

# 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

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Transcription factor 7-like 2 (*TCF7L2*) regulates genes involved in cell proliferation and differentiation. The *TCF7L2* gene is located on chromosome 10q25 in a region of replicated linkage to type 2 diabetes. Recently, a microsatellite marker in intron 3 (DG10S478) and five correlated single nucleotide polymorphisms (SNPs) were identified in Icelandic individuals that showed strong association with type 2 diabetes, which was replicated in Danish and European-American cohorts. We genotyped four of the SNPs (rs7901695, rs7903146, rs11196205, and rs12255372) in Amish subjects with type 2 diabetes ( $n = 137$ ), impaired glucose tolerance (IGT;  $n = 139$ ), and normal glucose tolerance (NGT;  $n = 342$ ). We compared genotype frequencies in subjects with type 2 diabetes with those with NGT and found marginal association for rs7901695 ( $P = 0.05$ ; odds ratio [OR] 1.51); comparison between NGT control subjects and the combined type 2 diabetes/IGT case group showed strong association with rs7901695 and rs7903146 ( $P = 0.008$ – $0.01$ ; OR 1.53–1.57) and marginal association with rs11196205 and rs12255372 ( $P = 0.07$  and  $P = 0.04$ , respectively). In an expanded set of 698 Amish subjects without diabetes, we found no association with insulin and glucose levels during a 3-h oral glucose tolerance test. We also genotyped these SNPs in nondiabetic, non-Amish subjects ( $n = 48$ ), in whom intravenous glucose tolerance tests were performed, and found an association between rs7901695 and rs7903146 and insulin sensitivity ( $P = 0.003$  and  $P = 0.005$ , respectively) and disposition index ( $P =$

0.04 and  $P = 0.007$ , respectively). These data provide replicating evidence that variants in *TCF7L2* increase the risk for type 2 diabetes and novel evidence that the variants likely influence both insulin secretion and insulin sensitivity. *Diabetes* 55:2654–2659, 2006

The transcription factor 7-like 2 (*TCF7L2*) gene is a member of the T-cell factor (TCF)/lymphoid-enhancing factor family of high mobility group box-containing transcription factors involved in the Wnt signaling pathway. This pathway is a key component to the regulation of cell proliferation and differentiation. Wnt signaling is initiated by the binding of Wnts to their receptor complex, which results in the release of  $\beta$ -catenin from its degradation complex and translocation to the nucleus. In the nucleus,  $\beta$ -catenin heterodimerizes with the TCF/lymphoid-enhancing factor family of transcription factors to regulate the expression of Wnt target genes (1,2).

The *TCF7L2* gene spans 215.9 kb on chromosome 10q25, a region of replicated linkage to type 2 diabetes in Mexican-American (3) and Icelandic (4) cohorts. Recently, Grant et al. (5) identified a microsatellite marker (DG10S478) in intron 3 of *TCF7L2* that showed strong association with type 2 diabetes in Icelandic, Danish, and European-American populations. The authors then genotyped the five single nucleotide polymorphisms (SNPs) from the CEPH Utah (CEU) HapMap samples with the strongest correlation to DG10S478 (rs12255372, rs7903146, rs7901695, rs11196205, and rs7895340) and showed association between all five SNPs and type 2 diabetes in all three cohorts. Although no evidence for linkage to type 2 diabetes was detected on chromosome 10q25 in our genome-wide scan in the Amish (average marker density, 9.7 cM; logarithm of odds, 0.0 between markers D10S597 and D10S190) (6), we genotyped four of the five SNPs in 835 subjects enrolled in the Amish Family Diabetes Study (AFDS). The rs7895340 SNP was not genotyped; however, according to the CEU HapMap (7), rs7895340 is in high linkage disequilibrium (LD) with rs11196205 ( $r^2 = 0.97$ ). All SNPs conformed to Hardy-Weinberg expectations. Figure 1 shows the *TCF7L2* gene structure, SNPs genotyped in the Amish, and pairwise LD ( $r^2$ ) among the SNPs.

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AFDS, Amish Family Diabetes Study; AIRg, acute insulin response to glucose; DI, disposition index; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; LD, linkage disequilibrium; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism; TCF, T-cell factor.

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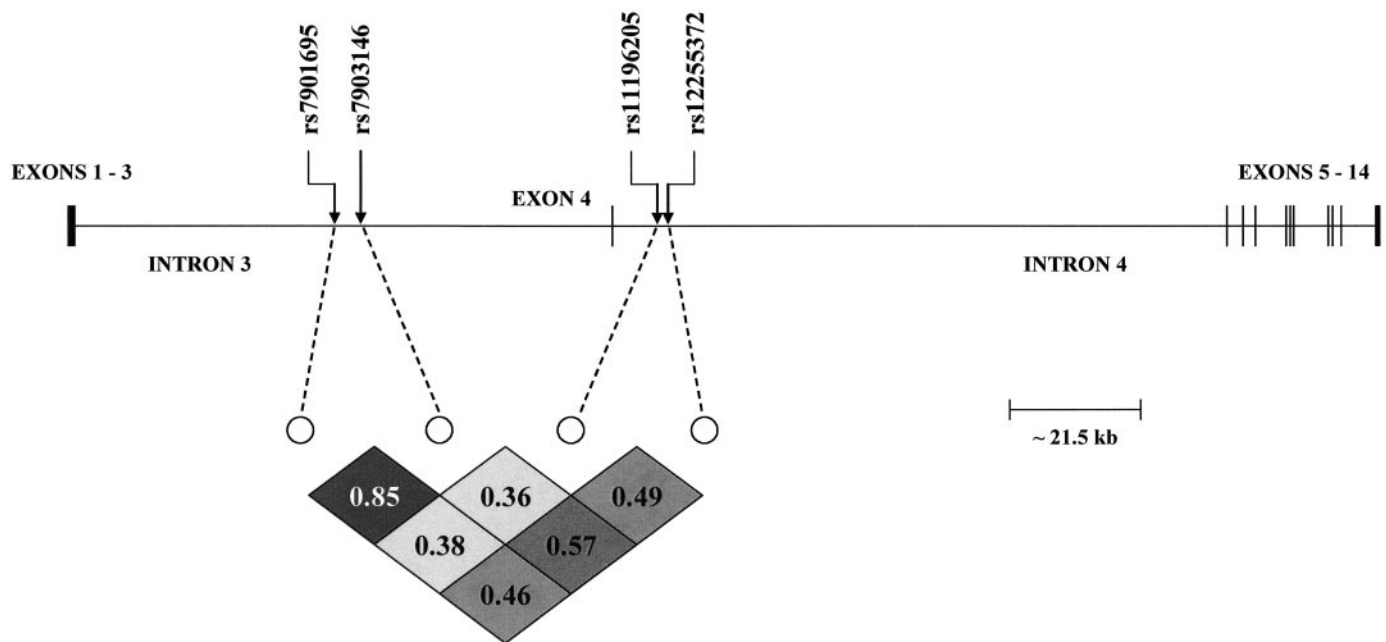


FIG. 1. Gene structure, location of polymorphic sites, and pairwise LD among SNPs in *TCF7L2*. The upper portion of the figure shows the gene structure and location of the polymorphisms genotyped in the Amish and Caucasian IVGTT subjects. Two of the SNPs are located in intron 3 and the other two SNPs are located in intron 4 of the 215.9-kb gene. The lower portion of the figure shows a schematic of the pairwise LD, calculated as  $r^2$ , among the SNPs in the Amish. The dotted lines connect each SNP name and position with the corresponding cell in the LD matrix. Increasing level of LD is shown by darker grayscale.

Table 1 summarizes the allele frequencies in subjects with type 2 diabetes ( $n = 137$ ), impaired glucose tolerance (IGT;  $n = 139$ ), and normal glucose tolerance (NGT;  $n = 342$ ) and the results of genotypic association analysis for each SNP. For this case-control analysis, subjects were considered to have NGT if they were aged  $\geq 38$  years at the time of testing to minimize misclassification given the age-dependent penetrance of type 2 diabetes. The comparison of genotype frequencies in subjects with type 2 diabetes to those with NGT showed marginal significance for rs7901695 ( $P = 0.05$ ; odds ratio [OR] 1.51). To increase power, we combined the subjects with type 2 diabetes and IGT into one case group. In previous studies, we have shown that the prevalence of type 2 diabetes is approximately one-half that of the National Health and Nutrition Examination Survey Caucasian population, despite similar BMI, while the prevalence of IGT is the same or greater than that of the National Health and Nutrition Examination Survey Caucasian population (8). It is likely that the high level of physical activity characteristic of the Amish

lifestyle protects genetically vulnerable individuals from progressing from IGT to type 2 diabetes. Indeed, our previously reported genome-wide linkage analysis has revealed some of our strongest linkages to the combined trait IGT/type 2 diabetes (6), and prospective studies of others (9) have demonstrated the potent effect of lifestyle interventions on progression from IGT to type 2 diabetes. Using this combined case group, we found that the genotype frequencies of rs7901695 and rs7903146 differed significantly between type 2 diabetic/IGT subjects and NGT control subjects ( $P = 0.01$  and  $P = 0.008$ , respectively), with the minor allele being the at-risk allele for type 2 diabetes/IGT (OR 1.53 and 1.57, respectively). These two SNPs are in high LD ( $r^2 = 0.85$ ) (Fig. 1). The rs12255372 and rs1196205 SNPs showed marginal differences in genotype frequency between the type 2 diabetes/IGT and NGT groups ( $P = 0.04$ , OR 1.40 and  $P = 0.07$ , OR = 0.74, respectively). Our results are similar to the findings by Grant et al. (5) in that the rs7903146 SNP showed the strongest association with type 2 diabetes in two (Icelandic

TABLE 1  
Allele frequencies and results of association analysis in Amish subjects with type 2 diabetes, IGT, and NGT\* for SNPs in *TCF7L2*

SNP name	Chromosome location	Major/minor allele	Minor allele frequency			Type 2 diabetes vs. NGT†		IGT vs. NGT†		Type 2 diabetes + IGT vs. NGT†	
			Diabetes ( $n = 137$ )	IGT ( $n = 139$ )	NGT ( $n = 342$ )	$P$	OR	$P$	OR	$P$	OR
rs7901695	114418675	T/C	0.40	0.35	0.28	0.05‡	1.51	0.06	1.46	0.01‡	1.53
rs7903146	114422936	C/T	0.38	0.36	0.29	0.07	1.46	0.03‡	1.55	0.008‡	1.57
rs11196205	114471634	C/G	0.41	0.40	0.46	0.36	0.83	0.05‡	0.69	0.07	0.74
rs12255372	114473489	G/T	0.42	0.41	0.37	0.11	1.38	0.12	1.36	0.04‡	1.40

\*NGT control group restricted to subjects aged  $\geq 38$  years to avoid misclassification given the age-dependent penetrance of type 2 diabetes. † $P$  values are based on genotyped frequencies, and ORs reflect the odds of disease associated with having two copies of the minor allele versus the odds of disease associated with having two copies of the major allele. The analysis models were adjusted for age, sex, and pedigree structure. Reported  $P$  values were derived using the additive model and were not adjusted for multiple comparisons. ‡ $P < 0.05$ .

TABLE 2

Mean fasting glucose, fasting insulin, glucose and insulin area under the OGTT curve, insulin secretion index, and homeostatis model assessment of insulin resistance by genotype in nondiabetic Amish subjects ( $n = 698$ ) for each *TCF7L2* SNP

	Mean trait value $\pm$ SE*			<i>P</i> †
	TT	CT	CC	
rs7901695				
<i>n</i>	321	304	58	
Fasting glucose (mmol/l)	5.11 $\pm$ 0.07	5.13 $\pm$ 0.06	5.04 $\pm$ 0.07	0.92
ln(fasting insulin) (mmol/l)	4.10 $\pm$ 0.06	4.10 $\pm$ 0.06	4.09 $\pm$ 0.06	0.92
GAUC	18.96 $\pm$ 0.52	19.23 $\pm$ 0.51	19.30 $\pm$ 0.53	0.82
IAUC	660.2 $\pm$ 66.7	630.7 $\pm$ 66.0	612.1 $\pm$ 67.2	0.36
$\sqrt$ IS	0.81 $\pm$ 0.04	0.76 $\pm$ 0.04	0.77 $\pm$ 0.09	0.69
HOMA-IR	2.79 $\pm$ 0.22	2.69 $\pm$ 0.21	2.59 $\pm$ 0.22	0.27
rs7903146				
<i>n</i>	304	300	60	
Fasting glucose (mmol/l)	5.10 $\pm$ 0.04	5.13 $\pm$ 0.04	5.03 $\pm$ 0.07	0.92
ln(fasting insulin) (mmol/l)	4.11 $\pm$ 0.03	4.09 $\pm$ 0.03	4.12 $\pm$ 0.06	0.92
GAUC	18.81 $\pm$ 0.32	18.99 $\pm$ 0.30	18.96 $\pm$ 0.51	0.28
IAUC	665.2 $\pm$ 41.0	637.7 $\pm$ 39.3	630.3 $\pm$ 66.4	0.54
$\sqrt$ IS	0.82 $\pm$ 0.04	0.77 $\pm$ 0.04	0.78 $\pm$ 0.09	0.39
HOMA-IR	2.81 $\pm$ 0.13	2.64 $\pm$ 0.12	2.78 $\pm$ 0.21	0.70
rs11196205				
<i>n</i>	127	341	193	
Fasting glucose (mmol/l)	5.09 $\pm$ 0.06	5.13 $\pm$ 0.04	5.07 $\pm$ 0.05	0.92
ln(fasting insulin) (mmol/l)	4.10 $\pm$ 0.05	4.09 $\pm$ 0.04	4.09 $\pm$ 0.04	0.92
GAUC	18.82 $\pm$ 0.43	19.14 $\pm$ 0.33	19.31 $\pm$ 0.37	0.27
IAUC	651.7 $\pm$ 55.3	661.3 $\pm$ 43.5	614.8 $\pm$ 46.8	0.44
$\sqrt$ IS	0.76 $\pm$ 0.08	0.81 $\pm$ 0.04	0.76 $\pm$ 0.05	0.08
HOMA-IR	2.72 $\pm$ 0.18	2.72 $\pm$ 0.14	2.81 $\pm$ 0.15	0.92
rs12255372				
<i>n</i>	240	329	92	
Fasting glucose (mmol/l)	5.13 $\pm$ 0.05	5.13 $\pm$ 0.04	4.98 $\pm$ 0.06	0.06
ln(fasting insulin) (mmol/l)	4.11 $\pm$ 0.04	4.07 $\pm$ 0.03	4.14 $\pm$ 0.05	0.92
GAUC	19.12 $\pm$ 0.35	19.13 $\pm$ 0.31	18.88 $\pm$ 0.46	0.92
IAUC	701.0 $\pm$ 45.0	631.1 $\pm$ 40.9	634.0 $\pm$ 60.3	0.13
$\sqrt$ IS	0.81 $\pm$ 0.05	0.76 $\pm$ 0.03	0.76 $\pm$ 0.08	0.57
HOMA-IR	2.79 $\pm$ 0.14	2.75 $\pm$ 0.13	2.72 $\pm$ 0.19	0.92

\*Adjusted for age, sex, and family structure. †*P* values were derived using the additive model. GAUC, glucose area under the OGTT curve; HOMA-IR, homeostatis model assessment of insulin resistance; IAUC, insulin area under the OGTT curve; IS, insulin secretion.

dic and Dutch) of their three study populations and in the combined analysis of all three cohorts. In addition, the same alleles for all four SNPs that were associated with increased type 2 diabetes risk in the cohorts studied by Grant et al. (5) were also associated with increased risk in the Amish.

We used Haploview 3.2 (10) to estimate four SNP haplotype frequencies and perform haplotype analysis. None of the haplotypes provided stronger evidence for association with type 2 diabetes/IGT than rs7903146 (data not shown). In addition, we genotyped the SNPs in an expanded set of 698 nondiabetic AFDS subjects (including 217 subjects with NGT aged <38 years) and tested for association with insulin and glucose levels during a 3-h oral glucose tolerance test (OGTT), including OGTT-derived homeostatis model assessment of insulin resistance and insulin secretion measurements, but we found no association with any of these traits (Table 2).

To shed light on a potential mechanism by which SNPs in *TCF7L2* contribute to type 2 diabetes susceptibility, we used regression analysis to examine the effects of *TCF7L2* SNP genotype on acute insulin response to glucose (AIRg), insulin sensitivity index ( $S_i$ ), and disposition index (DI) in a small sample of nondiabetic, non-Amish Caucasian subjects in whom intravenous glucose tolerance tests

(IVGTTs) were performed ( $n = 48$ ) (Table 3). Subject characteristics are shown in the online appendix Table 1 (available at <http://diabetes.diabetesjournals.org>). After adjustment for age and sex, we found that rs7901695 and rs7903146 were significantly associated with  $S_i$  ( $P = 0.003$  and  $P = 0.005$ , respectively) and DI ( $P = 0.04$  and  $P = 0.007$ , respectively), and the putative type 2 diabetes risk alleles, identified in the Amish case-control study and the study by Grant et al. (5), were associated with lower  $S_i$  and DI. SNP rs7903146 accounted for  $\sim 16\%$  of the variance in age- and sex-adjusted  $S_i$ , although the effect size estimate has a wide confidence band attached to it because of the relatively small sample size. The power to detect an effect this large in our data was 83%. Although the magnitude of the effects of rs7901695 and rs7903146 genotype on  $S_i$  ( $P = 0.02$  and  $P = 0.03$ , respectively) and DI ( $P = 0.09$  and  $P = 0.02$ , respectively) were reduced after adjustment for BMI, the association remained significant with the exception of the marginal effect of rs7901695 on DI. In addition, the risk allele of rs7903146 was associated with lower AIRg after adjustment for BMI ( $P = 0.05$ ). The rs12255372 SNP was also significantly associated with  $S_i$  ( $P = 0.02$ ), but after correction for BMI, the effect was only marginal ( $P = 0.08$ ). To reduce our reliance on distributional assumptions, we repeated analysis of  $S_i$ , AIRg, and DI using a

TABLE 3  
Mean  $S_i$ , AIRg, and DI by genotype in non-Amish Caucasians for each *TCF7L2* SNP

	Mean trait value $\pm$ SE*			Regression model $P$ value $\dagger$	Regression model $P$ value (BMI adjusted) $\dagger$	Spearman rank correlation $P$ value $\dagger$
	TT	CT	CC			
rs7901695						
<i>n</i>	29	13	5			
$S_i$ ( $10^{-5}$ [min $\times$ pmol/l] $^{-1}$ )	5.63 $\pm$ 0.45	4.22 $\pm$ 0.68	2.38 $\pm$ 1.05	0.003 $\ddagger$	0.02 $\ddagger$	0.0005 $\ddagger$
AIRg (pmol/l)	474.1 $\pm$ 45.6	576.0 $\pm$ 70.7	242.4 $\pm$ 122.5	0.47	0.38	0.74
DI ( $S_i \times$ AIRg)	2,558 $\pm$ 263	2,285 $\pm$ 399	797 $\pm$ 693	0.04 $\ddagger$	0.09	0.03 $\ddagger$
rs7903146						
<i>n</i>	31	13	4			
$S_i$ ( $10^{-5}$ [min $\times$ pmol/l] $^{-1}$ )	5.62 $\pm$ 0.44	3.77 $\pm$ 0.71	2.67 $\pm$ 1.18	0.005 $\ddagger$	0.03 $\ddagger$	0.002 $\ddagger$
AIRg (pmol/l)	510.9 $\pm$ 44.9	496.3 $\pm$ 77.6	244.6 $\pm$ 123.1	0.09	0.05 $\ddagger$	0.29
DI ( $S_i \times$ AIRg)	2,674 $\pm$ 249	1,941 $\pm$ 422	824 $\pm$ 670	0.007 $\ddagger$	0.02 $\ddagger$	0.006 $\ddagger$
rs11196205						
<i>n</i>	14	24	10			
$S_i$ ( $10^{-5}$ [min $\times$ pmol/l] $^{-1}$ )	4.84 $\pm$ 0.66	5.41 $\pm$ 0.52	3.62 $\pm$ 0.78	0.33	0.72	0.28
AIRg (pmol/l)	452.3 $\pm$ 64.4	556.3 $\pm$ 49.0	343.0 $\pm$ 80.4	0.49	0.41	0.60
DI ( $S_i \times$ AIRg)	2,013 $\pm$ 358	2,828 $\pm$ 278	1,509 $\pm$ 446	0.67	0.91	0.48
rs12255372						
<i>n</i>	30	15	3			
$S_i$ ( $10^{-5}$ [min $\times$ pmol/l] $^{-1}$ )	5.55 $\pm$ 0.46	3.92 $\pm$ 0.67	2.83 $\pm$ 1.39	0.02 $\ddagger$	0.08	0.009 $\ddagger$
AIRg (pmol/l)	486.2 $\pm$ 47.5	508.4 $\pm$ 73.3	355.4 $\pm$ 146.9	0.63	0.48	0.96
DI ( $S_i \times$ AIRg)	2,474 $\pm$ 266	2,300 $\pm$ 403	948 $\pm$ 810	0.13	0.26	0.09

\*Adjusted for age and sex.  $\dagger$ Analyses were adjusted for age and sex, and  $P$  values were derived using the additive model.  $\ddagger P \leq 0.05$ .

nonparametric approach (Spearman correlation) in which we assessed the additive effect of genotype on the ranked order of  $S_i$ , AIRg, and DI (while adjusting for age and sex). In these analyses, each SNP remained significantly associated with  $S_i$  and DI (Table 3).

Consistent with our results in the Amish, the rs7903146 SNP shows the strongest effects on insulin sensitivity in the non-Amish IVGTT subjects. The T allele, which was associated with increased risk for type 2 diabetes in the Amish, is associated with lower  $S_i$  and impaired AIRg in the non-Amish IVGTT subjects, suggesting both a reduction in insulin sensitivity and a defect in insulin secretion from the  $\beta$ -cell. Lower DI in individuals with the T allele demonstrates a failure of the  $\beta$ -cells to fully compensate for the degree of insulin resistance, providing additional evidence for defects in both hallmarks of type 2 diabetes.

Although the mechanism by which *TCF7L2* influences susceptibility to type 2 diabetes is unclear, over 60 target genes have been identified for the  $\beta$ -catenin/TCF complex (11), including CCAAT/enhancer-binding protein- $\alpha$  (*CEBPA*) and peroxisome proliferator-activated receptor- $\gamma$  (*PPARG*), two important regulators of adipogenesis (12,13), and cell/tissue type-specific regulation of the preproglucagon (*GCG*) gene, a gene involved in glucose homeostasis and satiety (11). Grant et al. (5) put forth the hypothesis that variants in *TCF7L2* influence type 2 diabetes susceptibility through altered transcriptional regulation of the insulinotropic hormone glucagon-like peptide-1 (GLP1), a peptide encoded by *GCG* and expressed in the brain and gut. This hypothesis was driven by observations of intestine-specific roles for *TCF7L2*, including a role in the development of colon cancer (14–17) and the observation that *TCF7L2*-null mice, which die shortly after birth, lack epithelial stem-cell compartments in the small intestine (18). An alternative hypothesis is that variants in *TCF7L2* disrupt adipogenesis and/or adipocyte function by altering transcriptional regulation of *CEBPA* and *PPARG*,

leading to deposition of triglycerides in peripheral tissues (i.e., liver and muscle) and resulting in insulin resistance. Our results in the non-Amish IVGTT subjects are consistent with one or both hypotheses in that we show defects in both insulin secretion and insulin sensitivity.

In summary, we found evidence for association between SNPs in *TCF7L2* and the type 2 diabetes/IGT trait in the AFDS. These results replicate the report of association by Grant et al. (5) between these SNPs and type 2 diabetes in three Caucasian cohorts. This replication of the same SNPs with similar magnitudes of ORs for the same risk alleles are characteristic of a true susceptibility gene and add *TCF7L2* to a growing list of bone fide type 2 diabetes susceptibility genes that includes P12A *PPARG*, E23K *KCNJ11*, and SNPs in *CAPN10*. In addition, we found significant association with  $S_i$ , AIRg, and DI in nondiabetic, non-Amish Caucasian subjects, suggesting for the first time a role for *TCF7L2* in regulating genes involved in insulin sensitivity and glucose-stimulated insulin release. Studies in other populations, as well as functional analyses, will be required to further elucidate the role of variation in *TCF7L2* in the pathogenesis of type 2 diabetes.

## RESEARCH DESIGN AND METHODS

The AFDS was initiated in 1995 with the goal of identifying susceptibility genes for type 2 diabetes in the Old Order Amish in Lancaster County, Pennsylvania. Details of the AFDS design, recruitment, phenotyping, and pedigree structure have been described previously (19). Briefly, probands with previously diagnosed type 2 diabetes (onset age 35–65 years) and all willing first- and second-degree relatives of probands and spouses aged >18 years were recruited. Phenotypic characterization of participants included medical and family history, anthropometry, and a 3-h, 75-g OGTT with insulin levels. Diabetes was defined by fasting plasma glucose level ( $\geq 7$  mmol/l), 2-h OGTT plasma glucose level ( $\geq 11.1$  mmol/l), random plasma glucose level ( $\geq 11.1$  mmol/l), the use of insulin or oral glucose-lowering agents, or a diagnosis of diabetes (with onset age  $\geq 35$  years) documented by a physician. IGT was diagnosed based on OGTT plasma glucose levels (2-h OGTT plasma glucose level between 7.8 and 11.1 mmol/l). NGT was defined based on fasting plasma

glucose level (<6.1 mmol/l) and 2-h OGTT plasma glucose level (<7.8 mmol/l). For case-control association analysis, we genotyped the *TCF7L2* SNPs in subjects with type 2 diabetes ( $n = 137$ ), IGT ( $n = 139$ ), and NGT ( $n = 342$ ). NGT subjects included in this analysis were required to be aged  $\geq 38$  years. For association analysis of quantitative traits, a larger set of 698 nondiabetic subjects (including the 481 NGT and IGT subjects described above and an additional 217 NGT subjects aged <38 years) was studied. Traits that were examined in this group included fasting glucose, fasting insulin, insulin and glucose area under the OGTT curve, insulin secretion index ( $[\text{insulin at 30 min} - \text{fasting insulin}]/[\text{glucose at 30 min} - \text{fasting glucose}]$ ), and homeostasis model assessment of insulin resistance ( $[\text{fasting insulin} \times \text{fasting glucose}]/22.5$ ).

We also genotyped the four *TCF7L2* SNPs in a set of nondiabetic, non-Amish Caucasian individuals in whom IVGTTs were performed ( $n = 48$ ). Subjects between the ages of 18 and 45 were recruited from the University of Maryland Baltimore campus and local workplace sites and underwent a standard 75-g, 2-h OGTT. If the OGTT demonstrated NGT, the subject underwent an IVGTT receiving at  $t = 0$ , glucose 0.3 g/kg i.v. and at  $t = 20$  min, insulin 0.025 units/kg i.v. AIRg and  $S_i$  were calculated using minimal model analysis. DI was calculated as the product of  $S_i$  and AIRg.

Informed consent was obtained from all study subjects, and the study protocols were approved by the institutional review board at the University of Maryland, Baltimore.

**Genotyping.** All SNPs were genotyped using TaqMan SNP Genotyping Assays (Applied Biosystems) according to the manufacturer's protocol. All genotyping included 8% duplicate samples to determine mistyping rates, which were 0–1%. **Statistical analysis.** Before analysis, genotypes were checked for Mendelian consistency using the PedCheck software program (20) in the extended Amish pedigree. Mendelian errors (0.5% of genotypes) were resolved or removed before analysis. Allele frequencies were calculated for each SNP by gene counting, and observed genotypes were tested for fit to the expectations of Hardy-Weinberg using the  $\chi^2$  test. Pairwise LD was computed between the SNPs, and haplotypes were inferred for each individual using Haploview 3.2 (10).

In the Amish, we evaluated the association between SNP genotype and disease status (type 2 diabetes versus NGT, IGT versus NGT, and type 2 diabetes/IGT versus NGT) using a variance component approach in order to account for the relatedness among study subjects. Using a liability threshold model, we modeled the probability that the subject was a case or control, as a function of the individual's age, sex, and genotype, conditional on the correlations in phenotype among relative pairs. Statistical testing was performed using the likelihood ratio test in which we compared the likelihood of the data under a model in which the genotype effect was estimated against the likelihood of a nested model, wherein the genotype effect was constrained to be zero. Parameter estimates (i.e.,  $\beta$ -coefficients) were obtained by maximum likelihood and ORs by taking the inverse log of the  $\beta$ -coefficient. The OR for the additive model was scaled to reflect the odds that a case was homozygous for the minor allele versus the odds that the case was homozygous for the major allele. Variance components analysis was performed using SOLAR (21).

Quantitative trait means were estimated according to *TCF7L2* genotypes in the nondiabetic AFDS subjects ( $n = 698$ ). To account for the relatedness among family members, the measured genotype approach was used (22) in which we estimated the likelihood of specific genetic models given the pedigree structure. Parameter estimates were obtained by maximum likelihood methods, and the significance of association was tested by likelihood ratio tests. Within each model, we simultaneously estimated the effects of age and sex. Quantitative trait analyses were conducted using SOLAR (21).

IVGTT quantitative traits were analyzed using multiple regression adjusted for age, sex, and with and without BMI as implemented in SAS version 9 (Cary, NC). Extreme outliers, defined as values greater than three SDs above or below the mean, were set to missing (one  $S_i$  value and one AIRg value). Values of DI corresponding to a missing AIRg or  $S_i$  value were also set to missing (two DI values). The SNPs were modeled as an additive effect, that is, quantitative predictor variables reflecting the number of "risk alleles" (0, 1, or 2) as defined previously (5). Analyses of the IVGTT traits were repeated using the nonparametric Spearman rank correlation test, in which we assessed the additive effect of genotype on the ranked order of  $S_i$ , AIRg, and DI adjusted for age and sex.

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## REFERENCES

- Nelson WJ, Nusse R: Convergence of Wnt, beta-catenin, and cadherin pathways (Review). *Science* 303:1483–1487, 2004
- Prunier C, Hocevar BA, Howe PH: Wnt signaling: physiology and pathology (Review). *Growth Factors* 22:141–150, 2004
- Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP: Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am J Hum Genet* 64:1127–1140, 1999
- Reynisdottir I, Thorleifsson G, Benediktsson R, Sigurdsson G, Emilsson V, Einarsdottir AS, Hjorleifsdottir EE, Orlygsdottir GT, Bjornsdottir GT, Saemundsdottir J, Halldorsson S, Hrafnkelsdottir S, Sigurjonsdottir SB, Steinsdottir S, Martin M, Kochan JP, Rhee BK, Grant SF, Frigge ML, Kong A, Gudnason V, Stefansson K, Gulcher JR: Localization of a susceptibility gene for type 2 diabetes to chromosome 5q34–q35.2. *Am J Hum Genet* 73:323–335, 2003
- Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadóttir 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
- Hsueh WC, St. Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, Bell CJ, Sakul H, Wagner MJ, Burns DK, Shuldiner AR: Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21–q24. *Diabetes* 52:550–557, 2003
- The International HapMap Consortium: The International HapMap Project. *Nature* 426:789–796, 2003
- Resnick HE, Harris MI, Brock DB, Harris TB: American Diabetes Association diabetes diagnostic criteria, advancing age, and cardiovascular disease risk profiles: results from the Third National Health and Nutrition Examination Survey. *Diabetes Care* 23:176–180, 2000
- Wylie-Rosett J, Herman WH, Goldberg RB: Lifestyle intervention to prevent diabetes: intensive and cost effective. *Curr Opin Lipidol* 17:37–44, 2006
- Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265, 2005
- Yi F, Brubaker PL, Jin T: TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. *J Biol Chem* 280:1457–1464, 2005
- Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA: Inhibition of adipogenesis by Wnt signaling. *Science* 289:950–953, 2000
- Bennett CN, Ross SE, Longo KA, Bajnok L, Hemati N, Johnson KW, Harrison SD, MacDougald OA: Regulation of Wnt signaling during adipogenesis. *J Biol Chem* 277:30998–31004, 2002
- Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW, Vogelstein B, Clevers H: Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. *Science* 275:1784–1787, 1997
- Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW: Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* 275:1787–1790, 1997
- van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H: The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 111:241–250, 2002
- Batlle E, Henderson JT, Beghtel H, van den Born MM, Sancho E, Huls G, Meeldijk J, Robertson J, van de Wetering M, Pawson T, Clevers H: Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell* 111:251–263, 2002

18. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, Clevers H: Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet* 19:379–383, 1998
19. Hsueh WC, Mitchell BD, Aburomia R, Pollin T, Sakul H, Gelder EM, Michelsen BK, Wagner MJ, St. Jean PL, Knowler WC, Burns DK, Bell CJ, Shuldiner AR: Diabetes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. *Diabetes Care* 23:595–601, 2000
20. O'Connell JR, Weeks DE: PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63:259–266, 1998
21. Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198–1211, 1998
22. Boerwinkle E, Chakraborty R, Sing CF: The use of measured genotype information in the analysis of quantitative phenotypes in man. I. Models and analytical methods. *Ann Intern Med* 50:181–194, 1986