TCF7L2 Is Not a Major Susceptibility Gene for Type 2 Diabetes in Pima Indians

Analysis of 3,501 Individuals

Tingwei Guo, Robert L. Hanson, Michael Traurig, Yunhua Li Muller, Lijun Ma, Janel Mack, Sayuko Kobes, William C. Knowler, Clifton Bogardus, and Leslie J. Baier

OBJECTIVE—The transcription factor 7-like 2 (TCF7L2) gene was initially reported to be associated with type 2 diabetes in Icelandic, Danish, and U.S. populations. We investigated whether TCF7L2 also has a role in type 2 diabetes susceptibility in Pima Indians.

RESEARCH DESIGN AND METHODS—The six variants reported to be associated with type 2 diabetes in the Icelandic study were genotyped in a population-based sample of 3,501 Pima Indians (1,561 subjects had type 2 diabetes, and 1,940 did not have diabetes). In addition, the coding and promoter regions of TCF7L2 were sequenced in 24 Pima subjects. The one variant identified by sequencing, 35 additional database variants positioned in introns, and the six variants reported in the Icelandic study were genotyped in Pima families to determine the haplotype structure of TCF7L2 among Pima Indians. Fourteen representative variants were selected and genotyped in 3,501 Pima Indians.

RESULTS—The six variants initially reported to be associated with type 2 diabetes were less common in Pima Indians compared with samples of European origin, and none were associated with type 2 diabetes. One representative variant, rs1225404, was nominally associated with type 2 diabetes in a general model (additive $P = 0.03$, dominant $P = 0.005$) but not in a within-family analysis (additive $P = 0.2$, dominant $P = 0.07$). However, several variants were associated with BMI; in particular, rs12255372 was associated in both general and within-family analyses (both $P = 0.0007$). Modest associations were also found with traits predictive for type 2 diabetes.

CONCLUSIONS—Variation within TCF7L2 does not confer major risk for type 2 diabetes among the Pima Indian population.
***Diabetes* 56:3082–3088, 2007**

From the Diabetes Molecular Genetics Section, Phoenix Epidemiology and Clinical Research Branch, National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona.

Address correspondence and reprint requests to Leslie J. Baier, PHD, NIDDK, NIH, 445 North 5th St., Phoenix, AZ 85004. E-mail: lbaier@phx.niddk.nih.gov.

Received for publication 7 May 2007 and accepted in revised form 18 September 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 1 October 2007. DOI: 10.2337/db07-0621.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-0621.

OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

A microsatellite marker (DG10S478) within intron 3 of the transcription factor 7-like 2 (TCF7L2) gene and five intronic single nucleotide polymorphisms (SNPs) have been reported to be highly associated with type 2 diabetes in subjects from Iceland, Denmark, and the U.S. (1). Associations with these specific variants and type 2 diabetes have subsequently been replicated consistently and robustly in multiple studies involving subjects of European origin (2–11), Asian Indians (12), and Japanese subjects (13,14). To investigate whether variation in TCF7L2 also has a major role in type 2 diabetes susceptibility in Pima Indians, a population with an extraordinarily high prevalence of type 2 diabetes, variants from the initial report (1), as well as 14 additional representative variants, were genotyped in a population-based sample of full-heritage Pima Indians for association analyses.

RESEARCH DESIGN AND METHODS

All subjects are Pima Indians who are participants in our ongoing longitudinal study of type 2 diabetes among members of the Gila River Indian Community (15). Initially, a family-based sample was genotyped to determine the haplotype structure in this population, and representative SNPs were subsequently genotyped in a population-based sample for association analyses. The family-based sample consisted of 1,037 subjects (578 with type 2 diabetes and 459 without diabetes) from 332 nuclear families in 112 pedigrees. The population-based sample consisted of 3,501 full-heritage Pima Indians for whom there was DNA and information on diabetes status and BMI. This sample consisted of 1,561 subjects with type 2 diabetes (580 of whom were male and 981 female, with mean ± SD BMI 38.5 ± 8.4 kg/m^2 and age of onset 37.2 ± 12.1 years) and 1,940 nondiabetic subjects (902 of whom were male and 1,038 female, with BMI 35.7 ± 8.2 kg/m^2, aged 31.1 ± 14.5 years), as defined by a 2-h oral glucose tolerance test (OGTT) (16). A total of 896 subjects overlapped between the family- and population-based samples. Among the nondiabetic subjects, a subset ($n = 372$) had additionally undergone metabolic phenotyping as inpatients in our clinical research center. Glucose tolerance was determined by a 75-g OGTT with measures of fasting plasma glucose; 30-, 60-, 120-, and 180-min plasma glucose; and insulin concentrations. The acute insulin response was measured by collecting blood samples before a 25-g glucose bolus infusion and 3, 4, 5, 6, 8, and 10 min afterward. Acute insulin response was calculated as the mean increment in plasma insulin concentrations from 3 to 5 min (17). Insulin sensitivity was assessed using a two-step hyperinsulinemic-euglycemic clamp (17). Body composition was estimated by underwater weighing or dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation) (18). All of the studies were approved by the Gila River Indian Community Council and the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases.

SNP identification and genotyping. Sixteen exons that encode the 10 transcripts of TCF7L2, all exon-intron boundaries extending >100 bp into each intron, the 5′ and 3′ untranslated regions, and 2 kb of the upstream (putative promoter) region were sequenced in DNA samples from 24 non–first-degree–related Pima Indians (of whom 12 had age of type 2 diabetes onset <25 years and 12 were confirmed to be without diabetes at age ≥45 years), using Big Dye terminator (Applied Biosystems) on an automated DNA

capillary sequencer (model 3730; Applied Biosystems). SNPs identified by sequencing and database SNPs positioned within unsequenced intronic regions were genotyped by the method of SNPlex (Applied Biosystems). Microsatellite marker DG10S478 was amplified and analyzed by capillary electrophoresis on an ABI 3730 DNA sequencer (Applied Biosystems).

Statistical analysis. The relationship between genotype and continuous variables was assessed by linear regression with adjustment for appropriate covariates. The association of SNPs with type 2 diabetes was assessed by logistic regression with adjustment for covariates. Both linear and logistic models were fit with generalized estimating equations to account for familial relationships (i.e., sibships). For regression modeling in the additive model, homozygotes for the major allele (1/1), heterozygotes (1/2), and homozygotes for the minor allele (2/2) were coded to a continuous numeric variable for genotype (0, 1, and 2). The dominant model was defined as 1/1 + 1/2 vs. 2/2 and the recessive model as 1/1 vs. 1/2 + 2/2. In addition to these general association tests, within-family tests of association were conducted by a modification of the method of Abecasis et al. (19) to control for potential population stratification. A sliding window analysis using a four-SNP window was used for haplotype analysis. This approach provides 16 different windows from the 19 SNPs. The MLINK program (20) was used to assign a probability of carriage of a given haplotype as previously described (21). An exhaustive analysis was done, testing all common (minor allele frequency >1%) haplotypes for all possible combinations of one, two, three, and four SNPs within each window. The haplotype frequencies were estimated with ILINK to account for the familial relationships (20).

RESULTS

Association with type 2 diabetes. The microsatellite marker DG10S478 and five SNPs (rs7901695, rs7903146, rs7895340, rs11196205, and rs12255372) that were highly associated with type 2 diabetes in the Icelandic study (1) were genotyped in a population-based sample of Pima Indians (n = 3,501). DG10S478 was monomorphic for the protective allele (designated as allele 0 in ref. 1) in full-heritage Pima Indians. The minor alleles of the five SNPs, which were the diabetes risk alleles in other populations, were less common in Pima Indians (frequencies from 0.01 to 0.1 in Pima Indians compared with 0.2 to 0.5 in Caucasians), and none were associated with type 2 diabetes under either a general or within-family model in these Native American samples (Table 1). In a recent meta-analysis containing >17,000 case and >29,000 control subjects from various populations, the odds ratio (OR) for diabetes per copy of the rs7903146 T-allele was 1.46 (95% CI 1.42–1.51) (22). In the present study, the 95% CI for the OR excludes an effect of this magnitude (OR per copy of the T-allele 1.04 [95% CI 0.82–1.32]), and the Pima OR is significantly different from the global estimate (Q = 7.62; P = 0.006); thus, the lack of association with rs7903146 in the present study does not reflect inadequate power. On the other hand, rs12255372 is so rare in Pima Indians that the power to detect an association is limited even with the present sample size. Although the OR suggests no association with rs12255372, the CIs are consistent with a fairly large effect (OR per copy of the T-allele 0.87 [0.41–1.87]).

The association of rs7903146 with type 2 diabetes in Pima Indians was not substantially changed when BMI was included as a covariate. The OR for the T-allele was 1.10 per copy (95% CI 0.85–1.41), P = 0.46, when controlled for BMI. If the analysis for type 2 diabetes was stratified by sextiles of BMI, the T-allele tended to be associated with lower type 2 diabetes prevalence among those in the lowest sextile (BMI <29.5 kg/m^2; n = 462, 125 with diabetes; OR 0.67 per copy of the T-allele) (supplemental Table 1 [available at http://dx.doi.org/10.2337/db07-0621]). Conversely, among those in the highest sextile of BMI (>44.8 kg/m^2; n = 458, 262 with diabetes), the T-allele tended to be associated with higher diabetes prevalence (OR 1.87). The P value for the rs7903146 genotype–BMI interaction is 0.002; however, given the absence of an overall association between genotype and diabetes in this population, it is unclear how to interpret a P value of this magnitude.

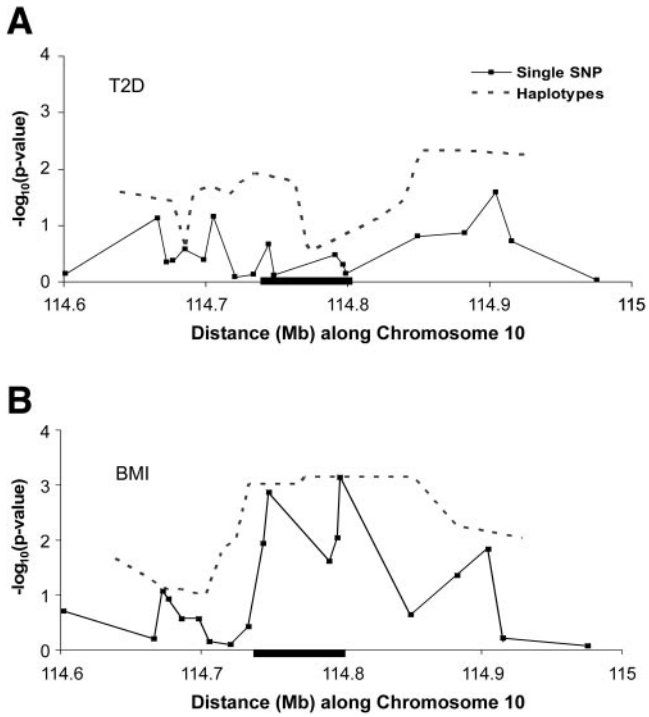

FIG. 1. Single SNP and sliding window haplotype analysis for association of TCF7L2 with type 2 diabetes and BMI. An exhaustive analysis was done, testing all common (minor allele frequency >1%) haplotypes for all possible combinations of one, two, three, and four SNPs within each window. The best *P* value within each window is plotted at the window midpoint (dashed line), along with the *P* value for the single SNP analysis (points along solid line). The box on the *x*-axis in *A* indicates the region of the Icelandic SNPs that are highly associated with type 2 diabetes in other populations. Each of the 19 SNPs listed in Tables 1 and 2 is shown as a square for the single SNP analysis.

To examine additional variation across this locus in Pima Indians, TCF7L2 was sequenced in 24 subjects, and one rare SNP predicting a Pro500Thr was identified (frequency of Thr-allele 0.03). The Pro500Thr SNP, the 5 Icelandic SNPs, and the 35 additional SNPs (all noncoding), detected either by sequencing or selected from public databases within intronic regions that were not sequenced, were genotyped in a family-based group of 1,037 Pima Indians to determine the haplotype structure across TCF7L2 (23). Genotypes from these 41 SNPs (supplemental Fig. 1) were used to select 14 representative SNPs. The Tagger algorithm (24) as implemented in Haploview was used to select representative SNPs for genotyping in the full population from among the 20 SNPs with minor allele frequency >0.2. In this analysis, $r^2 > 0.8$ was considered indicative of redundance. As had been done with the five Icelandic SNPs, the representative SNPs were genotyped in the population-based Pima Indian sample (n = 3,501). One SNP, rs1225404, was nominally associated with type 2 diabetes using a general analysis (additive P = 0.03, dominant P = 0.005) but not using a less powerful within-family analysis (additive P = 0.2, dominant P = 0.07) (Table 1).

Haplotypes were constructed from the 19 SNPs in Table 1. A sliding window approach using four SNPs per window was used for the haplotype association analysis (Fig. 1). Modest associations were observed with windows that

TABLE 1
Association between SNPs in TCF7L2 and type 2 diabetes in a Pima Indian population–based study

SNP (chr. position)	Allele 1/2	Freq. allele 2	Diabetic subjects			Diabetic subjects			*P* value: general (upper row) and within family (lower row)			
			1/1 (%)	1/2 (%)	2/2 (%)	1/1 (%)	1/2 (%)	2/2 (%)	Additive	Dominant	Recessive	Additive*
			1,394 (76.8)	390 (21.5)	32 (1.8)	1,130 (77.4)	309 (21.2)	21 (1.4)	0.71	0.30	0.94	0.97 (0.81–1.15)
(114601494)									0.73	0.34	0.98	0.95 (0.72–1.27)
rs10509966†	A/G	0.15	1,199 (70.0)	477 (27.9)	36 (2.1)	1,011 (74.1)	330 (24.2)	24 (1.8)	0.08	0.85	0.04	0.85 (0.71–1.02)
(114666170)									0.51	0.94	0.46	0.90 (0.67–1.22)
rs3862012†	T/C	0.26	964 (53.2)	704 (38.9)	141 (7.7)	815 (56.5)	523 (36.3)	102 (7)	0.44	0.74	0.42	0.95 (0.83–1.09)
(114672543)									0.85	0.71	0.64	0.98 (0.78–1.23)
rs11196152†	T/C	0.26	946 (53.4)	690 (38.9)	137 (7.7)	804 (56.5)	520 (36.5)	99 (7.0)	0.43	0.73	0.41	0.95 (0.82–1.09)
(114676795)									0.82	0.86	0.69	0.97 (0.77–1.23)
rs10509967†	A/C	0.26	909 (52.6)	690 (39.9)	130 (7.5)	775 (56.6)	506 (37.0)	88 (6.4)	0.27	0.49	0.30	0.92 (0.80–1.06)
(114685922)									0.81	0.77	0.63	0.97 (0.77–1.23)
rs3814570†	C/T	0.26	963 (53.6)	704 (39.2)	131 (7.3)	814 (56.4)	536 (37.1)	94 (6.5)	0.40	0.59	0.43	0.94 (0.82–1.08)
(114698500)									0.72	0.72	0.52	0.96 (0.77–1.20)
rs2094405†	G/A	0.17	1,244 (68.1)	517 (28.3)	65 (3.6)	1,028 (70.1)	397 (27.1)	42 (2.9)	0.07	0.11	0.15	0.87 (0.75–1.01)
(114705679)									0.79	0.42	0.97	1.03 (0.82–1.31)
rs12573128†	A/G	0.37	730 (40.8)	821 (45.9)	237 (13.3)	560 (39.1)	692 (48.3)	182 (12.7)	0.82	0.86	0.66	1.02 (0.90–1.15)
(114720787)									0.78	0.83	0.58	1.03 (0.83–1.29)
rs7895307†	A/G	0.31	889 (49.0)	744 (41.0)	180 (9.9)	690 (47.7)	634 (43.8)	123 (8.5)	0.74	0.82	0.77	1.02 (0.90–1.16)
(114733951)									0.42	0.26	0.76	0.91 (0.73–1.14)
rs7901695†	T/C	0.08	1,379 (84.9)	232 (14.3)	13 (0.8)	1,098 (83.8)	199 (15.2)	13 (1.0)	0.22	0.20	0.31	1.15 (0.92–1.44)
(114744078)									0.31	0.58	0.33	1.20 (0.85–1.69)
rs7903146†	C/T	0.08	1,415 (85.4)	226 (13.6)	16 (1.0)	1,124 (86.2)	169 (13.0)	11 (0.8)	0.76	0.66	0.83	1.04 (0.82–1.32)
(114748339)									0.92	0.94	0.89	1.02 (0.68–1.54)
rs7895340	G/A	0.10	1,482 (83.3)	280 (15.8)	18 (1.1)	1,164 (82.0)	241 (17.0)	15 (1.1)	0.33	0.40	0.41	1.11 (0.90–1.36)
(114791515)									0.49	0.67	0.54	1.12 (0.81–1.53)
rs11196205	G/C	0.10	1,514 (83.6)	279 (15.4)	17 (0.9)	1,212 (83.1)	231 (15.8)	15 (1.0)	0.49	0.26	0.66	1.07 (0.87–1.32)
(114797037)									0.76	0.70	0.82	1.05 (0.77–1.44)
rs12255372	G/T	0.01	1,747 (98.6)	26 (1.5)	0 (0)	1,407 (98.8)	18 (1.3)	0 (0)	0.72	NA	0.72	0.87 (0.41–1.86)
(114798892)									0.59	NA	0.59	0.70 (0.19–2.53)
rs7085532†	A/G	0.17	1,250 (68.6)	514 (28.2)	57 (3.1)	1,054 (72.4)	365 (25.1)	37 (2.5)	0.16	0.45	0.17	0.89 (0.76–1.05)
(114849453)									0.48	0.31	0.21	0.90 (0.68–1.20)
rs10787475†	C/T	0.44	553 (31.3)	870 (49.2)	346 (19.6)	473 (33.3)	681 (48.0)	265 (18.7)	0.14	0.18	0.25	0.91 (0.80–1.03)
(114882458)									0.43	0.80	0.35	0.92 (0.73–1.14)
rs1225404†	C/T	0.48	499 (27.4)	922 (50.6)	402 (22.1)	427 (29.2)	752 (51.4)	284 (19.4)	**0.03**	**0.005**	0.36	0.87 (0.78–0.98)
(114904655)									0.22	0.07	0.80	0.88 (0.72–1.08)
Pro500Thr	C/A	0.02	1,677 (96.7)	58 (3.3)	0 (0)	1,308 (94.7)	70 (5.2)	0 (0)	0.19	NA	0.19	1.31 (0.87–1.96)
									0.49	NA	0.49	1.27 (0.65–2.49)
rs911770†	T/A	0.35	753 (41.4)	834 (45.9)	231 (12.7)	618 (42.4)	657 (45.1)	181 (12.4)	0.92	0.99	0.89	1.01 (0.89–1.14)
(114975665)									0.93	0.71	0.90	0.99 (0.81–1.21)

P values were calculated using a general analytical model (upper value) and a within-family analytical model (lower value). *P* values adjusted for age, sex, birth year, and family membership. Significant *P* values ($P < 0.05$) are indicated by boldface. *OR (95% CI) is given per copy of allele 2; underlined alleles were the diabetes risk alleles in previous studies (ref. 1). †Representative SNP selected from supplemental Fig. 1; underlined SNPs had the strongest associations in the Icelandic study.

included the three SNPs rs7085532, rs10787475, and rs1225404, where the G- (rs7085532), T- (rs10787475), and T- (rs1225404) alleles were more common among the nondiabetic subjects compared with subjects with type 2 diabetes in the Pima population sample (frequency 0.08 vs. 0.06; additive $P = 0.005$; OR 0.68 [95% CI 0.52–0.89]). It is notable that rs1225404, which is part of this haplotype, was the only SNP nominally associated with type 2 diabetes in the single-marker analysis, where the T-allele was the protective allele (Table 1). Modest associations were also observed with windows including the C-, C-, and A-alleles for rs10787475, Pro500Thr, and rs911770, respectively, which were more common among nondiabetic subjects compared with type 2 diabetic subjects (frequency 0.22 vs. 0.20; additive $P = 0.006$; OR 0.79 [0.67–0.93]). However, none of the SNPs in these haplotypes overlapped with the most significant SNPs described by Grant et al. (1), and these modest haplotype associations could be attributed to multiple variant testing.

Association with BMI. The 19 SNPs (Icelandic and additional representative SNPs) and their haplotypes were also analyzed for associations with BMI in the Pima population sample (Fig. 1 and Table 2). Many of these subjects have been studied longitudinally and had multiple measurements of BMI; therefore, maximum BMI (without regard to diabetes status) was selected for analysis. The rare SNP rs12255372 had the strongest association with BMI, where individuals with the G-allele (frequency 0.99) had an ~4.5 kg/m^2 higher BMI than those without ($P = 0.0007$). The C-allele of rs7903146 (frequency 0.92) was also associated with increased BMI ($P = 0.001$), with a difference in BMI of ~1.2 kg/m^2 per copy of the C-allele. Although this C-allele is the low-risk allele for diabetes in most populations, it has previously been reported to be associated with higher BMI (11,25). In Pima Indians, the within-family association of higher BMI with the rs12255372 G-allele was also significant ($P = 0.007$), but this was not the case for the rs7903146 C-allele ($P = 0.35$).

Helgason et al. (25) identified a haplotype, termed HapA, that was strongly associated with BMI. This haplotype consisted of the rs10885406 A-allele and the rs7903146 C-allele. rs10885406 was not typed in the present study; however, it is in virtually complete concordance with rs7895340 in Asian populations; thus, in the current study, HapA was constructed using the rs7903146 C-allele and rs7895340 G-allele. By this definition, HapA was associated with higher BMI in Pima Indians (~1.1 kg/m^2 per copy of HapA; $P = 0.002$). However, a different haplotype, consisting of the rs7903146 C-allele and rs12255372 G-allele, provided the strongest association with BMI in the Pima Indian study (1.2 kg/m^2 per copy; $P = 0.0007$). The individual SNPs of this second haplotype, rs7903146 and rs12255372, were not highly concordant ($r^2 = 0.07$), and each remained significantly associated with BMI after controlling for the association of the other, although the statistical significance was attenuated. After controlling for the genotype at rs7903146, the rs12255372 G-allele remained associated with higher BMI ($P = 0.01$); likewise, after controlling for the rs12255372 genotype, the rs7903146 C-allele remained associated with higher BMI ($P = 0.01$). The fully parameterized haplotype model did not provide a significantly better fit than the model containing the additive effects of the two genotypes, suggesting that the information for the BMI association is in the genotypes from the two individual SNPs. In Pima Indians, the C-allele of rs7903146 was highly concordant with HapA ($r^2 = 0.91$), making it difficult to differentiate between the effects of HapA and the rs7903146 C-allele on BMI. Likewise, it is difficult to determine whether the associations of rs7903146 and rs12255372 with BMI represent the effects of a single or two distinct functional variants.

Association with pre-diabetic metabolic traits. Among the 3,501 Pima Indians in the population sample, 372 had been metabolically studied as inpatients in our clinical research center when they did not have diabetes. SNPs were analyzed for associations with quantitative traits that predict type 2 diabetes and obesity in these subjects (Table 3). The five Icelandic SNPs were not associated with any trait, with the exception of the 2-h plasma glucose level following a 75-g OGTT, which showed a nominal association ($P = 0.08–0.03$; data shown for rs7903146 and rs7895340 in Table 3), where the risk allele for type 2 diabetes in other populations had a higher 2-h plasma glucose level. Among the additional representative SNPs, the C-allele of rs7895307, which was nominally associated with higher BMI in the population sample ($P = 0.04$) (Table 2), was also nominally associated with higher percentage of body fat and BMI ($P = 0.03$ and 0.009, respectively) (Table 3) among the metabolically characterized subgroup. This C-allele was also nominally associated with a lower acute insulin response to an intravenous glucose tolerance test ($P = 0.02$). However, these associations are very modest and would not be significant if adjusted for multiple testing (19 SNPs analyzed for eight diabetes-related quantitative traits).

DISCUSSION

Although variants in TCF7L2, particularly rs7903146, have been reproducibly associated with type 2 diabetes in numerous populations (1–14), the present study shows that these variants are not strongly associated with type 2 diabetes among Pima Indians. However, HapA and the C-allele at rs7903146, which is the low-risk allele for type 2 diabetes among most populations, were associated with higher BMI in the Pima Indians, and similar associations with BMI have been reported in a few other studies (11,25). The Pima Indians have a high prevalence of obesity and type 2 diabetes, and it is possible that the higher BMI associated with the C-allele at rs7903146 overwhelms its protective effect for diabetes in this population. This seems an unlikely explanation for the lack of association between this SNP and type 2 diabetes in the Pima Indians, since the OR was largely unmodified by adjustment for BMI and since the C-allele tended to be associated with higher prevalence of type 2 diabetes among the leanest individuals in the present study, which is opposite the effect seen in most populations. It is possible that variants in TCF7L2 interact with other unidentified genetic or environmental risk factors that are highly prevalent in Pima Indians and that this results in no overall association in this population. Alternatively, since the functional consequences of alleles at rs7903146 are largely unknown, it remains possible that its association with type 2 diabetes in most populations reflects linkage disequilibrium with more distant functional alleles that have a different linkage disequilibrium pattern or are invariant in the Pima Indians. In the present study, additional representative SNPs across TCF7L2 were genotyped, and none were strongly associated with diabetes. Although it is possible that important functional variants in the unresequenced regions of the gene were not de-

TABLE 2
Association between SNPs in TCF7L2 and BMI in a Pima Indian population–based study

SNP	1/1 [1/1–2/2]	1/2 [1/1–1/2]	2/2 [1/2–2/2]	*P* value: general (upper row) and within family (lower row) Additive	Dominant	Recessive
rs477167*						
BMI (kg/m^2)	37.3 ± 8.5 (2,364)	36.8 ± 8.4 (652)	36.5 ± 8.3 (50)	0.19	0.37	0.24
Sibpair difference in BMI	1.4 ± 7.7 (17)	−0.2 ± 10.4 (546)	1.9 ± 9.1 (54)	0.94	0.55	0.83
rs10509966*						
BMI (kg/m^2)	37.3 ± 8.5 (2,064)	36.8 ± 8.5 (747)	37.7 ± 9.1 (58)	0.62	0.52	0.44
Sibpair difference in BMI	1.7 ± 8.5 (24)	−0.5 ± 10.9 (580)	0.8 ± 9.6 (51)	0.37	0.89	0.36
rs3862012*						
BMI (kg/m^2)	37.4 ± 8.6 (1,669)	37.0 ± 8.2 (1,141)	36.2 ± 8.8 (229)	0.08	0.08	0.21
Sibpair difference in BMI	−1.7 ± 10.2 (69)	0.4 ± 10.3 (661)	1.3 ± 9.0 (255)	0.94	0.15	0.51
rs11196152*						
BMI (kg/m^2)	37.4 ± 8.6 (1,641)	37.0 ± 8.3 (1,123)	36.5 ± 9.0 (222)	0.12	0.15	0.23
Sibpair difference in BMI	−2.2 ± 10.0 (68)	0.4 ± 10.3 (632)	1.5 ± 9.2 (237)	0.87	0.21	0.60
rs10509967*						
BMI (kg/m^2)	37.3 ± 8.6 (1,574)	37.0 ± 8.3 (1,107)	36.7 ± 9.0 (208)	0.27	0.41	0.34
Sibpair difference in BMI	−2.1 ± 10.5 (63)	0.5 ± 10.2 (623)	1.0 ± 9.2 (233)	0.94	0.64	0.86
rs3814570*						
BMI (kg/m^2)	37.4 ± 8.6 (1,664)	37.0 ± 8.2 (1,154)	36.7 ± 9.0 (214)	0.27	0.32	0.38
Sibpair difference in BMI	−1.5 ± 10.2 (70)	0.3 ± 9.9 (670)	1.0 ± 9.3 (244)	0.79	0.43	0.46
rs2094405*						
BMI (kg/m^2)	37.2 ± 8.3 (2,130)	37.1 ± 8.9 (853)	36.9 ± 8.1 (99)	0.69	0.99	0.66
Sibpair difference in BMI	−2.7 ± 8.4 (38)	−0.9 ± 11.2 (709)	−2.4 ± 10.0 (98)	0.15	0.50	0.19
rs12573128*						
BMI (kg/m^2)	37.2 ± 8.4 (1,209)	37.2 ± 8.7 (1,419)	36.7 ± 7.8 (385)	0.79	0.52	0.95
Sibpair difference in BMI	−1.3 ± 10.7 (87)	0.0 ± 10.7 (715)	1.0 ± 10.1 (580)	0.88	0.50	0.51
rs7895307*						
BMI (kg/m^2)	37.3 ± 8.7 (1,477)	37.2 ± 8.5 (1,295)	36.3 ± 7.5 (320)	0.37	0.07	0.87
Sibpair difference in BMI	1.3 ± 9.3 (75)	0.6 ± 10.6 (747)	2.5 ± 9.5 (273)	**0.04**	**0.0006**	0.5
rs7901695						
BMI (kg/m^2)	37.3 ± 8.5 (2,303)	36.4 ± 8.0 (401)	34.7 ± 7.4 (24)	**0.01**	0.06	**0.02**
Sibpair difference in BMI	2.7 ± 7.2 (27)	−0.4 ± 10.6 (310)	3.6 ± 7.8 (17)	0.39	**0.05**	0.62
rs7903146*						
BMI (kg/m^2)	37.4 ± 8.6 (2,367)	36.2 ± 8.1 (366)	34.2 ± 6.8 (25)	**0.001**	**0.01**	**0.004**
Sibpair difference in BMI	4.9 ± 7.5 (16)	0.7 ± 11.4 (278)	5.7 ± 7.7 (20)	0.35	**0.03**	0.56
rs7895340						
BMI (kg/m^2)	37.4 ± 8.6 (2,473)	36.4 ± 8.1 (488)	36.1 ± 7.8 (31)	**0.02**	0.29	**0.03**
Sibpair difference in BMI	3.3 ± 9.9 (26)	0.4 ± 10.7 (395)	3.8 ± 7.8 (31)	0.46	0.20	0.64
rs11196205						
BMI (kg/m^2)	37.3 ± 8.5 (2,549)	36.3 ± 8.1 (477)	36 ± 7.6 (30)	**0.009**	0.25	**0.01**
Sibpair difference in BMI	4.2 ± 9.5 (22)	0.7 ± 10.3 (396)	3.8 ± 8.4 (25)	0.28	0.19	0.38
rs12255372						
BMI (kg/m^2)	37.3 ± 8.5 (2,949)	32.8 ± 6.3 (41)	NA (0)	**0.0007**	NA	**0.0007**
Sibpair difference in BMI	NA (0)	9.5 ± 13.8 (35)	NA (0)	**0.007**	NA	**0.007**
rs7085532*						
BMI (kg/m^2)	37.3 ± 8.6 (2,148)	36.7 ± 8.2 (862)	37.3 ± 7.7 (89)	0.22	0.46	0.10
Sibpair difference in BMI	−3.6 ± 8.5 (31)	0.0 ± 10.6 (612)	−0.7 ± 10.6 (85)	0.97	0.16	0.54
rs10787475*						
BMI (kg/m^2)	37.4 ± 8.5 (944)	37.1 ± 8.6 (1,456)	36.9 ± 8.4 (583)	**0.04**	0.2	0.06
Sibpair difference in BMI	1.4 ± 9.0 (95)	0.4 ± 10.6 (547)	−0.7 ± 11.3 (463)	0.21	0.72	0.15
rs1225404*						
BMI (kg/m^2)	37.4 ± 8.5 (861)	37.3 ± 8.2 (1,570)	36.6 ± 9.1 (642)	**0.01**	**0.008**	0.19
Sibpair difference in BMI	−1.0 ± 9.6 (102)	0.4 ± 10.6 (547)	0.6 ± 10.5 (498)	0.27	0.16	0.81
Pro500Thr						
BMI (kg/m^2)	37.18 ± 8.4 (2,782)	37.32 ± 8.9 (123)	NA (0)	0.61	NA	0.61
Sibpair difference in BMI	NA (0)	1.4 ± 10.0 (97)	NA (0)	0.23	NA	0.23
rs911770*						
BMI (kg/m^2)	37.2 ± 8.2 (1,292)	37.1 ± 8.6 (1,386)	37.2 ± 8.9 (386)	0.84	0.65	0.56
Sibpair difference in BMI	−1.8 ± 10.6 (78)	−0.5 ± 10.5 (741)	−1.0 ± 10.6 (348)	0.58	0.25	0.93

Data are means ± SD (*n*) unless otherwise indicated. Alleles for each SNP are given in Table 1. For each SNP, the unadjusted mean BMI ± SD is given for subjects in each genotypic group (upper line). For within-family analyses (lower line) the mean± SD difference in BMI among genotypically discordant sib-pairs is given; the difference is computed as the BMI for the sib with the genotype listed first minus that for the sib with the genotype listed second. For rs12255372, for example, among 35 genotypically discordant sibling pairs, the BMI for the sib with the 11 (GG) genotype was on average 9.5 kg/m^2 higher than the BMI for the sib with the 12 (GT) genotype. *P* values were calculated using a general analytical model (upper value) and a within-family analytical model (lower value). *P* values adjusted for age, sex, birth year, and family membership. Significant *P* values ($P < 0.05$) are indicated by boldface. *A representative SNP selected from online Fig. 1; underlined SNPs had the strongest associations with type 2 diabetes in the Icelandic study.

TABLE 3
Association between SNPs in TCF7L2 and obesity and diabetes-related phenotypes among nondiabetic Pima Indians

	rs7903146				rs7895340				rs7895307			
	CC	CT	TT	*P*	GG	GA	AA	*P*	AA	AG	GG	*P*
Nondiabetic												
Male/females (*n*)	171/134	30/30	0/2		189/136	42/37	0/2		114/66	91/80	26/26	
Age (years)	26.6 ± 6.00	26.1 ± 6.16	28.2 ± 7.00		26.6 ± 6.01	26.4 ± 6.16	28.2 ± 7.00		26.8 ± 6.26	26.9 ± 6.04	25.5 ± 5.93	
Percent body fat*†‡	33.3 ± 8.57	33.8 ± 8.32	29.3 ± 7.92	0.24	33.1 ± 8.60	33.6 ± 8.30	29.3 ± 7.92	0.27	33.3 ± 8.54	33.1 ± 8.73	33.1 ± 8.23	**0.03**
BMI (kg/m^2)*†‡	34.3 ± 7.30	34.3 ± 7.89	26.1 ± 9.83	0.57	34.2 ± 7.33	34.4 ± 8.34	26.1 ± 9.83	0.53	34.9 ± 7.91	34.0 ± 7.42	32.5 ± 6.22	**0.009**
Fasting plasma glucose (mmol/l)*†‡§	5.01 ± 0.55	4.96 ± 0.57	5.36 ± 0.51	0.74	4.99 ± 0.55	4.99 ± 0.55	5.36 ± 0.51	0.88	4.98 ± 0.53	5.03 ± 0.57	4.97 ± 0.54	0.87
2-h plasma glucose (mmol/l)*†‡§	6.79 ± 1.69	7.13 ± 1.60	8.22	0.08	6.77 ± 1.69	7.19 ± 1.64	8.22	**0.03**	6.72 ± 1.66	7.05 ± 1.75	6.70 ± 1.56	0.82
Log_{10} fasting plasma insulin (μU/ml)*†‡§	1.57 ± 0.21	1.58 ± 0.22	1.47 ± 0.38	0.54	1.56 ± 0.21	1.56 ± 0.22	1.47 ± 0.38	0.8	1.57 ± 0.21	1.55 ± 0.21	1.58 ± 0.21	0.55
Log_{10} 2-h plasma insulin (μU/ml)*†‡§	2.20 ± 0.34	2.21 ± 0.36	2.51 ± 0.07	0.58	2.20 ± 0.34	2.18 ± 0.35	2.51 ± 0.07	0.94	2.22 ± 0.34	2.18 ± 0.34	2.18 ± 0.32	0.28
Log_{10} glucose disposal ($mg \cdot kg\ EMBS^{-1} \cdot min^{-1}$)*†‡§	0.54 ± 0.11	0.54 ± 0.12	0.57 ± 0.05	0.94	0.54 ± 0.12	0.54 ± 0.11	0.57 ± 0.05	0.8	0.54 ± 0.12	0.54 ± 0.11	0.56 ± 0.12	0.54
Normal glucose tolerant												
Male/female (*n*)	138/86	24/14	0/1		153/90	32/16	0/1		88/45	77/44	21/15	
Log_{10} AIR (μU/ml)*†‡§‖	2.36 ± 0.28	2.32 ± 0.27	2.01	0.06	2.35 ± 0.27	2.33 ± 0.27	2.01	0.07	2.33 ± 0.30	2.33 ± 0.25	2.45 ± 0.26	**0.02**
Log_{10} 30-min plasma insulin (μU/ml)*†‡§‖	2.35 ± 0.27	2.34 ± 0.24	2.10	0.15	2.35 ± 0.26	2.33 ± 0.23	2.10	0.09	2.35 ± 0.26	2.32 ± 0.23	2.42 ± 0.30	0.11

Data are raw means ± SD for each trait grouped by genotype. *P* values were calculated for the adjusted means. Covariates for adjustments are listed as: *age, †sex, ‡family membership, §percentage of body fat, ‖glucose disposal rate, and ¶30 min glucose levels. Significant *P* values (<0.05) are shown in boldface. Analysis of early insulin secretion (AIR and 30-min plasma insulin during an OGTT) is restricted to subjects with normal glucose tolerance. EMBS, estimated metabolic body size; IVGTT, intravenous glucose tolerance test.

tected, the present data do not support a major role for variants in TCF7L2 in the development of type 2 diabetes in this population.

ACKNOWLEDGMENTS

This study was supported by the intramural research program of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. M.T., Y.L.M., and L.M. are supported by grants from the American Diabetes Association.

REFERENCES

1. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K: Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38:320–323, 2006
2. Mayans S, Lackovic K, Lindgren P, Ruikka K, Agren A, Eliasson M, Holmberg D: TCF7L2 polymorphisms are associated with type 2 diabetes in northern Sweden. *Eur J Hum Genet* 15:342–346, 2007
3. Marzi C, Huth C, Kolz M, Grallert H, Meisinger C, Wichmann HE, Rathmann W, Herder C, Illig T: Variants of the transcription factor 7-like 2 gene (TCF7L2) are strongly associated with type 2 diabetes but not with the metabolic syndrome in the MONICA/KORA surveys. *Horm Metab Res* 39:46–52, 2007
4. Humphries SE, Gable D, Cooper JA, Ireland H, Stephens JW, Hurel SJ, Li KW, Palmen J, Miller MA, Cappuccio FP, Elkeles R, Godsland I, Miller GJ, Talmud PJ: Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. *J Mol Med* 84:1–10, 2006
5. Vliet-Ostaptchouk JV, Shiri-Sverdlov R, Zhernakova A, Strengman E, van Haeften TW, Hofker MH, Wijmenga C: Association of variants of transcription factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda cohort. *Diabetologia* 50:59–62, 2007
6. Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S, Balkau B, Charpentier G, Pattou F, Stetsyuk V, Scharfmann R, Staels B, Fruhbeck G, Froguel P: Transcription factor TCF7L2 genetic study in the French population: expression in human β-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 55:2903–2908, 2006
7. Damcott CM, Pollin TI, Reinhart LJ, Ott SH, Shen H, Silver KD, Mitchell BD, Shuldiner AR: Polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes* 55:2654–2659, 2006
8. Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, Duren WL, Chines PS, Stringham HM, Erdos MR, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Collins FS, Boehnke M: Association of transcription factor 7-like 2 (*TCF7L2*) variants with type 2 diabetes in a Finnish sample. *Diabetes* 55:2649–2653, 2006
9. Zhang C, Qi L, Hunter DJ, Meigs JB, Manson JE, van Dam RM, Hu FB: Variant of transcription factor 7-like 2 (*TCF7L2*) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. *Diabetes* 55:2645–2648, 2006
10. Groves CJ, Zeggini E, Minton J, Frayling TM, Weedon MN, Rayner NW, Hitman GA, Walker M, Wiltshire S, Hattersley AT, McCarthy MI: Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes* 55:2640–2644, 2006
11. Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D: TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355:241–250, 2006
12. Chandak GR, Janipalli CS, Bhaskar S, Kulkarni SR, Mohankrishna P, Hattersley AT, Frayling TM, Yajnik CS: Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. *Diabetologia* 50:63–67, 2007
13. Horikoshi M, Hara K, Ito C, Nagai R, Froguel P, Kadowaki T: A genetic variation of the transcription factor 7-like 2 gene is associated with risk of type 2 diabetes in the Japanese population. *Diabetologia* 50:747–751, 2007
14. Hayashi T, Iwamoto Y, Kaku K, Hirose H, Maeda S: Replication study for the association of TCF7L2 with susceptibility to type 2 diabetes in a Japanese population. *Diabetologia* 50:980–984, 2007
15. 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 Epidemiol* 108:497–505, 1978
16. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
17. 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
18. 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
19. Abecasis GR, Cardon LR, Cookson WOC: A general test of association for quantitative traits in nuclear families. *Am J Hum Gene* 66:279–292, 2000
20. Lathrop GM, Lalouel JM: Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 36:460–465, 1980
21. Vozarova de Courten B, Hanson RL, Funahashi T, Lindsay RS, Matsuzawa Y, Tanaka S, Thameem F, Gruber JD, Froguel P, Wolford JK: Polymorphisms in the adiponectin gene *ACDC* are not associated with type 2 diabetes in Pima Indians. *Diabetes* 54:284–289, 2005
22. Cauchi S, El Achhab YE, Choquet H, Dina C, Krempler F, Weitgasser R, Nejjari C, Patsch W, Chikri M, Meyre D, Froguel P: TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med* 88:777–782, 2007
23. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH, Knowler WC: An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet* 63:1130–1138, 1998
24. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D: Efficiency and power in genetic association studies. *Nat Genet* 37:1217–1223, 2005
25. Helgason A, Palsson S, Thorleifsson G, Grant SF, Emilsson V, Gunnarsdottir S, Adeyemo A, Chen Y, Chen G, Reynisdottir I, Benediktsson R, Hinney A, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Schafer H, Faruque M, Doumatey A, Zhou J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Sigurdsson G, Hebebrand J, Pedersen O, Thorsteinsdottir U, Gulcher JR, Kong A, Rotimi C, Stefansson K: Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet* 39:218–225, 2007