**TCF7L2** is a master regulator of insulin production and processing

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Received April 29, 2014; Revised June 30, 2014; Accepted July 7, 2014

Genome-wide association studies have revealed >60 loci associated with type 2 diabetes (T2D), but the underlying causal variants and functional mechanisms remain largely elusive. Although variants in **TCF7L2** confer the strongest risk of T2D among common variants by presumed effects on islet function, the molecular mechanisms are not yet well understood. Using RNA-sequencing, we have identified a **TCF7L2**-regulated transcriptional network responsible for its effect on insulin secretion in rodent and human pancreatic islets. **ISL1** is a primary target of **TCF7L2** and regulates proinsulin production and processing via **MAFA**, **PDX1**, **NKX6.1**, **PCSK1**, **PCSK2** and **SLC30A8**, thereby providing evidence for a coordinated regulation of insulin production and processing. The risk T-allele of rs7903146 was associated with increased **TCF7L2** expression, and decreased insulin content and secretion. Using gene expression profiles of 66 human pancreatic islets donors¹, we also show that the identified **TCF7L2-ISL1** transcriptional network is regulated in a genotype-dependent manner. Taken together, these results demonstrate that not only synthesis of proinsulin is regulated by **TCF7L2** but also processing and possibly clearance of proinsulin and insulin. These multiple targets in key pathways may explain why **TCF7L2** has emerged as the gene showing one of the strongest associations with T2D.

**INTRODUCTION**

**TCF7L2** harbors common genetic variants with the strongest effect on type 2 diabetes (T2D) risk yet described, i.e. the single-nucleotide polymorphism (SNP rs7903146) (¹–³). **TCF7L2** is a transcription factor (TF) in the Wnt-signaling pathway and expressed in many tissues including fat, liver and pancreatic islets of Langerhans (⁴). A majority of risk variants associated with T2D by GWAS seem to affect islet function (⁵,⁶). In concordance, the risk T-allele of rs7903146 is associated with impaired glucose-stimulated insulin secretion (GSIS) or other secretagogues-like GLP-1 (²⁻¹²). Risk T-allele carriers are further characterized by an elevated plasma proinsulin level and an increased proinsulin-to-insulin ratio suggestive of perturbed proinsulin processing (¹³–²¹). In support of a primary effect in pancreatic islets, the risk T-allele carriers show higher degree of open chromatin in pancreatic islets, but not in other tissues (²²). Several mouse models of **Tcf7l2** have been investigated (²³–²⁸), but the diabetic phenotype has not been replicated in all studies. Experiments in rodent islets where **TCF7L2** activity has been disrupted have usually demonstrated impaired GSIS (¹¹,²⁹,³⁰).

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Paradoxically, carriers of the risk T-allele show increased expression of TCF7L2 in human pancreatic islets (7,31). Plasmid construct carrying the risk T-allele, when compared with those with the C-allele, displayed a much stronger transcriptional activity, implying a higher enhancer activity of the T-allele (22,32). TCF7L2 may act both as a stimulator and a repressor of gene expression due to both differential splicing and interaction with different co-regulators in target gene recognition (33). However, the target genes of TCF7L2 in pancreatic islets or B-cells have not been described in detail. Our aim was to identify TCF7L2-target genes in pancreatic islets and to describe the regulatory network downstream of TCF7L2 responsible for its influence on insulin secretion.

RESULTS

**Tcf7l2 influences insulin synthesis and secretion**

Impaired *in vivo* (7) and *in vitro* (9,31) insulin secretion has been shown in risk T-allele carriers of rs7903146. We have replicated and extended these findings in pancreatic islets from human non-diabetic cadaver donors (n = 75, Fig. 1A; Supplementary Material, Table S3). We also showed reduced insulin content (27%) in risk T-allele carriers (n = 81, Fig. 1B), and that TCF7L2 mRNA expression is 16% higher than C-allele carriers (n = 66; RNA-seq, Fig. 1C). Further, TCF7L2 expression is negatively associated with proinsulin expression in human islets in C-allele carriers (n = 36), but not in T-allele carriers (n = 30, Fig. 1D; Supplementary Material, Table S3).

Silencing Tcf7l2 in a rat insulinoma cell line (INS1 832/13) using siRNA targeting the invariable exon 1, resulted in 84% knockdown of Tcf7l2 mRNA, 90% reduction of TCF7L2 activity and a reduced expression of the well-known TCF7L2-target genes Axin2 and Ccnd1 (Supplementary Material, Fig. S1A and B) (30). The decrease in Tcf7l2 resulted in a marked reduction of GSIS, i.e. 1.76-fold (Tcf7l2 silencing, P > 0.05) versus 6.97-fold (controls, P < 0.05, Fig. 1E) and a perturbed glucose-stimulated proinsulin secretion (GPS) (P < 0.05, Fig. 1F). Proinsulin-to-insulin ratio was higher under glucose stimulation compared with basal condition in Tcf7l2 silenced cells (P < 0.05, Fig. 1G) suggesting perturbed proinsulin processing. Immunohistochemistry (IHC) analysis of Tcf7l2 silenced INS1 cells showed reduced staining intensity for insulin (P < 0.001) and VAMP2 (P < 0.001), a marker of insulin-containing granules, in Tcf7l2 silenced cells (Fig. 1H). Furthermore, there was a reduction in insulin-containing granules close to the plasma membrane as assessed by total internal reflection fluorescence (TIRF) microscopy (P < 0.05, Fig. 1I). Together, these data suggest that TCF7L2 mediates key aspects of insulin production, processing and intra-cellular trafficking.

**Identification of TCF7L2-target genes influencing insulin synthesis and secretion**

The molecular mechanisms underlying these findings are not clear, but an altered expression of TCF7L2-target genes is likely part of the explanation. To identify genes regulated by TCF7L2 in B-cells, we screened for global differential expression using RNA-seq in Tcf7l2 silenced INS1 cells versus scramble-treated control. Overall, 10779 genes were actively expressed (30 aligned paired-end reads per gene, corresponding to >10 FPKM); 1680 genes were upregulated and 1885 genes were downregulated in Tcf7l2 silenced cells (5% false discovery rate, FDR, n = 4). Among the most differentially expressed genes were Il6r, Aldh1a1 and Syp14, but none of these has a known function in insulin secretion. Genetic variation in the IL6R-locus has been weakly associated with T2D (34), but this was not replicated (35) or supported by recent GWAS findings (21,36–42); therefore, Il6r was not studied further. Instead we categorized genes known to influence GSIS into three groups: (i) glucose sensing, (ii) proinsulin expression and maturation and (iii) exocytotic machinery (genes differentially regulated in the RNAseq screen are reported in Table 1). In addition, genes located proximal to SNPs with replicated associations to T2D and/or plasma proinsulin were also selected for further analysis (Supplementary Material, Table S2). Real-time quantitative PCR (QPCR) confirmed the RNA-seq results for many of the tested genes (Table 1), i.e. 18 out of 29 analyzed genes were replicated in independent samples at the mRNA level. To confirm these findings at the protein level, a selected set of 15 genes (with mRNA change >30%) was analyzed using western blot, with 11 genes having significant changes (Supplementary Material, Table S3, the complete list of 3565 potential target gene can be found in the Supplementary Material, Table S6).

**Tcf7l2 is a key regulator of proinsulin production and processing**

Twelve analyzed genes involved in proinsulin synthesis were downregulated in Tcf7l2 silenced cells, including proinsulin genes Ins1 and Ins2 (Table 1 and Fig. 2A). The reduction of all but one (Cpe) was confirmed using QPCR. The protein encoded by the Ins1 and Ins2 genes was decreased in Tcf7l2 silenced cells (Fig. 2A). We next replicated this finding in primary cells by measuring proinsulin protein expression in a Tcf7l2 homozygous null mouse model (Tcf7l2−/−) (26). Notably, proinsulin expression was reduced by 85% (n = 6) in pancreas from Tcf7l2−/− mice (postnatal day 0; P0) compared with wild-type mice (Fig. 2B). Given differences in the insulin gene between rodents and humans (43), we also replicated the findings in human pancreatic islets showing 49% reduced proinsulin mRNA expression after Tcf7l2 silencing (n = 5, Fig. 2C).

In the processing of proinsulin to insulin, the endoprotease prohormone convertase 1/3 (PCSK1) and prohormone convertase 2 (PCSK2) cleave the proinsulin molecule to yield mature insulin and C-peptide. We found reduced mRNA expression levels of both Pcsk1 and Pcsk2 in the RNA-seq screen of Tcf7l2 silenced INS1 cells (Table 1). This result was confirmed using QPCR and at the protein level in INS1 cells (Fig. 2A). No significant change in PCSK1 and PCSK2 protein expression was observed in the Tcf7l2−/− mouse pancreas (Fig. 2B). In Tcf7l2 silenced human pancreatic islets, PCSK1 and PCSK2 mRNA expression was reduced by 36 and 49%, respectively (Fig. 2C).

**Tcf7l2 is not a major regulator of genes involved in the exocytotic machinery or glucose sensing**

Of the 11 genes categorized as involved in the exocytotic machinery, mRNA expression of Cacna1a, Cacna1c, Cacna1d and Cacnb3 was reduced in Tcf7l2 silenced cells (Table 1;
Figure 1. The type 2 diabetes-associated SNP rs7903146 and TCF7L2 expression influences insulin synthesis and secretion. (A) GSIS in human islets (CC: \( n = 44 \), CT/TT: \( n = 31 \)). (B) Insulin content in human islets (CC: \( n = 45 \), CT/TT: \( n = 36 \)). (C) TCF7L2 mRNA expression measured by RNA-seq in human islets (CC: \( n = 36 \), CT/TT: \( n = 30 \)). (D) Expression (mRNA) of INS and TCF7L2 in human islets measured by RNA-seq stratified for rs7903146 genotype (CC: \( n = 36 \), CT/TT: \( n = 30 \)). (E) GSIS in INS1 cells after Tcf7l2 silencing. (F) GSPS in INS1 cells after Tcf7l2 silencing. (G) Proinsulin-to-insulin ratio in INS1 cells after Tcf7l2 silencing. (H) IHC staining of insulin and VAMP2 in INS1 cells after Tcf7l2 silencing. (I) Amount of insulin-containing granules measured by TIRF microscopy in INS1 cells after Tcf7l2 silencing. Data are represented as mean ± SEM. In (A)–(D), data were analyzed by linear regression with correction for age, gender and BMI. In (E)–(I), 2.8 versus 16.7 mM glucose were analyzed by paired Student’s \( t \)-tests, Tcf7l2 silencing versus controls by unpaired Student’s \( t \)-tests with \( n = 3 \). * \( P < 0.05 \), ** \( P < 0.01 \) and *** \( P < 0.001 \) (Supplementary Material, Tables S1 and S4).
Table 1. mRNA expression of selected genes influencing GSIS measured using RNA sequencing (RNAseq) in Tcf7l2 depleted INS1 832/13 cells versus scrambled control, with replication in independent experiments using real-time QPCR

<table>
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<th>Gene symbol</th>
<th>Gene ID</th>
<th>RNAseq LogFC</th>
<th>P-value corrected</th>
<th>QPCR LogFC</th>
<th>P-value</th>
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<td>Glud1</td>
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<td>G6pc</td>
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<td>Cacna1a</td>
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<td>Cacna1c</td>
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<td>Cacnb3</td>
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N/A, not analyzed using QPCR; LogFC, the logarithm of fold change, i.e. Tcf7l2 depleted/scrambled control; the P-value for RNAseq was corrected by FDR 5%. P-value for QPCR was calculated using Student’s t-tests; N = 4 (RNAseq), N = 3 (QPCR).

aVery low expression.

bPdx1 was not aligned in the RNA-seq analysis.

Supplementary Material, Fig. S1C). However, this was not confirmed at the protein level for Cav2.1, Cav1.2 or Cav1.3, either 72 h or 7 days after Tcf7l2 silencing (Supplementary Material, Fig. S1D and E). RNA expression of two out of the five genes involved in glucose sensing (Glud1 and Scl30a8/Slc2a2/Glut2) was reduced at the mRNA level in Tcf7l2 silenced cells (Table 1), and GLUD1 protein expression was measured but no change was observed (Supplementary Material, Fig. S1F).

Tcf7l2 is regulating proinsulin expression directly via Isl1, Ins1 and indirectly via Mafa, NeuroD1 and Pdx1

After establishing that TCF7L2 is a regulator of proinsulin expression and maturation, we investigated by which molecular mechanism this may occur. For this purpose, we interrogated the RNA-seq results for genes differentially expressed between Tcf7l2 silenced INS1 and control cells. Intriguingly, ISL1 LIM homeobox 1 (Isl1), v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog A (Mafa), pancreatic and duodenal homeobox 1 (Pdx1), neuronal differentiation 1 (NeuroD1), NK6 homeobox 1 (Nkx6.1) and NK2 homeobox 2 (Nkx2.2) were all expressed at lower levels in Tcf7l2 silenced cells (Table 1). All observations, except the Nkx2.2 protein, were confirmed using QPCR and western blot (Fig. 3A). The reduction of ISL1 and NEUROD1 was further confirmed using IHC (Supplementary Material, Fig. S1G and H). These results were confirmed in Tcf7l2−/− mice, with protein levels reduced relative to wild-type mice for ISL1, MAFA and NEUROD1 (Fig. 3B). Likewise, after TCF7L2 silencing in human islets, expression of most transcripts was downregulated, including ISL1, MAFA, PDX1, NEUROD1 and Nkx6.1 (Fig. 3C). In addition, in islets from CC-genotype carriers of rs7903146, TCF7L2 mRNA expression was negatively associated with that of ISL1, MAFA and Nkx6.1 but not for MAFA and Nkx6.1 in CT/TT-risk-genotype carriers (Supplementary Material, Table S4). The disruption of Tcf7l2 resulted in a marked reduction in GSIS compared with controls 1.93- (P > 0.05) versus 4.10-fold (P < 0.05) increases (Fig. 3D). The same was observed after disruption of Isl1, Mafa and NeuroD1 (Fig. 3D).

To delineate the proinsulin regulatory cascade among the identified TCF7L2-target genes, Isl1, Mafa, Pdx1, NeuroD1 and Nkx6.1 were sequentially silenced in INS1 cells using siRNA. First, knocking down Isl1 reduced both the mRNA and protein expression of Mafa, Pdx1, Nkx6.1, Pcsk1, Pcsk2, Ins1 and Ins2 (Fig. 4A). No effect of Isl1 knockdown was observed.
on Tcf7l2 or NeuroD1 mRNA expression (Fig. 4A). Previously, we have analyzed chromatin immunoprecipitation on tiling array (ChIP-on-Chip) using a TCF7L2 antibody identifying both the Isl1 and Ins1 promoters as directly bound by TCF7L2 (30). Together, these data indicate that Isl1 is a direct primary target gene for TCF7L2 and regulates proinsulin expression and processing via Isl1-dependent regulation of MafA, Pdx1, Nkx6.1, Pcsk1 and Pcsk2. In human islets, Isl1 expression is positively associated in C-allele carriers with that of MAFA, PDX1, NKK6.1, INS and SLC30A8 but in T-allele carriers only with PDX1 and NKK6.1 (Supplementary Material, Table S4).

Knocking down MafA reduced mRNA expression of Nkx6.1, Nkx2.2, Pcsk2, Ins1 and Ins2. These changes were confirmed at the protein level for all but NKKX6.1 and NKKX2.2 (Fig. 4B). In human islets, MAFA expression was positively associated with proinsulin gene expression both in C- and T-allele carriers (Supplementary Material, Table S4). Knocking down Pdx1 reduced the mRNA expression of MafA, Nkx6.1, Nkx2.2, Pcsk2, Ins1 and Ins2. These changes were confirmed at the protein level for all but PCSK2 (Fig. 4C). Knocking down NeuroD1 reduced the mRNA expression of Tcf7l2, Isl1, MafA, Pdx1, Nkx6.1, Nkx2.2, Pcsk1, Pcsk2, Ins1 and Ins2. This was,
Figure 3. TCF7L2 is regulating proinsulin expression via direct regulation of ISL1 and indirectly via MAFA, NEUROD1 and PDX1. (A) mRNA expression measured by QPCR in INS1 cells in Tcf7l2 silenced versus control cells, \( n = 3 \). Protein expression measured by western blot in INS1 cells in Tcf7l2 silenced versus control cells, \( n = 3 \). (B) Protein expression measured by western blot in Tcf7l2 allele-null versus wild-type mice \( n = 6 \). (C) mRNA expression measured by QPCR in human pancreatic islets in Tcf7l2 silenced versus control cells, \( n = 3 \). (D) GSIS in INS1 cells after Tcf7l2, Isl1, MafA and NeuroD1 silencing \( n = 3 \). Data are represented as mean ± SEM. \( * P < 0.05 \), \( ** P < 0.01 \) and \( *** P < 0.001 \) analyzed in (A)–(C) by unpaired Student’s \( t \)-tests. In (D), 2.8 versus 16.7 mM glucose were analyzed by paired Student’s \( t \)-tests (Supplementary Material, Table S1).
however, not reflected by changes in the ISL1, MAFA and PDX1 protein levels (Fig. 4D). Knocking down Nkx6.1 reduced the mRNA expression of Tcf7l2, Isl1, MafA, Pdx1, Nkx6.1, Nkx2.2, Pcsk1, Pcsk2, Slc30a8, Ins1 and Ins2. At the protein level, we could only confirm the reduction of MAFA and proinsulin (Fig. 4E).

In summary, these data together with previous ChIP-on-Chip results (30) indicate that ISL1 is a direct target of TCF7L2 and that ISL1, in turn regulates proinsulin production and processing via regulation of MAFA, PDX1, Nkx6.1, PCSK1 and PCSK2 expression.
TCF7L2 is a transcriptional regulator of key genes located in T2D-associated loci

Next, we examined whether silencing of Tcf7l2 was associated with changes in expression of rat orthologs of putative genes in T2D-associated loci (36,37,41,44–46) (Supplementary Material, Table S2). We observed differential mRNA expression for 23 genes, 17 out of these were replicated using QPCR and 3 at the protein level (i.e. Slc30a8, Ide and Bcl11a) (Figs 2 and 5). Of note, Tcf7l2 silencing in INS1 cells led to a decreased mRNA expression of Slc30a8 (Fig. 2A). In pancreata from Tcf7l2−/− mice, ZnT8 (Slc30a8) expression was reduced by 44% compared with wild-type control mice (n = 6, Fig. 2B). This was also replicated in human islets showing a 46% reduction of SLC30A8 mRNA expression after TCF7L2 silencing (Fig. 2C). ZnT8 protein expression was also reduced in INS1 cells after Isl1 and NeuroD1 silencing (Fig. 4A and D), but not after Pdx1, MafA and Nkx6.1 silencing (Fig. 4B, C and E).

Finally, expression (mRNA) of Grk5, Ide, Proxl, Kcnk16, Gipr, Adams19, Srebf1, Ankrd55, Jazf1, Cend2 and Tle1 was downregulated, whereas expression of Vps26a, Thada, Bcl11a and Bcl2 was upregulated after Tcf7l2 knockdown (Fig. 5; Supplementary Material, Tables S1 and S2).

DISCUSSION

The current report presents a genetic network controlled by TCF7L2 in pancreatic islets, providing a comprehensive view of the molecular mechanisms by which TCF7L2 regulates glucose metabolism. We demonstrate that TCF7L2 plays a central role in coordinating the expression of proinsulin and its subsequent processing to form mature insulin. We also show that TCF7L2 and Isl1 are key regulators of this transcriptional network and provide evidence that they regulate MAFA, PDX1, NEUROD1 and NKX6.1 (Fig. 6). The two prohormone convertases responsible for the processing of proinsulin to mature insulin were also targets of TCF7L2/ISL1 regulation. These functions seem to have been highly conserved during evolution as similar results were obtained in a rat cell line, primary mouse and human islets. In line with a previous report of an overrepresentation of TCF7L2 binding to genes in T2D-associated loci in HCT116 cells (47), we find that TCF7L2 regulates several of these genes also in pancreatic islets. Of note, SLC30A8 (encoding the zinc transporter ZnT8) is a downstream target of TCF7L2/ISL1 regulation. Zinc is important for the formation of insulin crystals (48), and a common non-synonymous SNP in the zinc transporter SLC30A8 has been associated with both T2D (40) and reduced proinsulin-to-insulin conversion.
SLC30A8 has previously been identified as a TCF7L2-target gene in mice (29), a finding now replicated in humans. Although the mechanisms by which the variant in SLC30A8 increases risk of T2D are not clear, recent data suggest that reduction in zinc concentrations in the portal vein after β-cell-specific disruption of Slc30a8 might influence hepatic insulin clearance (49). If confirmed, this would suggest that TCF7L2 might influence all key steps in (pro)insulin synthesis, processing and clearance.

Transcription of proinsulin in humans is under the control of many glucose responsive TFs that mainly bind a 400 bp conserved region (50,51). Among the most studied TFs are MAFA, PDX1 and NEUROD1, all shown here to be transcriptional targets of TCF7L2/ISL1 regulation. In support of this, ISL1 regulates MAFA expression by directly binding to the MAFA promoter (52), but its influence on Pdx1, Nkx6.1, Pcsk1 and Pcsk2 expression has not been described to our knowledge. Together with our recent demonstration of direct binding of TCF7L2 to the Isl1 promoter (30), these data indicate that TCF7L2 together with ISL1 forms a master regulatory circuit influencing both proinsulin expression and processing via regulation of MafA, Pdx1, Nkx6.1, Pcsk1 and Pcsk2. As ISL1 also can bind and influence the expression of other pancreatic hormones like glucagon (53) and somatostatin (54), this indicates that TCF7L2 may also have important regulatory functions in other islet cell types besides the B-cell. Relatively high expression of TCF7L2 in α-cells has also been reported in sorted human pancreatic islet cells (55). A recent study indicated that NEUROD1 regulates the expression of Ins1 but not Ins2 in
primary mouse β cells (56), whereas we observed altered expression of both proinsulin genes and Nkx6.1 in rat cell line. TCF7L2 may also regulate other β-cell functions essential for maintaining GSIS. A perturbation of intracellular [Ca^{2+}] was observed in one Tcf7l2^{-/-} mouse model, but no major effect on the expression of Ca^{2+} channels (29). Here, we find that although the mRNA levels of several Ca^{2+} channels important for GSIS were reduced in Tcf7l2 silenced cells, no changes of the corresponding proteins were observed either 72 h or 7 days after Tcf7l2 knockdown. An effect of TCF7L2 on Ca^{2+} handling in the B-cell should however not be excluded based upon these data as these proteins may have a very long half-life in the cell. An influence on cell viability and regulation of apoptosis are other well-documented effects of TCF7L2 in pancreatic islets (11,30,57). The T2D-associated SNP rs7903146 has also been associated with pancreatic islet morphology, i.e. with reduced islets density, reduction of islet size and an increased α- to β-cell ratio in risk-genotype carriers (31). In the same report, an association between the rs7903146 and GSIS was seen; but not with insulin content per islet equivalent as shown in our study. There may be several explanations for these discrepancies but a large number of high-quality human islets are probably needed to demonstrate a significant reduction in insulin content, as the state of the islets can have a large influence on insulin measurements.

Some recent reports have questioned the dogma that genetic variants in TCF7L2 exert its major effect on islets (23,58). Boj et al. recently reported reduced hepatic glucose production in a liver-specific Tcf7l2 knockout mouse model (23). We and others have shown an association between the rs7903146 variant and impaired suppression of hepatic glucose production (7,59,60), but it is not easy to disentangle these effects from the effects of a reduction in insulin secretion (61). Surprisingly, they reported no impaired effects on β-cell function in a β-cell-specific mice model (23). This finding is in sharp contrast with previous reports showing reduced β-cell mass, a perturbed incretin response and GSIS with reduced insulin expression (24,27). Although there are no apparent explanations for these discrepant results they could reflect the application of B-cell-specific versus whole-islet knockdown strategies, as TCF7L2 is also expressed in α-cells (55), or a difference between inducible versus congenital knockdown of TCF7L2, as Wnt signaling is important for pancreas development (62). An alternative explanation is a possible compensatory increased expression of other genes taking over the role of TCF7L2 in the β cells, in support is the reported lack of regulation of well-known TCF7L2-target genes, i.e. Axin2 and Sp5 (23). TCF7L2 is expressed in many tissues besides the pancreas and here is no doubt that it has central functions in the brain (58,63,64), liver (23,65,66), adipose tissue (67,68) and the gut (58,69). However, given the central role of insulin for maintenance of normal glucose tolerance it is unlikely that an extrapancreatic mechanism could exert an effect size of that seen for the rs7903146 in TCF7L2.

Interestingly, in human islets we observe a negative association between TCF7L2 expression and target gene expression in the proinsulin synthesis pathway (Fig. 1D), but also find that silencing total expression of TCF7L2 leads to reduced proinsulin expression (Fig. 2C). Experimental disruption of TCF7L2 activity has previously been associated with perturbed islet function (8,11,24,29–31) but the risk T-allele is suggested to lead to increased expression of TCF7L2 (Fig. 1C) (7,22,31,32) and perturbed GSIS in human islets (Fig. 1A) (2,7–12,31). These seemingly contradictory findings might reflect that total expression of TCF7L2 does not mirror TCF7L2 activity and rather it is the ratio of activating and inhibiting TCF7L2 isoforms that determine the activity and hence target gene regulation (33). The rs7903146 is located near the variable exon 4 (also named 3a), an exon that have particularly high expression in human islets compared with other tissues (4). This exon has also been shown to exhibit regulatory properties, inhibiting TCF7L2-target gene expression in different cell lines (70,71). Loss of exon 4 also promotes hepatic tumorigenesis (72). Future experiments would be warranted to investigate if increased expression of exon 4 containing or other isoforms (67) of TCF7L2 may propagate the risk of T2D conferred by the T-allele of rs7903146.

In summary, in rodents as well as human pancreatic islets, ISL1 is a direct target of TCF7L2 and ISL1, in turn, regulates pro-insulin production and processing via regulation of PCSK1, PCSK2, SLC30A8, MAFA, PDX1 and NKX6.1. Furthermore, TCF7L2 might also influence hepatic clearance of insulin via its effect on SLC30A8. Taken together, the current results provide some explanations for the large impact of TCF7L2 on the pathogenesis of T2D demonstrating that TCF7L2 is a key regulator of (pro)insulin synthesis, processing and possibly clearance.

MATERIALS AND METHODS

RNA sequencing

Sample preparation was made using Illumina TrueSeqâ™ RNA sample preparation kit with 1 µg of high-quality total RNA and was sequenced using a paired-end 101 bp protocol on the HiSeq2000 platform (Illumina). For INS1 cells, reads were aligned using Top Hat 2.0.0 (73) and Bowtie version: 2.0.5 (74) to the rat RGSC3.4 genome assembly. For human islet, 66 samples (all non-diabetic) were sequenced. Sequencing reads were aligned to the human reference genome (hg19) with STAR version 2.3.0e (75) and counted using HTSeq v0.5.4 and edgeR (76). The raw sequence reads have been deposited at European Nucleotide Archive (ERA261116) and at GEO (GSE50398) for INS1 and human islets, respectively.

Real-time QPCR and western blot

Total RNA was extracted using RNasy Plus Mini Kit (Qiagen), cDNA was prepared using RevertAidâ”¢ First Strand cDNA Synthesis Kit (Thermo Scientific). QPCR was performed using TaqMan assays (Supplementary Material, Table S5). mRNA was measured 48 h (INS1 cells) or 24 h (human islets) after silencing. For human islets, dispersed human islet cells in 804G-conditioned medium-coated dishes (77) were used. Protein (15–80 µg) was separated by SDS–PAGE, transferred to nitrocellulose membranes and detection was made using SuperSignalWestFemto Chemiluminescent Substrate (Thermo Scientific). Protein expression was measured 72 h or 7 days after silencing. Antibodies used can be found in Supplementary Material, Table S5.
Statistical analysis

Statistical analyses were performed in IBM® statistics version 21 (IBM Corporation, Armonk, NY, USA). Student’s t-test was used when two groups were compared and paired t-test was used for analysis of GSI, GSIPs and TIRFM data. P < 0.05 was regarded as significant and two-tailed P-values were calculated. FDR of 5% was used to correct for multiple comparisons. All results are given as mean ± SEM unless otherwise stated.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

ACKNOWLEDGEMENTS

We give our most sincere thanks to Mar González-Porta, Karl Bacos, Jens Lagerstedt, Anna-Maria Veljanovska Ramsay, Ulrika Krus, Jasmira Kravic, Lena Eliasson, Anders Rosengren, Emma Ahlvqvist and Peter Osmark and Alex Persson (Lund University) for constructive suggestions and technical assistance. The human islet was provided from the Human Tissue Laboratory at Lund University in collaboration with Olle Korsgren (Uppsala University) and the Nordic Network for Clinical Islet Transplantation.

Conflict of Interest statement. None declared.

FUNDING

This work has been supported through funding from the Swedish Research Council, grants (number 2007-4037, 2009-2001, 2007-5721, 2008-6589 and 2009-1039) the Knut and Alice Wallenberg Foundation (KAW 2009.0243), the Wallenberg foundation, the Lundberg foundation (grant nr. 359), the Swedish Diabetes Research Foundation (2009-060) and the European Commission’s Seventh Framework Program fund (the CEE3D consortium, Health-2008-223211), the UMAS fund, Novo Nordisk Foundation, Magus Bergvall’s Foundation, Craaford Foundation, Tore Nilson’s Foundation, the Hjelt Foundation, EFSD/MSD, the United States Public Health Service and by the National Institutes of Health and the Kovler Family Foundation. Funding to pay the Open Access publication charges for this article was provided by Swedish Research Council grants 2009-1039.

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