Diabetologia (2007) 50:1192–1200
DOI 10.1007/s00125-007-0656-6

ARTICLE

Variants of transcription factor 7-like 2 (*TCF7L2*) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion

J. Wang · J. Kuusisto · M. Vänttinen · T. Kuulasmaa · J. Lindström · J. Tuomilehto · M. Uusitupa · M. Laakso

Received: 1 December 2006 / Accepted: 15 February 2007 / Published online: 17 April 2007

Abstract
Aims/hypothesis We investigated the association of variants of the transcription factor 7-like 2 (*TCF7L2*) gene with: (1) incident diabetes in the Finnish Diabetes Prevention Study (DPS, Study I); (2) type 2 diabetes and impaired glucose regulation (i.e. IGT or IFG) in a cross-sectional study (Study II); and (3) insulin secretion, insulin sensitivity and adipose tissue expression of *TCF7L2* in offspring of type 2 diabetic probands (III).
Subjects and methods Study I (the DPS) included 507 individuals with IGT who were randomly allocated to control and intervention groups and followed for an average of 3.9 years to monitor for progression to diabetes. Study II was a population-based cross-sectional study of 1,766 men, aged 50–70 years, randomly selected from the population of Kuopio, eastern Finland. Study III included 238 non-diabetic offspring of patients with type 2 diabetes. Genotyping of rs12255372 and rs7903146 of *TCF7L2* was carried out.
Results In the DPS, the TT genotype of rs12255372 was significantly associated with an adjusted 2.85-fold risk (95% CI 1.17–6.95, p=0.021) of incident diabetes in the control group, but not in the intervention group. In Study II, the adjusted odds ratio in subjects with the TT genotype was 3.40 (1.45–7.97, p=0.005) for the comparison of diabetic subjects with normoglycaemic subjects. The T allele of rs12255372 was significantly associated with decreased insulin secretion (Studies II, III). Expression of *TCF7L2* in adipose tissue tended to be lower in subjects with the TT risk genotypes of rs12255372 and rs7903146.
Conclusions/interpretation The variant of rs12255372 of *TCF7L2* was associated with incident type 2 diabetes in the DPS and in a separate population-based cross-sectional study. Impaired insulin secretion is likely to be the main cause for our findings.

Keywords Genotyping · Impaired fasting glucose · Impaired glucose tolerance · Incidence · Insulin secretion · Insulin sensitivity · Lifestyle intervention · TCF7L2 · Type 2 diabetes · Variants

Abbreviations

DI	disposition index
DPP	Diabetes Prevention Program
DPS	Finnish Diabetes Prevention Study

J. Wang · J. Kuusisto · M. Vänttinen · T. Kuulasmaa · M. Laakso (✉)
Department of Medicine,
University of Kuopio and Kuopio University Hospital,
70210 Kuopio, Finland
e-mail: markku.laakso@kuh.fi

M. Uusitupa
Department of Clinical Nutrition,
University of Kuopio and Kuopio University Hospital,
Kuopio, Finland

J. Lindström · J. Tuomilehto
Department of Epidemiology and Health Promotion,
National Public Health Institute,
Helsinki, Finland

J. Tuomilehto
Department of Public Health, University of Helsinki,
Helsinki, Finland

J. Tuomilehto
South Ostrobothnia Central Hospital,
Seinäjoki, Finland

FPG	fasting plasma glucose
HOMA-IR	homeostasis model assessment for insulin resistance
IGR	impaired glucose regulation
LBM	lean body mass
2-h PG	2-h postload glucose
SNP	single-nucleotide polymorphisms
TCF7L2	transcription factor 7-like 2
WBGU	whole-body glucose uptake

Introduction

A study in Icelandic, Danish and US white cohorts recently reported that single-nucleotide polymorphisms (SNPs) of the transcription factor 7-like 2 (*TCF7L2*) gene, especially rs12255372 and rs7903146, were strongly associated with type 2 diabetes [1]. Subsequently, the Diabetes Prevention Program (DPP) Research Group confirmed that these two SNPs of *TCF7L2* predicted progression to diabetes in subjects with IGT [2], while other studies have reported associations of these SNPs with type 2 diabetes in white people and other populations [3–12]. Two of the previous studies have been longitudinal [2, 9], and none of them has investigated the functionality of SNPs of *TCF7L2* or examined their effects on insulin secretion measured by an IVGTT and insulin sensitivity measured by the euglycaemic clamp in non-diabetic offspring of type 2 diabetic patients.

The present study aimed to investigate the association of rs12255372 and rs7903146 of *TCF7L2* with incident type 2 diabetes in the Finnish Diabetes Prevention Study (DPS) [13, 14] and in a large cross-sectional study including 1,766 men. Furthermore, we investigated the mechanisms how SNPs of *TCF7L2* could increase the risk of type 2 diabetes. We did this by measuring insulin secretion and insulin sensitivity in offspring of type 2 diabetic patients and functionality of the variants of *TCF7L2* in adipose tissue samples.

Subjects and methods

Study I: The Finnish Diabetes Prevention Study

The study population has been described earlier in detail [13, 14]. In short, the DPS is a multicentre study with five participating centres in Finland. The diagnosis of diabetes and other categories of glucose intolerance were based on the criteria of the WHO in 1985 [15]. IGT was defined as fasting plasma glucose (FPG) <7.8 mmol/l and a 2-h plasma glucose in the range of 7.8–11.0 mmol/l (OGTT, glucose load 75 g). Altogether 522 overweight subjects (BMI 31.1 ± 4.6 kg/m^2) aged 40–68 years and with IGT were randomly allocated to one of the two treatment modalities, the intensive diet and exercise intervention group or the control group. The mean duration of follow-up was 3.9 years. DNA for genotyping of rs12255372 and rs7903146 of *TCF7L2* was available from 507 subjects (166 men, 341 women).

The intervention programme has been described previously [13, 14]. Briefly, subjects in the intervention group were given individually tailored dietary advice aimed to reduce weight and the intake of total and saturated fat and to increase the intake of dietary fibre. In addition, subjects in the intervention group were individually guided to increase their level of physical activity. The control group received general information on the benefits of weight reduction, physical activity and healthy diet.

The study protocol was approved by the Ethics Committee of the National Public Health Institute in Helsinki, Finland. All subjects gave written informed consent.

Study II: a population-based cross-sectional study of 1,766 men

An independent study population was collected to confirm the findings of Study I. The primary aim of this study is to investigate the effects of SNPs in genes of interest on the risk of type 2 diabetes and cardiovascular disease in a random sample of Finnish men, aged from 50 to 70 years and living in the town of Kuopio (population 90,000), eastern Finland. A total of 1,766 men from this ongoing population-based study were included. The WHO criteria in 1999 [16] for IGT, IFG and diabetes mellitus were used in the classification of subjects without previously known diabetes, which was based on FPG and 2-h postload glucose (2-h PG) levels in an OGTT conducted at baseline. Among the subjects, 228 had known or newly diagnosed diabetes, 356 had IFG or IGT (impaired glucose regulation [IGR]) and 1,182 had normoglycaemia. The protocol included a 1-day visit to the Clinical Research Unit of the University of Kuopio. Study II was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

Study III: a study on offspring of type 2 diabetic parents

Study III consisted of 238 non-diabetic offspring of 143 patients with type 2 diabetes (one to four offspring from each family) from our ongoing study [17]. Patients with type 2 diabetes were randomly selected from patients living in the catchment area of Kuopio University Hospital (population 250,000). On day 1, all subjects underwent an

OGTT to evaluate their glucose tolerance according to the WHO criteria [16]. All subjects had normal liver, kidney and thyroid function, no history of excessive alcohol intake and no severe chronic diseases. Adipose needle biopsy was taken from abdominal subcutaneous tissue for gene expression studies. Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

Methods

Clinical measurements (Studies I–III) Weight and height were measured. BMI was calculated as the weight in kilograms divided by the square of the height in meters.

Oral glucose tolerance test (Studies I–III) During a 2-h OGTT (75 g of glucose) samples for plasma glucose and insulin were drawn at 0 and 120 min in Study I, at 0, 30 and 120 min in Study II and at 0, 30, 60, 90 and 120 min in Study III to evaluate the degree of glucose tolerance and the insulin response to the oral glucose load. The trapezoidal method was used to calculate glucose AUC and insulin AUC during the OGTT.

Intravenous glucose tolerance test and the euglycaemic clamp (Study III) On day 2, metabolic studies were performed after an overnight fast. First, an IVGTT was performed to determine the first-phase insulin release as previously described [17, 18]. Immediately after an IVGTT at 60 min, the degree of insulin resistance was evaluated with the euglycaemic clamp technique [19]. After drawing of baseline blood, a priming dose of insulin (Actrapid 100 IU/ml; Novo Nordisk, Gentofte, Denmark) was administered during the initial 10 min to quickly raise the plasma insulin concentration to the desired level, where it was maintained by a continuous insulin infusion of 240 $pmol \cdot kg^{-1} \cdot min^{-1}$. Blood glucose was clamped at 5.0 mmol/l for the next 120 min by the infusion of 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals. The mean rates of glucose infusion during the last 60 min of the clamp were used to calculate the rates of insulin-stimulated whole-body glucose uptake (WBGU).

Body fat composition (Study II–III) Body composition and lean body mass (LBM) were determined by bioelectrical impedance (RJL Systems, Detroit, MI, USA) in the supine position after a 12-h fast [20].

Assays and calculation Plasma glucose levels in the fasting state and during the OGTT (Studies I–III) and IVGTT, and blood glucose levels during the euglycaemic clamp (Study III) were measured by the glucose oxidase method (2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH, USA). To determine plasma insulin, blood was collected in EDTA-containing tubes. After centrifugation, the plasma was stored at −70°C until analysis. Plasma insulin concentration was determined by a commercial double-antibody solid-phase RIA (Phadeseph Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden). Insulinogenic index ($\Delta I_{30-0}/\Delta G_{30-0}$) was calculated as the ratio of the increment of serum insulin 30 min after an oral glucose load to the increment of blood glucose concentration 30 min after the glucose load ([30 min insulin—fasting insulin] / [30 min glucose—fasting glucose]) (Studies II–III) [21]. Insulin AUC during the first 10 min of an IVGTT was calculated according to the trapezoidal rule (Study III). Three measures of insulin sensitivity were used: (1) homeostasis model assessment for insulin resistance (HOMA-IR) (FPG [mmol/l] × fasting serum insulin [pmol/l]/22.5) [22] (used in Study I); (2) insulin-sensitivity index, which is the reciprocal of fasting insulin concentrations (used in Studies II, III) [23]; and (3) the ratio of WBGU to LBM (WBGU:LBM) based on the euglycaemic clamp (Study III) [24]. Disposition index (DI) was calculated as insulin sensitivity × first-phase insulin secretion, which is a measure of the beta cell response to insulin sensitivity [25].

Genotyping of the SNPs in TCF7L2 Genotyping of rs12255372 (located in intron 4) and rs7903146 (intron 3) of *TCF7L2* was carried out using TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, CA, USA). Primers are available upon request from the authors. TaqMan genotyping reaction was amplified on a GeneAmp PCR system 2700 and fluorescence was detected using an ABI Prism 7000 sequence detector (Applied Biosystems). Genotyping success rate was 100%, the error rate in re-genotyped SNPs (7.1% of all genotypes) was 0%.

Gene expression studies Total RNA from subcutaneous adipose tissue of 86 offspring of type 2 diabetic patients (Study III) was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and a kit (Qiagen RNeasy Mini; Ambion, Austin, TX, USA), and transcribed to cDNA using random primers and a high-capacity cDNA archive kit (Applied Biosystems). Quantitative RT-PCRs were performed in a 7,500 Real-Time PCR System (Applied Biosystems) using 6 ng (RNA equivalents) of cDNA as template, gene-specific primers and probes (information on primers and probes available upon request from Applied Biosystems). *TCF7L2* expression was normalised to large ribosomal protein P0 (Hs99999902_ml, Applied Biosystems) using the standard curve method.

Statistical analyses Because of the skewed distribution, serum insulin, insulin AUC, the insulinogenic index, HOMA-IR, the insulin-sensitivity index and DI were log transformed for statistical analyses. χ^2 test, mixed linear model (adjusted for age, sex, family relationship and WBGU:LBM [Study III]) and univariate ANOVA adjusted for age, sex and BMI were applied to compare differences in characteristics between individuals with different genotypes (Studies I–III). The difference in the frequency of the genotypes by quartiles of the insulinogenic index and tertiles of first-phase insulin release was tested by the χ^2 test (linear-by-linear association). In Study I the effect of genotypes on progression to type 2 diabetes was tested with Cox proportional regression models adjusted for confounding factors. Survival curves were calculated to estimate the cumulative incidence of diabetes. The difference in the incidence of diabetes between subjects with different genotypes was tested with the log-rank test. In Studies II and III the effect of each genotype on diabetes or IGR was tested using multiple logistic regression analysis. To assess whether the effect of genotypes differed between the treatment groups (Study I), BMI (Study II), LBM (Study II) and the sexes (Study III), we analysed the first-level interactions between the genotypes of rs12255372 and these parameters. Genetic analyses were done according to the additive model, except where the sample size was small, in which case the dominant model was applied (Study III). All statistical analyses were performed with SPSS 14.0 (SPSS, Chicago, IL, USA).

Results

The distribution of rs12255372 and rs7903146 followed Hardy–Weinberg expectations in all three Studies. Linkage disequilibrium (r^2) between rs12255372 and rs7903146 was 0.72 in Study I, 0.72 in Study II and 0.68 in Study III.

Table 1 shows characteristics of 507 subjects of the DPS by the genotypes of rs12255372 and rs7903146 in the DPS (Study I). Subjects were middle-aged (age 55.3±7.1 years; mean ± SD), with BMI (31.3±4.5 kg/m^2) above the normal range. Carriers of the TT genotype of rs12255372 had higher levels of FPG than carriers of the GG or GT genotypes (p=0.019).

Table 2 shows hazard ratios of the rs12255372 and rs7903146 genotypes for progression to diabetes in the overall cohort (n=507), in the intervention group (n=259) and in the control group (n=248), respectively. There was no significant interaction between these SNPs and the treatment groups (intervention, control) or sexes (p>0.05). Genotypes of rs12255372 and rs793146 were not associated with incident diabetes in the overall cohort or in the intervention group. In the control group, carriers of the TT genotype of rs12255372 had an adjusted hazard ratio of 2.85 (95% CI 1.17–6.95, p=0.021) for incident diabetes compared with carriers of the GG genotype. SNP rs7903146 was not significantly associated with the risk of diabetes. Figure 1 (Study I) illustrates the estimated time to the cumulative incidence of diabetes for 248 subjects in the control group, based on the genotypes of rs12255372. Subjects with the TT genotype had a higher cumulative incidence of diabetes than carriers of the GG and GT genotypes (log-rank test, p=0.009).

Table 3 shows characteristics of 1,538 non-diabetic men (Study II) and 238 non-diabetic offspring of patients with type 2 diabetes (Study III) by rs12255372 the genotypes. Subjects in Study II were middle-aged men (age 58.5± 5.8 years; mean ± SD) with BMI of 27.1±3.8 kg/m^2. Among them, 1,182 had normoglycaemia, 140 had isolated IFG, 142 had isolated IGT, 74 had IFG and IGT, and 228 had type 2 diabetes. Non-diabetic men with the GT and TT genotypes had higher levels of 2-h PG (p=0.017), larger glucose AUCs during the OGTT (p=0.039) and lower DI (p=0.046). Subjects in Study III were middle-aged (age 35.3±6.4 years); their BMI was slightly above normal (26.3±4.8 kg/m^2) and they were non-diabetic on the basis of the OGTT. Among them, 198 had normoglycaemia, five had isolated IFG, 29 had isolated IGT and six had IFG and IGT. Carriers of the GT and TT genotypes were leaner (p= 0.005) and had decreased insulin AUC during the first 10 min of the IVGTT compared with carriers of the GG genotype; this applied even after adjustment for insulin sensitivity, measured by the euglycaemic clamp (p=0.045). In rs12255372 and rs7903146 genotypes no statistically significant effects on intra-abdominal fat area (measured by computed tomography, Study III) or fat percentage (Studies II, III) were observed (data not shown).

Table 4 shows odds ratios of the rs12255372 genotypes for an increased risk of IGR and type 2 diabetes among 1,766 men (Study II). There was no significant interaction between rs12255372 genotypes and BMI or LBM for the risk of type 2 diabetes. The GT and TT genotypes were associated with risk of diabetes compared with non-diabetic subjects. Adjusted odds ratios for subjects with the GT and TT genotypes were 2.11 (95% CI 1.51–2.94) and 3.40 (1.45–7.97), respectively, for type 2 diabetic subjects versus normoglycaemic subjects.

Figure 2a shows changes in insulin secretion (ΔI_{30-0}/ ΔG_{30-0}) as changes in insulin sensitivity (1/fasting insulin) among 1,538 non-diabetic male subjects (Study II) according to different genotypes of rs12255272. Carriers of the T allele had decreased insulin release at each level of insulin sensitivity compared with that of subjects with the GG genotype. In addition, the frequency of the T allele of rs12255372 non-significantly decreased according to the

Table 1 Baseline characteristics, by rs12255372 and rs7903146 genotypes, of 507 subjects participating in the Finnish Diabetes Prevention Study (Study I)

	rs12255372				rs7903146			
	GG (n=333)	GT (n=154)	TT (n=20)	p value[a]	CC (n=312)	CT (n=171)	TT (n=24)	p value[a]
Men/women[b]	113/220	47/107	6/14	0.729	104/208	56/115	6/18	0.693
Age (years)	55.7±7.1	54.5±7.0	54.9±6.3	0.190	55.6±7.2	54.9±6.9	54.2±7.1	0.413
BMI (kg/m^2)	31.1±4.4	31.6±4.8	30.7±4.5	0.613	31.0±4.2	31.5±4.9	32.2±6.0	0.499
FPG (mmol/l)	6.1±0.7	6.2±0.8	6.4±0.8	0.019	6.1±0.8	6.2±0.8	6.3±0.7	0.372
2-h PG (mmol/l)	8.8±1.5	8.9±1.5	9.5±1.4	0.183	8.8±1.5	8.9±1.5	9.3±1.2	0.347
Fasting serum insulin (pmol/l)	89±46	89±41	82±41	0.943	88±47	90±41	87±41	0.862
2-h serum insulin (pmol/l)	582±423	558±331	494±242	0.795	579±411	560±368	548±282	0.844
HOMA-IR	24±14	25±13	24±14	0.856	24±14	25±13	25±14	0.778

Data are expressed as means ± SD
[a] ANOVA adjusted for sex, age and BMI except for [c]
[b] χ^2 test

quartiles of the insulinogenic index (p=0.076) in 1,538 non-diabetic men (Fig. 2b). Figure 2c (Study III) shows that among offspring of probands with type 2 diabetes the frequencies of the T allele of rs12255372 significantly decreased according to tertiles of insulin AUC during the first 10 min of an IVGTT (p=0.019). Similar results were observed for rs7903146 (data not shown).

Finally, we examined *TCF7L2* expression in adipose tissue from 86 subjects participating (34 men, 52 women; age: 36.9±6.3 years; BMI: 26.0±4.6 kg/m^2) in Study III. Subjects with the GG genotype (n=61) of rs12255372 had *TFC7L2* expression of 1.08±0.29 and subjects with the GT (n=24) and TT genotypes (n=1) combined had 0.97±0.21, p=0.09 (GG vs GT, p=0.08). Subjects with the CC

Table 2 Hazard ratios of the rs12255372 and rs7903146 genotypes for incident diabetes (n=115) among 507 subjects with IGT who participated in the Finnish DPS during the 4-year follow-up (Study I)

	Hazard ratio (95% CI)[a]	p value	Hazard ratio (95% CI)[a]	p value
rs12255372	GT		TT	
Overall cohort (n=507)				
Unadjusted model	1.20 (0.81–1.78)	0.370	1.80 (0.83–3.91)	0.139
Adjusted model[b]	1.17 (0.78–1.74)	0.446	1.71 (0.78–3.73)	0.180
Intervention group (n=259)				
Unadjusted model	1.21 (0.65–2.25)	0.556	0.54 (0.07–3.96)	0.542
Adjusted model	0.89 (0.46–1.70)	0.714	0.61 (0.08–4.52)	0.624
Control group (n=248)				
Unadjusted model	1.23 (0.74–2.06)	0.425	3.38 (1.43–7.96)	0.005
Adjusted model	1.27 (0.76–2.13)	0.359	2.85 (1.17–6.95)	0.021
rs7903146	CT		TT	
Overall cohort (n=507)				
Unadjusted model	1.29 (0.88–1.89)	0.191	1.22 (0.53–2.81	0.643
Adjusted model[b]	1.29 (0.88–1.89)	0.197	1.14 (0.49–2.63)	0.763
Intervention group (n=259)				
Unadjusted model	1.19 (0.65–2.19)	0.577	ND[c]	ND[c]
Adjusted model	0.98 (0.52–1.83)	0.949	ND[c]	ND[c]
Control group (n=248)				
Unadjusted model	1.38 (0.84–2.25)	0.202	2.10 (0.89–4.96)	0.092
Adjusted model	1.42 (0.87–2.33)	0.161	1.98 (0.83–4.74)	0.125

Genetic analyses done according to the additive model.
[a] The GG genotype was the referent for rs12255372 and the CC genotype for rs7903146; adjusted model: adjusted for age, sex, BMI and FPG.
[b] Also adjusted for the treatment group (intervention group coded as 1; control group as 2).
[c] *ND*, not determined due to low number of subjects with the TT genotype (11 subjects).

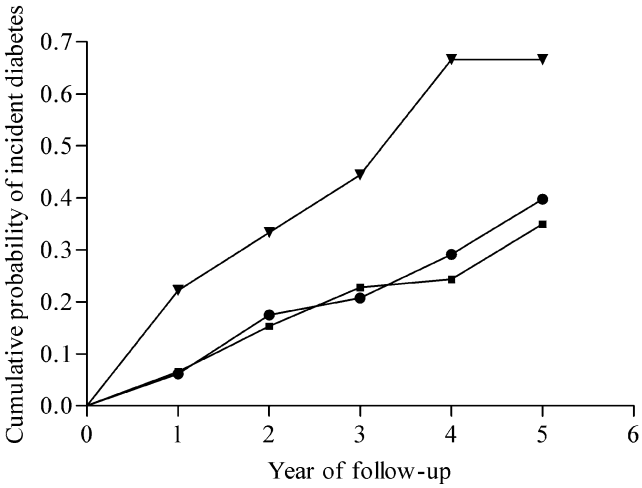

Fig. 1 Cumulative probability of incident diabetes by rs12255372 genotypes in the control group (n=248) during the 4-year follow-up (log-rank test, p=0.009) (Study I). *Inverted triangles*, TT genotype; *circles*, GT genotype; *squares*, GG genotype. Total number of subjects at risk: 248, 244, 225, 194, 136, 62 for years 1–5 post follow-up, respectively

genotype (n=57), the CT genotype (n=26) and the TT genotype (n=3) of rs7903146 had *TCF7L2* expression levels of 1.10±0.29, 0.97±0.23 and 0.88±0.20, respectively, (p=0.09) (CC vs CT, p=0.06).

Discussion

We found that the TT genotype of rs12255372 predicted incident diabetes in the control group of the DPS. In a separate large sample of 1,776 men, the TT genotype of rs12255372 was also associated with type 2 diabetes. Furthermore, the T allele of this SNP was associated with decreased first-phase insulin release in an IVGTT in non-diabetic offspring of type 2 diabetic patients. Finally, our study showed that rs12255372 and rs7903146 tended to be associated with altered gene expression of *TCF7L2* in adipose tissue samples.

Similarly to the DPP study [2], the DPS also showed that variants of *TCF7L2* were associated with incident diabetes in the control group, but not in the intervention group. In the DPP both rs12255373 and rs7903146 predicted progression to diabetes, whereas in the DPS only rs12255372

Table 3 Characteristics of non-diabetic subjects by the genotypes of rs12255372 (Studies II and III)

	Study II			Study III		
	GG (n=1,125)	GT + TT (n=413)	p value[a]	GG (n=164)	GT + TT (n=74)	p value[b]
Men/women (n)	1,125/0	413/0		76/88	36/38	0.780[c]
Age (years)	58.8±5.8	58.3±6.0	0.179	35.1±6.4	35.5±6.2	0.643
BMI (kg/m^2)	27.1±3.8	27.0±3.6	0.513	26.9±5.2	25.0±3.5	0.005
FPG (mmol/l)	5.5±0.5	5.5±0.5	0.706	5.2±0.4	5.2±0.5	0.605
2-h PG (mmol/l)	5.9±1.6	6.1±1.7	0.017	6.3±1.5	6.3±1.3	0.235
Fasting serum insulin (pmol/l)	50±37	49±35	0.835	57±28	49±20	0.334
2-h serum insulin (pmol/l)	318±317	336±317	0.298	283±205	258±187	0.580
OGTT						
Glucose AUC (mmol l^{-1} min^{-1})	858±131	874±140	0.039	841±1,169	832±144	0.754
Insulin AUC (pmol l^{-1} min^{-1})	39,302±28,608	42,285±70,501	0.853	34,940±20,192	30,970±17,865	0.713
Insulinogenic index [d]	101 (3,270)	100 (12,490)	0.122	98 (4,146)	77 (3,252)	0.689
Insulin-sensitivity index[e]	0.027 (0.15)	0.029 (0.09)	0.514	0.022 (0.05)	0.023 (0.04)	0.334
IVGTT, first 10 min						
Glucose AUC (mmol l^{-1} min^{-1})	ND	ND	–	124±12	121±11	0.293
Insulin AUC (pmol l^{-1} min^{-1})	ND	ND	–	2,980±1,849	2,230±1,131	0.045
WBGU:LBM (μmol kg^{-1} min^{-1})	ND	ND	–	54±18	58±17	0.155
Disposition index[f]	2.5 (165)	2.3 (128)	0.046	146±77	126±63	0.076

Data are expressed as means ± SD or medians (range).
Data for parameters of IVGTT and WBGU are available for 217 subjects.
ND, not determined
[a] ANOVA adjusted for age and body mass index.
[b] A mixed linear model, adjusted for age, sex, family relationship and WBGU:LBM, was used in all variables except for BMI, WBGU:LBM and DI (adjusted for age, sex and family relationship).
[c] χ^2 test.
[d] ($\Delta I_{30-0}/\Delta G_{30-0}$) ([pmol/l] ÷ [mmol/l])
[e] (1/fasting insulin) (pmol l^{-1})
[f] Study II: $\Delta I_{30-0}/\Delta G_{30-0}$× insulin-sensitivity index; Study III: insulin AUC during the first 10 min of an IVGTT×WBGU:LBM/1000.

Table 4 Odds ratios of the genotypes of rs12255372 for IGR (IFG or IGT) (n=356) and type 2 diabetes (n=228) among 1,538 non-diabetic and 228 diabetic men (Study II)

	Odds ratio (95% CI)							
	Unadjusted				Adjusted[a]			
	GT	p value	TT	p value	GT	p value	TT	p value
IGR vs NGT (n=1,538)	1.39 (1.07–1.82) (n=382)	0.014	1.06 (0.45–2.48) (n=31)	0.898	1.45 (1.10–1.90)	0.008	1.28 (0.53–3.08)	0.577
DM vs NGT (n=1,409)	2.00 (1.48–2.71) (n=360)	<0.001	2.74 (1.28–5.86) (n=34)	0.009	2.11 (1.51–2.94)	<0.001	3.40 (1.45–7.97)	0.005
DM vs non-DM (n=1,766)	1.84 (1.37–2.48) (n=466)	<0.001	2.71 (1.30–5.64) (n=41)	0.008	1.96 (1.43–2.69)	<0.001	3.10 (1.39–6.89)	0.006
DM vs IGR (n=584)	1.44 (1.01–2.05) (n=190)	0.046	2.59 (0.96–6.96) (n=17)	0.059	1.56 (1.08–2.25)	0.019	2.51 (0.90–7.03)	0.080

Genetic analyses done according to the additive model. The GG genotype was the referent for the comparisons.
DM, diabetes mellitus
[a] Adjusted for age and BMI

was significantly associated with the risk of diabetes. However, this result is consistent with another study in Finns showing that rs12255372 is more strongly associated with type 2 diabetes than is rs7903146 [4]. Results of the DPP and DPS studies are important for prevention strategies of type 2 diabetes, because they indicate that lifestyle-intervention can efficiently reduce the risk conferred by genetic factors, even when risk genotypes are related to impaired insulin secretion. There are two possible ways of explaining these results. First, lifestyle intervention improves insulin sensitivity as shown in the DPP [23] and DPS [26], making less insulin secretion from beta cells necessary to maintain non-diabetic glucose tolerance. Second, there is a possibility that lifestyle intervention, in addition to improving insulin sensitivity, could also improve insulin secretion capacity.

We also showed that in a cross-sectional study setting (Study II) the T allele of rs12255372 was associated with type 2 diabetes and IGR. This implies that *TCF7L2* not only regulates progression to type 2 diabetes, but also increases glucose concentrations at the population level. Therefore, cross-sectional case-control studies that have not evaluated glucose tolerance by an OGTT will probably underestimate the effect of *TCF7L2* on glucose metabolism. On the other hand intervention trials with IGT subjects only (e.g. DPP, DPS) might also underestimate the true effect of *TCF7L2* on the risk of diabetes.

Previous studies have suggested that rs12255372 and rs7903146 are associated with decreased insulin secretion [2, 6, 9, 27] or with decreased insulin secretion and insulin resistance [8]. In Study II with a large sample of subjects, we found that in an OGTT the T risk allele of rs12255372 was associated with glucose AUC and low DI, but not with insulin AUC. In a previous study [6] the T risk allele of rs7903146 was associated with low insulin AUC and low DI in an OGTT. In Study III we applied the euglycaemic clamp technique to measure insulin sensitivity [19] and an IVGTT to estimate first-phase insulin release. We found that the T risk allele of rs12255372 was associated with low first-phase insulin release (insulin AUC) in an IVGTT, but not with insulin sensitivity. Our results provide further evidence that the variants of *TCF7L2* are associated with decreased insulin secretion both in the general population, and in offspring of patients of type 2 diabetes, who have a high risk of developing future diabetes. No association of the variants with insulin resistance could be found in OGTT-derived measures of insulin resistance (Study II) or in clamp-determined insulin sensitivity (Study III).

The mechanisms by which *TCF7L2* regulates insulin secretion are not clear. A recent study found that *TCF7L2* is involved in the activation of mRNA expression of the proglucagon and the glucagon-like peptide-1 genes in gut endocrine cells [28]. Glucagon-like peptide-1, produced in the gut and brain, lowers blood glucose levels through: (1) stimulation of insulin secretion and biosynthesis; (2) inhibition of glucagon release and gastric emptying; (3) enhancement of peripheral insulin sensitivity; and (4) induction of satiety [29, 30]. Although previous studies [2, 6, 9] have suggested that the variants of *TCF7L2* were associated with impaired insulin secretion rather than with insulin resistance, they were based on results from an OGTT or fasting measurement. Our study showed that insulin response to an intravenous glucose bolus was impaired, while that to an oral glucose load was not significantly impaired, suggesting that a deficient ‘incretin

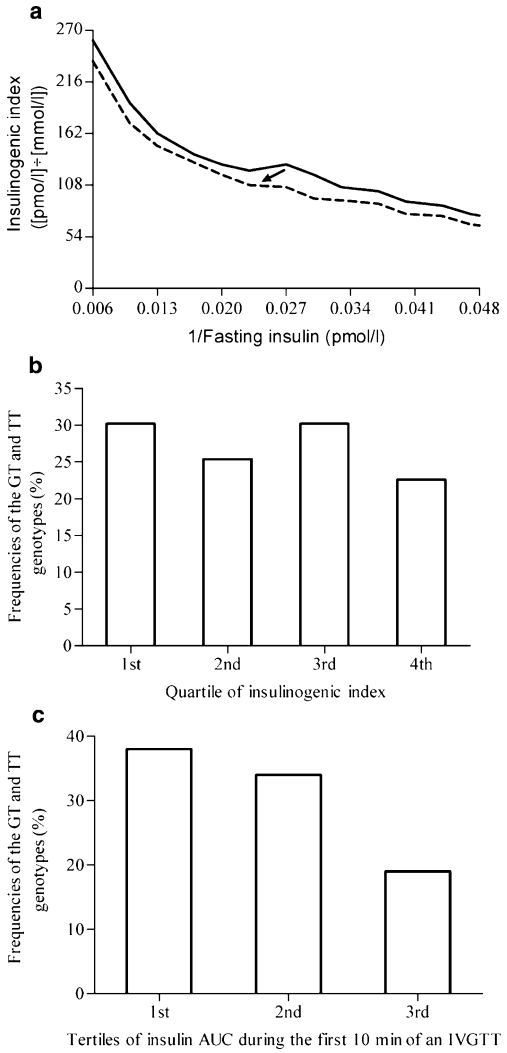

Fig. 2 **a** Among the 1,538 non-diabetic men in Study II, carriers of the GT and TT genotypes of rs12255372 had decreased insulin secretion at each level of insulin sensitivity; the curve shifts downwards and to the left (*arrow*) in those carriers (*dashed line*). *Bold line*, GG genotype; *dashed line*, GT and TT genotypes. **b** Frequencies of the GT and TT genotypes of s12255372 according to quartiles of the insulinogenic index ($\Delta I_{30-0}/\Delta G_{30-0}$) in 1,538 non-diabetic men in Study II, $p=0.076$. **c** Frequencies of the GT and TT genotypes of s12255372 according to tertiles of insulin AUC during the first 10 min of an IVGTT in 238 subjects in Study III, $p=0.019$

effect' is unlikely to be the only explanation why rs12255372 is associated with impaired insulin secretion.

A previous study has reported that *TCF7L2* is expressed in human pancreas [7], suggesting direct effects on beta cell development and/or function. Interestingly, in the same study, *TCF7L2* expression in human subcutaneous and omental fat tissue was lower in type 2 diabetic subjects than in that of normoglycaemic individuals. These findings were based on only three individuals per group and the results were not confirmed by quantitative PCR [7]. However, in agreement with these results, we showed that the T risk alleles of rs12255372 and rs7903146 tended to be associated with low expression of *TCF7L2* in subcutaneous tissue from 86 subjects. Due to a low number of rare homozygous subjects ($n=1$–3), our results were not statistically significant. These findings need to be further investigated, since *TCF7L2* is involved in the regulation of the Wnt signalling pathway [31]. In addition, and similarly to the findings of other studies [1, 2], we also found that the T allele of rs12255372 and rs7903146 was significantly associated with lower BMI in Study III. Therefore, it is possible that Wnt signalling is impaired in adipose tissue of individuals with the risk genotypes of *TCF7L2* and may contribute to weight change and hyperglycaemia [32].

A major problem in genetic association studies of complex diseases is inconsistent replication due to limited statistical power as a result of small sample size [33]. This was also the case in our study. In addition, multiple testing increases the likelihood of false positive p values. However, our results are consistent with previous studies on the effects of SNPs of *TCF7L2* on the risk of diabetes [1–12, 27]. We therefore believe that our major findings are likely to be true.

In conclusion, our study provides further evidence that common variants of *TCF7L2* are associated with type 2 diabetes and IGR. Our study also showed that, in the DPS, it was possible to lower the 'diabetogenic effect' of risk genotypes of rs12255372 by lifestyle intervention. Impaired insulin secretion attributable to the variants of *TCF7L2* is likely to be the main cause of why carriers of the risk genotypes have a high risk of developing type 2 diabetes.

Acknowledgements This work was supported by grants from the Academy of Finland, the European Union (EUGENE2 LSHM-CT-2004-512013) and The Kuopio University Hospital (EVO grant no. 5207) to M. Laakso.

Duality of interest None of the authors had any conflicting interests in connection with this study.

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