*Brief Genetics Report*

# The Role of Insulin Receptor Substrate-1 Gene (*IRS1*) in Type 2 Diabetes in Pima Indians

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**The insulin receptor substrate-1 (IRS1) is a critical element in insulin-signaling pathways, and mutations in the *IRS1* gene have been reported to have a role in determining susceptibility to traits related to type 2 diabetes. In gene expression studies of tissue biopsies from nondiabetic Pima Indians, *IRS1* mRNA levels were reduced in adipocytes from obese subjects compared with lean subjects, and *IRS1* mRNA levels were also reduced in skeletal muscle from insulin-resistant subjects compared with insulin-sensitive subjects (all $P$ < 0.05). Based on these expression differences and the known physiologic role of *IRS1*, this gene was investigated as a candidate gene for susceptibility to type 2 diabetes in Pima Indians, a population with an extremely high incidence and prevalence of type 2 diabetes. Thirteen variants were identified, and among these variants, several were in complete linkage disequilibrium. Four genotypically unique variants were further genotyped in 937 DNA samples from full-heritage Pima Indians. Three of the variants were modestly associated with type 2 diabetes ($P$ < 0.05), one of which was additionally associated with 2-h plasma insulin and glucose as well as insulin action at physiologic and maximally stimulating insulin concentrations (all $P$ < 0.05). The association of variants in *IRS1* with type 2 diabetes and type 2 diabetes–related phenotypes and the differential expression of *IRS1* in adipocytes and skeletal muscle suggest a role of this gene in the pathogenesis of type 2 diabetes in Pima Indians. *Diabetes* 52: 3005–3009, 2003**

Insulin receptor substrate-1 (IRS1) is a substrate of the insulin receptor tyrosine kinase and appears to have a central role in the insulin-stimulated signal transduction pathway. Therefore, the *IRS1* gene has been studied extensively as a candidate gene for type 2 diabetes. Laakso et al. (1) identified three variations that predict amino acid substitutions: Gly81Arg, Ser892Gly, and Gly971Arg. Almind et al. (2) proposed that the Gly971Arg substitution could impair insulin-stimulated signaling and contribute to insulin resistance in normal and diabetic populations. The same group also showed that subjects with the 971Arg allele had significantly lower fasting plasma insulin with C-peptide levels, and Clausen et al. (3) reported that the combination of obesity and the 971Arg allele was associated with a 50% reduction in insulin sensitivity. The Gly971Arg, however, was not associated with type 2 diabetes in Mexican Americans (4), and the IRS1 locus on chromosome 2q36 has been excluded as a major diabetogenic locus in linkage analysis of candidate regions for insulin resistance in type 2 diabetes (5). Consistent with these human studies, *IRS1*$^{-/-}$ mice are mildly insulin resistant and hyperinsulinemic but do not develop diabetes (6).

The Pima Indians of Arizona have an extremely high prevalence of type 2 diabetes (7), and prospective studies have indicated that insulin resistance and insulin secretory dysfunction are major predictors of type 2 diabetes (8,9). To investigate genetic factors involved in the control of type 2 diabetes, gene expression profile studies in lean and obese, as well as insulin-sensitive and -resistant subjects, have been carried out. Quantitative PCR measurements of mRNA obtained from skeletal muscle biopsies of insulin-sensitive and -resistant nondiabetic Pima Indians identified IRS1 as being differentially expressed; *IRS1* mRNA levels were twofold lower in skeletal muscle from insulin-resistant subjects (0.403 ± 0.0831 vs. 0.8201 ± 0.1981 normalized to cyclophilin; $P$ = 0.04 by $t$ test). Similarly, microarray analysis in adipocytes from obese and lean nondiabetic Pima Indians showed that *IRS1* expression was ~1.8-fold lower in adipocytes from obese subjects (relative expression 2,868 ± 164 vs. 5,121 ± 485; $P$ = 0.0001 by $t$ test). To determine whether genetic variation in *IRS1* itself caused the lower level of its expression in muscle from insulin-resistant and adipocytes from obese Pima Indians, the coding region and 8 kb of the putative promoter of the *IRS1* gene were sequenced in DNA from 24 nonrelated full-heritage Pima Indians. Each common, genotypically unique variant was further analyzed in a large cohort of Pima Indians ($n$ = 937; 610 diabetic subjects, 327 nondiabetic subjects; 365 sibships) and tested for associations with type 2 diabetes and its metabolic predictors.

In contrast to studies in Caucasian populations, screen-

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IRS1, insulin receptor substrate-1; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

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ing of *IRS1* in Pima Indians identified no sequence variants that would predict an amino acid substitution. However, eight single nucleotide polymorphisms (SNPs) were identified in the putative promoter region, three SNPs and an insertion/deletion variant were identified in the 5′ untranslated region, and one silent substitution was found at codon 804 (GCG/GCA; both predicting Ala) (Fig. 1). The more common variants (minor allele frequency >10% in 24 Pima Indians) (Fig. 1) were genotyped in 90 additional subjects to assess the extent of linkage disequilibrium (LD). Based on genotypic concordance, many of these SNPs were in complete LD: the −7329C/G variant was in 100% LD with the −7120G/A, −6087T/C, −5601G/A, and −5486C/T variants. Similarly, the −2223C/T variant was in 100% LD with the −632 insertion/deletion. The −446G/A variant and the silent substitution at codon 804 were unique. We selected a single representative SNP from each of the four common LD groups for a subsequent association analysis. For this analysis, DNA from 937 full-heritage Pima Indians was genotyped for the −7120G/A, −2223C/T, −446G/A, and the Ala804 SNP, as representative SNPs for the LD groups I, II, III, and IV, respectively (Fig. 1).

The −7120G/A, −446G/A, and Ala 804 SNP, but not the −2223C/T SNP, were modestly associated with type 2 diabetes ($P < 0.05$) (Table 1) in the large cohort of Pima Indians. For the SNPs at −7120 and −446, the minor allele was associated with increased prevalence of diabetes, whereas for the Ala804, the major allele was associated with increased prevalence of diabetes. Among the four SNPs, seven common haplotypes were identified and analyzed for diabetes associations (Table 2). Only one haplotype (IV) showed a significant difference in frequency between diabetic and nondiabetic subjects. As might be predicted from the single SNP analysis, this haplotype consisted of the minor alleles for SNPs −7120 and −446 (A and A, respectively) and the major allele for Ala804 (G). The four SNPs were further analyzed for metabolic predictors of type 2 diabetes in 235 normal glucose tolerant subjects from this group who had undergone a hyperinsulinemic-euglycemic clamp study to assess insulin resistance. Only the Ala804 variant was associated with 2-h plasma glucose and insulin concentrations during the oral glucose tolerance test (OGTT) and insulin action at physiologic and maximally stimulating insulin concentrations (all $P < 0.05$) (Table 3). The Ala804 variant was also modestly associated with fasting plasma insulin concentrations during the OGTT ($P = 0.06$) (Table 3).

Bjornholm et al. (10) reported that muscle IRS1 protein expression was not reduced in type 2 diabetes; however, our expression data in skeletal muscle are supported by other studies reporting decreased levels of IRS1 protein in muscle from obese humans (11) and animal models manifesting insulin resistance (12–14). It is also consistent with previous studies of Carvalho et al. (15), who proposed that individuals with type 2 diabetes and individuals at risk for developing diabetes could be identified via reduced *IRS1* mRNA and protein in their fat cells. The same authors (15) also report that low *IRS1* expression in fat cells is related to insulin resistance but is not secondary to hyperglycemia, obesity, or hyperinsulinemia.

Since insulin resistance and obesity are significant risk factors for type 2 diabetes, our data suggest that impaired

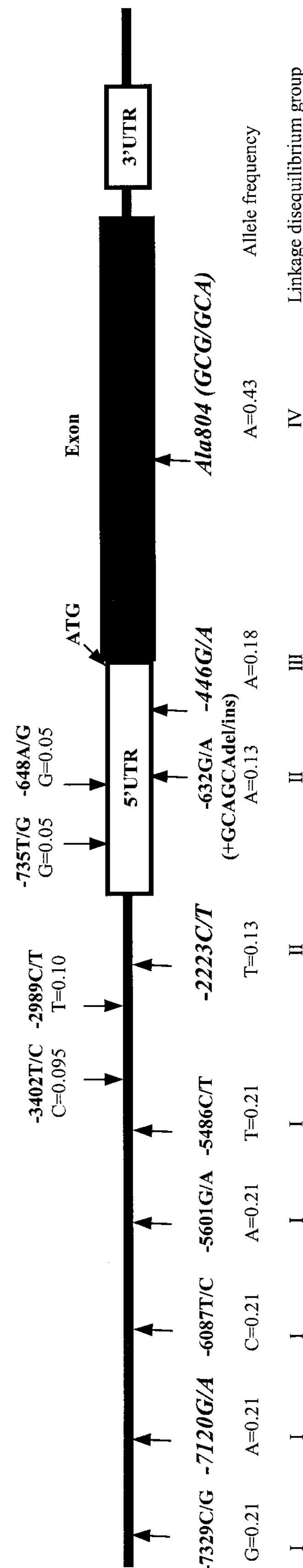


**FIG. 1. Genetic variants detected in the *IRS1* gene and flanking regions, their position, and frequency of minor allele. SNPs in italics were genotyped as the representative SNP for their LD group. Positions of variants are based on the genomic contig AC010735 (GenBank). D′ representing the degree of LD for genotyped SNPs was: 0.84 between −2223C/T and −7120G/A; 0.89 between −446G/A and −7120G/A; −0.66 between Ala804 and −7120G/A; 1.00 between −446G/A and −2223C/T; −1.00 between Ala804 and −2223C/T; and −1.00 between Ala804 and −446G/A. Allele frequencies for variants representative of LD groups were calculated from genotypes in 937 subjects; otherwise, 24 DNA samples were considered for estimating allele frequencies. D′ is expressed as a proportion of the maximum possible given the allele frequencies and the direction of association. D′ was calculated so that a positive value represents association among the more common alleles at each SNP, whereas a negative value represents association among the more common allele at one SNP and the less common allele at the other.**

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TABLE 1
Association of *IRS1* variants with type 2 diabetes in Pima Indians

| | Homozygote-common allele | Heterozygote | Homozygote minor allele | Odds ratio (95% CI) | *P* |
|---|---|---|---|---|---|
| −7120G/A | | | | | |
| Genotype | G/G | G/A | A/A | | |
| Diabetic | 326 (61) | 182 (34) | 30 (6) | | |
| Nondiabetic | 210 (66) | 96 (31) | 10 (3) | 0.70 (0.54–0.91) | 0.01 |
| −2223C/T | | | | | |
| Genotype | C/C | C/T | T/T | | |
| Diabetic | 413 (77) | 119 (22) | 7 (1) | | |
| Nondiabetic | 255 (79) | 63 (20) | 4 (1) | 0.81 (0.56–1.17) | 0.26 |
| −446G/A | | | | | |
| Genotype | G/G | G/A | A/A | | |
| Diabetic | 363 (65) | 182 (33) | 14 (2) | | |
| Nondiabetic | 237 (72) | 84 (26) | 6 (2) | 0.66 (0.48–0.90) | 0.01 |
| Ala804 (GCG/GCA) | | | | | |
| Genotype | G/G | G/A | A/A | | |
| Diabetic | 201 (34) | 299 (51) | 86 (15) | | |
| Nondiabetic | 91 (27) | 193 (56) | 59 (17) | 1.29 (1.02–1.63) | 0.04 |

Data are *n* (%). The odds ratio is given for a one-allele difference in the number of common alleles, adjusted for age and sex, and calculated by binomial generalized estimating equations that account for family membership; the *P* value is that for the null hypothesis of no difference in allele frequency between diabetic and nondiabetic individuals calculated by the Cochran-Armitage test for a linear trend in proportions.

transcription of *IRS1* might be involved in metabolic pathways ultimately leading to type 2 diabetes in the Pima population. In support of this, a variant within the *IRS1* gene was associated with in vivo insulin action and 2-h glucose and insulin levels as well as type 2 diabetes. However, it was not expected that the variant demonstrating the most consistent associations with insulin resistance and type 2 diabetes would be the silent Ala804 coding substitution. We believe that this silent substitution is unlikely to be the "causative" variant, but instead it is in relatively high LD with an unidentified causative variant in a distal regulatory site beyond the region that was sequenced. For example, mutations in a distal regulatory site located 45.6 kb from the previously characterized hepatocyte nuclear factor 4α (*Hnf4α*) promoter were recently shown to cosegregate with diabetes (16,17). This finding suggests that direct sequencing of all potential regulatory elements of a gene may, in some instances, be an enormous undertaking. In addition, LD can extend over large regions. Based on our preliminary analyses of pairwise LD between SNPs in Pima Indians, 5% of SNPs 1–2 Mb apart have D′ values >0.80, whereas 3% have D′ values >0.90. This makes it very difficult to rule out a more distal polymorphism as an explanation of an observed association. Consistent with our theory that the Ala804 genotype is not the actual causative variant, in 37 subjects the Ala804 genotype was not associated with *IRS1* mRNA levels measured in adipocytes or in skeletal muscle (data not shown), again suggesting that the Ala804 variant does not directly affect transcript levels. Moreover, our haplotype analysis showed that although the "G" allele at Ala804 is associated with higher prevalence of diabetes and diabetes-related traits in single SNP analyses, this is the only case when it is carried on the IV haplotype. This again leads us to speculate that Ala804 is not a causative variant, but instead is in high LD with a more distal functional variant(s). However, identification of distal variants and their functional analysis are necessary to prove this concept. In conclusion, we propose that genetically determined variation that decreases *IRS1* expression levels may increase susceptibility to type 2 diabetes in the Pima Indian population.

TABLE 2
Common haplotypes at *IRS1* and their association with type 2 diabetes in Pima Indians

| Haplotype | Composition | Frequency | Frequency (diabetes) | Frequency (nondiabetic) | Odds ratio (95% CI) | *P* |
|---|---|---|---|---|---|---|
| I | G_C_G_A | 0.39 | 0.38 | 0.42 | 0.78 (0.62–0.97) | 0.15 |
| II | G_C_G_G | 0.38 | 0.38 | 0.38 | 0.97 (0.78–1.20) | 1.00 |
| III | A_T_A_G | 0.10 | 0.10 | 0.10 | 1.00 (0.71–1.41) | 1.00 |
| IV | A_C_A_G | 0.05 | 0.06 | 0.03 | 2.44 (1.46–4.06) | <0.01 |
| V | A_C_G_A | 0.03 | 0.03 | 0.04 | 1.16 (0.65–2.07) | 0.99 |
| VI | A_C_G_G | 0.02 | 0.02 | 0.02 | 1.75 (0.79–3.92) | 0.67 |
| VII | G_T_A_G | 0.01 | 0.02 | 0.01 | 2.44 (1.04–5.71) | 0.22 |

A modification of the zero-recombinant haplotyping method was used to assess association between traits of interest and haplotypes of the four *IRS1* SNPs (23,24). Haplotypes are defined by the composition of alleles at each SNP in following order: −7120G/A_−2223C/T_−446G/A_Ala804. Odds ratios are calculated adjusted for age and sex for a difference of one in the number of the relevant haplotype using a binomial generalized estimating equation model that accounts for family membership; *P* values are adjusted for the multiple comparisons involved in analysis of 7 different haplotypes using the Bonferroni correction [corrected *P* value $= 1 - (1 - P)^6$].

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TABLE 3
Association of *IRS1* variants with diabetes-related traits in Pima Indians with normal glucose tolerance

| Genotype | −7120G/A | | | −2223C/T | | | −446G/A | | | Ala804 (GCG/GCA) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G/G | G/A + A/A | *P* | C/C | C/T + T/T | *P* | G/G | G/A + A/A | *P* | G/G | G/A | A/A | *P* |
| *n* | 143 | 76 | | 175 | 52 | | 164 | 66 | | 70 | 130 | 35 | |
| Age (years) | 27 ± 1 | 27 ± 1 | — | 27 ± 1 | 28 ± 1 | — | 27 ± 1 | 27 ± 1 | — | 27 ± 1 | 27 ± 1 | 26 ± 1 | — |
| Percent body fat | 32 ± 1 | 35 ± 1 | 0.97 | 32 ± 1 | 33 ± 1 | 0.96 | 33 ± 1 | 33 ± 1 | 0.73 | 34 ± 1 | 33 ± 1 | 30 ± 2 | 0.80 |
| Fasting plasma glucose (mg/dl) | 91 ± 1 | 92 ± 1 | 0.85 | 92 ± 1 | 92 ± 1 | 0.98 | 92 ± 1 | 92 ± 1 | 0.80 | 93 ± 1 | 91 ± 1 | 89 ± 1 | 0.09 |
| 2-h plasma glucose (mg/dl) | 125 ± 3 | 130 ± 3 | 0.54 | 127 ± 2 | 126 ± 4 | 0.74 | 126 ± 3 | 127 ± 3 | 0.99 | 133 ± 3 | 127 ± 3 | 109 ± 5 | 0.01 |
| $Log_{10}$ fasting plasma insulin (μU/ml) | 1.55 ± 0.02 | 1.59 ± 0.02 | 0.70 | 1.55 ± 0.02 | 1.58 ± 0.03 | 0.76 | 1.55 ± 0.02 | 1.57 ± 0.03 | 0.88 | 1.60 ± 0.03 | 1.56 ± 0.02 | 1.49 ± 0.06 | 0.06 |
| $Log_{10}$ 2-h plasma insulin (μU/ml) | 2.18 ± 0.03 | 2.23 ± 0.04 | 0.76 | 2.20 ± 0.03 | 2.21 ± 0.04 | 0.89 | 2.19 ± 0.03 | 2.21 ± 0.04 | 0.99 | 2.30 ± 0.04 | 2.17 ± 0.03 | 2.07 ± 0.08 | 0.03 |
| Basal glucose turnover (mg · kg $EMBS^{-1}$ · $min^{-1}$) | 1.93 ± 0.02 | 1.93 ± 0.03 | 0.78 | 1.92 ± 0.02 | 1.91 ± 0.03 | 0.86 | 1.92 ± 0.02 | 1.92 ± 0.03 | 0.79 | 1.93 ± 0.03 | 1.90 ± 0.02 | 1.93 ± 0.04 | 0.98 |
| Glucose disposal for low dose insulin clamp (mg · kg $EMBS^{-1}$ · $min^{-1}$) | 2.57 ± 0.08 | 2.53 ± 0.11 | 0.67 | 2.57 ± 0.07 | 2.52 ± 0.12 | 0.41 | 2.56 ± 0.07 | 2.58 ± 0.12 | 0.50 | 2.39 ± 0.09 | 2.59 ± 0.09 | 2.91 ± 0.20 | 0.04 |
| Glucose disposal for high dose insulin clamp (mg · kg $EMBS^{-1}$ · $min^{-1}$) | 8.62 ± 0.18 | 8.52 ± 0.22 | 0.94 | 8.64 ± 0.17 | 8.44 ± 0.28 | 0.80 | 8.57 ± 0.16 | 8.69 ± 0.27 | 0.55 | 8.00 ± 0.21 | 8.76 ± 0.19 | 9.04 ± 0.40 | 0.04 |

Data are given as means ± SE. *P* values were calculated after adjusting for age, sex, and nuclear family membership for the variable percent body fat; and age, sex, percent body fat, and nuclear family membership for the variables 2-h plasma glucose, 2-h plasma insulin, and $log_{10}$ glucose disposal for the low- and high-dose insulin clamps. EMBS, estimated metabolic body size; due to the low frequency of the rare alleles for −7120G/A, −2223C/T and −446G/A variants, for statistical analysis the homozygotes for each rare allele were combined with the heterozygotes; therefore, only a dominant effect on risk has been tested for the rare alleles.

## RESEARCH DESIGN AND METHODS

**Subjects.** The 937 subjects are participants of our ongoing longitudinal study of the etiology of type 2 diabetes among the Gila River Indian Community in Arizona (18). To determine diabetes status, a 75-g OGTT is given and the results are interpreted according to World Health Organization criteria (19). For detailed metabolic testing, nondiabetic individuals are admitted to our clinical research ward for 7–10 days, and only people found to be healthy by medical history, physical examination, and routine laboratory tests, and who were not taking medications, were studied. Oral glucose tolerance measurements and acute insulin response calculations are described in detail elsewhere (20). Insulin-stimulated glucose disposal (uptake) rates and basal glucose output are determined using the hyperinsulinemic-euglycemic clamp technique (21). Body composition was estimated by underwater weighing until January 1996 and thereafter by dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI). A conversion equation derived from comparative analyses was used to make estimates of body composition equivalent between methods (22). All studies were approved by the tribal council and the Institutional Review Board of the National Institutes of Diabetes and Digestive and Kidney Diseases.

**Sequencing of the IRS1 and genotyping of SNPs.** The exon, intron/exon boundaries, 5′ and 3′ untranslated regions, and 8 kb of the putative promoter region were sequenced in DNA samples from 24 (13 diabetic and 11 nondiabetic subjects) non–first-degree–related Pima Indians. Sequencing was performed using Big Dye Terminator (Applied Biosystems, Foster City, CA) on an automated DNA capillary sequencer (3700; Applied Biosystems). Sequence information for oligonucleotide primers is available upon request. Genotyping of selected SNPs in 937 DNA samples was done using the TaqMan assay (Applied Biosystems). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 9700 (95°C for 10 min, 95°C for 15 s, and 62°C for 1 min, for 38 cycles) and fluorescence was detected on an ABI PRISM 7700 sequence detector (Applied Biosystems).

**Muscle biopsy and RNA extraction.** Percutaneous skeletal muscle biopsies of the vastus lateralis muscle were performed in 12 nondiabetic, insulin-resistant and 13 insulin-sensitive Pima Indians (matched for age, sex, and percent body fat) in the morning after a 12-h overnight fast using Bergstrom needles (Depuy, Raynham, MA) under local anesthesia with 1% lidocaine. The biopsy was cleaned of any visible fat, rinsed in sterile 0.9% NaCl solution, frozen in liquid nitrogen, and stored at −70°C. Total RNA was isolated from the frozen muscle tissues homogenized in Trizol Reagent (Life Technologies, Gaithersburg, MD), and mRNA was subsequently isolated using oligo-dT latex beads (Qiagen, Santa Clarita, CA).

**Adipocyte isolation.** Adipose tissue was digested in Hank's balanced buffer solution containing 5.5 mmol/l glucose, 5% fatty acid–free BSA (Introgen, Houston, TX), and 3.3 mg/ml type I collagenase (Worthington Biochemical, Lakewood, NJ) for 30 min in a 37°C water bath. The digestion mixture was passed through a sterile 230-mm stainless steel tissue sieve (Thermo EC, Holbrook, NY), and the adipocytes were allowed to float by gravity. The

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supernatant containing adipocytes was collected, and total RNA was extracted using RNeasy Mini Kit from Qiagen (Valencia, CA).

**Microarray analysis.** GeneChip arrays (HG-U95 set; Affymetrix, Santa Clara, CA) containing ~12,000 genes and ~50,000 expressed sequence tags were used for global differential gene expression profiling in adipocytes isolated from lean ($n = 20$) and obese ($n = 19$) nondiabetic Pima Indians matched for age and sex.

**Quantitative RT-PCR.** Verification of transcript quantity of *IRS1* was performed using TaqMan Real Time PCR on cDNA synthesized from total RNA. The primer pairs and probe are available upon request. The quantification was performed using the standard protocol of ABI PRISM 7700 (Applied Biosystems). Each sample was run in duplicate, and the mean value of the duplicate was used to calculate transcript level. The transcript quantity in each cDNA sample was normalized to that of cyclophilin using the TaqMan Pre-Developed Assay Reagent for human endogenous control subjects (Applied Biosystems).

**Statistical analyses.** Statistical analyses were performed using the analysis system of the SAS Institute (Cary, NC). For continuous variables, the general estimating equation procedure was used to adjust for the covariates, including family membership, because some subjects were siblings. Plasma insulin concentrations and rates of glucose disappearance during the low-dose insulin infusion were log transformed before analyses to approximate a normal distribution.

## REFERENCES


1. Laakso M, Malkki M, Kekalainen P, Kuusisto J, Deeb SS: Insulin receptor substrate-1 variants in non-insulin-dependent diabetes. *J Clin Invest* 94:1141–1146, 1994
2. Almind K, Inoue G, Pedersen O, Kahn CR: A common amino acid polymorphism in insulin receptor substrate-1 causes impaired insulin signaling: evidence from transfection studies. *J Clin Invest* 97:2569–2575, 1996
3. Clausen JO, Hansen T, Bjorbaek C, Echwald SM, Urhammer SA, Rasmussen S, Andersen CB, Hansen L, Almind K, Winther K, et al: Insulin resistance: interactions between obesity and a common variant of insulin receptor substrate-1. *Lancet* 346:397–402, 1995
4. Celi FS, Negri C, Tanner K, Raben N, De Pablo F, Rovira A, Pallardo LF, Martin-Vaquero P, Stern MP, Mitchell BD, Shuldiner AR: Molecular scanning for mutations in the insulin receptor substrate-1 (IRS-1) gene in Mexican Americans with type 2 diabetes mellitus. *Diabete Metab Res Rev* 16:370–377, 2000
5. Elbein SC, Chiu KC, Hoffman MD, Mayorga RA, Bragg KL, Leppert MF: Linkage analysis of 19 candidate regions for insulin resistance in familial NIDDM. *Diabetes* 44:1259–1265, 1995
6. Araki E, Lipes MA, Patti ME, Bruning JC, Haag B 3rd, Johnson RS, Kahn CR: Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 372:186–190, 1994
7. Knowler WC, Bennett PH, Hamman RF, Miller M: Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. *Am J Epidemiology* 108:497–505, 1978
8. Bogardus C, Lillioja S, Howard BV, Reaven G, Mott D: Relationships between insulin secretion, insulin action, and fasting plasma glucose concentration in non-diabetic and noninsulin-dependent diabetic subjects. *J Clin Invest* 74:1238–1246, 1984
9. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 329:1988–1992, 1993
10. Bjornholm M, Kawano Y, Lehtihet M, Zierath JR: Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation. *Diabetes* 46:524–527, 1997
11. Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ, Dohm GL: Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *J Clin Invest* 95:2195–2204, 1995
12. Kerouz NJ, Horsch D, Pons S, Kahn CR: Differential regulation of insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. *J Clin Invest* 100:3164–3172, 1997
13. Anai M, Funaki M, Ogihara T, Terasaki J, Inukai K, Katagiri H, Fukushima Y, Yazaki Y, Kikuchi M, Oka Y, Asano T: Altered expression levels and impaired steps in the pathway to phosphatidylinositol 3-kinase activation via insulin receptor substrates 1 and 2 in Zucker fatty rats. *Diabetes* 47:13–23, 1998
14. Friedman JE, Ishizuka T, Liu S, Farrell CJ, Bedol D, Koletsky RJ, Kaung HL, Ernsberger P: Reduced insulin receptor signaling in the obese spontaneously hypertensive Koletsky rat. *Am J Physiol* 273:E1014–E1023, 1997
15. Carvalho E, Jansson PA, Axelsen M, Eriksson JW, Huang X, Groop L, Rondinone C, Sjostrom L, Smith U: Low cellular IRS 1 gene and protein expression predict insulin resistance and NIDDM. *FASEB J* 13:2173–2178, 1999
16. Hansen SK, Parrizas M, Jensen ML, Pruhova S, Ek J, Boj SF, Johansen A, Maestro MA, Rivera F, Eiberg H, Andel M, Lebl J, Pedersen O, Ferrer J, Hansen T: Genetic evidence that HNF-1alpha-dependent transcriptional control of HNF-4alpha is essential for human pancreatic beta cell function. *J Clin Invest* 110:827–833, 2002
17. Thomas H, Jaschkowitz K, Bulman M, Frayling TM, Mitchell SM, Roosen S, Lingott-Frieg A, Tack CJ, Ellard S, Ryffel GU, Hattersley AT: A distant upstream promoter of the HNF-4alpha gene connects the transcription factors involved in maturity-onset diabetes of the young. *Hum Mol Genet* 10:2089–2097, 2001
18. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns D, Almasy L, Blangero J, Garvey WT, Bennett PH, Bogardus C, Knowler WC: An autosomal genomic scan for loci linked to type 2 diabetes mellitus and body mass index in Pima Indians. *Am J Hum Genet* 63:1130–1138, 1998
19. World Health Organization: *WHO Expert Committee on Diabetes Mellitus. Second Report.* Geneva, World Health Org., 1980 (Tech. Rep. Ser., no. 646)
20. Baier LJ, Permana PA, Yang X, Pratley RE, Hanson RL, Shen GQ, Mott D, Knowler WC, Cox NJ, Horikawa Y, Oda N, Bell GI, Bogardus C: A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. *J Clin Invest* 106:R69–R73, 2000
21. Lillioja S, Mott DM, Zawadzki JK, Young AA, Abbott WG, Knowler WC, Bennett PH, Moll P, Bogardus C: In vivo insulin action is familial characteristic in nondiabetic Pima Indians. *Diabetes* 36:1329–1335, 1987
22. Norman RA, Tataranni PA, Pratley R, Thompson DB, Hanson RL, Prochazka M, Baier L, Ehm MG, Sakul H, Foroud T, Garvey WT, Burns D, Knowler WC, Bennett PH, Bogardus C, Ravussin E: Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. *Am J Hum Genet* 62:659–668, 1998
23. O'Connell JR: Zero-recombinant haplotyping: applications to fine mapping using SNPs. *Genet Epidemiol* 19 (Suppl. 1):S64–S70, 2000
24. Wolford JK, Gruber JD, Ossowski VM, Vozarova B, Antonio Tataranni P, Bogardus C, Hanson RL: A C-reactive protein promoter polymorphism is associated with type 2 diabetes mellitus in Pima Indians. *Mol Genet Metab* 78:136–144, 2003


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