Original Article

Variants in *ARHGEF11*, a Candidate Gene for the Linkage to Type 2 Diabetes on Chromosome 1q, Are Nominally Associated With Insulin Resistance and Type 2 Diabetes in Pima Indians

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A prior genome-wide linkage scan in Pima Indians indicated a young-onset (aged <45 years) type 2 diabetes susceptibility locus on chromosome 1q21-q23. *ARHGEF11*, which encodes the Rho guanine nucleotide exchange factor 11, was analyzed as a positional candidate gene for this linkage because this protein may stimulate Rho-dependent signals, such as the insulin signaling cascade. The *ARHGEF11* gene, and two adjacent genes *NTRK1* and *INSRR*, were sequenced in 24 Pima Indians who were not first-degree relatives. Sequencing of the coding regions, 5′ and 3′ untranslated regions and putative promoter regions of these genes, identified 28 variants in *ARHGEF11*, 11 variants in *NTRK1*, and 8 variants in *INSSR*. These 47 variants, as well as 84 additional public database variants within/between these genes, were genotyped for association analysis in the same group of Pima Indians who had participated in the linkage study (n = 1,228). An R1467H in *ARHGEF11*, and several additional noncoding variants that were in high linkage disequilibrium with this variant, were nominally associated with young-onset type 2 diabetes (P = 0.01; odds ratio 3.39) after adjusting for sex, family membership, and Pima heritage. The risk allele H had a frequency of 0.10. In a subgroup of 262 nondiabetic, full-heritage Pima Indians who had undergone detailed metabolic testing, the risk allele H also was associated with a lower mean insulin-mediated glucose disposal rate and a lower mean nonoxidative glucose storage rate after adjusting for age, sex, nuclear family membership, and percentage of body fat ($P \leq 0.01$). These findings suggest that variation within *ARHGEF11* nominally increases risk of type 2 diabetes, possibly as a result of increased insulin resistance. *Diabetes* 56:1454–1459, 2007

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IRS, insulin receptor substrate; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

The Pima Indians of Arizona have an extremely high prevalence of type 2 diabetes (1). Their diabetes is characterized by obesity, dysfunction of insulin secretion, insulin resistance (decreased insulin-mediated glucose disposal), and increased rates of postabsorptive endogenous glucose output (2). Studies (3–5) have shown that type 2 diabetes, insulin resistance, the acute insulin response, and obesity are highly heritable in this population.

A prior genome-wide linkage scan in Pima Indians indicated a susceptibility locus for young-onset (onset age <45 years) type 2 diabetes on chromosome 1q21-q23 at D1S1677 (6). Linkage to type 2 diabetes on chromosome 1q21–23 has subsequently been observed in seven other populations who have formed the International Type 2 Diabetes 1q Consortium (7–14). Within this region of linkage, there is a cluster of three genes (*ARHGEF11*, *NTRK1*, and *INSRR*) located between 153 and 154 Mb, which encode proteins that have putative roles in the insulin signaling system. *ARHGEF11* encodes the Rho guanine nucleotide exchange factor ARHGEF11 (also called PDZ-RhoGEF and KIAA0380). ARHGEF11 interacts with small GTPases (G-protein, guanine nucleotide-binding proteins), such as Rho, that function as molecular switches in signaling pathways that include the insulin signaling cascade (15,16). *NTRK1* encodes the neurotrophic tyrosine kinase receptor type 1, which recruits insulin receptor substrate (IRS)-1 and IRS-2 (17), and *INSRR* encodes the insulin receptor–related receptor, which potentially phosphorylates IRS-1 and IRS-2 (available at http://genecards.bcgsc.bc.ca/cgi-bin/carddisp?insrr). In this study, *ARHGEF11* and the two adjacent genes, *NTRK1* and *INSRR*, were analyzed as positional candidate genes for type 2 diabetes in the Pima Indians.

RESEARCH DESIGN AND METHODS

The subjects who were sequenced (n = 24) and genotyped (n = 1,228) were participants in our prior genome-wide linkage study for diabetes susceptibility loci in Pima Indians (6) and are part of our ongoing longitudinal study of the etiology of type 2 diabetes among the Gila River Indian Community in central Arizona (1). All individuals in the longitudinal study are invited to participate in a standardized health examination biannually. To determine diabetes

status, a 75-g orally administered glucose tolerance test is given and the results are interpreted according to the criteria of the World Health Organization (18). All studies were approved by the tribal council of the Gila River Indian Council and the institutional review board of the National Institutes of Diabetes and Digestive and Kidney Diseases.

Among 1,228 subjects that were genotyped, 262 of the nondiabetic subjects had additionally been studied for measures of pre-diabetic phenotypes in our clinical research center. Only individuals (aged 18–45 years) who are confirmed to be healthy by medical history, physical examination, and routine laboratory tests and are not taking medications are studied. Oral glucose tolerance is measured after 2–3 days on a weight-maintaining diet of mixed composition. Blood for plasma glucose and insulin measurements is drawn before ingesting 75 g of glucose and at 30, 60, 120, and 180 min thereafter. Subjects also receive a 25-g intravenous injection of glucose over 3 min to measure the acute insulin response. Blood samples were collected before infusion and at 3, 4, 5, 6, 8, and 10 min after infusion for determination of plasma glucose and insulin concentrations. The acute insulin response was calculated as the mean increment in plasma insulin concentrations from 3 to 5 min (19). The hyperinsulinemic-euglycemic clamp technique was used to determine basal glucose appearance and insulin-stimulated glucose disappearance (uptake) rates (19). Briefly, insulin was infused to achieve physiologic plasma insulin concentrations (137 ± 3 μU/ml) for 100 min. Plasma glucose concentrations were held constant at 100 mg/dl by a variable 20% glucose infusion. Tritiated glucose was infused for 2 h before the insulin infusion to calculate glucose disappearance rates during the insulin infusion. During the last 40 min of the insulin infusion, the rate of insulin-stimulated glucose disposal was calculated, adjusted for steady-state plasma glucose and insulin concentration, and normalized to estimated metabolic body size (fat-free mass plus 17.7 kg) as described (19,20). Ventilated-hood indirect calorimetry was used to estimate rates of glucose and lipid oxidation before and during the insulin infusions (20). Glucose and lipid oxidation rates were normalized to estimated metabolic body size (fat-free mass plus 17.7) as described (19,20). Body composition was estimated by underwater weighing until January 1996 and currently is measured by dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI). A conversion equation derived from comparative analyses is used to make estimates of body composition comparable between methods (21).

Single nucleotide polymorphism identification and genotyping. To identify sequence variants, all exons, exon-intron boundaries, 5′ and 3′ untranslated regions, and 2 kb of the putative promoter regions of *ARHGEF11*, *NTRK1*, and *INSRR* were PCR amplified and sequenced in DNA samples from 24 non–first-degree–related Pima Indians. Among 24 subjects, 12 developed diabetes before the age of 25 years and 12 were nondiabetic and were at least 45 years of age. Sequencing was performed using the Big Dye Terminator (Applied Biosystems) on an automated DNA capillary sequencer (model 3730xl; Applied Biosystems). Single nucleotide polymorphisms (SNPs) identified by sequencing were genotyped in DNA from 1,228 subjects using the TaqMan Allelic Discrimination Assay (Applied Biosystems), direct sequencing (as described above; Applied Biosystems), or SNPplex (Applied Biosystems) following the manufacture's protocol. Sequence information for all oligonucleotide primers and probes is available from the authors upon request.

Statistical analysis. For the association analysis with young-onset type 2 diabetes, only siblings who were confirmed to have developed type 2 diabetes before the age of 45 years ($n = 525$) or confirmed to be nondiabetic and at least 45 years of age ($n = 88$) were analyzed. Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Numeric variables are expressed as means ± SE. The association of genotypes with diabetes was assessed by logistic regression analysis. For continuous variables, linear regression analysis was used. Both linear and logistic models were fit with the general estimating equation procedure because some subjects were siblings. This procedure accounts for family membership by modeling the phenotypic variance/covariance matrix among siblings. For the present analyses, an exchangeable correlation matrix was assumed and the "empirical" SE, which is robust to the assumed correlation structure, was used.

Plasma insulin concentrations and glucose disposal rate during the physiological plasma concentration were log transformed before analyses to approximate a normal distribution. For analysis under an additive model, homozygotes for the major allele (1/1) and heterozygotes (1/2) and homozygotes for the minor allele (2/2) were coded to a continuous numeric variable for genotype (as 0, 1, and 2). The recessive model was defined as contrasting genotypic groups 1/1 vs. 1/2 + 2/2, where allele 1 is defined as the major allele. Although the general estimating equation approach accounts for the correlation among family members, it does not provide a specific within-family test of association and, thus, still is potentially confounded by population stratification. Therefore, data also were analyzed using a modification of the method of Abecasis et al. (22) to test for within-family association. In brief, this method partitions the association into between- and within-family components, represented, respectively, by the sibship mean of the continuous numeric variable for genotype and each individual's deviation from this mean. The test of the significance of the within-family components is a test of cotransmission among siblings, which is robust to population stratification (although it is less powerful than the more general test). Analyses of all traits were adjusted for potentially confounding variables (e.g., age, sex, percentage of body fat); all adjustments were specified a priori based on previous studies of the determinants of these traits. To examine pairwise linkage disequilibrium (LD), haplotype frequencies were estimated with the estimating haplotype (EH) program (Xie and Ott) (available at http://linkage.rockefeller.edu/ott/), and these haplotype frequencies were used to calculate D′ and Δ (2). *P* values of <0.05 were considered to be of nominal statistical significance.

RESULTS

Sequencing and genotyping of ARHGEF11. Sequencing of the 41 exons, exon-intron boundary regions, and 2 kb of the 5′ (putative promoter) region of *ARHGEF11* in 24 non–first-degree–related Pima Indians identified 28 variants, 3 of which were nonsynonymous amino acid substitutions (R293Q, S1456G, and R1467H). These 28 variants, and 66 additional database SNPs positioned outside of the regions that were sequenced, were genotyped for association analysis in the same Pima Indian subjects who were used for our prior type 2 diabetes linkage study (online appendix Table 1 [available at http://dx.doi.org/10.2337/db06-0640]). Three coding variants in *ARHGEF11* were nominally associated with young-onset type 2 diabetes. The H allele (frequency = 0.10) of R1467H was associated with young-onset type 2 diabetes ($P = 0.01$; odds ratio 3.4 [95% CI 1.29–8.93], recessive model) after adjusting for sex, family membership, and Pima heritage (Fig. 1; Table 1). The G allele (frequency = 0.26) of S1456G also was associated with young-onset type 2 diabetes ($P = 0.005$, 1.85 [1.20–2.85], additive model; $P = 0.012$, 1.93 [1.16–3.22], recessive model) after adjusting for sex, family membership, and Pima heritage (Fig. 1; Table 1). The Q allele of R293Q was extremely rare (frequency = 0.002), and although the association data for this variant are included in Table 1, these data may not be reliable due to the limited sample power. Thirteen noncoding SNPs also were nominally associated with young-onset type 2 diabetes (listed in Table 1 legend). All of these noncoding SNPs, with the exception of rs3753213 (shown in Table 1), were in high LD ($D' = 0.99$, $r^2 = 0.99$) with either R1467H or S1456G (online appendix Fig. 3).

We further assessed whether the R1467H and S1456G were associated with metabolic traits predictive of type 2 diabetes in Pima Indians who had not yet developed diabetes. In a subgroup of 262 nondiabetic, full-heritage Pima Indians who have undergone detailed metabolic testing, including measurements of body composition, oral glucose tolerance, insulin secretory function, insulin-stimulated glucose uptake, and indirect calorimetry, the risk allele (H) for R1467H was nominally associated with a lower mean glucose disposal rate ($P = 0.005$) and a lower mean nonoxidative glucose storage rate ($P = 0.01$) during a euglycemic-hyperinsulinemic clamp under physiologic concentrations of plasma insulin infusion, after adjusting for age, sex, nuclear family membership, and percentage of body fat (Table 2; Fig. 2). The R1467H was not associated with insulin secretion as assessed by the acute insulin response to a 25-g intravenous bolus injection of glucose or the insulin level at 30 min during an oral glucose tolerance test (Table 2). No association was found between S1456G and metabolic predictors of type 2 diabetes

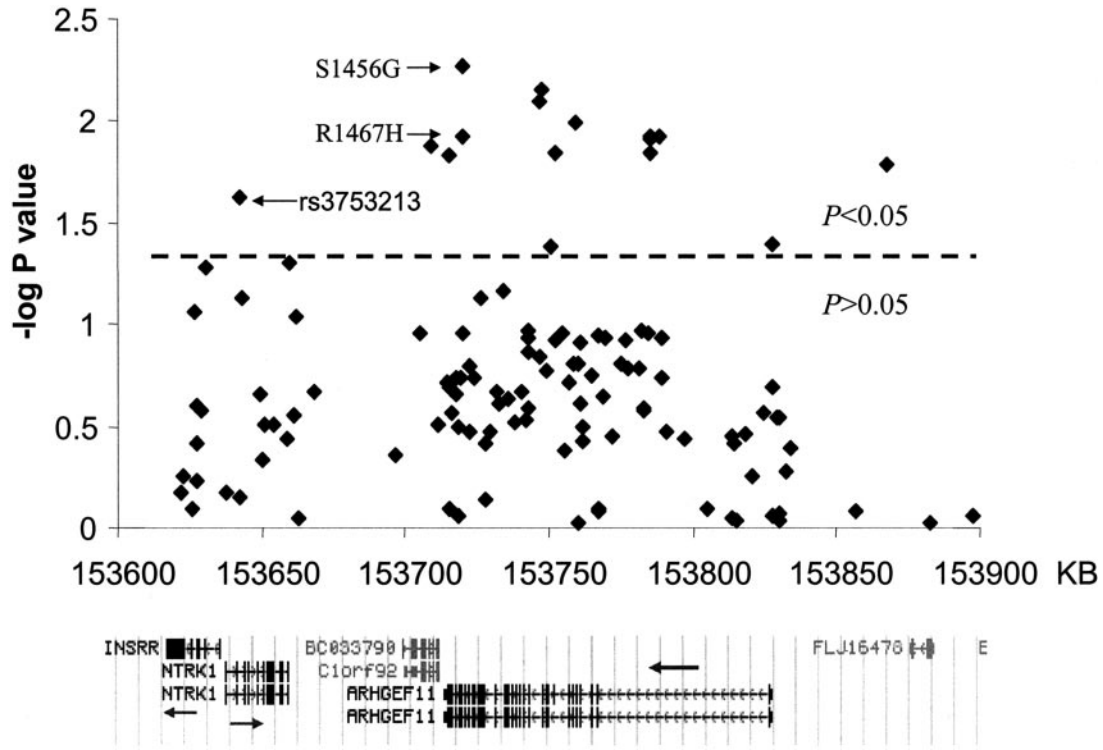

FIG. 1. Association plot of the −log *P* values (additive model) for SNPs with young-onset type 2 diabetes versus position along a 300-kb region of chromosome 1. The relative positions of exons for *INSRR*, *NTRK1*, and *ARHGEF11* within this region are shown beneath the plot.

in nondiabetic, full-heritage Pima subjects (data not shown).

Sequencing and genotyping of NTRK1 and INSRR. Direct sequencing of all exons, exon-intron boundary regions of *NTRK1* and *INSRR*, and 2.5 kb of the common 5′ flanking regulatory region between these two genes in 24 non–first-degree–related Pima Indians identified 19 variants (11 in *NTRK1* and 8 in *INSRR*; online appendix Table 1). Four of these variants predicted nonsynonymous amino acid changes (Phe316Leu, Arg869Leu, Pro928Leu, and Arg999STOP) located in *INSRR*. None of these coding variants were detected when *INSRR* previously was screened in Pima Indians using the technique of denaturing high-performance liquid chromatography on pooled DNA samples (23). The 19 variants, and 18 additional database SNPs positioned outside of the regions that were sequenced, were genotyped for association analysis in the same Pima Indian subjects who were used for the type 2 diabetes linkage study. None of the amino acid changes were associated with young-onset type 2 diabetes or metabolic predictors of type 2 diabetes in Pima Indians. Only SNP rs3753213, located in the shared common 5′ flanking regulatory region (2.5 kb) between *INSRR* and *NTRK1*, showed a nominal association with young-onset

TABLE 1
Representative* SNPs associated with young-onset type 2 diabetes in Pima Indians

SNP	SNP (1/2)	Gene locus	Minor allele frequency	Type 2 diabetes by genotype (*n*)†		Nondiabetic by genotype (*n*)‡		Association in general (upper) and within-family (lower, italics) models	
				11	12 + 22	11	12 + 22	*P* value	Odds ratio
rs3753213	**C**/T	INSRR/NTRK1 Intergenic	0.34	233	273	28	59	0.04 *0.18*	0.52 (0.27–0.96) *0.55 (0.23–1.32)*
R1467H	G/**A**	ARHGEF11 Exon 39	0.10	422	102	82	6	0.01§ *0.03*§	3.39 (1.29–8.93) *5.09 (1.20–21.55)*
S1456G	A/**G**	ARHGEF11 Exon 39	0.26	256	253	59	29	0.01 *0.24*	1.93 (1.16–3.22) *1.54 (0.76–3.18)*
R293Q	**G**/A	ARHGEF11 EXON 11	0.002	523	2	86	2	0.04 *NA*	0.18 (0.03–0.93) *NA*

SNP alleles are given as 1 = major and 2 = minor. The risk allele is given in bold. Due to the low frequencies of these SNPs, only the recessive analytical model is given, which is defined as comparing genotype groups 1/1 vs. 1/2 + 2/2. *P* values/odds ratios were adjusted for sex, family membership, and Pima heritage. Odds ratios were calculated by defining allele 2 as the defaulted risk factor. *R1467H is in nearly perfect LD (D′ = 0.99, r^2 = 0.99) with rs865239, rs822430, rs1336149, rs1572407, rs6664744, rs822577, rs822578, rs822579, hcv27852229, and rs4661014. S1456G is in nearly perfect LD with rs1336150 and rs4661077. †Subjects with type 2 diabetes diagnosed before the age of 45 years. ‡Subjects determined to be nondiabetic by oral glucose tolerance test after the age of 45 years. §The empirical *P* values obtained with 5,000 permutations were 0.011 for the general association test and *P* = 0.038 for the within-family test, respectively, which were very similar to the nominal values.

TABLE 2
Mean values for insulin action and insulin secretion among nondiabetic, full-heritage Pima Indians grouped by *ARHGEF11* R1467H genotypes

	R1467R	R1467H	*P* value*
n (male/female)	208 (121/87)	54 (35/19)	0.44
Age (years)	27 ± 0.4	26 ± 0.8	—
Percentage of body fat	33 ± 0.6	31 ± 1.3	0.08
Glucose disposal rate (mg · min^{-1} · kg EMBS^{-1})	3.61 ± 0.08	3.47 ± 0.14	*0.005*
Nonoxidative glucose storage (mg · min^{-1} · kg EMBS^{-1})	1.60 ± 0.07	1.47 ± 0.11	*0.01*
Glucose oxidation (mg · min^{-1} · kg EMBS^{-1})	2.02 ± 0.04	2.01 ± 0.07	0.42
Oral glucose tolerance test			
Fasting glucose (mg/dl)	91 ± 0.6	92 ± 1.3	0.62
2-h glucose (mg/dl)	128 ± 2.1	125 ± 4.2	0.95
$\log_{10}$ [fasting insulin (mIU/ml)]	1.56 ± 0.02	1.55 ± 0.03	0.20
$\log_{10}$ [2-h insulin (mIU/ml)]	2.21 ± 0.02	2.20 ± 0.05	0.40
$\log_{10}$ [acute insulin response (mIu/ml)] (n = 138 normal glucose tolerance)	2.32 ± 0.02	2.35 ± 0.04	0.69
$\log_{10}$ [insulin 30′ during oral glucose tolerance test (mIU/ml)] (n = 138 normal glucose tolerance)	2.33 ± 0.02	2.33 ± 0.05	0.99

Data are means ± SE. Among this subgroup there were no subjects homozygous for H1467H. Glucose disposal rate is measured as insulin-stimulated glucose uptake during a hyperinsulinemic-euglycemic clamp at the physiologic plasma insulin concentrations. Estimated metabolic body size (EMBS) equals fat-free mass plus 17.7 kg. *P value for sex distribution between two groups was calculated by χ^2. P value for percentage of body fat was assessed using the general estimating equation procedure after adjusting for age, sex, and family membership. P values for glucose disposal rate, oral glucose tolerance glucose, and insulin levels were obtained using the general estimating equation procedure after adjusting for age, sex, family membership, and percentage of body fat. P values for log-transformed acute insulin response was assessed in 138 normal glucose-tolerant full-heritage Pima subjects after adjusting for age, sex, percentage of body fat, family membership, and insulin action. P values for log-transformed insulin level at 30 min during an oral glucose tolerance test were obtained after adjusting for age, sex, percentage of body fat, family membership, insulin action, and glucose level at 30 min during oral glucose tolerance tests.

type 2 diabetes (P = 0.04; Table 1; Fig. 1), but this variant was not associated with any metabolic predictors of type 2 diabetes in nondiabetic, full-heritage Pima Indians (data not shown). The variants detected in *INSRR* and *NTRK1* are in a distinct LD block compared with variants in *ARHGEF11* in Pima Indians (online appendix Fig. 3).

Haplotype analyses for ARHGEF11 and INSRR. To detect an effect of multiple, potentially functional SNPs on the development of type 2 diabetes, haplotype analyses were done by combining the Arg1467His and Ser1456Gly in *ARHGEF11* and the Arg999STOP and Pro928Leu in *INSRR*. However, neither haplotype analysis provided additional information to the single SNP analyses (Table 3). The Arg193Gln in *ARGEF11* and the Phe316Leu and Arg869Leu in *INSRR* were not included in the haplotypes due to their extremely rare allele frequencies.

DISCUSSION

Our data suggest that the H allele at R1467H in *ARHGEF11* (or a variant that is carried on the H allele in Pima Indians) increases risk of type 2 diabetes by reducing insulin-stimulated glucose uptake. Among the nondiabetic, full-heritage Pima Indians, subjects with an H allele (homozygotes and heterozygotes) had a lower mean glucose disposal rate during a hyperinsulinemic-euglycemic clamp after adjusting for age, sex, nuclear family membership, and percentage of body fat. This lower glucose disposal rate mainly was attributed to reduced nonoxidative glucose storage because the other component of glucose disposal rate, glucose oxidation, was similar between subjects with an H allele and subjects who were homozygous for the R allele. These findings indicate that *ARHGEF11* may alter insulin-mediated glucose storage pathways, such as glycogen synthesis in the muscle and liver. *ARHGEF11* is ubiquitously expressed and is present in both liver and muscle, which are important organs for

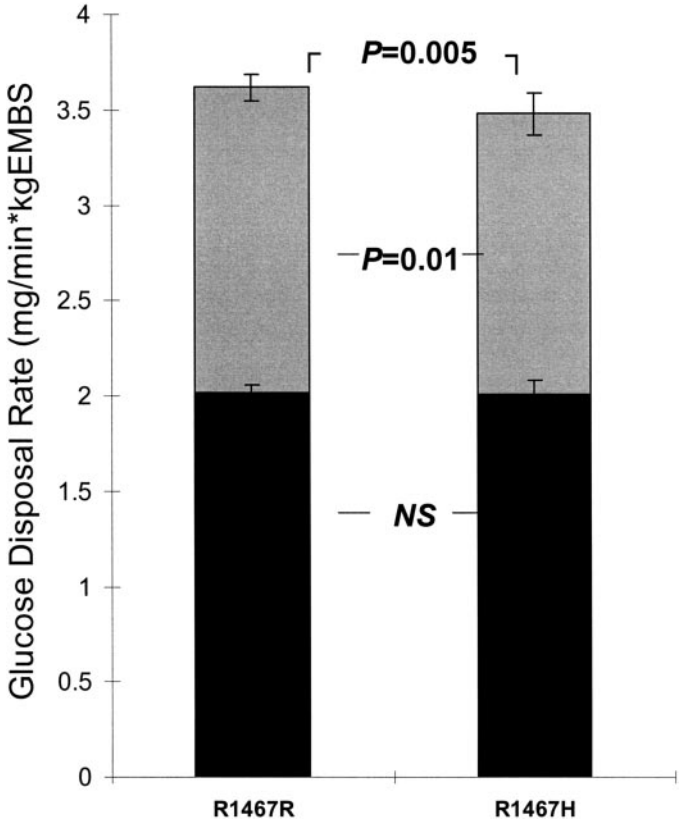

FIG. 2. Comparison of glucose disposal rates in nondiabetic, full-heritage Pima Indians grouped by R1467H genotypes. Insulin-mediated glucose disposal rate is measured during a euglycemic-hyperinsulinemic clamp during infusion of physiological concentrations of plasma insulin and is composed of nonoxidative glucose storage and glucose oxidation. ■, glucose oxidation; ▨, nonoxidative glucose storage.

TABLE 3
Haplotype analyses for AHRGEF11 (Arg1467His and Ser1456Gly) and INSRR (Arg999STOP and Pro928Leu)

Haplotype	Frequency (type 2 diabetes)*	Frequency (non–type 2 diabetes)†	Odds ratio (95% CI) (additive model)	*P* value (additive model)	Corrected *P* value
1467R-1456S	0.71	0.83	0.52 (0.32–0.86)	0.0106	0.0211
1467R-1456G	0.19	0.14	1.31 (0.80–2.16)	0.2870	0.4916
1467H-1456G	0.10	0.03	3.58 (1.28–10.03)	0.0151	0.0300
999R-928P	0.75	0.75	1.02 (0.66–1.59)	0.9295	0.9950
999R-928L	0.23	0.23	1.03 (0.66–1.62)	0.8975	0.9895
999STOP-928P	0.02	0.03	0.66 (0.24–1.95)	0.4815	0.7312

Corrected *P* is the *P* value corrected for multiple comparisons within haplotype combinations. The haplotype 1467H-1456S and 999STOP-928L were extremely rare and therefore were not included. *Subjects with type 2 diabetes diagnosed before the age of 45 years. †Subjects determined to be nondiabetic by oral glucose tolerance test after the age of 45 years.

insulin-mediated glucose metabolism (24). ARHGEF11 is a member of a subfamily of RhoGEFs that contain regulator of G-protein signaling domains. LARG (Leukemia-associated Rho guanine nucleotide exchange factor; also called ARHGEF12) also is a member of this regulator of G-protein signaling subfamily. We previously studied *LARG* as a candidate gene for type 2 diabetes and identified a functional Tyr1306Cys variant that also was associated with reduced insulin-stimulated glucose uptake among nondiabetic Pima Indians (16).

Although nominally significant associations were observed in our analysis of *ARHGEF11*, it should be cautioned that multiple comparisons inherent to association studies can lead to the discovery of false-positives, and for any given variant the prior probability of a true association is probably low. For these two reasons, particularly stringent *P* value thresholds for declaring a significant association have been proposed (25,26). The *P* values given in this study are unadjusted, and, if corrected for the 131 variants studied (which could be argued to be an overcorrection due to high LD among many of the variants), the *P* values of ~0.01 certainly would not be significant (*P* = ~0.5). Yet, the performance of a correction for multiple comparison proceeds on the assumption that the "global" null hypothesis (no association with any of the variants) is of interest, and it is not clear that this is appropriate for a physiologic candidate gene in a region with replicated evidence for linkage. In this context, its rank relative to other SNPs genotyped as part of our fine-mapping efforts across this region of linkage, along with potential functional consequences of the variant, may provide more relevant information about the importance of this gene. The *P* value of 0.01 for R1467H puts this variant within the top 2.5% of the >4,500 variants genotyped to date in Pima Indians across a 37-Mb region on chromosome 1q. The R1467H also is located in the COOH-terminal region of ARHGEF11 protein, which has been shown to regulate ARHGEF11 protein activity (27).

Replication of associations in other populations also can provide evidence that associations are not spurious. The region spanning *ARHGEF11*, *NTRK1*, and *INSRR* also has been sequenced and densely genotyped in the Amish population, where variants within *ARHGEF11* also were found to be associated with type 2 diabetes and oral glucose levels in response to an oral glucose tolerance test. However, the specific variants in *ARHGEF11* demonstrating the best associations differed between the Amish and the Pima populations (28). The chromosomal region spanning *ARHGEF11* also has been genotyped at a ~5-kb density as part of the fine-mapping efforts of the International Type 2 Diabetes 1q Consortium (29), but among the 75 database variants (which did not include R1467H) genotyped in the consortium's multiethnic subjects (n = 3,700) there was only weak evidence for an association with type 2 diabetes (lowest *P* value among 75 SNPs = 0.03). Therefore, we do not propose that the R1567H in *ARHGEF11* explains the linkage to type 2 diabetes observed in multiple populations on chromosome 1q21–23. However, the R1567H, or another variant carried on the H allele of *ARHGEF11*, may have played a subtle role in our ability to detect linkage in this region in Pima Indians. Adjustment of the original linkage peak, centered in D1S1677, for the effect of R1467H results in a 12% reduction in the evidence for linkage (logarithm of odds drops from 2.88 to 2.54), which is among the top 5% of the linkage reductions observed among >3,900 SNPs that have been analyzed, to date, within this region in Pima Indians.

In summary, we propose that *ARHGEF11* may have a minor, or modifying, role in influencing susceptibility to type 2 diabetes via its effect upon insulin action in Pima Indians. However, due to the relatively low frequency of risk allele H versus the high prevalence of type 2 diabetes in Pima Indians and the fact that this gene shows little evidence for association in other populations with linkage to type 2 diabetes on chromosome 1q21–23, except for the Amish, we speculate that there are additional type 2 diabetes susceptibility genes, with larger and more universal effects, on chromosome 1q21-q23.

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