

Unique splicing pattern of the *TCF7L2* gene in human pancreatic islets

P. Osmark · O. Hansson · A. Jonsson · T. Rönn ·
L. Groop · E. Renström

Received: 9 December 2008 / Accepted: 22 January 2009 / Published online: 27 February 2009
© Springer-Verlag 2009

Abstract

Aims/hypothesis Intronic variation in the *TCF7L2* gene exhibits the strongest association to type 2 diabetes observed to date, but the mechanism whereby this genetic variation translates into altered biological function is largely unknown. A possible explanation is a genotype-dependent difference in the complex splicing pattern; however, this has not previously been characterised in pancreatic or insulin target tissues. Here, the detailed *TCF7L2* splicing pattern in five human tissues is described and dependence on risk genotype explored.

Methods RT-PCR and quantitative real-time PCR were employed to characterise *TCF7L2* splicing in pancreatic islets, blood lymphocytes, skeletal muscle and subcutaneous and visceral adipose tissue from non-diabetic individuals.

Results The mapping of *TCF7L2* splice variants shows a specific pattern in pancreatic islets, with four predominant transcripts and high usage of the variable exons 4 and 15. The overall concentration of *TCF7L2* mRNA is highest in islets and fat and lower in blood and muscle. No significant difference in overall amount or splicing pattern was observed between carriers and non-carriers of the rs7903146 risk (T) allele. However, incorporation of exon 4 in islets correlates positively with plasma HbA_{1c} levels ($r=0.758$; $p=0.018$).

Conclusions/interpretation There were pronounced tissue-specific differences in the splicing of *TCF7L2* with forms containing exon 4 and 15 being most abundant in islets. The incorporation of exon 4 in islets correlated with HbA_{1c} levels. Further experiments will be needed to determine the direction of this correlation, and larger cohorts needed to unequivocally resolve whether there is a relationship between genotype and splicing in islets.

Keywords Genetics of type 2 diabetes · Human · Islets · Splicing · *TCF7L2* · Transcription factors

Abbreviations

CtBP1	C-terminal binding protein 1
SAT	Subcutaneous adipose tissue
SNP	Single-nucleotide polymorphism
TCF7L2	Transcription factor 7-like 2
VAT	Visceral adipose tissue

Introduction

Of all common risk variants identified for type 2 diabetes, variants in the *TCF7L2* gene, which encodes a transcription factor in the morphogenic wingless-type MMTV integration site family (WNT) signalling pathway, confer the highest risk of developing the disease (reviewed in [1]). The exact role of *TCF7L2* in the development of diabetes has not been determined, but several links between WNT signalling and insulin secretion and proliferation of human beta cells have been established (reviewed in [2]).

The *TCF7L2* gene consists of 17 identified exons (Fig. 1) and is known to display a complex pattern of splice variants with several alternative exons and splice sites [3]. A perturbed splicing pattern of *TCF7L2* has previously been

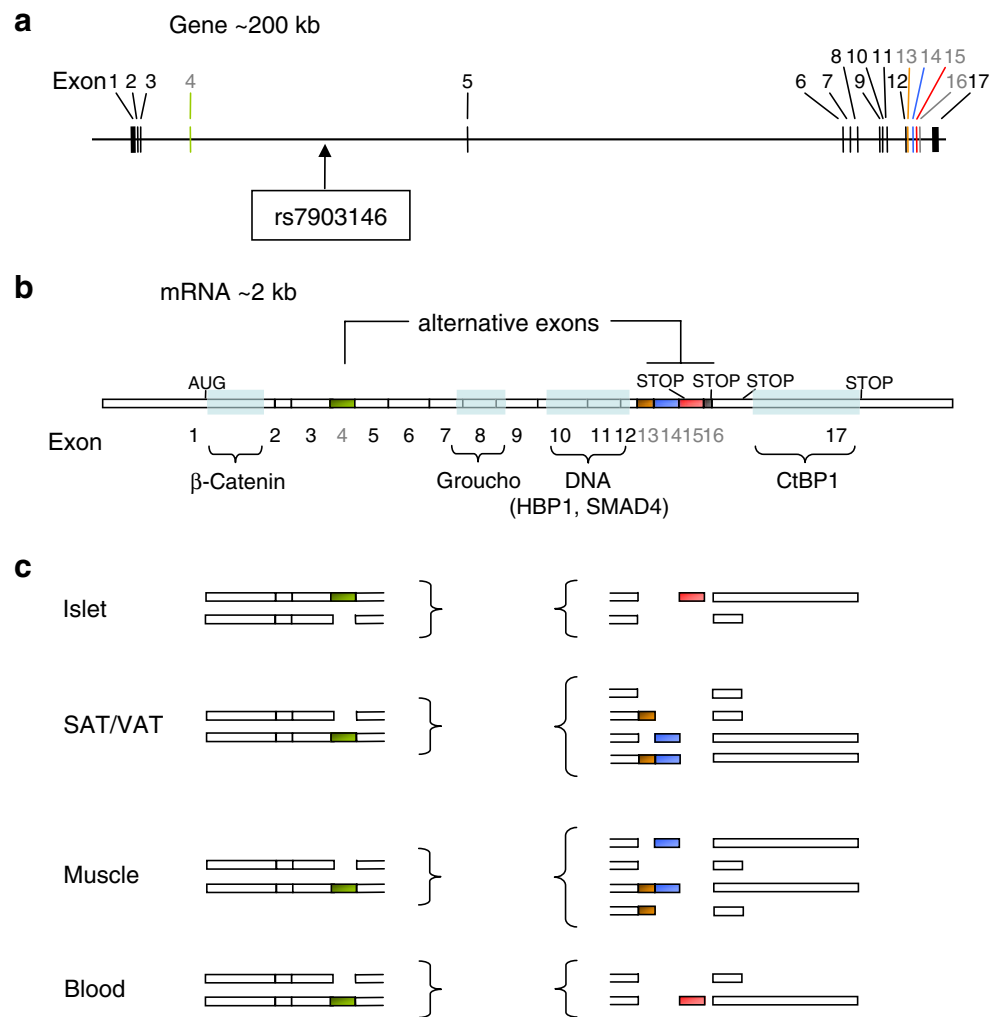
P. Osmark and O. Hansson contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-009-1293-z) contains supplementary material, which is available to authorised users.

P. Osmark · O. Hansson (✉) · A. Jonsson · T. Rönn · L. Groop ·
E. Renström

Department of Clinical Sciences, Diabetes and Endocrinology
Research Unit, CRC Malmö University Hospital,
Lund University,
205 02 Malmö, Sweden
e-mail: ola.hansson@med.lu.se

Fig. 1 Gene structure of *TCF7L2* (a); the location of the risk SNP, rs7903146, in intron 4 is indicated. Exon structure (b) of *TCF7L2*; known binding sites of the transcription factor are indicated by shading. The exact location of the binding sites of HBP1 and SMAD4 is not known. c Schematic overview of the open reading frames of the major *TCF7L2* splice variants in the five tissues investigated. The 3'-ends and the 5'-ends are shown separately (intervening sequences are invariant)



demonstrated in renal cell carcinomas and linked to *TCF7L2* target gene regulation [4]. Little is known about the splicing pattern of *TCF7L2* in human islets, nor whether splicing is influenced by risk genotypes. Previous studies on correlation of *TCF7L2* expression levels and risk genotypes have not taken into account the splicing pattern. Furthermore, most of these studies have investigated adipose and/or skeletal muscle tissue [5–9]. While experiments in vitro have shown that a reduction of *TCF7L2* expression leads to impaired beta cell function [10, 11] we have previously observed increased expression of *TCF7L2* in islets of Langerhans from diabetic patients [12]. However, in these previous studies no attempts were made to discriminate between different splice variants of *TCF7L2*, and differential expression of splice variants could be an explanation for these conflicting observations. Here, we present a detailed quantitative description of the *TCF7L2* splicing pattern in primary human lymphocytes, skeletal muscle and subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT), as well as human islets of

Langerhans, with particular focus on islets, and relate this to the rs7903146 genotype in the *TCF7L2* gene.

Methods

Tissues All tissue samples were from non-diabetic individuals. Islets were obtained from the Human Tissue Laboratory at Lund University Diabetes Centre from deceased donors (six female, 11 male), BMI 17.6–29.0 kg/m², aged 26–73 years. Purity varied from 13% to 90%. The islets were culture in CMRL 1066 (ICN Biomedicals, Costa Mesa, CA, USA) supplemented with 10 mmol/l HEPES, 2 mmol/l L-glutamine, 50 μ g/ml gentamicin, 0.25 μ g/ml Fungizone (GIBCO, BRL, Gaithersburg, MD, USA), 20 μ g/ml ciprofloxacin (Bayer Healthcare, Leverkusen, Germany), and 10 mmol/l nicotinamide at 37°C (5% CO₂) for 1–9 days prior to RNA preparation. HbA_{1c} levels were available for nine islet donors. SAT and VAT were obtained

from bariatric surgery of obese individuals (19 female, two male), BMI 32.6–55.5 kg/m², aged 20–61 years. Muscle biopsies and blood samples were collected from 18 individuals (all male), BMI 22.6–34.0 kg/m², aged 30–46 years. Informed consent was obtained from all study participants. All islet donors had given consent to donate organs for medical research. All procedures were approved by the ethical committees at Uppsala and Lund Universities.

RNA isolation RNA was isolated from islets using the AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA), from muscle using the RNeasy Fibrous Tissue Kit (Qiagen), from fat using the RNeasy Mini Kit (Qiagen), and from blood using the Tempus 12-Port RNA Isolation Kit and an ABI Prism 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA, USA). Concentration and purity was measured using a NanoDrop ND-1000 spectrophotometer ($A_{260}/A_{280} > 1.8$ and $A_{260}/A_{230} > 1.0$) (NanoDrop Technologies, Wilmington, DE, USA). No sign of degradation was observed using agarose gel electrophoresis and Experion DNA 1K gel chips (Bio-Rad, Hercules, CA, USA).

Analysis of expression and splicing of the TCF7L2 gene A detailed description of the procedure can be found in the Electronic supplementary material (ESM). Briefly, reverse transcription was performed using 1 μmol/l dT₁₈ oligomer

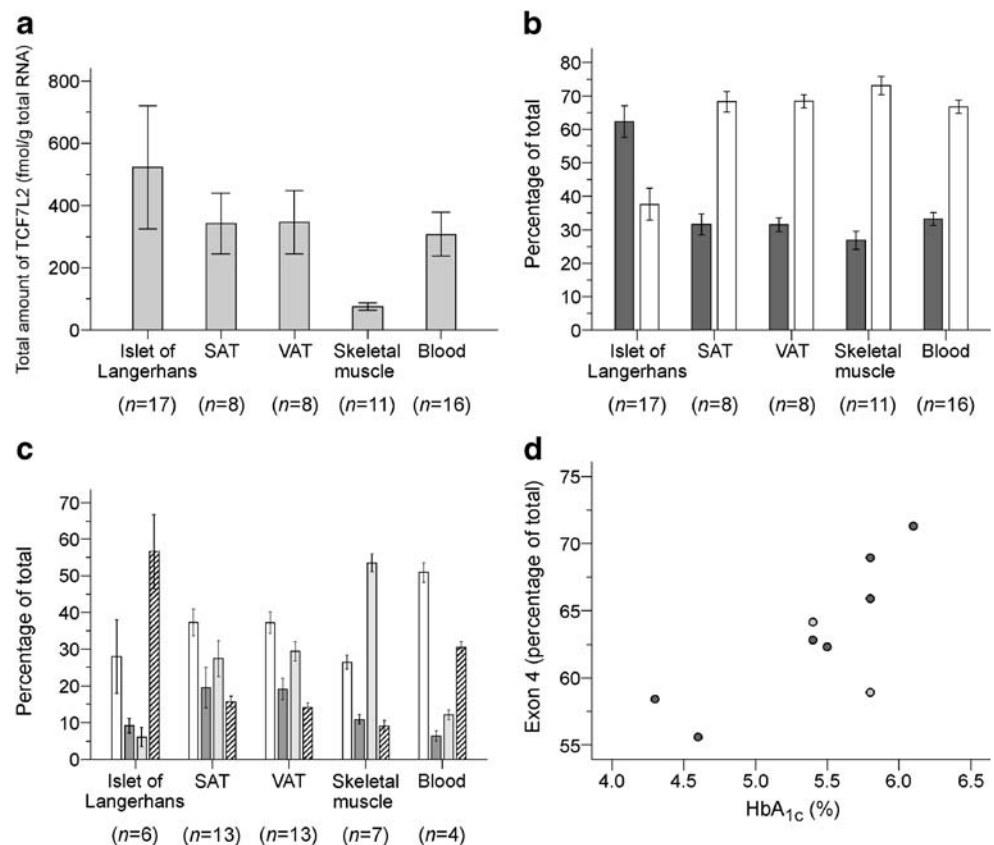
and 3 μmol/l random hexamer primers. Quantitative real-time PCR was performed using TaqMan chemistry according to the manufacturer's recommendation (Applied Biosystems) on an ABI 7900HT sequence detection system (see ESM Table 1). All samples were analysed in triplicates (maximum accepted variation in C_t value: 0.1 cycles). The absolute quantity was calculated using a dilution standard curve of an oligonucleotide template of known concentration [13] (see ESM Table 2).

Statistics Data are expressed as means±SD. Differences between genotypes and tissues were analysed using non-parametric Kruskal–Wallis and non-parametric Mann–Whitney *U* tests. Correlations were analysed using non-parametric Spearman's tests. In all tests $p < 0.05$ was considered statistically significant. Statistical tests were performed with SPSS 16.0 software (SPSS, Chicago, IL, USA).

Results

Large differences in splicing patterns between different tissues cDNA was prepared from human pancreatic islets, blood lymphocytes, skeletal muscle, SAT and VAT. Sequencing, RT-PCR and restriction cleavage analysis of

Fig. 2 The total amount (a) of *TCF7L2* in each tissue and relative distribution of *TCF7L2* splice variants determined by real-time PCR. **b** The 5'-end: real-time PCR over exon boundaries 3–4 (dark grey bars) and 3–5 (white bars). **c** The 3'-end: exon boundaries 12–17 (white bars), 12–13 (dark grey bars), 12–14 (light grey bars) and 12–15 (hatched bars). The main splice variants identified by each assay are listed in ESM Table 1. Data are presented as mean±SD. **d** Relative incorporation of *TCF7L2* exon 4 in islets plotted against plasma HbA_{1c} levels ($r=0.758$; $p=0.018$); white circles represent the rs7903146 CC genotype; grey circles represent the heterozygous (CT) genotype



TCF7L2 cDNA species reveals clear tissue-dependent differences in the splicing pattern (see ESM Figs 1 and 2, ESM Table 3).

The total amount of *TCF7L2* mRNA was highest in islets, followed by fat, blood and muscle (Fig. 2a; see ESM Table 4).

Human pancreatic islets show primarily splice isoforms containing exons 4 and 15 Pancreatic islets display a high incorporation of exon 4 (Fig. 2b and see ESM Table 4), which is located immediately upstream of the diabetes-associated polymorphism rs7903146 in the *TCF7L2* gene (Fig. 1a, b). In islets, exon 4 is present in $62.3 \pm 4.8\%$ of the transcripts compared with 26.9 ± 2.7 to $33.2 \pm 2.0\%$ in the other tissues ($p = 2.6 \times 10^{-9}$). In the 3'-end, exons 14 and 15 tend to be mutually exclusive and tissue-specific, so that exon 15 predominates in islets and blood, whereas exon 14 is more common in fat and muscle tissues (Fig. 2c and see ESM Table 5). An overview of the major splice variants in each tissue is given in Fig. 1c.

Exon 4 incorporation in islets correlates with HbA_{1c} levels In islets, neither BMI ($r = -0.109$; $p = 0.676$) nor age ($r = -0.173$; $p = 0.506$) was significantly correlated to incorporation of exon 4. However, exon 4 incorporation in human pancreatic islets was significantly and positively correlated with HbA_{1c} levels in blood ($r = 0.758$; $p = 0.018$; Fig. 2d) and with total amount of *TCF7L2* ($r = 0.583$; $p = 0.014$). Neither BMI ($r = 0.359$; $p = 0.157$) nor age ($r = 0.097$; $p = 0.711$) was correlated with the total amount of *TCF7L2* mRNA.

No significant differences in splicing pattern or total amount of *TCF7L2* in islets were observed between carriers of different rs7903146 genotypes (CC vs CT; see ESM Fig. 3, Tables 4 and 5).

Discussion

The incomplete knowledge of the complex and tissue-specific *TCF7L2* splicing pattern has hampered endeavours to detail the biological role of the transcription factor in glucose homeostasis and type 2 diabetes. We show that the tissues examined exhibit distinct variations in splicing patterns and that only a few of the theoretically possible variants are represented in each tissue. In the 5'-end there is a tissue-dependent variation in the presence or absence of exon 4. In the 3'-end, exon 14 and 15 largely appear to be mutually exclusive in the transcripts. It is noteworthy that these two exons are of the same length and share 63% identity at the mRNA level and 67% identity at the protein level, and the presence of both introduces a stop codon in exon 15. This leaves the impression that the two exons can

replace one another. Also, they tend to be tissue-specific, exon 15 being preferred in blood and islets and exon 14 in fat and muscle, which also display an enhanced incorporation of exon 13. A characteristic of the islets is the high incorporation of exon 15 and, most notably, of exon 4, which is retained in ~62% of the cDNA transcripts, compared with ~33% or less in the other tissues studied.

TCF7L2 interacts with numerous binding partners and several binding sites have been mapped to the sequence (reviewed in [14]; Fig. 1b). None of the known sites overlap with parts corresponding to alternative exons, but variants without either exon 14 or 15 are predicted to produce protein isoforms without the C-terminal binding protein 1 (CtBP1) site encoded by exon 17 (Fig. 1c). As CtBP1 is a co-repressor, these shorter forms will be expected to possess reduced repression capacities. Since exon 4 comprises 69 nucleotides its presence does not affect the reading frame or, consequently, the primary structure of the rest of the protein. However, a difference of 23 amino acids can influence the three-dimensional structure and, thereby, the interaction of *TCF7L2* with its binding partners.

The key issue addressed was whether the intronic single-nucleotide polymorphism (SNP) rs7903146 influences the splicing pattern. Although the splicing pattern differed between islets and other tissues with exon 4 being more predominant in islets, the splicing pattern was not significantly influenced by the rs7903146 genotype (ESM Fig. 3). The variation observed in islet tissue may reflect a number of factors, i.e. purity of the preparations, material from unmatched donors and differences in cultivation times. Significant correlations between *TCF7L2* expression levels and age and BMI were not found, and differences in purity of the islet preparations did not seem to have a major influence on *TCF7L2* splicing or expression levels (ESM). It is premature, though, to exclude an effect of genotype on splicing pattern as the power was limited to detect such a presumably subtle effect, and because no individuals were homozygous for the T allele. Also, the positive correlation between HbA_{1c} levels and retention of exon 4 in islets makes it likely that the genotype effect is revealed only after plasma glucose levels are taken into account. Although this correlation does not prove causality it suggests a link between *TCF7L2* splicing and plasma glucose levels.

We conclude that islets of Langerhans display a unique splicing pattern predominantly consisting of isoforms containing exons 4 and 15, the former being located immediately upstream of the rs7903146 polymorphism. However, we did not observe a significant effect of genotype on splicing pattern. Interestingly, the amount of exon 4 in islets correlated with HbA_{1c} levels, which suggests a possible link between glucose and *TCF7L2* expression.

Acknowledgements This work was supported by the Swedish Research Council, the Wallenberg Foundation, the Novo Nordisk Foundation, the UMAS Fonder, the Lund University Diabetes Centre (LUDC) and the Magn. Bergvalls Stiftelse and Syskonen Svenssons Fond. Human pancreatic islets were provided by the Nordic Network for Clinical Islet Transplantation by the courtesy of O. Korsgren, Uppsala, Sweden. The authors acknowledge the Human Tissue Laboratory at Lund University Diabetes Centre and J. Taneera for providing RNA from pancreatic islets, J. Hedenbro, M. Ridderstråle, L. Johansson and L. Johansson for providing RNA from fat tissues, K.-F. Eriksson and T. Elgzyri for muscle biopsies and blood samples and C. Ling and M. Dekker Nitert for assisting with RNA preparation. C. Ladenvall is thanked for assisting with statistical analysis. M. Svårdh, A. Berglund and M. Svensson are acknowledged for skilled technical assistance.

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

References

1. Florez JC (2007) The new type 2 diabetes gene TCF7L2. *Curr Opin Clin Nutr Metab Care* 10:391–396
2. Welters HJ, Kulkarni RN (2008) Wnt signaling: relevance to beta-cell biology and diabetes. *Trends Endocrinol Metab* 19:349–355
3. Duval A, Rolland S, Tubacher E, Bui H, Thomas G, Hamelin R (2000) The human T cell transcription factor-4 gene: structure, extensive characterization of alternative splicings, and mutational analysis in colorectal cancer cell lines. *Cancer Res* 60:3872–3879
4. Shiina H, Igawa M, Breault J et al (2003) The human T cell factor-4 gene splicing isoforms, Wnt signal pathway, and apoptosis in renal cell carcinoma. *Clin Cancer Res* 9:2121–2132
5. Wang J, Kuusisto J, Vanttinen M et al (2007) 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. *Diabetologia* 50:1192–1200
6. Wegner L, Hussain MS, Pilgaard K et al (2008) Impact of TCF7L2 rs7903146 on insulin secretion and action in young and elderly Danish twins. *J Clin Endocrinol Metab* 93:4013–4019
7. Ahlzen M, Johansson LE, Cervin C, Tornqvist H, Groop L, Ridderstråle M (2008) Expression of the transcription factor 7-like 2 gene (TCF7L2) in human adipocytes is down regulated by insulin. *Biochem Biophys Res Commun* 370:49–52
8. Kovacs P, Berndt J, Ruschke K et al (2008) TCF7L2 gene expression in human visceral and subcutaneous adipose tissue is differentially regulated but not associated with type 2 diabetes mellitus. *Metabolism* 57:1227–1231
9. Elbein SC, Chu WS, Das SK et al (2007) Transcription factor 7-like 2 polymorphisms and type 2 diabetes, glucose homeostasis traits and gene expression in US participants of European and African descent. *Diabetologia* 50:1621–1630
10. Shu L, Sauter NS, Schulthess FT, Matveyenko AV, Oberholzer J, Maedler K (2008) Transcription factor 7-like 2 regulates beta-cell survival and function in human pancreatic islets. *Diabetes* 57:645–653
11. Liu Z, Habener JF (2008) Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta cell proliferation. *J Biol Chem* 283:8723–8735
12. Lyssenko V, Lupi R, Marchetti P et al (2007) Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest* 117:2155–2163
13. Bustin SA (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* 25:169–193
14. Jin T, Liu L (2008) The Wnt signaling pathway effector TCF7L2 and type 2 diabetes mellitus. *Mol Endocrinol* 22:2383–2392