

***TCF7L2* is Not a Major Susceptibility Gene for  
Type 2 Diabetes in Pima Indians: An Analysis of 3501  
Individuals**

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**Running title: *TCF7L2* and type 2 diabetes in Pima Indians**

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## **ABSTRACT**

### **OBJECTIVE**

The transcription factor 7-like 2 gene (*TCF7L2*) was initially reported to be associated with type 2 diabetes (T2D) in Icelandic, Danish and U.S. populations. We investigated whether *TCF7L2* also has a role in T2D susceptibility in the Pima Indians.

### **RESEARCH DESIGN AND METHODS**

The 6 variants reported to be associated with T2D in the Icelandic study were genotyped in a population-based sample of 3501 Pima Indians (1561 subjects had T2D and 1940 were non-diabetic). In addition, the coding and promoter regions of *TCF7L2* were sequenced in 24 Pima subjects. The 1 variant identified by sequencing, 35 additional database variants positioned in introns, and the 6 variants reported in the Icelandic study were genotyped in Pima families to determine the haplotype structure of *TCF7L2* among Pimas. Fourteen representative variants were selected and genotyped in 3501 Pima Indians.

### **RESULTS**

The 6 variants initially reported to be associated with T2D were less common in Pimas as compared to samples of European origin, and none were associated with T2D. One representative variant, rs1225404, was nominally associated with T2D 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 body mass index (BMI); in particular, rs12255372 was associated in both a general and within family analysis (both  $P = 0.0007$ ). Modest associations were also found with traits predictive for T2D.

### **CONCLUSIONS**

Variation within *TCF7L2* does not confer major risk for T2D among the Pima Indian population.

A microsatellite marker (DG10S478) within intron 3 of the *TCF7L2* gene and 5 intronic single nucleotide polymorphisms (SNPs) have been reported to be highly associated with T2D in subjects from Iceland, Denmark, and the U.S. (1). Associations with these specific variants and T2D have subsequently been replicated consistently and robustly in multiple studies involving subjects of European origin (2-11), Asian Indians (12), and Japanese (13, 14). To investigate whether variation in *TCF7L2* also has a major role in T2D susceptibility in Pima Indians, a population with an extraordinarily high prevalence of T2D, 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

### Subjects and clinical characteristics

All subjects are Pima Indians who are participants in our ongoing longitudinal study of T2D 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 1037 subjects (578 with T2D, 459 non-diabetic) from 332 nuclear families in 112 pedigrees. The population-based sample consisted of 3501 full-heritage Pima Indians for whom there was DNA and information on diabetes status and BMI. This sample consisted of 1,561 subjects with T2D (male/female = 580/981; mean  $\pm$  SD BMI =  $38.5 \pm 8.4$  kg/m<sup>2</sup>, age of onset =  $37.2 \pm 12.1$  years) and 1,940 non-diabetic subjects (male/female = 902/1038; mean  $\pm$  SD BMI =  $35.7 \pm 8.2$  kg/m<sup>2</sup>, age = 31.1

$\pm 14.5$  years), as defined by a 2 hour oral glucose tolerance test (OGTT) (16). Eight hundred and ninety six subjects overlapped between the family and population-based samples. Among the non-diabetic 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, 30, 60, 120, and 180 min plasma glucose and insulin concentrations. The acute insulin response (AIR) was measured by collecting blood samples prior to a 25 g glucose bolus infusion and at 3, 4, 5, 6, 8, and 10 min afterwards. AIR 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 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 > 100bps into each intron, the 5' and 3'-UTRs, and 2kb of the upstream (putative promoter) region were sequenced in DNA samples from 24 non-first-degree related Pima Indians (12 with age of T2D onset < 25 years and 12 confirmed to be non-diabetic at the age  $\geq$  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 T2D 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. sibship). 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 versus 2/2 and the recessive model as 1/1 versus 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.* to control for potential population stratification (19). A sliding window analysis using a 4 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 described previously (21). An exhaustive analysis was done which tests all common (minor allele frequency > 1%) haplotypes for all possible combinations of 1, 2, 3, and 4 SNPs within each window. The haplotype frequencies were estimated with ILINK to account for the familial relationships (20).

## RESULTS AND DISCUSSION

### Association with T2D

The microsatellite marker DG10S478 and 5 SNPs (rs7901695,

rs7903146, rs7895340, rs11196205, and rs12255372) that were highly associated with T2D in the Icelandic study (1) were genotyped in a population-based sample of Pima Indians (N = 3501). DG10S478 was monomorphic for the “protective allele” (designated allele 0 in reference 1) in full-heritage Pima Indians. The minor alleles of the 5 SNPs, which were the diabetes-risk alleles in other populations, were less common in Pima Indians (frequencies from 0.01-0.1 in Pimas as compared to 0.2-0.5 in Caucasians), and none were associated with T2D under either a general or within family model in these Native American samples (Table 1, Icelandic SNPs underlined). In a recent meta-analysis containing > 17,000 cases and > 29,000 controls 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% confidence interval for the OR excludes an effect of this magnitude (OR = 1.04 per copy of the T allele, 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 Pimas that the power to detect an association is limited even with the present sample size. Although, the OR suggests no association with rs12255372, the confidence intervals are consistent with a fairly large effect (OR = 0.87 per copy of the T allele, 95% CI = 0.41-1.87).

The association of rs7903146 with T2D 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 T2D was stratified by sextiles of BMI, and the T allele tended to be associated with lower T2D prevalence among those in the

lowest sextile (BMI < 29.5 kg/m<sup>2</sup>, N = 462, 125 with diabetes, OR = 0.67 per copy of the T allele) (online Table 1 [available at <http://diabetes.diabetesjournals.org>]).

Conversely, among those in the highest sextile of BMI (> 44.8 kg/m<sup>2</sup>, 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 an unadjusted *P*-value of this magnitude.

To examine additional variation across this locus in Pima Indians, *TCF7L2* was sequenced in 24 subjects and 1 rare SNP predicting a Pro500Thr was identified (frequency of Thr allele = 0.03). The Pro500Thr SNP, the 5 Icelandic SNPs, and 35 additional SNPs (all non-coding) detected either by sequencing or selected from public databases within intronic regions that were not sequenced, were genotyped in a family-based group of 1037 Pima Indians to determine the haplotype structure across *TCF7L2* (23). Genotypes from these 41 SNPs (online Figure 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 redundancy. As had been done with the 5 Icelandic SNPs, the representative SNPs were genotyped in the population-based Pima Indian sample (N = 3501). One SNP rs1225404 was nominally associated with T2D 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 4 SNPs per window was used for the haplotype association analysis (Figure 1). Modest associations were observed with windows that included the 3 SNPs rs7085532, rs10787475, and rs1225404 where the G (rs7085532), T (rs10787475), and T (rs1225404) alleles were more common among the non-diabetic subjects as compared to the subjects with T2D 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 T2D 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 the non-diabetic subjects as compared to the subjects with T2D (frequency = 0.22 vs. 0.20; additive *P* = 0.006, OR = 0.79, 95% CI = 0.67-0.93). However, none of the SNPs in these haplotypes overlapped with most the significant SNPs described in (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 (Figure 1, Table 2). Many of these subjects have been studied longitudinally and had multiple measurements of BMI; therefore, maximum BMI (without regard to diabetic 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 ~ 4.5 kg/m<sup>2</sup> higher BMI than those without the allele (*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  $\sim 1.2 \text{ kg/m}^2$  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, so in the current study HapA was constructed using the rs7903146 C and rs7895340 G alleles. By this definition, HapA was associated with higher BMI in Pima Indians ( $\sim 1.1 \text{ kg/m}^2$  per copy of HapA,  $P = 0.002$ ). However, a different haplotype, consisting of the rs7903146 C and rs12255372 G alleles, provided the strongest association with BMI in the Pima study ( $1.2 \text{ kg/m}^2$  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 Prediabetic Metabolic Traits

Among the 3501 Pima Indians in the population sample, 372 had been metabolically studied as inpatients in our clinical research center when they were non-diabetic. SNPs were analyzed for associations with quantitative traits that predict T2D and obesity in these subjects (Table 3). The 5 Icelandic SNPs were not associated with any trait, with the exception of the 2 hour 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 T2D in other populations had a higher 2 hour 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 (IVGTT) ( $P = 0.02$ ). However, these associations are very modest and would not be significant if adjusted for multiple testing (19 SNPs analyzed for 8 diabetes-related quantitative traits).

Although variants in *TCF7L2*, particularly rs7903146, have been

reproducibly associated with T2D in numerous populations (1-14), the present study shows that these variants are not strongly associated with T2D among the Pima Indians. However, HapA and the C allele at rs7903146, which is the low risk allele for T2D among most populations, were associated with higher BMI in the Pimas, and similar associations with BMI have been reported in a few other studies (11,25). The Pimas have a high prevalence of obesity and T2D 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 T2D in the Pimas, since the OR was largely unmodified by adjustment for BMI and the C allele tended to be associated with higher prevalence of T2D among the leanest individuals in the present study which is opposite of 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

the Pimas 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 T2D in most populations reflects linkage disequilibrium with more distant functional alleles that have a different linkage disequilibrium pattern or are invariant in the Pimas. 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 un-resequenced regions of the gene were not detected, the present data do not support a major role for variants in *TCF7L2* in the development of T2D in this population.

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## Tables legends

### Table 1. Association between SNPs in *TCF7L2* and T2D in a Pima Indian Population-Based Study

\* indicates a representative SNP selected from Online Figure 1; underlined SNPs had the strongest associations in the Icelandic study. *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 ( $P < 0.05$ ) indicated in bold. #Odds Ratio (OR) is given per copy of allele 2. The underlined alleles were the diabetes-risk alleles in previous studies (1).

### Table 2. Association between SNPs in *TCF7L2* and BMI in a Pima Indian Population-Based Study

Alleles for each SNP are given in Table 1. \* indicates a representative SNP selected from Online Figure 1; underlined SNPs had the strongest associations with T2D in the Icelandic study. For each SNP, the unadjusted mean BMI  $\pm$  SD is given for subjects in each genotypic group (upper line). For within-family analyses (lower line) the mean  $\pm$  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<sup>2</sup> 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 ( $P < 0.05$ ) indicated in bold.

### Table 3. Association between SNPs in *TCF7L2* and obesity and diabetes-related phenotypes among non-diabetic Pima Indians

Data are raw means  $\pm$  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, ¶30 min glucose levels. Significant *P* values ( $< 0.05$ ) are shown in bold. Analysis of early insulin secretion (AIR and 30 minute plasma insulin during an OGTT) is restricted to subjects with normal glucose tolerance. IVGTT = intravenous glucose tolerance test. EMBS = estimated metabolic body size.

**TABLE 1.** Association between SNPs in *TCF7L2* and T2D in a Pima Indian Population-Based Study

SNP (Chr. Position)	Allele 1/2	Freq. Allele 2	Non-Diabetic			Diabetic			P value			General (upper) Within Family (lower) #OR (95% CI)
			1/1(%)	1/2(%)	2/2(%)	1/1(%)	1/2(%)	2/2(%)	Additive	Dominant	Recessive	Additive
rs477167* (114601494)	C/G	0.13	1394(76.8)	390(21.5)	32(1.8)	1130(77.4)	309(21.2)	21(1.4)	0.71	0.30	0.94	0.97(0.81,1.15)
rs10509966* (114666170)	A/G	0.15	1199(70.0)	477(27.9)	36(2.1)	1011(74.1)	330(24.2)	24(1.8)	0.73	0.34	0.98	0.95(0.72,1.27)6
rs3862012* (114672543)	T/C	0.26	964(53.2)	704(38.9)	141(7.7)	815(56.5)	523(36.3)	102(7)	0.08	0.85	0.04	0.85(0.71,1.02)
rs11196152* (114676795)	T/C	0.26	946(53.4)	690(38.9)	137(7.7)	804(56.5)	520(36.5)	99(7.0)	0.51	0.94	0.46	0.90(0.67,1.22)
rs10509967* (114685922)	A/C	0.26	909(52.6)	690(39.9)	130(7.5)	775(56.6)	506(37.0)	88(6.4)	0.44	0.74	0.42	0.95(0.83,1.09)
rs3814570* (114698500)	C/T	0.26	963(53.6)	704(39.2)	131(7.3)	814(56.4)	536(37.1)	94(6.5)	0.85	0.71	0.64	0.98(0.78,1.23)
rs2094405* (114705679)	G/A	0.17	1244(68.1)	517(28.3)	65(3.6)	1028(70.1)	397(27.1)	42(2.9)	0.43	0.73	0.41	0.95(0.82,1.09)
rs12573128* (114720787)	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	0.69	0.97(0.77,1.23)
rs7895307* (114733951)	A/G	0.31	889(49.0)	744(41.0)	180(9.9)	690(47.7)	634(43.8)	123(8.5)	0.27	0.49	0.30	0.92(0.80,1.06)
rs7901695* (114744078)	T/C	0.08	1379(84.9)	232(14.3)	13(0.8)	1098(83.8)	199(15.2)	13(1.0)	0.81	0.77	0.63	0.97(0.77,1.23)
rs7903146* (114748339)	C/T	0.08	1415(85.4)	226(13.6)	16(1.0)	1124(86.2)	169(13.0)	11(0.8)	0.40	0.59	0.43	0.94(0.82,1.08)
rs7895340 (114791515)	G/A	0.10	1482(83.3)	280(15.8)	18(1.1)	1164(82.0)	241(17.0)	15(1.1)	0.72	0.72	0.52	0.96(0.77,1.20)
rs11196205 (114797037)	G/C	0.10	1514(83.6)	279(15.4)	17(0.9)	1212(83.1)	231(15.8)	15(1.0)	0.07	0.11	0.15	0.87(0.75,1.01)
									0.79	0.42	0.97	1.03(0.82,1.31)
									0.82	0.86	0.66	1.02(0.90,1.15)
									0.78	0.83	0.58	1.03(0.83,1.29)
									0.74	0.82	0.77	1.02(0.90,1.16)
									0.42	0.26	0.76	0.91(0.73,1.14)
									0.22	0.20	0.31	1.15(0.92,1.44)
									0.31	0.58	0.33	1.20(0.85,1.69)
									0.76	0.66	0.83	1.04(0.82,1.32)
									0.92	0.94	0.89	1.02(0.68,1.54)
									0.33	0.40	0.41	1.11(0.90,1.36)
									0.49	0.67	0.54	1.12(0.81,1.53)
									0.49	0.26	0.66	1.07(0.87,1.32)
									0.76	0.70	0.82	1.05(0.77,1.44)

<u>rs12255372</u> (114798892)	G/T	0.01	1747(98.6)	26(1.5)	0(0)	1407(98.8)	18(1.3)	0(0)	0.72	NA	0.72	0.87(0.41,1.86)
rs7085532*	A/G	0.17	1250(68.6)	514(28.2)	57(3.1)	1054(72.4)	365(25.1)	37(2.5)	0.59	NA	0.59	0.70(0.19,2.53)
(114849453)									0.16	0.45	0.17	0.89(0.76,1.05)
rs10787475*	C/T	0.44	553(31.3)	870(49.2)	346(19.6)	473(33.3)	681(48.0)	265(18.7)	0.48	0.31	0.21	0.90(0.68,1.20)
(114882458)									0.14	0.18	0.25	0.91(0.80,1.03)
rs1225404*	C/T	0.48	499(27.4)	922(50.6)	402(22.1)	427(29.2)	752(51.4)	284(19.4)	0.43	0.80	0.35	0.92(0.73,1.14)
(114904655)									<b>0.03</b>	<b>0.005</b>	0.36	0.87(0.78,0.98)
Pro500Thr	C/A	0.02	1677(96.7)	58(3.3)	0(0)	1308(94.7)	70(5.2)	0(0)	0.22	0.07	0.80	0.88(0.72,1.08)
rs911770*	T/A	0.35	753(41.4)	834(45.9)	231(12.7)	618(42.4)	657(45.1)	181(12.4)	0.19	NA	0.19	1.31(0.87,1.96)
(114975665)									0.49	NA	0.49	1.27(0.65,2.49)
									0.92	0.99	0.89	1.01(0.89,1.14)
									0.93	0.71	0.90	0.99(0.81,1.21)

**TABLE 2.** Association between SNPs in *TCF7L2* and BMI in a Pima Indian Population-Based Study

SNP	BMI- Mean $\pm$ SD (N) (upper)			P Value	General (upper)		Within family (lower)
	[Sib-Pair Difference in BMI- Mean $\pm$ SD (N)] (lower)				family (lower)		
	1/1 [1/1-2/2]	1/2 [1/1-1/2]	2/2 [1/2-2/2]		Additive	Dominant	
rs477167*	37.3 $\pm$ 8.5 (2364)	36.8 $\pm$ 8.4 (652)	36.5 $\pm$ 8.3 (50)	0.19	0.37	0.24	
	[1.4 $\pm$ 7.7 (17)]	[-0. $\pm$ 10.4 (546)]	[1.9 $\pm$ 9.1 (54)]	0.94	0.55	0.83	
rs10509966*	37.3 $\pm$ 8.5 (2064)	36.8 $\pm$ 8.5 (747)	37.7 $\pm$ 9.1 (58)	0.62	0.52	0.44	
	[1.7 $\pm$ 8.5 (24)]	[-0.5 $\pm$ 10.9 (580)]	[0.8 $\pm$ 9.6 (51)]	0.37	0.89	0.36	
rs3862012*	37.4 $\pm$ 8.6 (1669)	37.0 $\pm$ 8.2 (1141)	36.2 $\pm$ 8.8 (229)	0.08	0.08	0.21	
	[-1.7 $\pm$ 10.2 (69)]	[0.4 $\pm$ 10.3 (661)]	[1.3 $\pm$ 9.0 (255)]	0.94	0.15	0.51	
rs11196152*	37.4 $\pm$ 8.6 (1641)	37.0 $\pm$ 8.3 (1123)	36.5 $\pm$ 9.0 (222)	0.12	0.15	0.23	
	[-2.2 $\pm$ 10.0 (68)]	[0.4 $\pm$ 10.3 (632)]	[1.5 $\pm$ 9.2 (237)]	0.87	0.21	0.60	
rs10509967*	37.3 $\pm$ 8.6 (1574)	37.0 $\pm$ 8.3 (1107)	36.7 $\pm$ 9.0 (208)	0.27	0.41	0.34	
	[-2.1 $\pm$ 10.5 (63)]	[0.5 $\pm$ 10.2 (623)]	[1.0 $\pm$ 9.2 (233)]	0.94	0.64	0.86	
rs3814570*	37.4 $\pm$ 8.6 (1664)	37.0 $\pm$ 8.2 (1154)	36.7 $\pm$ 9.0 (214)	0.27	0.32	0.38	
	[-1.5 $\pm$ 10.2 (70)]	[0.3 $\pm$ 9.9 (670)]	[1.0 $\pm$ 9.3 (244)]	0.79	0.43	0.46	
rs2094405*	37.2 $\pm$ 8.3 (2130)	37.1 $\pm$ 8.9 (853)	36.9 $\pm$ 8.1 (99)	0.69	0.99	0.66	
	[-2.7 $\pm$ 8.4 (38)]	[-0.9 $\pm$ 11.2 (709)]	[-2. 4 $\pm$ 10.0 (98)]	0.15	0.50	0.19	
rs12573128*	37.2 $\pm$ 8.4 (1209)	37.2 $\pm$ 8.7 (1419)	36.7 $\pm$ 7.8 (385)	0.79	0.52	0.95	
	[-1.3 $\pm$ 10.7 (87)]	[0.0 $\pm$ 10.7 (715)]	[1.0 $\pm$ 10.1 (580)]	0.88	0.50	0.51	
rs7895307*	37.3 $\pm$ 8.7 (1477)	37.2 $\pm$ 8.5 (1295)	36.3 $\pm$ 7.5 (320)	0.37	0.07	0.87	
	[1.3 $\pm$ 9.3 (75)]	[0.6 $\pm$ 10.6 (747)]	[2.5 $\pm$ 9.5 (273)]	<b>0.04</b>	<b>0.0006</b>	0.5	
<u>rs7901695</u>	37.3 $\pm$ 8.5 (2303)	36.4 $\pm$ 8.0 (401)	34.7 $\pm$ 7.4 (24)	<b>0.01</b>	0.06	<b>0.02</b>	
	[2.7 $\pm$ 7.2 (27)]	[-0.4 $\pm$ 10.6 (310)]	[3.6 $\pm$ 7.8 (17)]	0.39	<b>0.05</b>	0.62	
<u>rs7903146*</u>	37.4 $\pm$ 8.6 (2367)	36.2 $\pm$ 8.1 (366)	34.2 $\pm$ 6.8 (25)	<b>0.001</b>	<b>0.01</b>	<b>0.004</b>	
	[4.9 $\pm$ 7.5 (16)]	[0.7 $\pm$ 11.4 (278)]	[5.7 $\pm$ 7.7 (20)]	0.35	<b>0.03</b>	0.56	
<u>rs7895340</u>	37.4 $\pm$ 8.6 (2473)	36.4 $\pm$ 8.1 (488)	36.1 $\pm$ 7.8 (31)	<b>0.02</b>	0.29	<b>0.03</b>	
	[3.3 $\pm$ 9.9 (26)]	[0.4 $\pm$ 10.7 (395)]	[3.8 $\pm$ 7.8 (31)]	0.46	0.20	0.64	
<u>rs11196205</u>	37.3 $\pm$ 8.5 (2549)	36.3 $\pm$ 8.1 (477)	36 $\pm$ 7.6 (30)	<b>0.009</b>	0.25	<b>0.01</b>	
	[4.2 $\pm$ 9.5 (22)]	[0.7 $\pm$ 10.3 (396)]	[3.8 $\pm$ 8.4 (25)]	0.28	0.19	0.38	
<u>rs12255372</u>	37.3 $\pm$ 8.5 (2949)	32.8 $\pm$ 6.3 (41)	NA (0)	<b>0.0007</b>	NA	<b>0.0007</b>	
	[NA (0)]	[9.5 $\pm$ 13.8 (35)]	[NA (0)]	<b>0.007</b>	NA	<b>0.007</b>	
rs7085532*	37.3 $\pm$ 8.6 (2148)	36.7 $\pm$ 8.2 (862)	37.3 $\pm$ 7.7 (89)	0.22	0.46	0.10	
	[-3.6 $\pm$ 8.5 (31)]	[0.0 $\pm$ 10.6 (612)]	[-0.7 $\pm$ 10.6 (85)]	0.97	0.16	0.54	
rs10787475*	37.4 $\pm$ 8.5 (944)	37.1 $\pm$ 8.6 (1456)	36.9 $\pm$ 8.4 (583)	<b>0.04</b>	0.2	0.06	
	[1.4 $\pm$ 9.0 (95)]	[0.4 $\pm$ 10.6 (547)]	[-0.7 $\pm$ 11.3 (463)]	0.21	0.72	0.15	
rs1225404*	37.4 $\pm$ 8.5 (861)	37.3 $\pm$ 8.2 (1570)	36.6 $\pm$ 9.1 (642)	<b>0.01</b>	<b>0.008</b>	0.19	
	[-1.0 $\pm$ 9.6 (102)]	[0.4 $\pm$ 10.6 (547)]	[0.6 $\pm$ 10.5 (498)]	0.27	0.16	0.81	
Pro500Thr	37.18 $\pm$ 8.4 (2782)	37.32 $\pm$ 8.9 (123)	NA (0)	0.61	NA	0.61	
	[NA (0)]	[1.4 $\pm$ 10.0 (97)]	[NA (0)]	0.23	NA	0.23	
rs911770*	37.2 $\pm$ 8.2 (1292)	37.1 $\pm$ 8.6 (1386)	37.2 $\pm$ 8.9 (386)	0.84	0.65	0.56	
	[-1.8 $\pm$ 10.6 (78)]	[-0.5 $\pm$ 10.5 (741)]	[-1.0 $\pm$ 10.6 (348)]	0.58	0.25	0.93	

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

	rs7903146 Mean $\pm$ SD				rs7895340 Mean $\pm$ SD				rs7895307 Mean $\pm$ SD			
	CC	CT	TT	<i>P</i>	GG	GA	AA	<i>P</i>	AA	AG	GG	<i>P</i>
Non-diabetic												
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 $\pm$ 6.00	26.1 $\pm$ 6.16	28.2 $\pm$ 7.00		26.6 $\pm$ 6.01	26.4 $\pm$ 6.16	28.2 $\pm$ 7.00		26.8 $\pm$ 6.26	26.9 $\pm$ 6.04	25.5 $\pm$ 5.93	
Percent Body Fat <sup>**†</sup>	33.3 $\pm$ 8.57	33.8 $\pm$ 8.32	29.3 $\pm$ 7.92	0.24	33.1 $\pm$ 8.60	33.6 $\pm$ 8.30	29.3 $\pm$ 7.92	0.27	33.3 $\pm$ 8.54	33.1 $\pm$ 8.73	33.1 $\pm$ 8.23	<b>0.03</b>
BMI (kg/m <sup>2</sup> ) <sup>**†</sup>	34.3 $\pm$ 7.30	34.3 $\pm$ 7.89	26.1 $\pm$ 9.83	0.57	34.2 $\pm$ 7.33	34.4 $\pm$ 8.34	26.1 $\pm$ 9.83	0.53	34.9 $\pm$ 7.91	34.0 $\pm$ 7.42	32.5 $\pm$ 6.22	<b>0.009</b>
Fasting plasma glucose (mmol/l) <sup>**†‡§</sup>	5.01 $\pm$ 0.55	4.96 $\pm$ 0.57	5.36 $\pm$ 0.51	0.74	4.99 $\pm$ 0.55	4.99 $\pm$ 0.55	5.36 $\pm$ 0.51	0.88	4.98 $\pm$ 0.53	5.03 $\pm$ 0.57	4.97 $\pm$ 0.54	0.87
2 h plasma glucose (mmol/l) <sup>**†‡§</sup>	6.79 $\pm$ 1.69	7.13 $\pm$ 1.60	8.22	0.08	6.77 $\pm$ 1.69	7.19 $\pm$ 1.64	8.22	<b>0.03</b>	6.72 $\pm$ 1.66	7.05 $\pm$ 1.75	6.70 $\pm$ 1.56	0.82
Log <sub>10</sub> Fasting plasma insulin ( $\mu$ U/ml) <sup>**†‡§</sup>	1.57 $\pm$ 0.21	1.58 $\pm$ 0.22	1.47 $\pm$ 0.38	0.54	1.56 $\pm$ 0.21	1.56 $\pm$ 0.22	1.47 $\pm$ 0.38	0.8	1.57 $\pm$ 0.21	1.55 $\pm$ 0.21	1.58 $\pm$ 0.21	0.55
Log <sub>10</sub> 2 h plasma insulin ( $\mu$ U/ml) <sup>**†‡§</sup>	2.20 $\pm$ 0.34	2.21 $\pm$ 0.36	2.51 $\pm$ 0.07	0.58	2.20 $\pm$ 0.34	2.18 $\pm$ 0.35	2.51 $\pm$ 0.07	0.94	2.22 $\pm$ 0.34	2.18 $\pm$ 0.34	2.18 $\pm$ 0.32	0.28
Log <sub>10</sub> Glucose disposal (mg·kg EMBS <sup>-1</sup> ·min <sup>-1</sup> ) <sup>**†‡§</sup>	0.54 $\pm$ 0.11	0.54 $\pm$ 0.12	0.57 $\pm$ 0.05	0.94	0.54 $\pm$ 0.12	0.54 $\pm$ 0.11	0.57 $\pm$ 0.05	0.8	0.54 $\pm$ 0.12	0.54 $\pm$ 0.11	0.56 $\pm$ 0.12	0.54
Normal Glucose Tolerant												
Male/females (n)	138/86	24/14	0/1		153/90	32/16	0/1		88/45	77/44	21/15	
Log <sub>10</sub> AIR ( $\mu$ U/ml) <sup>**†‡§  </sup>	2.36 $\pm$ 0.28	2.32 $\pm$ 0.27	2.01	0.06	2.35 $\pm$ 0.27	2.33 $\pm$ 0.27	2.01	0.07	2.33 $\pm$ 0.30	2.33 $\pm$ 0.25	2.45 $\pm$ 0.26	<b>0.02</b>
Log <sub>10</sub> 30 min plasma insulin ( $\mu$ U/ml) <sup>**†‡§  ¶</sup>	2.35 $\pm$ 0.27	2.34 $\pm$ 0.24	2.10	0.15	2.35 $\pm$ 0.26	2.33 $\pm$ 0.23	2.10	0.09	2.35 $\pm$ 0.26	2.32 $\pm$ 0.23	2.42 $\pm$ 0.30	0.11

## Figure Legend

### **Figure 1. Single SNP and Sliding Window Haplotype Analysis for Association of TCF7L2 with T2D and BMI .**

An "exhaustive" analysis was done which tests all common (MAF > 1%) haplotypes for all possible combinations of 1, 2, 3 and 4 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 the upper panel 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."

Figure 1

