

Variants of the Insulin Receptor Substrate-1 and Fatty Acid Binding Protein 2 Genes and the Risk of Type 2 Diabetes, Obesity, and Hyperinsulinemia in African-Americans

The Atherosclerosis Risk in Communities Study

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We conducted a community-based case-control study of African-American men and women in the Atherosclerosis Risk in Communities Study. The allele frequencies of the Gly972Arg variant of the insulin receptor substrate-1 (*IRS-1*) gene and the Ala54Thr variant of the fatty acid binding protein 2 (*FABP2*) gene were compared in 992 normal control subjects and three patient groups: 1) 321 type 2 diabetic individuals, 2) 260 severely obese individuals, and 3) 258 markedly hyperinsulinemic individuals without diabetes. Allele frequencies of Gly972Arg *IRS-1* and Ala54Thr *FABP2* were 0.07 and 0.22, respectively; there were no differences in allele or genotype frequencies between patients and control subjects for either gene variant. In weighted linear regression of all patients and control subjects, the presence of the *IRS-1* gene variant was associated with a 0.85 (0.42) kg/m² higher BMI ($P = 0.04$). In addition, individuals with at least one *IRS-1* Arg972 allele and two *FABP2* Thr54 alleles had a BMI of 33.3 (7.9) kg/m², compared with 30.0 (6.3) kg/m² for those with neither allele ($P = 0.05$). These results suggest that in African-Americans, these variants in the *IRS-1* and *FABP2* genes are not associated with the risk of type 2 diabetes, severe obesity, or marked hyperinsulinemia, but that their independent and joint effects may be associated with small increases in BMI. *Diabetes* 48:1868–1872, 1999

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ARIC, Atherosclerosis Risk in Communities; FABP2, fatty acid binding protein 2; IRS-1, insulin receptor substrate-1; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

Few studies have addressed the genetic basis of type 2 diabetes in African-Americans, despite their high risk. Many candidate genes for type 2 diabetes have been proposed based on their role in insulin action or insulin resistance (1–4). Among these, the gene encoding the insulin receptor substrate-1 (*IRS-1*) protein located on chromosome 2q35–q36.1 has been studied extensively with inconclusive results (5–24). *IRS-1* is a signaling protein that acts as a docking and activation site for multiple signal transducing molecules that control cellular growth and metabolism (25). One of the most common mutations in the *IRS-1* gene is in codon 972, where a point mutation causes a change from glycine (GGG) to arginine (AGG) (14,18). This mutation is located between tyrosine phosphorylation motifs in the *IRS-1* gene, but is not within any of the tyrosine phosphorylation or ATP binding sites (17). The fatty acid binding protein 2 (*FABP2*) gene located on chromosome 4q28–31 is another candidate gene possibly implicated in the pathogenesis of type 2 diabetes, insulin resistance, and obesity (26–28). As a result of the excessive absorption of fatty acids attributed to the Thr54 allele, skeletal muscle begins to preferentially use fatty acids for fuel rather than glucose, thus resulting in an increase in plasma glucose levels. The increase in long-chain fatty acids may also contribute to insulin resistance because of the decrease in insulin-stimulated nonoxidative glucose uptake (29). Neither the *IRS-1* nor the *FABP2* gene variants have been studied in African-Americans, a group at high risk for type 2 diabetes.

Characteristics of the African-American patients and control subjects are shown in Table 1. The distribution of men and women in the patient and control groups was comparable. Patients with type 2 diabetes were the oldest and had the highest fasting insulin and fasting glucose levels. Patients with severe obesity had the highest BMI, weight gain since age 25 years, subscapular skin-fold thickness, triceps skin-fold thickness, and systolic blood pressure. Patients with marked hyperinsulinemia had the highest levels of LDL cholesterol and triglycerides. Control subjects were the youngest and had the lowest levels of fasting insulin, fasting glucose, BMI, sub-

TABLE 1

Characteristics of 1,748 African-Americans with type 2 diabetes, severe obesity, and marked hyperinsulinemia and control subjects

	Patients				Control subjects
	Type 2 diabetes	Severe obesity	Hyperinsulinemia		
<i>n</i>	321	260	258	992	
Age (years)	54.6 ± 6.0*	53.3 ± 5.7	54.1 ± 5.5†	52.9 ± 5.8	
Men (%)	125 ± 38.9	101 ± 38.8	101 ± 39.1	406 ± 40.9	
Fasting insulin (μU/ml)	46.81 ± 98.86*	24.38 ± 17.99*	33.06 ± 10.87*	10.06 ± 5.02	
Fasting glucose (mg/dl)	174.2 ± 76.1*	116.8 ± 47.5*	107.6 ± 12.4*	97.6 ± 10.1	
Total caloric intake (kcal/day)	1,444 ± 610*	1,556 ± 734‡	1,626 ± 660	1,677 ± 852	
BMI (kg/m ²)	30.83 ± 5.29*	40.63 ± 4.91*	33.54 ± 5.89*	26.99 ± 4.10	
BMI at age 25 years (kg/m ²)	24.54 ± 4.68*	27.69 ± 5.73*	23.55 ± 4.50†	22.31 ± 3.36	
ΔBMI since age 25 years (kg/m ²)	6.63 ± 5.49*	13.11 ± 6.87*	9.76 ± 5.66*	4.81 ± 4.30	
Subscapular skin-fold thickness (mm)	35.48 ± 12.58*	48.51 ± 11.53*	41.21 ± 12.69*	27.12 ± 11.84	
Triceps skin-fold thickness (mm)	27.70 ± 11.60*	40.16 ± 13.06*	32.98 ± 12.13*	23.90 ± 10.76	
Systolic blood pressure (mmHg)	133.0 ± 22.0*	134.2 ± 21.3*	128.0 ± 19.2	126.0 ± 20.3	
HDL cholesterol (mmol/l)	1.30 ± 0.37*	1.30 ± 0.35*	1.23 ± 0.31*	1.51 ± 0.48	
LDL cholesterol (mmol/l)	3.68 ± 1.20*	3.43 ± 1.04	3.75 ± 1.11	3.50 ± 1.12	
Triglycerides (mmol/l)	1.46 ± 0.68*	1.27 ± 0.59*	1.50 ± 0.66*	1.08 ± 0.53	

Data are means ± SD. Severe obesity is defined as BMI >38.3 kg/m². Hyperinsulinemia is defined as insulin >26 μU/ml. Data on BMI at and after age 25 years are missing for 35.5% of type 2 diabetic patients, 32.7% of severely obese patients, 31.0% of markedly hyperinsulinemic patients, and 27.9% of control subjects. **P* < 0.001, †*P* < 0.01, ‡*P* < 0.05 for patients compared with control subjects.

scapular skin-fold thickness, triceps skin-fold thickness, and triglycerides, but had the highest levels of total daily caloric intake and HDL cholesterol.

There were no statistically significant differences in allele frequencies between any of the patient groups and the control group (Table 2). The frequency of the IRS-1 Arg972 allele was 0.06 in the patients with type 2 diabetes, 0.08 in the patients with severe obesity, 0.06 in the patients with marked hyperinsulinemia, and 0.06 in the control subjects. Under the codominant model, the odds ratio for the association of the IRS-1 Gly972Arg gene variant with type 2 diabetes was 1.05 (95% CI 0.73–1.52), and with marked hyperinsulinemia was 1.02 (0.68–1.53). However, when compared with nonobese control subjects with BMI <25 kg/m², individuals with the IRS-1 Arg972

allele tended to be at greater risk of being severely obese than control subjects (odds ratio 1.36 [0.94–2.0]). Under a dominant model, individuals with the IRS-1 Arg972 allele had a 1.60 (0.98–2.61) greater risk of being severely obese than did nonobese control subjects.

The frequency of the FABP2 Thr54 allele was 0.22 in the patients with type 2 diabetes, 0.23 in the patients with severe obesity, 0.21 in the patients with marked hyperinsulinemia, and 0.23 in the control subjects. The odds ratio for the association of the FABP2 Ala54Thr gene variants with type 2 diabetes under the codominant model was 0.97 (0.78–1.20), with severe obesity 0.99 (0.79–1.25), and with marked hyperinsulinemia 0.90 (0.72–1.15). Under a recessive model, individuals with two FABP2 Thr54 alleles had a 1.82 (0.86–3.89)

TABLE 2

IRS-1 Gly972Arg and FABP2 Ala54Thr genotypes in 1,748 African-American patients and control subjects

	Patients				Control subjects
	Diabetes	Severe obesity	Hyperinsulinemia		
<i>n</i>	321	260	258	992	
IRS-1 genotype					
Gly/Gly	281 (87.5)	219 (84.2)	226 (87.6)	874 (88.1)	
Gly/Arg	39 (12.2)	40 (15.4)	32 (12.4)	115 (11.6)	
Arg/Arg	1 (0.3)	1 (0.4)	0 (0.0)	3 (0.3)	
<i>P</i> for codominant model	0.79	0.10	0.93	—	
<i>P</i> for dominant model	0.79	0.10	0.82	—	
FABP2 genotype					
Ala/Ala	190 (59.2)	159 (61.2)	164 (63.6)	587 (59.2)	
Ala/Thr	119 (37.0)	84 (32.3)	79 (30.6)	357 (36.0)	
Thr/Thr	12 (3.7)	17 (6.5)	15 (5.8)	48 (4.8)	
<i>P</i> for codominant model	0.76	0.95	0.41	—	
<i>P</i> for recessive model	0.41	0.27	0.52	—	

Data are *n* (%). Severe obesity is defined as BMI >38.3 kg/m². Hyperinsulinemia is defined as insulin >26 μU/ml. For IRS-1, the codominant model indicates test for trend across groups with 0, 1, or 2 Arg alleles, and the dominant model indicates comparison of Gly/Gly versus Gly/Arg and Arg/Arg. For FABP2, the codominant model indicates test for trend across groups with 0, 1, or 2 Thr alleles, and the recessive model indicates comparison of Ala/Ala and Ala/Thr versus Thr/Thr.

TABLE 3
Selected characteristics of 1,748 African-Americans by *IRS-1* Gly972Arg genotype

	Gly/Gly	Gly/Arg	Arg/Arg	<i>P</i> for codominant model	<i>P</i> for dominant model
<i>n</i>	1,531	212	5	—	—
Fasting insulin (μU/ml)	12.98 ± 8.58	13.96 ± 12.16	10.73 ± 7.19	0.31	0.26
Fasting glucose (mg/dl)	99.71 ± 14.61	99.72 ± 16.49	89.87 ± 92.94	0.71	0.86
BMI (kg/m ²)	28.98 ± 5.30	29.85 ± 5.98	29.29 ± 8.02	0.05	0.04
BMI at age 25 years (kg/m ²)	23.08 ± 3.78	23.09 ± 4.02	22.60 ± 3.04	0.98	0.99
Change in BMI since age 25 years (kg/m ²)	5.94 ± 4.91	6.78 ± 5.50	10.14 ± 5.84	0.04	0.05
Systolic blood pressure (mmHg)	128.24 ± 19.52	125.92 ± 16.43	121.23 ± 23.74	0.09	0.11
HDL cholesterol (mmol/l)	1.44 ± 0.42	1.47 ± 0.41	1.50 ± 0.50	0.31	0.31
LDL cholesterol (mmol/l)	3.57 ± 1.05	3.48 ± 1.04	3.65 ± 0.69	0.35	0.31
Triglycerides (mmol/l)	1.19 ± 0.55	1.17 ± 0.59	1.13 ± 0.56	0.31	0.32

Data are means ± SD from weighted linear regression (weights determined by sampling fractions of patients and control subjects). The codominant model indicates test for trend across groups with 0, 1, or 2 Arg alleles, and the dominant model indicates comparison of Gly/Gly versus Gly/Arg and Arg/Arg. Data for fasting insulin and glucose exclude type 2 diabetic patients. For triglycerides, *P* values given are for ln(triglycerides).

greater risk of being severely obese than did nonobese control subjects (BMI <25 kg/m²). Allele frequencies were the same in individuals who fulfilled two of the patient definitions and in the three main patient groups: diabetes, severe obesity, and hyperinsulinemia.

Assuming a dominant model of inheritance for *IRS-1*, in weighted linear regression, carriers of the Arg972 allele had a significantly higher BMI (*P* = 0.04) and a greater increase in BMI since age 25 years (*P* = 0.05) (Table 3). Under both codominant and dominant models, there was a trend for carriers of the *IRS-1* Arg972 allele to have lower systolic blood pressure. No significant associations were observed with the *FABP2* Thr54 allele (Table 4). However, homozygous carriers of the *FABP2* Thr54 allele had a 1.0 kg/m² higher BMI (*P* = 0.11). Possible confounders, such as age and sex, were not considered in these analyses because they were not significantly correlated with both the genotypes and the outcomes of interest. Similar results were obtained in unweighted multiple linear regression.

Individuals were classified by both their *IRS-1* and *FABP2* genotypes to examine the joint effect of the two genes on BMI (Table 5). The highest mean BMI was 33.30 kg/m² among those individuals who carried at least one copy of the *IRS-1* Arg972 allele and two copies of the *FABP2* Thr54 allele. The differences in mean BMI between all combinations of *IRS-1* and *FABP2* genotypes were statistically significant (*P* = 0.05). However, in weighted linear regression, the interaction term between the two gene variants was not statistically significant (*P* = 0.39). No statistically significant interactions were observed for BMI, leisure index, age, total caloric intake, and menopausal status (in analyses confined to women) with the *IRS-1* Gly972Arg gene variant nor with the *FABP2* Ala54Thr gene variant.

These data support the following three conclusions in African-Americans. First, neither the Gly972Arg variant of the *IRS-1* gene nor the Ala54Thr variant of the *FABP2* gene were associated with the risk of developing type 2 diabetes. Second, neither variant was associated with severe obesity or

TABLE 4
Selected characteristics of 1,748 African-Americans by *FABP2* Ala54Thr genotype

	Ala/Ala	Ala/Thr	Thr/Thr	<i>P</i> for codominant model	<i>P</i> for recessive model
<i>n</i>	1,045	616	87	—	—
Fasting insulin (μU/ml)	13.35 ± 9.45	12.56 ± 8.39	13.69 ± 9.13	0.42	0.59
Fasting glucose (mg/dl)	99.40 ± 14.27	99.91 ± 16.35	101.34 ± 9.91	0.32	0.37
BMI (kg/m ²)	29.09 ± 5.46	28.95 ± 5.22	30.08 ± 5.78	0.56	0.11
BMI at age 25 years (kg/m ²)	23.09 ± 3.96	23.03 ± 3.55	23.44 ± 3.78	0.84	0.50
ΔBMI since age 25 years (kg/m ²)	6.26 ± 4.99	5.65 ± 4.98	6.55 ± 4.94	0.28	0.47
Systolic blood pressure (mmHg)	128.02 ± 19.06	127.68 ± 18.94	128.91 ± 22.44	0.99	0.66
HDL cholesterol (mmol/l)	1.44 ± 0.42	1.44 ± 0.41	1.39 ± 0.39	0.37	0.29
LDL cholesterol (mmol/l)	3.53 ± 1.04	3.58 ± 1.06	3.64 ± 0.98	0.26	0.49
Triglycerides (mmol/l)	1.19 ± 0.58	1.19 ± 0.52	1.23 ± 0.50	0.21	0.36

Data are means ± SD from weighted linear regression (weights determined by sampling fractions of patients and control subjects). The codominant model indicates test for trend across groups with 0, 1, or 2 Thr alleles, and the recessive model indicates comparison of Ala/Ala and Ala/Thr versus Thr/Thr. Data for fasting insulin and glucose exclude type 2 diabetic patients. For triglycerides, *P* values given are for ln(triglycerides).

TABLE 5
BMI (kg/m²) for IRS-1 and FABP2 genotype combinations in 1,748 African-American patients and control subjects

FABP2	IRS-1	
	Gly/Gly	Gly/Arg and Arg/Arg
Ala/Ala	30.00 ± 6.33 (910)	30.81 ± 7.33 (135)
Ala/Thr	29.67 ± 6.11 (549)	31.07 ± 6.49 (67)
Thr/Thr	30.96 ± 7.03 (72)	33.30 ± 7.88 (15)

Data are means ± SD (*n*). F-statistic 2.19, 5 df, *P* = 0.05.

marked hyperinsulinemia. Third, there was a modest association of the IRS-1 Arg972 allele with higher BMI in middle age and BMI increase since age 25 years, especially in the presence of the FABP2 Thr54 variant. Strengths of the study that lend weight to these conclusions include its population-based design, its careful attention to quality controls, and its large sample size. In particular, its size yielded unusually precise risk estimates and afforded a unique opportunity to assess gene-by-gene and gene-by-environment interactions.

Few previous studies of IRS-1 that failed to show an association could exclude the possibility of mild to moderate gene effects (i.e., relative odds of 1.5–3.0). Many of these studies also had major limitations related to their study samples. First, the samples were generally not population based (5,7,9,14–16,24), introducing the possibility of selection bias. Second, the sample sizes were generally small (i.e., <200 patients and/or <100 control subjects) (5,7,10,13,14,18,24). FABP2 has been studied in four association studies (26–28), none of which found an association between the FABP2 Thr54 allele with type 2 diabetes. Because each of these studies examined different phenotypes and reached different conclusions, it is difficult to directly compare the results. Of these studies, three did not include detailed case-control comparisons (27–29) despite having large sample sizes. The fourth study had a sample size of only 40 patients and control subjects (26).

This study implies that there is little or no association of the IRS-1 Gly972Arg variant or the FABP2 Ala54Thr variant with type 2 diabetes, severe obesity, or hyperinsulinemia in African-Americans. Because of its population-based design and large sample size, the current study effectively rules out the possibility of moderately strong associations (i.e., odds ratios of 2.0), a possibility that previous studies of these variants could not exclude. Its large sample size also afforded the first opportunity to assess gene-by-gene interactions between these variants. This assessment suggested that the variants might act jointly to increase BMI. Despite the lack of association between these specific polymorphisms and the phenotypes in this study, these results do not preclude the possibility that other polymorphisms in the IRS-1 and FABP2 genes may have an effect on diabetes and related phenotypes. Large population-based association studies like this one will become increasingly valuable in determining the precise effects of the growing number of promising candidate gene variants on the risk of type 2 diabetes and related conditions.

RESEARCH DESIGN AND METHODS

Of the 4,268 African-Americans in the Atherosclerosis Risk in Communities (ARIC) Study, individuals were excluded if they were missing demographic, clinical, dietary, or laboratory data at baseline (*n* = 998). Definitions of type 2 diabetes

were adapted from World Health Organization criteria (30) and include self-report of physician diagnosis, insulin or sulfonylurea use, and/or fasting glucose 7.8 mmol/l (140 mg/dl) from information at baseline and at 3- and 6-year follow-up visits. Severe obesity and marked hyperinsulinemia were defined by baseline BMI in the top decile of ARIC participants (>38.3 kg/m²), and baseline fasting insulin in the top decile of ARIC participants (>26 μU/ml), respectively. Hyperinsulinemic patients were selected from individuals who did not fulfill the criteria for diabetes as stated above. Individuals who were both diabetic and severely obese or both severely obese and hyperinsulinemic were included in each of the individual case-control comparisons. The patient definitions of diabetes and hyperinsulinemia were mutually exclusive. Control subjects were randomly sampled from individuals who did not fulfill any of the criteria to be selected as a patient at baseline and excluded individuals who developed type 2 diabetes at the 3- or 6-year follow-up visits.

Genomic DNA was isolated from peripheral blood leukocytes stored as frozen buffy coat in accord with standard ARIC procedures (31). A 220-bp region encompassing codon 972 of the IRS-1 gene was detected by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis using upstream primer 5'-GCAGCCTGGCAGGAGAGCACT-3' and downstream primer 5'-CTCACCTCTCTGCAGCAATG-3'. PCR conditions were as follows: denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were digested with *Bst*NI (New England Biolabs, Beverly, MA) for 12 h at 60°C. The Gly972 allele resulted in one band sized 220 bp, while the Arg972 allele resulted in two bands sized 164 and 56 bp. Genotyping was performed by a technician who was blinded to case-control status. To assess the reproducibility of genotype results, we repeated the assay on a subsample of 42 homozygous Gly972 genotypes, 27 heterozygous genotypes, and 5 homozygous Arg972 genotypes. We found perfect agreement between the first and second genotypes (κ = 1.0).

A 180-bp region encompassing codon 54 of the FABP2 gene was also detected by PCR-based RFLP analysis. PCR amplification was performed using upstream primer 5'-ACAGGTGTTAATATAGTGAAAAG-3' and downstream primer 5'-TACCTGAGTTCAGTTCCTGTC-3'. PCR conditions were as follows: denaturation at 94°C for 5 min, followed by 27 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 1 min, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min. PCR products were digested with *Hha*I (Gibco BRL, Rockville, MD) for 6 h at 37°C. The Ala54 allele resulted in two bands sized 99 and 81 bp, while the Thr54 allele resulted in one band sized 180 bp. To assess the reproducibility of genotype results, we repeated the assay on a subsample of 31 homozygous Ala54 genotypes, 18 heterozygous, and 20 homozygous Thr54 genotypes. We found 99% agreement between the first and second genotypes (κ = 0.98, 95% CI 0.95–1.00).

The frequency of each marker allele was compared between each patient group to control subjects using the χ^2 test. The two-sample *t* test was used to test for differences in continuous demographic characteristics between patients and control subjects, and the χ^2 test was used to test for differences in categorical demographic characteristics between patients and control subjects. Logistic regression was used to test for significant associations between genotypes and the presence of type 2 diabetes, severe obesity, and marked hyperinsulinemia. Because many control subjects were obese, a subset of nonobese control subjects with BMI <25 kg/m² was used as a separate comparison group. In addition to analyzing the IRS-1 and FABP2 gene variants under the codominant model, we chose to analyze the data for the IRS-1 Gly972Arg gene variant under the dominant model because there were only five individuals who were homozygous for the Arg972 allele, and grouping them with the heterozygotes increased our power to detect a difference between carriers and noncarriers of the Arg972 allele. The codominant model for the IRS-1 gene variant tested for trend across groups with 0, 1, or 2 IRS-1 Arg972 alleles, and the dominant model compared the Gly/Gly genotype to Gly/Arg and Arg/Arg genotypes. For the FABP2 Ala54Thr gene variant, we chose the recessive model based on results that showed that the greatest differences appeared to be for homozygotes carrying the Thr54 allele compared with other genotypes. The codominant model for the FABP2 gene variant tested for trend across groups with 0, 1, or 2 FABP2 Thr54 alleles, and the recessive model compared the Ala/Ala and Ala/Thr genotypes to the Thr/Thr genotype.

To examine the association between genotypes and continuous outcomes of interest such as BMI, weighted linear regression using data from all patients and control subjects was performed. Weights were assigned based on the ratio of the sampling fraction of the control group to the sampling fraction of the relevant patient group, i.e., weight assigned to control subjects = 1.0 and patients = 1.0. Gene-by-gene and gene-by-environment interactions were examined using stratification, multiple logistic regression, and analysis of variance. SAS (Version 6.12) was used for all statistical comparisons (32). Two-tailed *P* values <0.05 were considered to be statistically significant. There were no corrections for multiple comparisons.

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