Association Analysis of Variation in/Near *FTO*, *CDKAL1*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *LOC387761*, and *CDKN2B* With Type 2 Diabetes and Related Quantitative Traits in Pima Indians

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OBJECTIVE—In recent genome-wide association studies, variants in *CDKAL1*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *CDKN2B*, *LOC387761*, and *FTO* were associated with risk for type 2 diabetes in Caucasians. We investigated the association of these single nucleotide polymorphisms (SNPs) and some additional tag SNPs with type 2 diabetes and related quantitative traits in Pima Indians.

RESEARCH DESIGN AND METHODS—Forty-seven SNPs were genotyped in 3,501 Pima Indians informative for type 2 diabetes and BMI, among whom 370 had measures of quantitative traits.

RESULTS—*FTO* provided the strongest evidence for replication, where SNPs were associated with type 2 diabetes (odds ratio = 1.20 per copy of the risk allele, $P = 0.03$) and BMI ($P = 0.002$). None of the other previously reported SNPs were associated with type 2 diabetes; however, associations were found between *CDKAL1* and *HHEX* variants and acute insulin response (AIR), where the Caucasian risk alleles for type 2 diabetes were associated with reduced insulin secretion in normoglycemic Pima Indians. Multiallelic analyses of carrying risk alleles for multiple genes showed correlations between number of risk alleles and type 2 diabetes and impaired insulin secretion in normoglycemic subjects ($P = 0.006$ and 0.0001 for type 2 diabetes and AIR, respectively), supporting the hypothesis that many of these genes influence diabetes risk by affecting insulin secretion.

CONCLUSIONS—Variation in *FTO* impacts BMI, but the implicated common variants in the other genes did not confer a significant risk for type 2 diabetes in Pima Indians. However, confidence intervals for their estimated effects were consistent with the small effects reported in Caucasians, and the multiallelic "genetic risk profile" identified in Caucasians is associated with diminished early insulin secretion in Pima Indians. ***Diabetes* 58: 478–488, 2009**

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Although it has been known for decades that both type 2 diabetes and obesity have a genetic basis (1), remarkably few susceptibility genes with robust and reproducible effects have been identified for these diseases. The recent introduction of large-scale, high-density genome-wide association (GWA) technology has revolutionized this field. Within the past year, six high-density (>300 K) GWA studies to identify genes affecting risk for type 2 diabetes among Caucasians have been published, and replicated associations were reported with single nucleotide polymorphisms (SNPs) in/near the genes transcription factor 7-like 2 (*TCF7L2*), CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), zinc transporter, member 8 (*SLC30A8*), hematopoietically expressed homeobox (*HHEX*), exostosin 2 (*EXT2*), insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), cyclin-dependent kinase inhibitor 2B (*CDKN2B*), *LOC387761* (a hypothetical gene), and fat mass and obesity associated (*FTO*) (2–7). In a prior study, we thoroughly examined the *TCF7L2* locus and determined that it was not a major gene for type 2 diabetes among Pima Indians who have a high prevalence of this disease and a high rate of obesity (8). We also recently reported results from our GWA study to identify genetic determinants for type 2 diabetes and obesity in Pima Indians (9), which did not identify SNPs in any of these genes as being among the strongest associations for type 2 diabetes. However, this prior GWA used the lower-density 100K Affymetrix array, and none of the reproducibly associated variants identified in the high-density GWA scans of Caucasians are well captured by the 100K array. Therefore, in the current study, we directly examine the specific SNPs reported in Caucasian studies and tag SNPs to examine alternative variation within *CDKAL1*, *SLC30A8*, and *IGF2BP2* to evaluate their potential role in affecting diabetic status, body weight, and quantitative metabolic risk factors for diabetes in the Pima Indian population.

RESEARCH DESIGN AND METHODS

All subjects are part of an ongoing longitudinal study of the etiology of type 2 diabetes in the Gila River Indian Community in Central Arizona (10). The population-based sample included every full-heritage Pima Indian from the longitudinal study for whom DNA was available, diabetes status was known, and height and weight were measured ($n = 3,501$). BMI was computed for those aged ≥15 years. Among these subjects, 1,561 had type 2 diabetes (37% men, mean age of onset 37.2 ± 12.1 years, and mean maximum BMI 38.5 ± 8.4 kg/m^2) and 1,940 were nondiabetic at their last exam (46% men, mean study

age 31.1 ± 14.5 years, and mean maximum BMI 35.7 ± 8.2 kg/m^2). Diabetes was diagnosed according to World Health Organization criteria (11) when the venous plasma glucose concentration was ≥200 mg/dl 2 h after a 75-g oral glucose load, the fasting plasma glucose was ≥126 mg/dl, or the diagnosis was made by clinical means. The BMI measurement that was used for association analysis was the maximum BMI measured when a longitudinally studied subject was nondiabetic. Measurements made at or after the diagnosis of diabetes were not included in the analysis. Subjects who were diabetic at their first exam were excluded; therefore, the BMI analyses are restricted to 2,458 subjects.

A subset of these full-heritage Pima Indian subjects (n = 370) was also studied as inpatients in our clinical research center when they were nondiabetic. All of these subjects (aged 18–45 years) were healthy by medical history, physical examination, and routine laboratory tests and were not taking medications. Subjects were fed a weight-maintaining diet for 2–3 days before they were administered an oral glucose tolerance test (OGTT). For the OGTT, subjects underwent an overnight fast and then ingested 75 g glucose; blood was drawn before ingesting the glucose and at 30, 60, 120, and 180 min thereafter for measurement of plasma glucose and insulin levels. On a different day, subjects received a 25-g intravenous glucose tolerance test (IVGTT) to measure acute insulin response (AIR). 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. AIR was calculated as one-half the mean increment in plasma insulin concentrations from 3 to 5 min as previously described (12). Analysis of AIR and 30-min insulin levels during the OGTT was restricted to subjects with normal glucose tolerance (n = 271) because impaired glucose tolerance is associated with secondary effects on these measures.

Insulin action was assessed at physiological insulin concentrations during the hyperinsulinemic-euglycemic clamp technique as previously described (12). Briefly, after an overnight fast, a primed (30 μCi), continuous (0.3μCi/min) 3-[^{3}H]glucose infusion was started to determine the rate of post-absorptive endogenous glucose production (EGP). Two hours after starting the isotope infusion, a primed, continuous intravenous insulin infusion was administered for 100 min at a rate of 40 mU/m^2 body surface area per min. This infusion achieved a steady-state insulin concentration of 144 ± 42 μU/l (mean ± SD). Plasma glucose concentrations were maintained at ~100 mg/dl with a variable infusion of 20% dextrose solution. Blood samples for measurement of 3-[^{3}H]glucose specific activity were collected at the end of the basal period and every 10 min during the final 40 min of the insulin infusion. The rate of total insulin-stimulated glucose disposal (M) was calculated for the last 40 min of the insulin infusion, and M was corrected for mean glucose and insulin concentrations and EGP during the final 40 min of the insulin infusion (12). All measurements derived from the glucose clamp were normalized to estimated metabolic body size.

Body composition was estimated by underwater weighing until January 1996 and by dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI) thereafter. A conversion equation derived from comparative analyses was used to make estimates of body composition equivalent between methods (13). Determination of adipose cell size has previously been described (14).

All studies were approved by the Tribal Council of the Gila River Indian Community and the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases.

SNP selection and genotyping. The present study prioritized genotyping of 16 specific SNPs (Table 1) reported to be associated with type 2 diabetes in prior genome-wide studies (2–7). Moreover, because associations with SNPs in *CDKAL1*, *SLC30A8*, and *IGF2BP2* replicated in multiple studies of Caucasians and mapped within biological candidate genes, additional SNPs in these three genes were also genotyped in Pima Indians to search for alternative variation within the same gene. However, because *CDKAL1*, in particular, is a large gene spanning 697.1 kb, tag SNPs were selected from the HapMap CHB population with a minor allele frequency ≥0.1 and an r^2 value ≥0.5 rather than the more customary r^2 value ≥0.8, allowing for potentially important variation to be missed. A total of 47 SNPs were genotyped in 3,501 population-based full-heritage Pima Indians by the method of SNPlex (Applied Biosystems) following the manufacturer's protocol. All SNPs were in Hardy-Weinberg equilibrium ($P > 0.008$); however, SNPs rs9300039 and rs17738231 were monomorphic in Pima Indians.

Statistical analysis. Statistical analyses were performed using the software of the SAS Institute (Cary, NC). The general association of genotypes with type 2 diabetes was assessed with logistic regression analysis and was adjusted for covariates (age, sex, and birth year). The model was fit with a generalized estimating equation to account for correlation among siblings. Analyses for type 2 diabetes and BMI are given for an "additive" model in which homozygotes for the major allele (1/1), heterozygotes (1/2), and homozygotes for the minor allele (2/2) were coded to a numeric variable for genotype (0, 1, and 2, respectively). A within-family association analysis among genotypically discordant siblings was also conducted by a modification of the method of Abecasis et al. (15) to control for any population stratification. The association of quantitative traits with genotypes was analyzed by linear regression using the general estimating equation procedure (GEE; SAS Institute, Cary, NC). Potentially confounding covariates were included in all models. BMI was adjusted for age, sex, and birth year. Percent body fat was adjusted for age and sex. Insulin-stimulated glucose disposal was adjusted for age, sex, and percent body fat. AIR was adjusted for age, sex, percent body fat, and insulin-stimulated glucose disposal rate. The 30-min plasma insulin concentration during an OGTT was adjusted for age, sex, percent body fat, insulin-stimulated glucose disposal rate, and 30-min plasma glucose concentrations. All covariates were specified a priori based on previous studies of the determinants of these traits. These models were also fit by generalized estimating equations to account for correlation among siblings. Plasma insulin concentrations, insulin-stimulated glucose disposal rates, and AIRs were log transformed before analysis to approximate a normal distribution. Linkage disequilibrium and haplotype block analysis were estimated by Haploview (version 3.32). P values <0.05 were considered statistically significant.

For the analysis of heterogeneity in the odds ratios (ORs) between Caucasians and Pima Indians, a summary OR was calculated for Pima Indians and Europeans as a weighted sum of the logarithms of the ORs for the individual studies with the inverse of the variance of the estimate of the logarithm of the OR as the weight (16). Variances for the European studies were inferred from the confidence intervals for published studies or, if these were not available, from the genotypic counts for case and control subjects. The Q statistic was used to test the null hypothesis that the Pima Indian and European ORs were equal. Power to detect heterogeneity under the assumption that the true OR in Europeans is the summary observed from published studies and that the true OR in the Pima Indians is 1 was calculated by the method of Hedges and Pigott (17).

The magnitude of the previously reported SNP associations in Caucasians has generally been modest (ORs ~1.15). To estimate the power to detect an association for each variant with the effect observed in Europeans, we conducted simulations for the present set of families given the observed Pima Indian allele frequencies under the assumption that the OR is constant with age. By these methods we estimate that the power of the present study to detect an OR of 1.15 at $P < 0.05$ is ~73% for a risk allele with frequency of 0.5 and ~37% for a risk allele with frequency of 0.1. To augment the power to detect an association, we assumed that most of these alleles do very modestly affect risk in the Pima population, and we therefore conducted a multiallelic analysis with the number of risk alleles carried by each individual. In these analyses, a sentinel SNP for each of the most strongly replicated regions in Europeans was selected [(rs7756992 in *CDKAL1*, rs13266634 in *SLC30A8*, rs1111875 in *HHEX*, rs4402960 in *IGF2BP2*, rs10811661 in *CDKN2B*, rs8050136 in *FTO*, and rs7903146 in *TCF7L2*, which was described in a previous publication [8]). The number of risk alleles ("risk" as defined in studies of Europeans) that each individual carried was summed over all of these sentinel SNPs, and the associations were analyzed in a fashion analogous to the individual SNPs. This procedure effectively gives equal weights to each of the risk variants.

RESULTS

***CDKAL1*.** Four SNPs in *CDKAL1* (rs4712523, rs10946398, rs7754840, and rs7756992) previously associated with type 2 diabetes (3–6) and 25 additional tag SNPs within *CDKAL1* were genotyped in the population-based sample of 3,501 full-heritage Pima Indians. The four previously reported SNPs fell into one linkage disequilibrium block 1, where rs7754840 was in complete linkage disequilibrium ($D' = 1$, $r^2 \geq 0.98$) with rs10946398 and rs4712523 and in high linkage disequilibrium ($D' = 0.98$, $r^2 = 0.68$) with rs7756992 (supplementary Fig. 1, available in an online appendix at http://dx.doi.org/10.2337/db08-0877). The allele frequencies of these SNPs were similar between Pima Indians and Caucasians (rs7754840 C allele = 0.26 in Pima Indians and 0.31 in Caucasians; rs7756992 G allele = 0.32 in Pima Indians and 0.26 in Caucasians). None of these previously reported SNPs or any of the additional tag SNPs were significantly associated with type 2 diabetes in Pima Indians (Table 1). However, the SNPs in complete linkage disequilibrium with rs7754840 were nominally associated with BMI, where Pima Indian subjects carrying the risk

TABLE 1
Association of SNPs in/near *CDKAL1*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *CDKN2B*, *LOC387761*, and *FTO* with type 2 diabetes and BMI in a population-based sample of full-heritage Pima Indians

					Subjects by genotype with type 2 diabetes (top row) and nondiabetic subjects (bottom row)					BMI by genotype (top row) and number of subjects (bottom row)			
SNP	Gene	Chromosome	Allele 1/2	A. 2. Frequency	1/1	1/2	2/2	Additive *P* value	Additive OR (95% CI)	1/1	1/2	2/2	Additive *P* value
rs6935317	*CDKAL1*	6	C/T	0.25	763	532	83	0.70	0.97 (0.85–1.12)	35.9 ± 8.1	36.6 ± 8.4	36.3 ± 8.8	0.13
				0.24	1,001	650	104			1,429	950	143	
rs1569660	*CDKAL1*	6	A/G	0.29	698	556	114	0.88	0.99 (0.87–1.13)	36.0 ± 8.1	36.4 ± 8.3	36.2 ± 8.4	0.34
				0.28	892	720	134			1,290	1,022	196	
rs2328528	*CDKAL1*	6	A/T	0.23	874	474	98	0.39	0.94 (0.81–1.08)	36.4 ± 8.4	35.9 ± 7.8	35.9 ± 7.6	0.13
				0.22	1,095	623	76			1,605	873	135	
rs7754840*†	*CDKAL1*	6	G/C	0.28	750	495	132	0.38	1.06 (0.93–1.22)	36.6 ± 8.6	35.9 ± 7.9	35.1 ± 7.1	0.01
				0.26	959	660	116			1,379	937	189	
rs7756992*	*CDKAL1*	6	A/G	0.34	610	574	178	0.15	1.10 (0.97–1.25)	36.5 ± 8.6	36.1 ± 7.8	35.5 ± 7.8	0.09
				0.32	820	744	184			1,153	1,072	280	
rs9295479	*CDKAL1*	6	T/A	0.44	448	734	266	0.57	1.03 (0.92–1.16)	36.0 ± 8.1	36.2 ± 8.0	36.6 ± 8.6	0.31
				0.44	568	862	366			809	1,273	531	
rs4351239	*CDKAL1*	6	T/C	0.12	1,068	287	17	0.11	1.16 (0.97–1.39)	36.2 ± 8.2	36.3 ± 8.2	36.3 ± 7.8	0.98
				0.14	1,303	409	33			1,903	565	41	
rs1004172	*CDKAL1*	6	C/T	0.20	874	418	61	0.18	1.10 (0.96–1.27)	36.0 ± 8.3	36.7 ± 8.1	36.0 ± 8.2	0.32
				0.21	1,078	579	79			1,585	798	104	
rs1498426	*CDKAL1*	6	T/C	0.24	840	461	101	0.16	0.91 (0.79–1.04)	36.5 ± 8.1	35.7 ± 8.2	36.7 ± 8.7	0.05
				0.22	1,063	604	82			1,552	851	132	
rs9465946	*CDKAL1*	6	A/G	0.18	969	392	59	0.11	0.88 (0.76–1.03)	36.4 ± 8.1	35.9 ± 8.4	37.6 ± 8.9	0.41
				0.17	1,233	507	46			1,791	706	78	
rs10946425	*CDKAL1*	6	C/T	0.18	909	416	43	0.16	1.12 (0.96–1.31)	36.3 ± 8.2	35.9 ± 8.2	36.4 ± 8.3	0.49
				0.19	1,151	519	80			1,660	751	99	
rs9465948	*CDKAL1*	6	G/A	0.03	1,289	88	1	0.32	0.84 (0.60–1.19)	36.2 ± 8.2	36.0 ± 8.8	38.2	0.73
				0.03	1,640	118	0			2,370	152	1	
rs9295488	*CDKAL1*	6	G/A	0.41	515	659	252	0.71	0.98 (0.86–1.10)	36.2 ± 8.2	36.3 ± 8.2	36.2 ± 8.2	0.40
				0.39	647	844	274			940	1,220	410	
rs4712580	*CDKAL1*	6	C/T	0.17	995	361	54	0.49	0.94 (0.80–1.11)	36.3 ± 8.1	36.2 ± 8.2	36.2 ± 8.9	0.13
				0.15	1,284	453	42			1,849	653	70	
rs9295491	*CDKAL1*	6	G/T	0.39	520	645	209	0.84	0.99 (0.88–1.11)	36.1 ± 8.3	36.1 ± 8.0	36.7 ± 8.6	0.69
				0.38	711	803	272			1,022	1,160	380	
rs6456396	*CDKAL1*	6	G/C	0.04	1,224	115	1	0.85	1.03 (0.76–1.39)	36.2 ± 8.2	35.1 ± 8.0	36.9 ± 6.1	0.03
				0.05	1,527	158	3			2,217	212	4	
rs4076112	*CDKAL1*	6	A/G	0.02	1,397	62	1	0.61	1.10 (0.75–1.61)	36.2 ± 8.2	35.6 ± 8.4	NA	0.28
				0.03	1,718	91	0			2,516	118	0	
rs12055489	*CDKAL1*	6	A/G	0.17	938	391	38	0.81	1.02 (0.86–1.21)	36.2 ± 8.2	36.2 ± 8.2	37.2 ± 7.5	0.53
				0.17	1,211	481	52			1,735	688	79	
rs9295496	*CDKAL1*	6	A/G	0.02	1,381	56	1	0.66	1.10 (0.73–1.63)	36.2 ± 8.2	35.6 ± 8.1	34.2	0.33
				0.02	1,700	77	1			2,489	100	1	
rs9295497	*CDKAL1*	6	G/A	0.02	1,382	53	1	0.77	1.07 (0.69–1.65)	36.2 ± 8.2	36.7 ± 7.6	NA	0.66
				0.02	1,690	66	0			2,482	90	0	
rs9465982	*CDKAL1*	6	C/T	0.18	893	401	42	0.83	1.02 (0.87–1.19)	36.4 ± 8.1	35.9 ± 8.5	35.2 ± 6.6	0.05
				0.18	1,145	508	55			1,644	739	70	

TABLE 1
Continued

SNP	Gene	Chromosome	Allele 1/2	A. 2. Frequency	Subjects by genotype with type 2 diabetes (top row) and nondiabetic subjects (bottom row) 1/1	1/2	2/2	Additive *P* value	Additive OR (95% CI)	BMI by genotype (top row) and number of subjects (bottom row) 1/1	1/2	2/2	Additive *P* value
rs9295501	*CDKAL1*	6	A/G	0.001	1,363	2	0	0.77	0.80 (0.18–3.52)	36.2 ± 8.2	35.1 ± 10.6	NA	0.77
				0.001	1,741	3	0			2,499	3	0	
rs12527222	*CDKAL1*	6	C/T	0.02	1,330	50	1	0.74	1.08 (0.70–1.66)	36.2 ± 8.2	37.0 ± 7.6	NA	0.42
				0.02	1,670	64	0			2,415	89	0	
rs898165	*CDKAL1*	6	C/T	0.16	1,029	382	37	0.63	0.96 (0.81–1.13)	36.1 ± 8.0	36.6 ± 8.6	35.8 ± 8.2	0.62
				0.14	1,310	442	32			1,897	654	52	
rs4710965	*CDKAL1*	6	G/C	0.08	1,221	204	16	0.23	1.14 (0.92–1.42)	36.3 ± 8.2	35.7 ± 8.4	34.8 ± 6.3	0.03
				0.10	1,485	312	24			2,172	421	33	
rs6937610	*CDKAL1*	6	A/G	0.32	621	618	133	0.47	1.05 (0.92–1.20)	36.5 ± 8.2	36.0 ± 8.2	35.9 ± 7.9	0.26
				0.35	744	754	222			1,073	1,122	292	
rs9460612	*CDKAL1*	6	G/A	0.41	471	659	233	0.46	0.95 (0.84–1.08)	36.2 ± 8.3	36.0 ± 8.2	36.9 ± 7.9	0.25
				0.40	600	878	252			868	1,246	373	
rs7002176	*SLC30A8*	8	T/A	0.25	756	500	84	0.34	0.93 (0.81–1.08)	36.3 ± 8.2	36.1 ± 8.1	35.6 ± 7.8	0.16
				0.24	977	599	96			1,404	876	146	
rs13266634*	*SLC30A8*	8	C/T	0.09	1,156	209	16	0.71	1.04 (0.84–1.29)	36.1 ± 8.2	36.5 ± 8.5	36.9 ± 8.0	0.98
				0.09	1,455	296	15			2,095	409	24	
rs1995222	*SLC30A8*	8	G/A	0.13	1,038	320	22	0.42	1.07 (0.90–1.27)	36.2 ± 8.2	36.4 ± 8.3	35.4 ± 8.3	0.38
				0.15	1,307	433	43			1,845	613	56	
rs1111875*	*HHEX*	10	T/C	0.40	499	661	214	0.51	1.04 (0.92–1.18)	36.3 ± 8.1	36.1 ± 8.1	36.6 ± 8.8	0.86
				0.38	659	841	248			923	1,223	366	
rs10509646	*HHEX*	10	T/C	0.46	414	680	291	0.02	1.15 (1.02–1.29)	36.1 ± 8.3	36.3 ± 8.2	36.2 ± 8.4	0.90
				0.49	467	879	418			694	1,271	568	
rs3740878*‡	*EXT2*	11	G/A	0.13	1,037	315	16	0.57	1.05 (0.88–1.27)	36.1 ± 8.1	36.5 ± 8.5	35.4 ± 8.8	0.53
				0.13	1,324	384	34			1,897	564	42	
rs6777038	*IGF2BP2*	3	C/T	0.02	1,328	48	2	0.19	1.31 (0.88–1.96)	36.2 ± 8.2	36.3 ± 7.9	46.2	0.94
				0.03	1,672	90	0			2,421	106	1	
rs16860234	*IGF2BP2*	3	A/C	0.06	1,199	156	4	0.74	0.96 (0.74–1.23)	36.1 ± 8.3	36.7 ± 7.9	37.4 ± 7.5	0.54
				0.06	1,525	189	7			2,207	261	8	
rs4402960*§	*IGF2BP2*	3	G/T	0.17	942	370	41	0.32	1.08 (0.93–1.26)	36.3 ± 8.2	35.9 ± 8.2	35.6 ± 7.3	0.14
				0.17	1,186	461	54			1,735	656	70	
rs10811661*	*CDKN2B*	9	T/C	0.06	1,214	149	3	0.38	1.13 (0.86–1.49)	36.1 ± 8.2	37.3 ± 8.6	36.2 ± 6.6	0.08
				0.06	1,544	204	3			2,211	294	6	
rs7480010*	*LOC387761*	11	A/G	0.16	976	368	33	0.75	1.03 (0.86–1.22)	36.1 ± 8.2	36.6 ± 8.3	37.0 ± 9.4	0.35
				0.14	1,302	418	40			1,827	639	56	
rs8050136*¶	*FTO*	16	C/A	0.15	1,071	357	44	0.03	1.20 (1.02–1.41)	36.1 ± 8.2	36.7 ± 8.0	37.7 ± 8.3	0.002
				0.14	1,360	435	30			1,975	624	55	

Data are *n* or means ± SD unless otherwise indicated. *SNP previously reported to be associated with type 2 diabetes in a Caucasian GWA study (2–6). For SNPs determined to be in complete linkage disequilibrium (D′ ≥ 0.99), only one representative SNP is shown. †rs7754840 (shown) is in complete linkage disequilibrium with rs10946398 and rs4712523 (not shown). ‡rs3740878 (shown) is in linkage disequilibrium with rs1113132 (not shown). §rs4402960 (shown) is in linkage disequilibrium with rs1470579 (not shown). ¶rs8050136 is in linkage disequilibrium with rs9939609 and rs7193144 (not shown). Data for monomophic SNPs rs9300039 and rs17738231 are not shown. *P* values were adjusted for age, sex, and birth year. ORs for type 2 diabetes are expressed per copy of the underlined allele; for previously reported variants, this is the risk allele identified in Europeans (2–7), whereas for other variants it is arbitrarily set as allele 1 (major allele). BMI for these longitudinally studied subjects is defined as the maximum BMI recorded from a nondiabetic exam. In addition to the general analysis shown in the table, all variants were analyzed using a within-family analysis. With the exception of variants in FTO (detailed in Table 3), no variant showed a significant association ($P < 0.05$) with either type 2 diabetes or BMI using a within-family analysis. NA, analytical model not applicable due to low frequency.

allele for type 2 diabetes reported among Caucasians (C for rs7754840) were less obese ($P = 0.01$, adjusted for age, sex, and birth year; Table 1). These variants in *CDKAL1*, in contrast to variants in the genes described below, had a significant interaction with BMI for diabetes risk (e.g., $P = 0.01$ for interaction with rs7756992, where the interaction is in the direction that heavier Pima Indians were at greater risk if they carried the Caucasian risk allele for diabetes). The four previously reported SNPs in *CDKAL1* were also modestly associated with impaired insulin secretion in Pima Indians (rs7756992 shown as representative in Table 2 and Fig. 1). Among Pima Indians with normal glucose tolerance, homozygotes for the G allele of rs7756992 (the type 2 diabetes risk allele in Caucasians) had a lower mean AIR to an IVGTT (dominant $P = 0.04$, adjusted for age, sex, percent body fat, and glucose disposal rate; Fig. 1*A*) and a lower early (30-min) mean plasma insulin level during an OGTT (dominant $P = 0.0004$, adjusted for age, sex, percent body fat, glucose disposal rate, and 30-min glucose levels; Fig. 1*D*). The reduced 30-min insulin response resulted an elevated trend for plasma glucose levels at 60 min (dominant $P = 0.09$, adjusted for age, sex, and percent body fat; Fig. 1*C*). However, insulin action as assessed by insulin-stimulated glucose uptake during a hyperinsulinemic-euglycemic clamp did not differ among the genotypic groups (dominant $P = 0.97$, adjusted for age, sex, and percent body fat; Fig. 1*B*). Two database tag SNPs (rs9295479 and rs2328528) in *CDKAL1*, each from different linkage disequilibrium blocks (supplementary Fig. 1), were also associated with both AIR and early (30-min) plasma insulin levels, the most notable being rs9295479 because of its common frequency (allele frequency = 0.50) among Pima Indians (adjusted dominant $P = 0.04$ for AIR, adjusted dominant $P = 0.003$ for 30-min insulin levels; data for rs9295479 shown in Table 2).

***SLC30A8*.** One SNP in *SLC30A8* (rs13266634) was associated with type 2 diabetes in multiple GWA studies (2–6). In Pima Indians, rs13266634 was not associated with type 2 diabetes or BMI (Table 1) and was not associated with insulin or glucose responses to an OGTT, insulin sensitivity, or insulin secretion among the 370 metabolically phenotyped nondiabetic Pima Indian subjects (data not shown; specific traits analyzed are listed in Table 2). The frequency of the C allele (the risk allele in the other studies) was higher among the Pima Indians (0.91) compared with Caucasians (0.61–0.70) and Chinese (0.52–0.56), but this allele was also very frequent among Africans (0.96–0.97). Three additional tag SNPs within/near *SLC30A8* (rs7002176, rs1995222, and rs17738231) were also genotyped in Pima Indians. Neither rs7002176 nor rs1995222 was associated with type 2 diabetes or BMI (Table 1), and rs17738231 was monomorphic.

***HHEX*.** A SNP near *HHEX* (rs1111875) was previously associated with type 2 diabetes in multiple studies of Caucasians (2,4–6,18) and Asians (19–22) but not African Americans (23). Based on these findings, rs1111875 and another SNP within this region (rs10509646) were genotyped in the Pima Indian subjects. Rs1111875 was not associated with type 2 diabetes or BMI (Table 1); however, among Pima Indians who were normal glucose tolerant, those homozygous for the previously reported risk allele (C/C) at rs1111875 had a decreased AIR and a reduced early (30-min) insulin response to an OGTT (adjusted *P* dominant = 0.02 and 0.01, respectively; Table 2). A nominal association with type 2 diabetes was observed with

TABLE 2
Association of *CDKAL1* SNPs rs7756992 and rs9295479 and *HHEX* SNP rs1111875 with acute and early (30-min) insulin response among full-heritage Pima Indians with normal glucose tolerance

	rs7756992 (*CDKAL1*)				rs9295479 (*CDKAL1*)				rs1111875 (*HHEX*)			
Trait	AA	AG	GG	*P* value	AA	AT	TT	*P* value	TT	TC	CC	*P* value
n (men/women)	85/53	67/25	11/10		45/25	86/47	44/24		61/33	83/44	24/11	
Age (years)	27 ± 6	27 ± 7	28 ± 6		27 ± 6	27 ± 7	27 ± 6		27 ± 6	27 ± 6	27 ± 6	
Percent fat*	32 ± 8	30 ± 8	32 ± 8	0.42	33 ± 8	32 ± 8	29 ± 9	0.003	31 ± 8	31 ± 8	30 ± 9	0.16
Log10 AIR (units/ml)†	2.35 ± 0.27	2.37 ± 0.29	2.23 ± 0.23	0.04	2.40 ± 0.29	2.37 ± 0.26	2.27 ± 0.27	0.04	2.38 ± 0.28	2.35 ± 0.28	2.25 ± 0.23	0.02
Fasting glucose (mg/dl)‡	89 ± 9	88 ± 9	89 ± 8	0.97	88 ± 8	89 ± 9	87 ± 8	0.56	88 ± 9	89 ± 8	90 ± 10	0.36
30-min glucose (mg/dl)‡	143 ± 27	141 ± 23	143 ± 24	0.81	144 ± 26	142 ± 25	140 ± 25	0.28	141 ± 27	141 ± 23	149 ± 27	0.08
60-min glucose (mg/dl)‡	139 ± 31	138 ± 28	152 ± 29	0.09	139 ± 31	139 ± 31	138 ± 30	0.90	139 ± 31	137 ± 27	146 ± 36	0.26
2-h glucose (mg/dl)‡	107 ± 20	111 ± 18	112 ± 19	0.64	108 ± 20	108 ± 20	109 ± 19	0.57	110 ± 18	108 ± 20	110 ± 20	0.67
Log10 fasting insulin (μU/ml)‡	1.52 ± 0.22	1.53 ± 0.23	1.51 ± 0.17	0.91	1.55 ± 0.21	1.53 ± 0.24	1.48 ± 0.21	0.99	1.55 ± 0.23	1.52 ± 0.22	1.47 ± 0.18	0.16
Log10 30-min insulin (μU/ml)§	2.37 ± 0.27	2.35 ± 0.23	2.19 ± 0.24	0.0004	2.40 ± 0.30	2.38 ± 0.23	2.25 ± 0.22	0.003	2.37 ± 0.26	2.36 ± 0.25	2.24 ± 0.25	0.01
Log10 2-h insulin (μU/ml)‡	2.08 ± 0.31	2.07 ± 0.29	2.11 ± 0.27	0.55	2.09 ± 0.33	2.10 ± 0.30	2.02 ± 0.32	0.51	2.12 ± 0.30	2.07 ± 0.30	2.02 ± 0.30	0.36
Log10 Insulin-stimulated glucose disposal (mg · kg EMBS^{-1} · min^{-1})‡	0.57 ± 0.12	0.57 ± 0.12	0.57 ± 0.11	0.97	0.55 ± 0.11	0.56 ± 0.11	0.60 ± 0.12	0.23	0.56 ± 0.12	0.57 ± 0.12	0.59 ± 0.10	0.32

Data are means ± SD. *P* values are given for a dominant model (rs7756992 AA + AG vs. GG; rs9295479 AA + AT vs. TT, and rs1111875 TT + TC vs. CC) and were calculated after adjusting for covariates that are listed as *age and sex; †age, sex, percent body fat, and glucose disposal rate; ‡age, sex, and percent body fat; and §age, sex, percent body fat, glucose disposal rate, and 30-min glucose levels. The AIR was assessed in response to a 25-g intravenous glucose bolus, and the early (30-min) insulin response was determined during a 75-g OGTT. Additional metabolic characteristics of these subjects include percentage of body fat, glucose, and insulin levels at intervals during the OGTT and insulin-stimulated glucose disposal rate as assessed during a hyperinsulinemic-euglycemic clamp.

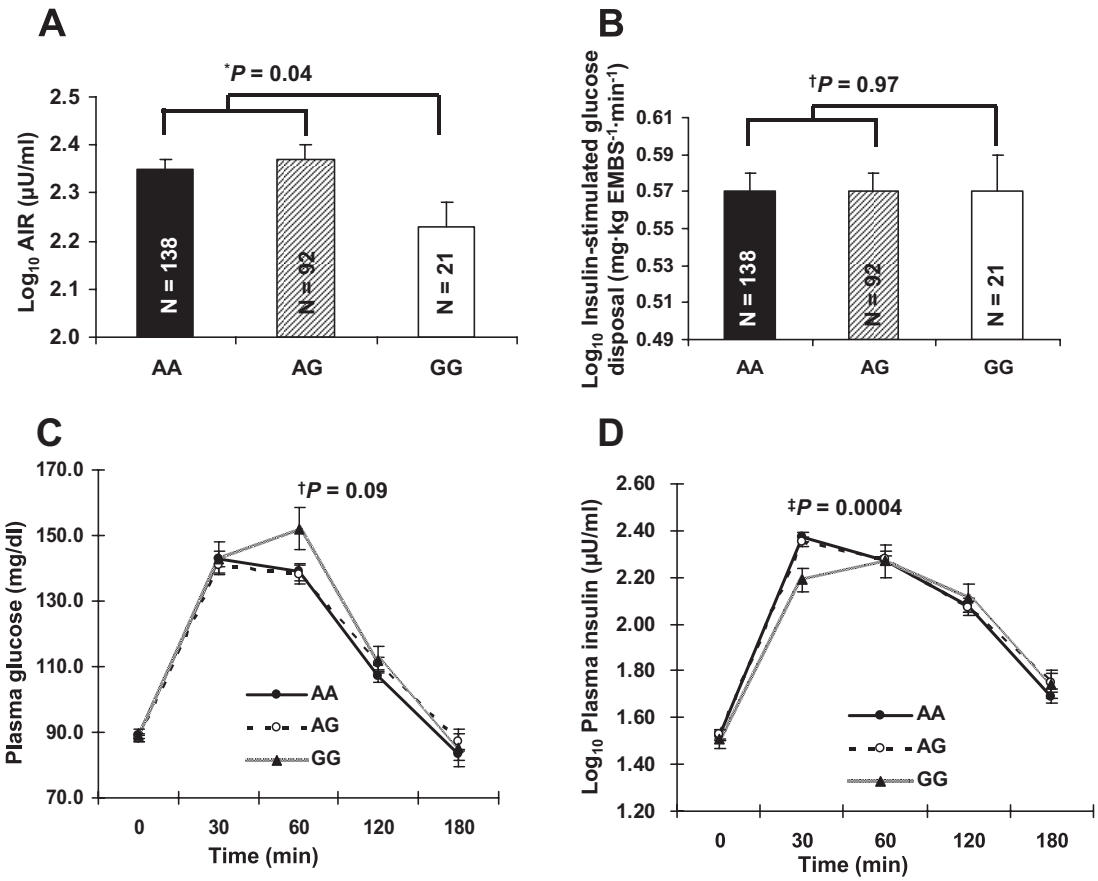

FIG. 1. Association of *CDKAL1* SNP rs7756992 with acute and early insulin secretion. Association of rs7756992 with the AIR to a 25-g bolus IVGTT (*A*), insulin-stimulated glucose disposal rate during a hyperinsulinemic-euglycemic clamp (*B*), plasma glucose levels during a 75-g OGTT (*C*), and plasma insulin levels in OGTT (*D*) among 251 normal glucose tolerant full-heritage Pima Indians. Data are given as means ± SE. *P* values are given for a dominant model (AA + AG vs. GG) under a general analysis. **P* value is adjusted for age, sex, percent body fat, and glucose disposal rate. †*P* value is adjusted for age, sex, and percent body fat. ‡*P* value is adjusted for age, sex, percent body fat, glucose disposal rate, and 30-min glucose levels.

rs10509646 (OR 1.15 [95% CI 1.02–1.29], $P = 0.02$, adjusted for age, sex, and birth year; Table 1).

EXT2. Two SNPs in *EXT2* (rs3740878 and rs1113132) that are in complete linkage disequilibrium in Caucasians and Chinese were associated with type 2 diabetes in a GWA study of French Caucasians (2) but were not replicated in other GWA studies of Caucasians (4–6) or Japanese populations (19,22). These SNPs were also in complete linkage disequilibrium in Pima Indians (supplementary Fig. 1) and were not associated with type 2 diabetes (rs3740878 shown in Table 1). However, among nondiabetic Pima Indians, these SNPs were associated with several measures of insulin resistance, including a lower insulin-stimulated glucose disposal rate in response to a hyperinsulinemic-euglycemic clamp ($P = 0.03$, adjusted for age, sex, and percent body fat; Fig. 2*A*), and elevated glucose and insulin levels during an OGTT ($P = 0.04$, 0.03, and 0.008 for 1-h glucose, 1-h insulin, and 2-h insulin levels, respectively, adjusted for age, sex, and percent body fat; Fig. 2*C* and *D*), despite no difference in percentage of body fat ($P = 0.32$, adjusted for age and sex; Fig. 2*B*). Pima Indians carrying the type 2 diabetes risk allele reported in the French Caucasians (A allele for rs3740878) were more insulin resistant. However, this risk allele is much less common among Pima Indians compared with Caucasians (frequency of A allele for rs3740878 = 0.13 and 0.70 in Pima Indians and Caucasians, respectively).

IGF2BP2. Two SNPs in *IGF2BP2* (rs4402960 and rs1470579) were associated with type 2 diabetes in several Caucasian GWA studies (4–6), and the association of rs4402960 with type 2 diabetes was subsequently replicated in a Danish population (18) and in Asians (21,22) but not in African Americans (23). These two SNPs were in high linkage disequilibrium in Pima Indians ($D' = 0.99$, $r^2 = 0.98$), and the risk alleles were less common among Pima Indians than among Caucasians (frequencies of T allele of rs4402960 and C allele of rs1470579 = 0.17 in Pima Indians and 0.29 and 0.30 in Caucasians). These SNPs and two additional tag SNPs within *IGF2BP2* (rs6777038 and rs16860234; supplementary Fig. 1) were genotyped in the Pima Indians, but none was associated with type 2 diabetes or BMI (Table 1) or any of the diabetes-related quantitative traits (data not shown).

CDKN2B. One SNP in *CDKN2B* (rs10811661) was associated with type 2 diabetes in several studies of Caucasians (4–6). This association was strongly replicated in a Danish population (18) and modestly replicated in Asians (19,21,22) but not replicated in African Americans (23). The Danish study also reported that nondiabetic carriers of the type 2 diabetes risk allele (T) had a lower insulin response and lower insulin levels at 30 and 120 min during an OGTT (18); however, a second study of subjects of European ancestry did not find an association between this SNP and insulin secretion or β-cell glucose sensitivity (24). In Pima Indians, rs10811661 was not associated with type 2 diabetes or BMI (Table 1) or any of the diabetes-related quantitative traits (data not shown; specific traits analyzed are listed in Table 2).

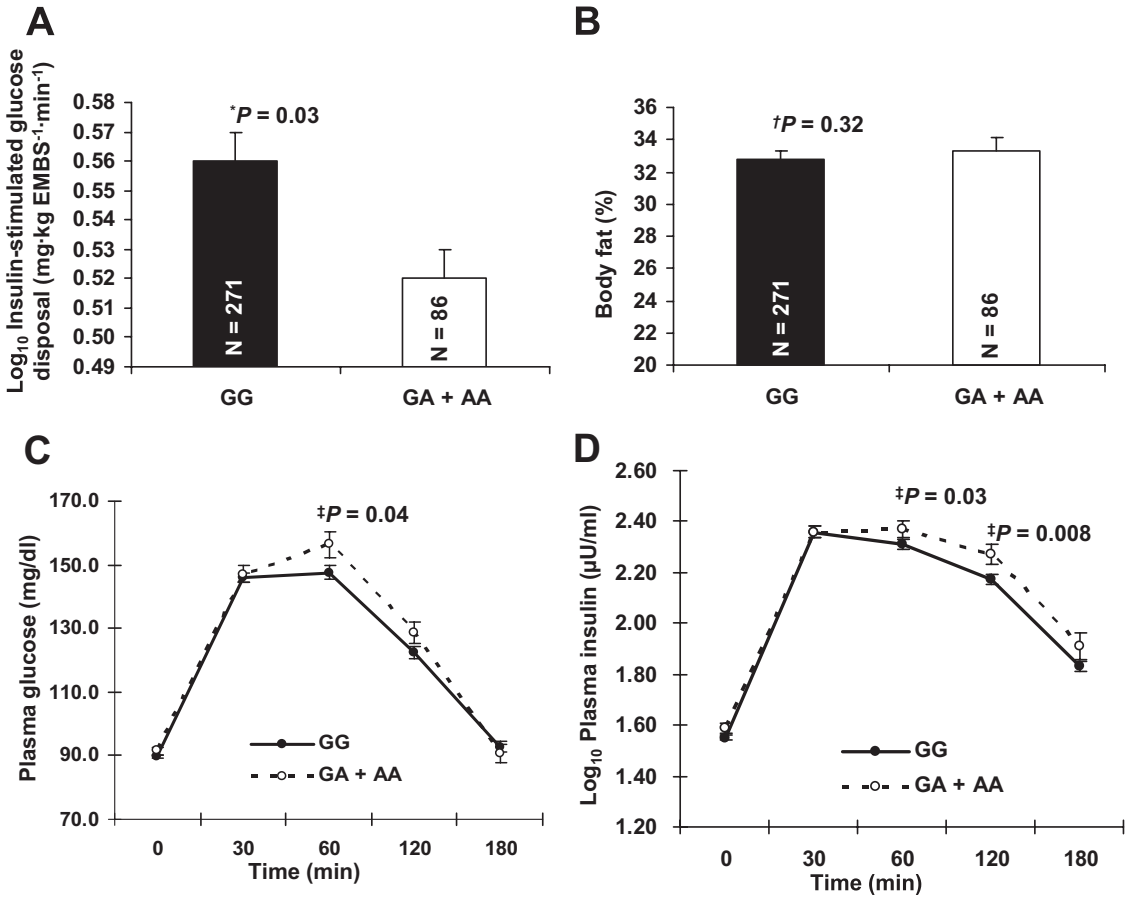

FIG. 2. Association of *EXT2* SNP rs3740878 with insulin action. Association of rs3740878 with insulin-stimulated glucose disposal rate during a hyperinsulinemic-euglycemic clamp (*A*), percent body fat (*B*), plasma glucose levels during a 75-g OGTT (*C*), and plasma insulin levels in OGTT (*D*) among 357 nondiabetic full-heritage Pima Indians. Data are given as means ± SE. The AA genotype was too rare ($n = 3$) for the mean to be reliable; therefore, data are presented as GG ($n = 271$) vs. GA + AA ($n = 86$). **P* value is adjusted for age, sex, and percent body fat. †*P* value is adjusted for age and sex. ‡*P* values are adjusted for age, sex, and percent body fat.

***LOC387761* and intergenic chromosome 11p.** One SNP in the predicted gene *LOC387761* (rs7480010) and a second SNP in an intergenic region on chromosome 11p at ~42 Mb (rs9300039) were highly associated with type 2 diabetes in two GWA studies of Caucasians (2,5). Among the Pima Indians, there was no evidence of association between rs7480010 and type 2 diabetes or BMI (Table 1) or a diabetes-related quantitative trait (data not shown; specific traits analyzed are listed in Table 2). SNP rs9300039 was monomorphic for the C allele.

***FTO*.** SNPs within a region of high linkage disequilibrium in intron 1 of *FTO* (rs8050136 and rs9939609) were initially reported to be associated with BMI and obesity in two studies of Caucasians (25,26). Although these SNPs were also associated with type 2 diabetes, their association with type 2 diabetes was due to the higher BMI of the diabetic subjects (25). The association of the SNPs with BMI has been widely replicated in additional studies of Caucasians and non-Caucasian populations (27–31), although there have been a few reports of a lack of association (32,33). The association of rs8050136, rs9939609, and rs7193144, which were in complete linkage disequilibrium among the Pima Indians (supplementary Fig. 1), with BMI was replicated in Pima Indians ($P = 0.002$ and 0.002 for the general and within-family analyses, respectively, where P is adjusted for age, sex, and birth year; Table 3). Subjects

TABLE 3
Association of rs8050136 in *FTO* with maximum BMI and fat cell size in full-heritage Pima Indians

Trait	Mean ± SD (top row) and number of subjects (bottom row)			*P* value general analysis (top row) and within-family analysis (bottom row)	
	CC	CA	AA	Additive	Recessive
BMI (kg/m^2)	36.1 ± 8.2	36.7 ± 8.0	37.7 ± 8.3	0.002	0.005
	1975	624	55	0.002	0.001
Fat cell size (ng lipid/cell)	0.76 ± 0.21	0.84 ± 0.20	0.86 ± 0.25	0.09	0.02
	142	36	6	0.006	0.002

P values are given for both additive and recessive models (CC vs. CA + AA) due to the small number of AA ($n = 6$) with measures of fat cell size. All *P* values were calculated after adjusting for age, sex, and birth year. *P* values for fat cell size were additionally adjusted for percent body fat. SNP rs8050136 is in linkage disequilibrium with rs9939609 and rs7193144 (not shown).

TABLE 4
Analysis of heterogeneity between Pima Indians and Caucasians in the association of sentinel SNPs in *CDKAL1*, *SLC30A8*, *HHEX*, *IGF2BP2*, *CDKN2B*, and *FTO* with type 2 diabetes

		Pima Indian risk allele			Caucasian risk allele		Heterogeneity	
SNP	Gene	Power	Frequency	OR (95% CI)	Frequency	OR (95% CI)	Power	*P* value
rs7756992	*CDKAL1*	0.59	0.32	1.10 (0.97–1.25)	0.25	1.14 (1.11–1.18)	0.49	0.59
rs13266634	*SLC30A8*	0.29	0.91	1.04 (0.84–1.29)	0.75	1.14 (1.11–1.18)	0.22	0.41
rs1111875	*HHEX*	0.58	0.39	1.04 (0.92–1.18)	0.56	1.13 (1.09–1.16)	0.49	0.20
rs4402960	*IGF2BP2*	0.43	0.17	1.08 (0.93–1.26)	0.29	1.13 (1.10–1.17)	0.33	0.59
rs10811661	*CDKN2B*	0.38	0.94	1.13 (0.86–1.49)	0.79	1.21 (1.16–1.26)	0.27	0.63
rs8050136	*FTO*	0.55	0.15	1.20 (1.02–1.41)	0.45	1.17 (1.12–1.22)	0.45	0.80

ORs and 95% CIs are given per copy of the risk allele, as determined in studies of Caucasians. Results for Pima Indians are derived from the present study, whereas those from Caucasians represent a combined estimate from other published studies (2–6,18) (see RESEARCH DESIGN AND METHODS). The heterogeneity power is defined as the power needed to detect significant heterogeneity at $P < 0.05$ given the Caucasian OR, the SE of its logarithm, and the SE of the logarithm of the Pima OR, under the assumption that the "true" OR in the Pima Indians is 1. The heterogeneity *P* value is given for the null hypothesis that the ORs in Pima Indians and Caucasians are the same.

homozygous for the risk allele (A for rs8050136) had a mean BMI that was 1.6 kg/m^2 greater than that of individuals homozygous for the nonrisk allele (C). The risk allele for high BMI is less common among Pima Indians than Caucasians (frequency of A allele of rs8050136 is 0.15 vs. 0.45, respectively). Among the metabolically phenotyped nondiabetic Pima Indians who had undergone abdominal subcutaneous adipose tissue biopsies, subjects carrying the risk allele for increased BMI (A allele for rs8050136) also had larger individual fat cells, even after adjustment for their higher percentage of body fat ($P = 0.02$ and 0.002 for the general and within-family analyses, respectively; Table 3). SNPs in *FTO* were also modestly associated with type 2 diabetes in Pima Indians (Table 1; OR 1.20 [95% CI 1.02–1.41], $P = 0.03$, adjusted for age, sex, and birth year); however, the association was weakened and no longer met statistical significance after adjusting for BMI (1.16 [0.98–1.38], $P = 0.08$), suggesting that the type 2 diabetes association was largely due to the effect on BMI.

Analysis of heterogeneity and multiallelic association. Associations of many of these SNPs in *CDKAL1*, *SLC30A8*, *HHEX*, *IGF2BP2*, *CDKN2B*, and *FTO* with type 2 diabetes have been well-replicated in populations of European origin; however, the magnitude of these associations has generally been modest (ORs ~1.15). To assess whether the effects seen in the present study of Pima Indians were consistent with those observed in previous studies of European ancestry, we conducted a test for heterogeneity of the ORs (Table 4). Although, with the exception of rs8050136 in *FTO*, none of the SNPs were associated with diabetes at $P < 0.05$ in Pima Indians, the confidence intervals for the OR in Pima Indians invariably included the point estimate for Caucasians. Furthermore, none of the ORs were significantly different between Pima Indians and Caucasians in a formal heterogeneity test. In contrast, omitted from Table 4 but shown previously (8), there was significant heterogeneity between Pima Indians and Caucasians in the ORs for diabetes and SNPs in *TCF7L2*, which were not associated with this disease in Pima Indians.

Results of the multiallelic analysis of all SNPs shown in Table 4 [plus rs7903146 in *TCF7L2* (8)] are shown in Fig. 3*A*. There was a modest but statistically significant increase in prevalence of type 2 diabetes among Pima Indians carrying increasing numbers of risk alleles, where risk is defined in Caucasian studies (2–7) (OR 1.10 per copy of a risk allele [95% CI 1.03–1.19], $P = 0.006$). If rs8050136 in *FTO* was excluded from the analysis, this effect was reduced (1.07 [95% CI 1.00–1.16], $P = 0.06$; supplementary Fig. 2*A*, available in the online appendix), and if rs7903146 in *TCF7L2*, which has been previously shown to have heterogeneous effects between Pima Indians and Caucasians, was further excluded, the OR was 1.09 (95% CI 1.01–1.17) ($P = 0.03$; supplementary Fig. 2*B*). These results were largely unmodified by further adjustment for BMI (e.g., 1.13 [1.05–1.22], $P = 0.001$ for multiallelic effect including all variants).

Results for the multiallelic analyses for percent body fat, insulin sensitivity, and AIR among individuals who had undergone extensive metabolic phenotyping are shown in Fig. 3*B–D*. There was a modest inverse relationship with body fat, such that an increased number of risk alleles was associated with lower percent body fat, but this was not statistically significant ($P = 0.07$). Similarly, an increased number of risk alleles had a nonsignificant trend toward increased insulin sensitivity as assessed by the hyperinsulinemic-euglycemic clamp ($P = 0.06$). In contrast, there was a marked decrease in the AIR associated with each copy of a risk allele ($P = 0.0001$). These results were largely unchanged when the SNPs in *FTO* and *TCF7L2* were excluded (supplementary Fig. 2*C* and *D*).

DISCUSSION

The common forms of type 2 diabetes and obesity are thought to be complex polygenic diseases. However, it remains unknown how many genes contribute to these diseases and whether any single susceptibility gene will be shared among all ethnic groups or whether all will show some degree of population specificity. Recent high-density GWA studies in humans have identified specific variants in several genes that are associated with type 2 diabetes or obesity in more than one population and some variants that are highly associated with type 2 diabetes in one group of subjects but not others. In our study, we sought to determine whether these variants and/or genes also contribute to type 2 diabetes or obesity in the Pima Indian population in Arizona. In addition, because type 2 diabetes is a complex heterogeneous disease, a single risk factor or diabetes-related quantitative trait may be influenced by fewer physiological pathways and thus be determined by fewer genetic loci than the development of type 2 diabetes itself. Therefore, we also sought to determine whether any of the variants associated with type 2 diabetes in another population were associated with a diabetes-related quantitative trait in Pima Indians. We have presented the *P*

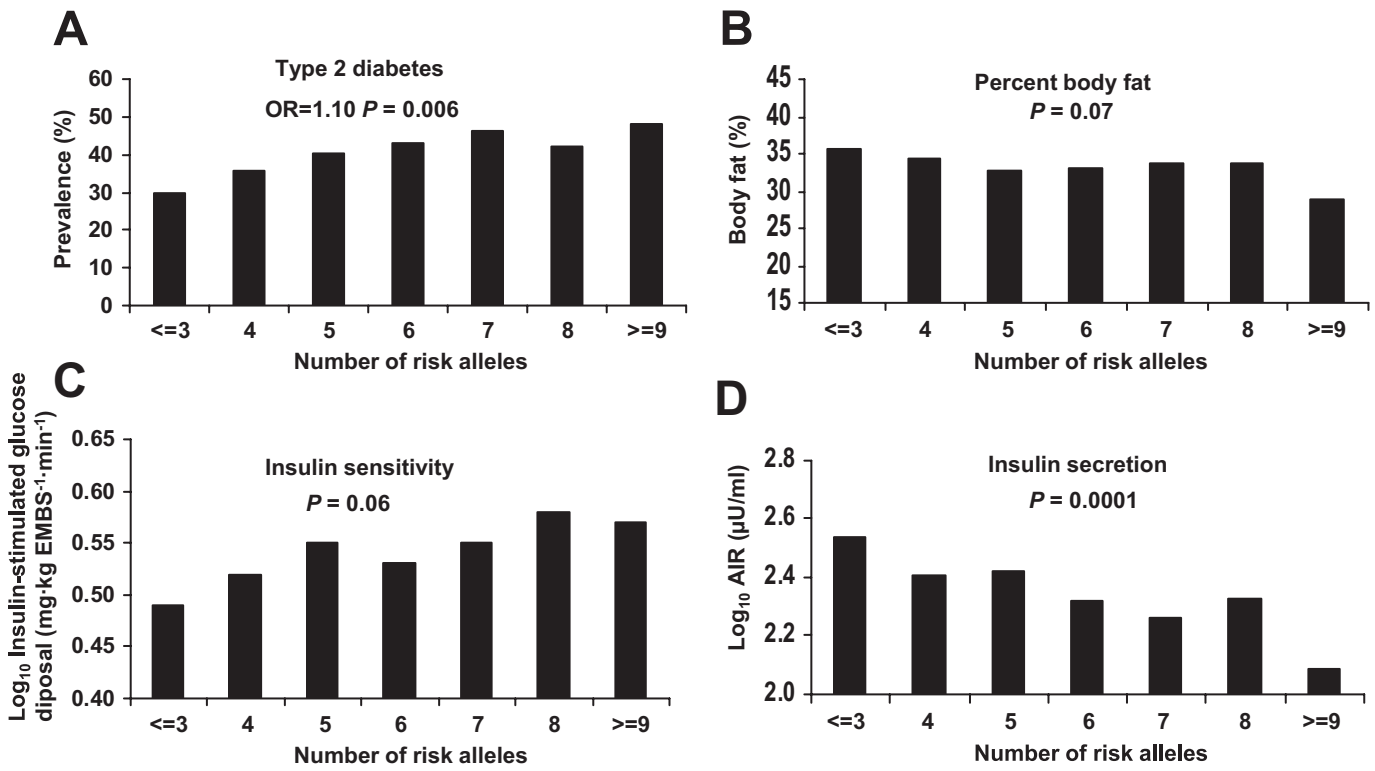

FIG. 3. Association of the number of risk alleles in sentinel SNPs with type 2 diabetes (*A*), percent body fat (*B*), insulin sensitivity (*C*), and AIR (*D*). Sentinel SNPs are defined as SNPs in *CDKAL1* (rs7756992), *SLC30A8* (rs13266634), *HHEX* (rs1111875), *IGF2BP2* (rs4402960), *CDKN2B* (rs10811661), *FTO* (rs8050136), and *TCF7L2* (rs7903146). Risk alleles are defined from studies in Caucasians (see Table 1). Numbers of individuals in each of the allelic groups (≤3, 4, 5, 6, 7, 8, and ≥9) are 55, 295, 668, 795, 524, 329, and 77, respectively, for type 2 diabetes; 9, 43, 82, 85, 49, 21, and 11 for percent body fat and insulin sensitivity; and 5, 32, 60, 59, 35, 14, and 8 for insulin secretion.

values in the present study without any adjustment for multiple comparisons. For SNPs that are well replicated in other populations (e.g., rs8050136 in *FTO*), the prior probability of a "true positive" is high and, thus, $P < 0.05$ is probably sufficient to conclude that these variants are also associated in this population. For the additional tag SNPs, however, this may not be the case, and none of the nominally significant results reported here would remain significant if a Bonferroni correction for the 31 tag SNPs were applied.

Genotyping of SNPs previously associated with type 2 diabetes in *CDKAL1*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *CDKN2B*, *LOC387761*, and an intergenic region on chromosome 11p provided no evidence that any of these are significantly associated with type 2 diabetes or obesity among full-heritage Pima Indians living in the Gila River Indian Community in Arizona. However, it remains possible that several of these genes have minor roles in the development of type 2 diabetes. Both low AIR and decreased insulin action (i.e., insulin resistance) are predictors of type 2 diabetes in Pima Indians (34). In the current study, the Caucasian type 2 diabetes risk alleles in *CDKAL1*, *HHEX*, and *EXT2* had nominal associations with either insulin secretion or insulin action in Pima Indians with normal glucose tolerance. Although AIR and insulin action are strongly predictive of type 2 diabetes, neither trait in itself is completely determinative; therefore, it is not surprising that a variant may have a relatively strong effect on AIR or insulin sensitivity and thus show a significant association, but a relatively weak effect and thus no significant association, on type 2 diabetes. It is also likely that other genes, with much larger effects on type 2 diabetes, exist in the Pima population and that the effect of these genes masks any minor role of *CDKAL1*, *HHEX*, and *EXT2* in increasing type 2 diabetes risk.

The nominal associations of SNPs in *CDKAL1* and *HHEX* with two independent measures of insulin secretion (namely, AIR assessed by an IVGTT and 30-min insulin assessed by an OGTT), where both of these measures were adjusted for insulin sensitivity, is consistent with prior association studies. Previous reports that homozygous carriers of the type 2 diabetes risk allele (G/G) for rs7756992 in *CDKAL1* had a lower corrected insulin response to an oral glucose load than carriers of the A allele (3) and that carriers of the type 2 diabetes risk allele (C) for rs10946398 in *CDKAL1* had a reduced 30-min insulin response (24) suggest a mechanism of impaired insulin secretion as the basis of the association with type 2 diabetes. Direct evidence that *CDKAL1* affects insulin secretion has recently come from a large study of European Caucasians who had undergone both measures of insulin secretion using an IVGTT and insulin sensitivity using a hyperinsulinemic-euglycemic clamp (35). Similarly, previous studies of *HHEX* in Caucasians have shown that type 2 diabetes risk allele (C) carriers of rs1111875 had a lower mean insulin response in Caucasians (18,24), which is also consistent with the known physiological role of *HHEX* in pancreatic development. *HHEX* is highly expressed in pancreatic islet tissue, and *HHEX* knockout mice have a complete loss of ventral pancreas (36).

The strongest replication in this study was the association of variation (rs8050136, rs9939609, and rs7193144, all in complete linkage disequilibrium) in *FTO* with BMI, where subjects homozygous for the previously reported risk allele were on average 1.6 kg/m^2 heavier than homozygotes for the nonrisk allele (heterozygotes were interme-

diate) among the population-based sample of Pima Indians. In addition, among the subset of subjects who had undergone subcutaneous adipose biopsies, subjects homozygous for the risk allele had larger individual adipocytes, even after adjusting for their higher percentage of body fat. *FTO* encodes a 2-oxoglutarate–dependent nucleic acid demethylase, which is highly expressed in hypothalamic nuclei and is thought to be regulated by fasting and feeding conditions (37). *FTO* is also expressed in adipose tissue, but *FTO* mRNA is not correlated with rs9939609 or rs8050136 (38,39). Healthy women homozygous for the obesity-protective *FTO* allele have an ~30% increased in vivo lipolytic activity (assessed as circulating glycerol corrected for total body fat), which is likely due to their increased spontaneous (basal) fat cell lipolysis (39). This finding is consistent with our observation of a difference in fat cell size by *FTO* genotype, where homozygotes for the protective genotype had significantly less lipid per cell (Table 3).

Most of the variants identified in previous GWA studies have fairly modest effects in Caucasian populations with ORs in the range of 1.1–1.2. Even with the present sample size of ~3,500 Pima subjects, there is only moderate power to detect (or exclude) associations of this magnitude. If most of these variants do have modest effects in Pima Indians, one would expect power to be increased in a multiallelic analysis that considers the total number of risk alleles across all variants. The present analyses did show a nominally significant, albeit modest, association of the number of risk alleles with type 2 diabetes. Furthermore, there was a marked association between number of risk alleles at these loci and a diminished AIR but not with other quantitative metabolic risk factors for type 2 diabetes, such as percent body fat or insulin sensitivity. These data are consistent with the hypothesis that many of the diabetes risk variants in Caucasians also have subtle effects in Pima Indians, although the statistical power of the present study is not sufficient to identify individual risk variants with confidence. In addition, the multiallelic analysis is consistent with the hypothesis that most of these variants influence the risk of type 2 diabetes through their effect on insulin secretion.

We have previously reported that variation in *TCF7L2*, which has shown the strongest association with type 2 diabetes across many populations, has minimal, if any, impact on this disease in Pima Indians (8). The variants presented in this paper, which also have well-replicated associations with both type 2 diabetes and measures of insulin secretion among Caucasians, do not have a major effect on type 2 diabetes in Pima Indians, although an association with AIR was observed. It is possible that these findings may be explained by a relatively smaller contribution of insulin secretory dysfunction to the occurrence of diabetes in Pima Indians compared with Caucasians, but insufficient data are available at present to evaluate this hypothesis. To determine whether additional variants exist with stronger effects in Pima Indians or other populations at high risk for diabetes among whom the relative contributions of obesity, insulin resistance, and insulin secretory dysfunction may differ will require mapping studies specific for these populations. In the present study, genotyping of additional tag SNPs in *CDKAL1*, *SLC30A8*, and *IGF2BP2* did not reveal alternative variants associated with type 2 diabetes in Pima Indians; however, important variation could have been missed because these genes were interrogated by tag SNPs with a criteria of $r^2 \geq 0.5$ rather than the more customary $r^2 \geq 0.8$.

In summary, when examined individually, variants in *CDKAL1*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *CDKN2B*, and *LOC387761* associated with type 2 diabetes in other populations were not significantly associated with type 2 diabetes in Pima Indians. However, taken together, they had a modest additive association with type 2 diabetes and a strong association with decreased insulin secretion. In addition, previously reported variation in *FTO* does have a role in determining BMI and type 2 diabetes in the Pima Indian population. A recent report by the Diabetes Genetics Replication and Meta-Analysis consortium has described six additional type 2 diabetes susceptibility loci identified via GWA methods (40). Studies of these loci in Pima Indians are ongoing.

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REFERENCES

1. Baier LJ, Hanson RL: Genetic studies of the etiology of type 2 diabetes in Pima Indians: hunting for pieces to a complicated puzzle. *Diabetes* 53:1181–1186, 2004
2. Sladek R, Rocheleau G, Rung J, et al.: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881–885, 2007
3. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, et al.: A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* 39:770–775, 2007
4. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, Novartis Institutes of BioMedical Research, et al.: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331–1336, 2007
5. Scott LJ, Mohlke KL, Bonnycastle LL, et al.: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345, 2007
6. Zeggini E, Weedon MN, Lindgren CM, et al.: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336–1341, 2007
7. The Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common disease and 3,000 shared controls. *Nature* 447:661–678, 2007
8. Guo T, Hanson RL, Traurig M, et al.: TCF7L2 is not a major susceptibility gene for type 2 diabetes in Pima Indians: analysis of 3,501 individuals. *Diabetes* 56:3082–3088, 2007
9. Hanson RL, Bogardus C, Duggan D, et al.: A search for variants associated with young-onset type 2 diabetes in American Indians in a 100K genotyping array. *Diabetes* 56:3045–3052, 2007
10. Knowler WC, Bennett PH, Hamman RF, et al.: Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. *Am J Epidemiol* 108:497–505, 1978
11. World Health Organization: *Diabetes Mellitus*: *Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
12. Lillioja S, Mott DM, Spraul M, et al.: 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
13. Norman RA, Tataranni PA, Pratley R, et al.: Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. *Am J Hum Genet* 62:659–668, 1998
14. Permana PA, Nair S, Lee YH, et al.: Subcutaneous abdominal preadipocyte differentiation in vitro inversely correlates with central obesity. *Am J Physiol Endocrinol Metab* 286:E958–E962, 2004

15. Abecasis GR, Cardon LR, Cookson WOC: A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 66:279–292, 2000
16. Petitti DB: Statistical methods in meta-analysis. In *Meta-Analysis, Decision Analysis and Cost-Effectiveness Analysis*: *Methods for Quantitative Synthesis in Medicine.* Petitti DB, Ed. Oxford, Oxford University Press, p. 94–118, 2000
17. Hedges LV, Pigott TD: The power of statistical tests in meta-analysis. *Psychol Methods* 6:203–217, 2001
18. Grarup N, Rose CS, Andersson EA, et al.: Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies *Diabetes* 56:3105–3111, 2007
19. Horikoshi M, Hara K, Ito C, et al.: Variations in the HHEX gene are associated with increased risk of type 2 diabetes in the Japanese population. *Diabetologia* 50:2461–2466, 2007
20. Furukawa Y, Shimada T, Furuta H, et al.: Polymorphisms in the IDE-KIF11-HHEX gene locus are reproducibly associated with type 2 diabetes in a Japanese population. *J Clin Endocrinol Metab* 93:310–314, 2008
21. Ng MC, Park KS, Oh B, et al.: Implication of genetic variants near TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2, and FTO in type 2 diabetes and obesity in 6,719 Asians. *Diabetes* 57:2226–2233, 2008
22. Omori S, Tanaka Y, Takahashi A, et al.: Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population. *Diabetes* 57:791–795, 2008
23. Lewis JP, Palmer ND, Hicks PJ, et al.: Association analysis in African Americans of European-derived type 2 diabetes single nucleotide polymorphisms from whole-genome association studies. *Diabetes* 57:2220–2225, 2008
24. Pascoe L, Tura A, Patel SK, et al.: Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic β-cell function. *Diabetes* 56:3101–3104, 2007
25. Frayling TM, Timpson NJ, Weedon MN, et al.: A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316:889–894, 2007
26. Dina C, Meyre D, Gallina S, et al.: Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P: Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 39:724–726, 2007
27. Scuteri A, Sanna S, Chen WM, et al.: Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 3:e115, 2007
28. Hinney A, Nguyen TT, Scherag A, et al.: Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. *PLoS ONE* 2:e1361, 2007
29. Chang YC, Liu PH, Lee WJ, et al.: Common variation in the fat mass and obesity-associated (FTO) gene confers risk of obesity and modulates BMI in the Chinese population. *Diabetes* 57:2245–2252, 2008
30. Marvelle AF, Lange LA, Qin L, et al.: Association of FTO with obesity-related traits in the Cebu Longitudinal Health and Nutrition Survey (CLHNS) Cohort. *Diabetes* 57:1987–1991, 2008
31. Hotta K, Nakata Y, Matsuo T, et al.: Variations in the FTO gene are associated with severe obesity in the Japanese. *J Hum Genet* 53:546–553, 2008
32. Li H, Wu Y, Loos RJ, et al.: Variants in FTO gene are not associated with obesity in a Chinese Han population. *Diabetes* 57:264–268, 2008
33. Ohashi J, Naka I, Kimura R, et al.: FTO polymorphisms in oceanic populations. *J Hum Genet* 52:1031–1035, 2007
34. Bogardus C: Metabolic abnormalities in the development of non-insulin dependent diabetes mellitus. In *Diabetes Mellitus*: *A Fundamental and Clinical Text.* LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott-Raven, p. 459–467, 1996
35. Stancáková A, Pihlajamäki J, Kuusisto J, et al.: Single-nucleotide polymorphism rs7754840 of CDKAL1 is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. *J Clin Endocrinol Metab* 93:1924–1930, 2008
36. Bort R, Martinez-Barbera JP, Beddington RS, et al.: Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. *Development* 131:797–806, 2004
37. Gerken T, Girard CA, Tung YC, et al.: The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* 318:1469–1472, 2007
38. Klöting N, Schleinitz D, Ruschke K, et al.: Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. *Diabetologia* 51:641–647, 2008
39. Wåhlén K, Sjölin E, Hoffstedt J: The common rs9939609 gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis. *J Lipid Res* 49:607–611, 2008
40. Zeggini E, Scott LJ, Saxena R, et al.: Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 40:638–645, 2008