

Variants of the *TCF7L2* gene are associated with beta cell dysfunction and confer an increased risk of type 2 diabetes mellitus in the ULSAM cohort of Swedish elderly men

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Abstract

Aims/hypothesis In a population-based cohort of elderly men with well-defined phenotypes and biochemical markers related to type 2 diabetes mellitus, we analysed two single nucleotide polymorphisms (SNPs), rs7903146 and rs12255372, in the transcription factor 7-like 2 gene (*TCF7L2*), which are associated with an increased risk of type 2 diabetes mellitus.

Materials and methods The 1,142 subjects were from the population-based Uppsala Longitudinal Study of Adult Men cohort study (see <http://www.pubcare.uu.se/ULSAM/>, last accessed in May 2007). Insulin sensitivity was assessed using a euglycaemic–hyperinsulinaemic clamp; fasting

intact and 32–33 split proinsulin, immunoreactive insulin and specific insulin were measured in plasma samples. The SNPs rs7903146 and rs12255372 were genotyped using a fluorescent homogeneous single base extension assay. The SNP genotypes were analysed against diabetes prevalence at age 70 using logistic regression and against quantitative biochemical measures using linear regression analysis.

Results We replicated the association with type 2 diabetes mellitus for both SNPs in this cohort of elderly males. The highest significant odds ratio (2.15, 95% CI 1.20–3.85) was found for SNP rs7903146. The odds ratio for SNP rs12255372 was 1.69 (95% CI 1.20–2.39). Both *TCF7L2* SNPs were found to be significantly associated with plasma proinsulin when adjusting for insulin sensitivity, both in the whole cohort and when the diabetic subjects were excluded. Analysis for fasting plasma insulin or insulin sensitivity did not give significant results.

Conclusions/interpretation The association between the risk alleles of the two SNPs studied and levels of proinsulin in plasma, identified when adjusting for insulin sensitivity using euglycaemic–hyperinsulinaemic clamp measurements in this study, is an important novel finding.

Keywords Impaired fasting glucose · Impaired glucose tolerance · Proinsulin · Single base primer extension · Single nucleotide polymorphisms · *TCF7L2* · Transcription factor 7-like 2 gene · Type 2 diabetes mellitus · Uppsala Longitudinal Study of Adult Men

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Abbreviations

IRI immunoreactive insulin
LD linkage disequilibrium
M/I insulin sensitivity index
SNP single nucleotide polymorphism
ULSAM Uppsala Longitudinal Study of Adult Men

Introduction

The search for genetic variants underlying type 2 diabetes mellitus has had limited success in the past [1, 2], until recently when Grant and colleagues showed a significant association between a microsatellite marker (DG10S478) located in the transcription factor 7-like 2 gene (*TCF7L2*) and the risk of type 2 diabetes mellitus [3]. The original association was found in an Icelandic cohort of type 2 diabetes mellitus patients and control subjects and was subsequently replicated in both Danish and American cohorts. In addition to the microsatellite marker DG10S478, the study identified several single nucleotide polymorphisms (SNPs) in *TCF7L2* that were in linkage disequilibrium (LD) with DG10S478 and also significantly associated with type 2 diabetes mellitus. Grant et al. suggested that two SNPs (rs12255372 and rs7903146) [3] be analysed for replication of their finding in other populations. Since the original publication, a number of studies have confirmed the association between *TCF7L2* and type 2 diabetes mellitus in European, Asian and American cohorts [4–15].

The objective of our study was to analyse the SNPs rs12255372 and rs7903146 in the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort. The participants in ULSAM have been particularly well characterised for phenotypes related to type 2 diabetes mellitus and several key biochemical markers for type 2 diabetes mellitus have been measured [16]. Insulin sensitivity was determined at age 70 using the euglycaemic–hyperinsulinaemic clamp [17]. The extensive phenotypic data available in ULSAM allowed us to investigate the possible biochemical aspects of associations between *TCF7L2* variants and the increased risk of type 2 diabetes mellitus in more depth than previous studies. While our study was in progress, the risk alleles of the two SNPs were shown to be associated with decreased levels of insulin in plasma and to affect progression to diabetes in subjects with impaired glucose tolerance [10]. Based on these findings, it has been suggested that the increased risk of type 2 diabetes mellitus associated with *TCF7L2* could be caused by impaired beta cell function affecting glucose-stimulated insulin secretion [7, 10, 11, 18].

Materials and methods

Participants ULSAM is a population-based cohort study of diabetes and cardiovascular disease in men (see <http://www.pubcare.uu.se/ULSAM/>, last accessed in May 2007). The study was initiated in 1970 when all men born between 1920 and 1924 and residing in Uppsala county, Sweden, were invited to a health survey, in which 2,322 men participated [19]. After 20 years, at around 70 years of age, the men were invited to a reinvestigation, which was

performed from August 1991 to May 1995, with 1,221 of the original 2,322 subjects participating [20]. All DNA samples ($n=1,142$) analysed in this study were collected from the 70-year-old (71 ± 0.6 years) cohort. The study was approved by the Ethics Committee of the Faculty of Medicine at Uppsala University and informed consent was obtained from all participants in the study.

Biochemical measurements The diagnosis of type 2 diabetes mellitus was made at age 70 according to the 1999 WHO criteria [21] with a 75 g OGTT [16]. Insulin sensitivity was calculated as the glucose disposal rate divided by the mean plasma insulin concentration (where the glucose disposal rate is the concentration of glucose infused per unit of time per unit of body weight) [16, 17]. Plasma samples were also analysed for immunoreactive insulin (IRI) during the OGTT, fasting intact and 32–33 split proinsulin and specific insulin [16]. At baseline (age 50) an IVGTT had been carried out using a glucose dose of 0.5 g/kg body weight in 77.2% of the subjects in the original cohort. The serum IRI concentrations during the IVGTT were measured in duplicate blood samples drawn before the glucose injection. The early insulin response was expressed as the mean value of the serum insulin concentrations at 4, 6 and 8 min after start of the glucose injection.

Genotyping Two SNPs were selected (rs12255372 and rs7903146) for genotyping based on the recommendations made by Grant et al. for replicating their findings that *TCF7L2* conferred risk of type 2 diabetes mellitus [3]. The selected SNPs were genotyped using a homogeneous single base extension assay with fluorescent polarisation detection [22] using in-house reagents. Fluorescence polarisation was recorded using an analysing device (Analyst AD; Molecular Devices, Sunnyvale, CA, USA). The genotype call rate of SNPs in the samples was 98% for both SNPs, with an accuracy, as determined by duplicate genotyping in independent experiments, of 100% according to 186 (8%) genotype comparisons for the SNP rs12255372 and 160 (7%) genotype comparisons for the SNP rs7903146. The genotypes conformed to Hardy–Weinberg equilibrium according to a χ^2 test ($p>0.05$).

Statistical analysis The statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). Genotype and allele frequencies for the SNPs were tested against diabetes (prevalence in the ULSAM cohort) as a dichotomous trait using logistic regression. For the quantitative biochemical measures, linear regression analysis was performed after excluding subjects with type 2 diabetes mellitus. For analysis including diabetic subjects, see Electronic Supplementary Material (ESM) Tables 1 and 2. Age was used as a covariate in all analyses. Adjustments

were made for the influence of insulin sensitivity using either euglycaemic–hyperinsulinaemic clamp measurements or BMI. All subjects receiving insulin by injections were excluded from the analysis of biochemical measurements that included diabetic subjects. The nominal significance level was 0.05. Correction for multiple testing using the effective number of SNPs and taking into account the LD between them resulted in a required critical p value of 0.033 for significance [23]. No correction was made for the multiple biochemical traits tested. Significant findings from the linear regression analysis were further analysed by two-tailed t testing on the mean values of biochemical measurements based on genotype.

Results

Logistic regression analysis of both *TCF7L2* SNPs showed a significant association with type 2 diabetes mellitus in the ULSAM cohort (Table 1). The highest significant odds ratio (2.15, 95% CI 1.20–3.85) was seen for SNP rs7903146 when comparing the TT against the CC genotype. Comparison of the CT against the CC genotype also resulted in a significant odds ratio of 1.88 (95% CI 1.32–2.67). An increased risk of type 2 diabetes mellitus was also observed for SNP rs12255372 with an odds ratio of 1.69 (95% CI 1.20–2.39) when comparing the GT against the GG genotype. The TT genotype, compared with the GG genotype, did not yield a significant odds ratio (1.52, 95% CI 0.81–2.84). The LD between the SNPs was moderate (D' =0.833, r^2 =0.675) based on the genotype data from the ULSAM cohort.

Table 1 Association analysis of SNP rs12255372 and rs7903146 genotypes with type 2 diabetes at age 70

SNP	Non-diabetic	Diabetic	p value	Odds ratio (95% CI)
rs7903146				
CC	496 (0.56)	67 (0.40)		CT vs CC
CT	327 (0.37)	83 (0.49)	0.0006	1.88 (1.32–2.67)
TT	62 (0.07)	18 (0.11)		TT vs CC 2.15 (1.20–3.85)
T allele	451 (0.25)	119 (0.35)	0.0002	
C allele	1,319 (0.75)	217 (0.65)		
rs12255372				
GG	498 (0.56)	73 (0.44)		GT vs GG
GT	327 (0.37)	81 (0.48)	0.011	1.69 (1.20–2.39)
TT	63 (0.07)	14 (0.08)		TT vs GG 1.52 (0.81–2.84)
T allele	453 (0.26)	109 (0.32)	0.0085	
G allele	1,323 (0.74)	227 (0.68)		

Logistic regression was performed to test for association with type 2 diabetes mellitus. Diabetic subjects were diagnosed with type 2 diabetes mellitus in accordance with WHO criteria [21]

Analysis of SNP genotypes against biochemical measures was performed by linear regression analysis. Analysis of the non-diabetic group revealed a significant association for SNP rs7903146 with plasma proinsulin levels (p =0.0049) when adjusting for age and insulin sensitivity (M/I) (Table 2). When adjusting for BMI, the association became non-significant (p =0.18), but then an additional significant association was observed for a low IRI measured at 30 min of the OGTT (p =0.03). Similar analyses for SNP rs12255372 showed significant associations for plasma proinsulin concentrations using M/I as covariate (p =0.004), but not for BMI (p =0.12). A significant association was also shown for rs12255372 and a low IRI measured at 30 min of the OGTT when adjusting for BMI (p =0.03). Similar analyses for both SNPs in the whole cohort, including diabetic subjects, are depicted in ESM Tables 1 and 2.

Table 3 shows the significant findings, by two-tailed t test, for rs7903146 and the mean concentrations of proinsulin and the 30 min oral insulin response to oral glucose according to genotype. The T allele of SNP rs7903146 was associated with higher plasma proinsulin concentrations when adjusting for M/I (p =0.001) and also associated with significantly lower IRI levels at OGTT 30 min when adjusting for BMI (p =0.01). Similar analyses for SNP rs12255372, as well as the mean values for all biochemical measures according to genotype in both subject groups, are shown in ESM Table 2. The distribution of mean values for the biochemical measures according to genotype for both SNPs are not shown here, but can be found in ESM Tables 3 and 4.

Utilising the longitudinal data in ULSAM, we calculated odds ratios for development of IFG between age 50 and 70. Comparison of the TT and CC genotypes of SNP rs7903146 resulted in an odds ratio of 2.55 (95% CI 1.23–5.28; p =0.0094). The same analysis for rs12255372 resulted in two significant odds ratios: (1) when comparing the TT and GG genotypes of SNP rs12255372 (odds ratio 3.25, 95% CI 1.62–6.50; p =0.0009); and (2) when comparing the TT and GT genotypes (odds ratio 2.34, 95% CI 1.15–4.72; p =0.0183).

In addition, in a subset of the cohort, measurements of early insulin response during an IVGTT [17, 24] performed at age 50 showed a lower insulin peak (mean 54.7 [SD 30.9] mU/l, n =794) in carriers of the TT genotype of SNP rs7903146, as compared with CC carriers (mean 68.4 [SD 50.8] mU/l, n =794) (p =0.004). This measure failed to reach significance (p =0.13) for carriers of the TT genotype of SNP rs12255372, in whom the mean value was 58.8 mU/l (SD 30.6; n =794), as compared with GG carriers (mean 65.6 mU/l [SD 44.2]; n =794).

A correlation matrix for the biochemical measurements analysed in this study can be viewed in ESM Table 5.

Table 2 Statistical analysis against quantitative biochemical measurements for all non-diabetic subjects at the age 70 investigation

	Proinsulin	Split proinsulin	Insulin	OGTT IRI 0 min	OGTT IRI 30 min	M/I
Study group						
All	8.4±8.3 (999)	10.8±11.6 (999)	51.6±36.8 (1,005)	76.8±42.7 (1,032)	341±218 (1,004)	4.7±2.3 (1,000)
Non-diabetic	6.9±5.0 (855)	9.1±9.0 (855)	48.2±32.7 (855)	72.4±38.2 (878)	353±224 (855)	5.1±2.2 (849)
Diabetic	16.8±15.7 (144)	21.0±18.0 (144)	71.9±50.9 (146)	101.6±56.8 (154)	271±167 (149)	2.7±1.6 (151)
SNP						
rs7903146						
M/I	0.005 (816)	0.21 (816)	0.67 (820)	0.91 (839)	0.27 (842)	–
BMI	0.18 (851)	–	0.06 (855)	0.49 (873)	0.03 (850)	–
rs12255372						
M/I	0.004 (818)	0.44 (818)	0.89 (822)	0.93 (841)	0.09 (844)	–
BMI	0.12 (854)	–	0.23 (858)	0.50 (876)	0.03 (853)	–

Values for biochemical measurements are means±SD, with number (*n*) of observations in parentheses. Euglycaemic–hyperinsulinaemic clamp measurements (M/I) values provided as $100 \times (\mu\text{mol}) (\text{kg body weight})^{-1} \text{min}^{-1} (\text{pmol/l})^{-1}$; all other biochemical measurements are given as pmol/l. All analyses used age as a covariate in addition to M/I or BMI. Subjects with type 2 diabetes mellitus were excluded (*n*=150)

Discussion

The association between the *TCF7L2* gene and type 2 diabetes mellitus is fast becoming a landmark achievement in genetic studies on human complex traits and diseases. Our study, along with many others, has successfully replicated the findings of Grant and colleagues regarding the significant association between the *TCF7L2* and type 2 diabetes mellitus [3]. We also found that the high-risk alleles for both SNPs, particularly TT homozygotes, were associated with a significantly increased odds ratio to convert from normal fasting glucose to IFG between age 50 and 70. To further investigate the possible mechanisms underlying the association between *TCF7L2* and type 2 diabetes mellitus, we utilised the unique phenotypic characteristics available in the ULSAM cohort. The euglycaemic–hyperinsulinaemic clamp measurements

provided means to very specifically adjust for insulin sensitivity and enabled us to focus on actual beta cell function, characterised by the 30 min insulin response to oral glucose and fasting proinsulin concentration.

A novel finding in our study was the clear association between the T alleles of both investigated SNPs and increased concentrations of proinsulin in plasma, when using M/I measured by the euglycaemic–hyperinsulinaemic clamp as covariate. This finding suggests that the increased risk of type 2 diabetes mellitus conferred by the *TCF7L2* variants is associated with dysfunction in the production of insulin within the beta cells, since we were able to compensate for the otherwise significant association between insulin resistance and elevated fasting plasma proinsulin (ESM Table 5). Thus we could distinguish the hyperproinsulinaemia associated with impending beta cell failure from that associated with the increased functional load imposed by insulin resistance.

We observed a significant association between the 30 min insulin response during the OGTT after adjustment for BMI at age 70 years and SNP rs7903146, an association also shown in other studies [7, 10, 18]. Analysis of a subset of the cohort, using the early insulin response data from IVGTT at age 50 years, showed a significantly lower acute insulin response in carriers of the high-risk allele of SNP rs7903146. These two observations made at 20 years interval, both after intravenous and oral administration of glucose, emphasise the association between *TCF7L2* genetic variants and first-phase insulin release. The results highlight the importance of impaired insulin secretion, which occurs decades before the clinical onset of type 2 diabetes mellitus in individuals at increased genetic risk [24]. WNT signalling through *TCF7L2* is linked to the production of the incretin glucagon-like peptide-1 in intestinal endocrine L cells [25]. In line with previous

Table 3 Analysis of SNP genotypes for significant findings from linear regression analysis using biochemical measurements from non-diabetic subjects at the age 70 investigation

SNP	Proinsulin (pmol/l) (<i>n</i>)	Two-tailed <i>t</i> test comparison	Proinsulin <i>p</i> value
rs7903146			
CC	6.8±5.0 (479)	CC vs CT	0.19
CT	6.9±4.1 (313)	CT vs TT	0.02
TT	8.3±8.2 (59)	CC vs TT	0.001
rs12255372			
GG	6.8±5.0 (475)	GG vs GT	0.52
GT	6.9±4.2 (316)	GT vs TT	0.004
TT	8.4±8.4 (60)	GG vs TT	0.0009

Values for proinsulin are means±SD. All analyses used age and euglycaemic–hyperinsulinaemic clamp measurements (M/I) as covariates. Subjects with type 2 diabetes mellitus were excluded (*n*=150)

reports [18], we found that insulin secretion stimulated by both oral and intravenous glucose is affected by the *TCF7L2* risk alleles; a finding that implicates mechanisms other than failure of beta cell stimulus, e.g. associations with defects in beta cell differentiation and proliferation. No association was observed between *TCF7L2* SNPs and BMI or M/I determined by the euglycaemic–hyperinsulinaemic clamp, nor with fasting concentrations of specific insulin or IRI, corroborating the notion that this genetic variation has no effect on insulin sensitivity, which has also been observed in the majority of other studies using homeostasis model assessment of insulin resistance or other models for assessment of insulin sensitivity.

Our study showed a significant association and increased risk between the two SNPs rs12255372 and rs7903146 in the *TCF7L2* gene and type 2 diabetes mellitus. Both SNPs gave significant *p* values, with the highest increased risk of disease being found for the T allele of SNP rs7903146, a finding consistent with observations in other populations [4, 6, 9]. For SNP rs12255372 the odds ratio was significant when comparing the GT and GG genotypes, while SNP rs7903146 showed an increased risk of type 2 diabetes for carriers of the T allele, both in heterozygous and homozygous forms. This difference between the two SNPs could possibly be due to lack of power in our cohort caused by the relatively low number of men with type 2 diabetes mellitus.

In summary, we found associations between the T alleles of both SNPs in the *TCF7L2* gene and type 2 diabetes mellitus, thus replicating the findings by Grant and colleagues [3] and later studies. Moreover, we present a novel finding of a significant association between both SNPs and the proinsulin concentrations in plasma, using insulin sensitivity assessed by the euglycaemic–hyperinsulinaemic clamp to allow a more precise analysis of the association with beta cell function measured as the early insulin response to oral or intravenous glucose. These findings highlight impairment of insulin secretion in the development of type 2 diabetes mellitus, but need to be replicated. Further clinical and molecular studies are required to fully understand the role of *TCF7L2* in the pathology and development of in type 2 diabetes mellitus. The discovery of the association between *TCF7L2* and type 2 diabetes mellitus is quickly establishing itself as one of the most important findings in the field of complex disease genetics and may not only lead to a greater understanding of the genetic components in type 2 diabetes mellitus, but also show the way for future investigations into the genetics of other complex traits and diseases.

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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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