***TCF7L2* variants are associated with increased proinsulin/insulin ratios but not obesity traits in the Framingham Heart Study**

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Abstract

Aims/hypothesis Common variants in the *TCF7L2* gene are associated with type 2 diabetes via impaired insulin secretion. One hypothesis is that variation in *TCF7L2* impairs insulin processing in the beta cell. In contrast, the association of related *TCF7L2* polymorphisms with obesity is controversial in that it has only been shown in cohorts susceptible to ascertainment bias. We reproduced the association of diabetes-associated variants with proinsulin/insulin ratios, and also examined the association of a *TCF7L2* haplotype with obesity in the Framingham Heart Study (FHS).

Methods We genotyped the *TCF7L2* single nucleotide polymorphisms rs7903146 and rs12255372 (previously associated with type 2 diabetes) and rs10885406 and rs7924080 (which tag haplotype A [HapA], a haplotype reported to be associated with obesity) in 2,512 FHS participants. We used age- and sex-adjusted linear mixed-

effects models to test for association with glycaemic traits, proinsulin/insulin ratios and obesity measures.

Results As expected, the T risk allele of rs7903146 was associated with higher fasting plasma glucose ($p=0.01$). T/T homozygotes had a 23.5% increase in the proinsulin/insulin ratio ($p=1 \times 10^{-7}$) compared with C/C homozygotes. There was no association of HapA with BMI ($p=0.98$), waist circumference ($p=0.89$), subcutaneous adipose tissue ($p=0.32$) or visceral adipose tissue ($p=0.92$).

Conclusions/interpretation We confirmed that the risk allele of rs7903146 is associated with hyperglycaemia and a higher proinsulin/insulin ratio. We did not detect any association of the *TCF7L2* HapA with adiposity measures, suggesting that this may have been a spurious association from ascertainment bias, possibly induced by the evaluation of obesity in separate groups of glycaemic cases and controls.

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Abbreviations

2 h plasma glucose	Plasma glucose 2 h after an OGTT
FHS	Framingham Heart Study
FPG	Fasting plasma glucose
Gutt's ISI	Gutt's 0–120 min insulin sensitivity index
HapA	Haplotype A
HOMA-IR	Insulin resistance by homeostasis model assessment
LD	Linkage disequilibrium
Mean BMI 35–50	Mean BMI between ages 35 and 50 years
Mean BMI 50–65	Mean BMI between ages 60 and 65 years
SAT	Subcutaneous adipose tissue
SNP	Single nucleotide polymorphism
VAT	Visceral adipose tissue

Introduction

Transcription factor 7-like 2 (*TCF7L2*) is a high mobility group box-containing transcription factor that serves as a nuclear receptor for β -catenin, which mediates the wingless-type MMTV integration site family (WNT) signalling pathway, a key developmental and growth regulatory mechanism of the cell. Variants in *TCF7L2* have been shown to be involved in the pathophysiology of type 2 diabetes [1]. There is evidence that polymorphisms in *TCF7L2* may predispose to type 2 diabetes by impairing pancreatic beta cell processing of proinsulin [2].

In the original report by Grant et al. [1], an initial microsatellite marker on 10q (DG10S478) within intron 3 of *TCF7L2* was strongly associated with type 2 diabetes ($p=2.1 \times 10^{-9}$). This finding was replicated in the same study using separate cohorts from Denmark and the USA. The non-coding single nucleotide polymorphisms (SNPs) rs12255372 and rs7903146 were in strong linkage disequilibrium (LD) with DG10S478 ($r^2=0.95$ and $r^2=0.78$, respectively) and showed similar associations with type 2 diabetes ($p<1 \times 10^{-7}$). Subsequent work has shown that the T allele of rs7903146 is probably the risk variant or the closest known correlate to the actual risk allele [3]. This association of the rs7903146 T risk allele has been replicated in multiple different populations, as shown in independent meta-analyses by Cauchi et al. [4], who analysed data from 29,195 controls and 17,202 cases to find an OR of 1.46 per risk allele ($p=5.4 \times 10^{-140}$), and Florez, who combined 50,000 samples and yielded a risk of 1.44 for the T allele ($p<1 \times 10^{-80}$) [5].

In addition, members of our group have shown in a prospective study that common variants in *TCF7L2* are associated with increased risk of diabetes in persons with impaired glucose tolerance, and that the risk genotypes are associated with impaired beta cell function but not with insulin resistance [6]. Furthermore, Loos et al. [2] have shown that the T allele of rs7903146 is associated with elevated fasting proinsulin ($p=4.6 \times 10^{-9}$) relative to total insulin levels, and Kirchhoff et al. [7] found a significantly high AUC proinsulin/insulin ratio ($p=0.02$) for the same polymorphism. Gonzalez-Sanchez et al. [8] have recently confirmed that the rs7903146 T allele is associated with type 2 diabetes (OR 1.29, $p=0.01$) and an increased proinsulin/insulin ratio after OGTT ($p=0.01$). These studies suggest that the *TCF7L2* risk allele may predispose to type 2 diabetes by impairing beta cell proinsulin processing.

Similarly, Helgason et al. [3] performed a phylogenetic reconstruction of the evolutionary relationships between haplotypes within the *TCF7L2* exon 4 LD block. Their analysis revealed two separate lineages: one contained all but one of the haplotypes carrying the diabetes risk allele rs7903146 T (called HapB_{T2D}), while the second major lineage was mostly characterised by the diabetes protective allele rs7903146 C (called HapA). This haplotype, tagged by either the combination of rs10885406 A and rs7903146 C, or rs7924080 T alone, was correlated with increased BMI ($p=0.001$) in separate groups of diabetic cases and non-diabetic controls. In a French case-control sample, the rs7903146 T allele was associated with a lower BMI in individuals with type 2 diabetes [9]. In contrast, this association of *TCF7L2* variants with BMI has not been found in studies that are free of ascertainment bias, for example in population samples such as Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) [10]. In a European population, it was found that *TCF7L2* does not increase the risk of obesity, but its effect on type 2 diabetes may be modulated by obesity [11]. In the population-based Framingham Heart Study (FHS), we have previously shown that a SNP included in the Affymetrix 100K fixed genotyping array and in strong LD with both rs7924080 and rs10885406 is not associated with BMI [12].

We therefore set out to confirm the association of rs7903146 with hyperglycaemia, reproduce the association of the same SNP with proinsulin/insulin ratios, and test directly the association of a previously reported haplotype with obesity traits in the population cohort of the FHS.

Methods

Population samples We used data from the FHS to study associations between variants in *TCF7L2* and quantitative diabetes- and obesity-related traits. The FHS is a community-

based, multi-generational, longitudinal study of cardiovascular disease and its risk factors, including diabetes. The FHS is comprised of the Original Cohort, Offspring Cohort, and Third Generation Studies. Participants described in this analysis consist of 2,512 individuals from the FHS Offspring Cohort. In this analysis, our quantitative traits of interest are from Offspring examination 7 (1998–2001) where phenotypic data from a 75 g OGTT are available for all Offspring without diagnosed diabetes, and where proinsulin and insulin levels were obtained for proinsulin/insulin ratios. The study was approved by Boston University's Institutional Review Board and written informed consent, including consent for genetic analyses, was obtained for all study participants. The demographic characteristics of the FHS study population are presented in Table 1.

An extensive array of diabetes- and obesity-related quantitative traits have been collected in the FHS. Diabetes quantitative traits measured in this study include: HbA_{1c}, fasting plasma glucose (FPG), fasting insulin, insulin resistance by HOMA (HOMA-IR), proinsulin/insulin ratio, Gutt's 0–120 min insulin sensitivity index (Gutt's ISI) and the time-averaged mean FPG level over examinations 3 to 7, spanning 16 years (mean FPG). Individuals being treated for diabetes with oral hypoglycaemic or insulin therapy were excluded from the analysis of the glycaemic traits; however, participants with diet-controlled diabetes were not excluded. Data on mean FPG and Gutt's ISI for the polymorphism rs7903146, using different association tests, have been presented previously in the context of a 100 K genome-wide association scan [12]. Laboratory methods for all quantitative traits have been described previously [13].

Obesity traits measured included: mean BMI 35–50 (which is the mean BMI between ages 35 and 50 years) and mean BMI

50–65 (which is the mean BMI between ages 50 and 65 years), and BMI, waist circumference, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) from examination 7. SAT and VAT were performed by multi-detector computed tomography assessment with adipose compartments delineated by manually tracing the abdominal wall as described previously [14], and were available in approximately one half of the participants for whom we had anthropomorphic measurements.

We used the 2003 American Diabetes Association clinical criteria to define diabetes, where a case was defined as use of oral hypoglycaemic or insulin therapy, or a FPG $\geq$ 7.0 mmol/l at the index examination and FPG $\geq$ 7.0 mmol/l on at least one prior examination [15].

SNP selection We selected four SNPs in *TCF7L2* that have been previously reported to be involved in type 2 diabetes and/or increased BMI: rs12255372, rs7903146, rs10885406 and rs7924080. As suggested by Helgason et al. [3], the haplotype designated HapA was tagged by two different methods: either by the combination of two SNPs, rs10885406 A and rs7903146 C, or by rs7924080 T alone. As shown below, they yielded essentially the same results.

Genotyping Genotyping was performed by allele-specific primer extension of multiplex amplified products with detection by matrix-assisted laser desorption/ionisation-time of flight mass spectroscopy on an iPLEX Sequenom platform (Sequenom, San Diego, CA, USA). Average genotyping call rates were 98%, and the average consensus rate based on 254 duplicate samples was 99%.

Statistical analysis The analysis of the quantitative traits was carried out with linear mixed-effects models that tested

Table 1 Demographic characteristics of the Framingham participants

Trait	N	Mean $\pm$ SD or %	Skewness	Kurtosis
Age at examination 7 (years)	2,464	61 $\pm$ 9.59		
Sex (% female)	2,512	53.0		
Type 2 diabetes (%)	2,512	9.6		
BMI (kg/m ²)	2,390	28.2 $\pm$ 5.36	1.17	2.91
FPG (mmol/l)	2,194	5.57 $\pm$ 0.99	5.23	53.53
HbA _{1c} (%)	2,021	5.5 $\pm$ 0.7	3.07	23.36
Mean FPG (mmol/l)	2,510	5.51 $\pm$ 1.15	3.91	20.65
Fasting insulin (pmol/l)	2,154	87 $\pm$ 50.1	3.63	36.63
HOMA-IR	2,154	3.7 $\pm$ 2.73	5.69	80.64
Gutt's ISI	812	21.7 $\pm$ 7.31	0.34	0.031
Waist circumference (cm)	2,373	99.8 $\pm$ 14.17	0.48	0.68
Proinsulin (pmol/l)	2,093	14.6 $\pm$ 12.39	4.34	32.71
Proinsulin/insulin ratio	2,065	1.0 $\pm$ 0.62	2.48	10.09
VAT (cm ³)	1,017	2,138.5 $\pm$ 1,100.47	0.73	0.45
SAT (cm ³)	1,017	2,984.8 $\pm$ 1,311.21	0.94	0.94

All quantitative traits, with the exception of mean FPG, were obtained during FHS examination 7 (1998–2001). Unrelated participants $n=1,435$; pedigrees $n=282$; sibpairs $n=985$; avuncular pairs $n=66$; cousin pairs $n=647$.

the association between Studentised residuals of the traits and the SNPs using an additive genetic coding. The following traits were log-transformed: HbA_{1c}, FPG, fasting insulin, HOMA-IR, mean FPG, proinsulin, mean BMI 35–50 and mean BMI 50–65. Traits that were not transformed were Gutt's ISI, waist circumference, SAT and VAT. Studentised residuals were generated for men and women separately, and two adjustment models were used: the first with age and age², and the second with age, age² and BMI (kg/m²). The adjustment for both age and age² allowed for a non-linear relationship between the trait and age. The covariates for each trait were taken from the examination at which the trait was measured, except for the mean FPG and mean BMI traits, in which case they were obtained over the same time period. The SOLAR software package was used to implement the linear mixed-effect models to account for within-family correlation of the traits [16]. The models included within-family random effects with a covariance structure determined by the degree of relatedness between each relative pair. For the association analysis we used a linear mixed-effects model to account for the relatedness between individuals, and for the survival analysis we used a frailty term in our model to account for the correlation (in diabetes survival) between family members.

An additional analysis was carried out to determine the association between the SNPs and the ratio of proinsulin to insulin. The participant-specific insulin/pro-insulin ratio was log-transformed and Studentised residuals were obtained using examination 7 covariates, and the analysis with linear mixed-effect models was performed as described above.

In order to distinguish whether a higher proinsulin/insulin ratio indicates an intrinsic defect in insulin processing or the hypersecretion of proinsulin by beta cells that cannot keep up with insulin demand, we assessed whether the association between rs7903146 and log(insulin/proin-

sulin) was jointly modified by BMI and HOMA-IR. If a higher proinsulin/insulin ratio stems from an intrinsic defect in insulin processing, the association should be present in both insulin-resistant and insulin-sensitive individuals; conversely, if it is due to a system that cannot meet the demands induced by high insulin resistance, the association should be much stronger in insulin-resistant than in insulin-sensitive individuals. To distinguish between these hypotheses, the sample was divided into tertiles of BMI and HOMA-IR: individuals in the highest tertile of both BMI and HOMA-IR were placed in a high BMI/high HOMA-IR group ($n=425$), while individuals in the lowest tertile of both BMI and HOMA-IR were placed in a low BMI/low HOMA-IR group ($n=415$). A linear mixed-effect model was run with log(insulin/proinsulin) as the outcome to test for interaction between BMI/HOMA-IR group and genotype at rs7903146. Using QUANTO software, with 840 participants included in the interaction analysis, we have 80% power to detect a genotype $\times$ group interaction explaining 0.9% of the variance in log proinsulin ratio [17].

We used the two-step approach proposed by Zaykin et al. [18] to test the association of our obesity-related quantitative traits with a haplotype, denoted HapA, defined by the A-C alleles of SNPs rs10885406 and rs7903146. First, haplotype frequencies were obtained for our sample using the haplo.stats software implemented in the software package R [19]. This software assumes that the sample is unrelated; although the haplotype frequencies observed in the whole sample were very similar to the haplotype frequencies observed in an unrelated subset of the sample. Individual dosages of the HapA haplotype, varying between 0 and 2, were obtained using posterior probabilities from the maximum likelihood estimation procedure. Linear mixed-effect models were run to assess the association between HapA and obesity-related quantitative traits. We tested the association between obesity (defined as BMI $\geq$

Table 2 Associations of rs7903146 with diabetes-related quantitative traits

Trait	CC/CT/TT	CC	CT	TT	<i>p</i> value	<i>p</i> value ^a	<i>p</i> value ^b
FPG	1060/893/223	5.52 $\pm$ 0.82	5.57 $\pm$ 1.09	5.77 $\pm$ 1.33	0.013	0.008	0.160
Mean FPG	1172/1036/258	5.36 $\pm$ 0.85	5.51 $\pm$ 1.29	5.59 $\pm$ 1.22	0.003	0.002	0.570
2 h plasma glucose	403/347/75	6.93 $\pm$ 2.46	7.33 $\pm$ 2.85	8.22 $\pm$ 3.16	0.002	0.005	0.040
HbA _{1c}	973/831/200	5.46 $\pm$ 0.59	5.54 $\pm$ 0.78	5.62 $\pm$ 0.81	0.026	0.021	0.210
Proinsulin	1007/854/215	14.21 $\pm$ 12.15	14.79 $\pm$ 13.0	15.87 $\pm$ 11.27	0.043	0.010	0.080
Fasting insulin	1036/879/221	89.52 $\pm$ 48.9	85.8 $\pm$ 52.86	81.84 $\pm$ 44.64	0.005	0.004	0.200
HOMA-IR	1036/879/221	3.8 $\pm$ 2.58	3.7 $\pm$ 2.98	3.63 $\pm$ 2.34	0.059	0.063	0.240
Gutt's ISI	391/341/75	22.15 $\pm$ 7.18	21.77 $\pm$ 7.51	19.22 $\pm$ 6.72	0.043	0.066	0.150

Association between genotype at SNP rs7903146 and glycaemic traits using an additive genetic model. The genotype counts are listed next to each quantitative trait

The minor allele frequency is 0.31 and is based on data from Framingham unrelated participants

All quantitative trait values are crude means $\pm$ SD, with all *p* values adjusted for sex, age, and age², and further adjusted for either ^aBMI or ^bproinsulin/insulin ratio

Mean FPG, FPG averaged over examinations 3 to 7

Table 3 Associations of *TCF7L2* SNPs with proinsulin/insulin ratios

SNP	MM/Mm/mm	Alleles M/m	MAF	M/M	M/m	m/m	<i>p</i> value	<i>p</i> value ^a
rs12255372	999/844/201	G/T	0.31	0.98±0.56	1.08±0.65	1.21±0.74	1.7×10 ⁻⁷	1.2×10 ⁻⁷
rs7903146	992/843/213	C/T	0.31	0.98±0.56	1.07±0.65	1.21±0.72	1.2×10 ⁻⁷	8.6×10 ⁻⁸
rs10885406	527/1017/452	A/G	0.48	1.0±0.58	1.00±0.57	1.18±0.71	3.7×10 ⁻⁵	3.4×10 ⁻⁵
rs7924080	518/1028/452	T/C	0.48	0.99±0.54	1.01±0.59	1.17±0.72	4.5×10 ⁻⁵	4.2×10 ⁻⁵

The proinsulin/insulin ratios for each genotype are given as a means ± SD

Genotype counts by SNP are given: M, major allele; m, minor allele

The minor allele frequencies (MAF) are based on data from Framingham-unrelated participants

^a*p* values are adjusted for BMI

30 kg/m²) and HapA using logistic regression generalised estimating equations, in which we used pedigree as a cluster and an independence correlation structure.

Results

Information about the four SNPs used in this analysis, including chromosomal position, major allele and minor allele frequency in both the HapMap and Framingham population is presented in the Electronic supplementary material [ESM] Table 1. The haplotype structures of the *TCF7L2* locus in the FHS and HapMap CEU (Centre d'Etude du Polymorphisme Humain trios originating from northern and western Europe living in UT, USA) populations are shown in ESM Figs 1 and 2. We examined whether individual SNPs in *TCF7L2* were associated with diabetes- and obesity-related quantitative traits in the FHS population using additive genetic models. We first focused our attention on SNP rs7903146: Table 2 presents mean trait levels with *p* values for association before and after adjustment for BMI. Several associations reached nominal levels of significance: we found associations of the minor allele (T) with higher FPG (*p*=0.01), mean FPG (*p*=0.003), plasma glucose 2 h after an OGTT (2 h plasma glucose; *p*=0.002), proinsulin (*p*=0.04), fasting insulin (*p*=0.005), Gutt's ISI (*p*=0.04) and HbA_{1c} (*p*=0.03); all of these associations remained nominally significant after adjusting for BMI except for Gutt's ISI. Interestingly, most of these associations lost statistical significance when adjusted for proinsulin/insulin ratio (Table 2). The association of the other three variants with glycaemic quantitative traits is shown in ESM Table 2 and their association with incident diabetes is shown in ESM Table 3. Despite a small number of incident cases in our sample (*n*=244), the minor alleles of all four variants were nominally associated with incidence of type 2 diabetes (ESM Table 3). As expected, the highest hazard ratio was obtained for rs7903146; adjustment for BMI attenuated the results.

We then explored the association between variants in *TCF7L2* and proinsulin/insulin ratios (Table 3). The minor

alleles of all four *TCF7L2* SNPs of interest were strongly associated with elevated proinsulin/insulin ratios: rs1225537 (*p*=1.7×10⁻⁷), rs7903146 (*p*=1.2×10⁻⁷), rs10885406 (*p*=3.7×10⁻⁵) and rs7924080 (*p*=4.5×10⁻⁵). These associations remained significant after adjustment for BMI. As a way to understand whether the high proinsulin/insulin ratio stemmed from an intrinsic defect in insulin processing or a more general dysfunction caused by increased insulin demand, we also examined the interaction between genotype at rs7903146 and BMI/insulin resistance by testing the association with proinsulin/insulin ratio in a high BMI/high HOMA-IR vs low BMI/low HOMA-IR group. We did not see a significant interaction between rs7903146 and BMI/HOMA-IR group (*p*=0.14). Within the limits of statistical power, this result indicates that the association of *TCF7L2* with the proinsulin/insulin ratio is the same in the obese/non-obese or insulin-resistant/insulin-sensitive group.

Finally, we tested the association between three *TCF7L2* SNPs and a haplotype previously reported to be associated with elevated BMI and obesity-related quantitative traits. Table 4 displays the associations between the *TCF7L2* variants and the HapA haplotype (consisting of SNPs rs10885406 A and rs7903146 C) and BMI, waist circumference, mean BMI 35–50, mean BMI 50–65, SAT and VAT. We did not find an association between HapA and obesity after adjusting for sex, age and age² (*p*=0.28). We did not detect any significant associations between any individual SNPs or between HapA and waist circumference, BMI, mean BMI, SAT or VAT (all *p* values>0.05). There was no significant association with obesity traits seen with the alternative method of tagging HapA, utilising only rs7924080 T.

Discussion

It has been previously reported that a composite risk allele of microsatellite DG10S478 within intron 3 of the *TCF7L2* gene is associated with type 2 diabetes in cohorts from Iceland, Denmark and the USA [1]. In the FHS, we have confirmed that the minor allele (T) of *TCF7L2* SNP

Table 4 Associations of *TCF7L2* SNPs and the HapA haplotype with obesity-related quantitative traits

SNP	Alleles M/m	MAF	Trait	M/M	M/m	m/m	<i>p</i> value
rs7903146	C/T	0.31	Waist circumference	99.8±14.22	99.9±14.45	99.6±12.92	0.62
			BMI	28.15±5.3	28.22±5.41	28.23±5.56	0.92
			Mean BMI 35–50	26.2±4.7	26.3±4.6	26.4±4.9	0.77
			Mean BMI 50–65	27.6±5.0	27.6±4.9	27.4±4.8	0.58
			VAT	2,113.0±1,111.2	2,146.5±1,115.8	2,215.7±1,060.2	0.38
			SAT	2,941.2±1,291.9	3,073.1±1,315.3	2,814.3±1,389.7	0.78
rs10885406	A/G	0.48	Waist circumference	98.9±14.12	100.5±14.58	99.1±13.33	0.89
			BMI	27.9±5.22	28.36±5.37	28.08±5.5	0.66
			Mean BMI 35–50	26.1±4.6	26.4±4.7	26.2±4.7	0.88
			Mean BMI 50–65	27.5±5.0	27.8±5.0	27.3±4.6	0.62
			VAT	2,097.3±1,071.7	2,170.4±1,141.6	2,106.6±1,058.9	0.86
			SAT	2,968.3±1,305.0	3,043.1±1,303.1	2,862.9±1,323.6	0.34
rs7924080	T/C	0.48	Waist circumference	98.9±14.17	100.5±14.55	99.2±13.41	0.79
			BMI	27.92±5.24	28.34±5.35	28.12±5.54	0.62
			Mean BMI 35–50	26.1±4.6	26.4±4.7	26.2±4.7	0.85
			Mean BMI 50–65	27.6±5.0	27.7±5.0	27.4±4.7	0.61
			VAT	2,082.0±1,070.7	2,176.3±1,140.7	2,108.5±1,057.9	0.68
			SAT	2,951.4±1,303.7	3,046.5±1,307.9	2,863.1±1,317.1	0.41
HapA			2 (HapA)	1 (HapA)	0 (HapA)		
	Waist circumference	98.8±13.97	100.6±14.73	99.3±13.46	0.89		
	BMI	28.0±5.2	28.3±5.4	28.1±5.5	0.98		
	Mean BMI 35–50	26.1±4.6	26.4±4.7	26.2±4.7	0.65		
	Mean BMI 50–65	27.6±5.0	27.7±5.0	27.3±4.6	0.41		
	VAT	2,088.8±1,066.0	2,188.1±1,144.5	2,105.4±1,046.5	0.92		
	SAT	2,977.0±1,312.1	3,056.9±1,297.4	2,862.5±1,338.4	0.32		

All quantitative trait values are unadjusted means ± SD

Association between SNPs and obesity traits in the FHS

The minor allele frequencies (MAF) are based on data from Framingham unrelated participants

p values are adjusted for sex and age

HapA is defined as the number of copies (0, 1, 2) of SNPs rs7903146-C and rs10885406-A (which corresponds to rs7924080-T)

rs7903146 is associated with elevated diabetes-related quantitative traits such as FPG, mean FPG, 2 h plasma glucose, fasting insulin and HbA_{1c}. This is consistent with other studies showing that the T allele of rs7903146 elevates the risk of type 2 diabetes [1–6].

Furthermore, previous studies have suggested that the diabetes-associated variants at *TCF7L2* may be involved in proinsulin to insulin processing in the pancreatic beta cell [2, 7, 8]. We have shown that these four SNPs are significantly associated with an elevated proinsulin/insulin ratio in the FHS population in directions consistent with their effect on diabetes risk; this finding was particularly robust with *p* values in the range 1×10^{-5} – 1×10^{-7} . The significantly elevated proinsulin/insulin ratios for all four SNPs probably reflects that they are in LD with the causal SNP for decreased insulin processing rather than each one being independently associated with this trait. Interestingly, the association of rs7903146 with hyperglycaemia becomes non-significant when adjusted for the proinsulin/insulin ratio, suggesting that it is mediated by the latter. The increased proinsulin/insulin ratios may be a non-specific marker of beta cell dysfunction in the insulin-resistant state

or a specific effect of *TCF7L2* on the insulin processing pathway. Lyssenko et al. [20] have shown that the risk T allele is associated with enhanced expression of *TCF7L2* and impaired insulin secretion in beta cells. There was no evidence of interaction between BMI/HOMA-IR and genotype at rs7903146 on the proinsulin/insulin ratio, indicating that the association of genotype at rs7903146 and proinsulin/insulin ratios may be independent of BMI and insulin resistance, and may therefore reflect an intrinsic defect in insulin processing. This negative result should be confirmed in samples with greater statistical power.

Other groups have also demonstrated that common variants associated with increased risk of type 2 diabetes in *CDKAL1* and *SLC30A8* are associated with impaired conversion of proinsulin to insulin from an unclear mechanism [7]. Whether this represents a general marker for beta cell dysfunction or a specific molecular defect remains to be determined.

We have also shown that three previously studied *TCF7L2* variants as well as the HapA haplotype are not associated with obesity-related traits in the FHS population cohort such as waist circumference, BMI, mean BMI, VAT

or SAT. These negative results may suggest that previous results of positive association are the result of ascertainment bias in the selection of cases and controls. Indeed, in a typical case–control study for type 2 diabetes, controls are selected to be normoglycaemic; since both obesity and the T allele of rs7903146 are associated with an elevated risk of diabetes, non-diabetic individuals carrying the T ‘diabetes risk’ allele who are in the control group will tend to be leaner than carriers of the C ‘diabetes protective’ allele. Similarly, in the case group carriers of the C ‘diabetes protective’ allele must have developed diabetes via alternative mechanisms, including a higher BMI than that of the T ‘diabetes risk’ allele carriers. Both of these scenarios may set up a false positive association whereby the C allele is spuriously associated with an elevated BMI. One method to avoid this is to test for association with obesity in a population free of glycaemic ascertainment constraints.

In summary, our study adds further evidence that the rs7903146 (T) minor allele increases risk of diabetes via an association with multiple intermediate traits. We have also confirmed that variants in *TCF7L2* are robustly associated with increased proinsulin/insulin ratios, and have attempted to illustrate that such an association is independent of insulin resistance, suggesting that dysfunctional insulin processing is part of the potential pathophysiological mechanism for this gene in type 2 diabetes. We found no evidence that polymorphisms in *TCF7L2* are associated with an increased risk of obesity.

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

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