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ARTICLE

Transcription factor 7-like 2 polymorphisms and type 2 diabetes, glucose homeostasis traits and gene expression in US participants of European and African descent

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Abstract
Aims/hypothesis We sought to determine: (1) the role of previously described transcription factor 7-like 2 (*TCF7L2*) variants in type 2 diabetes in African American individuals and in participants of European ancestry; (2) the physiological impact of these variants on glucose homeostasis; and (3) whether the non-coding variants altered *TCF7L2* expression in adipocytes and transformed lymphocytes.
Methods Association studies were conducted by genotyping 932 Europid and African American diabetic and control participants. Family studies were conducted in 673 members of 68 Europid families ascertained for at least two diabetic siblings. Metabolic studies were conducted in 585 non-diabetic individuals who had undergone frequently sampled intravenous glucose tolerance tests to determine insulin sensitivity and insulin secretion. Gene expression studies were conducted in 74 adipose samples and 64 muscle samples from non-diabetic individuals with known genotypes and also in 55 lymphoblastoid cell lines.
Results *TCF7L2* variants were associated with type 2 diabetes in a Europid case–control population and in families, but not in African Americans. Risk alleles increased the 60 min post-challenge glucose value in Europid families and reduced insulin sensitivity by 45% in Europids, but did not alter insulin secretion. *TCF7L2* expression was not altered by genotype and did not correlate with insulin sensitivity or BMI.
Conclusions/interpretation We confirmed *TCF7L2* as a risk factor in a population of European descent, where it reduced glucose tolerance and insulin sensitivity, but not insulin secretion. We found no role in African Americans and could not explain the association by altered adipocyte or muscle gene expression.

Keywords Allelic association · Gene expression · Insulin resistance · Insulin secretion · Single nucleotide polymorphism · SNP · Type 2 diabetes · WNT signalling

Abbreviations

AIR_g	acute insulin response to glucose
AIR_{max}	maximally potentiated insulin response to arginine
DI	disposition index
HOMA	homeostatic model assessment
OR	odds ratio
RR	relative risk
S_I	insulin sensitivity
SNP	single nucleotide polymorphism
WNT	wingless MMTV integration site family

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Introduction

Despite convincing evidence from twin and family studies that type 2 diabetes has a strong genetic component, unravelling the genetic predisposition has proved frustrating. Our laboratory and many others have reported significant associations in genes identified by positional cloning efforts and/or strong candidacy, but replication of these findings has been difficult to achieve. The most widely accepted type 2 diabetes susceptibility variants, Pro12Ala in the gene encoding peroxisome proliferator-activated receptor gamma (*PPARG*) [1] and Glu23Lys in the beta cell potassium channel gene potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*) [2, 3] confer only a modestly increased risk of type 2 diabetes in Europids. Recently, Grant et al. [4] identified a microsatellite in intron 3 of the transcription factor 7-like 2 gene (*TCF7L2*) that was strongly associated with type 2 diabetes, with a combined *p* value in Icelandic, Danish and US populations of 10^{-18} and a combined relative risk (RR) of 1.56. The single nucleotide polymorphisms (SNPs) rs12255372 and rs7903146 were in strong linkage disequilibrium with the microsatellite and showed similarly strong associations. Subsequent to this initial report, numerous published studies have examined this gene, with consistent replication among multiple cross-sectional Europid populations [5–10] and increased risk in several prospective studies [11–13]. These studies have resulted in extraordinarily low *p* values, odds ratios (ORs) of 1.4 to 1.9 and a risk of 1.4 per risk allele in Europid cohorts from prospective studies. Although no association was reported in African American [11] or African Caribbean [13] subsets, recently Helgason et al. [14] reported that SNP rs7903146 was associated with type 2 diabetes in families from west Africa (p=0.0002, RR 1.45).

TCF7L2 binds to β-catenin downstream of wingless MMTV integration site family (WNT) to regulate proglucagon gene expression in gut endocrine L cells, but reportedly not in pancreatic alpha cells [15]. Other studies report that *TCF7L2* is widely expressed, including in mature pancreatic beta cells as well as in peripheral and omental adipocytes [8]. The WNT pathway is important in adipogenesis [16]; thus, a broader physiological role for *TCF7L2* in glucose homeostasis is plausible. Both Florez et al. [11] and Saxena et al. [6] reported reduced insulin secretion in response to oral glucose in carriers of the risk allele. Damcott et al. [10] examined 48 unrelated Europid individuals by frequently sampled IVGTT and found a significant association with a reduced insulin sensitivity index (S_I) and the beta cell compensation for insulin resistance (disposition index [DI]), but only a marginal reduction in acute insulin response to glucose (AIR_g). Munoz et al. [17] reported reduced AIR_g and DI in the subset of 138 Europid and 118 African American women who were homozygous for the TT genotype at SNP rs12255372, albeit with statistical significance only in Europids and with no association for SNP rs7903146. In contrast, Scott et al. [7] reported no association with fasting insulin, glucose tolerance, S_I or DI in Finland–US Investigation of NIDDM Genetics (FUSION) families, while Cauchi et al. [18] found only non-significant trends towards reduced fasting insulin and homeostatic estimates of insulin secretion (homeostatic model assessment [HOMA]-B).

We sought to further explore the role of *TCF7L2* in type 2 diabetes and prediabetic traits by testing the association of previously reported variants in 932 individuals from Europid and African American case–control populations and in 693 members of Europid families ascertained for multiple diabetic siblings. To examine the physiological impact of *TCF7L2* variants, we examined 585 non-diabetic participants who had undergone frequently sampled IVGTT, including members of families ascertained in Utah and unrelated Europid and African American participants ascertained in Arkansas. Finally, we examined *TCF7L2* expression in adipose tissue, muscle tissue and transformed lymphocytes by genotype. Our results suggest ethnic differences in the risk of type 2 diabetes conferred by *TCF7L2* alleles. We provide support for a role of *TCF7L2* variants in insulin sensitivity and impaired glucose homeostasis in Europids, but we did not find significant evidence for genotypic effects on gene expression in three different tissues.

Methods

Study participants The study populations are summarised in Table 1. We tested for an association of *TCF7L2* variants with type 2 diabetes in a northern European (Europid) population primarily ascertained in Utah and in an African American population ascertained primarily in Arkansas. For both studies, diabetic participants were previously diagnosed and on pharmacological therapy or had clearly diabetic glucose tolerance tests. All diabetic patients had at least one first-degree relative with type 2 diabetes. Control participants had normal glucose tolerance tests or fasting or post-meal glucose levels below 5.6 mmol/l. Controls were selected for no family history of type 2 diabetes in parents, siblings or grandparents. Metabolic effects of *TCF7L2* variants were examined in three non-diabetic study populations. Non-diabetic members of northern European Utah families ascertained for at least two diabetic siblings underwent tolbutamide-modified, frequently sampled IVGTT, as described previously [19]. Europid participants and African American non-diabetic individuals ascertained in Arkansas were studied using either a tolbutamide-modified frequently sampled IVGTT (122 Europid, 69 African American) as described previously [20] or, because tolbutamide became unavailable during the study, an insulin-modified test (0.04 U/kg of

Table 1 Summary of study populations

Population	Description	Male/female (*n*)	BMI (kg/m^2)	Age (years)	Diagnosis age (years)
Europid case–control	Control	72/115	27.5 (18.5, 41.1)	51.0 (15.3)	–
	Case	134/57	31.1 (21.9, 44.2)	61.7 (10.7)	51.5 (12.1)
African American case–control	Control	91/94	29.5 (18.8, 46.3)	42.8 (13.3)	–
	Case	197/172	32.0 (20.8, 49.4)	54.8 (12.5)	42.8 (11.9)
Utah family study	Non-diabetic	175/215	27.1 (13.9, 40.5)	43.5 (16.0)	–
	Diabetic	124/159	29.9 (21.0, 42.6)	57.1 (14.2)	48.9 (12.2)
Utah Europid metabolic	Non-diabetic	50/72	27.5 (18.3, 41.3)	39.3 (10.5)	–
Europid metabolic	Non-diabetic	101/243	30.1 (20.3, 44.6)	38.9 (10.1)	–
African American metabolic	Non-diabetic	48/71	30.2 (20.5, 44.9)	37.5 (8.9)	–

Unless otherwise stated, values are means, shown as arithmetic means for normal variables and geometric means for skewed variables (BMI)
Age is shown as mean (SD), BMI as mean (95%CI) transferred to the linear scale from the ln-transformation

insulin at 20 min) using a similar protocol [21, 22] (217 Europids, 88 African Americans) (Table 2). Arkansas participants were ascertained for variable family history of type 2 diabetes and representing a range of weight, age at study, and glucose tolerance from normal to impaired [23, 24]. Participants underwent two visits: a standard 75 g 2 h OGTT with sampling at baseline, 30 min, 60 min, 90 min and 120 min for insulin and glucose and a frequently sampled IVGTT. A subset of 144 Europid and 60 African American participants underwent determination of the maximum insulin response to arginine (AIR_{max}). At the conclusion of the frequently sampled IVGTT, the glucose

Table 2 Descriptive statistics of unrelated, non-diabetic participants for metabolic studies

Trait	Europid	African American	*p* value
n	344	159	
Protocol (tolbutamide/insulin)	125/219	70/89	–
Sex (male/female)	101/243	54/105	–
Age (years)	38.9 (10.1)	38.4 (9.2)	NS
BMI (kg/m^2)	30.9 (6.1)	30.8 (6.1)	NS
WHR			
Male	0.951 (0.071)	0.927 (0.058)	0.04
Female	0.863 (0.074)	0.868 (0.094)	0.61
PFAT (%)	36.7 (9.1)	34.0 (10.7)	0.004
Systolic BP (mmHg)	115.8 (15.5)	111.3 (15.7)	0.003
Diastolic BP (mmHg)	75.5 (10.7)	68.3 (13.7)	2×10^{-8}
Cholesterol (mmol/l)	4.80 (1.01)	4.51 (1.04)	0.002
HDL-cholesterol (mmol/l)	1.30 (0.36)	1.42 (0.27)	0.0002
LDL-cholesterol (mmol/l)	2.83 (8.6)	2.64 (4.0)	0.005
Triacylglycerol (mmol/l)	1.50 (0.95)	1.03 (0.60)	4.5×10^{-12}
$AUC_{glucose}$ (mmol/l×min)	854.9 (172.7)	819.7 (178.4)	0.05
Fasting glucose (mmol/l)	4.74 (0.54)	4.75 (0.65)	0.76
2 h glucose (mmol/l)	6.44 (1.77)	6.13 (1.91)	0.08
$AUC_{insulin}$ (pmol/l×min)	46663 (36098)	54689 (36656)	0.03
Insulinogenic index (pmol/mmol)	116.9 (118.5)	254.1 (516.8)	0.00005
S_I ($\times10^{-4}$ min^{-1} [μU/ml]$^{-1}$)[a]	4.00 (4.80)	3.62 (3.55)	0.30
AIR_g (pmol/l×min)	2792 (2108)	4556 (3578)	1.2×10^{-7}
S_g (min^{-1})[b]	0.0174 (0.0088)	0.0190 (0.0082)	0.13
DI[c]	1430 (1213)	2168 (1893)	0.0002

Values are shown as means (SD).
Comparisons of Europid and African American participants were ascertained similarly in Arkansas for non-diabetic glucose tolerance tests. Comparisons were by two-tailed independent samples *t* test; skewed variables were ln-transformed prior to analysis.
BP: blood pressure, *PFAT*: per cent body fat from dual-energy X-ray absorptiometry measurement.
[a] Units are taken from the MINMOD program. To convert values to SI units ($\times10^{-4}$ min^{-1} [pmol/l]$^{-1}$) mutiply by 0.167.
[b] S_g is a measure of the ability of glucose to promote its own uptake.
[c] DI is calculated as $S_I\times AIR_g$ for individual values.

was increased over 10 min to 23 to 25 mmol/l, which was maintained for 30 min. At 30 min of hyperglycaemia, a 5 g intravenous arginine bolus was given over 30 s. Insulin response to arginine was measured at baseline (25 mmol/l glucose) and at 2, 3, 4, 6, 8 and 10 min.

Family-based analyses for type 2 diabetes, lipids and glucose homeostasis traits based on 75 g OGTTs were conducted in 673 members of 68 Utah families for which both genotypic and phenotypic data were available, as summarised in Table 1 and reported previously [25, 26]. A subset of non-diabetic participants from Arkansas who had had metabolic studies also underwent fat and muscle biopsies [27], including 28 participants with impaired glucose tolerance [23]. All participants provided written informed consent under approved Institutional Review Board protocols of either the University of Utah Health Sciences Center or the University of Arkansas for Medical Sciences.

Genotyping We typed rs7903146 and rs12255372 using pyrosequencing on a pyrosequencer (PSQ-96; Biotage, Uppsala, Sweden). Primer design was modified to permit use of a biotinylated universal primer. More than110 duplicate samples were included across all studies with 100% agreement for both SNPs. The microsatellite marker DG10S478 [4] was typed by using infrared dye labelled M13 primer on a sequencer (GR4200; LI-COR Biosciences, Lincoln, NE, USA) and scored using GeneImage IR software (version 3.5.6; Scanalytics, Fairfex, VA, USA). All primer sequences are available on request. Based on strong linkage disequilibrium between the SNPs and the dichotomised microsatellite [4], only the SNPs were typed for family and metabolic studies.

Gene expression RNA was extracted from Epstein–Barr virus-transformed lymphocytes grown under standard culture conditions using either Trizol (Invitrogen, Carlsbad, CA, USA) or RNEasy (Qiagen, Valencia, CA, USA). Adipose RNA was extracted using kits (RNeasy Lipid Tissue Minikit; Qiagen). Muscle RNA was extracted using RNA kits (Ultraspec; Biotecx Laboratories, Houston, TX, USA). Reverse transcription was performed using MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, CA, USA). Because of multiple splice forms for *TCF7L2* [28], we initially designed primer sets for both the 5′ (exons 1 and 2/3) and 3′ (exons 12 and 13/14) ends of the gene (Electronic supplementary material [ESM] Table 1, ESM Fig. 1). However, the 5′ primer set gave a single band in all tissues tested and detected all documented splice forms. Hence, we report only the results using this assay. Similar to findings in a previous report [8], we detected measurable levels of *TCF7L2* message in muscle but at 10-fold lower levels than in adipose. Real-time quantitative RT-PCR was conducted either on an ABI 7500 (Applied Biosystems) or on a Rotorgene 2000 (Corbett Life Science, Sydney, NSW, Australia) using SYBR Green (Applied Biosystems). All assays were normalised to 18S RNA.

Laboratory measures Insulin was measured by the General Clinical Research Center Core Laboratory using an immuno-chemiluminometric assay (MLT Assay, Cardiff, Wales, UK). Plasma glucose was measured by using a glucose oxidase method.

Statistical analysis Allele frequencies for the case–control study were compared using the Armitage Trend and Fischer Exact tests. Hardy–Weinberg equilibrium was tested separately in diabetic and control participants using the online DeFinetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Linkage disequilibrium and haplotype associations were tested in Haploview 3.32 [29]. S_I was estimated from glucose and insulin measures during the frequently sampled IVGTT using the MINMOD [30] or MONMOD Millenium programs [31]. AIR_g was calculated as the mean insulin response above basal from 2 to 10 min following the glucose bolus for the Utah family sample or as the AUC for the same time for Arkansas Europid and African American samples. DI was calculated as $S_I \times AIR_g$. Glucose and insulin AUCs ($AUC_{glucose}$, $AUC_{insulin}$) were calculated from the trapezoidal rule. Genotypic effects on glucose homeostasis traits (S_I, AIR_g, DI, $AUC_{glucose}$, $AUC_{insulin}$, insulinogenic index) and obesity traits (BMI, WHR, per cent fat) were tested using mixed effect, general linear models. Skewed variables were ln-transformed to normality prior to analysis. All models of glucose homeostasis traits included age and BMI as covariates and sex and genotype as fixed factors. Additionally, diagnosis and pedigree membership were included as fixed factors in analyses of family members from the Utah study. We adjusted for protocol type (tolbutamide or insulin) by including a numeric code as a fixed factor for participants studied in Arkansas [32]. Analyses of obesity traits were adjusted for age and sex. The significance of associations detected in the model was examined by comparison of marginal means using the least significant difference test. Based on the strong prior evidence and complicated correlations among analyses, we considered $p<0.05$ to be significant without presenting a correction for multiple testing. Gene expression ratios were tested after ln-transformation to normality using analysis of variance or mixed effects models. All analyses were conducted in SPSS for Windows, v 12 (SPSS, Chicago, IL, USA).

Family-based analyses were conducted using measured genotype analysis. The effect of each SNP was tested using logistic regression models for type 2 diabetes and normal densities for the quantitative traits using likelihood analysis implemented in the Java version of the Pedigree Analysis Package, jPAP [33]. Significance was tested using 2 *df* χ^2

statistics computed as twice the natural logarithm of the ratio of the likelihood upon estimating genotype-specific penetrances or means to the likelihood of a single penetrance or mean. In addition to genotype-specific penetrances or means, the effects of sex, age, BMI and polygenic inheritance were also estimated by maximum likelihood.

Power considerations Based on a Europid case and control population of 192 individuals each, a control allele frequency of 0.25 and an OR of 1.5, we had 70% power to detect an association at $p<0.05$ or 86% power to detect an OR of 1.6. Among African Americans, we had 85% power to detect an OR of 1.5 with a control allele frequency of 0.30 in 186 control and 369 diabetic participants at $p<0.05$ or 95% power at an OR of 1.6. For the quantitative trait study in Europids, we considered a simplified model, in which carriers and rare homozygotes were grouped and traits were ln-transformed for comparison by Student's *t* test. Notably, we used general linear regression with genotype for the actual analyses, but power analysis of that model was technically problematic. For S_I, we had 84% power to detect a 10% difference in ln S_I (mean±SD; 3.3±0.9 to 3.0±0.9) or a difference on the linear scale of 2.7 (95%CI 0.46–15.8) to 2.0 (95%CI 0.34–11.7). For AIR_g, we had 92% power to detect a 5% fall in lnAIR_g (mean±SD 6.0±0.8 to 5.7±0.8) or a difference on the linear scale from 403 (95%CI 84–1,935) pmol/l to 299 (96% CI 62–1,434) pmol/l.

Results

Associations of TCF7L2 with type 2 diabetes mellitus The now well-replicated association of the T allele at rs7903146 was replicated in Europids (OR 1.72, $p=0.0008$), with lesser associations at SNP rs12255732 and the microsatellite DG10S478 (OR 1.46, $p=0.02$). In contrast, none of these markers showed any evidence for an association with type 2 diabetes in African American participants ($p>0.5$). Results are summarised in ESM Table 2. Whereas the three markers were in strong linkage disequilibrium in Europids ($r^2>0.7$), little linkage disequilibrium was observed in African Americans ($r^2<0.3$) (ESM Tables 3 and 4). No significant association with BMI was seen for any SNP in Europids (data not shown), but the rs12255372 TT genotype was nominally associated with reduced BMI in African Americans (GG 31.1±16.2, GT 30.9±14.0, TT 28.4±7.0 kg/m^2; $p=0.03$).

Family-based studies of TCF7L2 SNPs in Europids Both phenotypic and genotype data for SNPs rs7903146 and rs12255372 were available in 673 members of 68 families. Although neither SNP showed excess transmission from parents to affected offspring using methods described previously [33–35], logistic regression methods showed a significant association of the TT genotype, for each of the two SNPs, with diabetes penetrance and with increased 60 min and 90 min glucose levels, but not with reduced insulin levels or altered HOMA estimates of insulin sensitivity (Table 3; data not shown). Among the subset of 122 non-diabetic family members who underwent frequently sampled IVGTT, neither SNP altered S_I, AIR_g or DI (data not shown).

Metabolic studies of TCF7L2 SNPs in unrelated, non-diabetic participants The characteristics of 159 non-diabetic African American and 344 non-diabetic Europid participants are shown in Table 2. The populations were similar for age and BMI, but African Americans had significantly greater insulin secretion (AIR_g, DI, insulinogenic index, $AUC_{insulin}$) and more favourable blood pressure and lipid profiles than Europids. Given the differences in linkage

Table 3 Measured genotype analysis in families for type 2 diabetes and glucose homeostasis traits

Trait	*n*	rs7903146				rs12255372			
		CC	CT	TT	*p* value	GG	GT	TT	*p* value
T2DM	668	0.43 (0.05)	0.48 (0.05)	0.61 (0.07)	0.037	0.42 (0.05)	0.48 (0.05)	0.64 (0.07)	0.017
60 min glucose	404	5.96 (5.78–6.14)	6.42 (6.15–6.70)	6.66 (6.03–7.36)	0.02	5.94 (5.78–6.10)	6.52 (6.35–6.71)	6.45 (5.81–7.18)	0.009
90 min glucose	187	5.34 (5.12–5.53)	5.81 (5.55–6.08)	6.13 (5.49–6.86)	0.034	5.32 (5.14–5.51)	5.87 (5.67–6.09)	6.08 (5.38–6.88)	0.022
60 min insulin	374	234 (123–447)	264 (105–663)	275 (218–346)	0.152	232 (129–419)	272 (104–711)	263 (206–335)	0.065

Means are shown from the measured genotype analysis for traits that were significant or approached significance. Type 2 diabetes (T2DM) is shown as the genotype-specific penetrance (values are mean (SD)). All other traits are shown as mean and 95%CIs, transformed to the linear scale from the ln-transformed results. All means were adjusted to male, age 80 years (type 2 diabetes) or 30 years (homeostasis traits) and BMI 20 kg/m^2.

n varied with trait availability and missing genotypes.

Glucose and insulin traits were tested only in non-diabetic individuals.

disequilibrium, metabolic profiles and association with type 2 diabetes, we analysed Europid and African American populations separately (Table 4, ESM Table 5). Risk alleles for both rs12255372 and rs7903146 were associated with reduced S_I in Europids ($p<0.007$), but not in African Americans. Consistent with reduced S_I in risk allele carriers, $AUC_{insulin}$ was increased. In contrast, no measure of insulin secretion (AIR_g, DI, AIR_{max}, insulinogenic index) differed significantly by genotype. $AUC_{glucose}$ showed a non-significant trend towards increasing values with heterozygosity and homozygosity for the risk allele (Table 4), but neither 2 h glucose nor glucose tolerance status (normal vs impaired glucose tolerance) differed by genotype at either marker ($p>0.7$; data not shown). Among African Americans, $AUC_{insulin}$ and the insulinogenic index were reduced in heterozygotes compared with common homozygous individuals, but not in the small number of minor allele homozygotes (Table 4, ESM Table 5). Neither SNP was significantly associated with BMI, waist circumference, WHR or per cent fat in either population (data not shown).

TCF7L2 gene expression We examined adipose tissue in 74 individuals for whom we had both *TCF7L2* expression and genotype (13 male, 61 female; 66 Europid, eight African American). Analyses were adjusted for age, sex, ethnicity and BMI using mixed effects regression models. Expression levels did not differ by genotype for either SNP (Table 5 for rs7903146), nor did levels influence S_I or AIR_g ($p>0.2$; data not shown). Glucose tolerance status was not a determinant of gene expression ($p>0.3$; data not shown). Conclusions were not altered when analyses were restricted to Europid samples. However, in exploratory analyses, significant interaction was found between genotype and BMI to determine expression levels for SNP rs12255732 in all samples ($p=0.003$) and for rs7903146 in Europid samples ($p=0.03$).

Muscle tissue was examined from 64 individuals for whom genotypes were available (58 Europid, six African American; eight male, 56 female). Data for rs7903146 are presented in Table 5. A non-significant trend towards lower expression in TT homozygotes was observed for both SNPs ($p>0.25$); again results did not differ if only Europid samples were included. Muscle *TCF7L2* expression was approximately tenfold lower than in adipose. Expression levels did not correlate with and were not significant determinants of S_I or AIR_g ($r^2<0.01$, $p>0.3$); again, they were not altered by glucose tolerance status ($p>0.2$; data not shown). Among the

Table 4 Metabolic studies for SNP rs7903146 in unrelated, non-diabetic individuals from Arkansas

	Europid				African American			
Genotype	CC	CT	TT	*p* value	CC	CT	TT	*p* value
n	177	136	23		84	66	7	
S_I ($\times10^{-4}$ min^{-1} $[\mu U/ml]^{-1}$)[a]	3.17 (2.79–3.60)	2.94 (2.54–3.40)	1.74 (1.24–2.44)	0.004	2.70 (2.35–3.10)	2.91 (2.46–3.43)	2.34 (1.45–3.77)	NS
AIR_g (pmol/l×min)	2183 (1925–2476)	2074 (1796–2394)	2501 (1793–3488)	0.56	3456 (2820–4230)	3018 (23270–3846)	3498 (1740–7026)	NS
S_g (min^{-1})[b]	0.0158 (0.0147–0.0169)	0.0167 (0.0153–0.0181)	0.0141 (0.0116–0.017)	0.21	0.0175 (0.0155–0.0197)	0.0170 (0.0147–0.0195)	0.0135 (0.009–0.0203)	NS
DI[c]	1152 (1001–1326)	1061 (903–1248)	726 (501–1052)	0.067	1596 (1278–1993)	1450 (1112–1890)	1361 (634–2922)	NS
$AUC_{glucose}$ (mmol/l×min)	834 (809–859)	867 (838–898)	875 (804–952)	0.16	794 (758–833)	821 (777–869)	863 (731–1020)	NS
$AUC_{insulin}$ (pmol/l×min)	34930 (31687–38504)	41361 (36948–46301)	48460 (36562–64230)	0.016	50277 (43594–57983)	38899 (33007–45842)	45851 (26597–79041)	0.06[d]
Insulinogenic index (pmol/mmol)	91.5 (81.9–102.1)	97.8 (86.1–111.1)	102.0 (74.3–140.0)	0.64	170 (137–212)	110 (85–141)	127 (55–291)	0.033[e]
AIR_{max} (pmol)[f]	1184 (1035–1354)	1356 (1162–1582)	1753 (1232–2495)	0.078	1123 (910–1385)	1111 (874–1412)	874 (465–1644)	NS

p values are shown for the general model.

[a] Units are taken from the MINMOD program. To convert values to SI units ($\times10^{-4}$ min^{-1} $[pmol/l]^{-1}$) multiply by 0.167.

[b] S_g (glucose effectiveness) is a measure of the ability of glucose to promote its own uptake.

[c] DI is calculated as $S_I \times AIR_g$ for individual values.

[d] C/C vs C/T, $p=0.018$

[e] C/C vs C/T, $p=0.009$

[f] Number of participants for AIR_{max} was reduced to 78/56/10 for CC/CT/TT genotypes at rs7903146 in Europids, to 28/29/3 in African Americans and to 75/63/6 for glucose tolerance test-related traits in African Americans, respectively.

Table 5 Gene expression levels in subcutaneous adipocytes and muscle from non-diabetic individuals by rs7903146 genotype

Tissue	CC	CT	TT
Adipose			
n	39	28	7
Ratio	0.948 (0.831–1.082)	0.980 (0.840–1.14)	0.921 (0.706–1.200)
Muscle			
n	30	30	4
Ratio	0.371 (0.284–0.486)	0.338 (0.246–0.464)	0.262 (0.136–0.501)

Numbers of participants for each tissue and with each genotype are shown.
Ratios of *TCF7L2* to 18S RNA are given as the mean and 95%CIs, obtained from marginal means in a mixed effect model adjusting for age and ln(BMI)
For data for rs12255372, see ESM Table 5.
No differences approach significance: $p>0.4$

38 individuals for whom both fat and muscle tissue were available, expression levels showed no significant correlation between tissues ($r^2=0.015$, $p=0.6$).

We next examined transformed lymphocyte cDNA from transformed lymphocytes from 25 Europid (10 non-diabetic, 15 diabetic) and 30 African American (10 non-diabetic, 20 diabetic) participants. African Americans had lower expression levels (0.94±0.58) than Europids (1.39±1.38; $p=0.03$), a difference not observed in the smaller number of adipose and muscle samples available from African American participants. Regardless of whether African American and Europid samples were considered separately or together, expression levels did not differ by diabetic status or by genotype (Table 6). In an exploratory analysis, rs7903146 interacted with diabetic status to reduce *TCF7L2* expression in non-diabetic TT homozygotes relative to common CC homozygotes, whereas levels were increased in CT and TT diabetic participants (Fig. 1).

Discussion

TCF7L2 is the best replicated and strongest genetic risk factor identified to date for type 2 diabetes in Europids, with SNP rs7903146 showing the most consistent and

Table 6 *TCF7L2* expression studies of transformed lymphocytes in diabetic and non-diabetic participants of Europid and African American ancestry

	Level			
Trait	CC (7 E/13 AA)	CT (8 E/13 AA)	TT (9 E/4 AA)	*p* value
Rs7903146 genotype				
All	0.89 (0.69–1.16)	0.92 (0.70–1.21)	0.70 (0.48–1.02)	0.48
Europid	1.03 (0.66–1.60)	1.07 (0.44–2.62)	1.02 (0.37–2.85)	0.97
African American	0.74 (0.18–3.04)	0.85 (0.23–3.14)	0.69 (0.27–1.77)	0.82
Diabetes				
Control (10 E/10 AA)				
Europid	0.87 (0.43–1.76)		—	0.063
African American	0.89 (0.29–2.73)		—	0.42
Type 2 diabetes (15 E/20 AA)				
Europid		1.34 (0.39–4.56)		0.063
African American		0.72 (0.20–2.85)		0.42
Ethnicity				
Control (10 E/10 AA)				
Europid ($n=24$)	1.02 (0.80–1.30)			0.03
T2DM (15 W/20 AA)				
African American ($n=30$)		0.68 (0.52–0.89)		

Comparison of ratios of *TCF7L2* to 18S RNA for Epstein–Barr virus transformed lymphocytes from Europid and African American participants across genotype and diabetes status.
All ratios were ln-transformed and are presented as geometric means with 95%CIs.
Numbers of participants are shown in parentheses for each category.
p values were calculated by analysis of variance.
For the presentation of marginal means from a mixed effects regression mode, see Fig. 1.
AA: African American, *E*: Europid

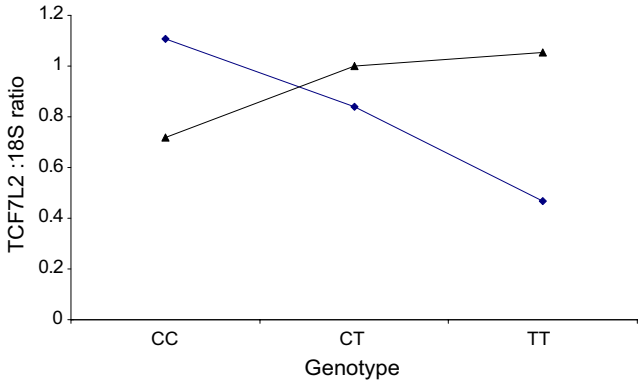

Fig. 1 Expression was evaluated in a mixed effects model with ethnicity and diagnosis (diabetic or non-diabetic) as co-factors. Expression ratio is for the 5′ primer set normalised to 18S RNA. Number of samples: *CC* (7 control, 13 diabetic); *CT* (8 control, 13 diabetic); *TT* (9 control, 4 diabetic). *diamonds*, control; *triangles*, diabetic

strongest association [6]. No coding variant explains the *TCF7L2* association with type 2 diabetes [4]. Our study replicates this association in Europids, in both a case–control study and in family-based analyses, with an OR for rs7903146 of 1.7, which is comparable with many previous studies [8]. Furthermore, we found significantly higher post-challenge plasma glucose levels in non-diabetic carriers of the *TCF7L2* risk alleles, both in members of high-risk families and in unrelated individuals. In contrast, we found no association in a similarly ascertained African American cohort. Few studies of *TCF7L2* in African Americans have been published. In the relatively small African American subset of the Diabetes Prevention Program [11] and in 385 Afro-Caribbean participants from the UK [13], *TCF7L2* was not associated with type 2 diabetes mellitus. Because the minor (T) allele frequency at rs7903146 is even higher in African Americans than in Europids, allele frequency cannot explain this discrepancy. In contrast, Helgason et al. [14] did replicate the association of rs7903146 in families from West Africa (RR 1.45, $p=0.002$ after correcting for relatedness and ancestry). Differences in linkage disequilibrium between populations of African and European ancestry are unlikely to explain this discrepancy, given that rs7903146 appears to be the causative SNP [14]. Using our African American sample size, an OR of 1.5 (close to the 1.45 estimated in West Africans) and the control minor allele frequency of 0.30, we had 85% power to detect an association at $p<0.05$. Were the OR only 1.3, our power would fall to 52%. Alternatively, gene–gene or gene–environment interactions could easily explain the differences among populations [36], particularly given the different lifestyles of West African and Arkansas African American individuals.

TCF7L2 binds to the promoter of the proglucagon gene in gut endocrine L cells [15], thus potentially acting through glucagon-like peptide 1 to affect insulin secretion. Indeed, some but not all studies have shown impaired insulin secretion to oral glucose in individuals with the rs7903126 TT genotype [6, 11, 18]. In contrast, we did not find evidence for such an effect. Although we found nominal evidence for reduced insulin secretion in African American carriers of the risk alleles, insulin secretion was higher among Europid individuals with risk alleles, consistent with reduced insulin sensitivity. Whereas we did observe the trend towards lower DI reported in smaller studies of Damcott et al. [10] and Munoz et al. [17]), this trend was driven by reduced S_I. However, post-challenge plasma glucose was increased with the risk allele among non-diabetic members of high-risk families (Table 3), with a similar trend among non-diabetic, unrelated individuals (Table 4). Nonetheless, neither 2 h glucose as a quantitative trait nor glucose tolerance (2 h glucose higher than 7.8 mmol/l) as a dichotomous trait was associated with *TCF7L2* genotype ($p>0.7$).

TCF7L2, which is widely expressed, acts downstream of WNT to complex with β-catenin, which in turn activates transcription of a wide variety of genes, including those implicated in cell proliferation and neoplasia [37, 38]. The WNT pathway also plays a role in adipogenesis [39]; hence *TCF7L2*, which is well expressed in adipose, may play a role in insulin action and insulin sensitivity. Damcott et al. [10] first reported reduced S_I in non-diabetic Europid participants with *TCF7L2* risk alleles. Our data confirm that association in a much larger Europid population, with a 45% reduction in S_I among TT homozygotes. In contrast, Munoz et al. found no association with S_I in 138 Europid women [17]; we found no association in 122 non-diabetic members of Utah families (the current study); and Scott et al. [7] found no association with S_I in members of Finnish families. Munoz et al. found a non-significant trend towards reduced S_I and reduced AIR_g among 118 African American women, which was not observed in our study. Differences in ascertainment, relatively small samples for published studies, particularly when considering the rare allele homozygote frequency, and possible gene–gene or gene–environment interactions may explain these differences. Neither the previously published studies nor we attempted to correct for multiple testing (two SNPs, several metabolic traits, with both the SNPs and traits showing correlation). Hence, some of the nominal p values ($0.01<p<0.05$) in our studies and published reports may be spurious.

Non-coding variants of *TCF7L2* may influence glucose homeostasis by changing transcription (regulation) or splicing and may do so in a tissue-specific fashion. To our knowledge, this is the first study to examine the role of ethnicity and genotype in *TCF7L2* gene expression or the role of muscle and adipocyte *TCF7L2* expression in insulin sensitivity. We found no genotypic effects on *TCF7L2* expression levels in adipose or muscle, nor any correlation

of *TCF7L2* mRNA levels with S_I or BMI. In contrast, we did find a trend towards genotype determination of gene expression in transformed lymphocytes, where expression was decreased in African American and Europid non-diabetic individuals, but increased in diabetic individuals with the TT risk genotype. As this study was small (24 non-diabetic individuals, 30 diabetic individuals) and included two ethnic groups that themselves show differences in expression, this unexpected finding requires further study. Because individuals for whom transformed lymphocytes were available were not the individuals who had undergone adipose and muscle biopsies, we were unable to examine the correlation in gene expression between these tissues. Given the multiple splice forms of *TCF7L2,* the lack of genotype effect on total *TCF7L2* expression measured in this study and the lack of correlation of adipocyte or muscle *TCF7L2* expression with S_I, we believe that tissue-specific splicing deserves further study.

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Duality of interest The authors declare that there is no duality of interest associated with this study.

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