Brief Genetics Report

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

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Recent data suggest that common variation in the transcription factor 7-like 2 (*TCF7L2*) gene is associated with type 2 diabetes. Evaluation of such associations in independent samples provides necessary replication and a robust assessment of effect size. Using four *TCF7L2* single nucleotide polymorphisms (SNPs; including the two most associated in the previous study), we conducted a case-control study in 2,158 type 2 diabetic subjects and 2,574 control subjects and a family-based association analysis in 388 parent-offspring trios all from the U.K. All SNPs showed powerful associations with diabetes in the case-control analysis, with strongest effects at rs7903146 (allele-wise relative risk 1.36 [95% CI 1.24–1.48], $P = 1.3 \times 10^{-11}$). Data were consistent with a multiplicative model. The family-based analyses provided independent evidence for association at all loci (e.g., rs4506565, 62% transmission, $P = 7 \times 10^{-5}$) with no parent-of-origin effects. The frequency of diabetes-associated *TCF7L2* genotypes was greater in cases ascertained for positive family history and early onset (rs4606565, $P = 0.02$); the population-attributable risk, estimated from the least-selected cases, is ~16%. The overall evidence for association for these variants ($P = 4.4 \times 10^{-14}$ combining case-control and family-based analyses for rs4506565) exceeds genome-wide significance criteria and clearly establishes *TCF7L2* as a type 2 diabetes susceptibility gene of substantial importance. *Diabetes* 55:2640–2644, 2006

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SNP, single nucleotide polymorphism.

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By common consent, the history of genetic association studies in type 2 diabetes has been a checkered one (1), and the number of variants so far shown to be reproducibly associated with type 2 diabetes is limited (2,3). The variants with the most convincing claims, such as the P12A variant in *PPARG* and the E23K variant in *KCNJ11* (4,5), lie in genes whose products were already established as players in pathways implicated in glucose and lipid homeostasis. The modest effect sizes associated with these variants restrict their value for the assignment of risk in the clinical setting. The objective of genome-wide strategies to diabetes susceptibility variant identification, whether based around linkage or association, is to characterize novel etiological pathways and to pinpoint variants with more substantial impacts on diabetes risk.

Recently, Grant et al. (6) reported that variation within the transcription factor 7-like 2 (*TCF7L2*) gene was strongly associated with type 2 diabetes in Icelandic subjects. These associations had been detected in the course of positional cloning efforts in a region of chromosome 10q previously linked to diabetes in Icelandic pedigrees (7) and were confirmed in case-control analyses of Danish and U.S. cohorts (6). Despite combined significance values for the associated variants well beyond those required for attesting genome-wide significance, the variants studied do not appear to account for the chromosome 10q linkage signal. Neither is it obvious that the single nucleotide polymorphisms (SNPs) examined are themselves etiological; in the Icelandic sample, for example, the strongest associations were observed at a nearby microsatellite (6).

Notwithstanding these compelling findings, past concerns over the performance of association studies in complex traits mandate independent replication (1,8). Given the large numbers of errors and biases that can compromise any individual study, replication ensures that the original findings are robust and provides a more accurate estimate of the likely effect size (9,10).

In the present study, we have typed four SNPs in *TCF7L2* in 6,736 subjects from well-characterized case, control, and family sample sets from the U.K. (Table 1). The samples typed for the main case-control analysis (all

TABLE 1
Characteristics of the subjects studied

	Case samples			Control samples	
	W2SP	W2C	W2TP*	58BC	HRC+
n	572	1,586	388	2,024	550
Male (%)	54.4	61.8	59.4	50.1	50.3
Age at examination (years)	64.1 ± 8.1	60.2 ± 8.2	46.3 ± 7.1	Not available	Not known
Age at diagnosis (years)	55.3 ± 8.4	51.4 ± 7.5	40.3 ± 7.7	Not applicable	Not applicable
BMI (kg/m^2)	28.4 (24.0–33.7)	31.5 (25.9–38.2)	32.3 (26.2–39.8)	Not available	Not known
Waist-to-hip ratio					
Men	0.95 (0.89–1.03)	0.98 (0.92–1.05)	0.98 (0.91–1.05)	Not available	Not known
Women	0.87 (0.80–0.93)	0.91 (0.84–0.99)	0.89 (0.81–0.98)	Not available	Not known
Treatment (ins/OHA/diet) (%)†	16/69/15	8/62/31	18/63/19	Not applicable	Not applicable

Data are means ± SD or geometric means (SD range). *Results given for all trio probands (n = 388). Of these, 350 were of British/Irish origin (60% male; age at diagnosis 40.3 ± 7.4 years; BMI 32.3 kg/m^2 [26.3–39.6]). †Treatment at the time of ascertainment. ins, insulin; OHA, oral hypoglycemic agent.

previously described) comprised *1*) 572 probands, all ascertained for positive family history, from the Diabetes U.K. Warren 2 sibpair collection (Warren 2 sibpair probands [W2SPs]) (11); *2*) 1,586 further type 2 diabetic cases from the Medical Research Council/Diabetes U.K. Case resource, ascertained for type 2 diabetes diagnosed before age 65 (Warren 2 cases [W2Cs]) (12); *3*) 550 U.K. control subjects (HRC+), 480 from the Human Random Control resource, with an additional 70 control samples from the same source (European Collection of Cell Cultures, Salisbury, U.K.); and *4*) 2,024 U.K. control subjects from the British Birth Cohort of 1958 (58BC). All cases were ascertained using similar criteria for a diagnosis of diabetes (based on usage of oral agents and/or insulin and/or biochemical evidence of hyperglycemia), with subtypes other than type 2 diabetes excluded using clinical, genetic, and/or immunological criteria (all are GAD antibody negative). Glucose tolerance status is not known for any of the control subjects. All subjects in the case-control analysis are of known British/Irish European origin.

For family-based association analyses, we typed 1,170 members of 390 complete parent-offspring trios (the Warren 2 trio families), each ascertained for a European proband with type 2 diabetes (13). Due to Mendelian errors in two of these, data are reported on 388 trios. Of the 388 Warren 2 trio probands (W2TPs), 350 have a three-generation history of exclusively British/Irish origin (13,14). For analysis of the effects of *TCF7L2* variation on the evidence for linkage to diabetes on chromosome 10q, we obtained genotypes from all 1,406 members of the 573 Warren 2 sibpair families (11). For further details concerning all subjects, see the online appendix (available at http://diabetes.diabetesjournals.org).

We genotyped the two variants displaying the strongest SNP association signal in the Icelandic study (rs12255372 and rs7903146), plus two other SNPs (rs4506565 and rs12243326) selected using Phase II HapMap data as the best-correlated proxies available. Genotyping was performed at KBiosciences (Hoddesdon, U.K.) using a fluorescence-based competitive allele-specific assay (KASPar) (details available from the authors). Call rates for all SNPs exceeded 95% overall (with no SNP in any sample below 91.7%). Genotype data performed well against stringent quality-control criteria, including a discrepancy rate on duplicate genotyping of 2/2,416 (0.04% error), two instances of Mendelian inconsistency in 963 families, and no evidence of departure from Hardy-Weinberg equilibrium (all $P > 0.05$) in control subjects.

Genotype frequency distributions for the four variants in case and control groups studied are shown in Table 2. In our samples, mutual r^2 values exceeded 0.70 for all pairwise combinations (see online appendix Table 1). As expected, SNPs rs4506565 and rs7903146 formed one pair of highly correlated variants, as did rs12255372 and rs12243326. To maximize the power of the main type 2 diabetic case-control comparison, case (W2SPs and W2Cs, but not W2TPs, to ensure independence of the case-control and family-based analyses) and control (those from the British Birth Cohort of 1958 and the Human Random Control resources) subjects were pooled after

TABLE 2
Genotype frequencies in the case-control sample groups studied

SNP	Genotype	W2SP	W2C	W2TP*	Combined Cases†	58BC	HRC+	Combined controls
rs4506565	AA	172 (31.8)	569 (38.0)	105 (33.2)	741 (36.4)	901 (45.5)	231 (45.0)	1,132 (45.4)
	AT	278 (51.5)	716 (47.9)	164 (51.9)	994 (48.2)	880 (44.5)	227 (44.3)	1,107 (44.4)
	TT	90 (16.7)	211 (14.1)	47 (14.9)	301 (14.8)	198 (10.0)	55 (10.7)	253 (10.2)
rs7903146	CC	192 (35.1)	579 (39.8)	112 (35.8)	771 (38.5)	932 (47.4)	243 (47.7)	1,175 (47.4)
	CT	274 (50.1)	686 (47.2)	158 (50.5)	960 (48.0)	867 (44.1)	217 (42.5)	1,084 (43.8)
	TT	81 (14.8)	189 (13.0)	43 (13.7)	270 (13.5)	167 (8.5)	50 (9.8)	217 (8.8)
rs12243326	TT	209 (38.3)	637 (43.0)	118 (37.3)	846 (41.7)	981 (49.7)	256 (48.7)	1,237 (49.5)
	CT	266 (48.7)	669 (45.2)	155 (49.1)	935 (46.1)	838 (42.5)	217 (41.3)	1,055 (42.2)
	CC	71 (13.0)	175 (11.8)	43 (13.6)	246 (12.1)	154 (7.8)	53 (10.1)	207 (8.3)
rs12255372	GG	208 (38.3)	628 (42.5)	117 (36.3)	836 (41.4)	969 (49.1)	251 (48.6)	1,220 (49.0)
	TG	266 (49.0)	675 (45.7)	162 (50.3)	941 (46.5)	842 (42.6)	215 (41.7)	1,057 (42.4)
	TT	69 (12.7)	175 (11.8)	43 (13.4)	244 (12.1)	164 (8.3)	50 (9.7)	214 (8.6)

Data are n (%). *W2TP genotype counts refer to the 350 probands from British/Irish families only. †Combined cases excluding W2TP.

TABLE 3
Estimates of the genotype and allele RRs for *TCF7L2* SNPs

SNP	Baseline genotype	RR (95% CI) for heterozygote*	*P*	RR (95% CI) for rare homozygote*	*P*	Allele RR (95% CI)	*P*
rs4506565	AA	1.37 (1.21–1.56)	9.4×10^{-7}	1.82 (1.49–2.21)	9.0×10^{-10}	1.35 (1.23–1.47)	1.6×10^{-11}
rs7903146†	CC	1.35 (1.19–1.53)	3.1×10^{-6}	1.90 (1.54–2.33)	3.6×10^{-10}	1.36 (1.24–1.48)	1.3×10^{-11}
rs12243326	TT	1.30 (1.14–1.47)	4.6×10^{-5}	1.74 (1.41–2.14)	1.2×10^{-7}	1.31 (1.19–1.43)	4.3×10^{-9}
rs12255372	GG	1.30 (1.15–1.47)	3.9×10^{-5}	1.66 (1.35–2.05)	1.1×10^{-6}	1.29 (1.18–1.41)	2.2×10^{-8}

P values are exact. These results are based on the data presented in Table 2 and compares type 2 diabetes case (W2SP and W2C but not W2TP) and control (from the British Birth Cohort of 1958 and the Human Random Control resources) subjects. *Genotype RRs compared with the baseline (common homozygote) genotype defined in the second column; †rs7903146: genotype RR for comparison of heterozygote and rare homozygote 1.41 (95% CI 1.15–1.72), $P = 8.6 \times 10^{-4}$.

first confirming homogeneity of genotype frequencies between subgroups on an SNP-by-SNP basis using standard contingency table methods (χ^2).

Allele and genotype frequency comparisons were conducted using standard contingency table analyses in Stata version 8 (Stata, College Station, TX) and StatXact 6 (Cytel, Cambridge, MA). All SNPs were significantly associated with type 2 diabetes. SNP rs7903146 showed the strongest single-point associations in the case-control analysis, with an allele-wise relative risk (RR) of 1.36 (95% CI 1.24–1.48, $P = 1.3 \times 10^{-11}$) for allele T. Heterozygous and homozygous carriers for allele T have genotype RRs of 1.35 (1.19–1.53, $P = 3.1 \times 10^{-6}$) and 1.90 (1.54–2.33, $P = 3.6 \times 10^{-10}$), respectively, relative to AA homozygotes (Table 3). These data are consistent with a multiplicative (or, equally, an additive) genetic model, in keeping with the original observations by Grant et al. (6). The population-attributable risk, as estimated using the least-selected cases (W2C) alone, was ~16%.

The associations described above do not include the W2TP samples (trio probands). Family-based association analysis within the full set of 388 trios (using TDTphase [15]) provided strong independent evidence for association in the form of substantial overtransmission of the high-risk alleles for all four variants (Table 4). In this analysis, the most extreme signal (62% transmission of the susceptibility allele, $P = 7.7 \times 10^{-5}$) was seen at rs4506565 rather than rs7903146. There was no evidence of parent-of-origin effects at any variant. In both the case-control and family-based studies, haplotype-based analyses did not generate additional evidence for association with disease (data not shown). Using Fisher's method to combine significance values from the case-control and family-based association analyses (16), the combined *P* value for rs4506565 is 4.4×10^{-14}.

When we considered all three case groups (but restricting the W2TP group to the 350 with British/Irish origin), case-control effects sizes were seen to be appreciably greater for those cases ascertained for familiality and/or early onset (i.e., the W2SPs plus the British/Irish W2TPs [$n = 922$]) than for the less-selected (W2C) cases ($n = 1{,}586$). For example, at rs4506565, the allele-wise RR for the familial/early-onset cases was 1.50 (95% CI 1.34–1.68, $P = 2.4 \times 10^{-12}$) compared with 1.28 (1.17–1.41, $P = 2.8 \times 10^{-7}$) for the W2Cs. There were modest differences in genotype frequencies between the two groups of case subjects ($P = 0.02$). However, we found no significant relationship between *TCF7L2* variants and age at diagnosis in any of the individual case samples ($P > 0.05$).

TABLE 4
Transmission disequilibrium analysis in 388 parent-offspring trios

	rs4506565	rs7903146	rs12243326	rs12255372
T/NT*	169/104	159/106	167/106	169/108
P	7.7×10^{-5}	1.1×10^{-3}	2.1×10^{-4}	2.3×10^{-4}

*Copies of the minor allele transmitted (T) and nontransmitted (NT) from heterozygous parents to affected offspring.

The genome-wide linkage scan undertaken in the Warren 2 sibpair families had identified chromosome 10q as one of the strongest linkage signals in the U.K. (11). Though *TCF7L2* maps ~25 Mb (29 cM) from the peak of this linkage signal and 19 Mb (19 cM) outside the 1–logarithm of odds support interval, we used the Genotype-IBD Sharing Test (17) to establish whether the typed variants in *TCF7L2* were in any way contributing to the linkage signal. Using rs7903146 genotypes exclusively from siblings with type 2 diabetes, we generated family-based weighted variables under dominant, recessive, and additive models. We found no evidence that *TCF7L2* variants contributed to the previously described linkage, whether we tested at the marker nearest *TCF7L2* itself (D10S597) or at the peak of linkage (D10S1765) (all $P > 0.6$).

Given increasing evidence of overlap between the genes implicated in type 2 diabetes susceptibility and those causative for related Mendelian subtypes of diabetes (18), we sought to establish whether *TCF7L2* mutations were involved in the pathogenesis of permanent diabetes arising during infancy (19). Accordingly, we studied 48 subjects (27 male) from an international cohort with permanent diabetes, all diagnosed before 6 months of age (20), and in whom mutations in the major neonatal diabetes genes (*KCNJ11*, *IPF-1*, and *GCK*) had been excluded (19). All 14 exons of *TCF7L2* were amplified using M13-tailed primers (details available from the authors) and sequenced using an ABI 3730 DNA sequencer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Four novel, rare, heterozygous variants were identified. Numbered with respect to adenine thymine guanine translation-initiation site these were c.A879G p.Pro294Pro (exon 8; in a single Asian subject), c.C1429A p.Thr488Pro (exon 14; in three subjects of Vietnamese, Chilean, and Swedish origin), c.A1579C p.M526M (exon 14; one Syrian subject), and c.T1735C p.Ser579Pro (exon 14; one Brazilian subject). In each case, the rare allele was also observed in an unaffected parent, effectively excluding an etiological role.

Several conclusions can be drawn from these data. First, the independent replication provided by this study confirms *TCF7L2* as a genuine type 2 diabetes susceptibility gene, with the magnitude of the association exceeding even the strictest criteria for genome-wide significance. Second, the strong association seen within the family-based association analysis discounts the, admittedly, re-

mote possibility that the original findings had arisen as a result of latent population substructure. Third, though estimates of effect size obtained in the present study, using the least-selected cases, are somewhat lower than those observed in the original study, the "winner's curse" effect at this gene is less pronounced than that seen for other complex trait susceptibility genes (21). The high population-attributable risk and the almost twofold difference in RR between homozygote groups clearly establish *TCF7L2* as the most important player yet identified in susceptibility to multifactorial type 2 diabetes. However, it is worth noting that all studies to date have been conducted in populations of Northern European origin and that unbiased estimates of population effect size will require analysis of more representative cohorts. Fourth, demonstration that the inherited component of individual type 2 diabetes susceptibility extends to an influence by common susceptibility variants with substantial effect sizes (here, a control minor allele frequency of 30% and allele-wise RR of ~1.3) augurs well for ongoing efforts to map additional etiological variants through genome-wide association methods and encourages the belief that such endeavors will generate information of clinical value (22). Fifth, the observation that the frequency of the diabetes-associated *TCF7L2* genotypes is greater among cases ascertained for familiality and early onset (boosting the allele-wise RR to almost 1.5), reaffirms the value of such case-enrichment strategies in disease-gene identification (23). Sixth, the lack of evidence that *TCF7L2* variation contributes to linkage signals in our data is not surprising, given the considerable distance between the gene and our nearest linkage peak. As with the Pro12Ala variant at *PPARG* (4), the large population risk associated with *TCF7L2* variants does not translate into a detectable linkage signal; the sibling RR attributable to the typed *TCF7L2* variants is only 1.02 and the increase in the mean allele-sharing statistic among affected sibpairs limited to a modest 50.4% (24). Seventh, despite indications that the known functions of *TCF7L2* point toward loss of β-cell function (rather than insulin action) as the anticipated consequence of gene disruption, we find no evidence that mutations within the coding regions of this gene are responsible for the β-cell phenotype of permanent neonatal or infancy-onset diabetes (19).

The present study does not contribute to efforts to specify which *TCF7L2* variants are etiological; it is likely that the SNPs typed here are not causal (6). Until such time as these are identified, measures of effect size derived from partially correlated variants may substantially underestimate the total contribution of *TCF7L2* variation to type 2 diabetes susceptibility. Nevertheless, our data clearly establish *TCF7L2* as a gene of particular importance to the development of type 2 diabetes. Unraveling of the mechanisms whereby changes in the function or regulation of this transcription factor lead to loss of β-cell performance and/or insulin sensitivity is likely to provide crucial new insights into disease pathogenesis.

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