# TCF7L2 polymorphisms modulate proinsulin levels and β-cell function in a British Europid population

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Abstract

Objective and methods: Rapidly accumulating evidence shows that common TCF7L2 polymorphisms confer risk of type 2 diabetes (T2DM) through unknown mechanisms. We examined the association between four TCF7L2 single nucleotide polymorphisms (SNPs), including rs7903146, and measures of insulin sensitivity and insulin secretion in 1697 Europid men and women of the population-based MRC-Ely study.

Results: The T-(minor) allele of rs7903146 was strongly and positively associated with fasting proinsulin ($p=4.55x10^{-9}$) and 32,33 split proinsulin ($p=1.72x10^{-4}$) relative to total insulin levels; i.e. differences between T/T- and A/A-homozygotes amounted to 21.9% and 18.4% respectively. Notably the insulin-to-glucose ratio (IGR) at 30min OGTT, a frequently used surrogate of first-phase insulin secretion, was not associated with the TCF7L2 SNP ($p>0.7$). However, the insulin response (IGR) at 60min OGTT, was significantly lower in T-allele carriers ($p=3.5x10^{-3}$). The T-allele was also associated with higher HbA1c concentrations ($p=1.2x10^{-2}$) and reduced β-cell function, assessed by HOMA-B ($p=2.8x10^{-2}$). Similar results were obtained for the other TCF7L2 SNPs. Of note, both major genes involved in proinsulin processing (PC1, PC2) contain TCF-binding sites in their promoters.

Conclusions: Our findings suggest that the TCF7L2 risk allele may predispose to T2DM by impairing β-cell proinsulin processing. The risk-allele increases proinsulin levels and diminishes the 60min, but not 30min insulin response during OGTT. The strong association between the TCF7L2 risk-allele and fasting proinsulin, but not insulin levels is notable as in this unselected and largely normoglycaemic population external influences on β-cell stress are unlikely to be major factors influencing the efficiency of proinsulin processing.

## INTRODUCTION

A common variant in the TCF7L2 gene was found to increase the risk of type 2 diabetes by 1.41 in heterozygotes and by 2.27 in homozygotes in an Icelandic population, a finding that was replicated in a Danish and a European-American population (1). The association is robust and has been consistently reproduced in geographically and ethnically diverse populations (2-12).

The mechanisms through which TCF7L2 affects the susceptibility to type 2 diabetes remain to be elucidated. It is known that TCF7L2 serves as a nuclear receptor for β-catenin, which mediates the Wnt signaling pathway (13). The bipartite transcription factor, cat/TCF7L2, activates many genes downstream of the Wnt signaling cascade (14). One of the genes transcriptionally regulated by cat/TCF7L2 is proglucagon which encodes the insulinotropic hormone glucagon-like peptide 1 (GLP1) (13). Because GLP1, in concert with insulin, plays a critical role in blood glucose homeostasis, it has been postulated that TCF7L2 gene variants may affect the susceptibility to type 2 diabetes by indirectly altering GLP-1 levels (13). Since the Wnt signaling pathway is involved in a variety of biological events and TCF7L2 transcriptionally regulates many genes, other mechanisms cannot be excluded.

Case-controls studies have been instrumental in confirming the association between TCF7L2 variants and type 2 diabetes. However, by design, these studies provide limited insight into the biological pathways through which the TCF7L2 exerts its effect, because the biochemical profile in cases is likely to have been altered by the disease status or by treatment. Cohort studies with detailed phenotypic characterisation can provide new insights to further elucidate the mechanisms that underlie the robust association.

The present study examines the association between four TCF7L2 polymorphisms and measures of fasting insulin sensitivity, β-cell function, insulin secretion during a 120-min OGTT in 1697 Europid men and women of the population-based Ely study.

## RESEARCH DESIGN AND METHODS

### Participants

The MRC Ely Study is a population-based cohort study of the aetiology of type 2 diabetes. Study participants were randomly selected from people living in Ely and surrounding villages (East Anglia, UK), an ethnically homogenous Europid population. The current analyses included 1697 men and women, aged 35-79 years, for whom genotypic and phenotypic data were available from phase 3 as previously described (15). None of the participants had diagnosed type 2 diabetes, although 139 individuals met the WHO criteria for type 2 diabetes. The study design, methods and measurements have been described in Supplementary Information.

In brief, all participants attended a clinical examination for anthropometric measurements, medical questionnaires and a 75-g OGTT. Glucose and insulin concentrations were measured at fasting, 30-min, 60-min and 120-min post-glucose load. Proinsulin and 32,33 split proinsulin was measured at fasting. We calculated the insulin:glucose ratio (IGR) at 30-min and 60-min as a measure of insulin secretion. HOMA-S and HOMA-B, as measures of insulin sensitivity and β-cell function respectively, were estimated by the homeostasis model using Levy's computer model (16).

Descriptive characteristics of the population are given in Supplementary Table 1.

### SNP selection & genotyping

We genotyped the two SNPs (rs12255372 and rs7903146) that showed strongest association in the Icelandic population (1) and added two neighbouring SNPs (rs4506565 and rs12243326) that were in high but not perfect LD with the first two SNPs according to the CEU HapMap data (Supplementary figure 1).

Genotyping was performed using Custom TaqMan® SNP Genotyping Assays (Applied Biosystems, Warrington, UK) (see Supplementary Information). The genotyping success rate exceeded 98% for all SNPs and genotype frequencies were in Hardy-Weinberg equilibrium (HWE) ($p>0.05$) (Supplementary table 2).

In silico analyses

In silico analyses were performed to identify TCF-binding sites in the proprotein convertase 1 and 2 (PC1, PC2) and carboxypeptidase E (CPE) gene sequences. Genomic sequence and annotation of each gene, including 5kb upstream were obtained for a number of species. A global alignment for each gene was performed. The consensus sequence for the TCF-binding site (WWCAAWG) was then used to search for any conserved sites within the alignment. The positions for all candidate sites were added to the human annotation file and the whole annotated alignment viewed (see Supplementary information).

Statistical analyses

A likelihood ratio test was performed to assess HWE. Linkage disequilibrium (LD) between SNPs was estimated using Haploview V3.2 (http://www.broad.mit.edu/mpg/haploview).

We tested for association between phenotypes and SNPs using a generalised linear model assuming an additive effect for the presence of each additional minor allele. All phenotypes were adjusted for age, sex and BMI (except BMI, fat percentage and waist). An estimate of the 'composite β-cell function' was obtained by adjusting baseline insulin secretion (IGR) to insulin sensitivity (HOMA-S) through linear regression of log-transformed variables, as described previously (17). All phenotypes were log-transformed to obtain normal distributions and tables represent geometric means.

Statistical analyses were conducted using SAS/Genetics 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

The four TCF7L2 SNPs were in high LD (supplementary figure 2), thus the results of the associations were similar. Here, we describe the results for rs7903146. More details on the other TCF7L2 SNPs can be found in supplementary table 3.

The strongest association was observed with fasting proinsulin concentrations (Table 1). The T-(minor) allele of rs7903146 showed a highly significant ($p=4.7x10^{-7}$) association with intact proinsulin concentrations; i.e. T/T-homozygotes had on average 15% higher proinsulin concentrations compared to A/T heterozygotes, whose concentrations were 9% higher than those of A/A homozygotes. Similar associations were observed for fasting 32,33 split proinsulin concentrations (Table 1). Although the significance of the association was less ($p=2.8x10^{-3}$), the magnitude of the differences was similar; i.e. T/T-homozygotes had 15% higher 32,33 split proinsulin concentration than A/T heterozygotes, whose concentrations were 6% higher than those of A/A homozygotes. When intact proinsulin and 32,33 split proinsulin concentrations are expressed as a percentage of fasting total insulin (sum of insulin, intact proinsulin and 32,33 split proinsulin) the associations were even stronger (intact proinsulin: $p=4.55x10^{-9}$; 32,33 split proinsulin: $p=1.72x10^{-4}$) (Figure 1).

IGR, as an estimate of insulin secretion, was significantly ($p=3.5x10^{-3}$) associated with the TCF7L2 variant, but only when measured after 60-min OGTT; i.e. IGR at 60-min decreased by 10% for each additional T-allele in the genotype. No association was observed with IGR at 30-min OGTT. Adjusting the IGR measures for insulin sensitivity (HOMA-S), as a measure of composite β-cell function (17), resulted in similar findings.

HbA1c concentration (p=0.012) was increased and β-cell function (HOMA-B) was reduced (p=0.028) in T/T-homozygotes compared to A-allele carriers. Also fasting glucose concentrations (p=0.04) and glucose AUC during OGTT (p=0.013) were significantly higher in T/T-homozygotes compared to A-allele carriers. No associations were observed with insulin concentrations, insulin sensitivity (HOMA-S), BMI, fat percentage and waist circumference.

Results were similar after excluding 139 individuals who met the WHO-criteria for type 2 diabetes (data not shown).

No association or interactions with BMI were observed.

Because of the strong association between the TCF7L2 variants and insulin precursors, we theorised that TCF7L2 might act through transcription of PC1 and PC2 and/or CPE that are required for processing proinsulin to insulin (18;19). We identified a TCF-binding site in PC1 between exon-7 and exon-8 (table 2) and three more binding sites in the PC1-promoter. We also found a TCF-binding site in the PC2-promoter region that was conserved across human, dog, mouse and opossum. Although potential consensus TCF-binding sequences were located in the 10kb region upstream of the human CPE gene, none were fully conserved across species.

## DISCUSSION

This population-based cohort study in British Europids provides new insights into the potential mechanisms through which common TCF7L2 gene variants increase the susceptibility to type 2 diabetes. Consistent with previous observations, we found that TCF7L2 variants might predispose to type 2 diabetes by impairing β-cell insulin secretion, rather than by changes in insulin sensitivity. In addition, our results suggest that TCF7L2 is associated, directly or indirectly, with impaired insulin synthesis and/or processing of insulin precursors. The minor-allele carriers have substantially increased intact proinsulin and 32,33 split proinsulin relative to insulin and demonstrate impaired 60-min OGTT, but not 30-min OGTT insulin secretion. The strong association between the TCF7L2 risk-allele and proinsulin, but not insulin is notable as in this unselected and largely normoglycaemic population external influences on β-cell stress are unlikely to be major factors influencing the efficiency of proinsulin processing.

So far, only four cohort studies have reported quantitative trait analyses (5;10;20;21), whereas others performed these analyses within a case-control (2;6;11;12) or intervention (3) design. The TCF7L2 polymorphisms were found to be associated with insulin secretion (2;3;5;10;11;21), and insulin sensitivity (2;5;11), whereas others found no associations (6;12;20). Thus, the quantitative trait results are less consistent and the statistical significance is less when compared to case-control studies.

Our study is the first population-based cohort study that tested for associations with precursor proteins of insulin. The associations between the TCF7L2 polymorphisms and intact proinsulin levels, and to a lesser extend for 32,33 split proinsulin levels, are the strongest so far reported for quantitative traits.

The strong associations with proinsulin suggest that TCF7L2 gene is associated with defects in insulin synthesis, processing and/or secretion of insulin precursors. This is consistent with findings in previous studies and with the theory that TCF7L2 variants might impair β-cell function and insulin secretion (2;3;5;6;10;11). Proinsulin levels predict the risk of type 2 diabetes independent of insulin levels (22). This supports the hypothesis that a reduced efficiency in β-cell proinsulin processing might be the mediating factor in the TCF7L2 - type 2 diabetes association.

Given that PC1, PC2 and CPE are key proteins in the processing of proinsulin to insulin (19;23), suggests that TCF7L2 variants affect proinsulin levels through transcriptional regulation of their genes. An impaired function of PC1 and PC2 would lead to an excess of intact proinsulin, whereas a defect in only PC1 or PC2 would result in accumulation of 65,66 split and 32,33 split proinsulin respectively. A defect in CPE would results in accumulation of both split proinsulin forms. Support for our hypothesis comes from the identification of at least four conserved TCF-binding sites for PC1 and at least one for PC2. This observation needs further investigation and confirmation from experimental data. Given that PC1 and PC2 are involved in the processing of several other prohormones and neuropeptide precursors, the potential effects of TCF7L2 could manifest itself in other phenotypes.

Furthermore, the minor allele was also associated with reduced insulin secretion (IGR) at 60-min, but not at 30-min during an OGTT. These findings suggest that, upon glucose stimulation, the first-phase insulin release from the insulin-storing granules is normal. However, the insulin release at 60-min, which takes place once the insulin-storing granules are depleted and requires insulin synthesis, processing and secretion, is impaired in the minor-allele carriers. This corroborates the observed associations between the TCF7L2 gene variants and increased intact proinsulin and 32,33 split proinsulin concentrations. In contrast, Saxena et al. (2) found association with insulin response at 30-min in a population that combined cases and controls. No data at 60-min OGTT was available.

TCF7L2 activates many genes downstream of the Wnt signaling pathway, a complicated regulatory network implicated in many biological events such as the development of the pancreas and islets during embryonic growth (14;24). Therefore, our results might reflect impaired β-cell function due to reduced cell mass or to defects in cell differentiation and maturation.

It has been suggested that TCF7L2 could influence the susceptibility to type 2 diabetes by altering the expression of GLP-1 in enteroendocrine cells as TCF7L2 regulates the expression of its preprotein, proglucagon (13). Also other pathways make GLP-1 a good candidate. GLP-1 is a β-cell mitogen, capable of promoting β-cell proliferation and inhibiting β-cell apoptosis in animal models (25). In addition, GLP-1 replenishes insulin stores via stimulation of proinsulin gene expression and translation (25). Interestingly, also the processing of proglucagon to GLP-1 and other peptides requires PC1 (25).

In summary, our findings confirm and provide further insight in the role of TCF7L2 in insulin secretion. The strong association with proinsulin concentrations and the association with insulin secretion at 60-min, but not at 30-min OGTT suggest TCF7L2 might be involved in insulin synthesis, processing and secretion. This needs confirmation by experimental data while other mechanisms cannot be excluded.

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TABLES

TABLE 1. Mean (± SEM) values for measures of body composition, fasting and OGTT insulin and glucose concentrations and measures of insulin secretion, insulin sensitivity and β-cell function by genotype in 1697 Europids for the rs7903146 TCF7L2 gene variant.

| | **C/C** | **C/T** | **T/T** | **p-value** |
|---|---|---|---|---|
| **n** | 830 | 699 | 147 | |
| | **Mean ± SEM** | **Mean ± SEM** | **Mean ± SEM** | |
| **BMI (kg/m2)** | 27.0 ± 0.2 | 26.8 ± 0.2 | 26.6 ± 0.4 | 0.24 |
| **Fat percentage (%)** | 32.0 ± 0.2 | 31.8 ± 0.2 | 31.9 ± 0.5 | 0.61 |
| **Waist circumference (cm)** | 92.9 ± 0.4 | 92.5 ± 0.4 | 91.3 ± 0.9 | 0.21 |
| **Fasting insulin (pmol/l)** | 49.5 ± 0.9 | 48.9 ± 1.0 | 48.6 ± 2.1 | 0.60 |
| **Insulin AUC (pmol/l)*** | 616 ± 11 | 594 ± 12 | 621 ± 26 | 0.54 |
| **Fasting Glucose (pmol/l)** | 5.49 ± 0.02 | 5.54 ± 0.03 | 5.61 ± 0.06 | ***0.042*** |
| **Glucose AUC (pmol/l)*** | 15.0 ± 0.1 | 15.3 ± 0.1 | 15.8 ± 0.3 | ***0.013*** |
| **Insulin/glucose ratio at 30 min** | 88.6 ± 2.5 | 86.1 ± 2.7 | 88.4 ± 5.9 | 0.70 |
| **Insulin/glucose ratio at 60 min** | 134.0 ± 4.5 | 120.2 ± 4.5 | 108.5 ± 8.6 | ***0.0035*** |
| **Fasting intact proinsulin (pmol/l)** | 3.56 ± 0.07 | 3.89 ± 0.08 | 4.46 ± 0.20 | ***0.00000047*** |
| **Fasting 32,33 split proinsulin (pmol/l)** | 3.85 ± 0.10 | 4.06 ± 0.11 | 4.69 ± 0.28 | ***0.0028*** |
| **Fasting HbA1c (%)** | 5.35 ± 0.02 | 5.38 ± 0.02 | 5.46 ± 0.04 | ***0.012*** |
| **HOMA-B** | 83.3 ± 1.1 | 81.0 ± 1.2 | 77.7 ± 2.4 | ***0.028*** |
| **HOMA-S** | 90.1 ± 1.6 | 91.3 ± 1.8 | 92.9 ± 4.0 | 0.45 |

Means are geometric means. All phenotypes were adjusted for age, sex and BMI, except BMI and fat percentage that were adjusted for age and sex

Figure 1 Percentage (mean and SEM) of fasting proinsulin and 32,33 split proinsulin of total fasting insulin concentration (intact proinsulin + 32,33 split proinsulin + insulin) by genotype in 1697 Europids for the rs7903146 TCF7L2 gene variant.

Figure 2 Consensus sequence (WWCAAWG) for TCF-binding sites in the proprotein convertase 1 (PC1) and proprotein convertase 2 (PC2) gene sequence.

Four consensus sites were identified in PC1. Part A shows 27 bp of the sequence upstream of start codon. The raw genomic position of the consensus site is 95,795,320 – 95,795,326 at -818 bp from the start codon. Part B shows 27 bp of the sequence upstream of the start codon. The raw genomic position of the consensus site is 95794987 – 95794993 at -485 bp from the start codon. Part C shows 27 bp of the sequence upstream of the start codon. The raw genomic position of the consensus site is 95794948 – 95794954 at -446 bp from start codon. Part D shows the 27 bp of the sequence between exons 7 and 8. The raw genomic position is 95772777 – 95772783, at 994bp from the start of intron 7.

One consensus site was identified in PC2. Part E shows 27 bp of the sequence upstream of the start codon. The raw genomic position of the consensus site is 17155321 – 17155327, at -630 bp from the start codon.

All alignments are composed of sequence taken 5' -> 3' from the forward strand and positions are as per the current build (NCBI 36 assembly) of the human genome (August 2006).

Figure 1

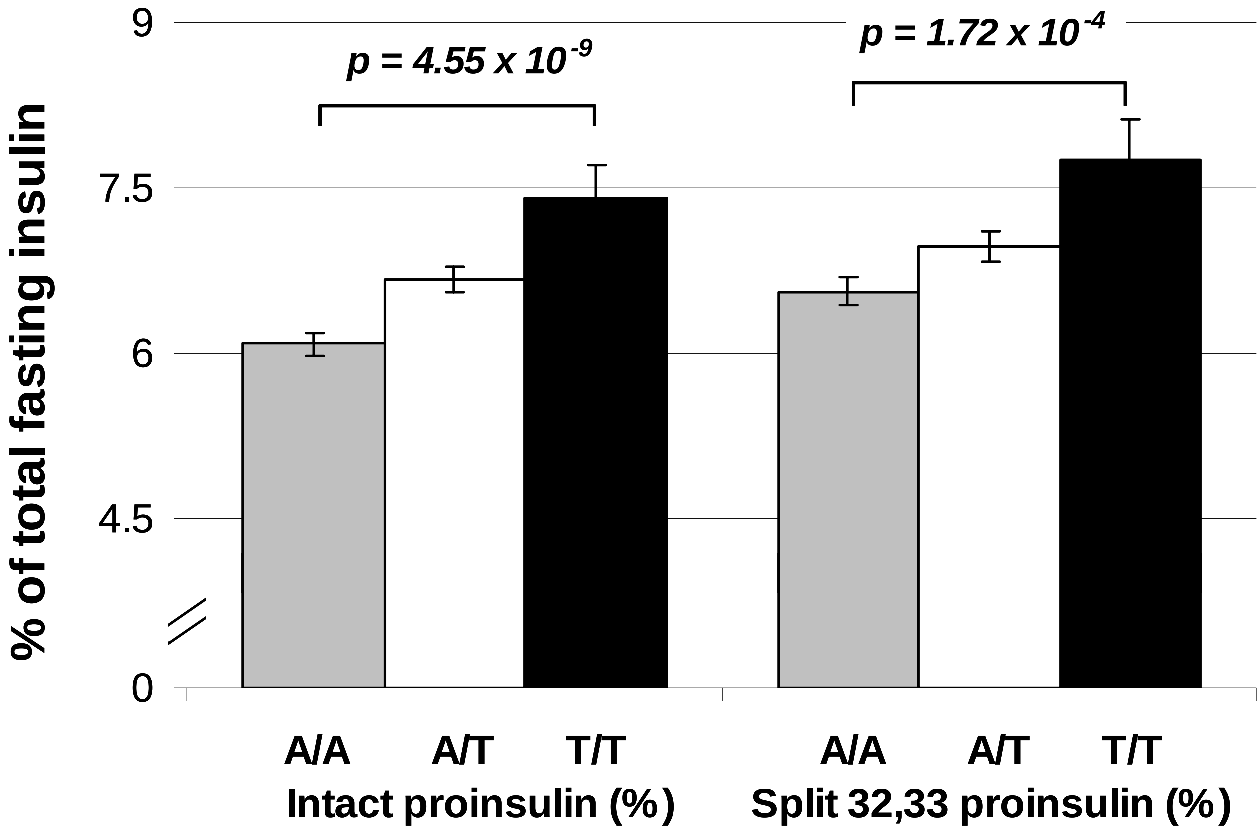
9
7.5
6
4.5
0
% of total fasting insulin
p = 4.55 x 10-9
p = 1.72 x 10-4
A/A
A/T
T/T
A/A
A/T
T/T
Intact proinsulin (%)
Split 32,33 proinsulin (%)

Figure 2

# Proprotein convertase 1

## A

```
                 -805         -818    -825
ATG <--
human   CCCTCCTCCCCTTTGTTTCTCAACCTC
cow     ---TTCTCTCCTCTGTTTCTTAACCTC
mouse   ---------CTCTTGCTTCTGACCCCT
rat     ---------CTCTTGCTTCTGAACACC
dog     ---TCCTCTCCTCAGTTTCTCCAC-TC
                 *    *  ****    *
```

## B

```
                 -480   -485          -500
ATG <--
human   ACTACCAAGAATCAAAGGCTGGGAGT-
cow     ACTACCAAGAATCAAAGACCTGGAGC-
mouse   ACTACCAAGATCCCAAGGCAGGAAGCA
rat     ACTACCAAGACTCTAAGGCAGAAAGCA
dog     ACTACCAAGAAGCAAAGGCTGGGAGTT
        **********  * ***.*   ..**
```

## C

```
                 -440   -446          -460
ATG <--
human   ATTTTCACTTTTCAAAGAGAAGTGCCC
cow     ACCTTCACTTCTCTTTGAGAAGTGCCC
mouse   ATCTCCATTCTTCAAAGAGAACCGCCT
rat     ATCTCCATTCTTCAAAGAGAACCGCCT
dog     ACCTTCACTTCTCAAAGAGAAGTGCCC
        *  * ** *  **   *****  ***
```

## D

```
        IVS7 +1010      IVS7 +994 IVS7 +990
                                         --> ATG
human   GCACTTAAATCTTTGAACTTCCTCTCT
cow     CCATTTGAGTCTTTGAACTTCCTCTCT
mouse   AGACTTGAATCTTTGAACTTCCTCTGT
rat     -----TGAATCTTTGAACTTCCTCTGT
dog     GTGTTTGAATCTTTGAACTTCCTCTCT
             * * **************** *
```

# Proprotein convertase 2

## E

```
          -640      -630      -620
                                         --> ATG
human     GATTCCCACAAACAATGACATCACTCC
dog       GCTCTCCACAAACAATGACATCACT-C
mouse     GATTTCCACAAACAATGACATCAC-CG
opossum   GATTTACACCAACAATGACATCAT---
          * *   *** *************
```