were inferred from genotypes generated using polymerase chain reaction–based assays as previously described (4,12).

Table 1—*Clinical characteristics of the study group subjects*

Characteristics	Men	Women	Total
n	202	251	453
Age (years)	39.3 ± 17.3	38.3 ± 16.3	38.7 ± 16.7
BMI (kg/m^2)	28.7 ± 6.0	30.4 ± 7.4*	29.6 ± 6.9
Waist (cm)	96.3 ± 15.6	94.1 ± 18.6	95.1 ± 17.3
Fat mass (kg)	20.7 ± 9.9	27.9 ± 12.9†	25.0 ± 12.3
ln fasting insulin (mU/l)‡	2.41 ± 0.85	2.42 ± 0.74	2.42 ± 0.79
ln fasting leptin (ng/ml)‡	2.03 ± 0.76	3.23 ± 0.65†	2.70 ± 0.91

Data are means ± SD, unless otherwise indicated. *$P < 0.05$ or †$P < 0.0001$ compared with the mean in men. ‡Values were transformed by their natural logarithm (ln).

Statistical analysis

Mean levels of obesity traits were estimated according to *ADRβ3* and *PPARγ2* genotypes. To account for the relatedness among family members, we used the measured genotype approach (18), in which we estimated the likelihood of specific genetic models given the pedigree structure. For example, we compared the likelihood of a full model, which allowed for genotypic-specific means, to that of a nested model in which genotypic means were restricted to be equal to each other. Parameter estimates were obtained by maximum likelihood methods. The significance of the association was tested by likelihood ratio tests, which compare the difference in the likelihoods between the full and the nested models. Two times the difference between the logarithm of the likelihoods of the two models is distributed asymptotically as a χ^2 statistic with degrees of freedom equal to the difference in the numbers of parameters in the models being compared. Within each model, we simultaneously estimated the effects of age, sex, and diabetes status (yes/no). In addition, we allowed for a residual heritability of the trait. Fasting insulin and leptin values were transformed by their natural logarithms (ln) to reduce skewness. Analyses of insulin levels were performed using data from nondiabetic subjects only. All analyses were conducted using the SOLAR program (19).

We tested for interaction between the *ADRβ3* Arg and the *PPARγ2* Ala variants by comparing the likelihood of a full model in which we parameterized the genetic effect into three components: an independent effect of having the ADRβ3 variant, an independent effect of having the *PPARγ2* variant, and a joint effect of having both variants (*ADRβ3* × *PPARγ2*) with the likelihood of a nested model in which we parameterized the genetic effect into two independent components—an effect of having the *ADRβ3* variant and an effect of having the *PPARγ2* variant. We simultaneously estimated the effects of age, sex, and diabetes status in each model and allowed for a residual heritable component of the trait.

We estimated the proportion of the total trait variance accounted for by these variants (both their main effects and interaction effects) by calculating the reduction in the total trait variance (after adjusting for age, sex, and diabetes status only) that occurs after also estimating the effects of these two variants and their interaction and then expressing this reduction as a proportion of the total trait variance. In other words, we estimated $(\sigma^2_{tot} - \sigma^2_{adj})/\sigma^2_{tot}$, where σ^2_{tot} equals the total trait variance (after adjusting for age, sex, and diabetes status only) and σ^2_{adj} equals the trait variance after further adjustment for genetic effects.

RESULTS — Clinical characteristics of the 453 study subjects are shown in Table 1. Of the study subjects, 55% were women, and the overall prevalence of diabetes was 15%. The frequencies of the ADRβ3 Arg and PPARγ2 Ala variants were 18 and 12%, respectively. The genotype frequencies did not deviate from those expected on the basis of the Hardy-Weinberg equilibrium. Mean ages of men and women were 39.3 and 38.3 years, respectively, whereas mean BMI was 28.7 kg/m^2 for men and 30.4 kg/m^2 for women ($P < 0.05$). Women had significantly higher mean fat mass and leptin levels

Table 2—*Adjusted means for obesity-related traits according to the ADRβ3 and PPARγ2 genotype**

	Variant in neither gene	ADRβ3 variant only		PPARγ2 variant only		Variant in both genes		
ADRB3 genotype	Trp/Trp	Trp/Arg	Arg/Arg	Trp/Trp	Trp/Trp	Trp/Arg	Arg/Arg	Trp/Arg
PPARγ2 genotype	Pro/Pro	Pro/Pro	Pro/Pro	Pro/Ala	Ala/Ala	Pro/Ala	Pro/Ala	Ala/Ala
Sample size (*n*)	234	115	6	61	5	23	5	4
BMI (kg/m^2)	29.55	28.81	28.84	30.01	30.60	32.73	30.58	32.70
Waist (cm)	98.00	95.51	94.14	94.81	101.19	104.36	94.02	103.21
Fat mass (kg)	23.74	25.20	18.98	24.47	25.14	26.19	24.00	26.86
Fasting insulin (mU/l)†	2.44	2.27	1.92	2.50	2.60	2.90	2.63	2.48
Leptin (ng/ml)†	2.68	2.59	2.11	2.78	2.82	3.14	2.84	3.13

*Means were adjusted for age, sex, and diabetes status (diabetic subjects excluded for insulin levels). †Values were transformed by their natural logarithm.

than men. There were no significant sex differences in waist circumference or insulin levels.

Initial association analyses of *ADRβ3* revealed that the obesity-related trait means did not differ significantly between subjects with and without the Arg variant (data not shown). In contrast, analysis of *PPARγ2* revealed that subjects with the Ala variant were significantly more obese (i.e., had larger waist circumference [$P = 0.03$] and higher leptin [$P = 0.009$] and insulin levels [$P = 0.03$]) than subjects homozygous for the more common Pro variant. The association of the *PPARγ2* Ala variant with obesity has been previously reported (6).

We next evaluated the effect of both variants on the variation of obesity-related traits simultaneously. Table 2 shows the adjusted mean levels of the obesity-related traits according to subjects' combined *ARDβ3* and *PPARγ2* genotypes. Only eight combinations are shown because none of the subjects were homozygous for both variants. There were 234 subjects who did not have either gene variant, 121 subjects who had the *ADRβ3* Arg variant only, 66 subjects who had the *PPARγ2* Ala variant only, and 32 subjects who had both variants. In general, mean levels of most traits (especially BMI, insulin, and leptin) tended to be higher among those with the *PPARγ2* Ala variant than in those without and higher still in those having both variants.

Because there was no indication that the allelic effects at either gene were additive, we collapsed the eight genotype combinations into four, depending on whether the subjects had a variant in neither of the two genes, in *ADRβ3* only, in *PPARγ2* only, or in both genes. Figure 1 shows the effect of each variant combination compared with the baseline group in which subjects did not have a variant in either gene. Mean BMI and mean concentrations of insulin and leptin were all higher in subjects with variants in both genes than in subjects having variants in only one of the two genes. The formal test for interaction revealed that the model in which we allowed for an interaction effect between these two variants (i.e., the model with three genetic effect parameters: *ADRβ3* Arg variant + *PPARγ2* Ala

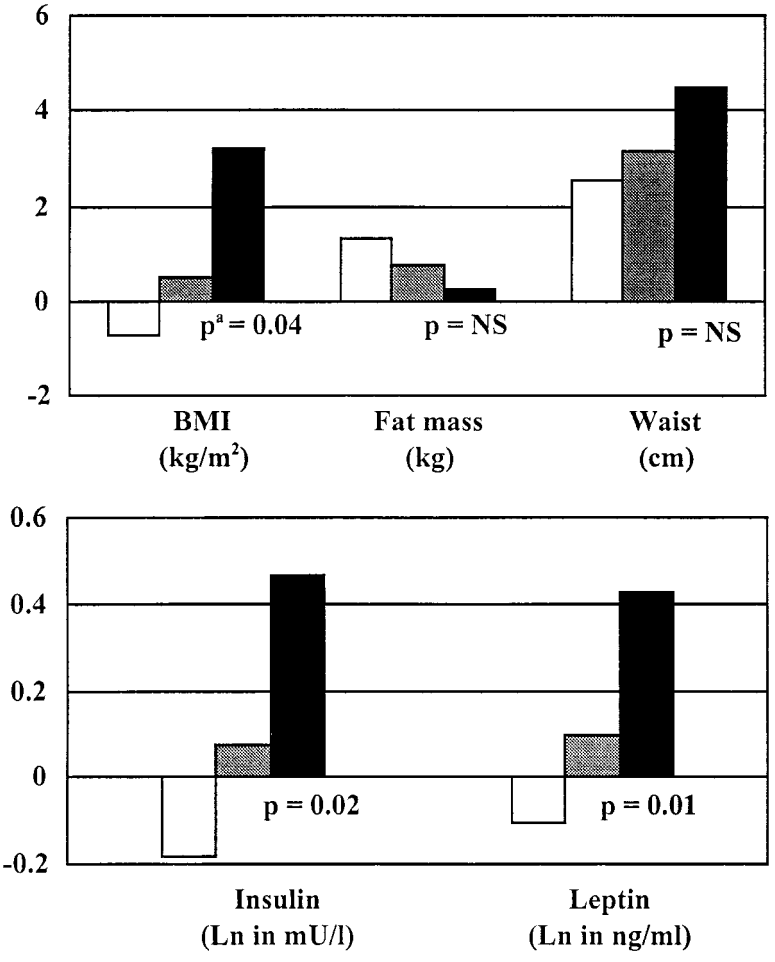

Figure 1—*Clinical characteristics of the study group subjects with and without the ADRβ3 and PPARγ2 variants. □, Variant in ADRβ3 only; ■, variant in PPARγ2 only; ■, variant in both genes. [a]P value for the significance of the interaction effect.*

variant + *ADRβ3* Arg variant × *PPARγ2* Ala variant) provided a significantly better fit to the data (i.e., higher likelihood) than the model in which we did not allow for an interaction (i.e., the model with only two independent genetic effect parameters: *ADRβ3* Arg variant + *PPARγ2* Ala variant). The age-, sex-, and diabetes-adjusted significance levels for the interaction effects were 0.04 for BMI, 0.02 for insulin, 0.01 for leptin, 0.26 for waist circumference, and >0.90 for fat mass. Further adjustment for the effects of smoking, level of physical activity, and total daily caloric intake altered these results very little ($P = 0.08$ for BMI, $P = 0.007$ for insulin, and $P = 0.02$ for leptin), and the results were not altered significantly when the 69 subjects with diabetes were removed from the analysis.

The effect of these two variants (both their independent and interaction effects) accounted for 2.6, 2.9, and 2.4% of the total phenotypic variation in BMI, insulin, and leptin concentrations, respectively, with the interaction accounting for about half of their total effects.

CONCLUSIONS — The present study builds on our previous observations by suggesting that the increased risk of obesity associated with the Ala variant at *PPARγ2* may not be uniform for all carriers of the Ala allele but may be restricted primarily to the group of subjects who also carry the Arg variant of *ADRβ3*. In fact, when we eliminated the 32 subjects carrying variants in both genes from the analysis, the association between the Ala variant and obesity was no longer statistically significant (adjusted *P* values: 0.28 for BMI, 0.22 for insulin, and 0.13 for leptin; data not shown). These results raise the intriguing speculation that the effect of the *PPARγ2* Ala variant on obesity might be expressed only in the presence of some other factors (such as the *ADRβ3* variant). Conversely, this speculation also suggests a possible explanation for the failure of many association studies to detect an effect of the *ADRβ3* Trp64Arg variant on obesity—namely that these studies may have contained an insufficient number of subjects with both factors (i.e., variants in both genes).

Emerging data suggest that *PPARγ2* is regulated by a complex set of factors, and possibly, the obese state itself may modify the expression of this gene (20). Expression of *PPARγ2* mRNA in adipose tissue is increased in obese humans but is downregulated while following a low-calorie diet (21). A potential role for the Ala variant in *PPARγ2* expression has been postulated (20), and epidemiological data provide some support. For example, Ek et al. (13) observed that in obese subjects, individuals homozygous for the Ala variant had significantly higher BMIs than other subjects, whereas in lean subjects, those homozygous for the Ala variant had significantly lower mean BMI. Several additional studies have also reported an association between the Ala variant and higher BMI in obese individuals (12,14), and an association is also observed in our study of a relatively obese population (6). On the other hand, Deeb et al. (7) studied a relatively leaner Finnish population and found that subjects with the *PPARγ2* Ala variant were less obese and had greater insulin sensitivity than subjects lacking the variant.

We did not observe an association between type 2 diabetes and the *PPARγ2* Ala variant in this Mexican-American population (6), although the power to detect a small variant effect in our sample may be limited. Somewhat paradoxically, however, several studies (7,22,23), including a recent meta-analysis (23), have reported the *PPARγ2* Ala variant to be associated with a decreased risk of type 2 diabetes. It is intriguing to speculate how the same mutation could be associated with both obesity and reduced risk of diabetes. Although epidemiological studies show a strong association between obesity and type 2 diabetes, it is important to realize that only 30% of obese subjects are diabetic, suggesting that obesity is not sufficient to cause diabetes. Indeed, some investigators (24) have even argued that not all types of obesity increase the risk of type 2 diabetes. Consistent with this view is the observation that enhanced insulin sensitivity, which is associated with a decreased risk of diabetes, is a predictor of weight gain (25). An increased number of adipocytes may not lead to increased risk of diabetes, and in fact, in mice with lipoatrophic diabetes, hyperglycemia was reversed after a surgical transplantation of adipose tissue (26). Adipocyte volume, on the other hand, is a predictor of type 2 diabetes, independent of body fat and body fat distribution, as shown in Pima Indians (27). These observations suggest that adipocyte tissue may promote antidiabetic effects via metabolic or endocrine mechanisms, such as increasing uptake of glucose, free fatty acid (FFA), or hormonal secretion to increase insulin sensitivity. However, if the adipocyte's ability to differentiate is compromised, its antidiabetic effect may be lost once it has reached its maximum capacity to store energy (e.g., glucose and FFA).

The mechanism(s) by which mutations in both *PPARγ2* and *ADRβ3* might interact to enhance obesity and insulin resistance is not yet proven. However, because both genes play important regulatory roles in adipocytes, there are several plausible mechanisms. *PPARγ* is a nuclear hormone receptor that stimulates transcription of multiple genes necessary for adipogenesis and insulin signaling, whereas the *ADRβ3* is a membrane receptor that increases intracellular cAMP concentrations and stimulates lipolysis. Singly, functional studies indicate that the Trp64Arg substitution in *ADRβ3* and the Pro12Ala substitution in *PPARγ2* result in decreased activity of their respective proteins (7–9,28,29). The most direct piece of evidence supporting a direct interaction between *ADRβ3* and *PPARγ2* expression comes from the recent work of Bakopanos and Silva (30), who observed that thiazolidinediones (TZDs), insulin sensitizers that exert their effects through the specific binding and activation of *PPARγs* (31), reduce *ADRβ3* expression at the transcriptional level. Furthermore, this effect was evident only on adipose cell lines containing *PPARγ2*. In a second experiment, the inhibition effect by TZDs was also observed in a cell line that lacked *PPARγ* but which was transfected by *PPARγ2*, and the inhibition increased in a dose-dependent manner with the amount of *PPARγ2* cDNA introduced in these cells.

Another study has indicated that adrenergic stimulation of rat brown adipose tissue (BAT) through cold exposure results in a marked increase in the expression of *PPARγ* (32). Because *ADRβ3* is the major adrenergic receptor subtype in rat BAT, these findings suggest a direct interaction between *ADRβ3* activation and *PPARγ* expression. Because the Trp64Arg substitution in *ADRβ3* leads to a decrease in intracellular signal transduction (28), it is possible that subjects with the Trp64Arg variant may have lower levels of expression of *PPARγ*, which may compound defects in *PPARγ* action caused by the Pro12Ala *PPARγ2* substitution. In-

deed, Gros et al. (33) demonstrated that forced expression of human *ADRβ3* in CHO/K1 fibroblasts resulted in a marked increase in PPARγ expression. The wild-type form (Trp64 *ADRβ3*) increased PPARγ expression, whereas the mutant form (Arg64 ADRβ3) did not. Together, these findings strongly suggest that the presence of both variants could result in significantly lesser *PPARγ* activity. The simplest model of *PPARγ* action (consistent with in vitro studies using *PPARγ* agonists) would suggest that lower *PPARγ* activity in vivo should associate with lesser obesity and lesser insulin sensitivity. However, it was shown recently that mice heterozygous for a knockout of *PPARγ* have surprisingly greater insulin sensitivity than the wild-type mice (34). This paradox makes it clear that the mechanisms whereby *PPARγ* influences obesity and insulin sensitivity in vivo are poorly understood and likely to be complex. Additional studies in humans will be required to clarify these and perhaps other mechanisms whereby these two genes may interact.

In summary, we observed that the *PPARγ2* Ala variant, but not the *ADRβ3* Arg variant, was significantly associated with obesity. However, subjects with variants in both genes were more obese than subjects with the *PPARγ2* Ala variant only, and, in fact, it appears that only those subjects carrying both the *PPARγ2* Ala and *ADRβ3* Arg variants have increased obesity. In terms of population significance, the effects of these two variants on obesity in this population are small. Together, they accounted for 2–3% of the total variation in BMI, insulin, and leptin, with the interaction accounting for about half of their total effects. Of course, we evaluated the effects of only two specific gene variants in this study. If there are other functional variants in these genes or in their regulatory regions, then the effects of these genes on obesity will be larger. More importantly, as there is an interaction between these two gene variants, it is likely that there are interactions among variants of other genes. Although these analyses are to some extent exploratory, they support the importance of these variants in obesity and reinforce the idea that gene effects will be underestimated if only their individual effects are considered. Incorporating interactions, whether gene by gene or gene by environment, may enhance our ability to detect genetic effects and may contribute to our understanding of the etiology of obesity.

Acknowledgments— This work was supported by research grants RO1-AR43351 and PO1-HL45522, awarded by the National Institutes of Health.

We are deeply grateful for the cooperation of the families participating in the San Antonio Family Heart Study.

A summary of this study was previously published as an abstract in *Am J Human Genet* 65 (Suppl. 4):A14, 1999.

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