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Association analyses of adrenergic receptor polymorphisms with obesity and metabolic alterations

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Abstract

Genes involved in the regulation of catecholamine function may be important in obesity because of the role catecholamines play in energy expenditure and lipolysis. To determine if common single nucleotide polymorphisms (SNPs) in β_1-adrenergic receptor (*ADRB1*), β_2-adrenergic receptor (*ADRB2*), β_3-adrenergic receptor (*ADRB3*), and α_2-adrenergic receptor (*ADRA2A*) genes associate with obesity and metabolic alterations, we recruited 74 healthy African American and 161 white men and women (age, 18-49 years) to participate in this case-control genetic association study. Genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism. Associations between genotype and body mass index (BMI), percentage of body fat (by measuring skinfold thickness in 7 different sites), fasting (12-hour) plasma glucose, insulin, potassium concentrations, glycated hemoglobin, and insulin resistance (homeostasis model assessment [$HOMA_{IR}$] score) were performed. Among whites, the *ADRB1* Arg389→Gly variant associated with insulin concentrations and $HOMA_{IR}$: mean ± SD values for insulin and $HOMA_{IR}$ in Arg389 homozygotes and carriers of the Gly were 10 ± 7.0 and 12 ± 9.4 μIU/mL (P = .02) and 2.1 ± 1.7 and 2.6 ± 2.2 (P = .057), respectively. Systolic blood pressure was higher in whites for carriers of the *ADBR1* Ser49 compared to Gly49 homozygotes (124 ± 12.6 vs 119 ± 11.3 mm Hg, respectively; P = .02). Subsequent analysis revealed that these associations were attributable to a higher BMI among obese participants. The *ADRA2A* G1780A SNP associated with BMI and percentage of body fat in African Americans (P = .05). Interactions were detected between *ADRA2A* C-1291G and *ADRB2* Gln27→Glu variants for obesity in African Americans and between *ADRA2A* C-1291G SNP and *ADBR1* haplotype for obesity in whites. We conclude that common SNPs in adrenergic receptor genes may be important susceptibility loci for obesity and related alterations. Because of the limited size of our populations, our results should be interpreted with caution and should be replicated in larger populations.

1. Introduction

Obesity is a major health problem in the United States. Sixty-five percent of American adults are overweight, and more than 30% are obese [1]. It is well accepted that obesity in some, but not in all, individuals leads to metabolic alterations including hyperinsulinemia and insulin resistance [2], which can lead to the development of type 2 diabetes mellitus and to cardiovascular diseases and some forms of cancer [3-6]. Although rare, obesity syndromes can be caused by mutations in single genes. However, the greatest proportion of obesity involves variants in multiple genes interacting with environmental factors, particularly diet [7,8].

Genes that are involved in the regulation of catecholamine function may be important in obesity because of the role catecholamines play in energy expenditure and lipolysis. Fat stored in the body as triglycerides is hydrolyzed to free fatty acids and glycerol through the process of lipolysis [9]. Activation of β-adrenergic receptors (ADRBs) expressed in adipocytes mediate lipolysis [3,10,11], whereas stimulation of α_2-adrenergic receptors (ADRA2) inhibit lipolysis [12]. Insulin is also an important inhibitor of

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catecholamine-stimulated lipolysis [13] by reducing the ADBR effects of epinephrine and by activating ADRA2 in adipocytes [14].

In addition, catecholamine-stimulated whole-body lipolysis and lipolysis in subcutaneous adipocytes are blunted in obesity [10,15,16], thereby limiting lipid mobilization and favoring fat accumulation. The mechanisms underlying lipolytic resistance to catecholamines in obesity are not clear and may include desensitization of ADRB2 function [17], increased activity of ADRA, and the hyperinsulinemia that accompanies obesity [18,19].

Several mutations in genes encoding ADRA and ADRB have been identified that could alter receptor expression and function. Given the important role that adrenergic receptors play in regulating energy expenditure and lipolysis, it is possible that common genetic polymorphisms in these genes contribute to obesity and to the accompanying metabolic alterations. Numerous studies have reported on the relationship between obesity and genetic variants in adrenergic receptors in different populations with conflicting results [20-22]. Few, however, have explored associations between adrenergic receptor polymorphisms and obesity and accompanying metabolic alterations in self-identified African Americans and whites. In the present study we tested the hypothesis that obesity and metabolic alterations associate with common polymorphisms in adrenergic receptor genes.

2. Methods

2.1. Study participants and outcome measures

All participants were between the ages of 18 and 49 years, healthy, nonsmokers, and were not taking any medications chronically. Body mass index (BMI) was calculated from height and weight using the formula: BMI = [body weight (pounds)/height (inches)^2] × 704.5. Obesity was defined as a BMI of 30 or more [1]. Percentage of body fat was determined by calculating the mean of 3 measurements of skinfold thickness in 7 different anatomical sites (triceps, biceps, subscapula, abdominal, suprailiac, thigh, and chest), using a Lange Skin fold Caliper (Beta Technology, Cambridge, MD) and equations as previously described [23]. All participants were fasted overnight. Systolic and diastolic blood pressures were measured manually with a mercury sphygmomanometer by auscultation using a stethoscope, after a 5-minute period of supine rest. Fasting plasma concentrations of glucose were quantified using the glucose oxidase method with a Glucose Analyzer II (Beckman, Fullerton, CA). Anthropometric measurements were performed by 1 of 2 registered nurses. Fasting plasma concentrations of insulin were quantified by radioimmunoassay (INSULIN RIA, Diagnostics Systems Laboratories, Webster, TX). Fasted glycated hemoglobin (HbA_{1c}) and potassium concentrations were quantified by a Beckman Autoanalyzer Model LX20 (Beckman, Fullerton, CA). Insulin resistance was assessed using the homeostasis model assessment score ($HOMA_{IR}$) [24]. Studies were performed in the Nemours Children's Clinic and the University of Florida Shands Jacksonville/SJCHC, Jacksonville, FL, and were approved by the Nemours Children's Clinic clinical research review committee, and Nemours Children's Clinic and University of Florida institutional review committees. All subjects gave informed written consent before entering the study.

2.2. Determination of genotype and haplotype

The genotype of common single nucleotide polymorphisms (SNPs) for the adrenergic receptors was determined using polymerase chain reaction and restriction fragment length polymorphism. Genomic DNA was extracted from whole blood using a DNA isolation kit (Gentra Systems, Minneapolis, MN). Primers were synthesized by Operon Technologies (Alameda, CA). The restriction enzymes were purchased from New England Biolabs (Beverly, MD). The following SNPs were typed: for *ADRB1* (NCBI RefSeq: NM_000684)—A145G (Ser49→Gly, rs1801252), C1165G (Arg389→Gly, rs1801253); for *ADRB2* (NCBI RefSeq: NM_000024)—T-47C (Cys19→Arg, rs1042711), G46A (Gly16→Arg, rs1042713), C79G (Gln27→Glu, rs1042714); for *ADRB3* (NCBI RefSeq: NM_000025)—T190C (Trp64→Arg, rs4994); and for *ADRA2A* (NCBI RefSeq: NM_000681)—C-1291G (rs1800544), G1780A (3′ UT, rs553668). The first nucleotide in the initiating codon of methionine for translation is referred as nucleotide position +1 in the reference sequences. Because of strong linkage disequilibrium (LD) between amino acids 49 and 389 [25,26], we inferred the following *ADRB1* haplotypes: Ser49/Arg389, Ser49/Gly389, and Gly49/Arg389. *ADRB2* haplotypes Cys19/Arg16/Gln27, Cys19/Gly16/Gln27, and Arg-19/Gly16/Glu27 were determined as previously described [27]. The following primers and restriction enzymes were used in polymerase chain reaction and restriction fragment length polymorphism: for *ADRB1* Ser49Gly—forward 5′-GTCCTGGGCGCCTCCGAG-3′ /reverse 5′-GATGGCCACGATCACCAGCAC-3′ and *Sau*96I; for *ADRB1* Arg389Gly—forward 5′-CGCTCTGCTGGCTG-CCCTTCTTCC-3′ /reverse 5′-GGTCTCCGTGGGTCG-CGTGG-3′) and *Bst*NI; for *ADRB3* Trp64Arg—forward 5′-CAATACCGCCAACACCAGTGG-3′ /reverse 5′-GGTCATGGTCTGGAGTCTCG-3′ and *Msp*I; for *ADRA2A* C-1291G—forward 5′-GGAGGTTACTTCCCTCG-3′ /reverse 5′-GGTACCTTGAGCTAGAGAC-3′ and *Msp*I; for A1780G—forward 5′-CAGAGCAGCACTGGACTAC-3′/ reverse 5′-TGGAAGGCATCTCTCCCAAG-3′ and *Dra*I.

2.3. Statistics

We estimated a sample size of 88 obese and 88 nonobese participants to address the hypothesis in this study. This estimate was based on the *ADRB2* haplotype frequencies [28] and on the assumption that the frequency of the Gly16/Glu27 haplotype is 20% higher in obese compared with that in nonobese participants. Normal Q-Q plots [29] with tests were

used to assess normality of distribution. The data were log-transformed when characteristics departed from normality in African Americans and whites. Hardy-Weinberg equilibria between expected and observed genotype distributions were calculated using χ^2 goodness-of-fit tests. Deviations between observed and expected values with probabilities larger than 10% was accepted as no statistical deviation from Hardy-Weinberg equilibrium. Linkage disequilibrium [30] was assessed by comparing expected and observed haplotype distributions, and analyzed by Haploview32 (www.broad.mit.edu/mpg/haploview/). The statistical significance of the estimated LD was tested by converting LOD score into an equivalent χ^2 statistic using 1 *df*.

The prevalence of obesity by sex and race were compared using Fisher exact test [31]. Nonparametric Spearman correlation coefficients [32] between BMI and metabolic characteristics were calculated based on ranking the 2 variables. Metabolic characteristics were compared between nonobese and obese participants, of which Student unpaired *t* test was used for continuous variables with normal distribution including body fat, HbA_{1c}, systolic blood pressure, and diastolic blood pressure; nonparametric method, Mann-Whitney test, was used for data that are significantly skewed including BMI, potassium, glucose, insulin, and insulin resistance.

Frequency distributions of SNP genotypes and haplotypes between African Americans and whites were compared using χ^2 test. General linear models [33] were used to assess qualitative and quantitative explanatory variables at the same time. Assuming a general linear model, logarithmic transformation was used to normalize the distributions of BMI, plasma potassium, glucose, insulin, and insulin resistance. The linear model was applied in all associations between the genetic variants with metabolic and clinical responses. Percentage of body fat and BMI were adjusted for age and sex; all other metabolic and clinical characteristics including potassium, glucose, insulin, HbA_{1c}, and insulin resistance were also adjusted for percentage of body fat.

Odds ratios were calculated to estimate the risk of obesity associated with candidate SNPs. Crude odds ratios were calculated by dividing the odds in the exposed group by the odds in the control group. Adjusted odds ratios for the risk of obesity were calculated by logistic regression model with adjustment of age and sex [31]. For each odds ratio, a 2-tailed *P* value and 95% confidence interval were estimated. Statistical significance was made at a cutoff value of $P < .10$ for screening risk factors [34]. Analysis of covariance was applied in associations between the numbers of haplotype copies and BMI adjusted for age and sex [33]. χ^2 test was used for testing obese prevalence differences between the numbers of haplotype copies. A general linear model with covariates of age and sex was used to assess interactions between *ADRA* and variants of *ADRB1* and *ADRB2*. All analyses were performed using SPSS (SPSS, Chicago, IL). *P* values of <.1 were considered significant.

3. Results

3.1. Participants

A total of 238 subjects participated in the study; 161 were self-identified whites (7 Hispanic, 154 non-Hispanic), 74 were African Americans, and the remaining participants were either Indian (2) or Asian (1). Because of the small numbers of Indians and Asians, analyses were performed on 235 participants; 161 self-identified whites (72.7% female) and 74 African Americans (80% female). Mean age ± SD for African Americans and whites were 30 ± 8.7 vs 30 ± 8.0 years, respectively ($P = .63$). Among African Americans, BMI and percentage of body fat were higher compared with those among whites: 33 ± 7.8 vs 29 ± 7.5 kg/m^2 ($P = .001$) and 36% ± 8.2% vs 32% ± 10%, respectively ($P = .008$). Among whites, 36% were classified as obese, compared with 61% in African Americans.

Table 1 compares anthropometric, metabolic, and other characteristics by ethnicity. For African Americans and whites, BMI, percentage of body fat, insulin concentrations, and values for $HOMA_{IR}$ were higher among obese individuals compared those among nonobese individuals. For

Table 1
Comparison of means ± SD for anthropometric measures and metabolic characteristics by ethnicity and body weight designation

Measurement	Whites					African Americans				
	Obese		Normal		*P*	Obese		Normal		*P*
	n	Mean ± SD	n	Mean ± SD		n	Mean ± SD	n	Mean ± SD	
BMI (kg/m^2)	58	37.02 ± 5.73	103	24.24 ± 3.26	<.0001	45	37.26 ± 6.07	28	25.27 ± 3.33	<.0001
Body fat (%)	58	40.30 ± 6.65	101	27.59 ± 7.73	<.0001	44	40.33 ± 5.26	28	28.52 ± 6.73	<.0001
Potassium (mEq/mL)	55	4.38 ± 0.57	98	4.37 ± 0.49	.959	45	4.17 ± 0.23	28	4.25 ± 0.53	.522
Glucose (mg/dL)	55	87.27 ± 10.40	99	82.23 ± 9.14	.015	45	86.13 ± 12.66	28	84.32 ± 11.11	.364
Insulin (μIU/mL)	54	15.52 ± 10.16	98	9.48 ± 10.81	<.0001	45	13.72 ± 7.94	28	8.44 ± 6.75	.001
HbA_{1c} (%)	51	5.35 ± 0.46	91	5.12 ± 0.41	.002	40	5.51 ± 0.49	26	5.42 ± 0.46	.464
$HOMA_{IR}$	54	3.46 ± 2.62	98	1.91 ± 2.04	<.0001	45	2.99 ± 1.88	28	1.80 ± 1.68	.001
Systolic	58	123.95 ± 10.58	103	118.30 ± 11.99	.003	44	123 ± 13.78	27	119.30 ± 12.23	.215
Diastolic	58	76.50 ± 8.50	103	74.29 ± 9.08	.132	44	78.00 ± 10.01	27	73.19 ± 8.21	.039

Student *t* test was used for variables of body fat, HbA_{1c}, systolic blood pressure, and diastolic blood pressure; Mann-Whitney *U* test was used for BMI, potassium, glucose, insulin, and $HOMA_{IR}$.

whites, resting glucose concentrations, HbA_{1c}, and systolic blood pressure were higher in obese participants compared with those in nonobese participants. Among African Americans, diastolic blood pressure was higher in obese compared with that in nonobese individuals. For African Americans, glucose concentrations and HbA_{1c} were similar between obese and nonobese individuals.

3.2. Relation between BMI and metabolic characteristics

Glucose, insulin, $HOMA_{IR}$, and HbA_{1c} were related to BMI in both African Americans and whites (Fig. 1). The relationship between glucose concentrations and BMI was weak with an R^2 value of 0.018 (P = .045). Insulin concentrations and $HOMA_{IR}$ values were more strongly related with obesity with R^2 values of 0.14 (P < .0001) and 0.18 (P < .0001), respectively (Fig. 1), demonstrating increased insulin resistance at normal glucose concentrations. Blood pressure was weakly associated with BMI: the R^2 values for systolic and diastolic blood pressures were 0.046 (P = .001) and 0.018 (P = .037), respectively. Systolic and diastolic blood pressures were not associated with age (P = .21 and .36, respectively [data not shown]).

3.3. Genotype and haplotype frequencies

A high rate of genotyping (> 98%) was achieved for all SNPs, which were in Hardy-Weinberg equilibrium when assessed by ethnicity except for the ADBR2 Gly16→Arg variant in whites (data not shown). The ADRB2 SNPs Cys19→Arg and Gln27→ Glu27 were in complete LD. ADRB1 SNPs Ser49→Gly and Arg389→Gly; ADRB2 SNPs amino acids Gln27→ Glu27; Gly16→Arg and Gln27→Glu; and ADRA2A SNPs C-1291G and G→1780A were in tight LD (data not shown). Table 2 compares genotype frequencies of the 8 adrenergic receptor SNPs between 2 ethnic groups. Ethnic differences for genotype frequencies were found for the *ADRB1* (Ser49→Gly), *ADRB2* (Cys19→Arg, Gln27→Glu), and *ADRA2A* (C-1291G) SNPs. Ethnic differences were also noted for *ADRB1* and *ADRB2* haplotype frequencies (Table 3).

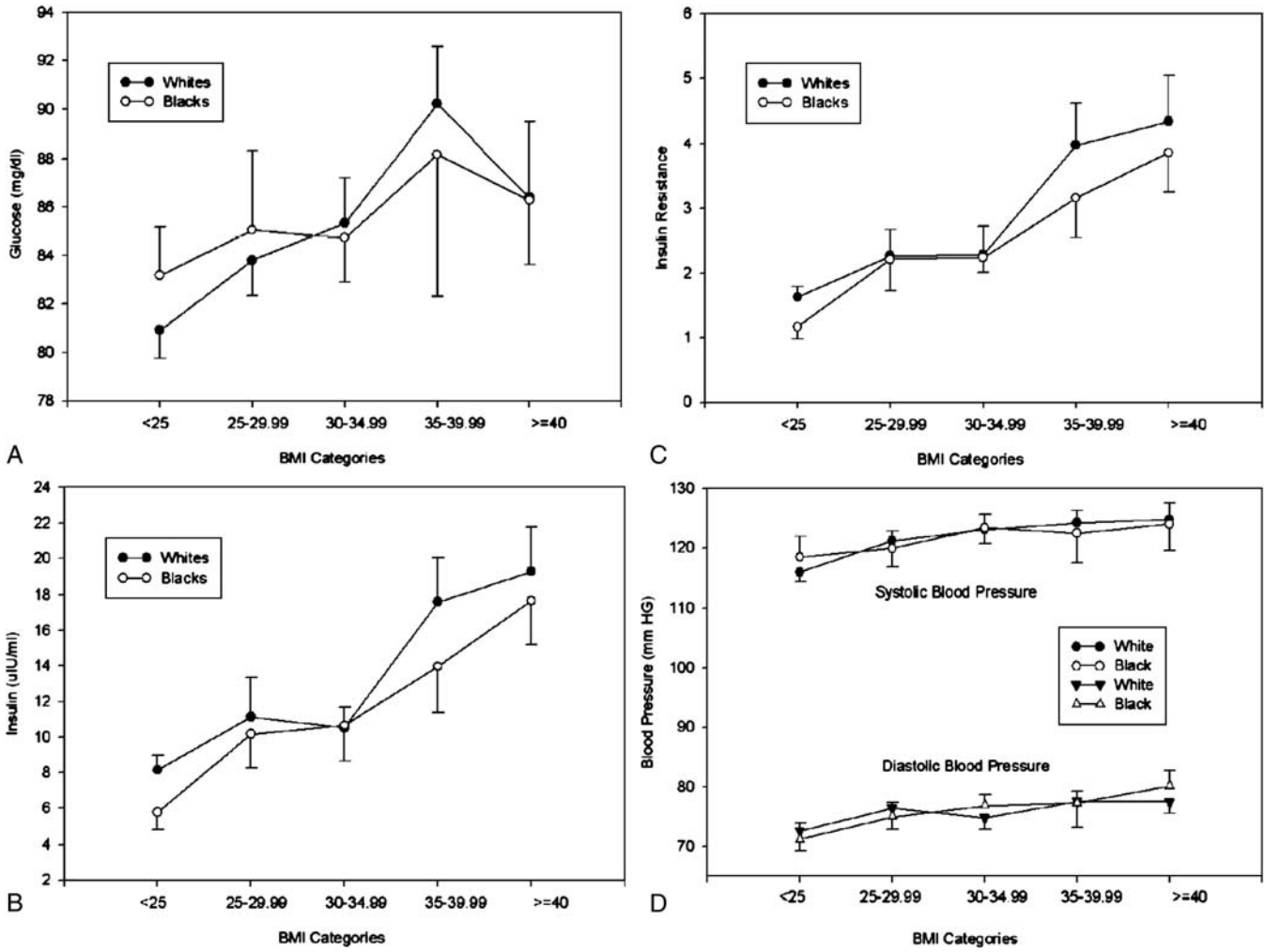

Fig. 1. Relationship between mean ± SD fasting glucose concentrations (A), insulin concentrations (B), and insulin resistance (C) as determined by $HOMA_{IR}$ and blood pressure (D) as a function of BMI in self-identified whites and African Americans.

Table 2
Adrenergic receptor polymorphism genotype frequencies in African American and white subjects

SNPs	Genotype	Frequency (%)		Pearson χ^2	P
		African American	White		
ADRB1 codon 49	A/A	44 (59.5)	120 (75.0)	7.440	.02
	G/A	25 (33.8)	37 (23.1)		
	G/G	5 (6.8)	3 (1.9)		
ADRB1 codon 389	C/C	30 (40.5)	85 (53.1)	3.614	.16
	C/G	35 (47.3)	63 (39.4)		
	G/G	9 (12.2)	12 (7.5)		
ADRB2 5′ LC-19	CC	4 (5.4)	31 (19.4)	15.91	<.0001
	TC	22 (29.7)	67 (41.9)		
	TT	48 (64.9)	62 (38.8)		
ADRB2 codon 16	CC	12 (16.2)	33 (20.6)	4.808	.09
	GC	39 (52.7)	60 (37.5)		
	GG	23 (31.1)	67 (41.9)		
ADRB2 codon 27	CC	48 (64.9)	62 (38.8)	15.905	<.0001
	GC	22 (29.7)	67 (41.9)		
	GG	4 (5.4)	31 (19.4)		
ADRB3	CC	3 (4.2)	2 (1.3)	3.454	.18
	TC	15 (20.8)	23 (14.7)		
	TT	54 (75)	131 (84.0)		
ADRA2A C-1291G	CC	10 (13.7)	85 (53.5)	39.827	<.0001
	CG	37 (50.7)	58 (36.5)		
	GG	26 (35.6)	16 (10.1)		
ADRA2A A2659G	AA	4 (5.5)	8 (5.0)	5.470	.07
	AG	29 (39.7)	40 (25.0)		
	GG	40 (54.8)	112 (70.0)		

3.4. Genetic association analyses

Among whites, significant associations were found for the *ADRB1* Arg389→Gly variant and insulin concentrations and $HOMA_{IR}$ (Fig. 2; Table 4): mean ± SD values for insulin and $HOMA_{IR}$ in Arg389 homozygotes and carriers of the Gly were 10 ± 7.0 and 12 ± 9.4 μIU/mL (P = .02) and 2.1 ± 1.7 and 2.6 ± 2.2 (P = .057), respectively, at similar values for BMI, percentage of body fat, and glucose concentrations. When we stratified by BMI (nonobese <30 kg/m^2 vs obese ≥30 kg/m^2), the associations between *ADRB1* Arg389→Gly variant and insulin and $HOMA_{IR}$ were lost (Table 4). However, among obese participants, BMI was significantly higher in carriers of the Gly allele compared to the Arg389 homozygotes (39 ± 6.5 vs 35 ± 4.0, P = .018), suggesting that the association between the Arg389→Gly genotype and insulin and insulin resistance (Fig. 2) was attributable to the higher BMI among carriers of the Gly16 allele. Systolic blood pressure was higher in whites for carriers of the *ADBR1* Gly49 compared to Ser49 homozygotes: 124 ± 12.6 vs 119 ± 11.3 mm Hg, respectively (P = .023). For nonobese participants, systolic blood pressures in Ser49 homozygotes (n = 74) and carriers of the Gly (n = 28) were 117 ± 11 and 122 ± 14, respectively (P = .19). For obese participants, systolic blood pressures in Ser49 homozygotes (n = 45) and carriers of the Gly (n = 12) were 123 ± 11 and 129 ± 8.9, respectively (P = .088).

Among African Americans, there was a significant gene dose-response relationship for the *ADRA2A* G1780A genotype with both BMI and percentage of body fat (Fig. 3). Compared to carriers of the *ADRB2* Glu27 (n = 25), Gln27 homozygotes (n = 48) had higher insulin concentrations (12.3 ± 8.0 vs 10.6 ± 7.7, P = .056) and were more insulin-resistant as determined by $HOMA_{IR}$ (2.7 ± 2.0 vs 2.2 ± 1.8, P = .06). There were no significant associations between the genotype of the ADRB3 Trp64→Arg SNP and anthropometric measures or metabolic characteristics in whites or African Americans (data not shown).

3.5. Gene-gene interactions

Among whites there was a significant interaction for BMI between *ADRB1* haplotype and *ADRA2A* C-1291G SNP (P = .038): 5 participants with the Ser49-Arg389/Ser49-Gly389 diplotype and C-1291G G homozygous genotype had the highest BMI: 39.4 ± 9.73 kg/m^2. Among African Americans, there was a significant interaction between the *ADRB2* Gln27→Glu variant and the *ADRA2A* C-1291G SNP (P = .010): 6 participants who were homozygous for Gln27 and homozygous for the C-1291 SNPs had the highest BMI compared with other combinations: 38.8 ± 5.98 kg/m^2.

4. Discussion

Obesity is a common complex disease that involves multiple genetic variants interacting with environmental and behavioral factors. Genes involved in the regulation of catecholamine function may be important in obesity because of the role catecholamines play in energy expenditure and lipolysis. Stimulation of ADRB increases lipolysis, which favors weight reduction, whereas ADRA2A inhibits lipolysis, which favors weight gain. In the present study we hypothesized that common SNPs in *ADRB* and *ADRA2A* associate with obesity and accompanying metabolic alterations. The results of our study demonstrate that common SNPs in *ADRB* and *ADRA2A* genes may contribute to obesity and to metabolic alterations in young healthy whites and African Americans, although in a complex manner. Among whites, genotype (and haplotype) of *ADRB* and *ADRA2A* SNPs contributed little or not at all to obesity, which is in keeping with

Table 3
Haplotype frequencies for ADRB1 and ADRB2 in African American and white subjects

Gene	Haplotype	Frequency		P
		African American (%)	White (%)	
ADRB1	Gly49-Arg389	35 (23.6)	43 (13.4)	<.0001
	Ser49-Arg389	60 (40.5)	194 (60.6)	
	Ser49-Gly389	53 (35.8)	83 (25.9)	
ADRB2	5′ LCCys19-Gly16-Gln27	55 (37.2)	65 (20.3)	<.0001
	5′ LCCys19-Arg16-Gln27	64 (43.2)	126 (39.4)	
	5′ LCArg19-Gly16-Glu27	29 (19.6)	129 (40.3)	

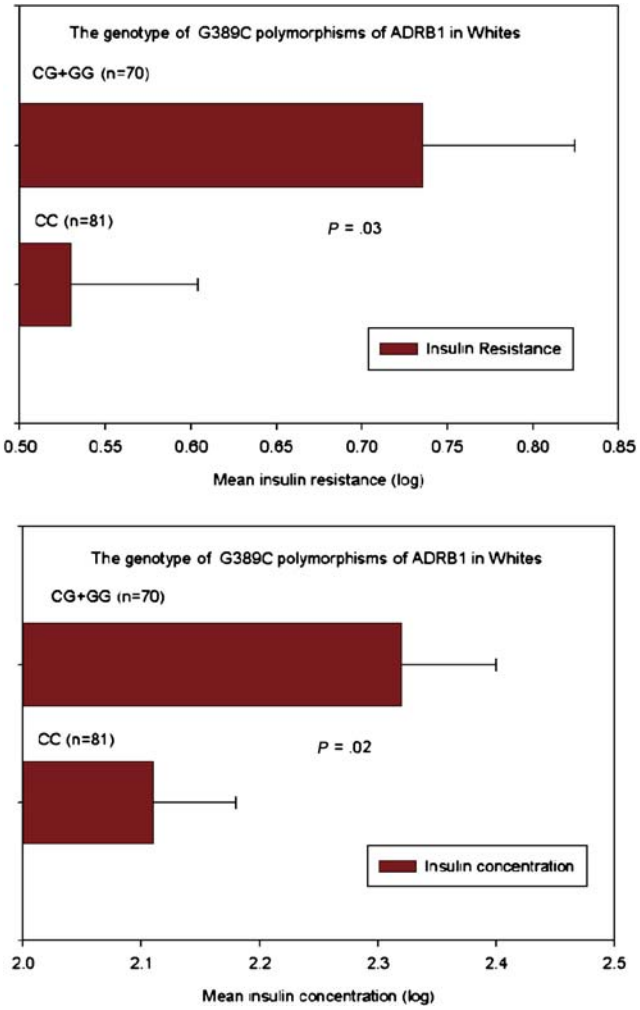

Fig. 2. Influence of the *ADRB1* Arg389→Gly (G1165C) polymorphism on insulin resistance and insulin concentrations in whites. Insulin concentrations and values for $HOMA_{IR}$ were log-transformed; means ± SE were determined and tested by *t* test in Arg389 homozygotes and Gly389 carriers.

previous studies in older adult women [26] and children [35], although in an earlier study, the Arg389 variant was weakly associated with obesity in older adult women (0.86 kd/m^2 per Arg allele) [36]. In the present study, white carriers of the *ADRB1* Gly389 (heterozygotes and Gly homozygotes) had higher insulin concentrations and were more insulin-resistant compared to Arg389 homozygotes (Fig. 2) at similar BMI and percentage of body fat values. To determine what drove this association, we stratified our cohort by obesity status and found that among obese participants (BMI ≥30 kg/m^2), BMI was significantly higher among Gly389 carriers compared to Arg389 homozygotes (Table 4). These data suggest that the association we found between the Gly389 variant and insulin resistance may, at least in part, be attributed to the higher BMI in this genotype. We therefore conclude that the *ADRB1* Arg389→Gly polymorphism contributes to obesity and to accompanying metabolic alterations in young healthy whites.

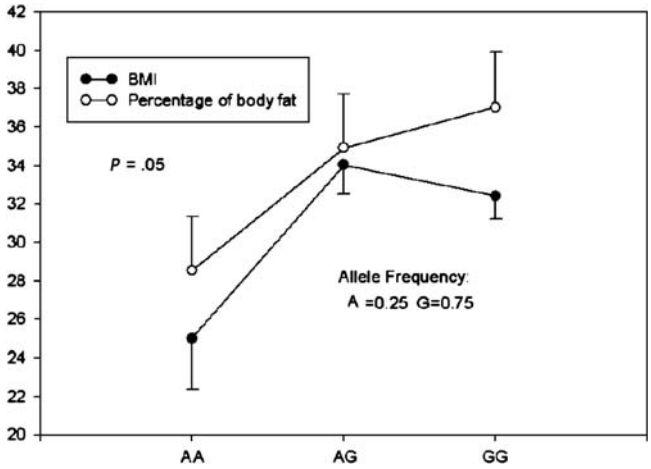

Fig. 3. Mean ± SE of BMI and percentage of body fat by *ADRA2A* A1780G genotypes in African Americans.

The functional basis underlying the association between the *ADRB1* Arg389→Gly variant with obesity and metabolic alterations may be related to the pivotal role the sympathetic nervous system (SNS) plays in obesity. Previous studies have reported that compared to the Gly389, the Arg389 form of the *ADRB1* mediated a higher isoproterenol-stimulated adenylyl cyclase activity and greater coupling [37], demonstrated a greater inotropic response to stimulation [38], and was more responsive to beta-blockade [25], although several studies report no differences between the 2 variants for several phenotypes (see reference

Table 4
Comparison of mean ± SD BMI, insulin, and insulin resistance (by $HOMA_{IR}$) by *ADRB1*389 genotype among obese and nonobese whites

Measurement (white)		ADRB1 codon 389 (mean ± SD)/n		
		CC	CG+GG	*P*
BMI (kg/m^2)		27.97 ± 6.04 (n = 85)	29.63 ± 8.72 (n = 75)	.29
Insulin (μIU/mL)		9.99 ± 6.96 (n = 81)	12.28 ± 9.36 (n = 69)	.041
BMI (kg/m^2)	Nonobese	24.41 ± 3.11 (n = 56)	24.04 ± 3.45 (n = 47)	.70
	Obese	34.86 ± 3.99 (n = 29)	39.02 ± 6.48 (n = 28)	.018
Insulin (μIU/mL)	Nonobese	7.77 ± 3.35 (n = 54)	9.50 ± 7.24 (n = 43)	.102
	Obese	14.43 ± 9.78 (n = 27)	16.87 ± 10.71 (n = 26)	.33
HOMA		2.13 ± 1.72 (n = 81)	2.63 ± 2.24 (n = 69)	.057
HOMA	Nonobese	1.59 ± 0.69 (n = 54)	1.93 ± 1.47 (n = 43)	.15
	Obese	3.20 ± 2.52 (n = 27)	3.78 ± 2.78 (n = 26)	.34

[21]). It has been hypothesized that SNS underactivity could lead to an inadequate thermogenic response to overeating, positive energy balance, and weight gain [39]. Alternatively, SNS activation that occurs with long-term overeating can lead to enhanced sympathetic stimulation [39,40]. Our finding is consistent with the former hypothesis as the Gly389 variant is the less active form of the ADBR1. Whatever the mechanism, it apparently does not involve lipolysis because lipolytic response to ADRB1 stimulation in human adipocytes did not differ by amino acid 389 variants [41].

Among whites, we found that carriers of the *ADRB1* Gly49 had higher systolic blood pressures compared to Arg49 homozygotes. Although we did not find associations between BMI or percentage of body fat with Ser49→Gly variant in obese vs nonobese participants, among obese individuals carrying the Gly49, systolic blood pressure was 6 mm Hg higher compared with obese Ser49 homozygotes ($P = .088$). These data suggest that the higher systolic blood pressure we observed in carriers of the Gly49 was driven by the higher BMI. These data are consistent with the study by Linne et al [42] who reported that the Gly49 was associated with long-term weight gain in women. In in vitro studies the Gly49 variant demonstrated higher basal and agonist-stimulated adenylyl cyclase activity compared to the Ser49 [43], and the Gly49 was down-regulated to a greater extent by continuous agonist exposure [43,44]. It is possible that increased sympathetic tone contributes to elevations in blood pressure in the obese state [40], which are consistent with our findings.

Among African Americans, we found a weak association between *ADRB2* Gln27→Glu and insulin: Gln27 homozygotes tended to have slightly higher insulin concentrations and were slightly more insulin-resistant. The *ADRB2* gene is highly polymorphic [45]. In studies of human adipocytes, the Glu27 variant has been associated with reduced lipolytic activity compared with the Gln27 [46]. Our results are not consistent with these studies. However, the results of several association studies of common SNPs (genotype and haplotype) with obesity, diabetes, and other common complex diseases are conflicting [21]. Taken together, and given the small numbers of African Americans in our study, the relevance of this finding is not clear.

Our data suggest that the *ADRA2A* gene may play an important role in obesity (Fig. 3). The *ADRA2A* gene is located on chromosome 10q24-q26, is intronless, and is highly polymorphic [47]. The C-1291G SNP is located in the promoter or enhancer region with the G allele having frequencies of 0.27 and 0.67 in whites and African Americans, respectively. The G1780A SNP is located in the 3′ UTR with the A allele having frequencies of 0.17 and 0.31, respectively [47]. In our study, the allele frequencies for the G-1291 allele in whites and African Americans were 0.28 and 0.61, respectively; for the A1780 SNP the allele frequencies were 0.18 and 0.25, respectively. These *ADRA2A* variants were selected because associations with the G1780A SNP and hypertension in African Americans [48], with obesity in whites [49,50], and with endurance in white athletes have been reported [51]. The C-1291G SNP has been associated with changes in glucose and diastolic blood pressure in response to dexamethasone [52]. In the present study, the G1780 allele was associated with obesity and percentage of body fat among African Americans. Moreover, we found evidence of interactions between *ADRA2A* and *ADRB1* genes among whites and between *ADRA2A* and *ADRB2* genes among African Americans with obesity. These data support a complex and important role for adrenergic receptor variants in obesity and the accompanying metabolic alterations and agree with the results of a study by Ukkola et al [53] that interactions among *ADRA2A* and *ADRB* genes contribute to obesity-related phenotypes. In the future, large studies should explore interactions for genes that regulate catecholamine-mediated effects on energy expenditure.

The epidemic of obesity in this country and worldwide has led to a marked increase in the incidence of metabolic syndrome, which is variably defined as a constellation of metabolic abnormalities including glucose intolerance (type 2 diabetes mellitus), insulin resistance, central obesity (BMI ≥ 30 kg/m^2), dyslipidemia, and hypertension (blood pressure $\geq 130/85$ mm Hg) [54]. The mechanism(s) underlying the development of the metabolic syndrome is not clear, but is thought to be largely attributable to insulin resistance with an excessive flux of fatty acids and the involvement of the proinflammatory state [55]. Although none of the obese individuals in our study could be characterized as having type 2 diabetes mellitus or metabolic syndrome, some, but not all, had higher concentrations of glucose and insulin, insulin resistance as assessed by $HOMA_{IR}$, and elevated HbA_{1c} and blood pressures compared with nonobese individuals (Table 1, Fig. 1). It is likely that many of these participants, if left untreated (ie, lifestyle modifications), would go on to develop type 2 diabetes mellitus, metabolic syndrome, and cardiovascular and other diseases. A few studies support a genetic basis for metabolic syndrome including the *ADRB2* Gly16→Arg and the Gln27→Glu variants [56] and others [57]. It is in this context that our findings that common SNPs in the *ADRB* and *ADRA* genes may associate with hyperinsulinemia, insulin resistance, and elevated systolic blood pressures portend significant clinical relevance.

We have uncovered several intriguing associations between common SNPs in *ADRB2A* and *ADRB* genes with obesity and related alterations. However, the present study has several limitations. Many gene variants contribute to obesity and related alterations and those in *ADRA2A* and *ADRB* may have modest effects, thus requiring large sample sizes to detect associations [58]. Our sample size was small, and it is possible that the associations we observed between *ADRA2A* and *ADRB* SNPs with obesity could represent

false positives. Because of the potential for population stratification [59], we stratified our population by self-identified ethnicity, which further exacerbates our problems with small numbers. In addition, our findings are further confounded by the fact that 73% and 80% of our white and African American population was female, although we adjusted for sex in our analyses. We did not correct for multiple hypothesis testing, which could also contribute to false positive associations [58]. We chose not to adjust for multiple comparisons because given the small numbers of participants, we reasoned that it is important not to dismiss differences that could be real. For these reasons, the results of our study should be interpreted with caution and should be regarded as exploratory, and underscore the need for replication in larger populations.

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