Insulin treatment and high-fat diet feeding reduces the expression of three *Tcf* genes in rodent pancreas

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Abstract

Specific single-nucleotide polymorphisms in intronic regions of human *TCF7L2* are associated with an elevated risk of developing type 2 diabetes. Whether *Tcf7l2* is expressed in pancreatic islets of rodent species at a considerable level, however, remains controversial. We used RT-PCR and quantitative RT-PCR to examine *Tcf7l2* expression in rodent gut, pancreas, isolated pancreatic islets, and cultured cell lines. The expression level of *Tcf7l2* was relatively lower in the pancreas compared to the gut or the pancreatic β -cell line Ins-1. Immunostaining did not detect a *Tcf7l2* signal in mouse pancreatic islets. Endogenous canonical Wnt activity was not

appreciable in the pancreas of TOPGAL transgenic mice. Both *Tcf7* and *Tcf7l1*, but not *Lef1*, were expressed in the pancreas. The expression of the three *Tcf* genes (*Tcf7*, *Tcf7l1*, and *Tcf7l2*) in the pancreas was reduced by treatment with insulin or high-fat diet feeding, in contrast to the stimulation of *Tcf7l2* expression by insulin in the gut. We suggest that hyperinsulinemia represses *Tcf* gene expression in the pancreas. Whether and how this reduction alters the function of pancreatic β cells during hyperinsulinemia deserves further investigation.

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Introduction

The major effector of the canonical Wnt signaling pathway is the bipartite transcription factor cat/TCF, formed by free β -catenin (β -cat) and a member of the TCF protein family (TCF-1/TCF7, LEF1, TCF-3/TCF7L1, and TCF-4/TCF7L2; Jin 2008). This pathway exerts important physiological and pathological functions in different cell lineages and organs, including organogenesis, tumor development, and metabolic homeostasis (Kinzler & Vogelstein 1996, He *et al.* 1998, Jin *et al.* 2008).

Grant *et al.* (2006) identified an association between *TCF7L2* polymorphisms and the risk of type 2 diabetes mellitus (T2D) among European and American populations. Since then, extensive genome-wide association studies have further confirmed their discovery globally and among ethnically diverse populations (Florez *et al.* 2006, Munoz *et al.* 2006, Scott *et al.* 2006, Cauchi *et al.* 2007, Dahlgren *et al.* 2007, Elbein 2007, Sladek *et al.* 2007, Jin 2008,

Jin & Liu 2008). Although several investigations have indicated that individuals who carry the risk of *TCF7L2* polymorphisms exhibited impaired insulin secretion and enhanced rate of hepatic glucose production (Florez *et al.* 2006, Lyssenko *et al.* 2007, Schafer *et al.* 2007, Kirchhoff *et al.* 2008), in mouse models and in humans, whether *Tcf7l2* regulates the function of β cells remains controversial (Jin 2008, Liu & Habener 2008, Krutzfeldt & Stoffel 2010). *Tcf7l2*^{-/-} mice that were generated more than a decade ago exhibit defects in the development of the gut (Korinek *et al.* 1998) and abnormal growth of the pituitary (Brinkmeier *et al.* 2007). However, these investigations did not find expression or function of *Tcf7l2* in pancreatic islets (Korinek *et al.* 1998, Brinkmeier *et al.* 2007). Very recently, Krutzfeldt & Stoffel (2010) reported that the canonical Wnt signaling pathway is not active in pancreatic islets of adult mice, whereas in two different insulin resistance mouse models, Wnt4 is abundantly expressed, and Wnt4 expression inhibits canonical Wnt signaling in pancreatic islets.

We show in this study that the expression level of *Tcf7l2* is relatively lower in rodent pancreas, compared with the gut. Furthermore, we observed the expression of *Tcf7* and *Tcf7l1* in the pancreas of rodent species. In addition, we found that the expression of *Tcf7*, *Tcf7l1*, and *Tcf7l2* in the rodent pancreas can be significantly downregulated by insulin treatment or high-fat diet (HFD) feeding, in contrast to the stimulation of *Tcf7l2* expression by insulin in the adult and fetal gut, as well as in the intestinal endocrine L-cell line GLUTag (Sun *et al.* 2010). Finally, we have also found a lack of cat/TCF-mediated reporter gene activity in the pancreas of TOPGAL transgenic mice (Krutzfeldt & Stoffel 2010), even at e12.5 and neonatal stage. These observations collectively suggest that further extensive investigations are required to clarify the role of the *Tcf* genes and Wnt signaling in the function of pancreatic β cells.

Materials and Methods

DNA sequencing

PCR products were verified by agarose gel electrophoresis, purified, and inserted into TA cloning vectors (Invitrogen). DNA sequences of the inserts were obtained by automatic sequencing. DNA sequences were aligned with the mouse *Tcf7l2* sequence from GenBank (NM_001142924) and analyzed with ClustalW2 (EBI) software.

Mice, rat, islet isolation and fetal rat intestinal cell culture preparation

TOPGAL mice were purchased from Jackson Laboratory (strain name Tg-Fos-lacZ-34Efu/J, stock no. 004623; Bar Harbor, ME, USA). Pancreas, tail, heart, lungs, intestines, neonatal mice, as well as mice at e12.5 were taken for LacZ staining. Briefly, mice were killed, and tissue samples were dissected, washed in cold PBS, and placed in fixing solution (0.5% glutaraldehyde, 0.5% formaldehyde, 5 mM EGTA, and 2 mM MgCl₂ in PBS at pH 7.3) for 10–20 min. Tissue samples were then washed three times (15 min each) with wash buffer (2 mM MgCl₂, 0.01% sodium deoxycholate, and 0.02% NP-40 in PBS at pH 7.3) at room temperature, then placed in staining solution (1 mg/ml X-gal, 2.12 mg/ml potassiumferrocyanide, and 1.64 mg/ml potassium ferricyanide in wash buffer) for 4 h shaking gently at 37 °C. Nine-week-old ($n=3$, male) adult Wistar rats, weighing ~300 g, were purchased from Charles River (St Laurent, Québec, Canada). Islets isolation was conducted as described earlier (Wang *et al.* 2009). Briefly, collagenase solution was injected into the pancreas through the common bile duct of the anesthetized rats. The distended pancreas was removed and incubated for 20 min in a 37 °C shaking water bath. Islets were separated after four centrifugations and a sedimentation period. The method for the generation of fetal rat intestinal cell (FRIC) cultures has been described elsewhere

(Yi *et al.* 2008). Briefly, intestines of 3-week gestation fetal Wistar rats were pooled together and incubated with collagenase, hyaluronidase, and DNase I. The dispersed cells were washed and placed into monolayer cultures. Mouse pancreas and gut samples were obtained from 6-week-old male C57BL6 mice ($n=3$) after being anesthetized. In addition, C57BL6 mice were fed with either HFD or regular chow diet for 2 months ($n=3$ for each group), and their pancreata were isolated. The generation of hyperinsulinemia and insulin resistance by HFD feeding was monitored by serum insulin detection and intravenous glucose tolerance tests. All animal procedures have been approved by Animal Resource Centre, Toronto General Research Institute, University Health Network.

Cell culture and western blotting

The mouse large intestine endocrine L-cell line GLUTag (Drucker *et al.* 1994) and the human colon cancer cell line SW-480 were cultured in DMEM medium (Sigma–Aldrich) as described earlier (Drucker *et al.* 1994, Sun *et al.* 2009). Rat pancreatic Ins-1 cell line was grown in RPMI-1640 (Sigma–Aldrich), supplemented with 10% FBS, 1% P/S, 1 mM sodium pyruvate, 2 mM L-glutamine, 10 mM HEPES, 0.05 mM 2-mercaptoethanol, and 11 mM glucose. Western blotting was conducted as described earlier (Sirek *et al.* 2009). Antibodies against TCF7L2 (H-125) [sc-13027] for C-terminal part of the protein and actin (C4) [sc-47778] were the products of Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Approximately, 30 µg of total protein was loaded into each lane. A rabbit polyclonal TCF7L2 antibody (SC13027; 1:1000 dilution) was used in this assay.

Immunohistochemistry

Mouse pancreatic tissues were processed and paraffin embedded as described earlier (Wang & Brubaker 2002). Triple staining was sequentially performed for insulin, glucagon, and *Tcf7l2*. The primary antibodies used were guinea pig anti-insulin (Dako, Mississauga, ON, Canada; 1:500), rabbit anti-glucagon (Dako; 1:500), and an anti-TCF7L2 antibody (SC8631, Santa Cruz Biotechnology Inc.; 1:200). The staining was visualized by secondary antibodies: FITC-conjugated donkey anti-guinea pig antibodies (Jackson, West Grove, PA, USA; 1:400), Cy3-conjugated donkey anti-rabbit antibodies, and aminomethyl coumarin-conjugated donkey anti-mouse antibody (Jackson; 1:200) using a Nikon fluorescence microscope (Eclipse TE 200).

RNA extraction, RT-PCR, and real-time RT-PCR

Total RNA was extracted using the Trizol method (Invitrogen Life Technology) according to the manufacturer's protocol. The quality of RNA was verified by agarose gel electrophoresis as described earlier (Jin & Drucker 1996). Approximately, 2 µg of total RNA (OD 260/280 ratio > 1.8) was used for first-strand cDNA synthesis using random

hexamers and Superscript RT II (Invitrogen Life Technology). PCRs were conducted with a Mastercycler Gradient thermocycler (Eppendorf Scientific Inc., Westbury, NY, USA). Real-time RT-PCR was performed with Rotor-Gene 3000 (Corbett Research, Dorval, Quebec, Canada) using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) as described earlier (Sun *et al.* 2009). The relative expression levels were normalized to the expression of β -actin or glyceraldehyde-3-phosphate dehydrogenase, which were used as internal controls. The relative mRNA transcript levels were calculated according to the $2^{-\Delta\Delta C_T}$ method, in which ΔC_T represents the difference in threshold cycle values between the target mRNA and the internal control, and $\Delta\Delta C_T$ represents the difference in threshold cycle values between the experimental and the control mRNAs (Sun *et al.* 2010). All the primers were synthesized by ACGT Corporation (Toronto, Ontario, Canada) and are presented in Table 1. All primers for *Tcf712* and the three other *Tcf* family members were designed so that each pair could amplify the corresponding cDNAs from both mouse (NM_001142924) and rat (XM_001072517).

Statistical analysis

All experiments were conducted at least three times. Results are indicated as the mean $\pm$ S.D., and the statistical significance between two experimental conditions was analyzed by the Student's *t*-test. Differences between groups are considered significant if $P < 0.05$.

Results

Design of primers for *Tcf712* detection by RT-PCR in mouse pancreas

Tcf712 mRNA is abundantly expressed in the mouse gut (Korinek *et al.* 1998), and extensive alternative splicing of the *Tcf712* gene has been documented (Duval *et al.* 2000b, Shiina *et al.* 2003, Prokunina-Olsson *et al.* 2009a,b). Based on these previous reports regarding alternative splicing of mouse *Tcf712*, and cDNAs and ESTs in the NCBI database, we designed five pairs of primers to portions of the various alternative transcripts (Table 1). The positions of these primers on the exon regions of the gene are illustrated in Fig. 1A. We found that all five pairs of primers could amplify *Tcf712* cDNA from mouse gut, but failed to generate a visible band with cDNA from mouse pancreas (data not shown). We also used these primers with cDNA from the pancreatic β -cell line Ins-1. Although these primer pairs failed to detect *Tcf712* cDNA from the pancreas, all of them could amplify cDNA for *Tcf712* from the Ins-1 cell line (data not shown). These observations suggested that if rodent pancreas does express *Tcf712*, then the level of expression will be lower than that observed in the gut or in the immortalized β -cell line Ins-1 (Supplementary Figure 1, see section on supplementary data given at the end of this article).

Next, we conducted RT-PCR using the primer pair 1F and 3R, which amplifies sequences from exons 1 to 18, and the primer pair 1F and 5R, which amplifies sequences from

Table 1 Nucleotide sequences of primers used in this study

Name	DNA sequence	Anticipated PCR product size (bp)
Pair 1	1F: 5'-GCT AAC GAC GAG CTG ATC TCC TTC-3' 1R: 5'-GTC TAC GTC AGC TGG TAA GTG CGG-3'	650
Pair 2	2F: 5'-CTG CTT GAT GTC CAA GCA GGA AGC-3' 2R: 5'-GTA CAG CTG CAT GTG AAG CTG TCG-3'	700
Pair 3	3F: 5'-TGA AAG AGA TGA GAG CGA AGG TGG-3' 3R: 5'-CTA GTA AGC TTC CAT CTG AAG AGG-3'	350
Pair 4	4F: 5'-GCT AAC GAC GAG CTG ATC TCC TTC-3' 4R: 5'-CCT CAC AAG AAG TCA CAG CGA CTC-3'	850
Pair 5	5F: 5'-TGA AAG AGA TGA GAG CGA AGG TGG-3' 5R: 5'-ACA TAA GAG TGG TCC TCC AAC CTC-3'	250
TCF7	F: 5'-AGG TCA GAT GGG TTG GAC TG-3' R: 5'-AGG GTG CAC ACT GGG TTT AG-3'	450
TCF7L1	F: 5'-GAG TGC GAA ATC CCC AGT TA-3' R: 5'-ATG CAT GGC TTC TTG CTC TT-3'	440
LEF1	F: 5'-CTC ATC ACC TAC AGC GAC GA-3' R: 5'-TGA GGC TTC ACG TGC ATT AG-3'	350
Gcg	F: 5'-GCC CAG GAC ACA CTC AAA GT-3' R: 5'-TGA CGT TTG GCA ATG TTG TT-3'	357
c-Myc	F: 5'-CAA ATC CTG TAC CTC GTC CG-3' R: 5'-GTT GTG CTG GTG AGT GGA GA-3'	307
β -Actin	F: 5'-TCA TGA AGT GTG ACG TTG ACA-3' R: 5'-CCT AGA AGC ATT TGC GGT G-3'	300
GAPDH	F: 5'-TGA AGG TCG GTG TGA ACG GAT TTG GC-3' R: 5'-CAT GTA GGC CAT GAG GTC CAC CAC-3'	982

Figure 1 Exon organization of the mouse *Tcf7l2* gene and locations of the five pairs of primers. (A) Scheme showing the exon structure of the *Tcf7l2* gene. Arrows indicate the positions of the five pairs of primers (detailed in Table 1). Schematic representation of alternatively spliced forms of *Tcf7l2* detected in the (B) mouse gut and the (C) rat pancreatic β -cell line Ins-1. Isoforms A1–A3 were detected using the primers 1F and 3R, whereas isoforms B1–B4 were detected using the primers 1F and 5R.

exons 1 to 13 (Fig. 1A). After sequencing 19 clones, we were able to identify five different alternatively spliced forms of *Tcf7l2* expressed in the mouse gut (Fig. 1B). Isoforms A1 and A2 end in exon 18, whereas isoforms B1, B2, and B3 end in exon 13. The same approach was then applied to the immortalized pancreatic β -cell line Ins-1, which led to the identification of four isoforms (Fig. 1C) from 20 sequenced clones, including two novel isoforms: A3, which ends in exon 18 but contains exon 14, and B4, which ends in exon 13 but with the deletion of exon 3. *Tcf7l2* isoforms A2 and B2 were found in both the gut and Ins-1 cDNA.

Since all of the isoforms that we obtained from Ins-1 cells contain intact exons 8, 9, 10, and 11, we designed a new pair of primers, namely 6F and 6R (Fig. 2A), and used them on cDNA generated from mouse gut and pancreatic tissue and islets. This pair of primers generated a DNA fragment of the anticipated size from gut, pancreatic, and islet cDNA, and was confirmed to be *Tcf7l2* by DNA sequencing. Although this 320 bp fragment could be amplified from the

gut, the pancreas, and the pancreatic islets cDNA, the intensity of the RT-PCR products from pancreatic tissue and islets was much weaker than those from the gut (Fig. 2B).

Expression of Tcf7l2 in pancreas and islets is low

Real-time quantitative RT-PCR (qRT-PCR) was then performed with the new pair of primers with cDNA from mouse gut, pancreas, and islets. As shown in Fig. 2C, the *Tcf7l2* expression level in pancreas and islets is approximately five times lower than that of the gut. Western blotting was then conducted with C-terminal antibody for *TCF7L2* and detected a protein that is slightly above the molecular mass of 55 kDa, matching the predicted size range of *Tcf7l2*, in the GLUTag, Ins-1, and SW-480 cell lines, as well as in mouse gut (Fig. 2D). Only a very weak band at the corresponding position was detected from the mouse pancreas (Fig. 2D). Densitometric analysis indicates that *Tcf7l2* protein expression level in the pancreas was 7% of that detected in the gut. This antibody also detected another protein from the three cell lines and two other proteins from the intestinal tissue that migrated slower than the presumed *Tcf7l2* band (Fig. 2D). The estimated sizes of these three proteins do not match the predicted sizes of any of the *Tcf7l2* isoforms identified in Fig. 1. When a *Tcf7l2* antibody was used in immunostaining of mouse pancreatic paraffin sections, we could not detect *Tcf7l2* in pancreatic islets (Fig. 2E), which is consistent with our previous observation (Yi *et al.* 2005). These observations collectively suggest that *Tcf7l2* is not abundantly expressed in the mouse pancreas or in pancreatic islets.

Both Tcf7 and Tcf7l1 are also expressed in the pancreas

Considering the suggested role of the Wnt signaling pathway in mouse pancreatic islet development and the lack of abundant expression of *Tcf7l2*, we examined the expression of the other three members of the *Tcf* family in the rodent gut and pancreas. Primers used in RT-PCR for the detection of expression of the *Tcf7*, *Tcf7l1*, and *Lef1* genes are shown in Table 1. PCR products were purified, inserted into TA cloning vectors, and verified by DNA sequencing. As shown in Fig. 3, in addition to *Tcf7l2*, both *Tcf7* and *Tcf7l1*, but not *Lef1*, were found to be expressed in the mouse (Fig. 3A) and rat (Fig. 3B) pancreas and gut.

Expression of Tcf7l2 in pancreatic tissue and islets is downregulated by insulin treatment

A recent study has shown that *Tcf7l2* expression in pancreatic islets can be downregulated by hyperinsulinemia, associated with the reduced expression of the incretin hormone receptors (Shu *et al.* 2009). We, however, have observed that in human and mouse intestinal cell lines, insulin stimulated *Tcf7l2* mRNA and protein expression (Sun *et al.* 2010). To investigate whether insulin affects the expression of *Tcf7l2* and other *Tcf7* family members in rodent

Figure 2 *Tcf7l2* expression level is lower in the mouse pancreas. (A) The positions of the primers 6F and 6R. (B) Detection of *Tcf7l2* expression in the mouse gut, the pancreas, and the islets using primers 6F and 6R. Both β -actin and GAPDH were used as loading controls. A representative gel from three experiments is shown. (C) Quantitative RT-PCR assessment of *Tcf7l2* expression in the mouse gut, the pancreas, and the isolated mouse pancreatic islets, using primers 6F and 6R. GAPDH (left panel) and β -actin (right panel) were used as normalization controls. Results are from three independent experiments, with triplicate samples per tissue from one mouse per experiment. (D) Western blotting with C-terminal antibody shows the very low expression level of *Tcf7l2* in mouse pancreas. A representative blot was shown (left panel), and densitometric graph (right panel) was generated from three independent experiments. (E) Immunohistochemistry of pancreatic section from a 9-week-old male cluster of differentiation 1 mouse stained for insulin (green), glucagon (red), and *Tcf7l2* (blue, C-terminal antibody). * $P < 0.05$; ** $P < 0.01$. Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-10-0044>.

pancreatic islets, we conducted the following investigations. First, we treated both the large and small intestines of the mouse with insulin (100 nM) for 3 h and assessed the expression of the *Tcf* family members by both RT-PCR and qRT-PCR. Results obtained from both of these methods indicate that *Tcf7l2* mRNA expression in the large intestine was significantly increased after insulin treatment; however, the mRNA levels of both *Tcf7* and *Tcf7l1* were not affected significantly by insulin treatment in the large intestine (Fig. 4A and B). Similar observations were made with the small intestine (Fig. 4C and D). Although an approximately threefold increase in *Tcf7l2* mRNA expression was observed in the small intestine after insulin treatment, no significant

effect of insulin on *Tcf7* or *Tcf7l1* mRNA expression levels was observed. We then expanded our investigation into FRIC cultures. Freshly prepared FRIC cultures were treated with 100 nM insulin for 3 h, followed by cell harvesting, RNA extraction, RT-PCR, and qRT-PCR. Consistent with our previous report (Yi *et al.* 2008), insulin treatment led to a 2.5-fold increase of proglucagon (gcg) mRNA expression, associated with a threefold increase in expression of c-Myc, a known downstream target of the canonical Wnt signaling pathway, and a twofold increase in *Tcf7l2* expression (Fig. 5A and B). In contrast, in the pancreas, a 3 h insulin treatment led to 80, 60, and 80% reductions in *Tcf7*, *Tcf7l1*, and *Tcf7l2* mRNA abundance respectively (Fig. 5C and D).

Figure 3 *Tcf7* and *Tcf7l1* are expressed in mouse and rat pancreas. cDNAs from (A) 6-week-old mouse ($n=3$) or (B) 9-week-old rat ($n=3$) pancreatic and gut RNA were subjected to RT-PCR with the TCF/LEF and β -actin primers listed in Table 1. For *Tcf7l2*, the primers 6F and 6R were used. Representative gels from three experiments are shown.

Finally, we isolated rat pancreatic islets. Approximately 400 islets were obtained from each rat ($n=4$) and were evenly split into two parts and treated with or without insulin for 3 h. Cells were collected and RNA was extracted for qRT-PCR. As shown in Fig. 6A, insulin treatment led to a 95, 85, and 90% reduction in the level of expression of *Tcf7*, *Tcf7l1*, and *Tcf7l2* respectively. Figure 6B shows that insulin treatment led to a significantly reduced expression of *gcg*, consistent with observation made by J Philippe two decades ago (Philippe 1989, 1991). Figure 6C shows that in the pancreas of HFD fed mice, the expression level of *Tcf7*, *Tcf7l1*, and *Tcf7l2* was significantly lower than those from mice fed with chow diet.

Endogenous canonical Wnt signaling is not active in the pancreas

Considering the lack of abundant *Tcf7l2* in the pancreas, we wondered whether the canonical Wnt signaling pathway is active in the pancreas of rodents. We used a transgenic mouse model, TOPGAL, for this study. In this model, the expression of the LacZ reporter is under the control of a *Tcf*/*Lef*-responsive promoter element. The expression of LacZ reporter would indicate canonical Wnt activity. Consistent with a recent report (Krutzfeldt & Stoffel 2010), we cannot detect LacZ reporter activity in the pancreas of adult TOPGAL mice (data not shown). Figure 7A shows that in the neonatal TOPGAL mice, LacZ expression was not detectable in the pancreas (left panel), although it was detectable in the hair follicles of the mouse tail (right panel), liver, lung, and certain areas in the heart (data not shown). In the TOPGAL mice at an age of e12.5, we still could not see appreciable LacZ expression in the area of pancreata (Fig. 7B).

Discussion

After the demonstration of a very strong association between the risk of T2D and specific polymorphisms in the *TCF7L2* gene (Grant *et al.* 2006), this effector of Wnt signaling has drawn global attention (Florez *et al.* 2006, Munoz *et al.* 2006,

Scott *et al.* 2006, Cauchi *et al.* 2007, Dahlgren *et al.* 2007, Elbein 2007, Sladek *et al.* 2007, Jin 2008, Jin & Liu 2008). How these single-nucleotide polymorphisms (SNPs) within intronic regions of the human *TCF7L2* gene led to an increase in the risk of the development of T2D is unknown because it is difficult to assess whether these SNPs actually affect *TCF7L2* expression levels. If these polymorphisms do affect *TCF7L2* expression, then how mechanistically is this regulation achieved? Nevertheless, Prokunina-Olsson *et al.* (2009b) have reported very recently that the expression levels of two specific alternatively spliced forms of *TCF7L2* mRNA in human pancreatic islets are expressed at relatively lower levels in individuals that carry the T2D-associated SNPs rs7903146 and rs12255372. Florez *et al.* (2006) found that these two *TCF7L2* SNPs are associated with an increased risk of diabetes among subjects with impaired glucose tolerance. Lyssenko *et al.* (2007) reported that the T risk allele of SNP rs7903146 associates with impaired insulin secretion, incretin effects, and an increased rate of hepatic glucose production. They reported that *TCF7L2* expression in human islets was increased fivefold in T2D patients, particularly in carriers of the TT genotype of the SNP rs7903146 (Lyssenko *et al.* 2007). Furthermore, overexpression of *TCF7L2* in human islets reduced glucose-stimulated insulin secretion (Lyssenko *et al.* 2007). These observations, however, were contradicted by a later report by Shu *et al.* (2008) who claimed a beneficial effect of *TCF7L2* on β -cell function from experiments with siRNA-mediated depletion, which suggested a role of *TCF7L2* in the stimulation of β -cell proliferation and a

Figure 4 Insulin increases the expression of *Tcf7l2* but not the other two *Tcf* genes in the large and small intestines of the mouse. cDNAs from large (A and B) and small intestines (C and D) of 6-week-old mouse ($n=3$) treated with 100 nM insulin (3 h) were subjected to (A and C) RT-PCR or (B and D) real-time qRT-PCR for assessing the expression of *Tcf7*, *Tcf7l1*, and *Tcf7l2*. For RT-PCR and qRT-PCR, β -actin was used as the internal control. RT-PCR gels are representatives of three independent experiments. qRT-PCR results are from three independent experiments, with triplicate samples per tissue from one mouse per experiment. * $P<0.05$; ** $P<0.01$.

Figure 5 Insulin stimulates the expression of *Tcf7l2* in FRIC cultures but represses the expression of *Tcf* genes in pancreas. (A and B) cDNAs from 100 nM insulin-treated FRIC cultures (3 h) were subjected to (A) RT-PCR or (B) real-time RT-PCR for the detection of expression of *gcg*, *c-Myc*, and *Tcf7l2*. β -Actin was used as the internal control. Results are from three independent experiments. (C and D) cDNAs from mouse pancreas ($n=3$) treated with 100 nM insulin (3 h) were subjected to (C) RT-PCR or (D) real-time RT-PCR for the detection of the expression of *Tcf7*, *Tcf7l1*, and *Tcf7l2*. Representative gels are from three independent experiments. qRT-PCR results are from three independent experiments, with triplicate samples per tissue. β -Actin was used as the normalizing control. * $P < 0.05$; ** $P < 0.01$.

reduction in β -cell apoptosis. Shu *et al.* (2009) also reported that in a T2D mouse model, *Tcf7l2* level was reduced instead of increasing, and this reduction was strongly associated with the reduced expression of the incretion hormone receptors, i.e. GLP1 receptor and GIP receptor. None of the above studies, however, have directly addressed the question of how mechanistically the *TCF7L2* risk SNPs rendered individuals more susceptible to T2D. These studies, however, have re-directed our attention to the expression and function of *Tcf7l2* and Wnt signaling in pancreatic islets, especially in the pancreatic β cells.

Tcf7l2 is abundantly expressed in the mouse intestinal epithelia and adipocytes (Barker *et al.* 1999, Ross *et al.* 2000), and in the central nervous system (Lee *et al.* 2009). Inconsistent findings about *Tcf7l2* expression in the mouse pancreas, however, can be found in the literature. Yi *et al.* (2005) reported the failure to detect *Tcf7l2* by an immunohistochemistry method in mouse pancreatic islets, which was consistent with previous reports by Korinek *et al.* (1998), Barker *et al.* (1999), and Duval *et al.* (2000a). *Tcf7l2* knockout mice or *Tcf7l2* and *Tcf7* double knockout mice showed a defect in gut development, but no abnormality was reported in the development of the pancreas (Korinek *et al.* 1998, Gregorieff *et al.* 2004). In this study, we show again the failure to detect *Tcf7l2* expression by immunohistochemistry,

although a low level of expression was detected by RT-PCR. It should be pointed out that in the pancreatic β -cell line Ins-1, *Tcf7l2* expression level was comparable to that observed in the mouse gut and two intestinal cell lines. This observation is consistent with a recent report by Liu & Habener (2008). We suggest that the abundant expression of *Tcf7l2* in this cell line cannot be taken as evidence that this gene is abundantly expressed in primary pancreatic islets. Through our study of the alternatively spliced forms of *Tcf7l2* in this cell line, we were able to design a pair of primers for

Figure 6 *Tcf* gene expression in rat pancreatic islets is downregulated by insulin treatment or HFD feeding. Freshly isolated rat islet cells were treated with insulin (100 nM) for 3 h, followed by real-time RT-PCR for the detection of (A) *Tcf7*, *Tcf7l1*, and *Tcf7l2* expressions or (B) *gcg* expression. β -Actin was used as the normalizing control. (C) HFD feeding leads to reduced *Tcf* gene expression. C57BL6 mice were fed with either HFD or chow diet for 2 months. The generation of hyperinsulinemia and insulin resistance by HFD feeding was monitored by serum insulin level and intravenous glucose tolerance test (data not shown). Pancreata were collected, and the expression of *Tcf7*, *Tcf7l1*, and *Tcf7l2* was assessed by qRT-PCR. GAPDH was used as the normalizing control for qRT-PCR. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 7 Cat/TCF-mediated LacZ expression is absent in the pancreas of TOPGAL mice. (A) No LacZ expression in the neonatal TOPGAL mouse pancreas, while the tail hair follicles show positive in LacZ staining. (B) No appreciable LacZ expression in the pancreata of TOPGAL mice at e12.5.

semi-quantitatively assessing *Tcf7l2* expression level in the mouse pancreas. We show in this study that the *Tcf7l2* expression level in rodent pancreas is relatively low. This was further supported by observations in both western blotting and immunostaining.

The role of nuclear β -cat and the canonical Wnt signaling pathway in the development of pancreatic islets and the function of pancreatic β cells have been documented recently in a number of transgenic mouse studies (Rulifson *et al.* 2007, Jin 2008, Jin & Liu 2008), suggesting that pancreatic islet cells may express certain *Tcf* family members. We show in this study that in rodent pancreas and rat pancreatic islets, in addition to a low level of expression of *Tcf7l2*, mRNAs for two other *Tcf* family members, *Tcf7* and *Tcf7l1*, were detectable. How these two *Tcf* proteins work with β -cat or other partners in mediating the effects of Wnt or other cell signaling pathways in pancreatic development and β -cell function deserves further investigation. In mice, it is possible that these *Tcf* members exert redundant functions since *Tcf7l2* knockout mice and *Tcf7* and *Tcf7l2* double knockout mice show no detectable defect in pancreatic development (Korinek *et al.* 1998, Brinkmeier *et al.* 2007).

An important finding of the current study is that though in the gut and intestinal endocrine L-cells, *Tcf7l2* expression can be substantially stimulated by insulin, the expression of all three *Tcf* genes that are expressed in pancreatic islets

was significantly repressed by insulin. The opposite effects of insulin on up- and downregulations of *gcg* expression in intestinal endocrine L-cells and pancreatic α cells respectively have been observed previously as well (Philippe 1991, Yi *et al.* 2005). The cell-type-specific response to insulin treatment is physiologically important, as the final active hormonal products of the pro-hormone, pro-glucagon, in pancreatic α cells and intestinal endocrine L-cells, exert opposite effects on blood glucose homeostasis. Interestingly, both insulin and lithium do not affect nuclear β -cat levels in a pancreatic α -cell line (Yi *et al.* 2005). These observations, along with the lack of appreciable canonical Wnt activation in the TOPGAL mice (Krutzfeldt & Stoffel 2010), made us wonder whether *Tcf7l2* or other TCF proteins can function as positive regulators of gene expression, independent of the participation of β -cat or of the formation of the bipartite transcription factor cat/TCE. If this is true, then our current view of the mechanistic function of Wnt signaling may have to change. At this stage, it is generally accepted that in the absence of β -cat, a TCF protein that binds to a downstream target gene promoter represses transcription through the recruitment of repressive partners (Jin & Liu 2008).

TCF7L2 is also expressed in adipocytes. A previous study showed that insulin reduced the *TCF7L2* expression level in cultured human adipocytes, which could be attenuated by the

incubation of these cells with the free fatty acids palmitate or oleate (Ahlzen *et al.* 2008). An *in vivo* study by the same group showed a significantly higher level of *TCF7L2* mRNA expression in subcutaneous adipose tissue in subjects with severe insulin resistance, although a correlation between *TCF7L2* mRNA expression and extent of obesity could not be established (Ahlzen *et al.* 2008). Furthermore, adipocyte-derived Wnt signaling is able to stimulate insulin secretion and β -cell proliferation (Schinner *et al.* 2008). How the downregulation of TCF expression by insulin in both pancreatic islets and adipose tissue collectively contributes to the development of T2D deserves intensive investigation.

In conclusion, we demonstrate in this study that in rodent pancreas, *Tcf7l2* expression level is relatively low, whereas the expression of the other two *Tcf* genes, *Tcf7* and *Tcf7l1*, is detectable. The expression of these three genes can be downregulated by insulin or HFD feeding. The contribution of *Tcf7* and *Tcf7l1* to pancreatic development and function of β cells is unknown and deserves further investigation. Since cat/TCF-mediated reporter gene activity is absent in adult (Krutzfeldt & Stoffel 2010) and e12.5 pancreas, Tcf proteins may exert their biological functions by a mechanism that falls outside the scope of the narrowly defined canonical Wnt signaling pathway that uses TCF/LEF DNA-binding *cis*-elements.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1677/JOE-10-0044>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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