Brief Genetics Report

Common Single Nucleotide Polymorphisms in *TCF7L2* Are Reproducibly Associated With Type 2 Diabetes and Reduce the Insulin Response to Glucose in Nondiabetic Individuals

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Recently, common noncoding variants in the *TCF7L2* gene were strongly associated with increased risk of type 2 diabetes in samples from Iceland, Denmark, and the U.S. We genotyped 13 single nucleotide polymorphisms (SNPs) across *TCF7L2* in 8,310 individuals in family-based and case-control designs from Scandinavia, Poland, and the U.S. We convincingly confirmed the previous association of *TCF7L2* SNPs with the risk of type 2 diabetes (rs7903146T odds ratio 1.40 [95% CI 1.30–1.50], $P = 6.74 \times 10^{-20}$). In nondiabetic individuals, the risk genotypes were associated with a substantial reduction in the insulinogenic index derived from an oral glucose tolerance test (risk allele homozygotes have half the insulin response to glucose of noncarriers, $P = 0.003$) but not with increased insulin resistance. These results suggest that *TCF7L2* variants may act through insulin secretion to increase the risk of type 2 diabetes. *Diabetes* 55:2890–2895, 2006

Type 2 diabetes is highly heritable, but known variants explain only a small fraction of the overall genetic risk in the population. Recently, Grant et al. (1) reported a strong association of variants in *TCF7L2* with increased risk of type 2 diabetes in an Icelandic sample, and this association was confirmed in Caucasian samples from Denmark and the U.S. (combined odds ratio [OR] 1.56, $P = 4.7 \times 10^{-18}$). Testing of this association in other well-phenotyped samples is needed to *1*) validate the association, *2*) estimate the true effect size, and *3*) identify effects on intermediate traits that may suggest how *TCF7L2* variants act (e.g., through changes in insulin secretion, insulin resistance, BMI, waist-to-hip ratio).

TCF7L2 has been implicated as a member of the Wnt signaling pathway and was previously well studied only in colon cancer. However, based on its role in intestinal cells (2), Grant et al. (1) proposed that variants of *TCF7L2* may alter levels of glucagon-like peptide 1, which influences insulin secretion from the β-cells of the pancreas. Thus, one hypothesis is that *TCF7L2* might influence the risk of type 2 diabetes by influencing insulin secretion. Alternatively, a gene increasing the risk of diabetes could act through insulin action or through currently unknown mechanisms.

To evaluate these questions, we selected tag SNPs to capture common variation in a 64.6-kb region of strong linkage disequilibrium surrounding the most significant association signal and spanning intron 3, exon 4, and intron 4 of *TCF7L2*. We genotyped 13 tag SNPs that capture 32 of 44 variants with $r^2 > 0.8$ (mean $r^2 > 0.985$) in the phase II HapMap CEU population (3); all 5 SNPs that were most highly correlated with the DG10S478 allele X in the original report (1) were directly genotyped.

The tag SNPs were genotyped in well-characterized family-based and case-control samples from Scandinavia, Poland, and the U.S.; phenotypic characteristics of all samples are described in Table 1. We included five previously described patient samples that have formed the basis of multiple previous publications from our research group: 333 Swedish and Finnish trios; 2 Scandinavian case-control samples with 918 and 1,010 subjects, respec-

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

AUC, area under the curve; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

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TABLE 1
Clinical characteristics of study samples

Sample	Sex (M/F)	Age (years)	BMI (kg/m^2)	Fasting plasma glucose (mmol/l)	Plasma glucose at 2-h OGTT (mmol/l)	Reference
Scandinavian trios						
Diabetes/IGT/IFG	176/157	39 ± 9	27 ± 5	7.2 ± 2.6	8.5 ± 2.9	4
NGT	187/192	31 ± 10	24 ± 5	5.2 ± 0.5	5.6 ± 1.1	
Scandinavian C/C						
Diabetes/sIGT	247/212	61 ± 10	28 ± 5	9.8 ± 3.4	15.0 ± 5.3	4
NGT	247/212	60 ± 10	26 ± 4	6.2 ± 1.8	6.8 ± 2.8	
Swedish C/C						
Diabetes/sIGT	267/247	66 ± 12	28 ± 4	8.5 ± 2.5	ND*	5
NGT	267/247	66 ± 12	28 ± 4	4.8 ± 0.7	ND	
Genomics Collaborative Poland C/C						
Diabetes	422/587	62 ± 10	30 ± 5	8.9 ± 4.0	ND†	6
NGT	422/587	59 ± 7	26 ± 4	4.8 ± 1.2	ND	
Genomics Collaborative U.S. C/C						
Diabetes	644/582	63 ± 11	33 ± 7	9.8 ± 3.0	ND‡	6
NGT	644/582	61 ± 10	27 ± 5	5.1 ± 0.9	ND	
Botnia C/C						
Diabetes	101/121	65 ± 10	28 ± 4	9.1 ± 2.8	14.7 ± 5.1	New
NGT	101/121	57 ± 10	27 ± 4	5.2 ± 0.5	5.2 ± 1.4	
Swedish/Finnish C/C						
Diabetes	63/70	66 ± 10	28 ± 4	9.6 ± 3.1	16.3 ± 4.4	New
NGT	63/70	55 ± 9	26 ± 4	5.3 ± 0.6	5.6 ± 1.0	
Swedish/Finnish sibs						
Diabetes	57/49	60 ± 11	30 ± 5	10.8 ± 3.7	15.2 ± 4.8	New
NGT	40/66	61 ± 12	27 ± 4	5.3 ± 0.4	6.1 ± 1.0	
Botnia sibs						
Diabetes	55/75	64 ± 12	28 ± 5	9.0 ± 2.7	14.8 ± 5.3	New
NGT	56/74	62 ± 12	26 ± 4	5.5 ± 0.5	6.1 ± 1.2	

Data are means ± SD. Plasma glucose was measured at fasting and 2 h after an OGTT. *HbA_{1c} (A1C) = 6.5 ± 1.5%; †A1C = 7.9 ± 1.3%; ‡A1C = 8.0 ± 3.1%. C/C, case/control subjects; ND, not determined; sIGT, severe IGT.

tively; a Polish case-control sample with 2,018 subjects; and a U.S. Caucasian case-control sample with 2,452 individuals (4–11). A smaller fraction of the samples studied have not previously been described: *1*) a case-control sample (444 subjects) and 130 discordant sibpairs from Botnia (a Swedish-speaking isolate of Finland) and *2*) a case-control sample (266 subjects) and 106 discordant sibpairs from Sweden and Finland. All Scandinavian case-control samples were matched for age, sex, BMI, and geographic region (described in more detail in RESEARCH DESIGN AND METHODS).

Association of SNPs in *TCF7L2* with type 2 diabetes was strongly confirmed in these samples (Table 2 and online appendix Table 1 [available at http://diabetes.diabetesjournals.org]). We found that rs7903146 was most significantly associated with the risk of type 2 diabetes (OR 1.40 [95% CI 1.30–1.50], $P = 6.74 \times 10^{-20}$) in agreement with the original report (best SNP) (1). Heterozygous and homozygous carriers of the risk allele had genotype relative risks of 1.40 (1.27–1.55) ($P = 3.22 \times 10^{-11}$) and 1.86 (1.55–2.23) ($P = 1.38 \times 10^{-11}$) relative to noncarriers, which is consistent with an additive model. Overall, given that the original report was highly significant ($P = 10^{-18}$) (1), our results provided an independent P value of 10^{-20}, and Groves et al. (unpublished observations) observed a P value of 10^{-14}; this association is by far the most convincing and broadly relevant risk factor for type 2 diabetes yet found in the human population.

TABLE 2
Association of *TCF7L2* SNP rs7903146 with risk of type 2 diabetes and model-free estimates of genotype relative risks

	Allele	OR (95% CI)	P
Total sample*	T	1.40 (1.30–1.50)	6.74×10^{-20}
Case/control subjects only†	T	1.39 (1.29–1.50)	1.55×10^{-17}
	Genotype	**Genotype relative risks (95% CI)**	P
Heterozygotes†	TC vs. CC	1.40 (1.27–1.55)	3.22×10^{-11}
Homozygotes†	TT vs. CC	1.86 (1.55–2.23)	1.38×10^{-11}
Homozygotes vs. Heterozygotes†	TT vs. TC	1.30 (1.08–1.57)	0.00269

Genotype relative risks are relative to noncarriers or to heterozygote carriers as indidcated. One-tailed P value: *results from all 9 samples or †6 case-control samples combined by Mantel-Haenszel meta-analysis.

TABLE 3
Association of rs7903146 with type 2 diabetes in each subsample

Sample	*n*	OR (95% CI)	One-tailed *P* value	MAF
Case-control group				
Scandinavian	946	1.27 (1.03–1.58)	0.0277	0.23
Swedish	966	1.45 (1.18–1.77)	2.84×10^{-4}	0.28
Genomics Collaborative				
Polish	1,942	1.38 (1.20–1.59)	1.04×10^{-5}	0.27
Genomics Collaborative U.S.	2,246	1.45 (1.27–1.64)	1.54×10^{-8}	0.32
Botnia	430	1.47 (1.06–2.03)	0.0195	0.22
Swedish/Finnish	260	1.02 (0.69–1.51)	0.921	0.25
All*	6,790	1.39 (1.29–1.50)	1.55×10^{-17}	0.25
Family-based group				
Botnia sibs	260	1.83 (0.75–1.63)	0.0863	
Swedish/Finnish sibs	212	1.56 (0.84–2.91)	0.160	
Scandinavian trios†	756	1.42 (1.09–1.86)	0.0101	
All	1,228	1.48 (1.17–1.87)	4.69×10^{-4}	
Combined (all samples)*	8,018	1.40 (1.30–1.50)	6.74×10^{-20}	

*Data combined by Mantel-Haenszel meta-analysis; no heterogeneity was observed ($P = 0.60$ by Breslow Day test). †Diabetes trios ($n = 91$) OR 1.79 (95% CI 1.09–2.93) ($P = 0.02$); impaired fasting glucose trios ($n = 163$) 1.43 (0.98–2.09) ($P = 0.06$); IGT trios ($n = 36$) 1.18 (0.53–2.64) ($P = 0.68$). MAF, minor allele frequency.

The strongest single variant (rs7903146) was individually significant ($P < 0.05$) in the six largest samples (including all previously published trio and case-control samples) and trended in the expected direction in the three smaller remaining samples (Table 3). We have previously published a lack of association to many candidate genes in these samples (4–8,10,11); replication of *TCF7L2* association in each subsample provides a positive control for those previous studies, clearly demonstrating that the samples can be used to distinguish true diabetes genes of this magnitude and robustness from statistical fluctuations. To test if the best result was the only signal of association observed at this locus, we performed logistic regression analysis conditional on rs7903146. No additional signal of association was observed (data not shown), suggesting that the entire signal observed stems from rs7903146 or from a closely correlated variant.

We tested for epistasis between *TCF7L2* rs7903146 and two other common variants known to be causally associated with the risk of type 2 diabetes: peroxisome proliferator–activated receptor γ P12A and Kir6.2 E23K (4,5,12). No significant genetic interactions were seen (online appendix Table 2).

We next examined the correlation of *TCF7L2* genotypes with covariates of sex, age of onset, and BMI in case and control subjects to test if *TCF7L2* variants contribute to risk of type 2 diabetes through an effect on these covariates. No heterogeneity by sex was observed in the case-control samples (male [$n = 3,288$] OR 1.46 [95% CI 1.31–1.62], $P = 8.75 \times 10^{-12}$ and female [$n = 3,424$] 1.32 [1.19–1.47], $P = 3.79 \times 10^{-7}$; $P_{\text{homogeneity}} = 0.20$). Furthermore, in case subjects, *TCF7L2* genotypes did not associate with the age of onset of diabetes ($n = 1,856$, mean [±SD] TT = 55 ± 11, TC = 55 ± 12, and CC = 56 ± 12; $P = 0.64$). In addition, no significant association to BMI was observed (Table 4). Thus, while some variants may prove

TABLE 4
Mean trait values by genotype

Phenotype	*n*	Mean trait values (±SD) by genotype			Two-tailed *P* value				
		TT	CT	CC	Additive	Recessive	TT vs. CC	TT vs. TC	TC vs. CC
BMI (kg/m^2)	8,258	28.4 ± 5.4	28.4 ± 5.3	28.2 ± 5.2	0.31				
n	995	42	322	631					
Ins index (mU/mmol)		10.9 ± 12.7	16.5 ± 50.5	18.1 ± 33.1	0.0030	0.0010	0.0009	0.0031	0.3767
Disp index (mU^2/l^2)		22.5 ± 28.9	35.8 ± 112.9	42.6 ± 79.9	0.0044	0.0011	0.0012	0.0019	0.6278
Fasting insulin (mU/l)		7.27 ± 4.24	9.19 ± 6.66	8.94 ± 6.02	0.3423	0.1405	0.1537	0.1340	0.7685
HOMA-IR (mmol · mU/l^2)		1.88 ± 1.29	2.30 ± 2.06	2.19 ± 1.63	0.2561	0.1286	0.1590	0.1007	0.5191
n	721	27	236	454					
AUC_{ins}*		3,911 ± 3,658	4,971 ± 3,176	5,229 ± 3,248	0.0007	0.0004	0.0002	0.0048	0.1250
AUC_{glu}*		339.8 ± 262.8	271.4 ± 214.5	270.0 ± 195.3	0.1775	0.0841	0.0778	0.1266	0.9297
AUC_{ins}/AUC_{glu}*		23.46 ± 39.75	24.33 ± 71.06	32.14 ± 243.05	0.0023	0.0025	0.0015	0.0135	0.1640

Traits were log transformed. *Four negative values of AUC_{ins} were removed, and 26 negative values of AUC_{ins}/AUC_{glu} were removed. Insulin and glucose units used for all calculations were mU/l and mmol/l, respectively. AUC, AUC during OGTT; Disp index, insulin disposition index; Ins index, insulinogenic index.

to be heterogeneous, associated only in substrata of sex, BMI, or other genotypes, common variation in *TCF7L2* is associated with type 2 diabetes across the measured covariates.

The order and relative contributions of defects in insulin resistance and insulin secretion to the pathogenesis of type 2 diabetes remain controversial (13,14). Some postulate that type 2 diabetes is caused primarily by defects in insulin resistance, followed by a failure of pancreatic β-cells to compensate for increased insulin demand (15). Conversely, type 2 diabetes genes identified thus far (the maturity-onset diabetes of the young genes that cause monogenic forms of type 2 diabetes and Kir6.2) act through reduced insulin secretion without insulin resistance in carriers before the onset of disease (16,17). Each additional gene that is truly associated with type 2 diabetes helps inform the relative contributions of these two mechanisms.

We found no effect of *TCF7L2* rs7903146 on oral glucose tolerance test (OGTT)-derived measures of insulin resistance in 995 nondiabetic individuals (Table 4). We did see a dramatic effect, however, on insulin secretion as measured by the OGTT (Table 4). The OGTT provides rough measures of insulin secretion and resistance and has been widely used in clinical investigations of type 2 diabetes. Individuals homozygous for the rs7903146 risk allele have a significant 50% reduction in insulinogenic index ($P = 0.003$) and insulin disposition index ($P = 0.004$). We also observed a significant reduction in the area under the curve (AUC) for insulin during the OGTT ($P = 0.0007$) and $AUC_{insulin}/AUC_{glucose}$ ($P = 0.002$), suggesting that the polymorphism not only influences the early insulin response to glucose but also could have an effect on the capacity of the β-cells to secrete insulin. These associations are stronger in risk allele homozygotes than in heterozygous carriers, and a trend is seen in heterozygous carriers compared with noncarriers.

Our results replicate the strong association of *TCF7L2* variants with the risk of type 2 diabetes. The risk model for the most significantly associated SNP, rs7903146, was slightly weaker than the original report, perhaps because the magnitude of the risk effect may have been overestimated by Grant et al. (1), which was expected due to the winners curse. Consistent with this, Groves et al. (unpublished observations) estimate an effect size similar to that in our study (OR 1.36 and 1.40, in both replications, vs. 1.54 for the same SNP in the original report). Nevertheless, *TCF7L2* variation contributes more powerfully to increased risk of type 2 diabetes than any other gene identified thus far. Consistent replication across European populations confirms that the causal *TCF7L2* variant influences disease risk reproducibly, without the need to yet invoke population-specific effects.

We observed an insulin secretion defect in nondiabetic individuals homozygous for risk alleles of *TCF7L2*, suggesting that as in maturity-onset diabetes of the young and the common E23K polymorphism in Kir6.2, the primary defect attributable to a common variation in the *TCF7L2* region is reduced insulin secretion rather than insulin resistance (12,16,17). However, OGTT measures used here provides only rough estimates of insulin secretion, and follow-up work will be necessary to understand the nature of the defect in insulin secretion and any possible effects on insulin action.

TCF7L2 has not previously been implicated in type 2 diabetes and would not have been an obvious diabetes gene candidate. (A PubMed search for the keywords "TCF7L2" or "TCF4" reveals 218 articles but none that share the term "diabetes" before the Grant et al. [1] publication.) The discovery of this gene reinforces that type 2 diabetes is an endocrine disease with its origin in multiple organ systems, now possibly including the intestine. Post hoc, because *TCF7L2* activates glucagon-like peptide 1 in a cell-specific manner, a putative mechanism to influence blood glucose homeostasis could be proposed, and reduced insulin secretion observed here is consistent with this mechanism (1). However, the causal variant or functional defect in *TCF7L2* has not yet been found. (It could be rs7903146 itself, a proxy within a broader region of this gene, or a proxy even in an adjacent gene.) More extensive genotyping and sequencing is clearly warranted, as are functional studies of the most associated alleles to document that they function through *TCF7L2* rather than some adjacent gene.

The identification of this gene has interesting implications for the several diabetes whole-genome association studies planned in the coming year. There has been much speculation that common variants responsible for common diseases such as type 2 diabetes, at most, exert extremely modest effects, owing to an underlying complex genetic architecture (18–20). Some have even questioned whether disease-influencing common variants exist or can be found with linkage disequilibrium approaches (21,22). Grant et al. (1) discovered an association to *TCF7L2* during a follow-up of a putative linkage peak, but as they state, the finding is likely coincidental since "the median allele-sharing LOD score generated with our previous familial samples is less than 0.1." That is, this variant could not possibly have generated the linkage peak but could be (and was) found by systematic studies of common variation for the association with type 2 diabetes. Unless *TCF7L2* is the only such gene in the genome, which is unlikely given that a focused search of ~10.5 Mb led coincidentally to its discovery, more diabetes genes of similar effect are likely to be found by ongoing whole-genome association studies.

RESEARCH DESIGN AND METHODS

The diabetic samples include a previously described sample of 333 Scandinavian parent-offspring trios (163 offspring with impaired fasting glucose, 36 with impaired glucose tolerance [IGT], and 134 with type 2 diabetes) (4) and two previously described Scandinavian case-control samples consisting of 954 and 1,028 subjects, individually matched for age, sex, BMI, and geographic region of origin (4,5). World Health Organization 1998 definitions of type 2 diabetes, IGT, impaired fasting glucose, and normal glucose tolerance (NGT) were used for these samples, and severe IGT was defined as 2-h OGTT plasma glucose >8.5 mmol/l but <10 mmol/l. This study also includes two previously described case-control samples from Poland (2,018 subjects) and the U.S. (2,452 subjects) individually matched for sex, age, and geographic origin, both collected by Genomics Collaborative (6,11). For these Polish and U.S. samples, case subjects met the American Diabetes Association 2003 criteria for type 2 diabetes, and control subjects had fasting plasma glucose <7 mmol/l. Finally, the study included four newly selected Scandinavian samples: *1*) a case-control sample (444 subjects) from Botnia (a Swedish-speaking isolate of Finland) individually matched by age, sex, and BMI; *2*) 130 sibpairs from Botnia, discordant for type 2 diabetes; *3*) a case-control sample from Sweden and Finland (266 subjects) individually matched by age, sex, BMI, and geographic origin; and *4*) 106 sibpairs discordant for type 2 diabetes from Sweden and Finland. For the new case-control and family-based samples, diabetic case subjects were defined by the American Diabetes Association 2003 criteria, had an age of onset ≥35 years, and were GAD antibody negative. Control subjects had NGT (fasting plasma glucose <6.1 mmol/l and 2-h OGTT plasma glucose <7.8 mmol/l). Age matching required that control subjects be normal glucose tolerant at age <5 years from onset age of matched case. For discordant sibpairs, the youngest sibling who fulfilled the case inclusion

criteria was matched with the eldest normal glucose tolerant sibling. Case and control subjects were recruited from the previously described Botnia Study, which includes families from the Botnia region on the western coast of Finland and families from other parts of Finland and Sweden (23). All patient samples were approved by the human subject institutional review board at respective institutions, and informed consent was obtained from all subjects. Insulin measures during the OGTT were available for a subset of individuals as previously described (7,11); genotype-phenotype correlation was examined for rs7903146 with fasting insulin, insulinogenic index as a measure of early insulin response to glucose ([Ins_{30} − fasting insulin]/[$Gluc_{30}$ − fasting glucose]), homeostatis model assessment of insulin resistance ([fasting glucose × fasting insulin]/22.5), insulin disposition index (insulinogenic index × homeostatis model assessment of insulin resistance), and $AUC_{insulin}$, $AUC_{glucose}$, and $AUC_{insulin}/AUC_{glucose}$ (AUCs determined by the trapezoidal method).

Genotyping. Patient DNA was isolated from whole blood, whole genome amplified using REPLI-G (Qiagen), and purified using the Nucleofast (Clontech). Genotyping was performed by primer extension of multiplex products with detection by MALDI-TOF mass spectroscopy using a Sequenom platform. Genotyping success rate was 99%, and concordance rate, based on 889 duplicate comparisons for each of the 13 SNPs, was 99.58%.

Statistical analysis. Tag SNPs were selected using Tagger (24); 12 SNPs were untested. Simple χ^2 analysis was used to test the association of SNPs with type 2 diabetes in the matched case-control subjects, a transmission disequilibrium test was performed in the trios (25), and the discordant allele test was carried out in the sibpairs (26). Results from each sample were combined by Mantel-Haenszel meta-analysis of ORs; homogeneity was tested using the Breslow Day test, and no heterogeneity was found. Logistic regression for each SNP with diabetes-affected status was performed conditionally on rs7903146 using Whap (http://pngu.mgh.harvard.edu/~purcell/whap). For epistasis analysis, pairwise combinations of SNPs rs7903146 with peroxisome proliferator–activated receptor γ P12A and Kir6.2 E23K were tested for association with type 2 diabetes using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink). Log-transformed quantitative traits were compared by ANOVA across three genotypic classes of rs7903146; two-tailed *t* tests were performed for other models/risk estimates. Unadjusted $P_{nominal}$ values are reported; results were similar after adjustment for sex, age, and BMI.

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