Brief Genetics Report

Variant of Transcription Factor 7-Like 2 (*TCF7L2*) Gene and the Risk of Type 2 Diabetes in Large Cohorts of U.S. Women and Men

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Emerging evidence indicates that variation in the transcription factor 7-like 2 (*TCF7L2*) gene may play a role in the pathogenesis of type 2 diabetes. In a prospective, nested, case-control study (n = 3,520) within the Nurses' Health Study (687 type 2 diabetic case and 1,051 control subjects) and the Health Professionals Follow-up Study (886 case and 896 control subjects), we examined the association of a common variant of the *TCF7L2* gene (rs12255372 [T/G]) with type 2 diabetes risk among Caucasians. Frequencies of the T-allele were significantly higher among case than control subjects; each copy of the T-allele was associated with a 1.32-fold (P = 0.0002) and 1.53-fold (P < 0.0001) increased type 2 diabetes risk in women and men, respectively. The odds ratios (95% CI) associated with homozygous carriers of the T-allele were 1.86 (1.30–2.67) and 2.15 (1.48–3.13) in women and men, respectively. Population-attributable risks for diabetes associated with the T-allele were 14.8 and 22.3% for women and men, respectively. In a meta-analysis of 3,347 case and 3,947 control sujects, each copy of the T-allele was associated with a 1.48-fold increased risk ($P < 10^{-16}$). Our findings confirm that the *TCF7L2* gene represents an important locus for predicting inherited susceptibility to type 2 diabetes. *Diabetes* 55:2645–2648, 2006

Type 2 diabetes is a complex metabolic disease with major genetic components (1). To date, only modest effects have been identified for variants of candidate genes for type 2 diabetes, and the associations identified have often been inconsistent. Currently, reproducible associations have been documented for the Pro12Ala polymorphism in the peroxisome proliferator–activated receptor (*PPARG*) gene and the E23K polymorphism in *KCNJ11*, which encodes the ATP-sensitive K^+ channel Kir6.2 (2,3). Most recently, within a region in linkage to type 2 diabetes on chromosome 10q (4,5), a set of single nucleotide polymorphisms (SNPs) and a microsatellite marker in a well-defined linkage disequilibrium (LD) block in the transcription factor 7-like 2 (*TCF7L2*) gene were found to be significantly associated with type 2 diabetes risk (6). Although the functional causal variant has not been pinpointed, the association is intriguing in that emerging evidence indicates that the *TCF7L2* gene may be implicated in the pathogenesis of type 2 diabetes through its roles in the Wingless-type (Wnt) signaling pathway (6–8).

In light of these novel findings and considering that replication is a key criterion for convincing genetic association (9), we examined the association of a reported common variant of the *TCF7L2* gene and the risk of type 2 diabetes in two large well-established cohorts: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). Furthermore, we evaluated whether this relationship was modulated by conventional risk factors for type 2 diabetes.

RESEARCH DESIGN AND METHODS

The NHS and HPFS are prospective cohort studies among 121,700 U.S. female registered nurses aged 30–55 years at baseline in 1976 (NHS) and 51,529 U.S. male health professionals aged 40–75 years at baseline in 1986 (HPFS) (10,11). A blood sample was requested from all living participants between 1989 and 1990 for NHS and between 1993 and 1995 for HPFS. Demographics and health status of participants who provided blood samples were generally similar to those who did not. Among participants who returned blood samples, we identified 738 women with incident type 2 diabetes through the year 2000 in NHS, and they were matched to 1,131 healthy control subjects by age (within 1 year), race, month and year of blood draw, and fasting status. A second control subject was matched according to BMI ($\pm$1 kg/m^2) for all cases that were diagnosed before or during 1996 and for cases that were diagnosed during or after 1997 and in the top 10% of the distribution of BMI (12). In HPFS, we identified 999 cases of type 2 diabetes (414 incident cases between 1994 and 2002 and 585 prevalent cases), and they were matched to 999 control subjects by age, race, month and year of blood draw, and fasting status. To minimize potential bias due to population stratification, we restricted our analyses to non-Hispanic Caucasians (697 case/1,070 control subjects in NHS; 920 case/922 control subjects in HPFS). The analyses using the overall sample yielded similar results.

Type 2 diabetic cases were defined as self-reported diabetes confirmed by a validated supplementary questionnaire. In a validation study, 98% of self-reported diabetic cases identified by the supplementary questionnaire were confirmed by medical record review (13). For cases diagnosed before 1998, the National Diabetes Data Group criteria was used (14). The American

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GLP-1, glucagon-like peptide 1; HPFS, Health Professionals Follow-up Study; LD, linkage disequilibrium; NHS, Nurses' Health Study; SNP, single nucleotide polymorphism; TCF-4, transcription factor 4.

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TABLE 1
Comparison of baseline characteristics of type 2 diabetes case and control subjects among U.S. Caucasian women and men

Characteristics	Women			Men		
	Cases	Control subjects	*P**	Cases	Control subjects	*P**
n	687	1,051	—	886	896	—
Age (years)	56.5 ± 6.8	56.4 ± 6.9	0.90	56.6 ± 8.4	56.6 ± 8.5	0.91
BMI (kg/m^2)	30.6 ± 5.7	27.4 ± 5.9	<0.0001	27.7 ± 4.1	25.0 ± 2.8	<0.0001
Family history of diabetes in first-degree relatives	46.6	22.7	<0.0001	35.2	13.2	<0.0001
Physical activity (MET hours/week)	12.4 ± 15.1	15.1 ± 18.0	0.0016	14.0 ± 18.0	22.4 ± 28.9	<0.0001
Alcohol consumption (g/day)	2.87 ± 6.6	5.31 ± 9.6	<0.0001	10.9 ± 16.5	12.3 ± 15.3	0.06
Never smokers	42.6	47.8	0.05	39.3	47.1	0.11
Postmenopausal status	81.9	79.8	0.26	—	—	—
History of hypertension	54.7	31.0	<0.0001	37.8	17.4	<0.0001
History of high cholesterol	48.5	37.8	<0.0001	17.5	11.2	<0.0001

Data are means ± SD or percent. *Variables were compared between case and control subjects using Student's *t* test or χ^2 test.

Diabetes Association diagnostic criteria were used to ascertain type 2 diabetes after the 1998 cycle (15,16).

Genotype determination and assessment of covariates. In a previous study of variants of the *TCF7L2* gene and the risk of type 2 diabetes, a microsatellite marker DG10S478 (0/X composite allele) conferred the highest risk (6). SNP rs12255372 (a T/G variation in intron 3) was in strong (nearly perfect) LD with DG10S478 0/X (r^2 = 0.95) in CEPH Utah (CEU) HapMap samples (17). DG10S478 (0/X), SNP rs12255372, and a set of another four SNPs are located to a well-defined LD block in the *TCF7L2* gene. By sequencing exon 4 and all other *TCF7L2* exons, the existence of synonymous or nonsynonymous variations within exon 4 or other exons that account for the association among CEU was previously excluded (6). We selected the rs12255372 SNP as a representative candidate loci of the *TCF7L2* gene, which may be associated with the risk of type 2 diabetes.

DNA was extracted from the buffy coat fraction of centrifuged blood using a QIAmp blood kit (Qiagen, Chatsworth, CA). DNA samples were genotyped using Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). Replicate quality control samples (10%) were included and genotyped with 100% concordance. Primers and probes are available on request. Genotype data for the rs12255372 SNP were available for 686 case (98.4% of all cases) and 1,047 control subjects (97.8% of all control subjects) in women and 886 case (96.3% of all cases) and 896 control subjects (97.2% of all control subjects) in men. Information about medical history, anthropometric data, lifestyle factors, and family history of diabetes in first-degree relatives was assessed by self-administered questionnaire (10,11).

Statistical analyses. To assess whether genotype distributions among control subjects departed from Hardy-Weinberg equilibrium, χ^2 tests were used. ANOVA was used to compare geometric mean levels of continuous characteristics across genotypes in control subjects. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% CIs adjusted for matching factors. In secondary analyses, we further adjusted for conventional risk factors of diabetes (i.e., physical activity [MET hours/week], smoking, family history of diabetes, and the history of hypertension). Allele-specific OR was calculated assuming a multiplicative model, as heterozygous carriers had greater risk than noncarriers and less risk than homozygous carriers.

Heterogeneity of results was assessed by a χ^2 statistic between women and men in the present study and across five populations, including the present study and one previous study (6), totaling 3,347 type 2 diabetic cases and 3,947 control subjects. Tests of heterogeneity were not statistically significant. Thus, fixed-effect models were used to pool the OR estimates. Population-attributable risk (PAR) was calculated as PAR = (X-1)/X, assuming the multiplicative model where $X = (1\text{-}f)^2 + 2f(1\text{-}f)\gamma + f^2\gamma^2$; γ is the estimated OR and f is the frequency of the risk allele (18). All reported *P* values were two-tailed, and statistical significance was defined at the $\alpha = 0.05$ level. Statistical analyses were performed using SAS statistical package (version 8.2 for UNIX; SAS Institute, Cary, NC).

RESULTS

Distributions of characteristics of case and control subjects in women and men are presented in Table 1. Distributions of genotype frequencies did not significantly deviate from Hardy-Weinberg equilibrium among control subjects ($P = 0.95$ for women, $P = 0.83$ for men). There were no significant differences in baseline characteristics among control subjects according to the *TCF7L2* genotypes in either women or men (Table 2).

Frequencies of the minor allele (T), heterozygous carriers (i.e., genotype GT), and homozygous carriers (i.e., genotype TT) were significantly higher among cases than control subjects (Table 3). Both the heterozygous and the homozygous carriers had significantly increased type 2 diabetes risk compared with noncarriers. When we pooled

TABLE 2
Association of *TCF7L2* rs12255372 (T/G) genotypes with covariates among diabetes-free control subjects in U.S. Caucasian women and men

Covariate	Women				Men			
	GG	GT	TT	*P**	GG	GT	TT	*P**
n	564	409	78		471	363	62	
Age (years)	56.5 ± 6.9	56.2 ± 6.7	57.4 ± 7.4	0.37	56.9 ± 8.6	56.5 ± 8.5	55.1 ± 7.0	0.26
BMI (kg/m^2)	27.3 ± 6.0	27.4 ± 5.8	27.1 ± 5.8	0.90	25.2 ± 2.9	24.9 ± 2.7	24.7 ± 2.8	0.32
Physical activity (MET hours/week)	16.0 ± 19.5	14.3 ± 16.4	13.2 ± 15.0	0.24	24.1 ± 32.4	20.8 ± 25.0	18.9 ± 19.4	0.16
Family history of diabetes in first-degree relatives	22.8	23.0	20.5	0.89	11.9	14.3	16.1	0.46
Never smokers	47.1	48.0	50.0	0.38	46.5	47.9	46.8	0.79
Postmenopausal status	79.3	79.9	82.0	0.61	—	—	—	
History of hypertension	31.6	32.4	20.5	0.11	18.1	15.4	24.2	0.21
History of high cholesterol	38.3	36.3	42.3	0.56	12.3	9.9	9.7	0.51

Data are means ± SD or percent. *ANOVA was used to compare geometric mean levels of continuous characteristics across genotypes.

TABLE 3
Association of the *TCF7L2* rs12255372 (T/G) SNP with the risk of type 2 diabetes among U.S. Caucasian women and men

Genotype*	Case	Control	OR (95% CI)†	*P*	PAR
Women					
n	687	1,051			
GG	317 (46.1)	564 (53.7)	1.00		
GT	290 (42.3)	409 (38.8)	1.25 (1.01–1.55)		
TT	80 (11.6)	78 (7.5)	1.86 (1.30–2.67)		
Allele T	450 (32.8)	565 (26.8)	1.32 (1.13–1.54)	0.0002	14.8%
Men					
n	886	896			
GG	373 (42.1)	471 (52.6)	1.00		
GT	415 (46.8)	363 (40.5)	1.63 (1.32–2.02)		
TT	98 (11.1)	62 (6.9)	2.15 (1.48–3.13)		
Allele T	611 (34.5)	487 (27.1)	1.53 (1.31–1.80)	<0.0001	22.3%
P for heterogeneity‡		0.19			
Women + men					
n	1,573	1,947			
GG	690 (43.8)	1,035 (53.2)	1.00		
GT	705 (45.0)	772 (39.6)	1.43 (1.23–1.66)		
TT	178 (11.2)	140 (7.2)	1.99 (1.54–2.59)		
Allele T	1,121 (33.7)	1,052 (27.0)	1.42 (1.27–1.59)	<0.0001	18.7%

Data are *n* (%) unless otherwise indicated. *Genotype distributions do not depart from Hardy-Weinberg equilibrium. †Allele-specific OR calculated assuming a multiplicative model adjusted for age, date of blood draw, fasting status, and BMI. ‡χ^2 test of heterogeneity for the association in women and men.

the risk estimates for women and men, each copy of the variant allele was related to a 1.42-fold elevated risk of type 2 diabetes ($P < 0.0001$), and homozygous carriers of the variant allele had a 1.99-fold increased risk ($P < 0.0001$) compared with noncarriers. Further adjustment for conventional risk factors of diabetes did not change the results materially. The association between *TCF7L2* SNP and type 2 diabetes risk did not change materially after excluding prevalent cases in HPFS. Assuming a population frequency of 26% for the T-allele as observed in the present study and in a previous study (6), PAR were 14.8% for women and 22.3% for men. Although point estimates for the association of the *TCF7L2* SNP with type 2 diabetes risk was slightly stronger in men than women, the test for heterogeneity was not statistically significant ($P = 0.19$). The PAR was 18.7% for women and men pooled. Further, there was no evidence that the association of the *TCF7L2* SNP with type 2 diabetes risk was significantly modified by other diabetes risk factors such as BMI and physical activity in either men or women (all *P* values for interaction >0.10).

In a meta-analysis of five populations (i.e., populations in the present study and one previous study [6]), tests of heterogeneity of associations of the T-allele and heterozygous and homozygous carriers of the variant allele with type 2 diabetes risk across these populations were not statistically significant. Each copy of the T-allele was associated with a 1.48 (95% CI 1.37–1.60)-fold increased risk for type 2 diabetes ($P < 10^{-16}$). Compared with noncarriers, heterozygous carriers and homozygous carriers had a 1.42-fold ($P = 5.16 \times 10^{-10}$) and a 2.11-fold ($P < 10^{-16}$) elevated risk, respectively.

DISCUSSION

In this large prospective study of U.S. women and men, we observed a strong association of the *TCF7L2* rs12255372 (T/G) SNP with an increased risk of type 2 diabetes. This association was not significantly modified by conventional risk factors for type 2 diabetes. The corresponding population-attributable risk of the genetic variant was 14.8% for women and 22.3% for men.

Previous studies of the *TCF7L2* gene have focused on its role in human oncogenesis (19) and cancer progression, especially in colorectal cell lines (20). Recently, Grant et al. (6) observed that five SNPs including the rs12255372 SNP and a microsatellite marker DG10S478, which were in a well-defined LD block of the *TCF7L2* gene, were significantly associated with increased risk of type 2 diabetes. Consistent with these findings, we observed a significant association between the variant allele of the rs12255372 SNP and type 2 diabetes risk in both men and women. The frequency of the variant allele in our control subjects was also similar to that reported previously (6). The microsatellite DG10S478 marker and other SNPs in the LD block were not measured in the present study. However, network analysis documented that haplotypes with the DG10S478 X allele belong to a single monophyletic lineage in populations of European ancestry (6). This lineage of closely related haplotypes can be defined by the T-allele of the SNP rs12255372, which is in strong (nearly perfect) LD with the DG10S478 0/X microsatellite marker ($r^2 = 0.95$) (6). Although we cannot determine whether the rs12255372 SNP is a causative variant or a surrogate for an underlying causal variant, consistent findings of a strong association of the *TCF7L2* SNP with type 2 diabetes risk in these independent populations minimize the possibility of a chance finding and indicate that variants in the LD block of the *TCF7L2* gene may be implicated in the pathogenesis of type 2 diabetes.

The exact mechanism by which the *TCF7L2* gene may be related to the risk of type 2 diabetes has yet to be determined. Several lines of evidence indicate that it could be through regulating glucagon-like peptide 1 (GLP-1) by its roles in the Wnt signaling pathway. Human T-cell transcription factor 4 (TCF-4), the *TCF7L2* gene product, is a high-mobility transcription factor that plays a pivotal role in the Wnt signaling pathway, which is one of the key developmental and growth regulatory mechanisms of the

cell (7,21). TCF-4 regulates the transcription of proglucagon gene in enteroendocrine cells, the gene encoding the insulinotropic hormone GLP-1 in vitro (8). Dominant-negative TCF-4 was shown to repress proglucagon gene mRNA expression and GLP-1 synthesis (8). GLP-1 has been demonstrated to exert critical effects on blood glucose homeostasis; GLP-1 can lower blood glucose levels through the stimulation of insulin secretion and biosynthesis, the inhibition of glucagon release and gastric emptying and the enhancement of peripheral insulin sensitivity (8). Therapeutic strategies for type 2 diabetes based on GLP-1 appears to be promising (22,23).

A potential limitation of our study is that some of our control subjects may have undiagnosed diabetes, which could have biased our results toward the null. However, due to the professional background of the study participants, the proportion of undiagnosed diabetes in our study of health professionals was much lower than that in the general population according to our previous validation study (24). Population stratification may affect the observed associations. However, because our case and control subjects were selected in well-characterized cohorts with a defined study base and the analyses were restricted to Caucasians, bias due to population stratification is unlikely.

In summary, a significant association of the *TCF7L2* variant with type 2 diabetes risk was observed in both large prospective cohorts of U.S. women and men and are in accordance with those from a previous study (6). Although the exact biological mechanism for the association between the *TCF7L2* gene and type 2 diabetes risk remains uncertain, these consistent findings indicate that *TCF7L2* gene represents an important locus for predicting inherited susceptibility to type 2 diabetes.

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